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RESEARCH**

APPLICATION NUMBER:

22-011

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY REVIEW

NDA: 22011 **SN:** 000 **DATE REVIEWED:** 10/23/06

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

NDA #: 22011

Serial #: 000

Applicant Name and Address: Idenix Pharmaceuticals, Inc.
60 Hampshire Street
Cambridge, MA 02139

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

Initial Submission Dates:

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Product Name(s):

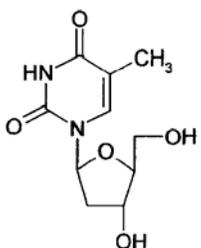
Proprietary: SEBIVO™

Non-Proprietary/USAN: Telbivudine

Code Name/Number: L-dT

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

Structural Formula:



Telbivudine

Molecular Formula: C₁₀H₁₄N₂O₅

Molecular Weight: 242.23

Dosage Form(s): 600 mg tablet

Route(s) of Administration: Oral

Indication(s): Treatment of chronic hepatitis B in patients with evidence of viral replication and active liver inflammation

Recommended Dosage: 600 mg once daily

Dispensed: Rx OTC (Discipline relevant)

Abbreviations: A549, human lung carcinoma; ABC, abacavir; ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AZT, zidovudine; BFU-E, burst-forming units-erythroid; CC₅₀, 50% cytotoxic concentration; CFU-GM, colony-forming units-granulocyte-macrophage; CV-1, African green monkey kidney fibroblast cell; Daudi, Burkitt's B cell

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lymphoma; d4T, stavudine; ddC, zalcitabine; ddI, didanosine; DHBV, duck hepatitis B virus; EBV, Epstein Barr virus; EC₅₀, 50% effective concentration; ELISA, enzyme-linked immunosorbent assay; ETV, entecavir; FIAU, fialuridine; FTC, emtricitabine; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HFF, human foreskin fibroblast; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IC₅₀, 50% inhibitory concentration; ITT, intent-to-treat; KB, human nasopharyngeal carcinoma; L-dT, telbivudine; LAM, lamivudine; MA-104, Rhesus monkey kidney epithelial cell; MDCK, canine kidney epithelial cell; mtDNA, mitochondrial DNA; NRTI, nucleoside reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; QS, quantitation standard; RT, reverse transcriptase; PDH, primary duck hepatocyte; RSV, respiratory syncytial virus; TFV, tenofovir; VZV, varicella zoster virus; WHsAg, woodchuck hepatitis virus serum antigen; WHV, woodchuck hepatitis virus; ZDV, zidovudine

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EXECUTIVE SUMMARY

Telbivudine, a synthetic thymidine nucleoside analog, demonstrated inhibitory activity against hepatitis B virus (HBV) replication with EC_{50} and EC_{90} values of 0.19 μ M and 0.98 μ M, respectively, in the HBV stably-transfected human liver HepG2.2.15 cell line. Like other nucleoside analogs, telbivudine is phosphorylated by cellular kinases to the active triphosphate form that can be incorporated into HBV DNA by competing with the natural substrate, thymidine 5'-triphosphate. Incorporation of telbivudine-TP causes DNA chain termination, resulting in inhibition of HBV DNA synthesis. In HepG2.2.15 liver cells, telbivudine inhibited both HBV first (EC_{50} value = 1.308 ± 1.567 μ M) and second strand synthesis (EC_{50} value = 0.237 ± 0.206 μ M). In biochemical assays telbivudine-TP inhibited the endogenous human, duck, and woodchuck hepatitis viral DNA polymerase (reverse transcriptase, RT) with IC_{50} values of 1.0 μ M, 0.46 μ M, and 0.24 ± 0.09 μ M, respectively, while no inhibition was observed with human cellular DNA polymerases α , β , or γ at concentrations up to 100 μ M.

Telbivudine was not cytotoxic to numerous cell lines of human and other mammalian origin at the highest concentration tested (50 to 2,000 μ M), including stationary HepG2.2.15 cells (CC_{50} value >2,000 μ M) and human PBMCs (CC_{50} value >200 μ M). In HepG2 cells exposed to telbivudine at concentrations up to 10 μ M, no appreciable mitochondrial toxicity was observed: no differences were observed in mitochondrial DNA content, lactic acid levels produced, and mitochondrial morphology.

Cell-based drug combination studies showed that the anti-HBV activity of telbivudine was additive in combination with adefovir and was not antagonized by the HIV NRTIs didanosine and stavudine. Telbivudine is not active against HIV-1 (EC_{50} value >100 μ M) and was not antagonistic to the anti-HIV activity of abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, or zidovudine.

Cell-based cross-resistance studies indicated significant cross resistance between lamivudine and telbivudine. Telbivudine was inactive against lamivudine-resistant HBV strains containing either the rtM204I mutation or the rtL180M/rtM204V double mutation (fold changes in EC_{50} values of $\geq 1,000$). However, telbivudine exhibited little to no loss of anti-HBV activity against the rtM204V single mutation with the calculated fold changes in EC_{50} values of 1.2 ± 0.4 . HBV containing the adefovir-resistance substitution rtA181V showed 3- to 5-fold reduced susceptibility to telbivudine, while HBV containing the adefovir-resistance substitution rtN236T remained susceptible to telbivudine.

The Phase III clinical study, Study NV-02B-007, examined the efficacy of telbivudine 600 mg once daily compared to lamivudine (LAM) 100 mg once daily 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. FDA analyses indicated that antiviral efficacy of telbivudine at Week 52 was not inferior to LAM in HBeAg-positive and HBeAg-negative patients, confirming the Applicant's conclusion. HBeAg-positive telbivudine-treated patients achieved a mean serum HBV DNA reduction of 6.44 ± 2.01 \log_{10} copies/mL, compared to a mean reduction of 5.46 ± 2.55 \log_{10} copies/mL for LAM-treated patients. In HBeAg-negative

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patients, the mean HBV DNA reductions were $5.18 \pm 1.90 \log_{10}$ copies/mL and $4.37 \pm 2.08 \log_{10}$ copies/mL for telbivudine and LAM, respectively.

In the HBeAg-positive patient population, 65.4% (291/445) of telbivudine recipients were suppressed with serum HBV DNA <1,000 copies/mL and maintained viral suppression by Week 52, compared to 45.9% (209/455) of HBeAg-positive LAM recipients. In the HBeAg-negative patient population, 91.1% (214/235) of telbivudine recipients and 77.6% (180/232) of LAM recipients were suppressed and maintained. More importantly, 57.8% (257/445) of HBeAg-positive telbivudine recipients achieved HBV DNA clearance to PCR nondetectable levels (≤ 300 copies/mL HBV DNA) and maintained viral clearance by Week 52, compared to 37.8% (172/455) of HBeAg-positive LAM recipients. In the HBeAg-negative patient population, maintained HBV DNA clearance to PCR nondetectable levels was achieved by 88.5% (208/235) of telbivudine recipients versus 70.7% (164/232) of LAM recipients.

The rates of virologic failure ($\geq 1,000$ copies/mL at Week 52) and virologic rebound ($\geq 1 \log_{10}$ increase of serum HBV DNA from nadir while on therapy) were lower for telbivudine 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 telbivudine recipients versus 53.2% (233/438) of LAM recipients. In the HBeAg-negative patient group, 8.4% (19/227) of telbivudine recipients and 21.5% (48/223) of LAM recipients experienced virologic failure. Virologic rebound was observed in 7.9% (34/430) of telbivudine 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 telbivudine recipients and 16.6% (37/223) of LAM recipients.

Of the 164 patients who showed evidence of virologic failure to telbivudine treatment, the paired amino acid sequences of HBV RT from the screen and on-treatment samples of the 115 evaluable patients were submitted with this application. Amino acid substitutions emerged in the HBV RT from the viruses of 87 patients out of the 115 patients with the average number of changes per patient of 3.4 ± 4.7 (391/115). The 3 most frequently observed changes were at codons 80, 181, and 204 that are highly conserved among HBV isolates. The mutations at codons 80 and 204 have already been associated with both genotypic and phenotypic resistance to LAM, and the mutation at codon 181 to adefovir.

Amino acid substitutions at codon 204 encoding rtM204 were detectable from the viruses of 46 patients (46/115, 40%): HBV variants with the rtM204I substitution were detectable from the viruses of 37 patients (80.4%) and the mixed variants, rtM204M/I or rtM204M/I/V, were present in the HBV DNA of 9 patients (19.6%). No rtM204V mutation, alone or in conjunction with the rtL180M mutation that is strongly associated with LAM resistance developed in response to telbivudine therapy. The rtM204I variants are strongly associated with virologic rebound: 34 patients of the 46 patients whose virus harbored the rtM204I substitution showed evidence of virologic rebound (73.9%). Of 46 patients with the rtM204I substitution, 44 patients had virus that carried other amino acid substitutions in the HBV RT domain. In particular, all patients whose HBV carried mutations at codons 80 (27 patients) or 229 (6 patients) were found to have the rtM204

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mutation.

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. Interestingly, the rtA181V variants, known to confer resistance to ADV and reduced susceptibility to telbivudine in cell culture, were not detected. Of 16 patients, 1 patient also carried the rtM204I/M mutation and showed evidence of virologic rebound.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for telbivudine is approvable with respect to microbiology for the treatment of chronic hepatitis B in patients with evidence of viral replication and active liver inflammation.

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

- Determine the anti-HBV cell culture combination activity relationships of telbivudine with entecavir.
- Determine the anti-HBV combination activity relationships of telbivudine in cell culture with the HIV NRTIs abacavir, emtricitabine, lamivudine, tenofovir, zalcitabine, and zidovudine.
- Determine the susceptibility to telbivudine and adefovir of the HBV rtA181 variants, rtA181T and rtA181S.
- Determine the susceptibility in cell culture of HBV harboring the following mutations of highly conserved amino acid residues among HBV isolates: 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 $\geq 1,000$ copies/mL at Week 52) to telbivudine therapy.
- Determine the mitochondrial toxicity of telbivudine in growing muscle cells, cell lines and primary cells, and primary hepatocytes with appropriate controls to validate the results.
- Continue to perform genotypic and phenotypic analyses of HBV DNA from patients who experience virologic failure to long-term telbivudine therapy (serum HBV DNA levels $\geq 1,000$ copies/mL) in ongoing clinical trials.

2. Summary of OND Microbiology Assessments

2.1. Nonclinical Microbiology

Telbivudine is a synthetic thymidine nucleoside analog and phosphorylated by cellular kinases to the active triphosphate form (telbivudine-TP). Telbivudine-TP has a half-

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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 telbivudine-TP causes DNA chain termination, resulting in inhibition of HBV DNA synthesis.

Telbivudine is an inhibitor of both HBV first and second strand synthesis (EC_{50} values of $1.308 \pm 1.567 \mu\text{M}$ and $0.237 \pm 0.206 \mu\text{M}$, respectively, in stably transfected HepG2.2.15 cells). Telbivudine did not inhibit the HBV priming reaction.

Telbivudine exhibited antiviral activity against HBV in stationary HepG2.2.15 cells, reducing extracellular HBV DNA with EC_{50} and EC_{90} values of $0.19 \mu\text{M}$ ($0.046 \mu\text{g/mL}$) and $0.98 \mu\text{M}$, respectively. The EC_{50} and EC_{90} values of telbivudine against DHBV in primary duck hepatocytes were $0.18 \mu\text{M}$ and $1.0 \mu\text{M}$, respectively.

Telbivudine was not effective against other human viruses tested including human immunodeficiency virus (HIV-1), suggesting that telbivudine is a specific inhibitor for HBV.

Telbivudine-TP did not inhibit human cellular DNA polymerases α , β , or γ in biochemical reactions at concentrations up to $100 \mu\text{M}$.

Telbivudine was not cytotoxic to numerous cell lines of human and other mammalian origin at the highest concentration tested, including stationary HepG2.2.15 cells (CC_{50} value $>2,000 \mu\text{M}$) and human PBMCs (CC_{50} value $>200 \mu\text{M}$). In addition, telbivudine at concentrations of $>10 \mu\text{M}$ had no inhibitory effect on the growth of human bone marrow progenitor cells. No mitochondrial toxicity was observed in HepG2 cells treated with telbivudine at concentrations up to $10 \mu\text{M}$.

Telbivudine exerted additive antiviral effects when combined with adefovir in a stably transfected cell line, HepG2 49-29. No evidence of cytotoxicity or antagonism was observed at the tested concentrations.

Cell-based drug combination studies demonstrated that telbivudine did not enhance or reduce the antiviral efficacy of all seven FDA-approved HIV NRTIs against HIV-1 with the calculated fold changes in EC_{50} values ranging from 0.8- to 1.5-fold. HIV NRTIs didanosine and stavudine exhibited no antagonistic effect on the cell culture antiviral activity of telbivudine against HBV.

2.2. Clinical Microbiology

In Study NV-02B-007, antiviral efficacy of telbivudine (600 mg once daily) was compared to lamivudine (100 mg once daily) separately in the HBeAg-positive and HBeAg-negative patient populations.

At Week 52, HBeAg-positive telbivudine-treated patients achieved a mean serum HBV DNA reduction of $6.44 \pm 2.01 \log_{10}$ copies/mL, compared to a mean reduction of $5.46 \pm 2.55 \log_{10}$ copies/mL for lamivudine-treated patients. In HBeAg-negative patients,

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The rates of virologic failure ($\geq 1,000$ copies/mL at Week 52) and virologic rebound ($\geq 1 \log_{10}$ increase of HBV DNA from nadir while on therapy) were lower for telbivudine recipients, compared to lamivudine 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 telbivudine recipients versus 53.2% (233/438) of lamivudine recipients. In the HBeAg-negative patient group, 8.4% (19/227) of telbivudine recipients and 21.5% (48/223) of lamivudine recipients experienced virologic failure. Treatment-emergent virologic rebound was observed in 7.9% (34/430) of telbivudine recipients, compared to 23.5% (233/455) of lamivudine recipients in the HBeAg-positive patient population, while in the HBeAg-negative patient population, it was observed in 4.9% (11/227) of telbivudine recipients and 16.6% (37/223) of lamivudine recipients.

In the paired sequence analysis of baseline and on-treatment samples, 75.7% (87/115) of patients with evidence of virologic failure had genotypic changes in the HBV reverse transcriptase, including amino acid substitutions rtL80I/V, rtL180M, rtA181T, rtM204I, and rtL229W/V. All patients whose virus carried mutations at codons 80 (27 patients), 229 (6 patients), or 180 (4 patients) were found to have the rtM204 substitution.

Of the 115 patients receiving telbivudine with virologic failure whose paired baseline and on-treatment genotypes were submitted with this application, 46 patients (40.0%) had mutations at codon 204, rtM204. The rtM204I variants were detectable by population nucleotide sequence analysis in the viruses from 37 patients (80.4%), and the mixed variants, rtM204M/I or rtM204M/I/V, were detectable in the HBV DNA from 9 patients (19.6%). There was no emergence of the rtM204V substitution in response to telbivudine therapy. The mutation profile for telbivudine in the subset of patients with the rtM204 mutation is similar to that for lamivudine with the exception of the rtM204V mutation.

Sixteen patients (13.9%) of the 115 patients had mutations at codon 181, rtA181: the mixed variants, rtA181T/A, were detectable from the viruses of 8 patients (50.0%),

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and the rtA181T and rtA181S variants were of 7 (43.8%) and of 1 (6.3%) patients, respectively. No variants with rtA181V were detected.

3. Administrative

3.1. Reviewer's Signature(s)

Sung S. Rhee, Ph.D.
Microbiologist

3.2. Concurrence

_____ Date: _____
HFD-530/Assoc Dir/J. Farrelly

_____ Date: _____
HFD-530/MicroTL/J. O'Rear

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

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OND MICROBIOLOGY REVIEW

1. Introduction and Background

Chronic hepatitis B virus (HBV) infection causes necroinflammatory liver disease, cirrhosis, and hepatocellular carcinoma. Chronic HBV infection, detected as chronic seropositivity for HBV surface antigen (HBsAg), remains a widely prevalent global health problem with an estimated 350-400 million carriers worldwide. The World Health Organization estimates that more than 4 million new cases of chronic HBV infection occur every year and approximately 25% of chronic HBV carriers eventually die from chronic active hepatitis, cirrhosis, or primary liver cancer. In China, Southeast Asia, and sub-Saharan Africa, as many as 10 to 15% of the population are chronically infected with HBV. In North America and northern Europe, HBV chronic carrier rates are much lower, usually below 1 %. Intermediate HBV carrier rates of 1 to 7% are found in parts of Southern and Eastern Europe, Central and South America, the Middle East, and parts of Japan (Moyer and Mast, 1994; Shapiro, 1993).

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

Four therapeutic agents have received regulatory approval for the treatment of chronic hepatitis B virus infection: interferon-alpha, adefovir dipivoxil, entecavir, and lamivudine. 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. Clearance of HBV is not thought to be possible with currently approved therapies.

The Applicant identified several L-nucleosides that are specific and potent inhibitors of HBV replication in cell culture (Bryant *et al.*, 2001). Two of these compounds, telbivudine (L-dT) and valtorcitabine (monoval-L-dC), are currently being evaluated in clinical trials under U.S. Investigational New Drug authorization and other global regulatory authorizations. Telbivudine is a synthetic nucleoside analogue that is phosphorylated to its active metabolite, telbivudine-5'-triphosphate, by cellular kinases. Telbivudine-5'-triphosphate inhibits HBV reverse transcriptase by competing with the

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natural substrate, deoxythymidine-5'-triphosphate. Incorporation of telbivudine-5'-triphosphate into viral DNA causes DNA chain termination, resulting in inhibition of HBV replication. The Applicant has conducted an extensive global development program for telbivudine in the treatment of chronic HBV infection.

The NDA package for telbivudine contains study reports and datasets for the pivotal Phase III study (NV-02B-007) conducted at multiple sites (112), both within the U.S. and at international sites. This 007 GLOBE study is a randomized, double-blind trial of telbivudine 600 mg once daily compared to lamivudine 100 mg once daily for a treatment period of up to 104 weeks in adults chronically infected with HBV and having compensated liver disease but never treated with lamivudine or an investigational anti-HBV nucleoside or nucleotide analog. The overall study hypothesis is that telbivudine treatment would provide superior clinical and virologic efficacy for patients chronically infected with HBV (both HBeAg-positive and HBeAg-negative), while exhibiting a clinical safety profile similar to, or better than, the safety profile of lamivudine. The primary data analysis was conducted after all patients had reached Week 52. Data from the second year of the study to Week 104 are considered important to assess the longer-term efficacy, safety, and viral resistance for telbivudine.

2. Materials and Methods

Quantification of Serum HBV DNA Levels

Serum HBV DNA levels were quantified using the COBAS Amplicor HBV Monitor™ PCR assay kit (Roche Laboratories) at (b) (4). The COBAS Amplicor HBV Monitor Test permits simultaneous PCR amplification of HBV target and HBV Quantitation Standard (QS) DNA. HBV QS DNA is a noninfectious linearized plasmid that contains the identical primer binding sites as the HBV DNA target and a unique probe binding region that allows the QS amplicon to be distinguished from an HBV amplicon. The COBAS Amplicor Analyzer calculates the HBV DNA levels in the test specimens by comparing the HBV signal to the QS signal for each specimen.

The COBAS Amplicor HBV Monitor™ PCR assay was reported by the manufacturer to give a linear response from 300 (lower limit of quantification, 2.48 log₁₀ copies/mL) HBV DNA copies/mL to at least 200,000 (upper limit of quantification, 5.3 log₁₀ 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.

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 lamivudine arm and 2 in the telbivudine arm) infected with HBV genotype F.

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Plasma or serum can be stored at room temperature (18-25°C) for up to 72 hours, 2-8°C for up to 7 days, or frozen at -20° to -80°C for at least 6 weeks. Specimens can be frozen and thawed up to 4 times without loss in copy number.

Nucleotide Sequence Analysis of the HBV Reverse Transcriptase (RT)

The complete RT-domain of the HBV polymerase gene was amplified by PCR using the Expand High Fidelity kit (Roche Laboratories). The kit permits amplification of PCR fragments of considerable length at a very low error level during amplification, which is necessary for the ~1200 bp RT domain of the HBV polymerase gene. Therefore, the kit provides highly reliable nucleotide sequence data.

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.

Excess PCR primers and nucleotides were removed from the PCR-amplified samples with ExoSap-IT® (GE Healthcare Life Sciences), which were then subjected to sequence analysis using a set of forward and reverse primers with the ABI BigDye® Terminators v1.1 cycle sequencing kit (Applied Biosystems). A full set of 6 forward and reverse primers was used to cover the entire fragment and generate highly reliable overlapping sequences. The sequencing reaction products were purified using DyeEx gel filtration columns (Qiagen) and then electrophoresed on a 16-channel Applied Biosystems 3100 Genetic Analyser using a 50 cm capillary and Pop-6 polymer (Applied Biosystems). The multiple overlapping sequence data were then assembled into a composite using the SeqScape software version 2.1.1.

For the assembly protocol, a reference HBV sequence [REDACTED] (b) (4) was used as a template. Since this reference sequence contained one ambiguity, the Applicant used a different genotype C reference sequence [REDACTED] (b) (4) for the SAS transport file for the NDA submission.

HBV Genotype Determination at Screen

HBV DNA was isolated from the sera obtained from all 007 patients at Screen and the entire RT domain of the HBV polymerase gene was subjected to nucleotide sequence analysis as described above.

The HBV genotype of the viruses isolated from each patient was determined based on the homology scores obtained at the nucleotide sequence level against the 8 reference sequences (Table 1, Study NV-02B-RES1, Page 57). The homology score of 800 to 1,000 was typically obtained when two closely matched sequences of the same genotype are compared. The homology score was lowered by ~400, when compared to the next closest genotype reference sequences. The genotype of the viruses was assigned to the genotype of the reference sequence that gives the highest homology

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score. When the difference between the two scores, the highest and second highest, was less than 100, the Applicant assigned the viruses to both genotypes.

Table 1: HBV reference sequences representing genotypes A-H

HBV Genotype	Reference Sequence	GenBank Accession Number
A		(b) (4)
B		
C		
D		
E		
F		
G		
H		

HBV e Antigen (HBeAg) Detection

HBeAg was qualitatively detected in serum or plasma samples of patients using the AxSYM HBe 2.0 microparticle enzyme immunoassay system (Abbott Laboratories) at

(b) (4)

Samples with S/CO (sample rate/cut-off rate) values greater than 1.0 were considered reactive and reported as HBeAg positive, while samples with S/CO values less than 0.82 were considered nonreactive and reported as HBeAg negative. Samples with S/CO values greater than 0.82 and less than 1.0 were reported as Indeterminate and retested in duplicate. For retesting a new sample was collected.

3. Nonclinical Microbiology

Mechanism of Action

Hepadnaviruses utilize a reverse transcription-mediated replication cycle to convert a pregenomic RNA template into a partially double-stranded circular DNA genome that is characteristic of hepatitis B viruses (HBV; Ganem, *et al.* 1994). Like other nucleoside analogs, telbivudine (L-dT), a synthetic thymidine nucleoside analog, is phosphorylated by cellular kinases to the active triphosphate form (telbivudine-TP) that can be incorporated into HBV DNA by competing with the natural substrate, thymidine 5'-triphosphate. Incorporation of telbivudine-TP causes DNA chain termination, resulting in inhibition of HBV DNA synthesis. Telbivudine-TP has a half-life of 14 ± 1.4 h in stationary HepG2 cells (Study 02-CP-001C). The IC_{50} values of telbivudine-TP in the endogenous human, duck, and woodchuck hepatitis viral DNA polymerase biochemical assays were 1.0 μ M, 0.46 μ M, and 0.24 ± 0.09 μ M, respectively.

(b) (4)

The *in vitro* priming assay with duck hepatitis B virus

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(DHBV) revealed no inhibitory effect of telbivudine-TP on the HBV priming reaction (Study IDIX-04-194). In stably transfected HepG2.2.15 liver cell systems harboring integrated HBV genomes and constitutively producing HBV virion particles (Study IDIX-04-194), telbivudine is an inhibitor of both HBV first (EC_{50} value = $1.308 \pm 1.567 \mu\text{M}$) and second strand synthesis (EC_{50} value = $0.237 \pm 0.206 \mu\text{M}$) using a strand-specific Southern hybridization analysis.

Anti-HBV Activity in Cell Culture

Telbivudine exhibited potent antiviral activity against HBV replication in stationary HepG2.2.15 cells, reducing extracellular HBV DNA with EC_{50} and EC_{90} values of $0.19 \mu\text{M}$ ($0.046 \mu\text{g/mL}$) and $0.98 \mu\text{M}$, respectively (Table 2, Study 02-CP-001A, Page 6). Intracellular HBV DNA replicative intermediates (RI) were also reduced with EC_{50} and EC_{90} values of $0.29 \mu\text{M}$ and $1.4 \mu\text{M}$, respectively. The EC_{50} and EC_{90} values of telbivudine against DHBV in primary duck hepatocytes were 0.18 and $1.0 \mu\text{M}$, respectively. In contrast, telbivudine was not effective against other human viruses tested including HIV-1 (Table 2), suggesting that telbivudine is a specific inhibitor for HBV and related Hepadnaviruses such as DHBV and woodchuck hepatitis virus (WHV).

Table 2: Antiviral Activity and Cytotoxicity of Telbivudine

Virus (Cell line)	EC_{50}^a (μM)	CC_{50} (μM)
HBV (2.2.15) ^b	0.19	> 2000
DHBV (PDH)	0.18	ND ^c
HIV-1 (PBMC)	> 100	> 100
HSV-1 ^e (HFF ^d)	> 100	> 100
HSV-2 ^e (HFF)	> 100	> 100
VZV ^e (HFF)	45.2	> 100
EBV ^e (Daudi)	> 50	> 50
HCMV ^e (HFF)	> 100	> 100
Influenza A/H1N1 (MDCK)	> 100	> 100
Influenza A/H3N2 (MDCK)	> 100	> 100
Influenza B (MDCK)	> 100	> 100
Measles (CV-1)	> 100	> 100
Parainfluenza type 3 (MA-104)	> 100	> 100
RSV type A (MA-104)	> 100	> 100

Telbivudine was 8.8-fold more potent in inhibiting the replication of HBV in growing HepG2 cells than in stationary phase (confluent) cells, while the antiviral activity of ADV (PMEA) and LAM was largely independent of cell growth conditions (Table 3, Study IDIX-04-190, Page 15). The mechanism behind the cell growth effect on the telbivudine antiviral activity has not been determined, but the Applicant speculated that it may be related to the activities of cellular kinases that convert telbivudine to the monophosphate product. This differential activity of telbivudine in growing cells compared to stationary cells may be important for the control of HBV infection under conditions where the immune-mediated clearance of infected liver cells is followed by the outgrowth of

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uninfected liver cells (Summers *et al.* 2003).

Table 3: Antiviral Activity of Telbivudine against HBV in Rapidly Growing versus Stationary Phase Cells

Growth Conditions	Drug			
	<i>LdT</i> ^a	<i>LdC</i> ^b	<i>Lam</i> ^c	<i>PMEA</i> ^d
	<i>EC</i> ₅₀ (μM)	<i>EC</i> ₅₀ (μM)	<i>EC</i> ₅₀ (μM)	<i>EC</i> ₅₀ (μM)
Growth	0.053 ± 0.162	0.137 ± 0.029	0.014 ± 0.006	0.106 ± 0.047
Confluent	0.468 ± 0.063	0.086 ± 0.072	0.015 ± 0.002	0.158 ± 0.122

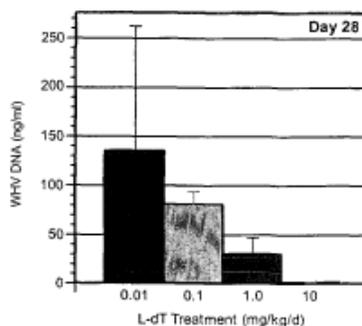
Numbers represent mean value ± SD derived from 2 to 5 independent experiments as indicated: ^a = 5; ^b = 3; ^c = 4; ^d = 2.

Antiviral Activity in the Woodchuck/WHV Model

HBV infects only humans and other higher primates making it difficult to study drugs that act against HBV. Woodchucks chronically infected with WHV have proven to be useful in the evaluation of anti-HBV agents (Genovesi *et al.* 1998) and is valued as a positive predictor of antiviral efficacy for therapies of chronic HBV infection (Korba *et al.* 2000). In addition, the woodchuck model has been shown to be a sensitive system for evaluation of the safety of nucleoside analogs (Tennant *et al.* 1998). In 4- (Study NC-NV-02B-007) and 12-week (Study NC-NV-02B-008) studies of WHV-infected woodchucks (>10¹¹ genome equivalents/mL serum), telbivudine significantly reduced serum WHV DNA levels.

In the 4-week study, oral doses of telbivudine ranging from 0.01 to 10 mg/kg/day once-daily for 4 weeks in woodchucks resulted in significant reductions in serum WHV DNA levels and a positive correlation of viral load reduction with increasing dose was observed (Figure 1, Study NC-NV-02B-007, Page 6). Notably, serum WHV DNA levels in the telbivudine 10 mg/kg/day treatment group (n=3) decreased by as much as 8 log₁₀ from baseline to below the limit of detection by the WHV PCR assay (<300 genome equivalents/mL serum). Following drug withdrawal, virus rebound occurred and reached near pre-treatment levels by 4 to 8 weeks post-treatment (Figure 2A, Study NC-NV-02B-008, Page 8).

Figure 1: Antiviral dose response of telbivudine at Day 28 of treatment in the WHV/woodchuck model of chronic HBV infection



Data are presented as mean (n=3) ± standard deviation. One ng of WHV DNA is equivalent to 2.7 x 10⁸ WHV genomes.

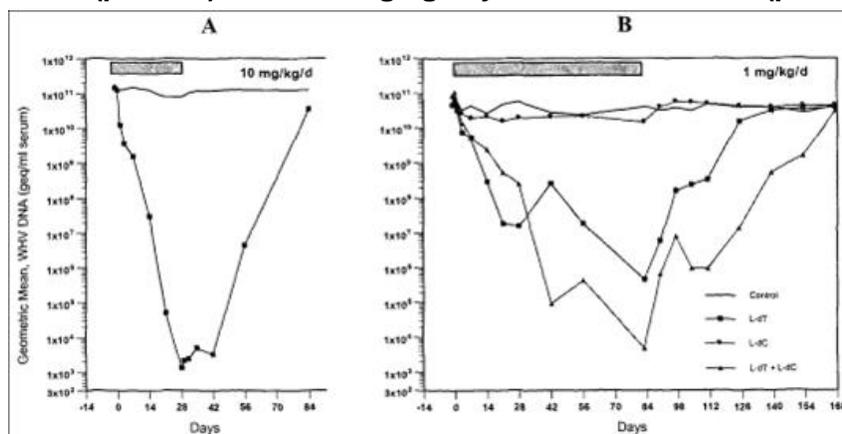
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When telbivudine (1 mg/kg/day) was given orally to woodchucks for 12 weeks (N=4), the decrease in serum WHV DNA was more gradual than in animals that received 10 mg/kg/day telbivudine for 4 weeks (Figure 2B, Study NC-NV-02B-008, Page 8). WHV viral load decreased 4 to 5 log₁₀ by the end of treatment and returned to near baseline by 6 weeks post-treatment. L-dC, a cytidine analog of telbivudine, showed minimal to no activity against WHV alone, but increased the reductions in serum WHV DNA over telbivudine alone. Serum WHsAg levels also declined throughout the 12-week treatment period, decreasing 0.5 to 1.5 log₁₀ from baseline levels.

Figure 2: Antiviral activity of telbivudine when administered at 10 mg/kg/day for 4 weeks (panel A) versus 1 mg/kg/day dose for 12 weeks (panel B)

**Cytotoxicity**

Conventional cell-based assays were used to assess the cytotoxicity of telbivudine and any metabolites. Telbivudine was not cytotoxic to numerous cell lines of human and other mammalian origin at the highest concentration tested (50 to 2,000 μ M; Table 2, Study 02-CP-001A, Page 6), including stationary HepG2.2.15 cells (CC₅₀ value >2,000 μ M) that is routinely used to determine the anti-HBV activity of potential antiviral agents and human PBMCs (CC₅₀ value >200 μ M).

Telbivudine-TP did not inhibit human cellular DNA polymerases α , β , or γ in biochemical reactions at concentrations up to 100 μ M (Study 02-CP-001A), approximately 100-fold higher than those required to inhibit HBV reverse transcriptase (RT).

Antiviral nucleoside analogs approved for HIV infection, such as didanosine (ddI; Yarchoan *et al.*, 1990), stavudine (d4T; Browne *et al.*, 1993), zalcitabine (ddC; Fischl *et al.*, 1993), and zidovudine (ZDV; Richman *et al.*, 1987), have been associated with clinically limiting delayed toxicities such as myopathy, pancreatitis, and peripheral neuropathy. These clinical adverse events have been attributed to inhibition of mitochondrial function due to nucleoside analog incorporation into mitochondrial DNA (mtDNA) and reduction in mtDNA content. In addition, a particular nucleoside analog, fialuridine (FIAU), caused hepatic failure, myopathy, neuropathy, pancreatitis, and lactic acidosis due to direct mitochondrial toxicity (McKenzie *et al.*, 1995). Drug-associated increases in lactic acid production are considered a marker of impaired mitochondrial

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function or oxidative phosphorylation. Thus, the potential of telbivudine to produce mitochondrial toxicity was assessed in the human liver cell line HepG2 by measuring lactic acid production and mtDNA content, and determining changes in morphology (e.g., loss of cristae, matrix dissolution and swelling, and lipid droplet formation) of mitochondrial ultrastructure. Results are summarized in Table 4 (Study 02-CP-001A, Page 7).

Table 4: Effect of Telbivudine on Hepatocyte Proliferation, Mitochondrial Function, and Morphology in HepG2 Cells

Compound	CC ₅₀ , μ M	Conc., μ M	% of Control		Lipid Droplet Formation	Mitochondrial Morphology
			L-Lactate	mtDNA		
Control		0	100	100	negative	normal
L-dT*	>2000	10	105 \pm 2	97 \pm 4	negative	normal
FIAU*	4	10	203	86	positive	abnormal
ZDV*	14	50	239	119	negative	abnormal
ddC*	20	1	95	13	negative	abnormal

Exposure of HepG2 cells for 14 days to telbivudine at concentrations up to 10 μ M had no effect on mtDNA content compared to an 87% reduction in the ddC-treated cells. No differences were observed in lactic acid levels produced in telbivudine-treated cells after exposure for 4 days and untreated control cells, whereas lactic acid production in the FIAU- and ZDV-treated cells increased by 100% compared to untreated control. No discernible changes in cell architecture or mitochondrial morphology were detected in telbivudine-treated cells after exposure for 14 days, while ZDV-treated cells showed typical swollen mitochondria and loss of cristae. Mitochondrial 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 telbivudine.

The potential myelosuppressive effects (e.g., anemia, neutropenia) of nucleoside analogs were tested by determining the inhibitory effect on the growth of human bone marrow progenitor cells in clonogenic assays. Zidovudine has been shown to directly inhibit human granulocyte-macrophage colony forming (CFU-GM) and erythroid burst forming (BFU-E) activity in cell culture at concentrations typically reached in patients (Berman *et al.*, 1989; Dornsife and Averett, 1996; Kurtzberg and Carter, 1990; Lerza *et al.*, 1997; Sommadossi and Carlisle, 1987; Weinberg *et al.*, 1998; Yoshida and Yoshida, 1990). Using human bone marrow clonogenic assays with zidovudine as a positive control, telbivudine was non-inhibitory of CFU-GM and BFU-E at concentrations of >10 μ M (Table 5, Study 02-CP-001A, Page 6).

Table 5: Cytotoxicity of Telbivudine in Granulocyte Macrophage Progenitor and Erythrocyte Precursor Cells

Compound	CFU-GM IC ₅₀ (μ M)	BFU-E IC ₅₀ (μ M)
L-dT	> 10	> 10
Lamivudine	> 10	> 10
Zidovudine	1.8	0.7

Values represent the results of three independent experiments in triplicate.

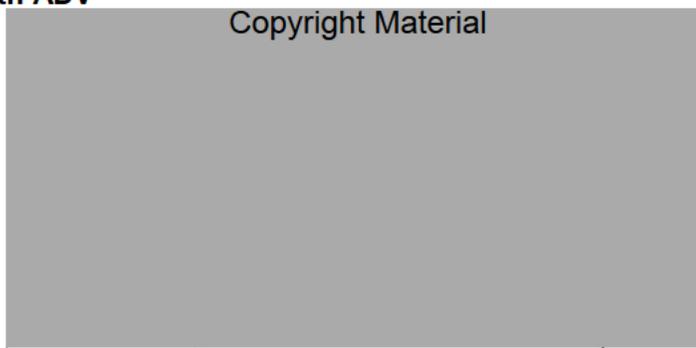
Serum Protein Binding

The *in vitro* plasma protein binding of telbivudine was determined using [¹⁴C]-telbivudine by ultrafiltration (Study 7245-106). Binding of telbivudine was low in rat, monkey, and human plasma with overall mean range of 3.3 to 7.5%, and was independent of telbivudine concentration over the range evaluated (0.4 to 40 µg/mL which corresponds to a 0.12- to 12-fold animal-to-human C_{max} exposure multiple).

Combination Activity Relationships of Telbivudine with Approved Drugs for Chronic HBV Infection

Recently, Delaney *et al.*, (2004) reported that telbivudine exerted additive antiviral effects when combined with ADV in a stably transfected cell line, HepG2 49-29, that expresses high levels of HBV replicative intermediates (Figure 3). No evidence of cytotoxicity or antiviral antagonism was observed at the tested concentrations. In these two-drug combination experiments, five concentrations were tested for each drug: the middle concentration was approximately equal to the EC₅₀ value for each drug (ADV EC₅₀ value = 0.215 ± 0.003 µM; telbivudine EC₅₀ value = 0.335 ± 0.042 µM); two higher concentrations corresponding to approximately 3 and 6 times the EC₅₀ values; and two lower concentrations corresponding to approximately 0.16 and 0.33 times the EC₅₀ values.

Figure 3: MacSynergy II Analysis of the Anti-HBV Activity of Drug Combinations Paired with ADV



Anti-HBV Combination Activity Relationships of telbivudine with Approved Antiretroviral NRTIs

With approximately 10% of HIV-1 infected individuals also co-infected with HBV, telbivudine may be co-administered with antiretroviral therapies including HIV NRTIs. Telbivudine 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. Telbivudine, like other nucleosides, does not interact with cytochrome P450 oxidases (Studies 7245-107 and 108) and is not therefore likely to influence the metabolism of drugs that do use this pathway.

Cell-based drug combination studies were performed to determine whether the inhibition

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of HBV replication by telbivudine is adversely affected when it is combined with HIV NRTIs using concentrations that meet or exceed the maximum concentration found in the blood of patients treated with these drugs. HIV NRTIs exhibited no antagonistic effect on the cell culture antiviral activity of telbivudine against HBV. Telbivudine combined with emtricitabine (FTC), LAM, tenofovir (TFV), or zidovudine (AZT) resulted in decreased HBV DNA levels with the calculated fold-changes in EC₅₀ values ranging from <0.06- to <0.41-fold (Study IDIX-05-115, Page 19).

In Study IDIX-05-115, the antiviral activity of telbivudine against HBV in the presence of HIV NRTIs was determined in a stably transfected HepG2-derived cell line, WT3/C1, constitutively producing HBV virion particles. Cells were treated for 10 days with telbivudine alone or in combination with one of the FDA-approved HIV NRTIs, and endogenous HBV RT activity was measured from cell lysates or intracellular HBV nucleocapsids. Results are summarized in Table 7 (Study IDIX-05-115, Pages 18-19).

Table 7: Anti-HBV Activity of Telbivudine in the Presence of HIV NRTIs

	Drug		
	<i>LdT</i>	<i>LdT + NRTI (low)</i>	<i>LdT + NRTI (high)</i>
	<i>EC₅₀ (μM)</i>	<i>EC₅₀ (μM)</i>	<i>EC₅₀ (μM)</i>
Lam	0.255 ± 0.115	<0.025 ± 0.007	<0.021 ± 0.000
FTC	0.385 ± 0.172	0.048 ± 0.005	<0.021 ± 0.000
TFV	0.329 ± 0.290	<0.185 ± 0.232	<0.021 ± 0.000
ABC	0.113 ± 0.032	0.223 ± 0.166	0.039 ± 0.012
AZT	0.277 ± 0.119	0.034 ± 0.018	<0.021 ± 0.000
d4T	0.383 ± 0.331	0.391 ± 0.384	0.030 ± 0.012
ddl	0.343 ± 0.375	0.332 ± 0.300	0.382 ± 0.393

Numbers represent mean values ± SD derived from 2 independent experiments.

Each NRTI was assayed at two fixed concentrations in the presence of a range of telbivudine concentrations. The “low” and “high” concentrations were individually determined in previous experiments. NRTIs with substantial anti-HBV activity (Group I) were assayed at 0.025 μM (low) and 0.25 μM (high) for FTC and LAM, and 0.25 μM (low) and 2.5 μM (high) for ABC and TFV. NRTIs without or with only weak anti-HBV activity (Group II) were assayed at two clinically relevant concentrations approximating C_{max} (low) and 5 times C_{max} concentrations (high), 1 and 5 μM for AZT, 6 and 30 μM for d4T, and 10 and 50 μM for ddl, respectively. AZT and d4T had measurable anti-HBV activity at concentrations of 1 μM for AZT or 30 μM for d4T, while ddl has no anti-HBV activity when tested up to 50 μM.

The mean EC₅₀ values for the seven telbivudine controls (no NRTI) ranged from 0.113 ± 0.032 to 0.385 ± 0.172 μM, in good agreement with previous results (EC₅₀ value = 0.43 ± 0.19 μM; Study IDIX-04-185). No negative interaction was observed between tested NRTIs and telbivudine. The Applicant did not provide data for the NRTIs alone so conclusions with respect to antagonism could not be made for these. Of note, the Applicant reported in the clinical phase IIb study (NV-02B-003) that the combination of telbivudine and LAM showed no advantage over telbivudine monotherapy.

Anti-HIV Combination Activity Relationships of telbivudine with Approved

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Antiretroviral NRTIs

Cell-based drug combination studies were performed to determine whether telbivudine adversely affected the anti-HIV activity of the 7 NRTIs approved for the treatment of HIV infection. Unlike ADV and LAM, which have activity against both HBV and HIV-1, telbivudine is inactive against HIV-1 and related viruses. Telbivudine did not enhance or reduce the antiviral efficacy of HIV NRTIs against HIV-1 with the calculated fold changes in EC₅₀ values ranging from 0.8- to 1.5-fold (Studies IDIX-05-114, Page 14).

Study IDIX-05-114 used MT-4 cells infected with HIV-1_{BH10}. Telbivudine was tested in these experiments at two fixed concentrations; at 13 μM, which approximates the mean steady-state C_{max} of telbivudine in patients receiving a 600 mg per day dose of telbivudine, and at 65 μM, representing about 5 times the mean C_{max} concentration value. Inhibition of virus propagation was determined 4 days after infection by quantifying HIV-1 p24 antigen in culture supernatants using an HIV-1 p24 ELISA assay (Table 6, Study IDIX-05-114, Page 13).

Table 6: Anti-HIV Activity of HIV NRTIs in the Presence of Telbivudine

Drug	NRTI ^a	NRTI + Ldt (13μM) ^b	NRTI + Ldt (65 μM) ^c
	EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)
lam	0.475 ± 0.393	0.445 ± 0.321	0.576 ± 0.230
ABC	0.968 ± 0.445	0.841 ± 0.045	1.214 ± 0.157
AZT	0.014 ± 0.004	0.013 ± 0.002	0.012 ± 0.002
d4T	0.233 ± 0.070	0.209 ± 0.059	0.323 ± 0.048
ddI	3.333 ± 1.014	3.003 ± 1.027	2.545 ± 0.412
FTC	0.133 ± 0.065	0.106 ± 0.030	0.109 ± 0.006
TFV	1.420 ± 0.673	1.597 ± 0.826	1.805 ± 0.469

Numbers represent mean values ± SD derived from two independent experiments.

When tested alone, the overall EC₅₀ values for each NRTI tested showed the expected anti-HIV activity in cell culture and were in good agreement with results reported in the NIAID chemical database. Telbivudine alone did not inhibit HIV-1 replication at either 13 or 65 μM, as reported previously (Standring *et al.*, 2001). The fold-changes in EC₅₀ values in the presence of 13 and 65 μM telbivudine are 1.0 and 1.5 for LAM, 1.0 and 1.4 for abacavir (ABC), 0.9 and 1.0 for AZT, 0.9 and 1.4 for d4T, 0.9 and 0.8 for ddI, 0.8 and 0.9 for FTC, or 1.1 and 1.3 for TFV, respectively. Thus, telbivudine does not appear to interfere with the anti-HIV activity of any of the FDA-approved NRTIs that are currently marketed for treatment of HIV-1 infection in patients.

Cross-Resistance with Approved Nucleoside Analogs for Chronic HBV Infection

There is currently no system for propagating HBV in cell culture. Therefore, preclinical resistance studies for new HBV antiviral agents are limited to determining the activity of the agent against transfected HBV genomes bearing mutations that confer clinically-relevant resistance to approved anti-HBV agents.

The single rtM204V and rtM204I amino acid substitutions found in the key YMDD active

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site motif of HBV RT, and the double mutants rtL180M/M204V and rtL180M/M204I appear to be the primary mutations seen to date that are responsible for resistance to LAM. When tested in cell culture, these mutants exhibited 153-fold (rtM204V) to >10,000-fold (rtM204I and rtL180M/M204V) resistance to LAM (Allen *et al*, 1998). The less resistant rtM204V mutant virus is thought to be a key intermediate in the major pathway for development of breakthrough resistance to LAM (Gauthier *et al*, 1999). Study IDIX-04-189 assessed the susceptibility of these LAM signature resistance mutations to telbivudine in stably transfected HepG2 clonal cell lines harboring either a wild-type or LAM-resistant HBV genome (genotype D, subtype ayw), compared to ADV (PMEA) and LAM as controls. The results indicate significant cross resistance between LAM and telbivudine (Table 8, Study IDIX-04-189, Page 22).

Table 8: Antiviral Activity of Telbivudine against LAM-Resistant HBV mutants

Cell Line	Virus	Drug			
		LdT	LdC	Lam	PMEA
		EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)
WT3/C1	WT	0.65 ± 0.28	0.18 ± 0.09	0.05 ± 0.03	0.33 ± 0.17
V1/C9	M204V	0.85 ± 0.48	0.36 ± 0.13	0.96 ± 0.36	1.02 ± 0.22
I2	M204I	≥1000	≥1000	≥1000	1.6 ± 1.12
MV5/B3	L180/M204V	≥1000	777 ± 99	≥1000	0.62 ± 0.3
MI4	L180/M204I	≥823 ± 307	≥1000	≥1000	1.49 ± 0.3

Numbers represents mean values ± SD derived from three independent experiments.

Telbivudine was largely inactive against the rtM204I mutation without (fold changes in EC₅₀ values of ≥1,360 ± 363) or with the compensatory rtL180M mutation (fold changes of ≥1,049 ± 226). It was also inactive against the rtM204V mutation in conjunction with the rtL180M mutation (fold changes of ≥1,360 ± 363). However, telbivudine exhibited little to no loss of antiviral activity against the rtM204V single mutation with the calculated fold changes in EC₅₀ values of 1.2 ± 0.4. The rtM204V mutant was not found in patients who showed evidence of virologic failure from the phase III telbivudine clinical study (see Clinical Resistance Analyses).

In the phase III telbivudine clinical study NV-02B-007, HBV substitutions at rtL80, rtA181, and rtM204 were found in patients who had shown evidence of virologic failure to telbivudine treatment (see Clinical Resistance Analyses). The rtL80V/I and rtM204V/I mutants have been typically associated with LAM resistance (Delaney *et al.*, 2001), whereas the rtA181V/T mutant has been associated with ADV resistance (Fung *et al.*, 2005). No substitutions were found at amino acid positions I169, T184, S202, and M250 associated with entecavir (ETV) resistance.

Studies IDIX-04-185 and IDIX-06-101 assessed the antiviral activity in cell culture of telbivudine against HBV harboring the ADV resistance-associated substitutions rtN236T and rtA181V, respectively. The rtN236T and rtA181V (alone or in conjunction with rtN236T) mutations appear to be the primary mutations seen in ADV breakthrough patients (Angus *et al.*, 2003): the rtN236T mutant occurs approximately four times more frequent than the rtA181V mutant (Locarnini *et al.*, 2005). The cumulative probability of ADV resistance (rtA181V/T and/or rtN236T) was 0%, 3%, 11%, 18%, and 30% at weeks 48, 96, 144, 192, and 240, respectively (Hadziyannis *et al.*, 2005; (b) (4))

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(b) (4)

In stably transfected HepG2 clonal cell lines harboring wild-type, rtN236T, or rtA181V mutant HBV genome (genotype D, subtype ayw), telbivudine was found to exhibit slightly (~2-fold) better antiviral activity against the rtN236T mutant (fold changes in EC₅₀ values of 0.5 ± 0.43; Table 9, Study IDIX-04-185, Page 21) and 3 to 5 times less against rtA181V mutant (fold changes of 3.72 ± 2.18; Table 10, Study IDIX-06-101, Page 24) than wild-type virus. Therefore, the ADV-resistant rtA181V mutant appears more cross resistant to telbivudine than the rtN236T mutant.

Table 9: Antiviral Activity of Telbivudine against ADV-Resistant rtN236T Mutant

Cell Line	Virus	Drug			
		LdT	LdC	Lam	PMEA
		EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)
WT3/C1	WT	0.43 ± 0.19	0.09 ± 0.04	0.03 ± 0.01	0.46 ± 0.22
N236T/E8	N236T	0.26 ± 0.29	0.06 ± 0.17	0.04 ± 0.01	1.76 ± 0.60

Numbers represents mean values ± SD derived from four independent experiments.

Table 10: Antiviral Activity of Telbivudine against ADV-Resistant rtA181V Mutant

Cell Line	Virus	Drug			
		LdT	LdC	Lam	PMEA
		EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)	EC ₅₀ (μM)
WT3/C1	WT	0.173 ± 0.143	0.143 ± 0.121	0.014 ± 0.004	0.171 ± 0.129
A181V/F11E4H3	A181V	0.675 ± 0.507	>2.5	0.084 ± 0.065	0.627 ± 0.635

Numbers represents mean values ± SD derived from four independent experiments.

In the phase III telbivudine clinical study NV-02B-007, HBV substitutions at rtA181, but no rtN236, were found in patients with evidence of virologic failure to telbivudine treatment (see Clinical Resistance Analyses).

4. Clinical Microbiology

(1) Antiviral Efficacy

For Study NV-02B-007, the efficacy analyses were conducted separately in the HBeAg-positive and HBeAg-negative patient populations. In HBeAg-positive patients, telbivudine (600 mg once daily) was superior to lamivudine (LAM, 100 mg once daily) in both Therapeutic Response (HBV DNA suppression <5 log₁₀ copies/mL with either HBeAg loss or ALT normalization at Week 52) in an intent-to-treat analysis, 75% versus 67%, and Histologic Response (≥2 point improvement in Knodell necroinflammatory score with no worsening in Knodell fibrosis score at Week 52) in an as-treated analysis, 69% versus 60%. However, in HBeAg-negative patients, telbivudine was non-inferior to LAM in both Therapeutic Response (75% versus 77%) and Histologic Response (69% versus 68%) at Week 52. Therapeutic Response was the primary endpoint of the study, while Histologic Response was the key secondary endpoint. Please refer to the reviews by Medical Officer Charlene Brown, M.D. and Statistician Fraser Smith, Ph.D. for a detailed analysis of the efficacy of telbivudine.

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Antiviral efficacy of telbivudine was also assessed compared to LAM for following secondary endpoints at Week 52: (1) reduction in serum HBV DNA level; (2) suppression of serum HBV DNA below 1,000 copies/mL; (3) clearance of circulating viral load to PCR nondetectable levels (serum HBV DNA <300 copies/mL as quantified by the COBAS Amplicor HBV Monitor PCR assay); (4) reduction in virologic failure ($\geq 1,000$ copies/mL at Week 52); and (5) reduction in treatment-emergent virologic rebound (virologic breakthrough with $\geq 1 \log_{10}$ increase of serum HBV DNA from nadir while on therapy). Results are summarized in Table 11.

Of note, virologic failure is defined in this review as failure to achieve HBV DNA suppression at Week 52. The FDA definition of HBV DNA suppression (HBV DNA <3 \log_{10} copies/mL on 2 consecutive visits or at the last visit) is different from that of the Applicant (HBV DNA <5 \log_{10} copies/mL on 2 consecutive visits or at the last visit). The 1,000 copies/mL cutoff was chosen because of technical limitations and this level was previously used for the resistance analysis in the adefovir NDA. Treatment-emergent virologic rebound was defined in this review as virologic breakthrough with $\geq 1 \log_{10}$ increase of HBV DNA from nadir while on therapy, which also differs from the protocol definition of virologic breakthrough the Applicant utilized for its virologic breakthrough analyses. The Applicant's definition is: (a) In patients with HBV DNA levels of $\geq 6 \log_{10}$ copies/mL at Baseline who subsequently achieved 2 consecutive HBV DNA values <5 \log_{10} copies/mL, and has (1) HBV DNA $\geq 5 \log_{10}$ copies/mL on 2 consecutive visits with no more than one subsequent value <5 \log_{10} copies/mL or (2) HBV DNA $\geq 5 \log_{10}$ copies/mL at the last treatment visit OR (b) In patients who never achieved 2 consecutive HBV DNA levels <5 \log_{10} copies/mL but achieved $\geq 2 \log_{10}$ copies/mL reduction from Baseline, and has (1) return of HBV DNA to within 1 \log_{10} copies/mL of Baseline on two consecutive visits with no more than one subsequent level >1 \log_{10} copies/mL below Baseline or (2) a single HBV DNA level within 1 \log_{10} copies/mL of Baseline at the last treatment visit.

Table 11: Antiviral Efficacy Outcomes at Week 52 in Study NV-02B-007

Outcome	HBeAg-positive		HBeAg-negative	
	LAM	Telbivudine	LAM	Telbivudine
Serum HBV DNA at Baseline (\log_{10} copies/mL)	9.58 \pm 1.96	9.57 \pm 1.81	7.40 \pm 1.49	7.65 \pm 1.72
Reduction of Serum HBV DNA (\log_{10} copies/mL)	-5.46 \pm 2.55	-6.44 \pm 2.01	-4.37 \pm 2.08	-5.18 \pm 1.90
HBV DNA Suppression (<1,000 copies/mL) ¹ , n/(%)	209/455 (45.9)	291/445 (65.4)	180/232 (77.6)	214/235 (91.1)
PCR Nondetectable HBV DNA (<300 copies/mL) ¹ , n/(%)	172/455 (37.8)	257/445 (57.8)	164/232 (70.7)	208/235 (88.5)
Virologic Failure ² , n/(%)	233/438 (53.2)	145/430 (33.7)	48/223 (21.5)	19/227 (8.4)
Virologic Rebound ² , n/(%)	103/438 (23.5)	34/430 (7.9)	37/223 (16.6)	11/227 (4.9)

¹The Intent-to-treat (ITT) population includes all randomized patients who received at least one dose of study medication with at least one observation after Baseline.

²The as-treated population includes all randomized patients who received at least one dose of study medication with at least one observation after Baseline but excludes patients with missing or problematic data.

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Serum HBV DNA levels at Baseline were generally comparable between the two treatment groups, but with much higher pretreatment HBV DNA levels 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 log₁₀ copies/mL.

At Week 52, HBeAg-positive telbivudine-treated patients achieved a mean serum HBV DNA reduction of 6.44 ± 2.01 log₁₀ copies/mL, compared to a mean reduction of 5.46 ± 2.55 log₁₀ copies/mL for LAM-treated patients. In HBeAg-negative patients, the mean HBV DNA reductions were 5.18 ± 1.90 log₁₀ copies/mL and 4.37 ± 2.08 log₁₀ copies/mL for telbivudine and LAM, respectively.

In the HBeAg-positive patient population, 65.4% (291/445) of telbivudine recipients were suppressed with serum HBV DNA <1,000 copies/mL as quantified by the COBAS Amplicor HBV Monitor PCR assay and maintained viral suppression by Week 52, compared to 45.9% (209/455) of HBeAg-positive LAM recipients. In the HBeAg-negative patient population, 91.1% (214/235) of telbivudine recipients and 77.6% (180/232) of LAM recipients were suppressed and maintained. More importantly, 57.8% (257/445) of HBeAg-positive telbivudine recipients achieved HBV DNA clearance to PCR nondetectable levels (≤300 copies/mL HBV DNA) and maintained viral clearance by Week 52, compared to 37.8% (172/455) of HBeAg-positive LAM recipients. In the HBeAg-negative patient population, maintained HBV DNA clearance to PCR nondetectable levels was achieved by 88.5% (208/235) of telbivudine recipients versus 70.7% (164/232) of LAM recipients.

The rates of virologic failure and treatment-emergent virologic rebound were lower for telbivudine recipients, 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 telbivudine recipients versus 53.2% (233/438) of LAM recipients. In the HBeAg-negative patient group, 8.4% (19/227) of telbivudine recipients and 21.5% (48/223) of LAM recipients experienced virologic failure. Treatment-emergent virologic rebound was observed in 7.9% (34/430) of telbivudine 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 telbivudine recipients and 16.6% (37/223) of LAM recipients.

In summary, telbivudine showed greater serum HBV DNA reduction, greater proportion of patients achieving HBV DNA suppression (to <1,000 copies/mL) and viral clearance to PCR nondetectable levels (<300 copies/mL), and reduced virologic failure and treatment-emergent virologic rebound, compared to LAM in both HBeAg-positive and HBeAg-negative patients. Overall, the FDA analyses confirmed the Applicant's conclusion that telbivudine offers antiviral efficacy not inferior to LAM in HBeAg-positive and HBeAg-negative patients. However, there are differences in results for the proportion of patients with treatment-emergent virologic rebound. In both telbivudine and LAM treatment groups, FDA analyses yielded much higher virologic rebound rates, although these proportional differences did not affect the conclusions of this study. Detailed analyses are described below.

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(2) Clinical Resistance Analyses

For Study NV-02B-007, virologic treatment failures and responders were separated based upon a viral load cutoff of 1,000 copies/mL using the last available patient sample (usually 52 weeks).

Response to Telbivudine Treatment by Baseline HBV DNA Levels

The correlation between HBV DNA levels at Baseline and virologic treatment response was analyzed in the HBeAg-positive and HBeAg-negative patient populations (Table 12). Higher rates of virologic failure and treatment-emergent virologic rebound were observed in the subgroup of patients with higher baseline viral load in both HBeAg-positive and HBeAg-negative telbivudine-treated patients. Of the subjects in the HBeAg-positive patient populations with baseline viral DNA levels $\geq 8 \log_{10}$ copies/mL, 37% developed virologic failure to telbivudine treatment, compared to 12.3% of the subjects with viral DNA levels $< 8 \log_{10}$ copies/mL. In the HBeAg-negative patient populations, 15.1% of the subjects with baseline viral DNA levels $\geq 8 \log_{10}$ copies/mL developed virologic failure to telbivudine treatment, compared to 3.7% of the subjects with viral DNA levels $< 8 \log_{10}$ copies/mL.

Table 12: Correlation between Baseline HBV DNA Level and Virologic Response to Telbivudine Treatment

HBV DNA at Baseline (\log_{10} copies/mL)	Virologic Failure		Virologic Rebound	
	HBeAg-positive (n=430)	HBeAg-negative (n=227)	HBeAg-positive (n=430)	HBeAg-negative (n=227)
<6, n/(%)	1/17 (5.9)	1/31 (3.2)	0/17 (0.0)	1/31 (3.2)
6 to <8, n/(%)	6/40 (15.0)	4/103 (3.9)	2/40 (5.0)	4/103 (3.9)
8 to <10, n/(%)	68/238 (28.6)	9/77 (11.7)	15/238 (6.3)	4/77 (5.2)
10 to <12, n/(%)	34/80 (42.5)	5/11 (45.5)	8/80 (10.0)	2/11 (18.2)
≥ 12 , n/(%)	36/55 (65.5)	0/5 (0.0)	9/55 (16.4)	0/5 (0.0)

Response to Telbivudine Treatment by HBV Genotype

The distribution of HBV isolates by genotypes at Baseline is shown by HBeAg-status and treatment group in Table 13. The majority (77.8%) of patients were infected with HBV genotypes B or C as would be expected in a study in which most patients were of Asian ethnicity (77.0%). Overall, there were similar proportions of genotypes A, B, C, and D between telbivudine and LAM-treated patient populations, and between HBeAg-positive and HBeAg-negative patient populations with the exception of genotypes C and D (56.9% and 9.2% versus 39.8% and 26.7% for the HBeAg-positive and the HBeAg-negative patients, respectively). The proportion of patients (<1%) enrolled in this study infected with HBV genotypes E, F, and G was minimal. The most common genotypes in the US are A (36%), D (32%), and B (24%; Wai *et al.*, 2005).

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Table 13: Distribution of HBV Genotype

Genotype	HBeAg-positive		HBeAg-negative	
	LAM (n=438)	Telbivudine (n=430)	LAM (n=223)	Telbivudine (n=227)
A, n/(%)	28/438 (6.4)	21/430 (4.9)	14/223 (6.3)	13/227 (5.7)
B, n/(%)	109/438 (24.9)	126/430 (29.3)	58/223 (26.0)	59/227 (26.0)
C, n/(%)	246/438 (56.2)	248/430 (57.7)	86/223 (38.6)	93/227 (41.0)
D, n/(%)	49/438 (11.2)	31/430 (7.2)	63/223 (28.3)	57/227 (25.1)
E, n/(%)	1/438 (<0.5)	0/430 (0.0)	2/223 (0.9)	2/227 (0.9)
F, n/(%)	1/438 (<0.5)	1/430 (<0.5)	0/223 (0.0)	1/227 (<0.5)
G, n/(%)	3/438 (0.7)	2/430 (<0.5)	0/223 (0.0)	1/227 (<0.5)
Others, n/(%)	1/438 (<0.5)	1/430 (<0.5)	0/223 (0.0)	1/227 (<0.5)

The response to telbivudine treatment at week 52 by HBV genotype is shown in Table 14. Virologic failure and treatment-emergent virologic rebound occurred in 27% to 58.1% and 3.2% to 19.1%, respectively, of the HBeAg-positive patients infected with HBV genotypes A, B, C, and D, while of the HBeAg-negative patients, 0% to 19.3% and 0% to 8.8% experiences virologic failure and virologic rebound, respectively. The average rates of virologic failure and virologic rebound were 33.7% and 7.9% in the HBeAg-positive patient population, and 8.4% and 4.9% in the HBeAg-negative patient group, respectively (Table 11). These limited data suggest that the response to telbivudine is not remarkably affected by HBV genotype.

Table 14: Response to Telbivudine Treatment by HBV Genotype

Genotype	Virologic Failure		Virologic Rebound	
	HBeAg-positive (n=430)	HBeAg-negative (n=227)	HBeAg-positive (n=430)	HBeAg-negative (n=227)
A, n/(%)	12/21 (57.1)	0/13 (0)	4/21 (19.1)	0/13 (0)
B, n/(%)	45/126 (35.7)	4/59 (6.8)	12/126 (9.5)	2/59 (3.4)
C, n/(%)	67/248 (27.0)	3/93 (3.2)	16/248 (6.5)	3/93 (3.2)
D, n/(%)	18/31 (58.1)	11/57 (19.3)	1/31 (3.2)	5/57 (8.8)
E, n/(%)	0/0 (0.0)	0/2 (0.0)	0/0 (0.0)	0/2 (0.0)
F, n/(%)	1/1 (100)	0/1 (0.0)	0/1 (0.0)	0/1 (0.0)
G, n/(%)	1/2 (50)	0/1 (0.0)	0/2 (0.0)	0/1 (0.0)
Others, n/(%)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)

Telbivudine Treatment-Emergent Amino Acid Substitutions in HBV RT

Of the 164 patients who showed evidence of virologic failure to telbivudine treatment, the complete amino acid sequences of HBV RT from the paired screen and on-treatment samples of the 115 evaluable patients were submitted with this application. The viral sequences present at Week 48 (or at discontinuation, if prior to Week 48) in these patients were compared to the sequences present at Screen to identify treatment-emergent genotypic changes (mutations) in HBV RT. This analysis employed direct

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nucleotide sequence analysis of the HBV RT domain (344 amino acids) in PCR fragments that were amplified from the sera by nested PCR.

When paired baseline and on-treatment samples were compared, amino acid substitutions emerged in the HBV RT from the viruses of 87 patients out of the 115 patients with evidence of virologic failure to 600 mg telbivudine treatment by Week 48. There were no treatment-emergent genotypic changes found from samples of 28 patients. As summarized in Table 15, the actual number of amino acid substitutions identified per patient ranged from 0 to 28 with the average number of changes per patient of 3.4 ± 4.7 (391/115). The most frequent outcome per patient was the presence of 0 or 1 mutation per patient. In the subgroup of patients with virologic rebound, amino acid substitutions identified per patient also ranged from 0 to 26 with the average number of changes per patient of 5.0 ± 4.2 (195/39). Three or 4 mutations were found most frequently per patient.

Table 15: Telbivudine Treatment-Emergent Genotypic Changes in HBV RT per Patient in Paired Sequences

Number of mutations	0	1	2	3	4	5	6	7	8	9	10	11	12	25	26	28
Number of Patients	Virologic Failure (n=115)															
	28	19	12	16	13	7	6	3	2	2	2	1	1	1	1	1
	Virologic Rebound (n=39)															
	2	0	5	8	8	5	4	1	2	1	1	0	1	0	1	0

Isolates of 3 patients, 019-003, 035-003, and 120-048, had 26, 25 and 28 amino acid substitutions, respectively, more than twice the number of mutations seen for the patient with the next highest number of changes (12). This extent of changes might be anticipated for an HBV genotype change (the sequence variation between genotypes is around 8% or 28 changes per 344 amino acids in the RT domain). Indeed, patient 019-003 went from genotype B/C at Screen to B at follow up and 035-003 went from C (at Screen) to B. An rtM204I substitution was not detected in the viruses of these 2 patients. No change of genotype but rtM204M/I substitution was observed with patient 120-048.

A total of 119 codons (or 34.6% of the codons in the HBV RT domain) were found to be mutated in the viruses of patients with virologic failure to telbivudine treatment (see Appendix 1). Most (78 codons) were only mutated once or twice. Some of these may be clinically significant but the low number of occurrences precludes making definitive conclusions. Table 16 shows codons that were changed in 5 or more patients.

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Table 16: Codons Changed from Screen to Week 48 in 5 or More Patients with Virologic Failure to Telbivudine Treatment in Paired Sequences

Codon	Number of Occurrences			
	Virologic Failure (n=115)			Virologic Rebound (n=39)
	Total (n=115)	with M204 change (n=46)	without M204 change (n=69)	
204	46	46	0	34
80	27	27	0	21
181	16	1	15	1
91	13	10	3	10
134	13	7	6	8
55	9	5	4	4
78	9	3	6	3
221	8	5	4	4
222	8	6	2	6
124	7	1	6	1
135	7	5	2	6
267	7	4	3	4
118	6	2	4	2
131	6	3	3	2
207	6	5	1	5
229	6	6	0	5
256	6	4	2	4
38	5	3	2	2
238	5	2	3	2
269	5	3	2	4
271	5	2	3	2

The 3 most frequently observed changes were at codons 80, 181, and 204 (27, 16, and 46 occurrences each) in the highly conserved domains A, B, and C of HBV RT, respectively. Detailed analyses of these mutations are described in subsequent sections, Telbivudine Treatment-Emergent rtM204 mutations and Telbivudine Treatment-Emergent rtA181 mutations. Six domains (A-F) were identified as highly conserved within HBV RT and implied to play key roles in the binding of nucleosides as well as in the catalysis of DNA synthesis (Das *et al.*, 2001; Poch *et al.*, 1989; Stuyver *et al.*, 2001); mutations that confer resistance to ADV (Angus *et al.*, 2003), ETV (Tenney *et al.*, 2004), and LAM (Allen *et al.*, 1998) in the preclinical or clinical studies to date have been localized within these domains. The mutations at codons 80 and 204 have already been associated with both genotypic and phenotypic resistance to LAM (Allen *et al.*, 1998; Delaney *et al.*, 2001), and the mutation at codon 181 to ADV (Fung *et al.*, 2005). In cell culture, telbivudine was inactive (>1,000 fold reduced susceptibility) against HBV harboring the rtM204I substitution, and had 3- to 5-fold reduced susceptibility against HBV harboring the rtA181V substitution (See Nonclinical Microbiology). Telbivudine was active against HBV harboring the rtM204V substitution alone, but inactive against HBV with both the rtL180M and rtM204V substitutions.

The other 18 amino acid changes observed in 5 or more patients were at codons 38, 55,

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78, 91, 118, 124, 131, 134, 135, 207, 221, 222, 229, 238, 256, 267, 269, and 271. These amino acids with the exception of rtS78 are not positioned within the conserved domains of HBV RT, showing a high variability between genotypes as well as within genotypes. The frequency of variants at these positions was $39.7 \pm 24\%$ at Screen when compared to the reference sequence (genotype C), and individuals with these variants had the same response rate (HBV DNA $<3 \log_{10}$ copies/mL), $72.5 \pm 4.8\%$, as the overall population. Although rtS78 is in the conserved domain A, T78 and mixed T/S78 and S/C78 were also detected at this position at Screen (3.2%), and the viral loads in 59.1% of the patients with these variants at Screen were suppressed. Thus, the mutations at these positions detected in the viruses of patients with virologic failure to telbivudine treatment are unlikely to contribute significantly to reduced telbivudine susceptibility.

Interestingly, of the 119 codons mutated in the viruses of patients with virologic failure to telbivudine treatment (Appendix 1), the 16 codons at positions 22, 29, 58, 69, 82, 99, 119, 173, 180, 181, 200, 209, 240, 244, 254, and 261 are not polymorphic in the viruses from patients in the telbivudine arm at Screen (n=680). Amino acids at positions 22, 58, 69, 82, 99, 180, 209, 240, 254, and 261 are highly conserved among HBV isolates. Isolates from 34 of the 115 patients with virologic failure in paired sequences have mutations at these positions (each codon change, with the exception of rtA181T/S, was observed in 2 to 4 patients). The rtA181T/S and rtM204I substitutions were also found in the viruses of 30 patients out of the 34 patients. Thus, the contribution of these amino acid substitutions to decreased telbivudine susceptibility can not be determined with the limited clinical data (003-211, 008-046, 116-050, and 125-002; Table 17) where the substitutions developed on telbivudine treatment in the absence of the rtA181T/S and rtM204I mutations (see Telbivudine Treatment-Emergent Mutations in HBV RT in the Absence of the rtA181 and rtM204 Mutations).

Table 17: Mutations Found in Patients (n=4) without dominant rtA181 and rtM204 Mutations in Paired Sequences

Subject ID	HBV DNA (\log_{10} copies/mL)			Amino Acid Substitutions in HBV RT Developing on Telbivudine
	Baseline	Week 24	Week 52	
003-211	9.61	3.82	3.56	W58G/W
008-046	9.99	3.58	4.09	L69P/L, A/S113A, N/T118N, T/A222A, A307A/V
116-050	9.72	4.43	3.92	L29L/V, L91I/L, N134N/D, S135Y/S, V173M/V, N226N/H, L331L/F
125-002	13.23	4.89	4.09	T54H/Y, T/S78S, S116del, S117del, N118del, S119del, R120del, I121del, L122del, N123del, N124del

Telbivudine Treatment-Emergent rtM204 mutations

Of the 115 patients with virologic failure to telbivudine treatment whose paired baseline and on-treatment genotypes were submitted with this application, the isolates of 46 patients (40.0%) had mutations at codon 204 encoding rtM204. The rtM204I variants were detectable from the viruses of 37 patients (80.4%) by population nucleotide sequence analysis and the mixed variants, rtM204M/I or rtM204M/I/V were present in

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the HBV DNA of 9 patients (19.6%). There is no emergence of a pure rtM204V mutant population in response to telbivudine therapy.

The rtM204 mutation, rtM204I variant in particular, are strongly associated with virologic rebound: 34 patients of the 46 patients whose virus harbored the rtM204 mutation showed evidence of virologic rebound (73.9%). Thirty-three patients out of the 37 patients whose virus harbored the rtM204I variants had virologic rebound (89.2%), while 1 patient out of the 9 patients whose virus harbored M/I/V mixed variants at codon 204 had virologic rebound (11.1%).

The mutation profile for telbivudine in the subset of patients with the rtM204 mutation is similar to that for LAM with the exception of rtM204V mutation (Table 18). In addition, no rtM204V mutation in conjunction with the rtL180M mutation that is strongly associated with LAM resistance developed through the M204V pathway (Gauthier *et al.*, 1999) developed on telbivudine treatment. There were 4 patients whose virus harbored the rtL180M or rtL180M/L variants found in conjunction with the rtLM204I, rtLM204M/I, or rtLM204M/I/V mutations.

Table 18: Combinations of Mutations Found in Patients with the rtM204 Mutation in Paired Sequences

Mutation Combination		Virologic Failure		Virologic Rebound	
		LAM (n=50)	Telbivudine (n=46)	LAM (n=50)	Telbivudine (n=34)
M204I	Only, n/(%)	7 (14)	15 (32.6)	7 (14)	13 (38.2)
	+ L80 mutations, n/(%)	16 (32)	20 (43.5)	16 (32)	18 (52.9)
	+ V173 mutations, n/(%)	1 (2)	0	1 (2)	0
	+ L80 + L180 mutations, n/(%)	1 (2)	2 (4.4)	1 (2)	2 (5.9)
	+ L80 + V173 + L180 mutations, n/(%)	1 (2)	0	1 (2)	0
M204V	+ L180 mutations, n/(%)	13 (26)	0	13 (22)	0
	+ V173 + L180 mutations, n/(%)	1 (2)	0	1 (2)	0
M204M/I or M204M/I/V	Only, n/(%)	0	4 (8.7)	0	0
	+ L80 mutations, n/(%)	3 (6)	3 (6.5)	3 (6)	0
	+ L180 mutations, n/(%)	3 (6)	0	3 (6)	0
	+ L80 + L180 mutations, n/(%)	4 (8)	2 (4.4)	4 (8)	1 (2.9)

Of 46 patients whose virus harbored an rtM204 mutation, 44 patients had virus that carried other mutations in the HBV RT domain: only 2 patients (010-002 and 083-003) had the rtM204I/M mixed variants without other detectable codon changes. The most frequent accompanying substitutions were at codons 55 (5/46), 80 (27/46), 91 (10/46), 134 (7/46), 135 (5/46), 207 (5/46), 221 (5/46), 222 (6/46), and 229 (6/46). These mutations appeared tightly associated with the rtM204 mutation: the frequency of occurrence of each mutation to be detected together with the rtM204 mutation ranged from 55.6 to 100% (Table 16). In particular, all patients that carry mutations at codons 80 (27 patients) or 229 (6 patients) were found to have the rtM204 mutation. The amino acids at codons 55, 91, 134, 135, 207, 221, and 222 are highly variable between genotypes as well as within genotypes with the frequency of variants of 31.7 ± 22.8% at

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Screen when compared to the reference sequence (genotype C). The viral loads in 66.4 ± 3.1% of the patients with these variants at Screen were suppressed. Thus, it is difficult to evaluate the contribution of these changes to the rtLM204I-associated resistance to telbivudine.

Of note, there were 3 patients with pre-existing rtM204 variants at Screen: 2 patients were in the LAM treatment group and 1 patient in the telbivudine arm (Table 19). As expected, these patients were completely refractory to LAM and telbivudine therapy.

Table 19: Patients with Pre-Existing rtM204 Variants at Screen

Subject ID	Treatment	Virologic Failure	Virologic Rebound	HBV DNA (log ₁₀ copies/mL)			Key Mutations detected in HBV RT at Screen
				Baseline	Week 24	Week 52	
109-040	LAM	Yes	Yes	9.98	10.03	12.06	L180M, M204V
109-048	LAM	Yes	No	7.16	7.18	7.00	L180M, M204V
116-025	Telbivudine	Yes	yes	10.05	9.64	12.20	L80V, M204I

Telbivudine Treatment-Emergent rtA181 mutations

Of the 115 patients with virologic failure to telbivudine treatment whose paired baseline and on-treatment genotypes were submitted with this application, substitutions at rtA181 developed in 16 patients (13.9%; Table 20). Eight of the 16 treatment emergent variants had mixtures of T and A at codon 181 in place of the consensus rtA181, 7 variants had a pure rtA181T single change, and one had an rtA181S change. Interestingly, no variants with rtA181V were seen. The rtA181V mutation is known to confer resistance to ADV and reduced susceptibility to telbivudine in cell culture (3- to 5-fold increase in susceptibility; see Nonclinical Microbiology). No data are available to evaluate susceptibility to telbivudine in cell culture of HBV harboring rtA181T or to rtA181S mutations. Of 16 patients, 1 patient 116-059 also carried the rtM204I/M mutation and showed evidence of virologic rebound.

Table 20: Mutations Found in Patients (n=16) with the rtA181 Mutation in Paired Sequences

Subject ID	HBV DNA (log ₁₀ copies/mL)			Amino Acid Substitutions in HBV RT Developing on Telbivudine
	Baseline	Week 24	Week 52	
003-085	8.93	4.10	3.85	S78T/S, Q/H124H, E/D134D, A181T
005-002	13.29	6.08	5.75	A181T/A
035-003	9.09	3.84	4.42	E1D, Y9H, H13R, I16T, K/T38T, S53N, H55R, P109S, T118N, N121I, Y124H, G127R, D131N, K/N/E/D139N, L145M, F151Y, A181T , E/V214V, F221Y, T/A238H, T259S, R270K, Q271S, I/V278V, C332S,
054-030	12.61	3.73	3.79	A181T/A
057-030	10.22	3.96	3.03	Q/P130P, A181T
057-067	8.73	3.86	3.27	T/78S, Y124Q, A181T , V214A

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057-088	13.13	5.38	5.03	A181T/A
059-001	12.15	4.95	4.28	K/R18R, Q/R153R, A181T/A
068-021	8.38	3.98	3.75	T/S78S, A181T
079-008	10.24	5.89	5.56	A181T/A
094-001	9.98	6.20	5.66	Y54H/Y, R110R/G, A181T/A
104-001	10.21	4.75	4.39	K/R138R, A181T, P325P/S
108-059	10.27	4.29	5.17	A181S, E/D263E
116-059	12.24	5.00	4.18	S78T/S, S116A/S, A181T/A, M204I/M, P261P/L
119-007	10.09	4.29	3.82	A181T
122-004	15.48	5.81	5.46	L/F164F, A181T/A

Telbivudine Treatment-Emergent Mutations in HBV RT in the Absence of the rtA181 and rtM204 Mutations

Of the 115 patients with virologic failure to telbivudine treatment whose paired baseline and on-treatment genotypes were submitted with this application, the most frequently emerging RT substitutions at rtA181 and rtM204 were not detected in the viruses from 54 patients (47%). All of these patients were HBeAg-positive. The 54 patients experienced reductions in viral load by week 52 with a mean serum HBV DNA reduction of $6.07 \pm 2.06 \log_{10}$ copies/mL, but failed to achieve virologic suppression with the exception of 1 patient (118-008). Patient 118-008, experienced virologic rebound after achieving nondetectable HBV DNA levels (Table 21).

Of the 54 patients, 28 patients showed no treatment-emergent genotypic changes, while RT substitutions appeared in 26 patients at conserved (Table 21, highlighted in yellow) and polymorphic sites. A total of 69 codons (or 20.1% of the codons in the HBV RT domain) were found to be mutated in the viruses of the 26 patients and most (61 codons) were only mutated once or twice, unlikely to be of any statistical significance. The other 8 changes observed in 3 or more patients were at codons 55, 78, 91, 118, 124, 134, 137, and 267. These amino acids with exception of rtS78 are not positioned within the conserved domains of HBV RT, showing a high variability between genotypes as well as within genotypes. The frequency of variants at these positions was $46.9 \pm 24.5\%$ at Screen when compared to the reference sequence (genotype C) and $72.9 \pm 1.5\%$ of the patients with these variants showed evidence of virologic suppression (HBV DNA $<3 \log_{10}$ copies/mL). Although rtS78 is in the conserved domain A, T78 and mixed T/S78 and S/C78 were also detected at this position at Screen (3.2%) and 59.1% of the patients with these variants at Screen were with virologic suppression. Thus, insufficient data are available to evaluate the contribution of these changes to reduced susceptibility to telbivudine.

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Table 21: Mutations Found in Patients (n=26) without the rtA181 and rtM204 Mutations in Paired Sequences

Subject ID	HBV DNA (log ₁₀ copies/mL)			Amino Avid Substitutions in HBV RT Developing on Telbivudine
	Baseline	Week 24	Week 52	
003-211	9.61	3.82	3.56	W58G/W
008-046	9.99	3.58	4.09	L69P/L, A/S113A, N/T118N, T/A222A, A307A/V
019-003	10.23	7.97	7.21	N/S/H/R13R, T/I16T, N/S53N, H/R55R, I/L91L, S106S/C, P/S109S, N/T118N, N/I121I, N/Y124N, R/G127R, N/D131N, N/D134N, S/F135F, M/L145M, Y/F151Y, Y/F221Y, T/A222A, I/V224V, N/H238H, R/S242R, Q/L267Q, I/L269I, K/I/M/Q/L271M, S/C332S, N/H337N
025-008	10.80	6.98	5.72	E1Q
041-027	9.65	5.83	4.29	A342T/A
057-095	12.65	4.75	4.55	S137T/S, Q/L267Q, Q/H/R319H/R
058-015	8.96	4.51	4.37	S137P/S, K/N139N, S238N
065-018	8.11	4.48	3.40	N124K
068-001	10.09	5.17	6.79	N/D76N, N131N/H, E/V142V, K/Q149K, K/Q241K, H246H/P/Y/S, S/C256S
068-017	9.64	3.61	5.87	Y141H/Y
071-024	9.92	5.25	4.64	C314R/C
071-043	10.83	5.58	5.38	L/V231L
084-012	9.33	4.46	3.56	T/S78T, T/S116S, T/I128T, Q149K,
105-009	12.39	4.20	4.48	I163I/V
106-024	9.05	3.82	3.04	H/R55R, S219A, I/V224I, H/D271H
108-039	9.31	4.25	3.83	I/L91I, Y/F141F, G295E/G
108-060	12.48	4.59	4.26	N/S134N
108-062	9.62	4.19	4.34	L220I/L
112-006	12.65	4.05	4.18	R55H, N134D, S137T, S256C, Q267L, L269I, I282V, Q/H316Q, K/R318K
112-023	9.64	3.18	3.32	K275K/Q
114-030	15.95	5.49	4.98	F221Y/F
116-050	9.72	4.43	3.92	L29L/V, L91I/L, N134N/D, S135Y/S, V173M/V, N226N/H, L331L/F
118-008	10.29	2.18	4.02	S78T/S, E/D134D, V207I/M/V
122-015	9.97	3.33	3.17	A329T
122-016	10.03	3.19	4.91	T38T/A, M/L146L
125-002	13.23	4.89	4.09	T54H/Y, T/S78S, S116del, S117del, N118del, S119del, R120del, I121del, L122del, N123del, N124del

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Summary

The findings from the telbivudine clinical resistance analyses of Study NV-02B-007 can be summarized as follows:

- In the ITT analysis, the mean serum HBV DNA reductions at Week 52 were $6.44 \pm 2.01 \log_{10}$ and $5.46 \pm 2.55 \log_{10}$ copies/mL for telbivudine and LAM, respectively. In HBeAg-negative patients, the mean HBV DNA reductions at Week 52 were $5.18 \pm 1.90 \log_{10}$ and $4.37 \pm 2.08 \log_{10}$ copies/mL for telbivudine and LAM, respectively.
- In the ITT analysis, 65.4% (291/445) of treatment-naïve HBeAg-positive and 91.1% (214/235) of treatment-naïve HBeAg-negative patients receiving telbivudine 600 mg once daily achieved virologic suppression (serum HBV DNA levels <1,000 copies/mL) by Week 52.
- In the ITT analysis, 57.8% (257/445) of treatment-naïve HBeAg-positive and 88.5% (208/235) of treatment-naïve HBeAg-negative patients receiving telbivudine 600 mg once daily achieved nondetectable serum HBV DNA levels (<300 copies/mL) by Week 52.
- In the as-treated analysis, 33.7% (145/430) of treatment-naïve HBeAg-positive and 8.4% (19/227) of treatment-naïve HBeAg-negative patients receiving telbivudine 600 mg once daily failed to achieve virologic suppression by Week 52.
- In the as-treated analysis, 7.9% (34/430) of treatment-naïve HBeAg-positive and 4.9% (11/227) of treatment-naïve HBeAg-negative patients receiving telbivudine 600 mg once daily experienced virologic rebound ($\geq 1 \log_{10}$ increase of HBV DNA from nadir while on therapy) by Week 52.
- Higher rates of virologic failure (serum HBV DNA levels $\geq 1,000$ copies/mL) and virologic rebound were observed in the subgroup of patients with higher baseline viral load in both HBeAg-positive and HBeAg-negative telbivudine-treated patients.
- In the paired sequence analysis of baseline and on-treatment samples, 75.7% (87/115) of patients with evidence of virologic failure had genotypic changes in the HBV RT.
- Amino acid substitutions rtL80I/V, rtL180M, rtA181T, rtM204I, and rtL229W/V were associated with virologic failure to telbivudine therapy: these changes were detected in 48% (49/103) of the HBeAg-positive patients and in 100% (12/12) of the HBeAg-negative patients.
- Forty percent of patients (46/115) had mutations at codon 204, rtM204: the rtM204I variants were detectable from the viruses of 37 patients (80.4%) and the mixed variants, rtM204M/I or rtM204M/I/V were of 9 patients (19.6%).
- Amino acid substitutions rtL80I/V, rtL180M, and rtL229W/V appeared tightly associated with the rtM204 mutation: all patients that carry mutations at codons 80 (27 patients), 180 (4 patients), or 229 (6 patients) were found to have the rtM204 mutation.
- In the subset of patients with the rtM204 mutation, the mutation profile for telbivudine is similar to that for LAM with exception of rtM204V mutation.
- The rtM204V mutation in conjunction with the rtL180M mutation was not detected.
- 13.9% of patients (16/115) had mutations at codon 181, rtA181: the mixed variants, rtA181T/A, were detectable from the viruses of 8 patients (50.0%), and the rtA181T and rtA181S variants were of 7 (43.8%) and of 1 (6.3%) patients, respectively.

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- No variants with rtA181V were detected.

5. Conclusion

This NDA is approvable with respect to microbiology for the treatment of chronic HBV. Telbivudine showed greater serum HBV DNA reduction, greater proportion of patients achieving HBV DNA suppression (to <1,000 copies/mL) and viral clearance to PCR nondetectable levels (<300 copies/mL), and reduced virologic failure and treatment-emergent virologic rebound, compared to LAM in both HBeAg-positive and-negative patients. Overall, telbivudine offers antiviral efficacy not inferior to LAM in HBeAg-positive and HBeAg-negative patients. Genotypic analysis revealed that amino acid substitutions rtL80I/V, rtL180M, rtA181T, rtM204I, and rtL229W/V were associated with virologic failure to telbivudine therapy. Amino acid substitutions rtL80I/V, rtL180M, and rtL229W/V appeared tightly associated with the rtM204 mutation. Studies evaluating virologic responses and resistance to telbivudine are continuing to be monitored post 52 weeks. Post 52-week follow-up data of telbivudine-treated patients is needed to obtain long term resistance data.

6. Recommendations

- Determine the anti-HBV cell culture combination activity relationships of telbivudine with entecavir.
- Determine the anti-HBV combination activity relationships of telbivudine in cell culture with the HIV NRTIs abacavir, emtricitabine, lamivudine, tenofovir, zalcitabine, and zidovudine.
- Determine the susceptibility to telbivudine and adefovir of the HBV rtA181 variants, rtA181T and rtA181S.
- Determine the susceptibility in cell culture of HBV harboring the following mutations of highly conserved amino acid residues among HBV isolates: 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 $\geq 1,000$ copies/mL at Week 52) to telbivudine therapy.
- Determine the mitochondrial toxicity of telbivudine in growing muscle cells, cell lines and primary cells, and primary hepatocytes with appropriate controls to validate the results.
- Continue to perform genotypic and phenotypic analyses of HBV DNA from patients who experience virologic failure to long-term telbivudine therapy (HBV DNA levels $\geq 1,000$ copies/mL) in ongoing clinical trials.

7. Microbiology Package Insert

Mechanism of Action

Telbivudine is a synthetic thymidine nucleoside analogue with activity against HBV DNA

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polymerase. It is phosphorylated by cellular kinases to the active triphosphate form, which has an intracellular half-life of 14 hours. Telbivudine 5'-triphosphate inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5'-triphosphate. Incorporation of telbivudine 5'-triphosphate into viral DNA causes DNA chain termination, resulting in inhibition of HBV replication. Telbivudine is an inhibitor of both HBV first strand (EC_{50} value = $1.3 \pm 1.6 \mu\text{M}$) and second strand synthesis (EC_{50} value = $0.2 \pm 0.2 \mu\text{M}$). Telbivudine 5'-triphosphate at concentrations up to $100 \mu\text{M}$ did not inhibit human cellular DNA polymerases α , β , or γ . No appreciable mitochondrial toxicity was observed in HepG2 cells treated with telbivudine at concentrations up to $10 \mu\text{M}$.

Antiviral Activity

The antiviral activity of telbivudine was assessed in the HBV-expressing human hepatoma cell line 2.2.15, as well as in primary duck hepatocytes infected with duck hepatitis B virus. The concentration of telbivudine that effectively inhibited 50% of viral DNA synthesis (EC_{50}) in both systems was approximately $0.2 \mu\text{M}$. The anti-HBV activity of telbivudine was additive with adefovir in cell culture, and was not antagonized by the HIV NRTIs didanosine and stavudine. Telbivudine is not active against HIV-1 (EC_{50} value $>100 \mu\text{M}$) and was not antagonistic to the anti-HIV activity of abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, or zidovudine.

Resistance

In an as-treated analysis of the Phase III 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 telbivudine 600 mg once daily achieved nondetectable serum HBV DNA levels (<300 copies/mL) by Week 52.

At Week 52, 145/430 (34%) and 19/227 (8%) of HBeAg-positive and HBeAg-negative telbivudine recipients, respectively, had evaluable HBV DNA ($\geq 1,000$ copies/mL). Genotypic analysis detected one or more amino acid substitutions associated with virologic failure (rtM204I, rtL80I/V, rtA181T, rtL180M, rtL229W/V) in 49 of 103 HBeAg-positive and 12 of 12 HBeAg-negative patients with amplifiable HBV DNA and ≥ 16 weeks of treatment. The rtM204I substitution was the most frequent mutation and was associated with virologic rebound ($\geq 1 \log_{10}$ increase above nadir) in 34 of 46 patients with this mutation.

Cross-Resistance

Cross-resistance has been observed among HBV nucleoside analogues. In cell-based assays, lamivudine-resistant HBV strains containing either the rtM204I mutation or the rtL180M/rtM204V double mutation had $\geq 1,000$ -fold reduced susceptibility to telbivudine. Telbivudine retained wild-type phenotypic activity (1.2-fold reduction) against the lamivudine resistance-associated substitution rtM204V alone. The efficacy of telbivudine against HBV harboring the rtM204V mutation has not been established in clinical trials. HBV encoding the adefovir resistance-associated substitution rtA181V showed 3- to 5-

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fold reduced susceptibility to telbivudine in cell culture. HBV encoding the adefovir resistance-associated substitution rtN236T remained susceptible to telbivudine.

8. Appendices

Appendix 1

Identity and Frequency of Individual Codon Changes in Paired Sequences of Samples from Patients with Virologic Failure to Telbivudine treatment

Codon	Number of Occurrences			
	Virologic Failure (n=115)			Virologic Rebound (n=39)
	Total (n=115)	with rtM204 change (n=46)	without M204 change (n=69)	
204	46	46	0	34
80	27	27	0	21
181	16	1	15	1
91	13	10	3	10
134	13	7	6	8
55	9	5	4	4
78	9	3	6	3
221	8	5	3	4
222	8	6	2	6
124	7	1	6	1
135	7	5	2	6
267	7	4	3	4
118	6	2	4	2
131	6	3	3	2
207	6	5	1	5
229	6	6	0	5
256	6	4	2	4
38	5	3	2	2
238	5	2	3	2
269	5	3	2	4
271	5	2	3	2
9	4	3	1	2
13	4	2	2	2
109	4	2	2	2
121	4	1	3	1
137	4	1	3	1
139	4	2	2	1
180	4	4	0	3
200	4	4	0	4
278	4	3	1	1
1	3	1	2	1
16	3	1	2	1
53	3	1	2	1
116	3	1	2	0
122	3	2	1	2
127	3	1	2	1
141	3	1	2	1
145	3	1	2	1
151	3	1	2	1
224	3	1	2	2
332	3	1	2	1
11	2	2	0	2
18	2	1	1	0

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29	2	1	1	0
35	2	2	0	1
42	2	2	0	1
54	2	0	2	0
106	2	1	1	2
110	2	1	1	1
123	2	1	1	1
138	2	1	1	0
149	2	0	2	0
163	2	1	1	1
164	2	1	1	1
191	2	2	0	1
214	2	0	2	0
219	2	1	1	1
223	2	2	0	1
226	2	1	1	1
242	2	1	1	1
279	2	2	0	2
309	2	2	0	1
314	2	1	1	0
318	2	1	1	1
337	2	1	1	2
2	1	1	0	1
3	1	1	0	1
22	1	1	0	1
24	1	1	0	0
27	1	1	0	1
41	1	1	0	0
58	1	0	1	0
62	1	1	0	0
69	1	0	1	0
76	1	0	1	0
82	1	1	0	1
99	1	1	0	1
113	1	0	1	0
117	1	0	1	0
119	1	0	1	0
120	1	0	1	0
125	1	1	0	1
128	1	0	1	0
130	1	0	1	0
142	1	0	1	0
146	1	0	1	0
148	1	1	0	1
153	1	0	1	0
162	1	1	0	1
173	1	0	1	0
209	1	1	0	1
215	1	1	0	1
220	1	0	1	0
231	1	0	1	0
240	1	1	0	1
241	1	0	1	0
244	1	1	0	0
246	1	0	1	0
248	1	1	0	0
254	1	1	0	1
259	1	0	1	0
261	1	1	0	0
263	1	0	1	1
266	1	1	0	0
270	1	0	1	0
275	1	0	1	0

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282	1	0	1	0
295	1	0	1	0
307	1	0	1	0
316	1	0	1	0
317	1	1	0	1
319	1	0	1	0
320	1	1	0	0
322	1	1	0	0
325	1	0	1	0
329	1	0	1	0
331	1	0	1	0
333	1	1	0	0
342	1	0	1	0

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