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APPLICATION NUMBER:

21-449

MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 21,449; SN-000 Review completed 09/10/02

Reviewer: LALJI MISHRA, Ph.D.
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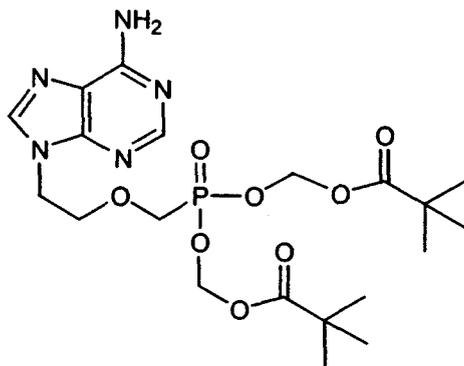
Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Product Name(s):

Proprietary: Hepsera

Non-proprietary: Adefovir dipivoxil (bis-POM PMEA)

Chemical: 9-2-[[bis[(pivaloyloxy)methoxy]phosphinyl]methoxy]ethyl]adenine



Route of Administration/Dosage Form: Oral/Tablet, capsule
Indication: Treatment of chronic hepatitis B virus infection

Additional submissions reviewed:

Supplement #	Date of Correspondence	Date of Receipt
N21449 C	June 13, 2002	June 14, 2002
N21449 BM	July 8, 2002	July 10, 2002
N21449 NC	July 8, 2002	July 10, 2002
N21449 BL	August 19, 2002	August 21, 2002
N21449 BZ	August 23, 2002	August 26, 2002

BACKGROUND

Gilead Sciences, Inc. (GSI) has submitted a new drug application NDA # 21449 and seeks marketing approval of 10 mg adefovir dipivoxil (ADV) tablets once daily for the treatment of HBV infection.

ADV (9-2-[[bis[(pivaloyloxy)methoxy]phosphinyl]methoxy]ethyl]adenine) is an oral prodrug of adefovir 9- [2(phosphonomethoxy)-ethyl]adenine. Adefovir is an acyclic

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nucleotide analogue of adenosine 5' monophosphate. Adefovir has demonstrated in vitro and in vivo antiviral activity against HBV.

In the current application, GSI has submitted results of two pivotal studies: GS-98-437 and GS-98-438, and supporting studies GS-98-435 and GS-00-461, to support the use of ADV for the treatment in chronic hepatitis patients. Resistance (genotypic and phenotypic analyses) data were obtained for HBV from patients enrolled in studies GS-98-437, and GS-98-438 and these data are reviewed here. Study GS-98-435 supports the use of ADV against lamivudine (3TC)-resistant HBV. Resistance data for study 461 were not provided for review. GSI has also provided preliminary data on adefovir resistance from a pilot study in patients co-infected with HIV-1 and HBV (GS-00-460i). For efficacy results of the above-mentioned studies, please see the Medical Officer's review. The pertinent microbiology data are summarized below.

1. Metabolism

Following oral administration, ADV is rapidly converted to adefovir. Adefovir is actively transported into HeLa cells by a 50-kD plasma membrane protein (Cihlar et al., 1995), but this does not appear to be efficient. The metabolism of ADV and adefovir was studied in MT-2 cells labeled with 1 μM [^3H] ADV or 10 μM [^3H] adefovir (Srinivas et al., 1993). ADV was rapidly converted to the parent compound adefovir inside cells. Nearly 21% of the [^3H] ADV appeared within the cells, and essentially all the intracellular radioactivity was associated with adefovir and its metabolites, adefovir monophosphate and adefovir diphosphate. No radioactivity was detected as intact prodrug. In contrast, only a small proportion (1%) of the labeled adefovir, which was distributed primarily in adefovir, adefovir monophosphate and adefovir diphosphate appeared within adefovir-treated cells.

Adefovir is phosphorylated to its active metabolite adefovir diphosphate by cellular kinases. Adenylate kinase (AK) is responsible for the phosphorylation of adefovir to adefovir monophosphate. Approximately 56%, 44%, and <0.1% of cellular AK activity in CEM cells was localized in the mitochondrial, cytosolic, and microsomal fractions, respectively (Robbins, et al., 1995). Thus, the major phosphorylating activity for adefovir in CEM cells was associated with mitochondria. The second phosphorylation to generate adefovir diphosphate is carried out by a non-specific nucleotide diphosphate kinase. The intracellular half-life of adefovir diphosphate in quiescent peripheral blood mononuclear cells (PBMCs) was found to be 30 hours (Bischofberger, 1999).

II. Mechanism of action

Adefovir diphosphate preferentially inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate deoxyadenosine triphosphate (dATP). Because it lacks a 3' hydroxyl group, adefovir diphosphate also causes chain termination of viral DNA synthesis upon its incorporation into the nascent viral DNA strand. The inhibition constant (K_i) for adefovir diphosphate against recombinant HBV polymerase was estimated to be 0.1 μM (Table 1). Activated calf thymus DNA served as

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primer-template for the HBV DNA polymerase assay (Xiong *et al.*, 1998) and the human DNA polymerases α , β , and γ enzymatic assays (Cherrington *et al.*, 1995). Adefovir diphosphate is a weak inhibitor of cellular DNA polymerases α and γ . Polymerase α /primase initiates the synthesis of both the leading and lagging strand during replication of chromosomal DNA. DNA polymerase γ is responsible for the replication of mitochondrial DNA.

Table 1: K_i values of adefovir diphosphate and K_m value of dATP against HBV DNA polymerase and human DNA polymerases (Sources: Xiong *et al.*, 1998; Cherrington *et al.*, 1995).

	Adefovir diphosphate (K_i) μM	DATP (K_m) μM
HBV DNA polymerase	0.1	0.38
Human DNA polymerase α	1.18	2.7
Human DNA polymerase β	70.4	5.6
Human DNA polymerase γ	0.97	0.72

III. Anti-HBV activity of adefovir

III (a). Anti-HBV activity of adefovir in vitro

A suitable cell culture system is not available for the evaluation of potential anti-HBV drugs. Recently a human hepatoma cell line (HB611) has been described that accumulates a significant amount of HBV DNA replicative intermediates (Yokata *et al.*, 1994). Inhibition of the synthesis of these replicative intermediates has been used as a measure of anti-HBV activity. The concentration of adefovir which inhibited intracellular HBV DNA synthesis by 50% (IC_{50}) was 0.2 μM in HB611 cells. The concentration of adefovir which inhibited cell growth by 50% (CC_{50}) was 107 μM resulting in a therapeutic index (TI) of 535 (Yokata *et al.*, 1994).

Adefovir inhibited extracellular HBV DNA levels in HepG2 2.2.15 cells and HB611 cells. The IC_{50} values were 0.7 and 1.2 μM , respectively (Heijtkink *et al.*, 1993). The CC_{50} value as measured by the inhibition of [^3H -methyl] deoxythymidine incorporation was 150 μM for the two human hepatoma cell lines. Adefovir reduced intracellular episomal HBV DNA production as measured by Southern blot hybridization (relaxed circle and single-strand bands). The IC_{50} values ranged from 1.0 -2.5 μM in the two cell lines (Heijtkink *et al.*, 1993).

III (b) Anti-HBV activity of adefovir in combination with tenofovir (TNV) in vitro

Tenofovir disoproxil fumarate (TDF), an oral prodrug of tenofovir, is approved for the treatment of HIV-1 infection. Both adefovir and TNV are structurally-related analogues of adenosine monophosphate. The purpose of this experiments was to determine the *in vitro* antiviral activity of combinations of adefovir and TNV against hepatitis B virus.

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HepG2 cells were transfected with pHY49, a plasmid encoding a 1.1 unit-length HBV genomic recombinant DNA clone under the control of the CMV immediate early promoter and the neomycin phosphotransferase (neo^r) gene under the control of the SV-40 promoter. Cells taking up plasmid DNA were selected using G418. G418 resistant transformants were expanded into 24-well plates and screened for the production of hepatitis B virus e antigen (HBeAg) using an colorimetric immunoassay kit

Several transformants which expressed high levels of HBeAg were expanded and assayed for other markers of viral replication. One clone, HepG2 49-27, was chosen for combination studies since it produced high levels of all markers of viral replication (approximately 35-fold greater than those produced by the reference cell line, HepG2 2.2.1.5). HepG2 49-27 cells were seeded at approximately 2.5×10^5 cells per well in 24-well plates the day prior to the experiment. Starting one day post seeding, cells were treated every other day for one week with media containing various concentrations of adefovir and TNV. After one week, cells were harvested and the DNA was extracted, subjected to electrophoresis, and transferred to nylon membrane (Southern blot). HBV replicative intermediates were detected and quantified by nucleic acid hybridization with a ³²P-labelled HBV-specific probe and a

Results showed that the average IC₅₀ values of adefovir and TNV calculated from single drug dose responses were 0.22 and 0.19 μM, respectively. These values agree well with previous results of 0.18 to 2.5 μM (NDA 21,449, vol 80, pages 342-353).

The concentrations of drug tested during these experiments ranged between 0.04 and 1.5 μM for both adefovir and tenofovir including a combination of 0.08 μM adefovir and 0.75 μM TNV. The *in vivo* C_{max} values for adefovir following a 10-mg dose of ADV, and for tenofovir following a 300-mg dose of TDF were determined to be 0.07 μM and 0.8 μM, respectively. At a combination of 0.08 μM adefovir plus 0.75 μM tenofovir, observed levels of viral replication were close to those predicted by an additive interaction. These results suggest that combinations of adefovir and TNV at physiological relevant concentration of drug produce an additive antiviral effect against HBV *in vitro*.

III (c). Activity of adefovir against 3TC-resistant HBV in vitro

HBV mutants with reduced susceptibility to 3TC have been obtained from patients receiving long term therapy. Genotypic analysis has shown that these isolates contain an amino acid substitution in the conserved tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV reverse transcriptase. These amino acids are involved in the nucleotide binding in the catalytic domain of the viral reverse transcriptase. HBV mutants with reduced susceptibility to 3TC contain a substitution of valine or isoleucine for the methionine at position 552 (M552V or M552I) in the YMDD motif of the reverse transcriptase (Ling *et al.*, 1996; Allen *et al.*, 1998; Melagari *et al.*, 1998; Ono-Nita *et al.*, 1999). The mutation M552V is most frequently accompanied by a substitution of methionine for leucine at position 528 (L528M). Mutation L528M lies in the B domain of HBV reverse transcriptase.

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The *in vitro* activity of adefovir diphosphate and 3TC triphosphate against wild type and 3TC-resistant strains of HBV was evaluated using an enzymatic assay based on recombinant HBV polymerase expressed in insect cells infected with a recombinant baculovirus. Results are shown in Table 2.

These data indicate that the M552V and M552I mutations confer resistance to 3TC with an increase in inhibition constants of 20- and 8-fold, respectively, compared with that of wild-type HBV DNA polymerase. The double mutation L528M/M552V conferred a 25-fold increase. The F514L mutation is also associated with resistance to 3TC with inhibition constants increasing 6-fold for F514L alone and 10.4-fold in combination with L528M. These mutants all remained sensitive to adefovir, with the inhibition constants ranging from 0.79 to 2.3 fold compared with wild-type.

Table 2: Inhibition constants of adefovir diphosphate and 3TC triphosphate for wild-type and mutant HBV DNA polymerases containing mutations associated with resistance to 3TC.

Mutations/Strains	Adefovir Diphosphate		Lamivudine Triphosphate	
	K _i (μM)	Fold increase*	K _i (μM)	Fold Increase*
Wild-type	0.10	1.0	0.25	1.0
M552I	0.13	1.3	2.0	8.0
M552V	0.22	2.2	4.9	19.6
L528M	0.23	2.3	0.64	2.6
L528M/M552V	0.079	0.79	6.3	25.2
F514L	0.19	1.9	1.5	6.0
F514L/L528M	0.11	1.1	2.6	10.4

*Fold increase = (mutant K_i/wild-type K_i)

In addition, adefovir was effective in suppressing HBV replication in HuH7 cells transfected with 3TC-resistant, recombinant HBV containing the M552V, or M552I, or L528M/M552V mutations. In a separate study, adefovir was evaluated for its antiviral activity against wild type and 3TC-resistant HBV mutants L528M, M552I and L528M/M552V in transfected Hep2 cells. This study showed that HBV encoding the M552V, M552I, or L528M + M552V mutations caused 100- to 10,000-fold resistance to 3TC but remained sensitive to adefovir with an IC₅₀ changed by less than 8.4-fold compared to wild-type HBV.

III (d). Anti-HIV-1 activity of adefovir in combination with other antiviral agents

The interaction of adefovir with nucleoside analogs used in the treatment of HIV-1 was previously reviewed

Adefovir exhibited synergistic to additive *in vitro* anti-HIV-1 activity in combination with most of the FDA approved nucleoside analogue reverse transcriptase inhibitors

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(NRTIs), non-nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs).

Clinical Study

Study GS-98-437

Title: Genotypic and Phenotypic Analyses of HBV from Patients in a Phase III Study of ADV for the Treatment of Hepatitis B e Antigen Positive Chronic HBV Infection

Study GS-98-437 is a randomized, double blind, placebo-controlled phase III clinical study of the safety and efficacy of ADV for the treatment of patients with HBeAg+ chronic HBV infection. A total of 515 patients were enrolled and randomized to receive placebo or adefovir (ADV) 10 mg or 30mg qd at a ratio of 1:1:1. Inclusion criteria included requirements for the presence of HBsAg, HBeAg and serum HBV DNA levels $>10 \times 10^6$ copies/mL. The primary efficacy endpoint was changes in liver histology for the 10-mg ADV daily compared to placebo. Secondary efficacy endpoints included: 1) changes in liver histology for the 30-mg ADV daily compared to placebo, 2) the efficacy of each of two doses of ADV (10 mg or 30 mg) compared to placebo in enhancing anti-HBe seroconversion, decreasing serum HBV DNA, and decreasing ALT levels.

Virology Substudy (Study GS-98-437)

A virology substudy of Study GS-98-437 included all patients in the ITT population (n=511).

Objectives

1. To identify mutations in the HBV polymerase gene from HBV patient isolates that are potentially associated with virological resistance to ADV during the first 48 weeks of clinical study GS-98-437.
2. To evaluate the effects of these mutations on the clinical response to ADV therapy.
3. To determine whether these mutations alter antiviral susceptibility to adefovir in vitro.

IV. HBV DNA levels in serum of patients treated with ADV

The median baseline HBV DNA for patients randomized to placebo group (n= 167), 10 mg ADV (n=171) or 30 mg ADV (n=173) were 8.3, 8.4, or 8.3 \log_{10} copies/mL, respectively. The median change in HBV DNA from baseline to week 48 for placebo group (n=164) was -0.5; for ADV 10 mg (n=131), -2.8; and for ADV 30 mg (n=83), -4.0 \log_{10} copies/mL (NDA 21449; vol 84; Appendix 9, page 288, Table A2). For analysis of histologic improvement and other efficacy markers, please see reviews of the Medical Officer and Statistician.

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mutants (R366K/R, S467A/S, V562A, E566K, H582Q, and K589R) were within 0.3 to 3.6 fold of that for wild-type HBV. None of the mutant IC₅₀ values for adefovir were significantly different from that of wild-type HBV, indicating that these conserved site substitutions did not cause resistance to adefovir *in vitro*. The HBV carrying the H481L substitution was replication defective in cell culture. Thus, this substitution was subsequently evaluated in an enzymatic assay.

Table 3: Conserved site substitutions in HBV polymerase (Study GS-98-437)

Substitution	Patient ID	Treatment arm	Reversion to wild-type Residue
R366K/R	047-6030	Placebo	No
G458R	0511-4063	30 mg	Yes
S467A/S	0329-1072	10 mg	No
H481H/L	0456-7006	30 mg	No
V562A	0517-7019	30 mg	No
A562V	0338-3018	10 mg	Yes
E566E/K	0471-6079	Placebo	No
H582Q	0510-4001	10 mg	No
V583L/V	0340-3014	30 mg	Yes
K589R	0370-2030	Placebo	No

Table 4: Phenotypic analyses of recombinant HBV expressing novel conserved site substitutions

Conserved Site substitutions	Treatment Group	IC ₅₀ (μM)#	Fold Increase
Wild-type		0.24	1
R366K	Placebo	0.07	0.3
S467A	10 mg	0.15	0.6
H481L	30 mg	r.d.*	r.d.
V562A	30 mg	0.26	1.1
E566K	Placebo	0.36	1.5
H582Q	10 mg	0.86	3.6
K589R	Placebo	0.21	0.9

#= All IC₅₀ values are the average of at least two separate experiments.

+= An adefovir resistance phenotype is predefined by the sponsor as ≥ 50-fold increase in IC₅₀ values for a mutant HBV compared to wild type HBV.

r.d.* = replication defective in cell culture

The inhibition constant (K_i) of adefovir diphosphate for H481L HBV polymerase was determined to be 0.18 μM in the enzymatic assay (NDA 21,449; vol 084, page 26, Table 8). The K_i of adefovir diphosphate for the mutant enzyme was increased by only 1.8-fold compared to the wild-type enzyme, indicating that the H481L HBV polymerase mutant remained sensitive to adefovir diphosphate.

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Comments

1. The sponsor defined adefovir resistance for HBV isolates as ≥ 50 -fold increase in IC_{50} exhibiting (NDA, 21449; vol 84; page 65). However, this IC_{50} value for adefovir resistance is too high. At present, there is no defined IC_{50} value of adefovir for HBV resistance. However, an HBV isolate exhibiting >10 -fold increase in IC_{50} would be considered resistant under the experimental condition used since other study has shown that lamivudine resistant mutant with the M552V mutation exhibited a 8-fold increase in IC_{50} and was sensitive to adefovir. The clinical significance of fold changes in IC_{50} has not been established.
2. The phenotypic assay used by the sponsor may not be able to detect drug resistance caused by multiple-substitutions in the specific viral genetic background of individual patients. The patient-derived full-length HBV clones may provide a better system to determine adefovir susceptibility in cell culture.
3. A shift in adefovir susceptibility of 3.6-fold for HBV isolate with H582Q conserved site mutation was not considered significant because a 5.9 log reduction in serum HBV DNA level from baseline at week 48 was found for the patient 0510-4001 with this mutation (Table 5). Additionally, phenotypic susceptibility for adefovir was determined using HBV transfected HepG2 cells. The transfection efficiency and assay variability would affect the IC_{50} values determined by this assay.

Each of the four ADV-treated patients had a >3.3 \log_{10} decrease in HBV DNA levels at week 48 and had no evidence of rebound during the first 48 weeks of therapy (Table 5).

Table 5: HBV DNA responses in ADV-treated patients with conserved site substitutions in HBV polymerase

Patient ID	Amino acid substitutions	Treatment arm	Changes in HBV DNA at week 48 (\log_{10} copies/mL)	HBV DNA Rebound ¹
0329-1072	S467A/S	10 mg	-4.1	No
0456-7006	H481H/L	30 mg	-3.3	No
0517-7019	V562A	30 mg	-4.4	No
0510-4001	H582Q	10 mg	-5.9	No

¹HBV DNA rebound is defined as a 1.0 \log_{10} copies/mL increase in serum HBV DNA at any time from nadir to the last HBV DNA measurement up to 48 weeks.

Results in Table 5 showed that HBV variants with S467A/S, H481H/L, V562A and H582Q mutations were responding to ADV treatment with a reduction of 3.3 to 5.9 \log_{10} copies/ml in viral DNA at week 48. In addition, HBV DNA rebound was not observed in these patients suggesting that these mutations do not cause viral DNA rebound.

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Comment

Genotypic and phenotypic analyses of HBV from patients in study GS-437 failed to identify mutations that may confer a reduction in susceptibility to adefovir in vitro.

VI. Viral DNA rebound (Study GS98-437)

There were 88 patients whose viral load rebounded. Of these, 44 patients received placebo. The remaining 44 patients received either ADV 10 mg or 30 mg. For patients receiving 30 mg ADV, there was a dose reduction for 15 patients and these patients were excluded for rebound analysis. In addition, three other patients in the 30-mg treatment group were also excluded for analysis because of drug compliance. Only 26 patients were identified as rebounders; n=19 for the ADV 10-mg group and n= 7 for ADV 30-mg group.

These 7 patients from ADV 30 mg with HBV DNA rebound were 0334-1061; 0339-3054; 0381-1133; 0454-3049; 0491-6068; 0499-3040; 0534-1067. Data on the viral DNA rebound, changes in HBV DNA (\log_{10} copies/mL) from baseline to week 48 and polymorphic mutations identified in the polymerase gene are shown in Table 6.

Table 6 shows the following:

1. No mutations were observed in post therapy isolates of 23 patients. Therefore, the HBV DNA rebound observed in these patients could be due to natural fluctuations or drug compliance issues and not due to development of drug resistance.
2. Polymorphic substitution in the HBV polymerase gene exhibiting HBV DNA rebound were obtained in two patients in the ADV 10-mg group (patients 0496-6048 and 0518-2014) and from 1 patient (0491-6068) in the ADV 30-mg group. Another patient 0474-6086 with a polymorphic substitution was excluded from analysis because of documented non-compliance.
3. The serum HBV DNA levels for 3 of the patients who rebounded with emerging amino acid substitutions are shown in Figure 2 (NDA 21449; vol 84, page 37). HBV DNA levels for these patients (n= 3) were marginally above the rebound criteria (week 48-nadir = $1.0 \log_{10}$ copies/mL). A total of 13 amino acid substitutions were observed in HBV polymerase gene from 3 patients with HBV DNA rebound in ADV-treated patients (n=2 with ADV 10 mg and 1 from ADV 30 mg).

To evaluate if any of these mutations conferred resistance to adefovir, HBV DNA data in patients carrying each of the amino acid substitution at baseline were analyzed. In other ADV-treated patients carrying HBV with the specified amino acids at baseline, the median HBV DNA reduction at week 48 were in the range of 3.5 to 4.8 (NDA 21449, vol 84, page 36, Table 17) indicating that none of these amino acids individually conferred resistance to ADV. The sponsor stated that the observed HBV DNA rebound in ADV-treated patients appeared not to be due to the specific emergence of any of these substitutions.

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Table 6: Viral DNA rebound (NDA 21,449, vol 84, pages 252-268 and Appendix 9, Listing 1, pages 294-300).

Patient ID	Treat- Ment	HBV DNA log ₁₀ copies/mL			HBV DNA nadir at week	Δ from wk 48 to nadir	Δ from baseline to wk 48	Genotype (Substi- tution)
		Baseline	Nadir	Week 48				
0030-1062	10 mg	6.52	2.89	4.64	Wk 28	1.75	-1.88	No
0329-1084	10 mg	7.44	4.48	6.75	Wk 16	2.27	-0.68	No
0330-1073	30 mg	9.43	4.45	5.46	Wk 28	1.01	-3.96	No
0330-1102	30 mg	9.95	2.60	3.96	Wk 28	1.36	-5.99	Unmat- Ched
0334-1053	10 mg	9.27	7.14	8.61	Wk 40	1.47	-0.66	No
0334-1061	30 mg	8.40	6.65	8.30 (wk 32)	Wk 28	1.65 (wk 32)	-0.10 (wk 32)	No
0339-3017	30 mg	7.79	2.60	7.87	Wk 32	5.27	+0.08	No
0339-3054	30 mg	8.72	4.58	7.41	Wk 24	2.83	-1.31	No
0340-3024	30 mg	8.37	4.62	6.17	Wk 36	1.55	-2.2	No
0341-2001	10 mg	8.25	5.79	7.20	Wk 24	1.41	-1.05	No
0370-2042	30 mg	8.36	4.32	5.99	Wk 20	1.67	-2.37	No
0381-1133	30 mg	8.55	4.90	8.51	Wk 8	3.61	-0.04	No
0454-3049	30 mg	7.98	4.80	6.45	Wk 24	1.65	-1.53	No
0456-7001	10 mg	8.72	6.54	7.80	Wk 32	1.26	-0.92	No
0456-7009	30 mg	7.50	2.60	6.10	Wk 28	3.5	-1.40	Y483S
0465-3057	30 mg	8.70	5.29	6.38 (wk 36)	Wk 20	1.09 (wk 36)	-2.32	No
0468-6032	30 mg	8.72	4.43	7.29	Wk 32	2.86	-1.43	No
0468-6064	30 mg	7.43	2.60	6.05	Wk 12	3.45	-1.38	No
0469-6117	10 mg	10.16	7.39	8.85	Wk 24	1.46	-1.31	No
0474-6062	10 mg	7.39	4.18	5.24	Wk 40	1.06	-2.15	No
0474-6086	30 mg	6.00	3.41	5.86	Wk 12	2.45	-0.14	S454C/S
0486-6006	10 mg	9.52	7.26	9.3 (wk 44)	Wk 8	2.04	-0.22	No
0487-6091	10 mg	8.78	5.61	6.65	Wk 44	1.04	-2.13	No
0491-6068	30 mg	8.33	2.60	3.82	Wk 44	1.22	-4.52	H474Y N619H, R667Q

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Patient ID	Treat- Ment	HBV DNA log ₁₀ copies/mL			HBV DNA nadir at (week)	Δ from wk 48 to nadir	Δ from baseline to wk 48	Genotype (Substi- tution)
		Baseline	Nadir	Week 48				
0494-6109	30 mg	8.52	3.46	4.55	Wk 36	1.10	-3.97	No
0496-6048	10 mg	7.80	3.96	5.07	Wk 32	1.11	-2.73	T364I, H472Y, I614R, V626I, S661A
0497-6105	30 mg	8.75	2.93	5.91	Wk 36	2.98	-2.84	No
0499-3035	30 mg	8.22	3.12	5.10	Wk 28	1.98	-3.12	E482D/E
0499-3040	30 mg	7.36	4.36	6.40	Wk 8	2.04	-0.96	No
0505-6127	10 mg	8.14	6.51	9.23	Wk 4	2.72	+1.09	No
0511-4032	10 mg	8.98	5.94	6.99	Wk 20	1.05	-1.99	No
0511-4038	30 mg	8.36	3.87	4.96	Wk 36	1.09	-3.40	No
0511-4060	30 mg	8.79	4.89	6.27	Wk 24	1.38	-2.52	No
0514-7028	30 mg	8.63	3.29	5.14	Wk 28	1.85	-3.49	No
0517-7011	10 mg	8.90	7.45	8.90	Wk 20	1.45	0	No
0518-2010	10 mg	9.57	6.59	7.95	Wk 16	1.36	-1.62	No
0518-2014	10 mg	6.38	2.60	4.14	Wk 28	1.54	-2.24	I470T, T476N/ S567A/S T586A I626I/V
0519-1059	10 mg	7.80	5.07	6.79	Wk 24	1.72	-1.01	No
0522-1094	30 mg	9.32	3.99	8.37	Wk 28	4.38	-0.95	No
0532-1043	10 mg	9.49	4.72	5.86	Wk 36	1.14	-3.63	No
0534-1067	30 mg	8.12	4.43	8.15	Wk 8	3.72	+0.03	No
0535-1085	30 mg	9.20	4.25	5.72	Wk 40	1.47	-3.48	No
0548-1039	10 mg	8.95	6.12	7.24	Wk 44	1.42	-1.71	No
0696-1007	10 mg	8.09	6.77	8.77	Wk 8	2.0	+0.68	No

VII. Clinical Study (Virology Substudy) GS-98-438

GS-98-438 is a double-blind, randomized, placebo-controlled Phase III study of ADV for the treatment of patients with HBeAg-/anti-HBe/ HBV DNA+ chronic HBV infection. Inclusion criteria included requirements for the presence of HBsAg, anti-HBe, and serum HBV DNA $\geq 1 \times 10^5$ copies/mL. One hundred eighty-four patients were enrolled and randomized to receive placebo or ADV 10 mg qd at a ratio of 1:2. The primary efficacy endpoint is an improvement in liver histology at week 48 and monthly measurement of serum HBV DNA, ALT and HBV serology utilized in secondary assessments.

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Objectives:

1. To identify mutations in the HBV polymerase gene that are potentially associated with resistance to ADV during the first 48 weeks of clinical study GS-98-438.
2. To evaluate the effects of these mutations on the clinical response to ADV therapy.
3. To determine whether these mutations alter antiviral susceptibility to adefovir in vitro.

Precore-HBV mutants

Molecular analysis of the HBV species isolated from patients with HBeAg-/HBe+/HBV DNA showed mutations that reduce the expression of the precore gene product, the precursor of HBeAg. This type of infection is also termed precore mutant HBV infection. In this setting HBeAg is undetectable despite high level HBV replication. The precore region of the HBV genome consists of 87 nucleotides upstream of, and in frame with, the core open reading frame. If translation begins at the translational start codon (ATG) located at the beginning of the precore region, the HBeAg protein is produced following synthesis and proteolytic processing and secreted in wild-type HBV hepatocytes. In hepatocytes infected with precore mutants, the synthesis and secretion of HBeAg protein is prevented or reduced. The precore mutation observed in 90% of patients is G1896 to A, creating a stop codon. In many patients a second mutation G1899 to A has also been found. The stop codon mutation is found only in patients infected with genotypes that bear a thymidine at nucleotide 1858, i.e., genotypes B, C, D, and E; thus, accounting for the lower incidence in the USA and northern Europe where genotype A predominates.

The regulation of HBeAg expression is controlled by the HBV basal core promoter (BCP) that has been mapped to nucleotides 1742 to 1849. The double mutations of A1762T and G1764A in the BCP have been correlated with decreased HBeAg production in vitro and in patients (NDA 21449; vol 85: page 12).

VII (a). Genotypic and Phenotypic Analysis

Matched baseline and week 48 HBV sequences were obtained from 117 of the 184 patients. Sixty-seven of 123 patients receiving ADV 10 mg had serum HBV DNA levels <400 copies/mL at week 48 and were not genotyped. Therefore, matched baseline and week 48 HBV genotypes were available from 56 patients receiving ADV 10 mg, and from 61 patients receiving placebo. None of the HBV sequences from 56 patients treated with ADV 10 mg at week 48 showed any conserved sites mutation in the HBV polymerase gene (NDA 21449; vol 85, page 20, Table 4). However, conserved site HBV polymerase mutations emerged in HBV variants from 3 patients receiving placebo at week 48. A polymorphic substitution V562P emerged in the HBV isolates from a patient (0624-1528) in the ADV treatment arm. In vitro phenotypic analysis showed that the IC₅₀ value of adefovir for V562P mutant HBV variant was 0.20 μM, similar to wild type HBV. A patient who developed a V562P mutation had a 3.9 log₁₀ decrease in HBV DNA (NDA 21449; vol 85, page 26, Table 7).

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VIII. Viral DNA Rebound (Study GS-98-438)

HBV DNA rebound was observed in five patients treated with ADV 10 mg (Table 7) and 19 patients from placebo group. Two of the five patients in the ADV treatment group developed HBV polymerase substitutions. The viral load rebound observed in patients with no amino acid substitution in polymerase could be due to natural fluctuation of HBV DNA levels. The two ADV-treated rebounder patients developed 3 and 11 HBV polymerase substitutions, respectively. In ADV-treated patients carrying HBV with a specific amino acid substitution at baseline, the median viral load reduction at week 48 ranged from 3.1 to 4.3 log₁₀ indicating that none of these amino acid substitution individually conferred resistance to ADV (NDA 21,449; vol 85; page 34, Table 12).

Table 7: Viral load rebound (Source: NDA 21,449, vol 85, Appendix 7, pages 208-214; and Appendix 9, Listing 1, pages 234-236 and Appendix 9, Listing 1, pages 294-300).

Patient ID	Dose-Reduction	HBV DNA log ₁₀ copies/mL			HBV DNA nadir at (week)	Δ from wk 48 to nadir	Δ from baseline to wk 48	Genotype (Substitution)
		Baseline	Nadir	Wk 48				
0470-5514	No	6.77	2.98	4.56	Wk 24	1.58	-2.21	No
0474-5508	No	7.52	5.36	6.42	Wk 8	1.06	-1.10	T426S F499Y K681K/N
0510-4515	No	6.20	2.60	4.58	Wk 8	1.98	-1.62	No
0624-1503	No	6.15	2.60	5.70	Wk 32	3.1	-0.45	No
0625-1544	No	6.70	2.60	6.61	Wk 32	4.01	-0.09	Yes (see below)

0625-1544 Substitution in HBV Pol gene at wk 48: E349Q, A386E, Y402H, S454T, R458G, L463V, Y472H, H474R, S483Y, R614I, E619Q

IX. HBV polymerase substitutions associated with other agents (study GS-98-437)

Hepatitis B immunoglobulin (HBIG) is indicated to prevent HBV re-infection in patients following liver transplantation. Hepatitis B surface antigen (HBsAg) mutants were found to be associated with recurrence of HBV infection in liver transplant patients treated with HBIG. The open reading frame coding for HBV surface antigen overlaps completely with that of HBV polymerase. Thus, nucleotide changes encoding HBIG-resistant surface antigen mutations can simultaneously cause mutations in HBV polymerase. Several HBIG-associated HBV polymerase mutations have been reported including T476N, V490E, V490T, W501K, W501Q, and I535L. The T476N mutation, in combination with M552I or additionally with L528M, has been reported to increase the replication efficacy

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of HBV in vitro. All famciclovir-associated mutations tested to date remain sensitive to adefovir in cell based and enzymatic assays.

Three substitutions previously reported to be associated with famciclovir (V555I) or hepatitis B immunoglobulin (HBIg)-escape (T476N and R501Q) resistance were observed to develop (Study GS-98-437). None of these patients had received famciclovir or HBIg prior to or during the GS-98-437 clinical study.

These three substitutions were tested in cell culture for their susceptibility to adefovir. In vitro phenotypic analysis indicated that all three HBV mutants remained fully susceptible to adefovir with IC₅₀ values increased by ≤1.5 fold compared to that of wild-type HBV (NDA 21449; vol 084, page 31, Table 13). All four patients (Table 8) had a decrease of 2.2 to 6.3 log₁₀ copies/mL in HBV DNA at week 48. Table 8 shows that HBV polymerase substitutions associated with HBIg -escape did not confer cross-resistance to adefovir.

Table 8: HBV polymerase substitutions associated with other antiviral agents (GS-98-437)

Substitutions	Patient ID	Treatment Arm	Change of HBV DNA at week 48 (log ₁₀ copies/mL)
T476N	0518-2014	10 mg	-2.2
R501Q	0511-4063	30 mg	-3.4
V55I	0339-3019	10 mg	-2.2
	0487-6099	30 mg	-6.3

X. Antiviral activity of ADV against different genotypes of HBV (study GS-98-437)

The anti-HBV activity of ADV against A-D genotypes of HBV is summarized in Table 9. Patients enrolled in study GS-98-437 were grouped according to their genotypes and change in HBV DNA log₁₀ copies from baseline to week 48 was tabulated (Source: NDA 21449; vol 84, Appendix 7 pages 251-268).

Table 9: Antiviral activity of ADV against HBV genotypes

HBV genotypes	HBV DNA (log ₁₀ copies/ml) change from baseline at week 48							
	-0 to 1.0	-0 to -1.0	-1 to -2	-2 to -3	-3 to -4	-4 to -5	-5 to -6	≥-6
A (n=45)	2	2	8	9	8	3	6	7
B (n=37)	0	3	5	9	7	8	4	1
C (n=59)	0	1	7	15	10	12	13	1
D (n=23)	0	1	2	6	8	3	2	1

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Table 9 shows that adefovir demonstrated anti-HBV activity against all genotypes (A, B, C, D) tested. There were very few patients from genotype E and F. Since the HBV polymerase catalytic site is conserved in all strains, adefovir is expected to be active against all HBV genotypes.

2. If a decrease in HBV DNA of 1.0 log₁₀ copies/mL from baseline is considered as a criterion, then ADV efficacy was 91% against genotype A, 91% against genotype B, 98% against genotype C and 96% against genotype D.

Study GS-98-435

Title: Genotypic Analyses of Baseline HBV and Response to Adefovir Dipivoxil Therapy from Patients in an Open-Label Study of Adefovir Dipivoxil for the Treatment of Liver Disease Due to Lamivudine Resistant HBV in Liver Transplant Patients.

OBJECTIVES:

1. To determine the patterns of 3TC resistance mutations in liver transplant patients at baseline.
2. To assess the anti-HBV efficacy of ADV in patients with different patterns of 3TC resistant mutations at baseline.

Study Design

One hundred and thirty-one post liver transplantation patients who failed 3TC therapy were enrolled and received at least one dose of ADV in GS-98-435 between May 1999 and February 2001. ADV 10 mg once daily was added to the existing HBV therapy. ADV was dose adjusted for patients with renal impairment at baseline. The primary endpoint for the antiviral efficacy of ADV 10 mg daily was HBV DNA suppression. Secondary endpoints included: changes in liver histology, changes in Childs-Pugh score, the proportion of patients with HBeAg loss or HBsAg loss or seroconversion, the proportion of patients with HBV DNA below 400 copies/mL.

Inclusion Criteria

1. Positive serum HBV DNA ($\geq 1 \times 10^6$ copies/mL by Roche Amplicor™ PCR assay or
2. Prior liver transplantation or wait listed for liver transplantation.
3. Prior 3TC therapy.
4. ALT ≥ 1.2 x upper limit of the normal range.
5. Compensated or mildly or moderately decompensated liver disease.
6. Adequate renal function
7. Adequate hematological function.

Three cohorts of patients were enrolled.

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1. Cohort 1 included patients meeting all eligibility criteria.
2. Cohort 2 included patients previously enrolled in study GS-98-451i (physician held IND, in which the patients received open-label ADV).
3. Cohort 3 included liver transplant patients who did not meet the eligibility criteria of cohort 1 due to several medical conditions and life-threatening HBV disease.

Each of the three cohorts contained two groups of patients; those who had received liver transplant (group A) and those waitlisted to receive transplant (group B).

Prior to enrollment in GS-98-435, all patients had received 3TC therapy. In addition, patients also had received HBIG (33%), famciclovir (33%), interferon (13%), ganciclovir (10%) and adefovir dipivoxil (5%).

Virology substudy (GS-98-435)

The virology substudy of GS-98-435 included all patients (n = 131) defined for the interim analyses. Baseline resistance-associated mutations in HBV polymerase were identified by DNA sequence analysis of PCR-amplified HBV specific DNA from baseline patient samples. The effect of specific mutations on viral load reduction by ADV were also evaluated. Serum HBV DNA was measured by a PCR assay (LLQ= 400 copies/mL) in a central laboratory. Viral load was analyzed for patients in Cohort A and 3A patients (n= 121).

XI (a). Baseline HBV polymerase mutations:

Baseline HBV sequences were obtained for 122 patients (NDA 21449, vol 86, page 90, Table 3). Of the 122 patients with genotyping data, 119 patients (98%) had 3TC resistance mutations in the YMDD motif in the HBV polymerase at baseline (Table 10).

Table 10: Baseline mutational patterns in HBV isolates from patients in GS-98-435

Pattern	Number of Patients
Patients with YMDD Mutations	119 (100%)
528M+552V	73 (61%)
521L+528M+552V	23 (19%)
552I	11 (9%)
528M+552I	11 (9%)
Total	118 (99%)

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XI (b): Effect of baseline 3TC-associated mutations on HBV DNA reduction by ADV

Treatment with ADV 10 mg once daily resulted in sustained viral load reduction in the overall treatment population. Following 24 and 48 weeks of treatment with ADV, the median reductions in HBV DNA were 3.5 and 4.4 log₁₀ copies/mL (Table 11).

ADV showed similar efficacy against 3TC- resistant HBV regardless of the patterns of mutations associated with 3TC- resistance (Table 12).

Table 11: Change from baseline in serum HBV DNA (log₁₀ copies/mL) to each study visit (Source : NDA 21449; vol 86, Appendix 1, Table 16, pages 107-110).

Cohort

	1A (N= 79)	3A (N= 42)	Total (N=121)
<u>Log₁₀HBV DNA at baseline</u>			
Mean (Std Dev)	8.2 (1.1)	7.7 (1.6)	8.0 (1.3)
Median	8.3	8.1	8.2
N	68	35	103
Missing	11	7	18
Assays below LLQ	1	1	2

**Changes in Log₁₀HBV DNA
To week 24**

Mean (Std Dev)	-3.8 (1.2)	-3.8 (1.4)	-3.8 (1.3)
Median	-3.5	-3.7	-3.5
N	55	26	81
Missing	24	16	40

**Changes in Log₁₀HBV DNA
to week 48**

Mean (Std Dev)	-4.5 (1.2)	-4.1 (1.8)	-4.4 (1.4)
Median	-4.4	-3.9	-4.4
N	28	15	43
Missing	51	27	78

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Table 12: Viral Load responses by baseline patterns of 3TC-resistant HBV

Pattern	Change in HBV DNA at Week 24 (log ₁₀ copies/mL)		Number of Patients
	Mean	Median	
528M+552V	-4.0	-3.7	41
521L+528M+552V	-3.5	-3.3	19
552I	-3.6	-3.8	9
528M+552I	-3.9	-3.5	7

Table 12 showed that there was no statistical difference in the viral load responses of patients with either the 552V or the 552I mutation to adefovir dipivoxil. The presence of the 521L or 528M mutations in HBV did not affect viral load reduction by ADV.

XII. GS-00-460i

Title: Genotypic Analyses of HBV and HIV from Patients in an Open-Label Study of Adefovir Dipivoxil for the Treatment of Chronic HBV Infection in Patients Co-infected with HIV.

Study GS-00-460i was an open-label (single center) pilot study to evaluate the safety and efficacy of a 10 mg once daily dose of ADV for the treatment of chronic hepatitis B in HIV-1/HBV co-infected patients with lamivudine resistant HBV. During this study, ADV 10 mg administered daily demonstrated significant anti-HBV efficacy with a mean 3.4 log₁₀ copies/mL decrease in serum HBV DNA at week 24, and 4.0 log₁₀ copies/mL at week 48.

Match baseline and week 48 HBV genotypes were available for 20 patients. However, genotypic data for HIV-1 were not available since all patients had HIV-1 RNA of <400 copies/mL. All patients had 3TC -resistant mutations (L528M+ M552V; n=30, or L528M+M552I; n=4, or M552I alone; n=1) at baseline. Genotypic analysis of baseline matched HBV isolates at week 48 from 20 patients showed the following:

- HBV isolates from 18 patients retained 3TC -resistant mutations (L528M+ M552V; n=17, or L528M+M552I; n=1) in the HBV polymerase gene at week 48 (GSI E-mail dated 10/15/2002, NDA 21449, vol 86, appendix I, pages 156-158). In addition, amino acid isoleucine (552I) present at baseline in the HBV polymerase from one patient  changed to valine (552V) at week 48. Lamivudine resistant mutation (M552V) present at baseline in the HBV polymerase gene of another patient  reverted to wild type residue (V552M) at week 48.
- A new mutation R462G in the HBV polymerase gene was detected in HBV isolates from 7/20 patients at week 48. HBV isolates from 5 of these 7 patients with R462G mutation in the polymerase gene retained baseline 3TC -resistant mutations (L528M + M552V) at week 48. Similarly an HBV isolate from 1 patient  with R462G mutation in the polymerase gene retained baseline 3TC -resistant mutations (L528M+ M552I). However, an HBV isolate from another patient  with R462G

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mutation in the polymerase gene had an isoleucine to valine substitution (I552V) at week 48. The baseline isolate from this patient contained 3TC -resistant mutation L528M+ M552I in the HBV polymerase gene. HBV DNA rebound was not observed in 4 of the 5 patients with R462G mutation and 3TC-resistant mutations L528M + M552V (NDA 21449, vol 86, page 149, Figure 2). However, HBV DNA from one of the 5 patients with R462G mutation and 3TC-resistant mutations L528M + M552V fluctuated during therapy. HBV DNA level for this patient was below the baseline HBV DNA level during therapy. Similarly, HBV DNA rebound was not observed for the patient with R462G mutation and 3TC -resistant mutation L528M+ M552I (NDA 21449, vol 86, page 150, Figure 3). HBV DNA from the patient with R462G and 3TC -resistant mutations L528M + I552V fluctuated during therapy (weeks 36 to 48), but remained below the baseline HBV DNA level. Phenotypic analysis data for patients with R462G mutation alone, or in combination with 3TC -resistant mutations were not available.

- Besides R462G, other mutations were detected in the HBV polymerase gene from 4/20 patients at week 48. Mutations N470T and N470L were detected in the HBV polymerase gene from 2 of these 4 patients at week 48. A substantial reduction in HBV DNA was observed in these 4 patients. The role of these mutations for development of adefovir resistance is not known.

CONCLUSIONS

With respect to microbiology, this NDA is approvable. ADV has demonstrated ample antiviral activity in HBeAg+ and HBeAg- chronic hepatitis patients. A median decrease of HBV DNA of 3.52 log₁₀ copies/mL from baseline at week 48 was observed in HBeAg+ patients treated daily with 10 mg of ADV (study 437). Similarly, a median decrease of HBV DNA of 3.91 log₁₀ copies/ml from baseline at week 48 was observed in HBeAg- patients treated daily with ADV 10 mg (study 438). Approximately, 21% of patients (n= 171; study 437) and 51% patients (n= 123; study 438) had serum HBV DNA levels of <400 copies/mL at week 48 (p= <0.001). Limited data available suggested that ADV exhibited similar antiviral activity against HBV genotypes A, B, C and D.

Genotypic analysis of HBV from ADV (10 mg or 30 mg) treated HBeAg⁺ patients (n = 215; study 437) and HBeAg⁻ patients (n=56; study 438) at baseline and week 48 failed to identify mutations in the HBV polymerase gene that may confer reduced susceptibility to adefovir. HBV DNA from twenty-six patients in study GS-98-437 (ADV 10 mg group = 19 and ADV 30 mg group n= 7) rebounded (unconfirmed 1 log₁₀ increase in HBV DNA between the nadir and Week 48). No mutations in the HBV polymerase gene were observed in any of the HBV isolates from 23 patients. Post-therapy isolates from 3 patients with virologic rebound contained several polymorphic mutations in the HBV polymerase gene. In study GS-98-438, five of the 56 patients showed viral DNA rebound. Of these, HBV from two patients contained 3 and 11 polymorphic mutations in the polymerase gene. However, the molecular basis and clinical significance of the observed viral rebound are not known.

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Recombinant HBV variants containing lamivudine-resistance-associated mutations (L528M, M552I, M552V, L528M +M552V) in the HBV DNA polymerase gene were susceptible to adefovir in vitro. Adefovir has also demonstrated anti-HBV activity (a median reduction of HBV DNA of 3.5 log₁₀ copies/mL) against clinical isolates of HBV containing lamivudine-resistance-associated mutations (study GS98-435). Seven of the 20 patients co-infected with HIV-1 and HBV and treated with ADV for 48 weeks developed an R462G mutation in the HBV polymerase gene. Data on the in vitro drug susceptibility of HBV variants with R462G mutation were not available. Therefore, the contribution of this mutation towards adefovir resistance is not known. HBV variants with DNA polymerase mutations T476N and R501Q associated with resistance to hepatitis B immunoglobulin were susceptible to adefovir in vitro.

METHODOLOGY

Collection of Serum Samples

Blood samples were collected from all patients at screening, baseline, monthly visits and at the completion of dosing (study 437 & 438). A portion of the serum samples, at least 5 aliquots of 1 mL, was banked. Serum samples were maintained by the _____ and shipped to _____ for genotypic analysis.

Genotypic analysis

The nucleotide sequence of the polymerase/reverse transcriptase (pol/RT) domain of HBV polymerase (nucleotides 1045 to 2076, amino acid 349 to 692) was determined for all qualified patient samples. All genotypic analyses were performed by the reference _____ according to the standard laboratory procedure (NDA21449, vol 84; Appendix 3, pages 112-145) and are briefly summarized here.

HBV DNA extraction

Serum samples were thawed and HBV DNA was extracted and purified from 200 µl aliquots using the _____

Amplification

Purified HBV DNA was used for nested PCR. The first round of PCR yielded a 1.7 kb DNA fragment and the second round PCR yielded a 1.4 kb DNA fragment containing HBV pol/RT domain. The first round of PCR used primers HBV-7.5 and CHBV-4 (NDA 21449, vol 84, page 62, Table1) and the second round used primers CHBV-3 and HBV-SEQ-17 rev (NDA 21449, vol 84, page 62, Table 1).

Sequence analysis

The product of the second round of nested PCR was sequenced on both strands using

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5 sequencing primers in cycle sequencing reactions containing

The sequencing reaction products were purified by ethanol precipitation and analyzed on an ABI-377 DNA sequencer. During the validation process the minority sequence in a mixed population of sequences was detectable at a level of ~30%. The results of the 5 sequencing reactions were used to assemble a single consensus contiguous sequence using the analysis program

The contiguous sequences from serum samples at baseline and week 48 from the same patient were aligned using the MegAlign sequence alignment program

Any changes in the nucleotide sequence of the HBV polymerase domain present in post-treatment samples not present in baseline sample was investigated further. If the post-treatment sample contained a mixture of nucleotides not present in the baseline sample, the development of a mutation was reported. Amino acid sequences containing emergent mutations were aligned with the amino acid sequences of HBV polymerases from 70 individual isolates of HBV representing all 7 HBV genotypes (A-G) from Genbank (NDA 21,449; vol 84, Appendix 4, pages 137-145) to determine whether any mutations observed at the amino acid level occur at polymorphic sites in the coding sequence. Alignment revealed that 134 out of the 344 amino acid residues in the polymerase /reverse transcriptase domain of the HBV polymerase gene were non-conserved or polymorphic in the population of 70 wild-type HBV isolates. Amino acid substitutions emerging during therapy at conserved residues in the HBV polymerases were assessed for their ability to confer an adefovir resistant phenotype *in vitro* and for their association with virological rebound *in vivo*.

Phenotypic analysis

All novel substitutions at conserved sites within HBV polymerase were evaluated for adefovir susceptibility in an *in vitro* cell-based assay for HBV replication. Briefly, nucleotide mutations coding for specific amino acid substitutions were engineered by site-directed mutagenesis into a plasmid vector encoding a standard laboratory strain of HBV. HBV containing the substitutions was expressed by transient transfection into HepG2 cells. Transfected cells were treated with various concentrations of adefovir for 7 days and the amounts of intracellular replicating virus DNA were then quantified by Southern blot hybridization to determine adefovir sensitivity and the results expressed as a fold change in IC₅₀ from wild type.

Measurement of Serum HBV DNA

Amplicor HBV Monitor™ test was used for the measurement of serum HBV. This assay is not FDA approved.

Amplicor HBV Monitor™ test is based on competitive PCR with a quantitation standard. In the PCR assay, a fixed amount of quantitation standard is added to each of the tubes containing either the HBV sample or standard HBV DNA. HBV and the quantitation standard DNA are thus co-amplified with similar efficiency. The amount of HBV DNA

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originally present in the sample is determined in a quantitative hybridization assay by measuring the post-amplification ratios of HBV to the quantitation standard. The number of copies/mL is derived from the standard curve (established in each run). The Amplicor HBV Monitor™ assay can quantify HBV-DNA between 400 and 10⁷ copies/mL.

The Amplicor HBV Monitor™ assay was more sensitive than the b-DNA and hybrid – capture assays (Pawlotsky *et al.*, 2000). Amplicor HBV Monitor™ assay gave positive results for 4 samples that were negative in the other two assays, and on retesting with the Monitor assays (specificity = 97%). The sensitivity of Roche Amplicor HBV Monitor™ test was compared with Digene Hybrid -Capture™ and branched DNA (b-DNA) Quantiplex™ HBV DNA assays. The clinical sensitivities (the ability to detect and quantify HBV DNA) of these three assays were compared by testing 200 HBsAg-positive serum samples. Amplicor HBV Monitor™ assay was more sensitive than the b-DNA and hybrid-capture assays (Pawlotsky *et al.*, 2000).

MICROBIOLOGY LABEL

Mechanism of action:

Adefovir is an acyclic nucleotide analog of adenosine 5'-monophosphate. Adefovir is phosphorylated to the active metabolite, adefovir diphosphate, by cellular kinases. Adefovir diphosphate inhibits HBV polymerase (reverse transcriptase) by competing with the natural substrate deoxyadenosine triphosphate and by causing DNA chain termination after its incorporation into viral DNA. The inhibition constant (K_i) for adefovir diphosphate for HBV DNA polymerase was 0.1 μM. Adefovir diphosphate is a weak inhibitor of human DNA polymerases α and γ with K_i values of 1.18 μM and 0.97 μM, respectively.

Antiviral activity: The *in vitro* antiviral activity of adefovir was determined in HBV DNA-transfected human hepatoma cell lines. The concentration of adefovir that inhibited 50% of viral DNA synthesis (IC₅₀) varied from 0.2 to 2.5 μM.

Drug Resistance:

Clinical studies 437 & 438

Genotypic and phenotypic analyses of HBV from adefovir (10 mg or 30 mg) treated HBeAg⁺ patients (n = 215; study 437) and HbeAg- patients (n=56; study 438) at baseline and week 48 did not identify mutations in the HBV polymerase gene that may confer reduced susceptibility to adefovir. An unconfirmed increase of ≥ 1 log₁₀ copies/mL in serum HBV DNA was observed in some patients. The molecular basis and clinical significance of the observed increase in viral DNA from nadir to week 48 are not known.

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Cross-resistance:

Recombinant HBV variants containing lamivudine-resistance-associated mutations (L528M, M552I, 552V, L528M +M552V) in the HBV DNA polymerase gene were susceptible to adefovir in vitro. Adefovir has also demonstrated anti-HBV activity (median reduction in serum HBV DNA of 4.3 log₁₀ copies/mL) against clinical isolates of HBV containing lamivudine-resistance-associated mutations (study GS98-435). HBV variants with DNA polymerase mutations T476N and R501Q associated with resistance to hepatitis B immunoglobulin were susceptible to adefovir in vitro.

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DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
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