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APPLICATION NUMBER:

21-797

21-798

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21797/21798	Submission Date(s): 30SEP2004
Brand Name	BARACLUDE
Generic Name	Entecavir
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OCPB Division	DPE III
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Applicant	Bristol-Myers Squibb
Relevant IND(s)	IND 52196
Submission Type; Code	Priority (1P)
Formulation; Strength(s)	Entecavir 0.5 and 1.0 mg film-coated tablets and 0.05 mg/mL oral solution
Indication	Treatment of chronic hepatitis B (HBV) infection in adults

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1. EXECUTIVE SUMMARY

Entecavir is a guanosine nucleoside analog with selective activity against hepatitis B virus (HBV). Entecavir is proposed for the treatment of chronic HBV infection in adults with evidence of [redacted]. Entecavir 0.5 mg once daily was studied in nucleoside-naïve patients with compensated liver disease, and entecavir 1.0 mg once daily was studied in lamivudine-refractory patients.

The safety, tolerability, and pharmacokinetics (PK) of entecavir were evaluated in nineteen (19) clinical pharmacology studies and in one Phase 2 study in post-orthoptic liver transplant patients with HBV infection. In addition, a population PK analysis was performed using data from three Phase 2 studies, and modeling and simulations were conducted for determination of dosing recommendations in renally impaired patients. Efficacy of entecavir is supported by four (4) pivotal efficacy studies, two studies assessing the efficacy of the 0.5 mg dose in nucleoside naïve HBV patients (studies AI463022 and AI463027) and two studies assessing the efficacy of the 1.0 mg dose in lamivudine-refractory HBV patients (studies AI463014 and AI463026). Efficacy data are also available from seven supportive Phase 2 studies.

1.1. Recommendation

The Clinical Pharmacology and Biopharmaceutics information provided by the Applicant is acceptable, aside from the following clinical pharmacology and biopharmaceutics issue identified upon review of this submission:

- Inadequate dosing recommendations for hemodialysis and CAPD patients. Based on simulations of exposures in patients with varying degrees of renal impairment, the Applicant proposes [redacted].

  The submitted data supports a dosage adjustment of 0.05 mg for nucleoside-naïve and 0.1 mg QD for lamivudine-refractory patients on hemodialysis or CAPD. The revised dosing recommendations for renal impairment have been accepted by the Applicant.

1.2. Phase IV Commitments

- Conduct and submit a final study report for a study assessing the pharmacokinetics, safety, and efficacy of entecavir in children [redacted] of age through adolescence with chronic HBV. Use entecavir exposure information from pediatric patients to support dose-selection for the efficacy and safety assessment. Pediatric efficacy should be based on the results of a variety of virologic, biochemical, serologic, and composite endpoints over at least 48 weeks of dosing.

1.3. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Entecavir, a cyclopentyl guanosine analog, is a potent and selective inhibitor of hepatitis B virus (HBV) deoxyribonucleic acid (DNA) polymerase. By competing with the natural substrate deoxyguanosine triphosphate, entecavir triphosphate is a potent and selective

inhibitor of all three functional activities of the viral polymerase (priming, reverse transcription, and DNA-dependent DNA synthesis). Studies on the mechanism of action of entecavir demonstrate that in addition to competing directly with deoxyguanosine, the natural substrate for the HBV polymerase, entecavir-triphosphate (entecavir-TP) is a terminator of HBV DNA chain elongation. Entecavir demonstrates selective and potent inhibition of wild-type HBV, with an *in vitro* effective concentration for inhibition of 50% of virus yield (EC_{50}) of 0.00375 μM.

The clinical pharmacology characteristics of entecavir have been defined in healthy subjects and HBV-infected patients. These studies show entecavir demonstrates the following clinical pharmacology characteristics:

Exposure-Response:

- Entecavir dose selection was based on an assessment of efficacy, as measured by quantitative HBV DNA reduction in Phase 2 dose-ranging studies, and safety, as demonstrated by clinical adverse events. Significantly greater and sustained viral suppression was demonstrated by the 0.5 and 1.0 mg doses in the Phase 2 dose-ranging studies. Because an increased incidence of CNS events was observed with the 1.0 mg dose, 0.5 mg was carried forward in nucleoside-naïve patients in Phase 3. In lamivudine-refractory patients, entecavir 1.0 mg demonstrated significantly greater reductions in HBV DNA versus the 0.5 mg dose. In addition to this significant dose-response relationship, lamividine-resistant virus demonstrated reduced sensitivity to entecavir in vitro. Therefore, the 1.0 mg dose was carried forward in lamivudine-refractory patients in Phase 3. An assessment of exposure-response in the population PK/PD analysis of Phase 2 data supported dose selection. Change in viral load over time was well described by an Emax model. A majority of subjects with greater exposure (dose or steady state AUC) had greater maximal reductions in HBV DNA. Prior treatment with lamivudine was a significant covariate for anti-HBV activity of entecavir. No clear relationship between entecavir exposure (Cmax, AUC, or Cmin) and the severity of headache or selected CNS (headache, photophobia, blurred vision, somnolence, lethargy, and dizziness) or GI (nausea, vomiting, and dyspepsia) adverse events was discerned.

- No dose- or concentration-dependent relationships between QT interval (with Bazett's or Fridericia's correction) or change in QTc were observed following entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg in healthy volunteers. In contrast, a slight concentration-dependent effect on PR interval was observed following entecavir doses of up to 20 mg for 14 days (slope = 0.124 msec/ng/mL). The slight prolongation in PR in this retrospective analysis is not expected to be clinically significant.

Pharmacokinetics Summary:

- An integrated summary of entecavir single and multiple dose pharmacokinetic parameters following administration of the proposed therapeutic doses (0.5 mg and 1.0 mg) as a tablet/capsule and oral solution in the fasted state are presented in the following table.

Table 1.3-1 Summary of Entecavir Single and Multiple Dose Pharmacokinetic Parameters

Dose (mg)	Day	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC[b] (ng•h/mL)	$t_{½}$ (hr)	CL/F (mL/min)	CL_r (mL/min)
Tablet and Capsule[c]							
0.5	1	N =158 4.09 (30.1)	N =158 0.75 —	N =158 9.77 (27.2)	N =23 83.24 (40.4)	NA	NA
	14	N =12 5.22 (35.0)	N =12 0.88 —	N =12 16.21 (14.7)	N =12 113.25 (25.0)	N =12 520.74 (94.7)	N =12 368.20 (60.0)
1.0	1	N =172 8.72 (29.2)	N =172 0.75 —	N =172 19.00 (24.0)	N =107 95.61 (44.1)	N =49 557.48 (108.9)	N =49 379.65 (98.5)
	14	N =11 9.83 (27.1)	N =11 0.75 —	N =11 31.15 (17.2)	N =11 108.68 (39.0)	N =11 543.23 (102.8)	N =11 409.83 (109.8)
Oral Solution							
0.5 N=22	1	3.70 (26.6)	0.50 —	16.43 (22.7)	91.35 (59.4)	516.98 (103.2)	NA
1.0 N=6	1	8.31 (20.8)	0.63 —	33.29 (17.0)	113.48 (64.5)	506.99 (88.7)	376.04 (51.7)

The integrated summary includes fasting data for the tablet, capsule, and solution formulations from all clinical pharmacology and biopharmaceutics studies except studies AI463011 (renal impairment), AI463018 (no electronic data available), AI463032 (hepatic impairment), and elderly subjects from AI463042.
Data presented as geometric mean (CV%) unless otherwise specified.
NA Not available
[a] Data presented as median (minimum, maximum).
[b] AUC is AUC(0-T) on Day 1 and AUC(TAU) on Days 7 & 14 for tablet and capsule; AUC(INF) for oral solution
[c] Tablet and capsule are bioequivalent.

- Following the administration of entecavir at the clinically relevant doses of 0.5 or 1.0 mg, the systemic exposure demonstrated approximately 2-fold accumulation. Entecavir has an apparent terminal half-life of approximately 130 hours and an effective half-life for accumulation of approximately 24 hours. Trough concentrations indicated that steady-state was attained by approximately 9 to 10 days following once-daily dosing.

Absorption:

- Entecavir exposure decreased by approximately 20% following administration with a high-fat or light meal compared to fasted conditions. The proposed label recommends entecavir be administered on an empty stomach (at least 2 hours before or at least 2 hours after a meal).

- Following administration of a 1.0 mg dose of [^{14}C]-entecavir, 75% of the total radioactivity administered was recovered in the urine and 6% was recovered in the feces. Approximately 70% of the administered entecavir dose was excreted as unchanged drug in urine over 14 days of collection, suggesting an estimated bioavailability ≥ 70%.

- Entecavir is not a substrate for P-glycoprotein (P-gp).

Distribution:

- The protein binding of entecavir in human serum is low (approximately 13%), and entecavir uniformly distributes between plasma and red blood cells (RBCs) in whole human blood.

Metabolism:

- In vitro studies indicate that entecavir is not a substrate, inhibitor, or inducer of the cytochrome P450 enzyme system. The only metabolites detected in human plasma, urine, and feces were minor amounts of phase 2 metabolites, namely, glucuronide and sulfate conjugates. No oxidative metabolites of entecavir were detected indicating that CYP450 does not play a role in the metabolic clearance of entecavir.

Excretion:

- Renal excretion of unchanged drug is the primary route of entecavir elimination, while biliary excretion plays a minor role. Values for renal clearance of entecavir were greater than the glomerular filtration rate, indicating that the excretion of entecavir by the kidneys occurs via a combination of glomerular filtration and net tubular secretion.

Intrinsic Factors:

- In subjects with selected degrees of renal impairment, as renal function declined mean apparent total body clearance and renal clearance of entecavir decreased. This decrease in clearance resulted in a longer half-life and greater exposure to entecavir, as compared to subjects with normal renal function. Additionally, a 4-hour hemodialysis session removed approximately 13% of the entecavir dose, while continuous ambulatory peritoneal dialysis (CAPD) removed < 1% over 7 days in subjects with severe renal impairment. Based on these findings, dosage reduction of entecavir is warranted in the presence of renal impairment. Modeling and simulation of multiple-dose administration of the proposed dosage recommendations in patients with varying degrees of renal function was performed. Based on the safety margin defined by the Phase 1 program, a target range of exposure was defined for purposes of simulation as two times the geometric mean steady state AUC value in subjects with normal renal function (maximum) and the lowest predicted value for subjects with normal renal function (minimum). The Applicant's proposed dosage recommendations based on renal function are as follows.

Based upon Agency review of the submitted data, the following dosage adjustment recommendations for entecavir in patients with renal function impairment have been proposed by the Agency. The recommendations have been accepted by the Applicant.

Creatinine Clearance (mL/min)	Nucleoside-Naïve Patients	Lamivudine-Refractory Patients
≥ 50	0.5 mg once daily	1 mg once daily
30 to <50	0.25 mg once daily	0.5 mg once daily
10 to <30	0.15 mg once daily	0.3 mg once daily
Hemodialysis or CAPD*	0.05 mg once daily	0.1 mg once daily

- Hepatic impairment had a negligible impact on entecavir exposure, and no dose modification based on the presence of hepatic impairment is necessary.

- Entecavir pharmacokinetics differed between Asian and non-Asian populations. Cmax and AUC following multiple 0.5 mg dosing of entecavir were approximately 50% and 20% higher, respectively, in healthy Asian subjects versus healthy non-Asian subjects. Weight-normalized CL/F values were comparable between the Japenese and non-Asian study populations (clearance for Chinese subjects not available), suggesting the ethnic differences in exposure between Asian and non-Asian populations may be attributable to differences in body weight, but small sample sizes across these study populations preclude definition of an effect of race on entecavir pharmacokinetics.

- Entecavir exposure was approximately 29% higher in elderly compared to young subjects, a disparity attributable to differences in renal function.

- No significant gender-related differences in entecavir pharmacokinetics were observed.

- The population PK/PD analysis of Phase 2 studies revealed differences in entecavir exposure between healthy and HBV-infected subjects. In comparison to healthy subjects, entecavir AUC was approximately 30% and 71% higher after multiple daily dosing of 0.5 mg and 1.0 mg, respectively. In HBV subjects post-orthoptic liver transplant (OLT), mean Cmax was increased by approximately 42% and the mean AUC was increased by approximately 116% compared to healthy subjects following 14 days of oral 1.0 mg entecavir. This increase in Cmax and AUC in OLT patients was consistent with the degree of renal impairment in these subjects.

Extrinsic Factors:

- There were no significant pharmacokinetic interactions between entecavir and lamivudine, adefovir, or tenofovir in Phase 1 drug interaction studies. In addition, an in vitro study showed that co-administration of stavudine, didanosine, abacavir, zidovudine, lamivudine, or tenofovir with entecavir had no effect on anti-HBV and/or anti-HIV-1 activity of any of the compounds.

2. QUESTION BASED REVIEW

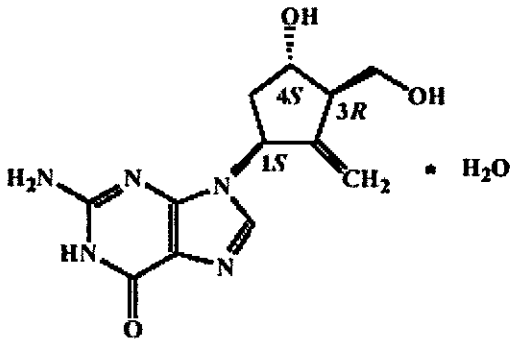
2.1. General Attributes of the Drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

The chemical structure and physical-chemical properties of entecavir are shown below:

Structural Formula: $C_{12}H_{15}N_5O_3 \bullet H_2O$

Chemical Structure:



Chemical Name: 2-Amino-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-ethylenecyclopentyl]-1,9-dihydro-6*H*-purin-6-one, monohydrate

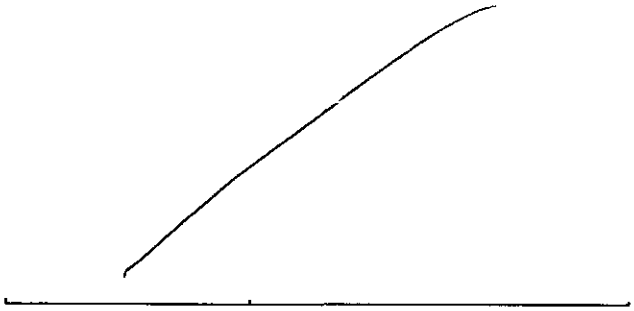
Molecular Weight: 295.3

Solubility Profile:

Solvent(s)	Solubility at 25 ± 0.5°C (mg/mL)	USP Definition
Water	2.4	Slightly

pH-solubility Profile:

pH	Solubility at 25 ± 0.5°C (mg/mL)



Ionization Constant (p*Ka*):

- pKa_1 – 2.75
- pKa_2 – 9.59

Partition Coefficient ($P_{O/B}$):

2.1.2. What is the proposed mechanism of drug action and therapeutic indication?

Entecavir, a cyclopentyl guanosine analog, is a potent and selective inhibitor of hepatitis B virus (HBV) deoxyribonucleic acid (DNA) polymerase. By competing with the natural substrate deoxyguanosine triphosphate, entecavir triphosphate is a potent and selective inhibitor of all three functional activities of the viral polymerase (priming, reverse transcription, and DNA-dependent DNA synthesis). Studies on the mechanism of action of entecavir demonstrate that in addition to competing directly with deoxyguanosine, the natural substrate for the HBV polymerase, entecavir-triphosphate (entecavir-TP) is a terminator of HBV DNA chain elongation. Entecavir is proposed for the treatment of chronic HBV infection in adults with evidence of

2.1.3. What is the proposed dosage and route of administration?

The proposed oral dose of entecavir in adults and adolescents older than 16 years is 0.5 mg once daily. For lamivudine-refractory patients [patients with evidence of viremia while on therapy with lamivudine or the presence of lamivudine-resistant (YMDD) mutations], the recommended dose is 1 mg once daily. The proposed label recommends entecavir be administered on an empty stomach (at least 2 hours before or at least 2 hours after a meal).

Patients with Renal Impairment:
In patients with renal impairment, the apparent oral clearance of entecavir decreases as creatinine clearance decreases. The Applicant's proposed dosage recommendations based on creatinie clearance, including patients on hemodialysis and CAPD are as follows.

Based upon Agency review of the submitted data, the following dosage adjustment recommendations for entecavir in patients with renal function impairment have been proposed by the Agency.

Creatinine Clearance (mL/min)	Nucleoside-Naïve Patients	Lamivudine-Refractory Patients
≥ 50	0.5 mg once daily	1 mg once daily
30 to <50	0.25 mg once daily	0.5 mg once daily
10 to <30	0.15 mg once daily	0.3 mg once daily
Hemodialysis or CAPD*	0.05 mg once daily	0.1 mg once daily

2.2. General Clinical Pharmacology

2.2.1. What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The dose selection for entecavir pivotal clinical trials was based on results from Phase 2 dose-ranging studies, the design features of which are summarized below. A population analysis (AI463017) used data from these three randomized, double-blind Phase 2 studies (AI463004, AI463005, and AI463014), and the results of the population analysis supported the use of the doses selected for the Phase 3 program (0.5 mg QD in nucleoside-naive subjects and 1.0 mg QD in LVD-refractory subjects).

- Study AI463004: a dose-escalating trial assessing safety and antiviral activity of four doses of entecavir (0.05, 0.1, 0.5, and 1.0 mg QD for 28 days) in adults with chronic HBV infection (42 subjects treated: 10 subjects in the 0.05 and 0.1 mg cohorts, 11 subjects in the 0.5 and 1.0 mg cohorts).
- Study AI463005: three doses of entecavir were investigated (0.01, 0.1, and 0.5 mg QD given for 24 weeks) and compared to lamivudine (LVD; 100 mg QD) in adults with HBV infection with well-compensated liver disease (177 subjects treated: 54, 36, and 46 subjects in the entecavir 0.01, 0.1, and 0.5 mg groups, respectively, and 41 in the LVD group).
- Study AI463014: entecavir (0.1, 0.5, and 1.0 mg QD) was compared with continued LVD therapy (100 mg QD) for up to 76 weeks in a LVD-refractory population (181 subjects treated: 42, 47, and 47 subjects in the entecavir 0.1, 0.5, and 1.0 mg groups, respectively, and 45 in the LVD group). This Phase 2 study served as one of four pivotal clinical trials (see below).

Efficacy of entecavir is supported by four pivotal efficacy studies, two studies assessing the efficacy of the 0.5 mg dose in nucleoside naïve HBV patients (studies AI463022 and AI463027) and two studies assessing the efficacy of the 1.0 mg dose in LVD-refractory HBV patients (studies AI463014 and AI463026). All were multinational, randomized, double-blind studies that compared entecavir versus LVD in subjects with chronic HBV infection and compensated liver disease. Evidence for hepatic inflammation was required for entry into all three Phase 3 studies (AI463022, AI463026, and AI463027) for which histology was the primary endpoint, whereas eligibility for the Phase 2 study (AI463014) was based on disease activity as assessed by viremia. The two studies in nucleoside-naïve subjects (AI463022 and AI463027) compared entecavir 0.5 mg QD with LVD 100 mg QD over a period of at least 52 weeks in subjects who had received ≤12 weeks of prior nucleosides/nucleotide exposure (709 subjects treated in AI463022: 354, entecavir; 355, LVD; 638 subjects treated in AI463027: 325, entecavir; 313, LVD). The Phase 3 study in LVD-refractory subjects (AI463026) compared entecavir 1.0 mg QD with continued LVD 100 mg QD (286 subjects treated: 141, entecavir; 145, LVD). The Phase 2 LVD-refractory study (AI463014) was a dose-ranging study and compared three doses of entecavir (0.1 mg, 0.5 mg and 1.0 mg) with continued LVD (see sample size above).

In addition, two studies support efficacy and safety in special populations with chronic, LVD-refractory HBV infection: an open-label Phase 2 study in post-orthoptic liver transplant (OLT) HBV patients receiving 1.0 mg entecavir QD (AI463015), and a double-blind, randomized, placebo-controlled Phase 2 study in HIV/HBV co-infected patients receiving 1.0 mg entecavir QD (AI463038) and continued LVD at anti-HIV doses (300 mg/day) throughout the study.

2.2.2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics [PD]) and how are they measured in clinical pharmacology and clinical studies?

Liver biopsies provide direct assessment of HBV-related necroinflammation and fibrosis and have served as an endpoint for measuring efficacy in other hepatitis B development programs. The proportion of subjects with histologic improvement was the primary measure of efficacy in the three pivotal Phase 3 studies (AI463022, AI463026, and AI463027). Histologic improvement was defined as improvement (≥ 2-point decrease) in the Knodell necroinflammatory score with no worsening of fibrosis (worsening was defined as ≥ 1-point increase in the Knodell fibrosis score) at the Week 48 liver biopsy compared with baseline. Secondary endpoints in these Phase 3 studies focused on: ALT as a biochemical correlate of hepatic inflammation, virologic response (reduction from baseline in HBV DNA by bDNA and PCR and proportions below the assigned cutoff level for each assay); and serologic response (loss of HBeAg and HBeAb seroconversion) in subjects who were HBeAg-positive at screening. Efficacy endpoints in the pivotal Phase 2 study, AI463014, included the biochemical, virologic, and serologic endpoints mentioned above, but liver biopsy was not required for this study.

2.2.3. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Entecavir concentrations in human plasma and urine samples were determined by validated liquid chromatographic methods using LC/MS/MS. The assays are acceptable. See section 2.6 for further details.

2.2.4. Exposure-Response

Entecavir dose selection was based on an assessment of exposure-response for efficacy, as measured by quantitative HBV DNA reduction in Phase 2 dose-ranging studies, and for safety, as demonstrated by clinical adverse events. Significantly greater and sustained viral suppression was demonstrated by the 0.5 and 1.0 mg doses in the Phase 2 dose-ranging studies. Because an increased incidence of CNS events was observed with the 1.0 mg dose, 0.5 mg was carried forward in nucleoside-naïve patients in Phase 3. In lamivudine-refractory patients, entecavir 1.0 mg demonstrated significantly greater reductions on HBV DNA versus the 0.5 mg dose. In addition to this significant dose-response relationship, lamividine-resistant virus demonstrated reduced sensitivity to entecavir in vitro. Therefore, the 1.0 mg dose was carried forward in lamivudine-refractory patients in Phase 3. Further details of the exposure-response findings for entecavir are presented in sections 2.2.4.1 and 2.2.4.2 below.

2.2.4.1. What are the characteristics of exposure-response relationships (dose-response, concentration-response) for efficacy?

In the double-blind, randomized, placebo-controlled Phase 2 pilot study AI463004 investigating a range of entecavir doses (0.05, 0.1, 0.5, and 1.0 mg once daily for 28 days) in adults with chronic HBV infection (mixed nucleoside-naïve and LVD-refractory patients), all entecavir doses studied exhibited significant antiviral activity compared to placebo following 28 days of treatment, as depicted in the following figure showing HBV viral load reductions by treatment. At 8 weeks (4 weeks post-dosing period), the two higher entecavir doses of 0.5 and 1.0 mg were associated with significantly greater viral suppression than the lower doses ($p = 0.004$ and 0.0051, respectively), indicative of sustained anti-HBV activity.

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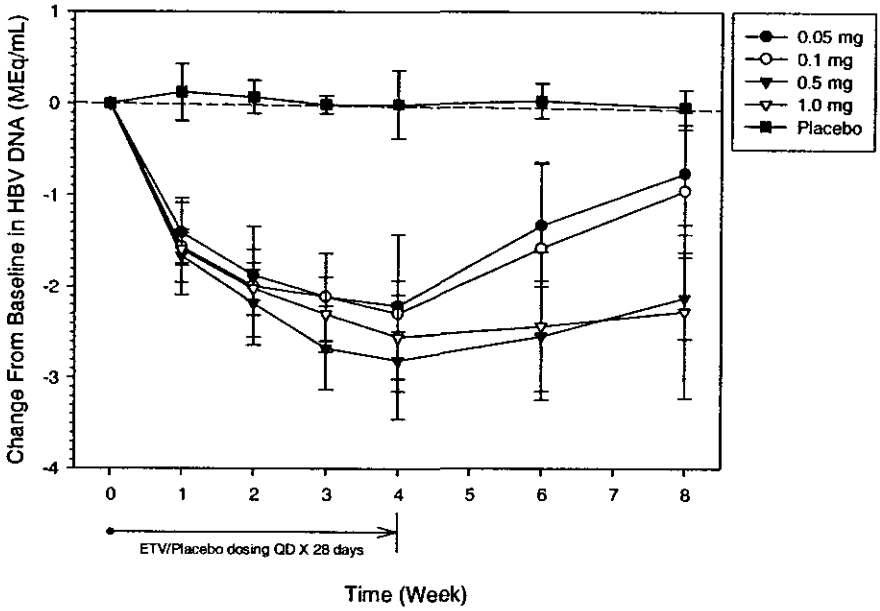
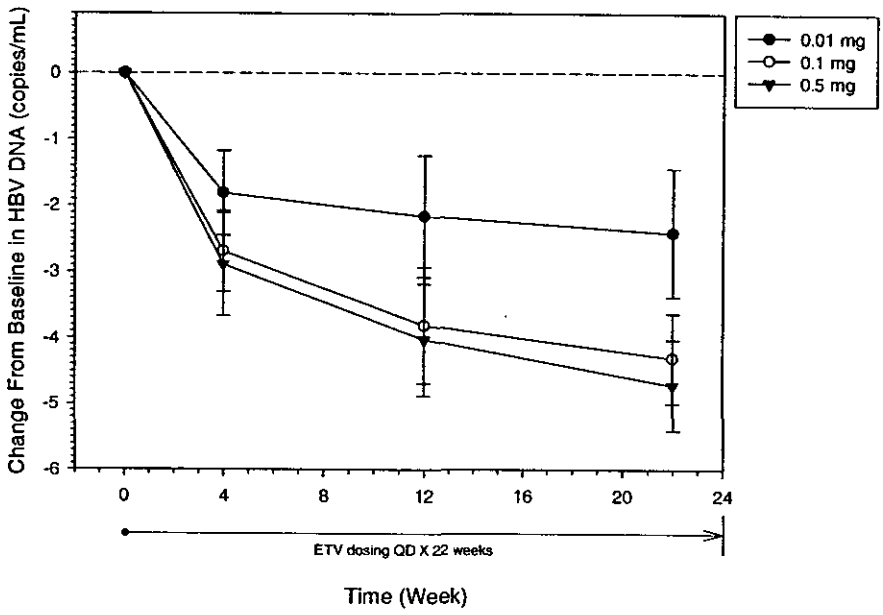
Figure 2.2.4.1-1 Change From Baseline in HBV DNA following Multiple Daily Doses of Entecavir and Placebo in Study AI463004



Source: AI463004 Clinical Study Report, Appendix 10.1A

A dose-response relationship was demonstrated in the double-blind, randomized Phase 2 study AI463005 investigating a range of entecavir doses (0.05, 0.1, and 0.5 mg once daily for 24 weeks) compared to lamivudine (100 mg QD) in adults with chronic HBV infection with well-compensated liver disease, as depicted in the following figure of HBV viral load reductions by treatment. The 0.1 and 0.5 mg doses of entecavir, with 4.31 and 4.72 $\log_{10}$ reductions in HBV DNA, respectively, displayed greater activity than the 0.01 mg dose ($p < 0.0001$ for both the comparisons of 0.01 mg entecavir to 0.1 and 0.5 mg). Reduction of HBV DNA by PCR was significantly greater following 22 weeks of 0.5 mg entecavir QD versus the 0.1 mg dose ($p = 0.018$ at Week 22).

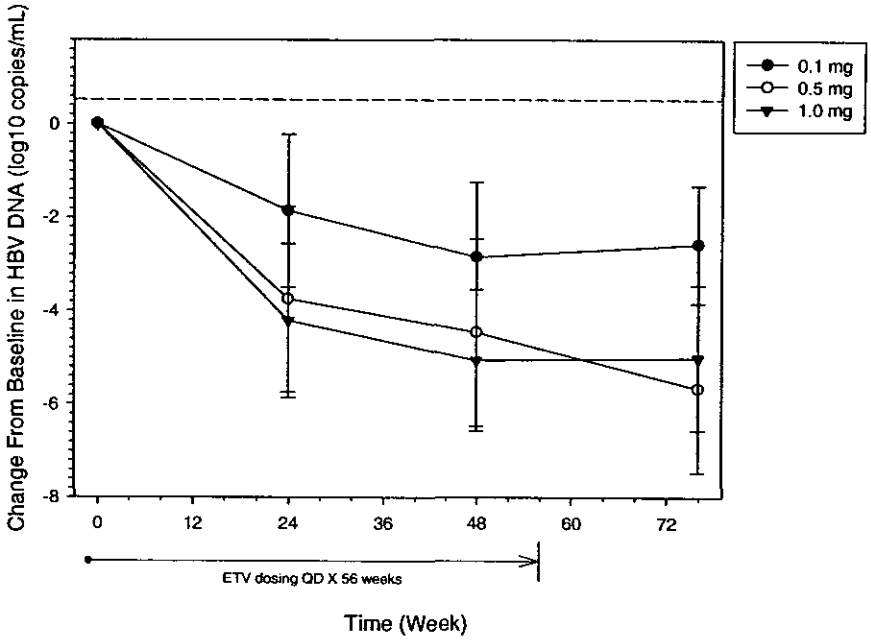
Figure 2.2.4.1-2 Change From Baseline in HBV DNA following Multiple Daily Doses of Entecavir (0.01, 0.1 and 0.5 mg) in Study AI463005



Source: AI463005 Clinical Study Report, Table 10.1.1A

In the double-blind, randomized Phase 2 study AI463014, three doses of entecavir (0.1, 0.5, and 1.0 mg once daily for 56 weeks) were investigated in subjects with chronic HBV infection with viremia while treated with lamivudine. The dose response for entecavir in this lamivudine-refractory population is displayed in the following figure showing HBV viral load reductions by treatment. A linear regression model applied to the reduction from baseline in HBV DNA by PCR assay showed a significant dose response over the dose range of 0.1 to 1.0 mg ($p < 0.0001$). Entecavir 1.0 mg was superior to 0.5 mg for the primary endpoint, HBV DNA < LOQ by bDNA assay at Week 24 ($p < 0.01$). The Cochran linear trend test applied to the primary endpoint also showed a significant dose response over the dose range of 0.1 to 1.0 mg ($p < 0.0001$).

Figure 2.2.4.1-3 Change From Baseline in HBV DNA following Multiple Daily Doses of Entecavir (0.1, 0.5 and 1.0 mg) in Study AI463014



Number of subjects for the 0.1, 0.5, and 1.0 mg treatment groups, respectively, were 46, 43, and 40 for Week 24; 33, 40, and 38 for Week 48; 4, 13, and 14 for Week 76.

Source: AI463014 Clinical Study Report, Table S.10.2.1B

In the population PK/PD analysis of Phase 2 data from studies AI463004, AI463005, and AI463014, change in viral load over time was well described by a direct effect inhibitory maximum effect (Emax) model. Subjects with greater exposure (dose or steady state AUC) had faster and greater maximal reductions in HBV DNA, as demonstrated in the following figure of the effect of entecavir dose on the time course of HBV DNA reduction for LVD naïve subjects.

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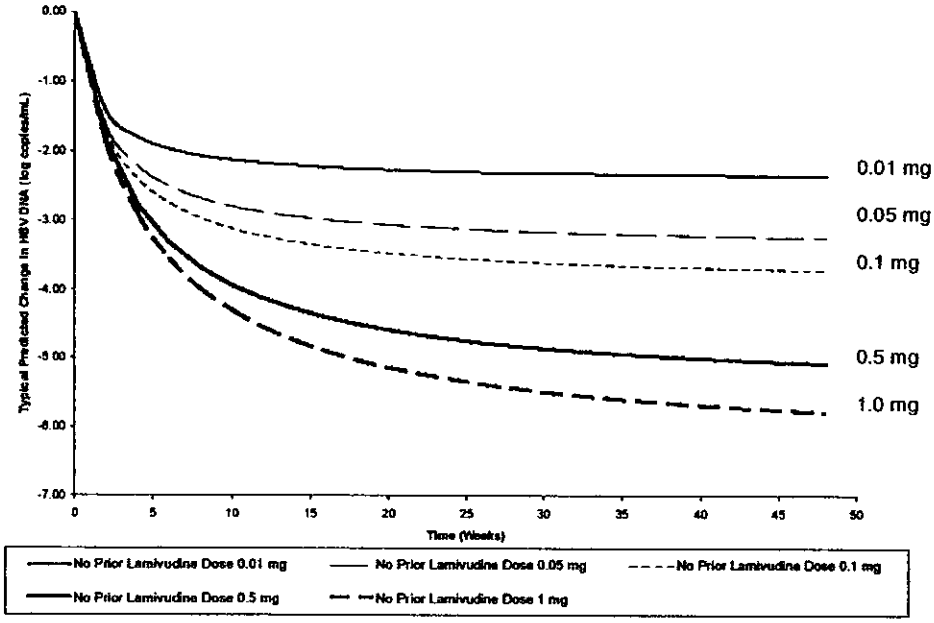
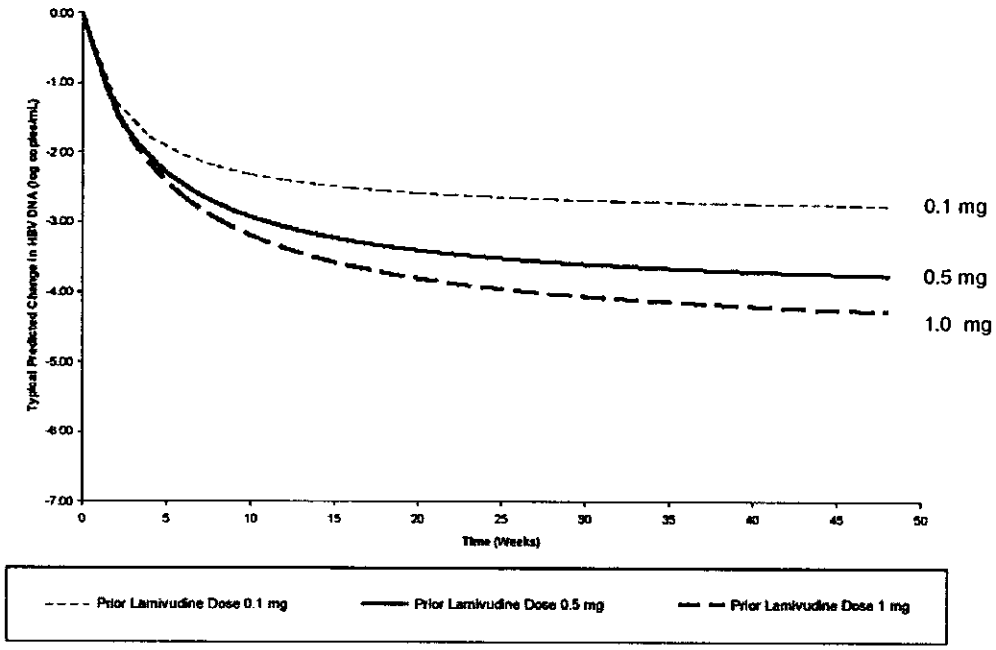
Figure 2.2.4.1-4 Effect of Entecavir Dose on the Time Course of HBV DNA Reduction for Treatment-Naïve Subjects



Source: AI463017 Clinical Study Report

Subjects who were LVD-refractory had a decreased maximal reduction in HBV DNA at a given dose compared to treatment naïve subjects, and prior treatment with lamivudine was a significant covariate for anti-HBV activity. The following figure illustrates the effect of entecavir dose on the time course of HBV DNA reduction for LVD-refractory subjects.

Figure 2.2.4.1-5 Effect of Entecavir Dose on the Time Course of HBV DNA Reduction for LVD-Refractory Subjects



Source: AI463017 Clinical Study Report

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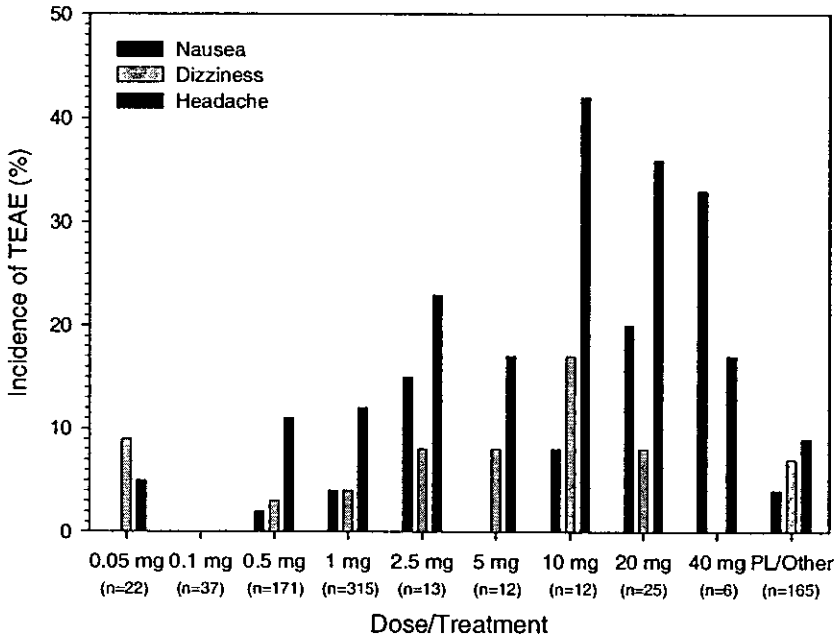
2.2.4.2. What are the characteristics of exposure-response relationships (dose-response, concentration-response) for safety?

Adverse Events

An integrated assessment of safety data from the Phase 1 clinical pharmacology and biopharmaceutics studies for entecavir suggests the incidence of all adverse events increased with increasing doses of entecavir ranging from 0.5 to 40 mg. Specifically, incidences of headache and nausea, two of the most common (≥ 5% incidence) treatment emergent adverse events (TEAEs), were higher at the higher entecavir doses. The following figure shows the most common TEAEs in the Phase 1 studies for entecavir (pooled) by dose.

Figure 2.2.4.2-1 Most Commonly Occurring Treatment Emergent Adverse Events in the Phase 1 Development Program for Entecavir by Dose

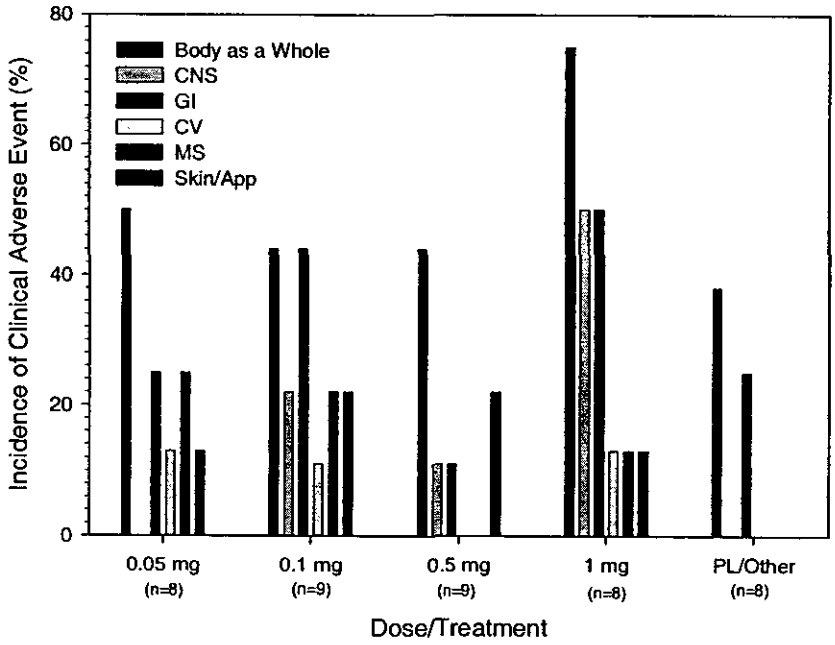


Source: Integrated Analysis of Clinical Safety for Biopharmaceutics and Clinical Pharmacology Studies, Table 1.2.1.1B

Based on an assessment of Phase 1 safety data, overall incidences of adverse events with the oral entecavir doses proposed in the application (0.5 mg once daily in treatment-naïve patients and 1 mg once daily for LVD-refractory patients) are comparable to placebo (40% versus 38%, respectively). Overall, entecavir demonstrated a wide safety margin following multiple doses of up to 20 mg QD for 14 days in Phase 1 healthy volunteer studies.

Clinical adverse event data from the double-blind, randomized, placebo-controlled Phase 2 pilot study AI463004 suggests the incidence of pooled CNS events and 'body as a whole' events were noticeably increased at the highest entecavir dose studied (1.0 mg QD × 28 days), as demonstrated in the following figure showing adverse events reported in the dosing phase by dose and body system.

Figure 2.2.4.2-2 Summary of the Most Commonly Occurring Adverse Events in Study AI463004 by Dose/Treatment and Body System

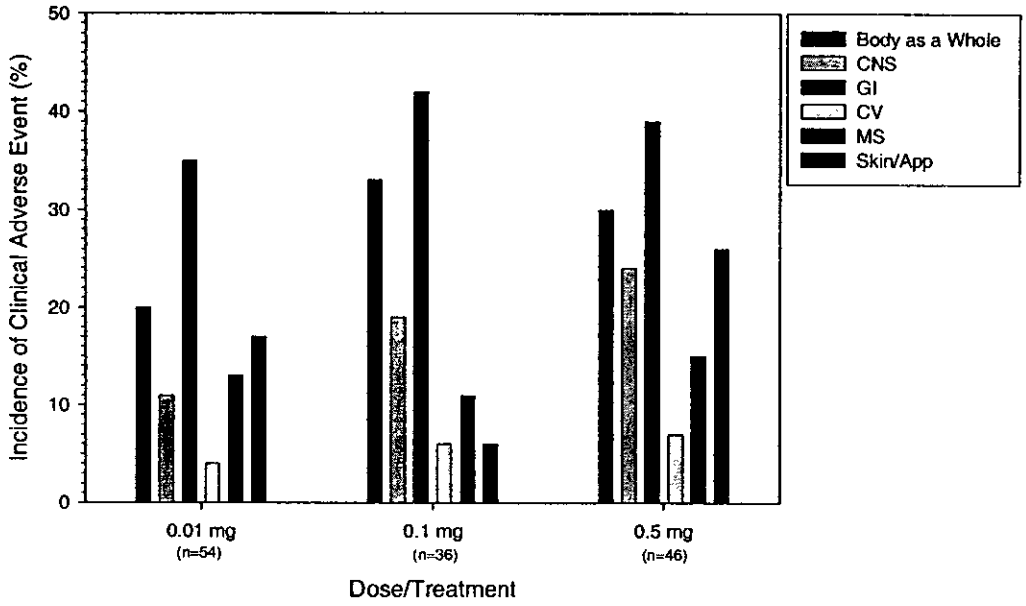


Abbreviations: CNS, central nervous system; GI, gastrointestinal or digestive system; CV, cardiovascular system; MS, musculoskeletal system; Skin/App, skin/appendages

Source: Clinical Study Report AI463004, Table 12.1.1

Clinical adverse event data from the double-blind, randomized Phase 2 study AI463005 suggests the incidence of pooled CNS events increases with increasing entecavir doses, with trends of greater frequencies of dizziness and insomnia in the highest dose group studied (0.5 mg). In addition, incidences of headache and rash showed a trend towards increase with increasing dose. Incidences of adverse events across the three entecavir doses studied (0.01, 0.1, and 0.5 mg QD × 24 weeks) are presented in the following figure by body system.

Figure 2.2.4.2-3 Summary of the Most Commonly Occurring Adverse Events in Study AI463005 by Dose/Treatment and Body System

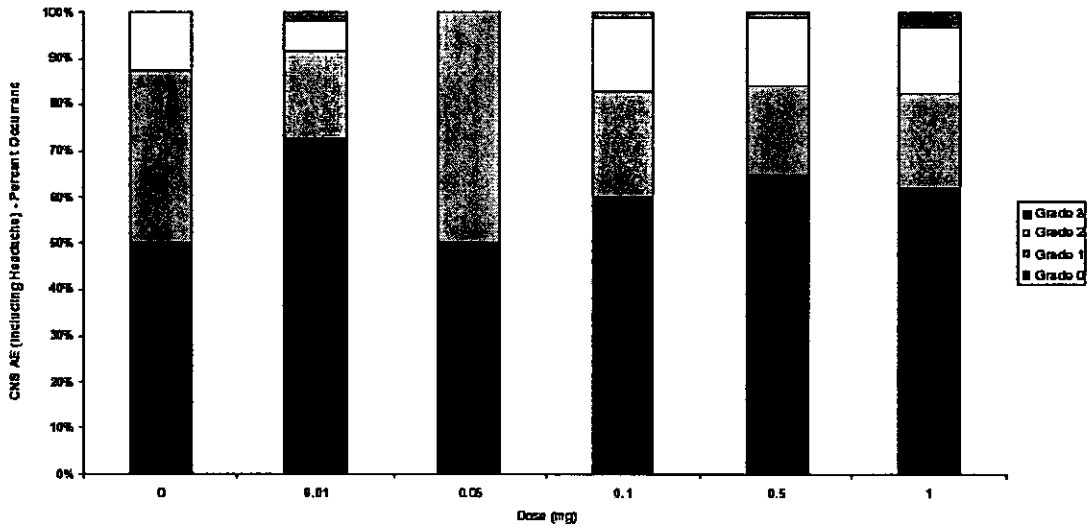


Source: Clinical Study Report AI463005, Table 12.1

In the double-blind, randomized Phase 2 study AI463014, no apparent dose-response relationship in the overall incidence of adverse events was discernable.

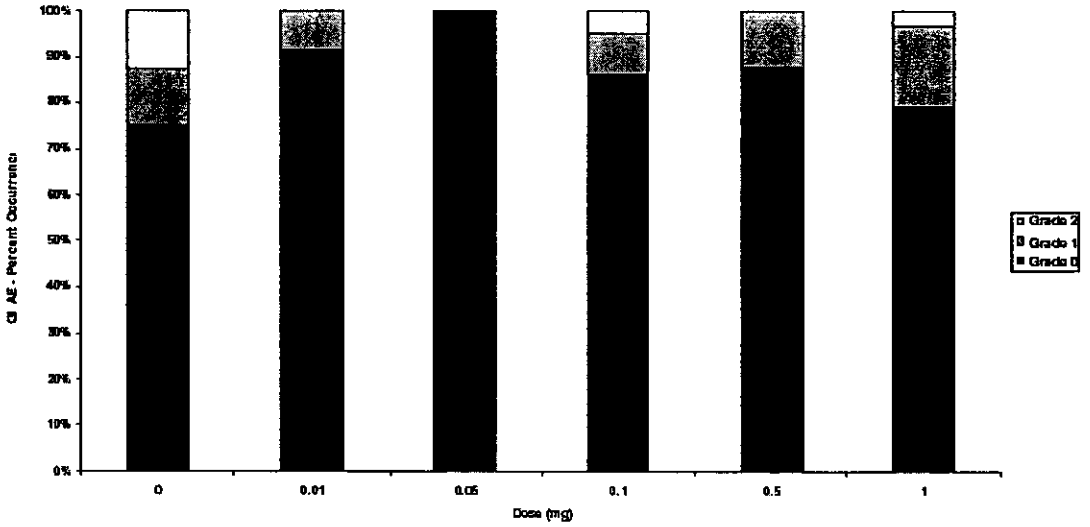
In the Applicant's population PK/PD analysis of Phase 2 data from studies AI463004, AI463005, and AI463014, no clear relationships between the doses administered and the severity of pooled adverse events were observed, specifically for CNS and GI events as presented in the following figures, respectively. Similarly, no relationships between predicted entecavir exposure (Cmax, AUC, or Cmin) from the population model and the severity of headache or selected CNS (headache, photophobia, blurred vision, somnolence, lethargy, and dizziness) or GI (nausea, vomiting, and dyspepsia) adverse events was observed. The lack of any demonstrable relationship between increased entecavir exposure and AE severity, as modeled over a dose range up to 1.0 mg of entecavir, suggests that this therapeutic range falls below any toxicity-defined dose-limit for entecavir based on observed, short-term clinical events.

Figure 2.2.4.2-4 Relationship Between Dose and CNS Adverse Events in the Population PK/PD analysis



Source: AI463017 Clinical Study Report

Figure 2.2.4.2-4 Relationship Between Dose and GI Adverse Events in the Population PK/PD analysis



Source: AI463017 Clinical Study Report

Cardiovascular Safety

In vitro investigations were conducted to assess the cardiovascular safety of entecavir. These included an evaluation of the potential for entecavir to interfere with cardiac potassium and calcium currents and evaluation of effects on electrophysiologic parameters in isolated rabbit and canine Purkinje fibers.

- In in vitro hERG assay studies, entecavir was tested at concentrations of 3, 10, and 30 μM (approximately 0.8, 2.8, and 8 μg/mL, respectively) against a vehicle and positive control (terfenadine, 60 μM). Entecavir results were comparable to the vehicle control versus terfenadine which produced marked inhibition of hERG current.
- In an in vitro calcium channel patch-clamp assay, entecavir was tested for effects on cardiac L-type calcium channel currents in canine ventricular myocytes at a concentration of 30 μM (approximately 8 μg/mL). Entecavir did not have any biologically significant effects on L-type calcium currents in this assay.
- In a rabbit Purkinje-fiber assay, entecavir at concentrations of 3, 10, and 30 μM had minimal effects on Purkinje fiber APD_{90}.
- In a canine Purkinje fiber assay, entecavir at concentrations of 3, 10, and 30 μM did not have any biologically meaningful effects on Purkinje fiber action potential parameters, including resting membrane potential, overshoot, maximal upstroke velocity (Vmax), and time to 50% and 90% repolarization (APD_{50} and APD_{90}).

To further define the potential for entecavir to cause untoward cardiac effects, a retrospective analysis (AI463041) of ECGs collected in five Phase 1 randomized single and multiple dose studies for entecavir (AI463001, AI463002, AI463010, AI463033, and AI463034) was conducted. These studies evaluated single and multiple doses of entecavir administered in capsule or tablet formulation at 0.1 mg, over the proposed therapeutic dose range (0.5 and 1.0 mg), and at doses significantly higher than the proposed therapeutic doses (up to 40 mg). The primary objective of the retrospective ECG analysis was to assess the effect of entecavir on the QT interval corrected for heart rate using Bazett's formula (QTcB). Secondary and tertiary objectives included assessing the effect of entecavir QT interval corrected for heart rate using Fridericia's formula (QTcF), PR, RR, the relationship between the QT and RR, QRS, absolute QT, and heart rate (HR).

No dose- or concentration-dependent relationships between QT interval (with Bazett's or Fridericia's correction) or change in QTc were observed following entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg in healthy volunteers. Based on regression analysis, for each additional 10 ng/mL of plasma concentration measured at the time of the ECG (CECG), the estimated increase in change in QTcB ranged between −1.22 and 0.02 msec. The estimated slope of the linear regression on Day 1 was slightly greater than zero, whereas the estimated slope on Days 7 and 14 were negative, as evidenced in the following summary of the linear regression analyses of change from baseline in QTcB on entecavir concentration following multiple daily dosing.

the expected time of Cmax (1 to 2 hours). Although no borderline or prolonged changes in QTc (QTc > 470 msec for females, > 450 msec for males or change > 60 msec) were observed in the retrospective analysis, instances of borderline and prolonged change in QTc were observed in entecavir clinical pharmacology studies. The retrospective analysis included subjects who experienced asymptomatic first-degree AV block in the five studies selected for analysis, but failed to capture additional cases of first-degree AV block in Phase 1 studies not included in the analysis, including treatment emergent cases and one case leading to discontinuation of study. These findings illustrate that the selection criteria for inclusion of data in the Applicant's retrospective analysis of ECGs collected in Phase 1 studies was not sufficient for identifying outliers for cardiac safety in the entecavir Phase 1 population. In addition, data used for describing the concentration-response relationships in this analysis do not include these outlier observations; therefore, the quantitative descriptions of concentration-response should be interpreted with caution. Despite these limitations, the results of the retrospective analysis are supportive of the in vitro cardiovascular safety studies and the cardiac adverse event profiles obtained in the Phase 3 program.

2.2.4.3. Does entecavir prolong QT or QTc interval?

Data from the retrospective analysis of ECGs collected in five Phase 1 studies for entecavir (AI463001, AI463002, AI463010, AI463033, and AI463034) and supportive preclinical data suggest the potential for entecavir to prolong QT or QTc interval is minimal. For a summary of the characteristics of the exposure-response relationships (dose-response, concentration-response) for ECG parameters, please refer to section 2.2.4.2.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dose and dose regimen of 0.5 mg once daily in treatment-naïve HBV patients and 1 mg once daily for lamivudine-refractory patients is consistent with the exposure-response relationships described in the entecavir application (see discussion of exposure- response in sections 2.2.4.1 and 2.2.4.2).

The entecavir 0.5 mg dose was selected for nucleoside-naïve chronic HBV subjects based on significantly greater antiviral activity versus the 0.1 mg dose, as evidenced by reduction of HBV DNA by PCR after 22 weeks of treatment ($p = 0.018$), and an acceptable safety profile (AI463005). The dose was confirmed in the pivotal Phase 3 studies in nucleoside-naïve populations (AI463022 and AI463027). In these studies entecavir 0.5 mg demonstrated superiority over lamivudine for the proportion of subjects exhibiting histologic improvement and key endpoints for antiviral activity response, including proportion of subjects with HBV DNA < 0.7 MEq/mL by bDNA assay, change from baseline in HBV DNA, proportion of subjects who achieved HBV DNA < 400 copies/mL by PCR assay, and proportion of subjects who achieved HBV DNA < 200 copies/mL by PCR assay.

The entecavir 1.0-mg dose was selected for LVD-refractory chronic HBV subjects based on reduced in vitro activity of entecavir against LVD-resistant virus, significantly greater antiviral activity versus the 0.5 mg dose, as evidenced by reduction of HBV DNA < LOQ by bDNA assay after 24 weeks of treatment ($p < 0.01$), and an acceptable safety profile

in LVD-refractory subjects (AI463014). The dose was confirmed in the pivotal Phase 3 study AI463026 in subjects with incomplete response to current lamivudine therapy. Entecavir 1.0 mg demonstrated superiority over lamivudine for the proportion of subjects exhibiting histologic improvement and the composite primary endpoint [the proportion of subjects who had undetectable HBV DNA by bDNA assay (< 0.7 MEq/mL) and normalization of serum ALT (< 1.25 × ULN)], as well as for key endpoints for antiviral activity response, including proportion of subjects with HBV DNA < 0.7 MEq/mL by bDNA assay, change from baseline in HBV DNA, and proportion of subjects who achieved HBV DNA < 400 copies/mL by PCR assay.

Entecavir doses greater than 1.0 mg in treatment naïve or lamivudine refractory HBV patients have not been studied.

2.2.5. What are the PK characteristics of entecavir?

2.2.5.1. What are the single and multiple dose PK parameters?

An integrated summary of entecavir single and multiple dose pharmacokinetic parameters following administration of the proposed therapeutic doses (0.5 mg and 1.0 mg) as a tablet or capsule and oral solution in the fasted state in healthy subjects are presented in the following table.

Table 2.2.5.1-1 Summary of Entecavir PK Parameters in Healthy Subjects

Dose (mg)	Day	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC[b] (ng•h/mL)	$t_{½}$ (hr)	CL/F (mL/min)	CL_r (mL/min)
Tablet and Capsule[c]							
0.5	1	N =158 4.09 (30.1)	N =158 0.75	N =158 9.77 (27.2)	N =23 83.24 (40.4)	NA	NA
	7	N =12 5.29 (30.1)	N =12 0.75	N =12 15.57 (13.9)	NA	N =12 540.62 (85.15)	N =12 398.60 (58.7)
	14	N =12 5.22 (35.0)	N =12 0.88	N =12 16.21 (14.7)	N =12 113.25 (25.0)	N =12 520.74 (94.7)	N =12 368.20 (60.0)
1.0	1	N =172 8.72 (29.2)	N =172 0.75	N =172 19.00 (24.0)	N =107 95.61 (44.1)	N =49 557.48 (108.9)	N =49 379.65 (98.5)
	7	N =13 10.62 (27.7)	N =13 0.75	N =13 30.10 (18.4)	NA	N =13 563.63 (114.7)	N =12 433.37 (135.5)
	10	N =80 10.13 (20.1)	N =80 0.75	N =80 27.89 (15.9)	NA	N =80 605.70 (103.0)	N =79 373.05 (107.5)
	14	N =11 9.83 (27.1)	N =11 0.75	N =11 31.15 (17.2)	N =11 108.68 (39.0)	N =11 543.23 (102.8)	N =11 409.83 (109.8)
Oral Solution							
0.5 N=22	1	3.70 (26.6)	0.50	16.43 (22.7)	91.35 (59.4)	516.98 (103.2)	NA
1.0 N=6	1	8.31 (20.8)	0.63	33.29 (17.0)	113.48 (64.5)	506.99 (88.7)	376.04 (51.7)

Data presented as geometric mean (CV%) unless otherwise specified.
NA Not available
[a] Data presented as median (minimum, maximum).
[b] AUC is AUC(0-T) on Day 1 and AUC(TAU) on Days 7 & 14 for tablet and capsule; AUC(INF) for oral solution
[c] Tablet and capsule are bioequivalent.
Source: Summary of Clinical Pharmacology

Single doses of entecavir ranging from 1.0 to 40 mg were studied in healthy volunteers enrolled in the first in human sequential dose escalation study AI463001. Multiple daily doses of entecavir up to 20 mg were studied in the two multiple dose escalation studies AI463002 and AI463033. Single and multiple dose PK parameters for entecavir in healthy volunteers enrolled in the two multiple dose escalation studies are summarized in the following table.

Table 2.2.5.1-2 Summary of Entecavir Single and Multiple Dose Pharmacokinetic Parameters in Healthy Subjects Enrolled in Dose Escalation Studies AI463002 and AI463033 (N=6)

Day	Dose (mg)	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC_{tau} (ng•h/mL)	$t_{1/2}$ (hr)	CL/F (mL/min)	CL_r (mL/min)
Study AI463033[b]							
1	0.1	0.51 (28)	0.63	0.94 (23)	-	-	-
	0.5	3.19 (30)	0.75	8.22 (18)	-	-	-
	1.0	6.80 (24)	0.75	16.37 (14)	-	-	-
7	0.1	0.65 (24)	0.75	2.25 (12)	-	782.90 (92.47)	499.94 (131.81)
	0.5	4.30 (20)	0.88	14.30 (14)	-	589.68 (93.28)	429.17 (51.11)
	1.0	9.08 (21)	0.75	25.61 (15)	-	657.76 (99.91)	482.65 (181.37)
14	0.1	0.60 (29)	1.00	2.51 (21)	127.69 (91.44)	678.03 (148.70)	426.66 (149.26)
	0.5	4.23 (9)	1.00	14.78 (17)	129.90 (17.28)	571.74 (110.76)	360.03 (64.23)
	1.0	8.24 (16)	0.75	26.38 (12)	148.89 (39.50)	636.06 (80.40)	471.36 (138.14)
Study AI463002							
1	2.5	17.3 (5.0)	0.75	38.1 (6.9)	-	-	513 (127)
	5	44.8 (12.1)	0.75	98.9 (12.5)	-	-	430 (83)
	10	99.4 (12.0)	0.75	247.3 (28.6)	-	-	417 (144)
	20	187.0 (54.2)	1.00	500.2 (112.6)	-	-	380 (152)
7	2.5	25.2 (3.3)	0.75	65.8 (1.8)	-	-	375 (73)
	5	60.4 (7.6)	0.88	164.5 (18.5)	-	-	348 (74)
	10	85.4 (20.5)	0.75	265.1 (34.9)	-	-	469 (39)
	20	153.7 (14.2)	1.00	476.5 (109.9)	-	-	508 (142)
14	2.5	22.8 (5.7)	0.75	71.6 (10.3)	115.7 (37.2)	594 (102)	387 (97)
	5	46.2 (6.4)	0.88	145.8 (28.4)	91.3 (57.9)	592 (127)	403 (92)
	10	99.9 (13.7)	0.75	304.3 (35.6)	127.5 (41.8)	554 (69)	396 (43)
	20[c]	179.8 (34.8)	1.00	545.6 (57.9)	142.5 (55.5)	617 (68)	430 (103)

Data presented as mean (SD) unless otherwise specified.
- Not calculated
[a] Data presented as median (minimum, maximum).
[b] Data presented as geometric mean (CV%) for Cmax, Tmax, and AUCtau.
[c] N=5
Source: AI463002 and AI463033 Clinical Study Reports

Trough concentrations following 14 days of multiple dosing indicated that steady-state was attained by approximately 9 to 10 days following once-daily dosing. Entecavir displayed a terminal half-life of approximately 130 hours in multiple dose studies (AI463002 and AI463033), a value considerably higher than half-lives observed in the single dose study AI463001, due to limited sampling in AI463001 and increased assay sensitivity and duration of quantitation in the multiple-dose studies. Following the administration of entecavir at the clinically relevant doses of 0.5 or 1.0 mg, the systemic exposure demonstrated approximately 2-fold accumulation resulting in an effective half-life of approximately 24 hours. In healthy subjects administered the proposed clinical doses of 0.5 and 1.0 mg, single and multiple dose half-lives were comparable.

2.2.5.2. How does the PK of entecavir in healthy volunteers compare to that in patients?

In general, exposure to entecavir is greater in HBV patients versus healthy subjects administered multiple comparable doses. This finding is supported by the population PK/PD analysis of Phase 2 data from studies AI463004, AI463005, and AI463014 and the pilot Phase 2 study in liver transplant recipients re-infected with HBV (AI463015).

In the population PK/PD analysis of Phase 2 data from studies AI463004, AI463005, and AI463014, differences in predicted entecavir exposure were observed in HBV-infected subjects versus healthy subjects. Estimates for AUCss, Css,min and Css,max in HBV-infected patients are presented in the following table. In comparison to healthy subjects, entecavir AUC was approximately 30% and 71% higher after multiple daily dosing of 0.5 mg and 1.0 mg, respectively.

Table 2.2.5.2-1 Summary of Derived Pharmacokinetic Parameters in HBV-Infected Patients

Parameter	Mean	SD	Minimum	Maximum
	0.5 mg QD Dose (n=75)			
AUCss (ng.h/mL)	21.3	9.03		
Css,min (ng/mL)	0.53	0.32		
Css,max (ng/mL)	4.17	1.13		
	1.0 mg QD Dose (n=29)			
AUCss (ng.h/mL)	53.9	28.8		
Css,min (ng/mL)	3.50	2.11		
Css,max (ng/mL)	9.70	3.26		

Source: Clinical Study Report AI463017

Entecavir pharmacokinetics were evaluated in nine (9) post-orthoptic liver transplant (OLT) patients with HBV infection on stable doses of tacrolimus of cyclosporine in study AI463015. In OLT patients infected with HBV, mean Cmax was approximately 42% greater and mean AUC was approximately 116% greater than exposures in healthy subjects following 14 days of oral 1.0 mg entecavir (via cross-study comparison), as displayed in the following table.

Table 2.2.5.2-2 Summary of Entecavir Single and Multiple Dose Pharmacokinetic Parameters in Healthy Subjects and HBV-Infected Post-Orthoptic Liver Transplant Patients

Population	Day	C_{max} (ng/mL)	T_{max}* (hr)	AUC_{tau} (ng•h/mL)	CL/F (mL/min)
Healthy Subjects (AI463033)	1	6.80 (24)	0.75	16.37 (14)	NA
	14	8.24 (16)	0.75	26.38 (12)	636.06 (80.40)
OLT HBV Patients (AI463015)	1	8.26 (37)	1.00	25.50 (34)	NA
	14	13.34 (38)	1.00	63.50 (37)	284.35 (129.81)

Data presented as geometric mean (CV%) unless otherwise specified.
NA Not available
* Data presented as median (minimum, maximum).
Source: Summary of Clinical Pharmacology

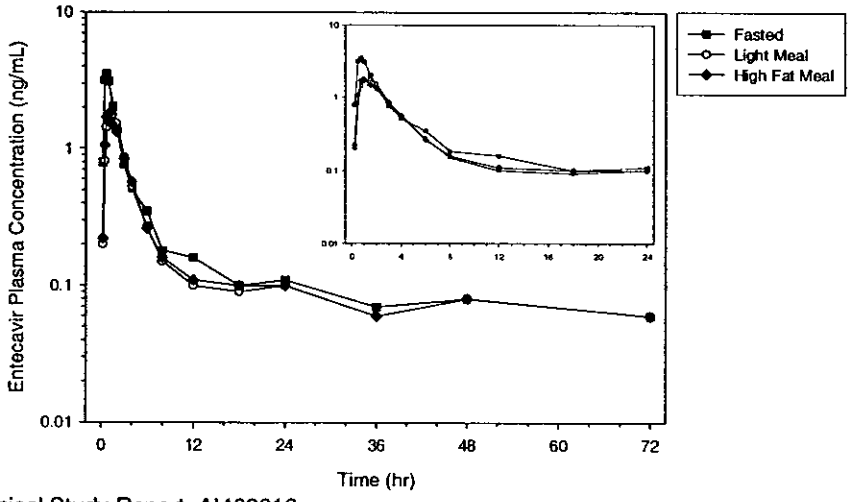
In this patient population, estimated creatinine clearance (CLcr) values ranged from 44 to 119 mL/min. Thus, the subjects in this study would be classified as having renal function ranging from moderately impaired (CLcr 30 to 50 mL/min) to normal (CLcr > 80 mL/min). The increased Cmax and AUC in OLT patients with HBV infection were consistent with the degree of renal impairment in these subjects.

2.2.5.3. What are the characteristics of drug absorption?

Following administration of a single 1.0 mg dose of [^{14}C]-entecavir, 70% of the dose was recovered unchanged in urine; therefore, the oral bioavailability of entecavir is ≥ 70%. Peak plasma concentrations (Cmax) of entecavir occur within 1 hour of drug administration.

The effect of food on entecavir pharmacokinetics is illustrated in the plasma concentration-time profiles below.

Figure 2.2.5.3-1 Mean Concentrations of Entecavir Following a Single Oral 0.5 mg Dose in the Fasted and Fed (High Fat and Light Meals) States in Healthy Subjects



Source: Clinical Study Report AI463016

Following administration of 1.0 mg entecavir in the fed state (either with a light or high fat meal), food decreased the rate and extent of entecavir absorption compared to the 1.0 mg dose administered in the fasted state. The light and high fat meals significantly reduced Cmax by 44 and 46% and AUC by 20 and 18%, respectively.

The relative bioavailability of the entecavir tablet compared to the oral solution is essentially 100% (see section 2.5.2 for further details).

2.2.5.4. What are the characteristics of drug distribution?

The apparent volume of distribution during the terminal phase (Vdβ/F) of entecavir is much larger than total body water (TBW), indicative of extensive extravascular distribution (AI463002). Mean values of Vdβ/F ranged between approximately 4000 to 8000 L in healthy subjects administered multiple daily doses up to 20 mg.

Protein binding of entecavir was evaluated by ultrafiltration across a range of entecavir concentrations (50, 500, and 5000 ng/mL). In general, protein binding of entecavir is low, approximately 13%, and independent of concentration, as detailed in the following table.

Table 2.2.5.4-1 Summary of Entecavir Protein Binding and Red Blood Cell Distribution in Humans

Concentration (ng/mL)	Protein Binding (%)	Mean (SD) RBC Distribution (%) N=3
50	13.8	56.5 (0.30)
500	13.9	52.1 (0.07)
5000	11.9	47.5 (0.15)
Overall Mean	13.2	52.0

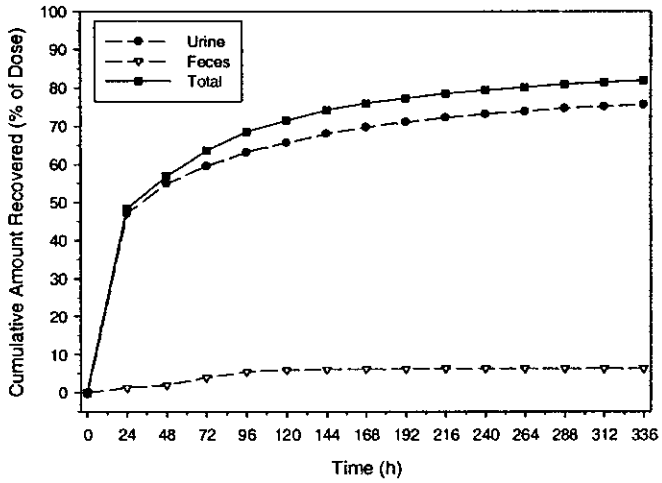
Source: Study MAP004

Entecavir uniformly distributes between plasma and red blood cells (RBCs) in whole human blood.

2.2.5.5. Does the mass balance study suggest renal or hepatic as the major route of elimination?

Renal elimination is the major route of elimination for entecavir, as supported by the mass balance study in six (6) healthy male subjects following a single 1.0 mg radiolabeled dose (Protocol AI463031). Entecavir plasma concentrations were slightly lower than those for radioactivity, and the geometric mean C_{max} value of 8.31 ng/mL for entecavir was 88% of the C_{max} value of radioactivity (9.28 ng-equiv/mL), suggesting that unchanged entecavir is the predominant circulating moiety in plasma. The following figure depicts mean cumulative total radioactivity recovered following administration of a single 1 mg dose of [^{14}C]-entecavir, demonstrating approximately 82% of the administered dose was recovered following the single 1.0 mg dose.

Figure 2.2.5.5-1 Mean Cumulative Total Radioactivity Recovered in Urine and Feces Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects (n=6)



Source: Clinical Study Report AI463031

Urinary and fecal recovery of radioactivity and radioprofiling of plasma, urine and feces suggest renal excretion of unchanged drug is the primary route of entecavir elimination, while biliary excretion plays a minor role. Approximately 70% of the administered entecavir dose was excreted as unchanged drug in urine. The relative percent distribution of radioactivity among parent and metabolites in human plasma, urine, and feces following administration of a single dose of 1 mg [^{14}C]-entecavir in healthy male subjects are presented in the following table.

Table 2.2.5.5-1 Relative Percent Distribution of Radioactivity Among Various Peaks in the Radiochromatographic Profiles of Pooled Human Plasma, Urine, and Feces Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects

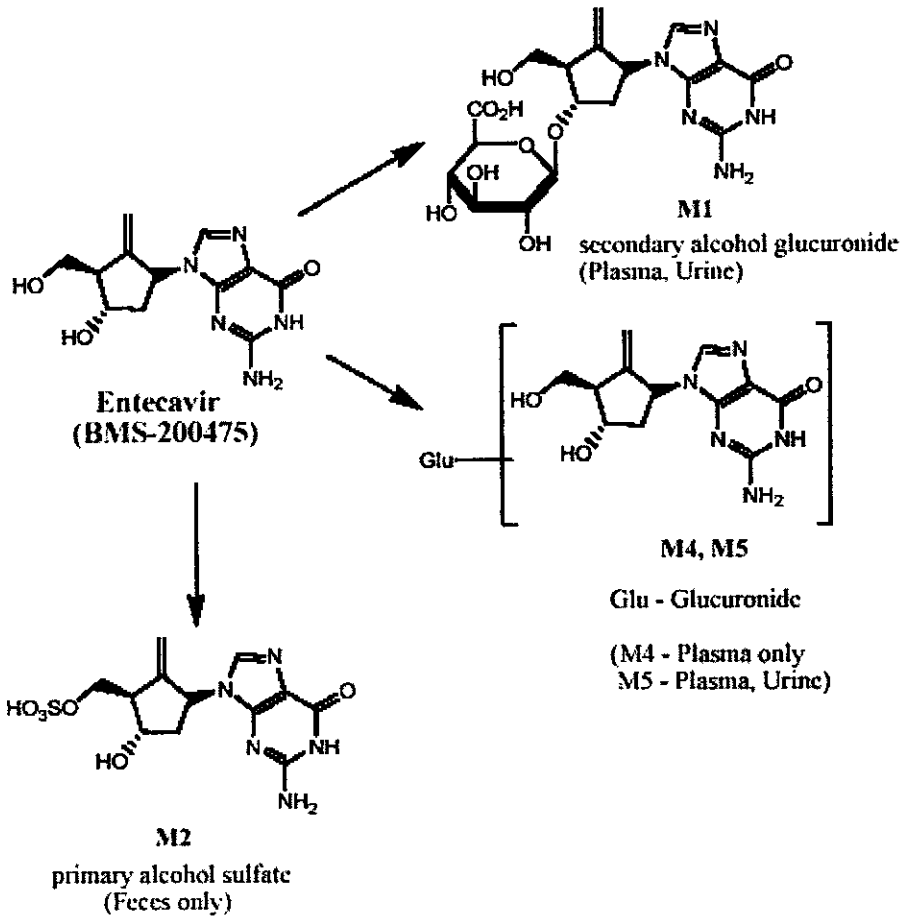
Identity	% Relative Distribution of Radioactivity in Pooled Sample			
	Plasma (1 h)	Plasma (2 h)	Urine (0-336 h)	Feces (0-336 h)
M1	8.7	20.1	5.7	-
M2	-	-	-	15.2
M4	4.5	3.8	-	-
M5	6.2	5.2	4.1	-
Parent	79.3	70.3	87.3	66.0
Others*	1.3	0.6	2.9	18.8
Total	100	100	100	100

* Others includes several unidentified peaks, each of which were ≤ 2% of the total radioactivity in that matrix.

2.2.5.6. What are the characteristics of drug metabolism?

Entecavir was not significantly metabolized in humans after a single oral dose of 1 mg [^{14}C]-entecavir in six (6) healthy male subjects (Protocol AI463031). The proposed metabolic pathway of entecavir is as follows.

Figure 2.2.5.5-2 Proposed Metabolic Pathway of Entecavir



Entecavir was metabolized in humans to three glucuronide conjugates (M1, M4, and M5), which were the only metabolites detected in plasma and urine samples, and a sulfate conjugate (M2) observed only in feces. No phase I oxidative metabolites of entecavir were observed in plasma, urine or feces, indicating that CYP450 does not play a role in the metabolic clearance of entecavir.

2.2.5.7. What are the characteristics of drug excretion?

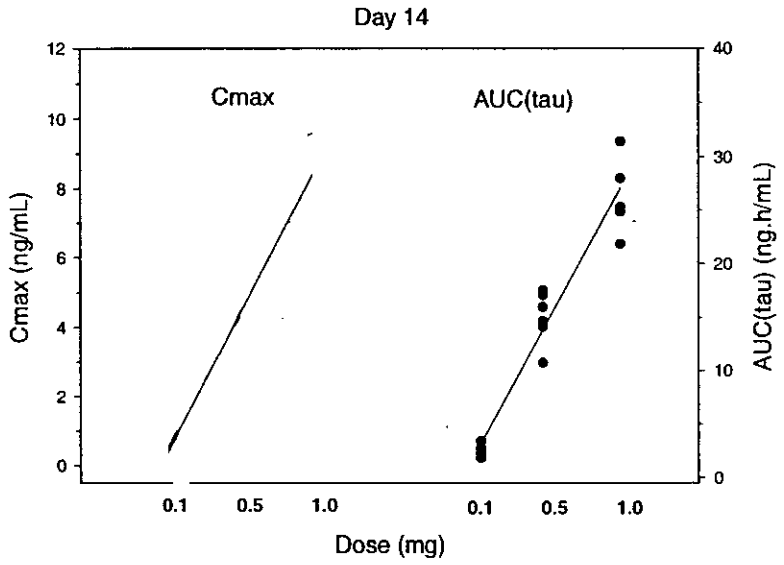
Approximately 70% of an administered entecavir radiolabeled dose was excreted as unchanged drug in urine. Therefore, renal excretion of unchanged drug is the primary route of entecavir elimination. Biliary excretion plays a minor role in excretion of entecavir, as 6.3% of dosed radioactivity in the mass balance study was recovered in feces. Values for renal clearance of entecavir in healthy subjects administered single and multiple doses of entecavir were consistently greater than glomerular filtration rate, suggesting renal excretion of entecavir occurs via a combination of glomerular filtration and net tubular secretion.

2.2.5.8. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Following oral administration in 14-day multiple-dose studies, the steady state exposure of entecavir was approximately dose-proportional at the clinically relevant doses of 0.5 and 1.0 mg, as well as at doses up to 20 mg. There were greater than proportional increases in Cmax and AUC following single-dose oral administration (AI463001), a finding most likely attributable to the limited sampling in this study and inadequate characterization of the terminal phase of elimination.

Cmax and AUC(TAU) versus dose following administration of multiple oral daily doses of 0.1, 0.5, and 1.0 mg entecavir for 14 days in healthy subjects in study AI463033 are presented in the following figure. Linear increases in concentration and exposure were observed with increasing doses of entecavir (r^2 of 0.9466 for Cmax and 0.9457 for AUC, respectively).

Figure 2.2.5.8-1 Plots of Cmax and AUC(TAU) Versus Dose Following Administration of Multiple Oral Daily Doses of 0.1, 0.5, and 1.0 mg Entecavir for 14 Days in Healthy Subjects. (n=6 per dose group)

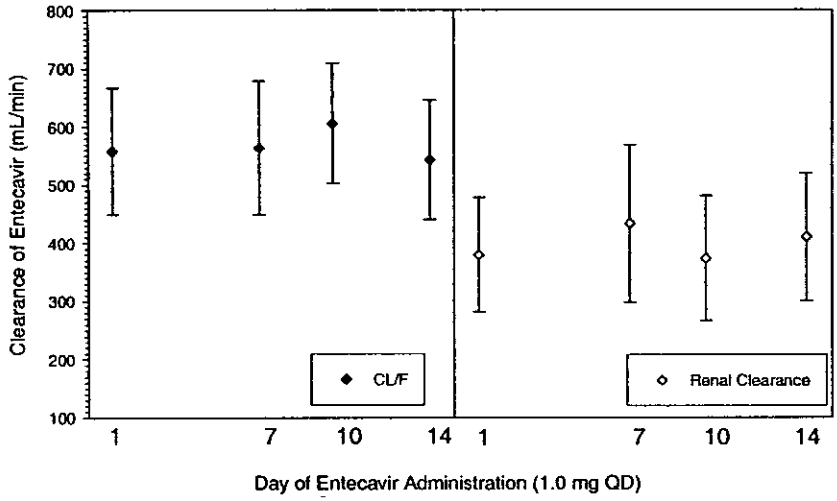


Symbols represent individual values.
Source: AI463033 Clinical Study Report

2.2.5.9. How do PK parameters change with time following chronic dosing?

Clearance of entecavir does not appear to change following chronic dosing, as demonstrated in the following graph of apparent oral clearance and renal clearance across 14 days of daily entecavir administration in healthy subjects receiving the highest proposed clinical dose of 1.0 mg.

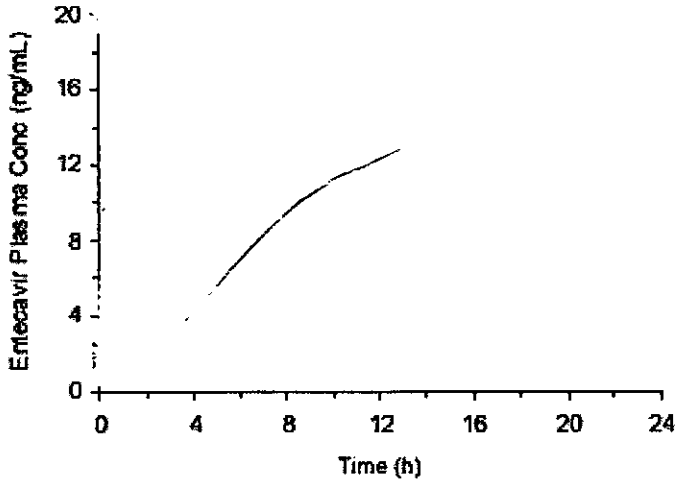
Figure 2.2.5.9-2 Apparent Oral Clearance and Renal Clearance Across 14 Days of Daily Entecavir 1.0 mg Administration in Healthy Subjects



Source: Summary of Clinical Pharmacology

In post-orthoptic liver transplant (OLT) patients with HBV infection receiving multiple doses of entecavir 1.0 mg QD in study AI463015, individual entecavir plasma concentrations from random sampling at Weeks 4, 12, 24, 36, and 48 of therapy were all within the range of entecavir plasma concentrations observed on Day 14 (presumed steady state) in each subject, as displayed in the following figure.

Figure 2.2.5.9-3 Entecavir Plasma Concentrations Obtained From Random Sampling in HBV-Infected Orthoptic Liver Transplant Patients



Source: AI463015 Clinical Study Report

This finding suggests that no marked changes in entecavir exposure occurred over the subsequent 46-week dosing period in OLT HBV patients.

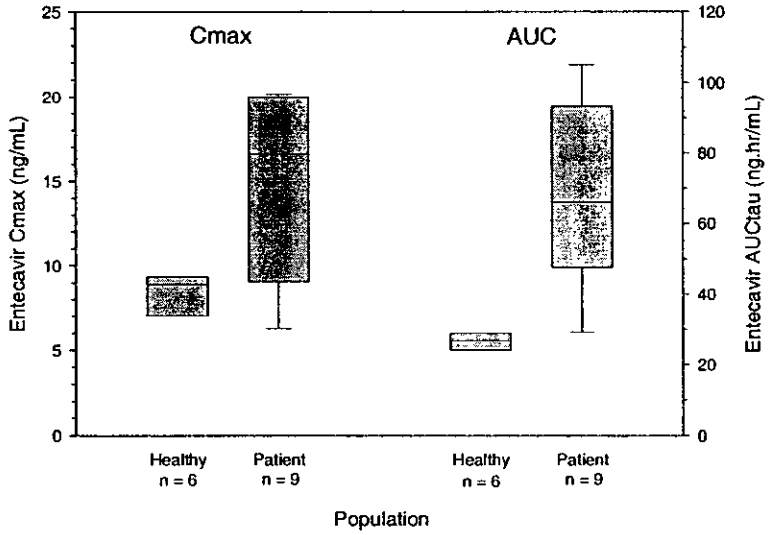
2.2.5.10. What is the inter- and intra-subject variability in volunteers and patients, and what are the major causes of variability?

In phase 1 studies in healthy subjects, entecavir exhibited a low and consistent degree of variability in apparent oral clearance following single and multiple dosing across the dose range studied. Mean %CV for CL/F following the proposed clinical doses (0.5 and 1.0 mg) administered in the fasted state was approximately 10 to 20%. Variability in entecavir trough concentrations following up to 14 days of dosing was comparable, with %CVs ranging between approximately 11 to 32%.

Administration of entecavir in the fed state (following either a light or high fat meal) slightly increased the variability of observed exposures in healthy subjects, as %CV for Cmax and AUC were 34 and 20% for the fasted state, 36 and 22% following a light meal, and 41 and 23% following a high fat meal, respectively.

Greater variability in exposure and apparent oral clearance was observed in HBV-infected OLT patients receiving 1.0 mg QD of entecavir compared to healthy subjects, as presented in the following figure showing a comparison of Cmax and AUC values following 14 days of multiple dosing.

Figure 2.2.5.10-1 Comparison of Entecavir Exposure Between Healthy Subjects and HBV-Infected Orthoptic Liver Transplant Patients



Lines represent median values; boxes, 25th and 75th percentiles; whiskers, 5th and 95th percentiles.
Source: AI463033 and AI463015 Clinical Study Reports

In the population PK/PD analysis of Phase 2 data from studies AI463004, AI463005, and AI463014, inter-individual variability in apparent oral clearance was estimated to be approximately 40%.

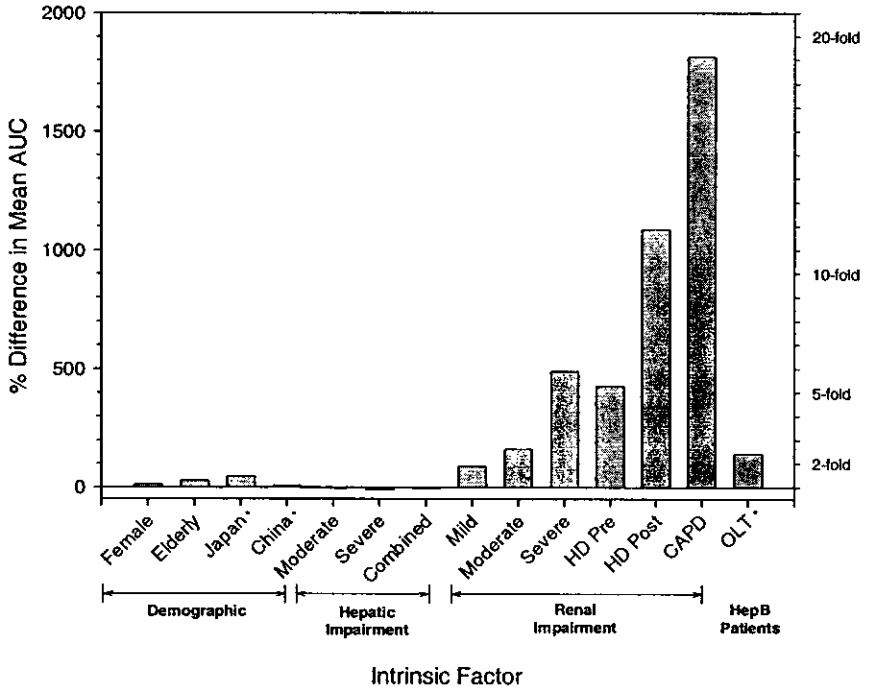
Variability in entecavir pharmacokinetics is most notably attributable to differences renal function. The effects of various intrinsic and extrinsic factors on entecavir exposure are discussed in detail in sections 2.3 and 2.4. Other causes of variability are not known.

2.3. Intrinsic Factors

2.3.1. What intrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The following graph provides an overview of the effects of various intrinsic factors on entecavir exposure following a single 1 mg dose. Dose adjustment is warranted in patients with renal impairment, but not for other intrinsic covariates.

Figure 2.3.1-1 Overview of the Influence of Intrinsic Factors on Entecavir Exposure



* % Difference in Mean AUC based on a cross study comparison.

2.3.2. Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

2.3.2.1. Elderly

The effect of age on the pharmacokinetics of entecavir was evaluated following administration of a single 1.0 mg oral dose in the age/gender study AI463032 (n=14 per age/gender group). Elderly subjects in this study displayed greater AUC(INF), 29.3% higher compared to young subjects, but adjustment for CLcr and body weight reduced this difference in exposure to 12.5%, indicating the impact of age on the systemic exposure to entecavir is most likely attributable to changes in renal function and/or body

weight. As the differences in body weight between elderly and young subjects in this study was minimal (< 6% for the mean values), its influence on the alteration in systemic exposure is most likely negligible. Average creatinine clearance was lower (21.5%) in elderly subjects versus young subjects in this study. Therefore, the disparity in exposure between elderly and young subjects was most likely attributable to differences in renal function. Dosage adjustment of entecavir should be based on the renal function of the patient, rather than age (for further recommendations, see section 2.3.2.5).

2.3.2.2. Pediatric Patients

The pharmacokinetics of entecavir have not been studied in subjects < 16 years of age in support of this application.

2.3.2.3. Gender

The effect of gender on the pharmacokinetics of entecavir was evaluated following administration of a single 1.0 mg oral dose in the age/gender study AI463032 (n=14 per age/gender group). No clinically significant gender-related differences in entecavir pharmacokinetics that warrant dose adjustment were observed, as the adjusted geometric mean Cmax and AUC(INF) were 17.2% and 14% higher, respectively, for female versus male subjects.

The incidence of AEs was more than 6-fold greater in female subjects (19/26, 73.1%) compared to male subjects (3/26, 11.5%) in the study (AI463032). No clear relationship between measures of exposure [Cmax and AUC(INF)] and the most frequently reported treatment emergent adverse event (TEAE) in females (headache) was discerned. All digestive-related adverse events reported in the age/gender study occurred in the female treatment group but showed no discernable relationship with entecavir exposure.

2.3.2.4. Race

The following table summarizes entecavir pharmacokinetics in healthy Chinese, Japanese, and US subjects following multiple daily doses of entecavir 0.5 mg.

Table 2.3.2.4-1 Comparison of Entecavir Exposure in Healthy Chinese, Japanese and US Subjects

Population	Day	C_{max} (ng/mL)	AUC_{tau} (ng•h/mL)	CL/F (mL/min)	CL/F (mL/min/kg)
Chinese Subjects (AI463018)[a] N=8	9	6.36 (1.43)	17.42 (2.83)	ND	ND
Japanese Subjects (AI463029)[b] N=6	14	6.43 (34.8)	17.78 (7.4)	468.67 (7.4)	8.17 (8.6)
US (Non-Asian) Subjects (AI463033)[b] N=6	14	4.23 (9.0)	14.78 (17)	571.74 (110.76)	7.74 (25.0)

[a] Data presented as arithmetic mean (SD).
[b] Data presented as geometric mean (%CV).
ND No data available
Source: AI463018, AI463029, and AI463033 Clinical Study Reports

This cross-study comparison of pharmacokinetic parameters in healthy Chinese, Japanese, and US subjects following multiple daily doses of entecavir 0.5 mg revealed differences in exposure between the three study populations. Cmax and AUC following multiple 0.5 mg dosing of entecavir were approximately 50% and 20% higher in healthy Asian subjects versus healthy non-Asian subjects. Weight-normalized CL/F values were comparable between the Japanese and non-Asian study populations (clearance for Chinese subjects not available), suggesting the ethnic differences in exposure between Asian and non-Asian populations may be attributable to differences in body weight, but small sample sizes across these study populations preclude definition of an effect of race on entecavir pharmacokinetics.

2.3.2.5. Renal Impairment

Dosage reduction of entecavir is warranted in the presence of moderate and severe renal impairment. Entecavir pharmacokinetics in subjects with varying degrees of renal function are presented in the following table.

Table 2.3.2.5-1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 1.0 mg in Subjects with Renal Impairment.

Parameter	A (N=6)	B (N=6)	C (N=6)	D (N=6)	E1 (N=6)	E2 (N=6)	F (N=4)
Cmax (ng/mL)	8.06 (30.70)	10.43 (37.2)	10.53 (22.7)	15.3 (33.8)	12.12 (41.1)	15.37 (56.4)	16.56 (29.7)
AUC(INF) (ng•h/mL)	29.15 (25.0)	54.94 (23.80)	75.77 (25.3)	171.65 (29.2)	153.61 (21.0)	346.14 (40.8)	558.32 (92.5)
AUC(0-T) (ng•h/mL)	27.90 (25.6)	51.46 (22.8)	69.49 (22.7)	145.66 (31.5)	127.10 (20.2)	233.91 (28.4)	221.80 (11.6)
Tmax[a] (hr)	0.75	0.88	0.63	0.88	0.88	0.75	1.00
$t_{½}$[b] (hr)	77.39 (29.88)	113.21 (13.67)	130.65 (25.74)	162.34 (33.49)	155.55 (33.16)	276.34 (143.40)	802.16 (872.37)
CLT/F[b] (mL/min)	588.11 (153.73)	309.18 (62.61)	226.26 (60.11)	100.58 (29.05)	110.69 (24.64)	50.61 (16.54)	35.66 (19.58)
CLR[b] (mL/min)	383.18 (101.80)	197.90 (78.11)	135.57[c] (31.55)	40.27 (10.11)	NA	NA	NA

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
[c] N=5
Group A: Subjects with normal renal function (CLcr > 80 mL/min)
Group B: Subjects with mild renal function impairment (CLcr > 50 ≤ 80 mL/min)
Group C: Subjects with moderate renal function impairment (CLcr 30-50 mL/min)
Group D: Subjects with severe renal function impairment (CLcr < 30 mL/min)
Group E1: Subjects with severe renal function impairment managed with hemodialysis; dosed 2 hours prior to dialysis
Group E2: Subjects with severe renal function impairment managed with hemodialysis in period 2; dosed immediately following dialysis
Group F: Subjects with severe renal function impairment managed with CAPD
Source: AI463011 Clinical Study Report

In subjects with renal impairment (study AI463011), as renal function declined, mean apparent total body clearance and renal clearance of entecavir decreased resulting in a longer half-life and greater entecavir exposure, as compared to subjects with normal renal function. The extent of renal function impairment is predictive of the renal clearance and total body clearance of entecavir, as demonstrated in the following linear regressions of creatinine clearance and CLr and CLT/F. Additionally, hemodialysis removed approximately 13% of the entecavir dose, while continuous ambulatory

for various dosage regimens and compared with a "clinically meaningful" AUCss target (reference) range. The lower limit of the reference range exposure was selected based on the lower limit of the predicted AUCss values determined in subjects with normal renal function. The upper limit of the reference range was set to two (2) times the geometric mean AUCss value in subjects with normal renal function. The goal was to dose adjust so that at least 75% of simulated AUCss values fell within the target range for a given degree of renal impairment. For purposes of simulation, dialysis patients were assigned a creatinine clearance value of 5 mL/min. Although underestimation of exposure is possible with this arbitrary value, it is a reasonable value for purposes of simulation. Percentages of simulated AUCss values outside the established target range before dosage adjustment are presented in the following table.

Table 2.3.2.5-2 Percent of Simulated AUCss Values for 1 mg QD Outside the Target Exposure Limits – Prior to Dose Adjustment

Renal Function Classification	CL_{Cr} (mL/min)	Percent Simulated AUCss Values Lower than Lower Target Value	Percent Simulated AUCss Values Higher than Upper Target Value
Normal	> 80	0	0
Mild impairment	> 50 - ≤ 80	0	21.7
Moderate impairment	>30 - ≤ 50	0	65.0
Severe impairment not requiring dialysis	< 30	0	98.3
ESRD receiving regular dialysis	5*	0	100

* Assigned value
Source: 930007867 Clinical Study Report

Simulations of exposure following various dosage regimens for groups with subjects falling outside the established criteria (moderate and severe impairment and dialysis) are presented in the following figures. The figure display results of the inverse prediction analysis for dose regimen adjustment for the 1 mg QD regimen in relation to the target range (represented by the long dashed lines). For nucleoside-naïve patients, the dose is reduced by 50%.

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Figure 2.3.1-4 Predicted Exposures for Patients with Moderate Renal Impairment

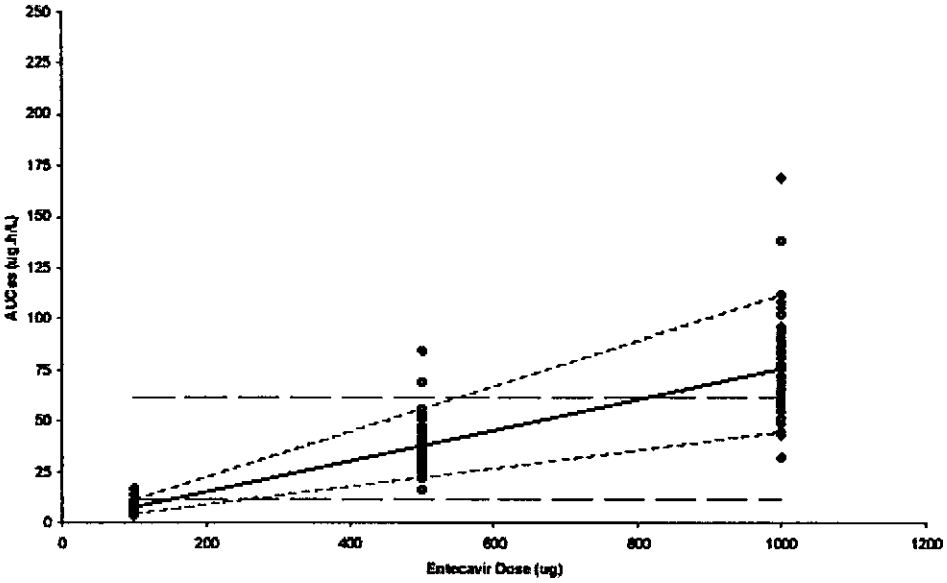


Figure 2.3.1-5 Predicted Exposures for Patients with Severe Renal Impairment

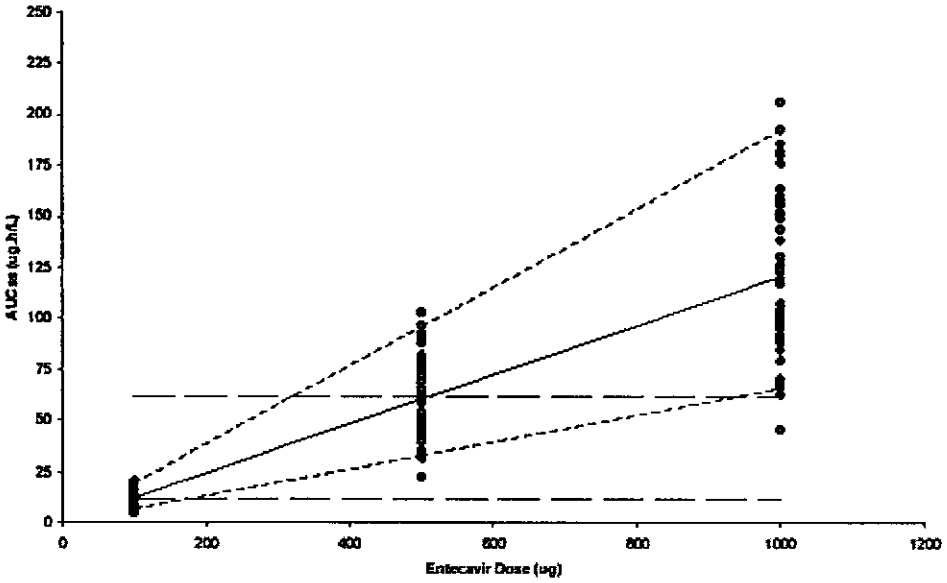
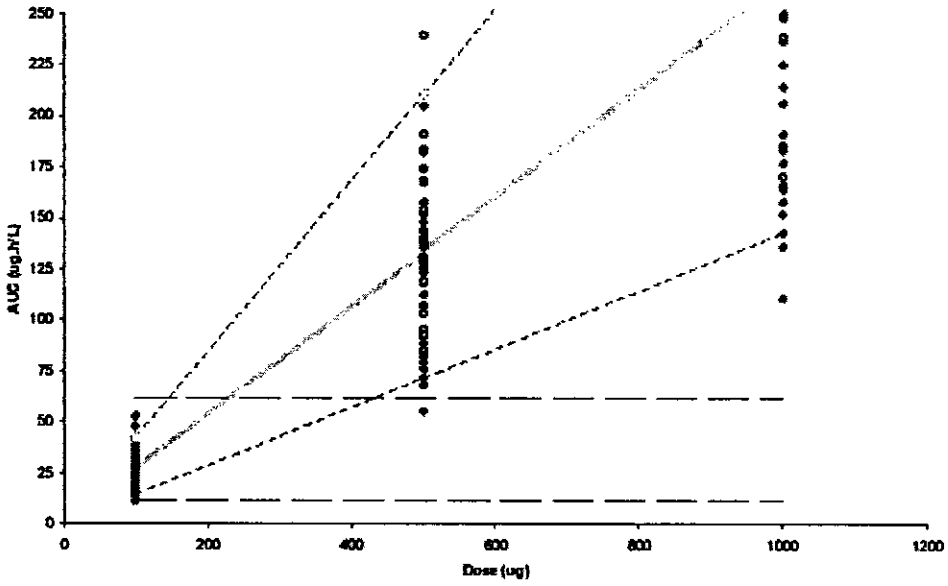


Figure 2.3.1-6 Predicted Exposures for Patients with ESRD Requiring Hemodialysis



Source: 930007867 Clinical Study Report

Based on the percentages of simulated AUCss values outside the established target range and the simulation results for the three groups falling outside the established criteria, the following dose reductions were recommended by the Applicant.

Table 2.3.2.5-3 Recommended Reduction in Daily Entecavir Dose by Renal Impairment Group

Renal Function Classification	CL_{Cr} (mL/min)	Recommended Dose Reduction
Normal	> 80	100% recommended dose
Mild impairment	> 50 - ≤ 80	100% recommended dose
Moderate impairment	>30 - ≤ 50	50% recommended dose
Severe impairment not requiring dialysis	< 30	30% recommended dose
ESRD receiving regular dialysis	5*	20% recommended dose

* Assigned value
Source: 930007867 Clinical Study Report

Percentages of simulated AUCss values outside the established target range after the proposed dosage adjustments are presented in the following table.

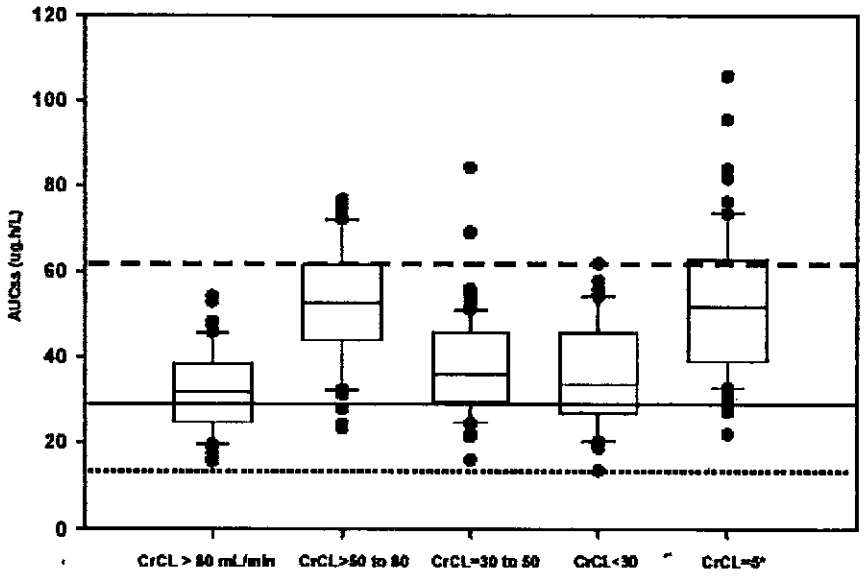
Table 2.3.2.5-4 Percent of Simulated AUCss Values Outside the Target Exposure Limits – After Dose Adjustment

Renal Function Classification	Proposed Dose	Percent Simulated AUCss Values Lower than Lower Target Value	Percent Simulated AUCss Values Higher than Upper Target Value
Normal	1.0	0	0
Mild impairment	1.0	0	21.7
Moderate impairment	0.5	0	3.33
Severe impairment not requiring dialysis	0.3	1.67	0
ESRD receiving regular dialysis	0.2	0	25.0

Source: 930007867 Clinical Study Report

Box plots of expected AUCss values for subjects with varying degrees of renal function, based on the Applicant's proposed dosage recommendation for LVD-refractory HBV infected patients, are depicted in the figure below.

Figure 2.3.1-7 Expected Exposures Based on Applicant Dose Recommendations for Patients with Varying Degrees of Renal Function



The solid horizontal line represents the geometric mean of the normal renal function group, the upper dashed horizontal line represents 2 times the geometric mean of the normal renal function group (ie, upper limit of predefined target range), and the lower dotted horizontal line represents the lowest predicted value for subjects with normal renal function (ie, lower limit of predefined target range).

Source: 930007867 Clinical Study Report

The Agency recommends the Applicant adjust the doses for renal impairment subjects with hemodialysis or CAPD to 10% of the dose required for subjects with normal renal function, as this population has not been studied in either Phase II /Phase III clinical trials and has greater variability on entecavir exposure. Altering the dose to 10% of the recommended dose for patients with normal renal function will provide entecavir exposures closer to that in patients with normal renal function following the full dose,

based on the simulated AUCss from population PK analysis and the AUCinf of entecavir from noncompartmental model.

In summary, based on analysis results from the renal impairment study AI463011 and the modeling and simulations performed in study 930007867, the following dosage adjustment recommendations for entecavir in patients with renal function impairment have been proposed by the Applicant.

Creatinine Clearance (mL/min)	Nucleoside-Naïve Patients	Lamivudine-Refractory Patients
≥ 50	0.5 mg once daily	1 mg once daily
30 to <50	0.25 mg once daily	0.5 mg once daily
10 to <30	0.15 mg once daily	0.3 mg once daily
Hemodialysis or CAPD*	[redacted]	[redacted]

* Administer after dialysis

Based upon Agency review of the analyses from the renal impairment study AI463011 and the modeling and simulation study 930007867, the following dosage adjustment recommendations for entecavir in patients with renal function impairment have been proposed by the Agency. The recommendations have been accepted by the Applicant.

Creatinine Clearance (mL/min)	Nucleoside-Naïve Patients	Lamivudine-Refractory Patients
≥ 50	0.5 mg once daily	1 mg once daily
30 to <50	0.25 mg once daily	0.5 mg once daily
10 to <30	0.15 mg once daily	0.3 mg once daily
Hemodialysis or CAPD*	0.05 mg once daily	0.1 mg once daily

* Administer after dialysis

2.3.2.6. Hepatic Impairment

The pharmacokinetics of a single 1.0 mg oral dose of entecavir was compared between 16 healthy subjects and 16 subjects with hepatic impairment in an open-label, single-dose, non-randomized study (AI463032). Hepatic impairment had a negligible impact on entecavir exposure, and no dose modification based on the presence of hepatic impairment is necessary.

2.4. Extrinsic Factors

2.4.1. What extrinsic factors influence dose-exposure and/or –response, and what is the impact of any differences in exposure on response?

The following extrinsic factors were evaluated in Phase 1 studies in healthy subjects to determine their influence on entecavir dose-exposure and dose-response (safety): drugs (lamivudine, adefovir, tenofovir) and diet (light and high-fat meals). The effects of co-administered medications on entecavir exposure are discussed in section 2.4.2. The effects of light and high-fat meals on entecavir exposure are summarized in section 2.5.3.

2.4.2. Drug-Drug Interactions

2.4.2.1. Is there any in vitro basis to suspect in vivo drug-drug interactions?

Several in vitro studies with human hepatic microsomes, liver S-9 fractions, precision cut liver slices, expressed cytochrome P450 (CYP), and cultures of primary human hepatocytes indicate that entecavir is not a substrate, inhibitor, or inducer of the cytochrome P450 enzyme system. For further description of in vitro information, see section 2.4.2.3.

2.4.2.2. Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

The only metabolites detected in human plasma, urine, and feces following a 1 mg radiolabeled dose of entecavir were minor amounts of phase 2 metabolites, namely, glucuronide and sulfate conjugates. Consistent with pre-clinical results, no oxidative metabolites of entecavir were detected in samples from healthy subjects, indicating that, in vivo, CYP450 does not play a role in the metabolic clearance of entecavir.

2.4.2.3. Is the drug an inhibitor and/or inducer of CYP enzymes?

An in vitro study was conducted to evaluate the potential for entecavir to inhibit CYP catalytic activity, specifically CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (study 930004689). The following probe substrates were utilized: phenacetin (1A2), [^{14}C]-*S*-mephenytoin (2B6, 2C19), diclofenac (2C9), bufuralol (2D6), p-nitrophenol (2E1), and testosterone (3A4). Entecavir concentrations tested ranged from 0.01 to 300 μM. At clinically relevant concentrations (approximately < 0.1 μM or 30 ng/mL), entecavir inhibited catalytic activities no greater than 9% for all enzymes studied.

The effect of entecavir on the expression of cytochrome P450 enzymes 1A2, 2B6, 2C9, 2C19 and 3A4/5, in primary cultures of human hepatocytes was compared to protypical inducers in study XT033016. At concentrations ranging from 0.1 to 10 μM, entecavir did not cause an increase in the activity of any of the enzymes, suggesting that entecavir is not an inducer of any of the CYP enzymes examined.

In addition, multiple-dose entecavir did not affect the urinary 6β-hydroxycortisol-to-cortisol ratio in healthy subjects, confirming that, in vivo, entecavir is not an inducer of CYP3A4 (AI463066).

2.4.2.4. Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

An in vitro transport experiment was conducted to evaluate entecavir as a substrate of human P-glycoprotein (P-gp) using Caco-2 cells. Both the apical-to-basolateral permeability (absorptive direction) and basolateral-to-apical permeability (secretory direction) of entecavir was very low (<15 nm/sec), indicating entecavir is a poor substrate for P-gp.

The potential for entecavir to inhibit P-gp was not evaluated.

2.4.2.5. Are there other metabolic/transporter pathways that may be important?

The primary route of elimination of entecavir is by renal excretion of unchanged drug. Renal clearance of entecavir is greater than GFR, suggesting renal excretion of entecavir occurs via a combination of glomerular filtration and net tubular secretion. As renal tubular secretion is an active process governed by a number of transporters (ie anionic and cationic) and this process is energy-dependent and saturable, drugs that share the same secretory renal tubular transporter may compete with entecavir, potentially resulting in decreased clearance of either or both drugs. Clinical studies evaluating the potential for pharmacokinetic interaction involving drugs eliminated via active tubular secretion (specifically lamivudine, adefovir and tenofovir) were conducted in the entecavir development program. There were no clinically significant drug interactions. See section 2.4.2.7 for further description of drug interaction study results.

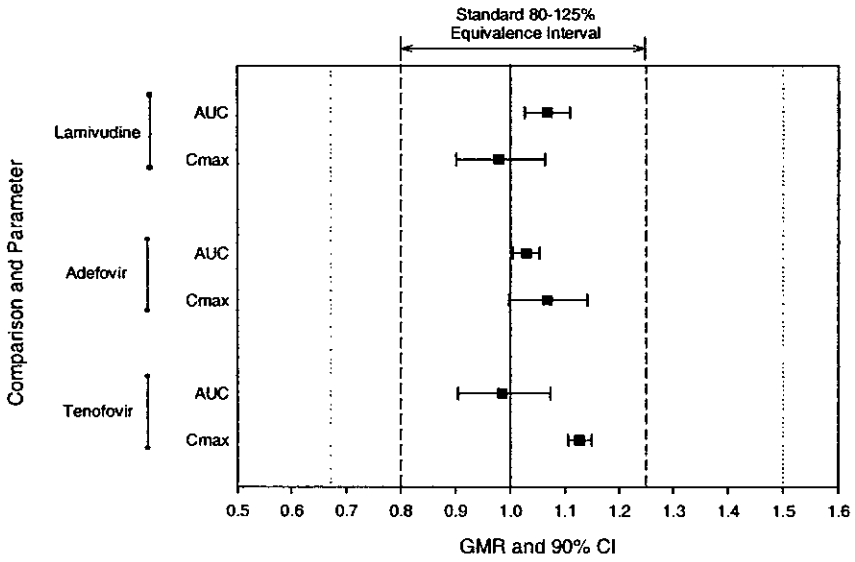
2.4.2.6. What other co-medications are likely to be administered to the target patient population?

In the treatment of HBV infection, combination therapy with two drugs in patient populations who fail monotherapy and/or in treatment-naïve patients is a potentially viable option for therapy, specifically with nucleoside and nucleotide analogs.
In addition, in patient populations co-infected with HBV and HIV-1, multiple drug regimens are anticipated. Therefore, the focus of the drug-drug interaction evaluation for entecavir included antivirals indicated for the treatment of HBV, as well as HIV-1, and that have demonstrated potential for a mechanistic-based intereaction (renal elimination by active processes).

2.4.2.7. Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

Clinical studies evaluating the potential for drug interaction following multiple-dose co-administration of entecavir with lamivudine, adefovir, or tenofovir were conducted in healthy subjects. A summary of the effects of co-administration of these agents on entecavir exposure are summarized in the following figure.

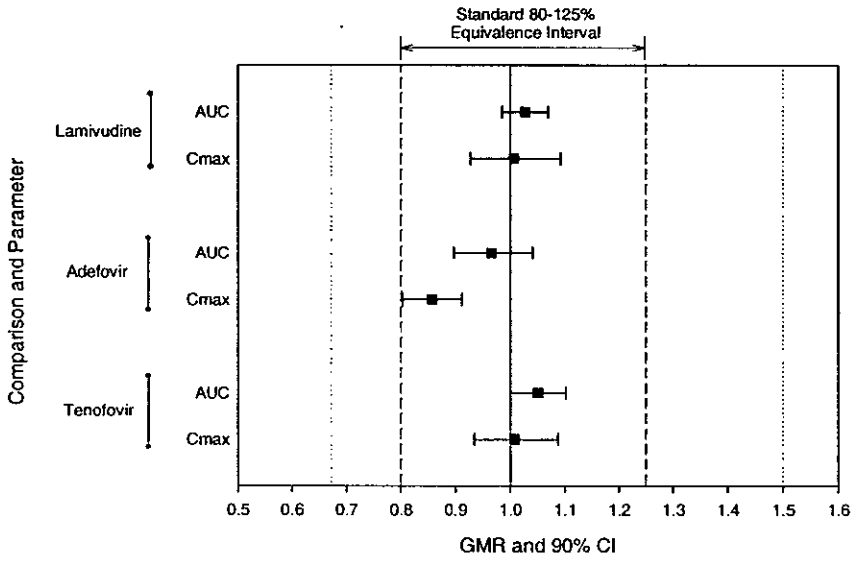
Figure 2.4.2.7-1 The Effects of Lamivudine, Adefovir, and Tenofovir Co-Administration on Entecavir Exposure



Source: AI463058, AI463063, and AI463066 Clinical Study Reports

A summary of the effects of entecavir co-administration on lamivudine, adefovir, and tenofovir exposure are summarized in the following figure.

Figure 2.4.2.7-2 The Effects of Entecavir Co-Administration on Lamivudine, Adefovir, and Tenofovir Exposure



Source: AI463058, AI463063, and AI463066 Clinical Study Reports

No statistically significant pharmacokinetic drug-drug interaction between entecavir and lamivudine, adefovir, or tenofovir was observed in healthy subjects.

2.4.2.8. Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

In an in vitro study (study 930002741), the antiviral activity of various nucleoside reverse transcriptase inhibitors (NRTIs; specifically abacavir, lamivudine, stavudine, tenofovir, didanosine, and zidovudine) and entecavir was measured to determine whether the inhibition of HBV replication by entecavir is adversely affected by the co-administration of NRTIs, and, conversely, whether entecavir adversely affects the anti-HIV activity of these NRTIs. EC_{50} values were determined at concentrations that met or exceeded the maximum concentrations in patients. In HBV replication assays, the antiviral activity of entecavir was not affected by the presence of stavudine, didanosine, abacavir, and zidovudine, at either Cmax or 5 times the Cmax for each HIV NRTI. For lamivudine and tenofovir (both compounds with inherent activity against HBV), their addition to HBV assays containing entecavir at its EC_{50} concentration did not lead to any reduction in anti-HBV activity over a wide range of concentrations. In HIV antiviral assays, there was no effect of entecavir on the in vitro antiviral activity of the six other NRTIs against HIV at > 4 times the Cmax of entecavir.

2.4.2.9. Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

In the mass balance study conducted in six (6) healthy male subjects administered 1.0 mg [^{14}C]-entecavir (Protocol AI463031), approximately 18% of the total radioactivity administered was not recovered after 14 days. The Applicant speculates that the unrecovered portion may have been retained in the body and was being eliminated slowly due to extensive penetration to a deep compartment or partially due to the uptake of entecavir and/or its metabolites by purine salvage pathways and/or intracellular inter-conversion of entecavir nucleotides (mono-, di-, and triphosphates). The mean urinary recovery of radioactivity in the last 4 collection intervals (Days 11 to 14) remained quite steady (approximately 0.5% to 0.8% per day). The Applicant surmises that this trend would have probably continued and the total recovery of radioactivity would be higher than 82% had urine collection continued beyond Day 14. The fate of the remainder of the entecavir dose administered to healthy subjects in the mass balance study remains unclear.

2.4.3. What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

Aside from Applicant concurrence with Agency proposed dosage recommendations in patients with renal impairment, no unresolved issues related to entecavir dose, dosing regimens, or administration have been identified.

2.5. General Biopharmaceutics

The quantitative composition of the to-be-marketed entecavir tablets is shown in the following table:

Table 2.5-1 Composition of Commercial Entecavir Tablets

Component	Compendial Reference	Function	Quantity per unit dose (mg/tablet)	
			0.5 mg	1.0 mg
			Product Identification Number	
			200475-K0X5-073[a]	200475-K001-075[b]
Entecavir[c]	NC	Active pharmaceutical ingredient	0.5	1.0
Lactose Monohydrate[d]	NF			
Microcrystalline Cellulose	NF			
Crospovidone	NF			
Povidone	USP			
Magnesium Stearate[e]	NF			
Total weight	---		206	412

[a] Triangular, white film coated tablet with "BMS" debossed on one side and "1611" debossed on the other side
[b] Triangular, pink film coated tablet with "BMS" debossed on one side and "1612" debossed on the other side
[c] Amount of entecavir is based on a theoretical potency of
[d]
[e] An amount in the range of of theoretical tablet weight can be used (preferred amount is w/w).
sing
NC = non compendial
NF = United States National Formulary

The quantitative composition of the to-be-marketed entecavir solution is shown in the following table:

Table 2.5-1 Composition of Commercial Entecavir Solution

Component	Compendial Reference	Concentration (mg/mL)
Entecavir[a]	NC	0.05
Methylparaben	NF	
Propylparaben	NF	
Maltitol	NF	
Orange Flavor	NC	
Citric Acid	NF	
Sodium Citrate Dihydrate	NC	

[a] Amount of entecavir is based on a theoretical potency of
[b]
[c]
[d]
NC = non compendial
NF = United States National Formulary

2.5.1. Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Based on available solubility and permeability information, entecavir may be classified as BCS Class 3 (high solubility-low permeability). The highest tablet strength for the proposed commercial formulation is 1 mg. Per FDA guidance, a drug substance is considered *highly soluble* when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1 to 7.5. Thus, for entecavir to be considered highly soluble, its solubility should exceed 0.004 mg/mL. The solubility of entecavir across a wide range of pH is presented in the following table, suggesting entecavir is highly soluble.

Table 2.5.1-1 Solubility Profile for Entecavir

pH	Solubility at 25 ± 0.5°C (mg/mL)
2.1	
2.3	
2.6	
2.9	
3.1	
3.4	
4.8	
7.9	
8.7	
9.1	
9.2	
9.6	
9.8	
10.0	

Per FDA guidance, the permeability class of a drug substance can be determined by mass balance, absolute BA, or intestinal perfusion studies. Based on mass balance determination, a drug substance is considered to be *highly permeable* when the extent of absorption in humans is determined to be 90% or more of an administered dose. Approximately 70% of the administered radiolabeled entecavir dose in the mass balance study was excreted as unchanged drug in urine over 14 days of collection, suggesting an estimated extent of absorption ≥ 70%. The applicant has not submitted any data that indicate entecavir is a high permeability drug.

2.5.2. What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial formulation?

The proposed to-be-marketed formulations include a 0.5 mg triangular film-coated tablet, 1.0 mg triangular film-coated tablet, and 0.05 mg/mL oral solution. Formulations used in the four (4) pivotal efficacy studies (studies AI463022, AI463027, AI463014, and AI463026) were 0.1 and 0.5 mg . film-coated clinical tablets and are summarized in the following table; capsules were used in one pivotal study.

Table 2.5.2-1 Entecavir Formulations Used in Pivotal Clinical Trials

Study	Formulation	Batch Numbers
AI463022	0.5 mg white tablets	N01020, N01030, N01032, 8MCE136, 8MDE141, 8MFE193, 2L61047
AI463027	0.5 mg white tablets	N01020, N01028, N01030, 8MCE136, 8MFE193
AI463014	0.1, 0.5, and 1.0 mg capsules	0.1 mg: N99047, N99113 0.5 mg: N99049, N00115 1.0 mg: N00003, N01071
AI463026	0.5 mg white tablets	N01026, N01030, 8MCE136,

The basis of approval for the three formulations are as follows.

- 0.5 mg oral tablet: The 0.5 mg film-coated tablet clinical trial formulation used in multiple pivotal clinical trials was identical to the proposed to-be-marketed 0.5 mg tablet, with the exception of a change in shape. The proposed commercial 0.5 mg film-coated tablet formulation is triangular in shape and identical in composition to the 0.5 mg clinical tablet. The difference in shape (versus triangular) is considered a minor formulation change and does not raise any concerns from a chemistry, manufacturing, and controls perspective. Exposure data for the 1.0 mg triangular tablet and comparative dissolution for the 1.0 and 0.5 mg triangular tablets support the change in shape.

- 1.0 mg oral tablet: The Applicant was granted a biowaiver of in vivo bioequivalence for the 1.0 mg commercial tablet. Rationale for the biowaiver is presented in Section 2.5.2.1. The proposed 1.0 mg commercial tablet is compositionally proportional to the 0.5 mg commercial tablet, and dissolution profiles for the 0.5 mg clinical tablet and the 0.5 and 1.0 mg commercial tablets are comparable.

- 0.05 mg/mL oral solution: Supported by results of bioequivalence study AI463035 (to-be-marketed 0.05 mg/mL solution versus 0.5 mg tablet clinical trial material).

The Applicant conducted three bioequivalence studies in support of the three proposed commercial formulations (0.5 mg triangular film-coated tablet, 1.0 mg triangular film-coated tablet, and 0.05 mg/mL oral solution), as follows.

- AI463034: Bioequivalence Study of Entecavir Tablets Relative to Entecavir Capsules in Healthy Subjects
- AI463035: Bioequivalence Study of Entecavir Oral Solution Relative to Entecavir Tablet in Healthy Subjects
- AI463065: Bioequivalence Study of a Single Entecavir 1.0 mg Tablet Relative to Two Entecavir 0.5 mg Tablets in Healthy Subjects

Formulations used in the three (3) bioequivalence studies conducted for approval (studies AI463034, AI463035, and AI463065) are summarized in the following table.

Table 2.5.2-2 Entecavir Formulations Used in Bioequivalence Studies

Study	Formulation	PIN/Batch Numbers
AI463034	0.5 mg white — film-coated tablets	200475-K0X5-039 (N01024)
	0.5 mg — capsules	200475-R0X5-014 (N00242)
AI463035	0.5 mg white — film-coated tablets	200475-K0X5-039 (N01030)
	0.05 mg/mL oral solution	200475-JX05-058 (8MHE234)
AI463065	2 × 0.5 mg white — film-coated tablets	200475-K0X5-039 (N01030)
	1.0 mg pink triangular film-coated tablets	200475-K001-049 (8MEE101)

The bioequivalence of a single entecavir 0.5 mg — film-coated oral tablet relative to a single entecavir 0.5 mg capsule was investigated in study AI463034. The 0.5 mg entecavir — film-coated tablet formulation was bioequivalent to 0.5 mg entecavir capsule formulation (geometric mean ratios [90%CI] for tablet vs. capsule: Cmax, 1.0186 [0.9535, 1.0881] and AUC, 1.0177 [0.9999, 1.0358]).

The proposed dose for LVD-refractory patients is 1.0 mg, and in the Phase 3 clinical trials 2 × 0.5 mg tablets and capsules were used to provide the 1.0 mg dose. A 1.0 mg tablet is a more desirable formulation because it provides LVD-refractory patients with a lower pill burden and may facilitate treatment adherence. The bioequivalence of a single entecavir 1.0 mg commercial triangular film-coated oral tablet relative to two entecavir 0.5 mg — film-coated tablets was investigated in study AI463065. Following exclusion of an outlier subject from the statistical analysis for the bioequivalence (for further description of the outlier see section 2.5.2.2), the 1.0 mg tablet was bioequivalent to the 2 × 0.5 mg tablets (geometric mean ratios [90%CI] for 1.0 tablet vs. 2 × 0.5 mg tablets: Cmax, 0.906 [0.837, 0.981] and AUC, 0.953 [0.918, 0.990]). A DSI inspection was conducted for study AI463065, and based on inspection findings, the anomolous Period 2 results for the outlier subject may be partially due to incomplete entecavir absorption or failure to ingest the tablets (for further description of the outlier subject, see Section 2.5.2.2.) Aside from the anomalous subject, DSI concluded the data from study AI463065 are not acceptable pending demonstration of reproducibility of the entecavir assay and — stability for — (for further description of the bioanalytical DSI findings, see Section 2.6.3). In conclusion, bioequivalence study AI463065 was not acceptable. The basis of approval for the 1.0 mg triangular, film-coated commercial tablet is supported by composition and dissolution data, as presented in Section 2.5.2.1.

An oral solution formulation of entecavir was developed for use in special populations, such as subjects who require dose modification (ie, pediatric subjects and subjects with renal impairment) and subjects who have difficulty with tablet administration. The bioequivalence of entecavir oral solution relative to the 0.5 mg entecavir — film-coated tablet was investigated in study AI463035. Following exclusion of an outlier subject from the statistical analysis for the bioequivalence (for further description of the outlier see section 2.5.2.1), entecavir 0.5 mg solution formulation is bioequivalent to the entecavir 0.5 mg tablet formulation (geometric mean ratios [90%CI] for oral solution vs. tablet formulations: Cmax, 0.974 [0.920, 1.031] and AUC, 0.968 [0.918, 1.021]). A DSI inspection was conducted for study AI463035, and based on inspection findings, the anomolous Period 2 results for the outlier subject may be partially due to incomplete

entecavir absorption or failure to ingest the tablets (for further description of the outlier subject, see Section 2.5.2.2.) The data from bioequivalence study AI463035 was acceptable and provides basis of approval for the 0.05 mg/mL oral solution.

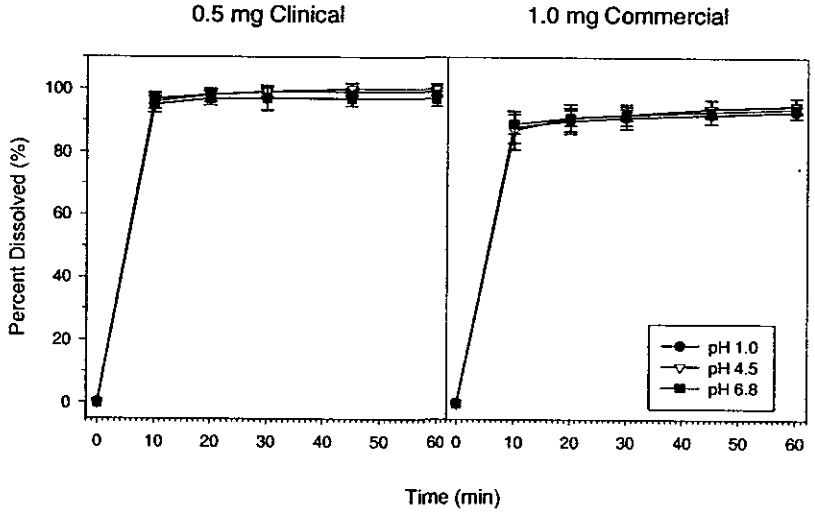
The composition of entecavir oral solution, 0.05 mg/mL, used in the clinical BE study (AI463035) is identical to the proposed commercial formulation except for the solution fill volume.

2.5.2.1. What data support a waiver of in vivo BE data?

The Applicant was granted a biowaiver of in vivo bioequivalence data for the 1.0 mg triangular, film-coated commercial tablet based on the following rationale:

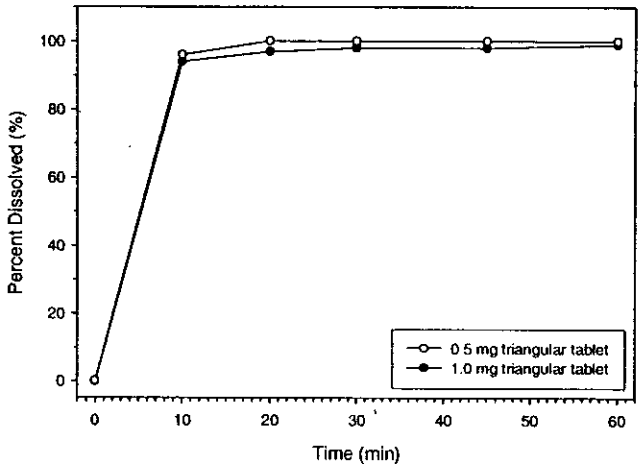
- The 1.0 mg commercial tablet is compositionally proportional to the 0.5 mg clinical trial material and commercial tablets.
- Comparative dissolution information for the 0.5 mg clinical and 1.0 mg commercial tablets suggest similar and rapid dissolution as presented in the following figure:

Figure 2.5.2.1-1 Dissolution Profiles in Three Media for Entecavir Clinical and Commercial Tablet Formulations



- As stated previously, the 0.5 mg , film-coated tablet clinical trial formulation was identical to the proposed to-be-marketed 0.5 mg tablet, with the exception of a change in shape (considered a minor CMC change). Although exposure data for the 0.5 mg triangular film-coated tablet is not available, the 0.5 and 1.0 mg formulations are proportionally similar in composition and dissolution profiles for the two triangular tablet formulations are comparable, as presented in the following figure.

Figure 2.5.2.1-2 Dissolution Profiles for Entecavir Triangular Film-Coated Tablet Formulations



Source: CMC Section 3.2.P

2.5.2.2. What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

Two bioequivalence studies failed to meet the 90% CI using equivalence limits of 80-125% upon initial analysis due to the inclusion of outlier subjects.

- In study AI463065, entecavir plasma concentrations for one subject (Subject 6) were significantly lower in Period 2 (1.0 mg tablet) compared to Period 1 (2 × 0.5 mg tablet). Throughout the concentration-time profile following dosing with the 1.0 mg tablet (Test, Period 2), this subject's plasma concentrations were approximately 5% of the magnitude of the concentrations observed in other subjects following dosing with the 1.0 mg tablet, while the shape of the concentration-time curve was consistent with other subjects' profiles. This subject had the expected exposure to entecavir during the first treatment period following administration of the 2 × 0.5 mg tablet (Reference, Period 1). Carryover from dosing of the 2 × 0.5 mg tablet treatment in Period 1 was negligible. Because of this unexpected finding, all aspects of the study conduct and analysis were reviewed, including subject compliance, bioanalysis of the plasma samples, and release data for the formulations. These aspects of study conduct were deemed unlikely to be the cause of the anomalous results for the study outlier. A review of pharmacokinetic data (Cmax and AUC) from 15 previously conducted Phase I clinical pharmacology studies for entecavir revealed no other subjects with similar magnitudes of exposure, regardless of formulation and demography. When all subjects were included in the statistical analyses, the point estimate of the adjusted geometric means ratios for AUC(0-T) and Cmax indicated that these parameters for the 1.0 mg tablet were approximately 14 and 21% lower, respectively, when compared to 2 × 0.5 mg tablets. However, when the outlier subject was excluded from the statistical analyses, bioequivalence criteria were met for AUC(0-T), AUC(INF) and Cmax. In addition, the Applicant included a bioequivalance analysis using a nonparametric method, with the rationale that the outlier violated the assumption of normality for parametric methods. The

results from the nonparametric analysis of both full and reduced data sets indicate that the 1.0 mg tablet is bioequivalent to the 2 × 0.5 mg tablet, and is consistent with the conclusion based on the parametric analysis after data from the outlier subject was excluded. The lower exposure observed following administration of the 1.0 mg tablet in the outlier subject appear to be anomalous due unexplainable reason(s).

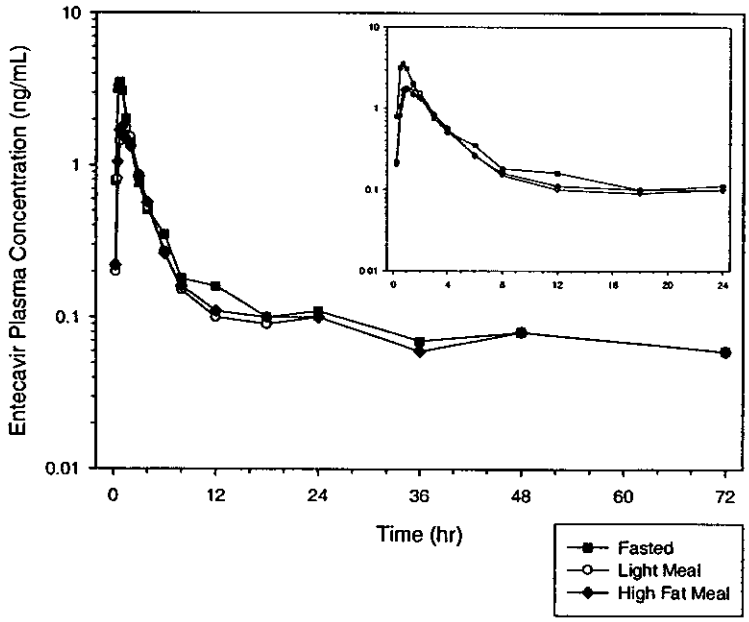
- In study AI463035, for one subject (Subject 1), none of the plasma samples obtained following the 0.5 mg tablet treatment (Period 2) contained quantifiable concentrations of entecavir. This subject had the expected exposure to entecavir during the first treatment period following administration of 0.5 mg entecavir as the solution. In addition, all aspects of the study conduct and analysis were reviewed, including subject compliance, bioanalysis of the plasma samples, and release data/drug product performance for the formulations. These aspects of study conduct were deemed unlikely to be the cause of the anomalous results for the study outlier. The lack of exposure observed following administration of the 0.5 mg tablet in Period 2 in the outlier subject suggests that the subject did not swallow the 0.5 mg entecavir tablet. Exclusion of this subject from the statistical analysis for the bioequivalence assessment demonstrates that the entecavir oral solution is bioequivalent, with respect to both AUC and Cmax, to the entecavir tablet formulation.

Upon DSI investigation of the anomalous results in studies AI463065 and AI463035, the original assays and the confirmatory assays had no detectable flaws in the respective analytical batches. The inspections did not reveal any discrepancies in randomization of treatments or sample handling. There was no documentation of mouth checks at the clinical sites. DSI review of studies AI463065 and AI463035 concluded that the anomalous Period 2 results for Subject 1 in AI463035 and Subject 6 in AI463065 may potentially be due to incomplete entecavir absorption or failure to ingest the tablets. In general, these anomalous results are not indicative of a greater safety or efficacy concern for entecavir.

2.5.3. What is the effect of food on the bioavailability (BA) of entecavir from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The effect of food on entecavir pharmacokinetics is illustrated in the plasma concentration-time profiles below.

Figure 2.5.3-1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 0.5 mg Entecavir in the Fasted and Fed (High Fat and Light Meals) States in Healthy Subjects.



Source: Clinical Study Report AI463016

Following administration of 1.0 mg entecavir in the fed state (either with a light or high fat meal), food decreased to rate and extent of entecavir absorption compared to the 1.0 mg dose administered in the fasted state. The light and high fat meals significantly reduced Cmax by 44 and 46% and AUC by 20 and 18%, respectively. Overall, Tmax values were comparable across treatments. Therefore, the proposed label recommends entecavir be administered on an empty stomach (at least 2 hours before and at least 2 hours after a meal). The recommendation is acceptable.

2.5.4. How do the dissolution conditions and specifications ensure in vivo performance and quality of the product?

The final dissolution method used for the film-coated clinical and commercial tablets of entecavir is presented in the following table. Concurrence on the use of this proposed regulatory method for dissolution testing was obtained from the FDA prior to submission.

Table 2.5.4-1 Dissolution Method for Entecavir Clinical/Commercial Tablets

Strengths	0.5 mg and 1.0 mg
Apparatus	USP, Apparatus 2. Paddles
Medium/Temperature	
Volume	
Speed of Rotation	
Sampling Time	
Brief Description of the Analytical Method	

The proposed dissolution specification for entecavir 0.5 and 1.0 mg tablets is Q = [ ] in 30 minutes. This specification is acceptable.

The solubility of entecavir increases significantly [ ] . The solubility of entecavir between pH [ ] remains unchanged at approximately [ ] Comparative dissolution testing was conducted in three media: [ ] In all three media, the dissolution of the film coated tablets was rapid, as presented in the following table.

Table 2.5.4-2 Comparative Dissolution of Entecavir 0.5 and 1.0 mg Tablets

Time (min)	0.5 mg Film Coated Tablet						1 mg Film Coated Tablet					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	97	1.9	96	2.4	95	2.6	88	5.1	87	6.0	89	3.0
20	98	1.4	98	2.1	97	2.3	90	4.2	91	4.3	91	2.4
30	99	1.5	99	2.0	97	3.9	91	3.5	92	3.4	92	1.8
45	99	1.3	100	1.8	97	2.4	92	2.7	94	2.8	93	1.2
60	99	1.4	100	1.7	97	2.5	93	1.9	95	2.5	94	1.0

[ ] using paddles at [ ], n=12
[ ] using paddles [ ], n=12
[ ] using paddles [ ], n=12

Source: CMC Section 3.2.P

For the dissolution method, sink conditions for evaluating 0.1, 0.5, and 1.0 mg tablets are met using [ ] Complete drug release is achieved using a [ ] paddle speed, [ ] . Thus, the [ ] was selected as the dissolution medium with paddles at [ ]

Dissolution profiles for the tablet and capsule formulations in [ ] of dissolution media using paddles at [ ] or each of the bioequivalence studies (AI463034, AI463035, and AI463065) are presented in the following figures. Symbols represent mean of 6 dosage units ± range.

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✓ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

_____ § 552(b)(5) Draft Labeling

2.5.5. What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?

As described in sections 2.5.2.1 and 2.5.2.2, bioequivalence studies AI463035 and AI463065 are currently under DSI review.

2.6. Analytical Section

2.6.1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Entecavir was measured in plasma, urine, and dialysate using liquid chromatography/ mass spectrometry/mass spectrometry (LC/MS/MS) methods. The rationale for measuring entecavir alone is two-fold. Entecavir plasma concentrations were slightly lower than those for radioactivity in the mass balance study (entecavir was 88% of the Cmax value for radioactivity), suggesting that unchanged entecavir is the predominant circulating moiety in plasma. In addition, entecavir is metabolized to a minor extent, and the metabolites are believed to be inactive glucuronide(s) and a sulfate metabolite(s). Therefore, assays were not developed to quantify entecavir metabolites in the clinical pharmacology studies.

2.6.2. For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Because protein binding of entecavir is low (approximately 13%) and independent of concentration, total entecavir concentrations were measured in the clinical pharmacology studies.

2.6.3. What bioanalytical methods are used to assess concentrations?

Plasma, urine, and dialysate samples from clinical studies were analyzed for entecavir (BMS-200475) using LC/MS/MS methods. The assays were sensitive and specific, using solid phase extraction for sample preparation prior to chromatographic separation and selected reaction monitoring for quantitation. A structural analogue (BMS-180194, lobucavir) was used as the internal standard for all studies. Reversed-phase separations with various gradient elutions were used, necessitated by the selection of separation column or different lots of extraction columns. The same ion transitions were monitored in all analyses for the various studies performed at different laboratories using a variety of mass spectrometers.

Plasma concentrations of lamivudine were measured in two drug-drug interaction studies, AI463010 and AI463058. Lamivudine was measured by LC/MS/MS in the former study and by high performance liquid chromatography with UV detection (HPLC/UV) in the latter study. In both studies, it was demonstrated that there was no quantifiable interference from the presence of entecavir in the matrix. Concentrations of adefovir in plasma and urine were determined in Study AI463063 using an LC/MS/MS assay. Similarly, concentrations of tenofovir in plasma and urine were determined in Study AI463066 using the same LC/MS/MS assay.

Validation of the various bioanalytical methods used for the determination of the concentrations of entecavir, lamivudine, adefovir, and tenofovir in the entecavir clinical pharmacology studies and performance of the validated assays in each of the clinical pharmacology studies are presented in the following tables.

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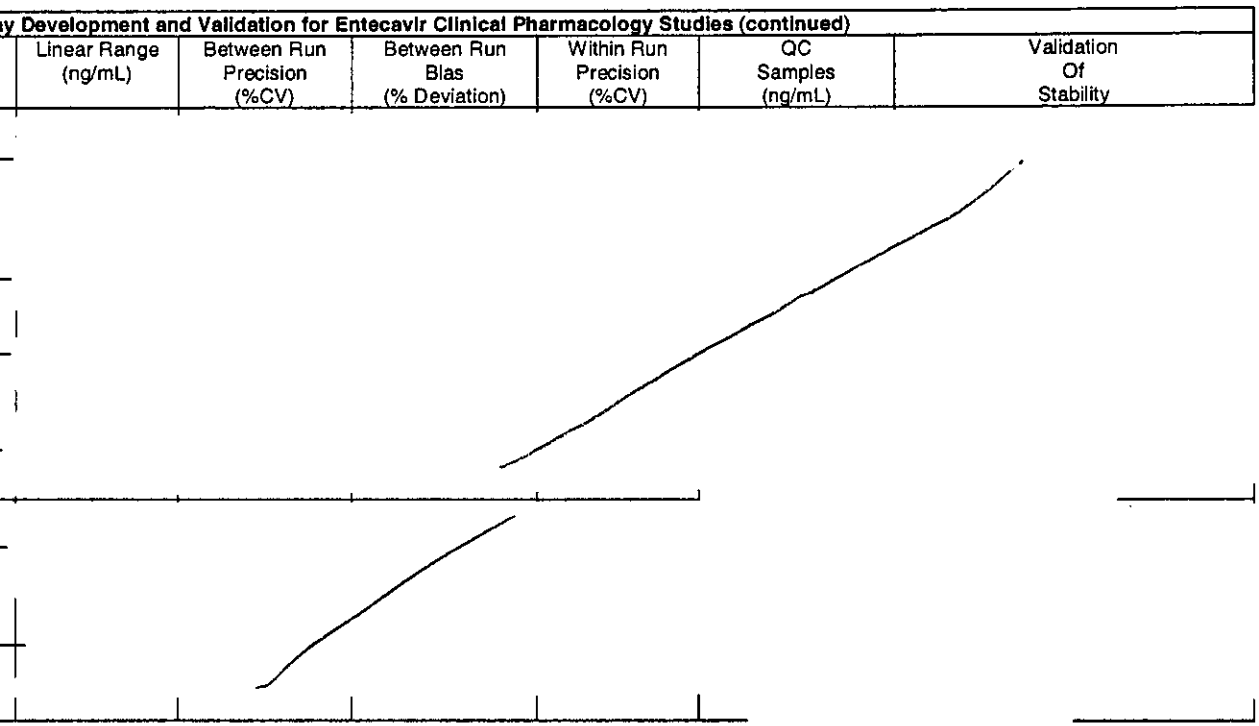
Table 2.6.3-1 Validation of Bioanalytical Methods Used for the Determination of Entecavir, Lamivudine, Adefovir, and Tenofovir Concentrations in the Entecavir Clinical Pharmacology Studies

Assay Development and Validation for Entecavir Clinical Pharmacology Studies									
Matrix	Method	Study	Analyte	Linear Range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	Within Run Precision (%CV)	QC Samples (ng/mL)	Validation Of Stability
Plasma	LC/MS/MS	002 003	Entecavir						
	LC/MS/MS	011 016 021 029 032 034 042	Entecavir						
	LC/MS/MS	033	Entecavir						
	LC/MS/MS	031 035 058 063 066	Entecavir						
	LC/MS/MS	065	Entecavir						
	LC/MS/MS	010	Entecavir						
			Lamivudine						
	HPLC/UV	058	Lamivudine						
	LC/MS/MS	063 066	Adefovir						
			Tenofovir						

DMT Deviation of the mean from theoretical
NR Not reported
RSD Relative standard deviation

Assay Development and Validation for Entecavir Clinical Pharmacology Studies (continued)

Matrix	Method	Study	Analyte	Linear Range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	Within Run Precision (%CV)	QC Samples (ng/mL)	Validation Of Stability
Urine	LC/MS/MS	002 003	Entecavir						
	LC/MS/MS	011 021 029 032 042	Entecavir						
	LC/MS/MS	033	Entecavir						
	LC/MS/MS	031 058 063 066	Entecavir						
	HPLC/UV	058	Lamivudine						
	LC/MS/MS	063 066	Adefovir						
			Tenofovir						
Dialysate	LC/MS/MS	011	Entecavir						



DMT Deviation of the mean from theoretical
NR Not reported
RSD Relative standard deviation

Table 2.6.3-1 Performance of Validated Assays in Entecavir Clinical Pharmacology Studies

Assay Performance in Entecavir Clinical Pharmacology Studies								
Matrix	Method	Study	Analyte	Linear Range (ng/mL)	Within Run Precision (%CV)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	Comments
Plasma	LC/MS/MS	002 003	Entecavir					
	LC/MS/MS	011 016 021 029 032 034 042	Entecavir					
	LC/MS/MS	033	Entecavir					
	LC/MS/MS	031 035** 058 063 066	Entecavir					
	LC/MS/MS	065**	Entecavir					
	LC/MS/MS	010	Entecavir					
			Lamivudine					
	HPLC/UV	058	Lamivudine					
	LC/MS/MS	063	Adefovir					
		066	Tenofovir					

DMT Deviation of the mean from theoretical
NR Not reported
RSD Relative standard deviation

Assay Performance in Entecavir Clinical Pharmacology Studies (continued)								
Matrix	Method	Study	Analyte	Linear Range (ng/mL)	Within Run Precision (%CV)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	Comments
Urine	LC/MS/MS	002	Entecavir					
	LC/MS/MS	011 021 029 032 042	Entecavir					
	LC/MS/MS	033	Entecavir					
	LC/MS/MS	031 058 063 066	Entecavir					
	HPLC/UV	058	Lamivudine					
	LC/MS/MS	063	Adefovir					
		066	Tenofovir					
Dialysate	LC/MS/MS	011	Entecavir					

DMT Deviation of the mean from theoretical
NR Not reported
RSD Relative standard deviation

2.6.3.1. What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

For linear ranges of assays used in each clinical pharmacology study, see tables in section 2.6.3. Standard curves were fitted by both weighted linear and quadratic regression models in the entecavir assays. The Applicant states the range of the assay was generally sufficient to measure entecavir concentrations in plasma, urine, or dialysate for the intended purposes. In general, entecavir plasma concentrations following multiple 0.5 and 1.0 mg doses of entecavir in healthy subjects are well within the established range of linearity for the assays (the highest concentrations observed with the 1 mg dose were < —. For the multiple-dose ranging study AI463002, the standard curves were linear over the concentration range — Multiple doses as high as — were studied in AI463002, and concentrations achieved with doses greater than 5 mg exceeded the linear range.

In the drug interaction study AI463010, both entecavir and lamivudine plasma concentrations in healthy subjects exceeded the established range of linearity for the LC/MS/MS assay used in this study (— ng/mL for entecavir — ng/mL for lamivudine). The second lamivudine interaction study (AI463058), entecavir was measure by a different LC/MS/MS method and lamivudine was measured by HPLC/UV. Entecavir and lamivudine concentrations were within the established range of linearity for the assays in the subsequent study.

Adefovir plasma concentrations in drug interaction study AI463063 were within the established range of linearity for the assay — ng/mL).

Tenofovir plasma concentrations in drug interaction study AI463066 exceeded the established range of linearity for the LC/MS/MS assay used in this study (— ng/mL). The range of maximum plasma concentrations of tenofovir observed in this study was — ng/mL, and approximately 20% of the tenofovir concentrations sampled in this study were — ng/mL. Tenofovir Cmax and AUC values in study AI463066 were comparable to historical data observed following 300 mg QD dosing in HIV-infected patients. Based on this comparison, the impact of the inadequate linear range for the tenofovir assay is likely minimal.

2.6.3.2. What are the lower and upper limits of quantification (LLOQ/ULOQ)?

Lower limits of quantitation of the entecavir LC/MS/MS assays were generally between — — and the upper limits ranged from — ng/mL in plasma. Similar LC/MS/MS procedures applied to the analysis of urine samples and human dialysate had dynamic ranges of — ng/mL and — ng/mL, respectively.

2.6.3.3. What are the accuracy, precision, and selectivity at these limits?

For further information on validation and performance, see section 2.6.3.

2.6.3.4. What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

For information on validated stability conditions, see section 2.6.3.

2.6.3.5. What is the QC sample plan?

For individual QC sample plans for each validated assay, see section 2.6.3.

5 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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cc: HFD-530 /NDA 21-797
/MO/Lewis/Murata
PM/Holloman
HFD-880 /Bergman
HFD-880 /TL/KReynolds

4. APPENDICES

4.1. Individual Study Reviews

4.1.1. General Pharmacokinetics

4.1.1.1. Pharmacokinetics and metabolism of [^{14}C]-entecavir in healthy male subjects (Protocol AI463031).

Objectives:

- Primary: to assess the pharmacokinetics, metabolism, and routes and extent of elimination of a single oral dose of [^{14}C]-entecavir in healthy male subjects.
- Secondary: to assess the safety of a single oral dose of [^{14}C]-entecavir.

Study Design:

This was an open-label, non-randomized, single-dose study in 6 healthy male subjects. Subjects were admitted to the clinical facility the evening prior to dosing (Day -1). On Day 1, subjects received a single oral dose of 1 mg [^{14}C]-entecavir containing approximately 100 μCi of total radioactivity (TRA). Subjects were required to remain in the clinical facility for the duration of the study. Blood was collected for pharmacokinetic and biotransformation analyses at selected time points over a 14-day period. Complete urinary and fecal output was collected over the 14-day period and analyzed for TRA. Subjects were discharged from the clinic on the morning of Day 15 provided that the Day 14 measurement of radioactivity in the urine was ≤ 1% of the administered radioactivity.

Formulations:

Subjects received 1 mg of entecavir containing [^{14}C]-entecavir as an oral solution. The [^{14}C]-entecavir was packaged in 120-mL sealed glass bottles (Batch Number 2D62412; Product Number 200475-ROX5-014-0; Expiration Date 30-Nov-2002). Each multiple-dose bottle contained 12 mg (strength 0.2 mg/mL; approximately 100 μCi/mg) [^{14}C]-entecavir. The actual radioactive dose was 108.1 μCi.

Pharmacokinetic Measurements:

Beginning on Study Day 1, blood (plasma), urine and fecal samples were collected at specified times for the analyses of concentrations of entecavir and/or TRA and for biotransformation analysis.

- Blood samples for plasma entecavir and TRA were obtained predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hours following the radiolabeled entecavir dose.
- Blood samples for biotransformation analysis were obtained predose and 1, 2, 4, 8, and 96 hours following the radiolabeled entecavir dose.
- Urine samples were collected continuously over 24-hour intervals (8-hour intervals on Day 1) throughout the study.
- Fecal samples were collected continuously over 24-hour intervals throughout the study.

Pharmacokinetic/Statistical Analysis:

Single-dose pharmacokinetics of entecavir and TRA were derived from plasma concentration versus time data, as well as urinary and fecal excretion data. Pharmacokinetic parameters were listed and descriptive statistics calculated. Total radioactivity recovered in urine and feces was tabulated and descriptive statistics calculated by collection interval and cumulative over the

entire period of collection. Biotransformation analyses were performed in order to identify major metabolites of entecavir.

Study Population Results:

- Six (6) male subjects enrolled in and completed the study.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463031	
Age (yr)	36 (23 – 42)
Weight (kg)	82.7 (70.5 – 95.9)
Height (cm)	179.4 (167.0 – 190.0)
Body Mass Index (BMI) (kg/m^2)	25.6 (22.6 – 29.7)
Race N (%)	3 White (50%) 3 Black (50%)

* Data presented as mean (range).

Pharmacokinetic Results:

Pharmacokinetic results are summarized in the tables and figures below.

Figure 1 Mean Concentrations of Entecavir (ETV) and Total Radioactivity (TRA) in Plasma Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects (n=6). Upper panel: 0-8 hours, lower panel: 0-336 hours

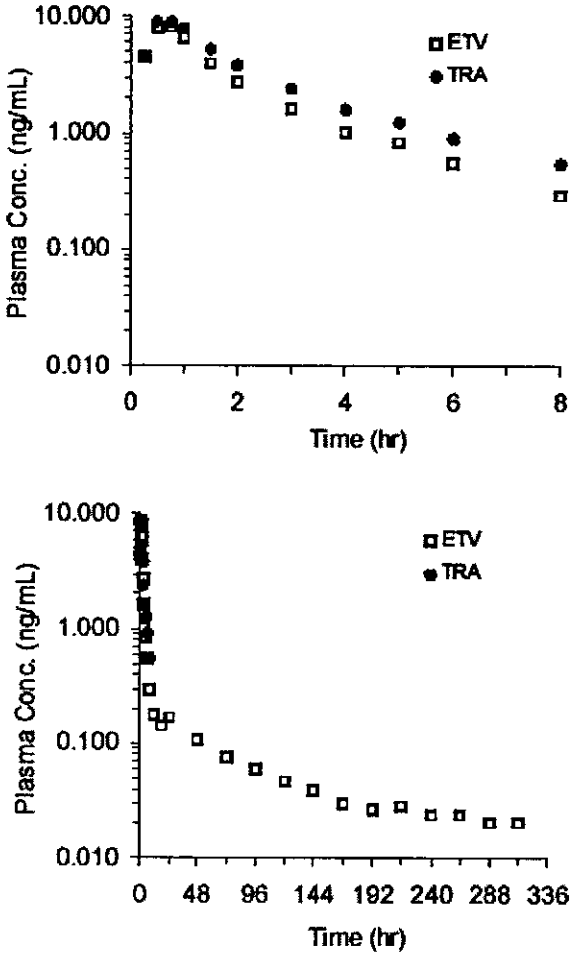


Table 1 Summary of Entecavir and Total Radioactivity (TRA) Plasma Pharmacokinetic Parameters Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects (n=6)

Parameter	Statistic	Entecavir[a]	TRA
C_{max} (µg/mL)[b]	Geometric Mean (%CV)	8.31 (20.8)	9.28 (19.1)
$AUC_{(0-\infty)}$ (ng•h/mL)[b]	Geometric Mean (%CV)	33.29 (17.0)	NR
$AUC_{(0-8)}$[c] (ng•h/mL)[b]	Geometric Mean (%CV)	15.77 (21.7)	20.83 (17.0)
T_{max} (hr)	Median (Min – Max)	0.63	0.63
$t_{½}$ (hr)	Mean (SD)	113.48 (64.5)	NR
Cl_r (mL/min)	Mean (SD)	376.04 (51.7)	NR
UR (%)	Mean (SD)	70.38 (3.9)	NR

NR Not reported
N=6

[a] C_{max}, $AUC_{(0-8)}$, $AUC_{(0-\infty)}$, and %UR were adjusted by a factor of 2 due to only 50% of 1 mg entecavir dose assayed using the LS/MS/MS method. Fifty percent (50%) of the administered entecavir dose contained [^{14}C]-entecavir, which is two mass units higher than non-labeled entecavir. The LC/MS/MS assay method is specific for non-labeled entecavir, therefore, entecavir concentrations and derived relevant pharmacokinetic parameters were multiplied by a factor of 2.

[b] Units for TRA parameters: C_{max}, ng-Equiv/mL and $AUC_{(0-8)}$ and $AUC_{(INF)}$, ng-Equiv•h/mL.

[c] Due to limited sensitivity of the assay method for quantitation of radioactivity (no quantifiable observations after 8 hours), $AUC_{(0-\infty)}$ and half life for radioactivity are not reported.

Figure 2 Mean Cumulative Total Radioactivity (TRA) Recovered in Urine and Feces Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects (n=6)

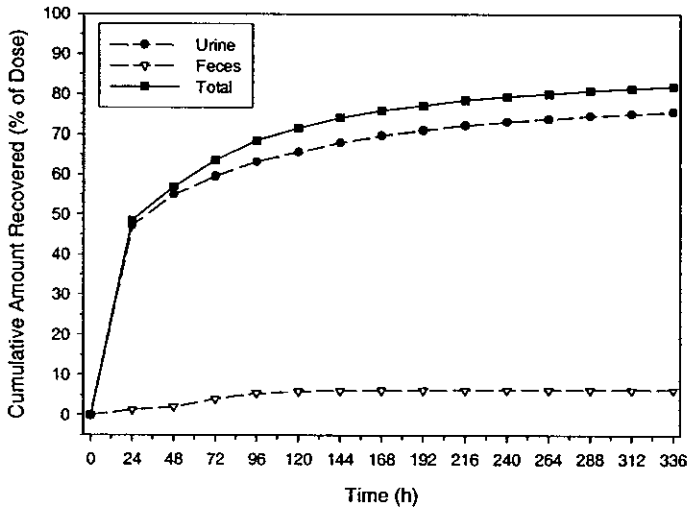


Figure 3 Proposed Pathway for Biotransformation of Entecavir in Human

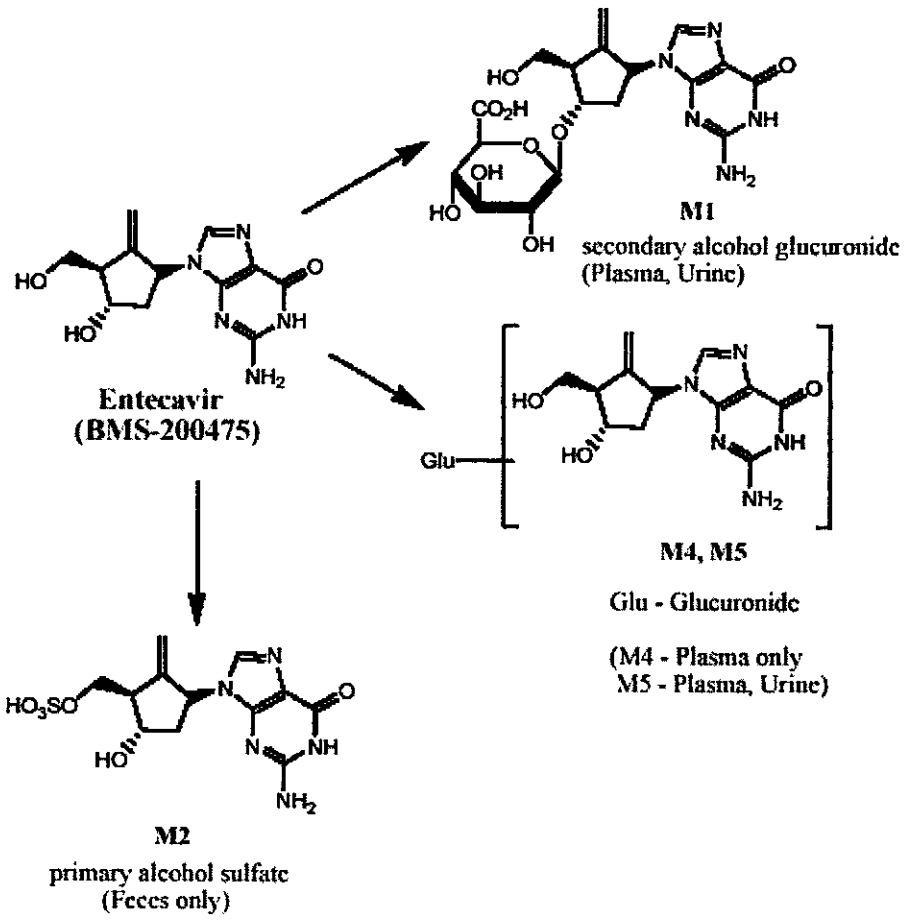


Table 2 Relative Percent Distribution of Radioactivity Among Various Peaks in the Radiochromatographic Profiles of Pooled Human Plasma, Urine, and Feces Following Administration of a Single Dose of 1 mg [^{14}C]-Entecavir in Healthy Male Subjects

Identity	% Relative Distribution of Radioactivity in Pooled Sample			
	Plasma (1 h)	Plasma (2 h)	Urine (0-336 h)	Feces (0-336 h)
M1	8.7	20.1	5.7	-
M2	-	-	-	15.2
M4	4.5	3.8	-	-
M5	6.2	5.2	4.1	-
Parent	79.3	70.3	87.3	66.0
Others*	1.3	0.6	2.9	18.8
Total	100	100	100	100

* Others includes several unidentified peaks, each of which were ≤ 2% of the total radioactivity in that matrix.

Assessment/Conclusion:

- Tmax for entecavir and radioactivity was less than 1 hour following oral administration of 1 mg entecavir as a solution. This finding is similar to observations in previous studies using entecavir capsule formulation, which demonstrated a rapid in vitro dissolution profile.
- Mean plasma TRA and plasma entecavir concentration versus time profiles indicate that concentrations of entecavir were slightly lower than those of TRA, and the geometric mean C_{max} value of 8.31 ng/mL for entecavir was 88% of C_{max} value of radioactivity (9.28 ng-equiv/mL). These findings suggest that unchanged entecavir is the predominant circulating moiety in plasma. This finding is consistent with results from pre-clinical metabolism studies indicating that entecavir was metabolized to only Phase II conjugated metabolites to a minimal extent in different animal species, while no detectable metabolism was observed upon incubation using human liver microsomes, S-9 fraction, or liver slices.
- Over the entire collection period (0 to 336 hours), 75.6% of the dosed radioactivity was recovered in urine and 6.3% was recovered in feces. Approximately 70% of the administered entecavir dose was excreted as unchanged drug in urine over 14 days of collection, suggesting an estimated bioavailability ≥ 70%. These results indicate that elimination of entecavir is predominantly via urinary excretion of unchanged drug, while elimination via biotransformation route(s) is minimal.
- Approximately 18% of the TRA administered was not recovered after 14 days. The fate of the remainder of the entecavir dose remains unclear. The Sponsor speculates that the unrecovered portion may still be retained in the body and was being eliminated slowly due to extensive penetration to a deep compartment or partially due to the uptake of entecavir and/or its metabolites by purine salvage pathways and/or intracellular inter-conversion of entecavir nucleotides (mono-, di-, and triphosphates). The mean urinary recovery for TRA in the last 4 intervals (Days 11 to 14) remained quite steady (~0.5% - 0.8%/day). The Sponsor surmises that this trend would probably continue and the total recovery of TRA would be higher than 82% had urine collection continued beyond Day 14.
- Entecavir was not significantly metabolized in humans after a single oral dose of 1 mg. Entecavir was metabolized in humans to three glucuronide conjugates (M1, M4, and M5), which were the only metabolites detected in plasma and urine samples, and a sulfate conjugate (M2) observed only in feces. No phase I metabolites of entecavir were observed in plasma, urine or feces.

4.1.1.2. Randomized, Double-Blind, Placebo-Controlled, Multiple-Dose, Dose-Escalation Study of the Safety and Pharmacokinetics of BMS-200475 in Healthy Volunteers (Protocol AI463002).

Objectives:

- to evaluate the safety and tolerance of multiple oral doses of BMS-200475; and,
- to assess the pharmacokinetics of BMS-200475 in healthy volunteers.

Study Design:

This was a randomized, double-blind, placebo-controlled, multiple-dose study in 33 normal subjects. Subjects were assigned to one of four dose cohorts, and then randomized in a 3:1 ratio to receive an assigned dose of BMS-200475 (2.5, 5, 10, or 20 mg) or placebo, respectively (six BMS-200475 and two placebo subjects in each dose cohort). For 14 consecutive days

(Days 1-14), subjects received a single oral dose of double-blind study drug. Blood and urine samples were obtained from each subject on Days 1, 7, and 14 and at follow-up visits for pharmacokinetic evaluation. Subjects received study drug on Days 1, 7, and 14 in the fasted state.

Formulations:
BMS-200475 and placebo for oral administration were supplied as identical capsules, as follows:

- BMS-200475 2.5mg (Batch Number N97045)
- BMS-200475 10 mg (Batch Number N97047)
- Placebo (Batch Number N96158)

Pharmacokinetic Measurements:
Blood (plasma) and urine samples were collected at specified times for the analysis of concentrations of BMS-200475.

- Blood samples for plasma BMS-200475 were obtained as follows:
  - Days 1 and 7: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 3-6 and 9-13: prior to dosing (trough samples); and,
  - Day 14: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 (Day 15), 36, 48 (Day 16), 72 (Day 17), 96 (Day 18), 168 (Day 21), and 336 (Day 28) hours following dosing.
- Urine samples were collected over the following intervals relative to drug administration:
  - Day 1: prior to dosing and 0-3, 3-6, 6-12, and 12-24 hours following dosing;
  - Day 7: 0-3, 3-6, 6-12, and 12-24 hours following dosing; and,
  - Day 14: 0-3, 3-6, 6-12, 12-24, 24-48, 48-72, and 72-96 hours following dosing.

Pharmacokinetic/Statistical Analysis:
BMS-200475 plasma concentration versus time data were analyzed via noncompartmental method, and total urinary recovery was assessed. Pharmacokinetic parameters were listed and descriptive statistics calculated. Dose proportionalty was evaluated by ordinary least-square regression, and an analysis of variance was performed on Cmax and AUCtau to assess drug accumulation.

Study Population Results:

- Thirty-three (33) subjects enrolled in the study, 25 randomized to BMS-200475 and 8 to placebo. All subjects were male.
- Thirty-one (31) subjects completed the study. One subject withdrew consent, and one subject discontinued due to adverse event on Day 12.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463002[a]			
Demographic	**BMS-200475 (n=25)**	**Placebo (n=8)**	**Total (n=33)**
Age (yr)	33 (22 – 43)	34 (26 – 41)	33 (22 – 43)
Weight (kg)	78.0 (64.4 – 100.5)	86.4 (74.4 – 96.0)	80.0 (64.4 – 100.5)
Height (cm)	178.3 (163 – 193.5)	180.1 (169.0 – 188.5)	178.7 (163 – 193.5)
Race	11 White (44%) 10 Black (40%) 3 Hispanic (12%) 1 Other[b] (4%)	4 White (50%) 4 Black (50%)	15 White (45.5%) 14 Black (42.4%) 3 Hispanic (9.1%) 1 Other[b] (3%)

[a] Data presented as mean (range).
[b] American Alaskan Native

Pharmacokinetic Results:
Pharmacokinetic results are summarized in the table and figures below.

Figure 1 Mean Concentrations of BMS-200475 in Plasma Following Administration of Multiple Oral Daily Doses of 2.5, 5, 10, or 20 mg BMS-200475 for 14 Days in Healthy Male Subjects. (n=6 per dose group; Inset: 0 to 24 hours)

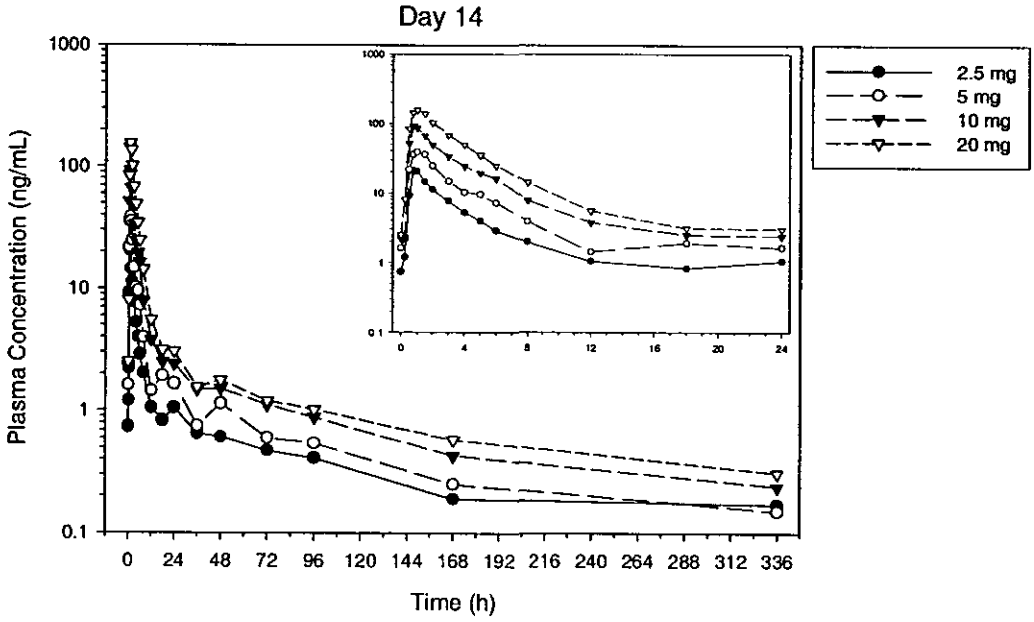


Figure 2 Mean Trough Concentrations of BMS-200475 in Plasma Following Administration of Multiple Oral Daily Doses of 2.5, 5, 10, or 20 mg BMS-200475 for 14 Days in Healthy Male Subjects. (n=6 per dose group)

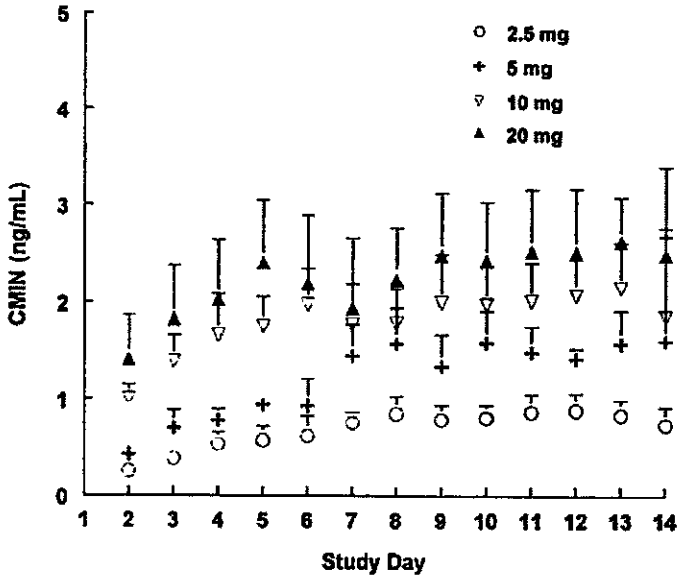
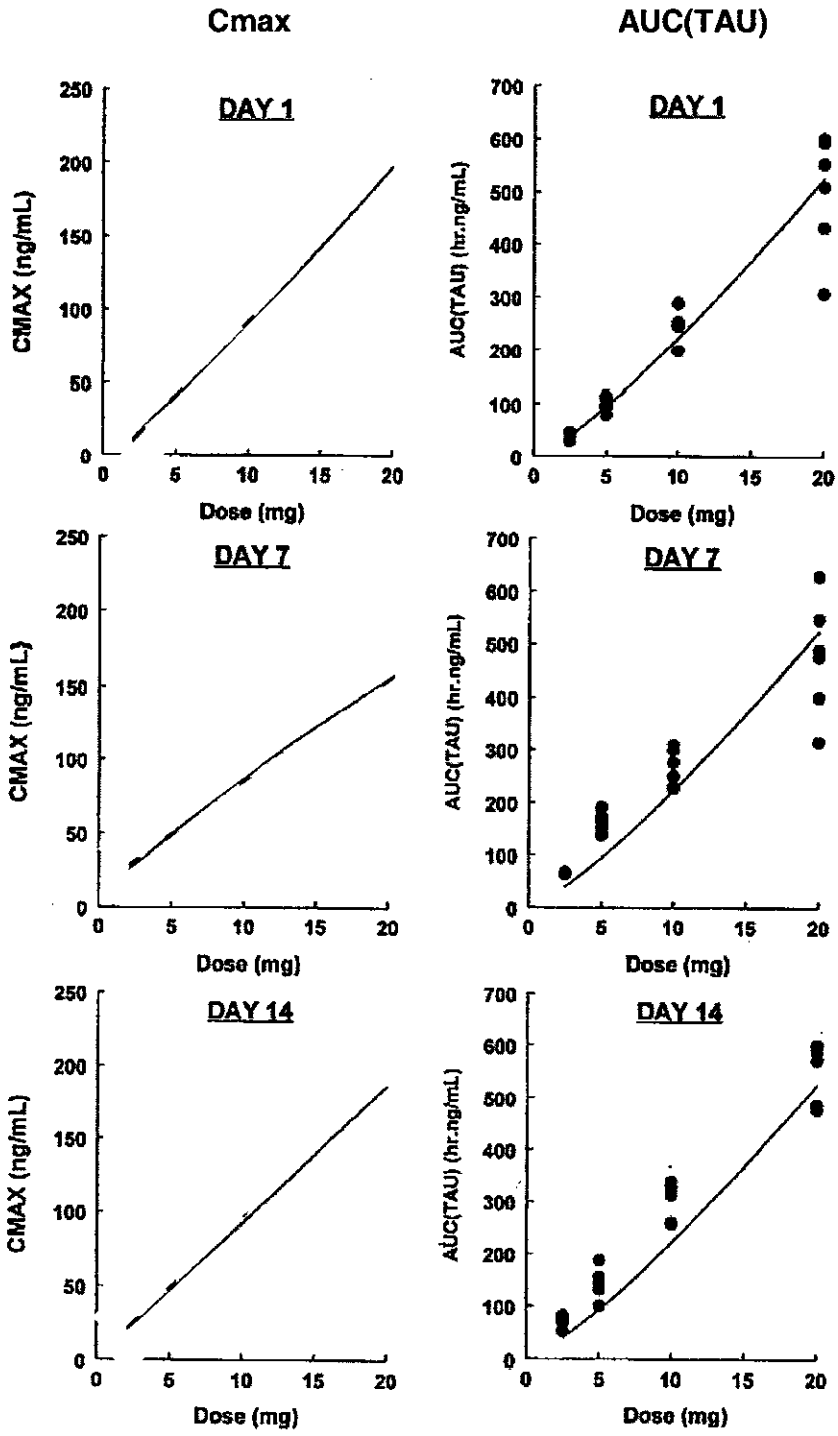


Table 1 Summary of BMS-200475 Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 2.5, 5, 10, or 20 mg BMS-200475 in Healthy Male Subjects. (n=6 per dose group)

Day	Dose (mg)	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC_{tau} (ng•h/mL)	$t_{½}$ (hr)	CL/F (mL/min)	CL_r (mL/min)	Vdβ/F (L)	UR (%)	R
1	2.5	17.3 (5.0)	0.75	38.1 (6.9)	-	-	513 (127)	-	45.2 (5.8)	-
	5	44.8 (12.1)	0.75	98.9 (12.5)	-	-	430 (83)	-	50.6 (9.0)	-
	10	99.4 (12.0)	0.75	247.3 (28.6)	-	-	417 (144)	-	59.8 (13.3)	-
	20	187.0 (54.2)	1.00	500.2 (112.6)	-	-	380 (152)	-	54.8 (17.3)	-
7	2.5	25.2 (3.3)	0.75	65.8 (1.8)	-	-	375 (73)	-	58.9 (10.0)	1.78 (0.32)
	5	60.4 (7.6)	0.88	164.5 (18.5)	-	-	348 (74)	-	67.5 (10.1)	1.67 (0.19)
	10	85.4 (20.5)	0.75	265.1 (34.9)	-	-	469 (39)	-	74.2 (9.3)	1.07 (0.10)
	20	153.7 (14.2)	1.00	476.5 (109.9)	-	-	508 (142)	-	69.7 (10.7)	0.96 (0.07)
14	2.5	22.8 (5.7)	0.75	71.6 (10.3)	115.7 (37.2)	594 (102)	387 (97)	5790 (1390)	64.8 (10.5)	1.94 (0.47)
	5	46.2 (6.4)	0.88	145.8 (28.4)	91.3 (57.9)	592 (127)	403 (92)	4397 (2536)	68.0 (5.7)	1.47 (0.15)
	10	99.9 (13.7)	0.75	304.3 (35.6)	127.5 (41.8)	554 (69)	396 (43)	6176 (2380)	71.7 (5.5)	1.24 (0.13)
	20[b]	179.8 (34.8)	1.00	545.6 (57.9)	142.5 (55.5)	617 (68)	430 (103)	7708 (3219)	69.0 (9.9)	1.04 (0.23)

Data presented as mean (SD).
N=6 unless otherwise specified.
- Not calculated
[a] Data presented as median (minimum, maximum).
[b] N=5

Figure 3 Plots of Cmax and AUC(TAU) Versus Dose Following Administration of Multiple Oral Daily Doses of 2.5, 5, 10, or 20 mg BMS-200475 for 14 Days in Healthy Male Subjects



Symbols represent individual values.
Line represents the best fit model of Cmax or AUC(TAU) = $\alpha Dose^{\beta}$.

Assessment/Conclusion:

- Tmax for BMS-200475 was approximately 1 hour following oral administration of 2.5, 5, 10, and 20 mg BMS-200475 as the capsule formulation.
- Dose proportional increases in Cmax and AUC(tau) were observed following administration of multiple oral daily doses of 2.5, 5, 10, or 20 mg BMS-200475 for 14 days.
- Applicant states that steady-state was achieved by Day 5 after administration of multiple oral daily doses of 2.5, 5, 10, or 20 mg BMS-200475. Based on visual inspection of Cmin data in Figure 2, consistency in Cmin concentrations was not observed until after Day 9. This assessment of steady-state at Day 9 following multiplte dosing of BMS-200475 is confirmed in the multiple dose study AI463033 (please refer to the Clinical Pharmacology review of Study AI463033 for further discussion). No statistical analysis of Cmin concentrations was planned or performed.
- BMS-200475 displayed a terminal half-life of approximately 119 hours. This value was considerably higher than previously reported mean values, due to increased assay sensitivity and duration of quantitation. Upon multiple dosing of BMS-200475, less than 2-fold accumulation was observed, resulting in an effective half-life of ≤ 24 hours.
- BMS-200475 is eliminated primarily as unchanged drug in the urine. Renal clearance was greater than GFR, suggesting tubular secretion plays a significant role in the elimination of BMS-200475.

4.1.1.3. Placebo-Controlled, Ascending Multiple-Dose Study to Evaluate the Safety and Pharmacokinetics of Entecavir in Healthy Subjects (Protocol AI463033).

Objectives:

- Primary: to assess the safety and tolerability of multiple oral doses of entecavir in healthy subjects.
- Secondary: to assess the pharmacokinetics of entecavir following the first oral dose and on Days 7 and 14.

Study Design:

This was a randomized, double-blind, placebo-controlled, dose escalation study in healthy subjects. Eight (8) subjects were assigned to each of 3 sequential panels (0.1 mg, 0.5 mg or 1.0 mg). Within each dose panel, subjects were randomized in a 3:1 ratio to receive entecavir or placebo. Subjects were admitted to the clinical facility the evening prior to dosing (Day -1) and were confined until at least 7 days after their last dose of study drug. On Days 1 to 14 subjects received a once daily oral dose of either entecavir (N = 6) or placebo (N = 2). Subjects were discharged from the study on Day 28. Blood and urine samples were obtained from each subject on Days 1, 7, and 14 for pharmacokinetic evaluation. Subjects received study drug on Days 1, 7, and 14 in the fasted state.

Formulations:

BMS-200475 and placebo for oral administration were supplied as [redacted] capsules packaged in bottles of 25 capsules/bottle, as follows:

Drug	Strength	Formulation	Route	Batch Number	BMS List No	Expiration Date	Description
Entecavir	0.1 mg	capsule	PO	N99047	200475-ROX1-011-0	31-May-2002	—
Entecavir	0.5 mg	capsule	PO	N00242	200475-ROX5-014-0	30-Nov-2001	—
Placebo	—	capsule	PO	N99060	200475-ROOO-003-0	31-May-2003	—

Pharmacokinetic Measurements:
Blood (plasma) and urine samples were collected at specified times for the analysis of concentrations of entecavir.

- Blood samples for plasma entecavir were obtained as follows:
  - Days 1 and 7: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 3-6 and 9-13: prior to dosing (trough samples); and,
  - Day 14: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 (Day 15), 36, 48 (Day 16), 72 (Day 17), 96 (Day 18), 120 (Day 19), 144 (Day 20), 168 (Day 21), and 336 (Day 28) hours following dosing.
- Urine samples were collected up to 24 hours post-dose on Days 1, 7, and 14 over the following intervals relative to drug administration:
  - Prior to dosing (Day 1 only) and 0-3, 3-6, 6-12, and 12-24 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Entecavir pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Summary statistics were tabulated for each of the pharmacokinetic parameters.

Study Population Results:

- Twenty-six (26) subjects were enrolled and randomized to treatment in the study. Of the 26 subjects, 24 (92.3%) subjects completed the study. Both non-completer subjects discontinued due to adverse events (one subject developed catheter-related cellulitis and one subject developed infection secondary to accidental injury).

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463033*	
Age (yr)	35 (21 – 45)
Gender N (%)	26 Males (100%) 0 Females (0%)
Weight (kg)	79.0 (57.7 – 102.2)
Height (cm)	177.0 (163.0 – 191.0)
BMI (kg/m^2)	25.2 (21.7 – 30.3)
Race N (%)	7 White (27%) 13 Black (50%) 4 Hispanic/Latino (15%) 1 Euroasian (4%) 1 Not specified (4%)

Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:
Pharmacokinetic results are summarized in the table and figures below.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily Doses of 0.1, 0.5 or 1 mg Entecavir for 14 Days in Healthy Subjects. (n=6 per dose group)

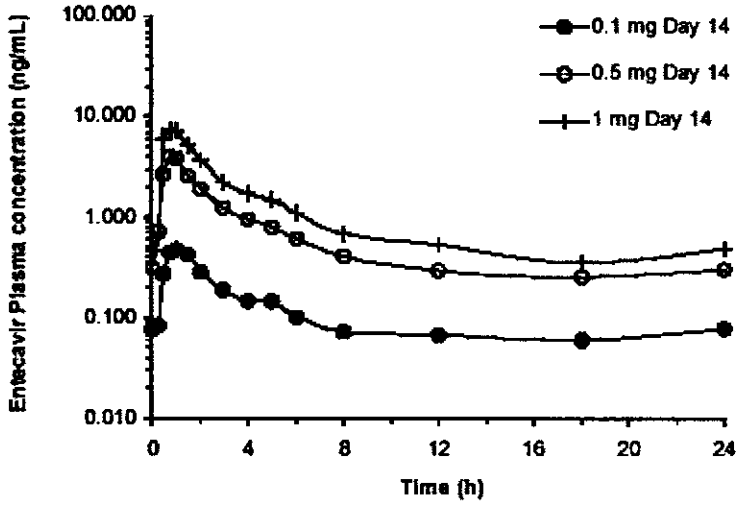


Figure 2 Mean (SD) Trough Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily of 0.1, 0.5 or 1 mg Entecavir for 14 Days in Healthy Subjects. (n=6 per dose group)

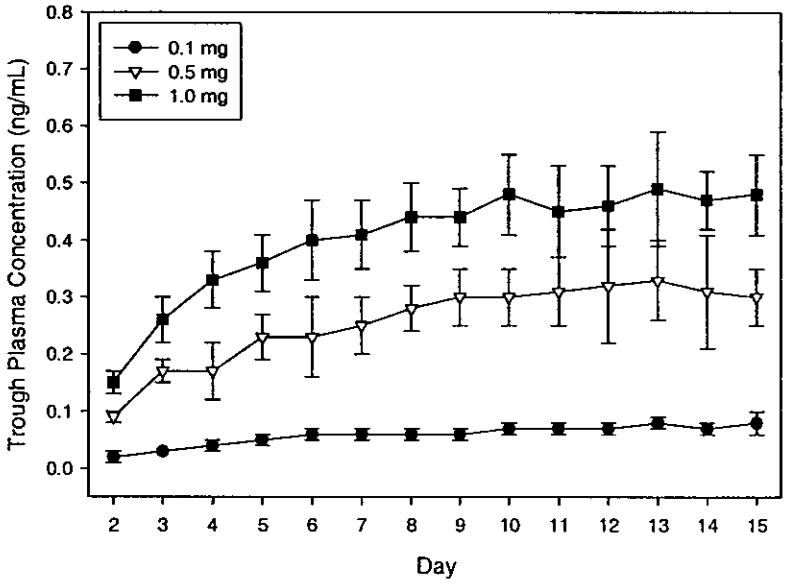
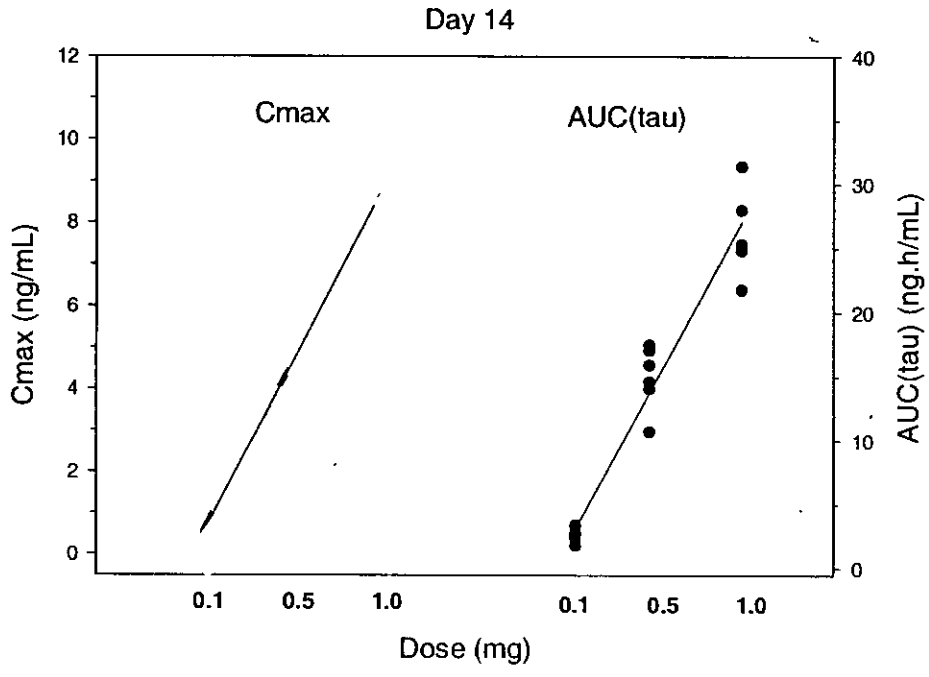


Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 0.1, 0.5, and 1.0 mg Entecavir in Healthy Subjects. (n=6 per dose group)

Day	Dose (mg)	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC_{tau} (ng•h/mL)	$t_{½}$[b] (hr)	CL/F[b] (mL/min)	CL_r[b] (mL/min)	UR[b] (%)	R
1	0.1	0.51 (28%)	0.63	0.94 (23%)	NA	NA	NA	NA	NA
	0.5	3.19 (30%)	0.75	8.22 (18%)	NA	NA	NA	NA	NA
	1.0	6.80 (24%)	0.75	16.37 (14%)	NA	NA	NA	NA	NA
7	0.1	0.65 (24%)	0.75	2.25 (12%)	NA	782.90 (92.47)	499.94 (131.81)	63.45 (12.98)	2.40 (28%)
	0.5	4.30 (20%)	0.88	14.30 (14%)	NA	589.68 (93.28)	429.17 (51.11)	74.47 (14.73)	1.74 (12%)
	1.0	9.08 (21%)	0.75	25.61 (15%)	NA	657.76 (99.91)	482.65 (181.37)	72.30 (17.85)	1.56 (15%)
14	0.1	0.60 (29%)	1.00	2.51 (21%)	127.69 (91.44)	678.03 (148.70)	426.66 (149.26)	62.19 (12.15)	2.67 (19%)
	0.5	4.23 (9%)	1.00	14.78 (17%)	129.90 (17.28)	571.74 (110.76)	360.03 (64.23)	65.22 (16.27)	1.80 (13%)
	1.0	8.24 (16%)	0.75	26.38 (12%)	148.89 (39.50)	636.06 (80.40)	471.36 (138.14)	73.30 (14.66)	1.61 (15%)

Data presented as geometric mean (CV%) unless otherwise specified.
NA Not applicable
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Figure 3 Plots of Cmax and AUC(TAU) Versus Dose Following Administration of Multiple Oral Daily Doses of 0.1, 0.5, and 1.0 mg Entecavir for 14 Days in Healthy Subjects. (n=6 per dose group)



Symbols represent individual values.

Assessment/Conclusion:

- In contrast to the multiple-dose escalation study AI463002, this study assessed the pharmacokinetics of entecavir doses over a range encompassing the clinically relevant therapeutic doses 0.5 and 1.0 mg, for nucleoside naive and nucleoside experienced and/or lamivudine failure HBV infected patients, respectively.
- As observed in the previous multiple dose study AI463002, Tmax for entecavir was approximately 1 hour following oral capsule administration.
- Following multiple doses of entecavir 0.1, 0.5, and 1.0 mg, approximate dose-proportional differences in entecavir Cmax and AUC(tau) were observed over the dose range studied.
- On Day 14, total clearance, renal clearance, terminal half-life, and urinary excretion are comparable to those found in the previous multiple dose study AI463002, suggesting linear kinetics of entecavir over the dose range up to 20 mg. The renal clearance data support the suggestion that tubular secretion plays a significant role in the elimination of entecavir. Upon multiple dosing of entecavir, approximately 2-fold accumulation was observed, resulting in an effective half-life of ≤ 24 hours. This finding is consistent with AI463002. Accumulation ratios appear to decrease with increasing doses.
- Trough entecavir concentrations suggest entecavir reached steady state 10 days after once daily dosing of 0.1-1 mg entecavir. In contrast, the Applicant concluded in the previous multiple-dose study AI463002 that entecavir reached steady-state after 5 days of once daily dosing. Visual inspection of Cmin data from both studies suggests the time to reach steady state for entecavir following once daily dosing is 9 to 10 days. No statistical analysis of Cmin concentrations was planned or performed.

4.1.2. Intrinsic Factors

4.1.2.1. Effects of Age and Gender on the Single Dose-Pharmacokinetics of Entecavir in Healthy Subjects (Protocol AI463442).

Objectives:

- Primary: to assess the effects of age and gender on the single-dose pharmacokinetics of entecavir.
- Secondary: to assess the safety and tolerability of a single dose of entecavir in elderly (≥ 65 years) and young (18 to 40 years) males and females.

Study Design:

This was an open-label, single-dose, 2x2 factorial designed study. Fifty-six (56) subjects were to be enrolled into the following four demographic groups (14 subjects per group): young males (18 to 40 years), elderly males (≥ 65 years), young females (18 to 40 years), and elderly females (≥ 65 years). Subjects were admitted to the clinical facility the evening prior to dosing (Day -1) and received a single oral dose of 1.0 mg entecavir on Day 1 in the fasted state. Blood and urine samples were collected at specified times on Days 1-15 for pharmacokinetic analysis. Subjects were discharged from the study on Day 15.

Formulations:

Entecavir tablets were supplied as 0.5-mg - , white — tablets in bottles of 25 tablets/bottle. The product batch number for entecavir tablets was N01030 and the label batch number was 2F59620. The expiry date of the tablets was 29-Feb-2004.

Pharmacokinetic Measurements:
Blood (plasma) and urine samples were collected at specified times for the measurement of concentrations of BMS-200475.

- Serial blood samples (5 mL) for measurement of plasma BMS-200475 were obtained at pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 12, 18, 24, 36, 48, 72, 96, 144, 216, and 336 hours post-dose.
- Urine samples were collected at pre-dose on Day 1 and on Days 1 to 5 at the following intervals post-dose: 0-6, 6-12, 12-24, 24-48, 48-72, and 72-96 hours.

Pharmacokinetic/Statistical Analysis:
Single-dose PK of orally administered entecavir were derived from plasma concentration versus time and urinary excretion data. Because the protein binding of entecavir is low (approximately 13%), only total plasma concentrations were determined and all derived pharmacokinetic parameters are based on total plasma concentrations. Absence of age or gender effect on Cmax or AUC(INF) was concluded if the corresponding 90% CI for the elderly-to-young ratio of population geometric means was contained within an equivalence interval from 67% to 150% for Cmax or 80% to 125% for AUC(INF). Presence of effect was concluded if the corresponding 90% CI was entirely outside the equivalence interval. These CIs were constructed from the results of the ANOVA with factor for main effects and interactions of age group and gender. Summary statistics were tabulated by age/gender group for all pharmacokinetic parameters.

Reviewer Comment: The Applicant defined "absence of effect" as the 90% CIs for the ratios of geometric means falling between 67-150% for Cmax and 80-125% for AUC(INF). These ranges were based on clinical safety data and PK/PD modeling from previous studies in healthy subjects and patients, which suggested that a 20% reduction in AUC is not expected to have clinical consequences. The wider range for Cmax was established to accommodate the additional variability of that parameter. The presence of an effect was concluded if the corresponding 90% CI was entirely outside the equivalence interval. The rationale supporting the chosen CI ranges is acceptable.

Study Population Results:
A total of 52 subjects were enrolled and randomized in the study. Of the 52 randomized subjects, 51 (98.1%) completed treatment; 1 (1.9%) male in the 18-40 age group (Subject AI463042-1-9, 20 years old) discontinued from the study on Day 4 due to personal reasons.

Demographic and Baseline Characteristics:
The demographic characteristics of each age/gender group were as follows:

Characteristic	Variable	Age/Gender Group (N = 52)			
		Females Aged 18-40 Years (n = 14)	Males Aged 18-40 Years (n = 14)	Females Aged ≥ 65 Years (n = 12)	Males Aged ≥ 65 Years (n = 12)
Age, years	Mean	29	25	69	74
	SD	7	4	4	5
	Range	20-40	20-33	65-79	67-83
Race, n (%)	White	12 (86)	13 (93)	12 (100)	12 (100)
	Black	2 (14)	1 (7)	—	—
Weight, kg	Mean	66.0	74.6	68.1	80.7
	SD	7.4	7.6	7.1	12.1
	Range	57.6-85.5	62.1-91.8	54.5-80.6	65.3-104.4
Height, cm	Mean	167.5	178.3	162.5	178.4
	SD	6.8	5.9	4.6	5.1
	Range	151.1-176.5	167.6-189.2	157.5-170.2	171.5-188.0
Body Mass Index, kg/m^2	Mean	23.8	23.7	26.1	25.5
	SD	2.4	2.3	2.6	2.7
	Range	20.3-27.9	20.2-27.7	21.5-29.9	21.5-29.9
CLcr (measured) mL/min[a]	Mean	122.21	134.69	91.00	110.33
	SD	15.4	26.7	28.1	14.9
	Range	100-150	93-182	36-133	80-135

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 1.0 mg in healthy subjects are presented by age/gender group in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 1.0 mg in healthy subjects are presented by age/gender group and by age and gender separately in Tables 1 and 2, respectively. A summary of the results from the statistical analysis of the effects of age and gender on entecavir PK is presented in Table 3.

Reviewer Comment: Of note, no statistically significant age-gender interactions in any of the analyses were observed, therefore age comparisons are presented pooled across gender groups and gender comparisons are presented pooled across age groups.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects by Age/Gender Group (N=14 per age/gender group)

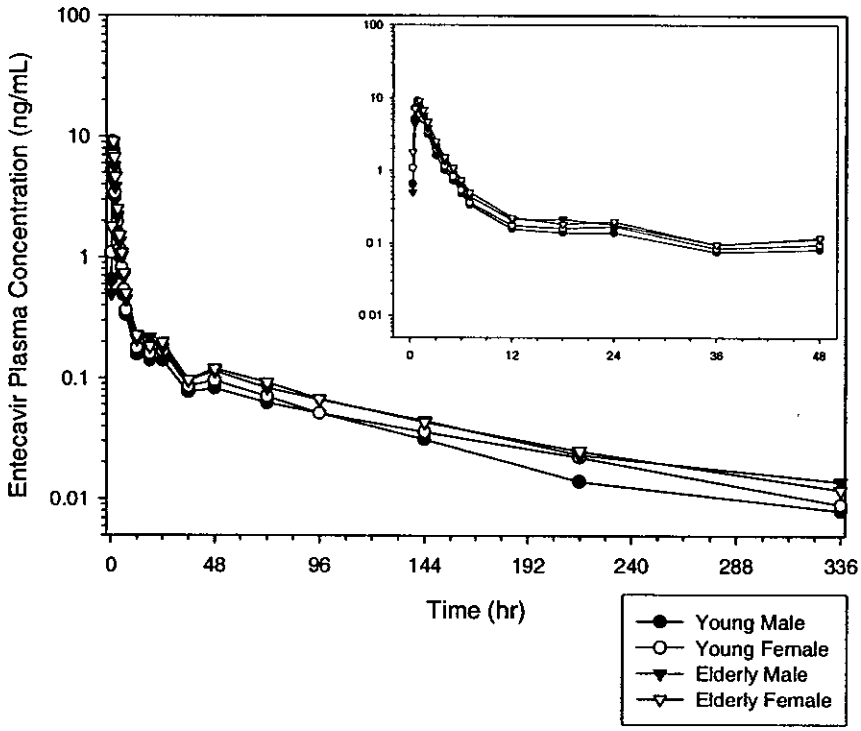


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Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects by Age/Gender Group.

Parameter	Young Male (N=13)	Elderly Male (N=12)	Young Female (N=14)	Elderly Female (N=12)
Cmax (ng/mL)	8.53 (17.5)	8.18 (16.8)	9.50 (27.8)	10.09 (23.5)
AUC(INF) (ng•h/mL)	27.87 (18.2)	37.12 (13.7)	32.71 (14.9)	41.08 (15.9)
AUC(0-T) (ng•h/mL)	27.10 (18.5)	35.08 (12.4)	31.65 (15.1)	41.08 (15.9)
Tmax[a] (hr)	0.75	0.75	0.75	0.75
$t_{½}$[b] (hr)	76.29 (19.7)	111.27 (25.0)	88.50 (14.5)	105.90 (20.6)
CLT/F[b] (mL/min)	606.33 (102.8)	452.69 (60.6)	514.98 (79.2)	410.77 (68.6)
UR[b] (%)	51.98 (11.3)	49.08 (4.6)	59.42 (5.7)	50.06 (12.4)
CLR[b] (mL/min)	385.04 (100.4)	297.91 (59.9)	379.33 (44.4)	265.90 (82.6)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Table 2 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects by Age and Gender.

Parameter	Males (N=25)	Females (N=26)	Young (N=27)	Elderly (N=24)
Cmax (ng/mL)	8.36 (17.0)	9.77 (25.5)	9.02 (24.7)	9.09 (24.0)
AUC(INF) (ng•h/mL)	31.98 (21.0)	36.34 (19.2)	30.28 (17.9)	39.05 (15.6)
AUC(0-T) (ng•h/mL)	30.67 (19.6)	34.97 (18.8)	29.37 (18.0)	37.12 (15.3)
Tmax[a] (hr)	0.75	0.75	0.75	0.75
$t_{½}$[b] (hr)	93.08 (28.3)	96.53 (19.3)	82.62 (18.0)	108.58 (22.6)
CLT/F[b] (mL/min)	532.58 (114.5)	466.88 (90.2)	558.96 (100.9)	431.73 (66.8)
UR[b] (%)	50.59 (8.7)	55.10 (10.4)	55.84 (9.5)	49.57 (9.2)
CLR[b] (mL/min)	343.22 (93.0)	326.98 (85.8)	382.08 (75.1)	281.90 (72.5)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Table 3 Statistical Analysis of Age and Gender Effects on Entecavir Pharmacokinetics in Healthy Subjects.

Parameter for Age Effect Analysis	Adjusted Geometric Mean		Elderly/Young Ratio	
	Young (N=27)	Elderly (N=24)	Point Estimate	90%CI
Cmax (ng/mL)	9.001	9.085	1.009	(0.896, 1.137)
Cmax (ng/mL) Adjusted for CLcr and BW	8.954	9.145	1.021	(0.881, 1.184)
AUC(INF) (ng•h/mL)	30.194	39.052	1.293	(1.201, 1.393)
AUC(INF) (ng•h/mL) Adjusted for CLcr and BW	32.264	36.308	1.125	(1.059, 1.196)
Parameter for Gender Effect Analysis	**Adjusted Geometric Mean**		**Female/Male Ratio**	
	Males (N=26)	**Females (N=25)**	**Point Estimate**	**90%CI**
Cmax (ng/mL)	8.354	9.789	1.172	(1.041, 1.320)
Cmax (ng/mL) Adjusted for CLcr and BW	8.549	9.579	1.120	(0.966, 1.299)
AUC(INF) (ng•h/mL)	32.166	36.657	1.140	(1.058, 1.227)
AUC(INF) (ng•h/mL) Adjusted for CLcr and BW	33.802	34.656	1.025	(0.965, 1.089)

Assessment/Conclusion:

- As the elimination of entecavir in humans is predominantly through urinary excretion, the impact of differences in renal function based on age and gender on entecavir PK were investigated in this study. The ANOVA demonstrated CLcr had a significant effect on AUC(INF) ($p< 0.001$), but not Cmax. Of note, body weight did not have a statistically significant effect on Cmax or AUC(INF).
- Age did not affect entecavir Cmax. In contrast, entecavir AUC(INF) was 29.3% higher in the elderly compared to young subjects, and the upper bound of the 90% C.I. for the elderly-to-young ratio (1.201, 1.393) was above the pre-specified 0.80-1.25 criterion for absence of effect. Adjustment for CLcr and body weight reduced this difference in AUC(INF) to 12.5%, and the resulting 90% C.I. (1.059,1.196) satisfied the criterion for no-effect. These results indicate that the impact of age on the systemic exposure to entecavir is attributable to changes in renal function and/or body weight. The decreased creatinine clearance (21.5%) observed in elderly subjects (mean CLcr of 100.67 mL/min vs. mean CLcr of 128.22 mL/min in young subjects) is expected to have an impact on the total systemic exposure to entecavir since elimination of entecavir depends primarily on urinary excretion. As the differences in body weight between elderly and young subjects was minimal (< 6% for the mean values), its influence on the alteration in systemic exposure is most likely negligible. Ideally, Cmax and AUC(INF) could have been corrected for Clcr and weight separately, as weight is a component of the Cockcroft-Gault formula used for calculation of CLcr in this study. Correcting for both weight and CLcr simultaneously may overcorrect for their effects.
- No clinically significant gender differences in entecavir pharmacokinetics were observed in the current study. The Applicant concluded differences in CLcr and body weight between genders explain the observed numerical differences in exposure between the gender groups. Ideally, Cmax and AUC(INF) could have been corrected for Clcr and weight

separately, as weight is a component of the Cockcroft-Gault formula used for calculation of CLcr in this study. Correcting for both weight and CLcr simultaneously may overcorrect for the effects of these two covariates. Upon further review of the data, no discernable differences between males and females in weight-normalized entecavir CL/T were evident, as the geometric means (CV%) for weight normalized clearance (mL/min/kg) were 6.77 (25%) and 6.88 (22%) for males and females, respectively. In addition, differences in CLcr between males and females were similar to the differences in CLcr observed between the young and elderly [geometric mean (CV%) 120.75 (20%) mL/min in males and 103.61 (25%) mL/min in females, yet corresponding differences in entecavir exposure were not consistent, suggesting differences in CLcr between the males and females may not be the source of the slight pharmacokinetic differences observed in this study.

- Safety results show that the incidence of AEs was more than 6-fold greater in female subjects (19/26, 73.1%) compared to male subjects (3/26, 11.5%). Regarding exposure-response, no clear relationship between measures of exposure (Cmax and AUC(INF)) and the most frequently reported treatment emergent adverse event (TEAE) in females (headache) was discerned. All digestive-related adverse events occurred in the female treatment group but showed no discernable relationship with entecavir exposure.
- Although the effect of age on the PK of drugs that are primarily cleared by renal excretion may warrant dose adjustment, age-related differences in entecavir PK can be accounted for by CLcr, and dosage adjustment of entecavir should be based on the renal function of the patient. Gender differences in entecavir PK do not warrant dose adjustment.

4.1.2.2. Single Dose Phase I Study of Entecavir in Japanese Healthy Volunteers (Protocol AI463021).

Objectives:

- Primary: to evaluate the safety and tolerability of single oral doses of BMS-200475 in Japanese healthy male subjects.
- Secondary: to determine the pharmacokinetics of BMS-200475 following single oral doses in Japanese male subjects.

Study Design:

This was a randomized, double-blinded, placebo-controlled, sequential, ascending single-dose study. Eight subjects were assigned to each of 5 sequential panels: 0.05, 0.1, 0.5, 1.0, and 2.5 mg or matched placebo. For each dose level all subjects received a single oral dose of either BMS-200475 (N = 6) or placebo (N = 2) administered as a capsule formulation in the fasted state. Venous blood and urine samples were obtained from each subject for pharmacokinetic evaluation.

Pharmacokinetic Measurements:

Blood (plasma) and urine samples were collected at specified times for the measurement of concentrations of BMS-200475.

- Venous blood samples for measurement of plasma BMS-200475 were obtained at pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 hours post-dose.
- Urine samples were collected for intervals of 0-3, 3-6, 6-12, 12-24, and 24-48 hours.

Pharmacokinetic/Statistical Analysis:
Entecavir pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Summary statistics were tabulated for each of the pharmacokinetic parameters.

Pharmacokinetic Results:
Pharmacokinetic results are summarized in the table and figure below.

Figure 1 Mean Concentrations of BMS-200475 in Plasma Following Administration of Single Oral Doses of 0.05, 0.1, 0.5, 1.0, and 2.5 mg BMS-200475 in Healthy Japanese Subjects. (n=6 per dose group)

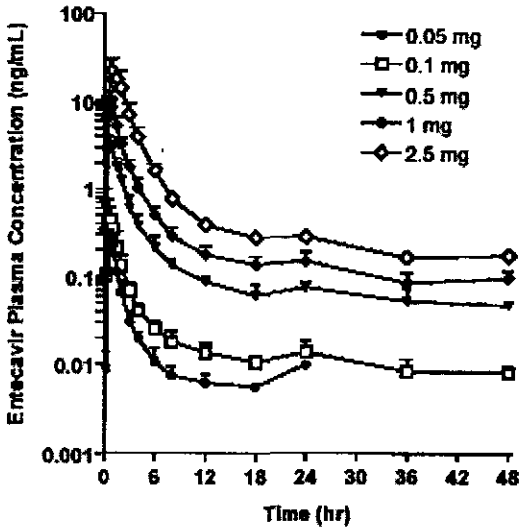


Table 1 Summary of BMS-200475 Plasma Pharmacokinetic Parameters Following Administration of Single Oral Doses of 0.05, 0.1, 0.5, 1.0, and 2.5 mg BMS-200475 in Healthy Japanese Subjects. (n=6 per dose group)

Dose (mg)	Cmax (ng/mL)	Tmax[a] (h)	AUC(0-T) (ng•h/mL)	AUC(INF) (ng•h/mL)	$t_{½}$[b] (h)	CLT/F[b] (mL/min)	CLR[b] (mL/min)	Vdβ/F (L)	UR[b] (%)
0.05	0.29 (31.9)	0.75 —	0.41 (34.4)	0.56 (53.8)	19.82 (21.28)	1488.35 (53.0)	349.56 (21.1)	1346.53 (127.1)	29.49 (5.60)
0.1	0.55 (27.1)	0.63	1.16 (17.7)	1.81 (21.9)	53.31 (27.25)	923.31 (22.0)	534.58 (21.6)	3697.33 (56.9)	37.31 (3.17)
0.5	3.53 (21.5)	0.75 —	8.74 (10.9)	11.36 (10.1)	39.08 (5.06)	733.82 (10.2)	505.59 (11.8)	2466.59 (17.1)	53.29 (5.41)
1.0	9.87 (26.6)	0.75 —	21.86 (22.1)	27.01 (23.3)	38.24 (7.39)	617.16 (23.3)	357.22 (66.4)	2014.93 (22.8)	50.31 (16.21)
2.5	27.29 (13.3)	1 —	61.90 (7.7)	69.84 (9.8)	32.05 (6.98)	596.58 (9.8)	441.06 (12.5)	1624.86 (16.0)	65.83 (6.70)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Assessment/Conclusion:

- Tmax for BMS-200475 was approximately 1 hour following oral capsule administration in healthy Japanese male subjects.
- Following single doses of BMS-200475 0.05, 0.1, 0.5, 1.0, and 2.5 mg in healthy Japanese subjects, greater than dose proportional changes in Cmax and AUC(tau) were observed. Differences in CLT/F across doses did not appear congruent with changes in renal clearance across doses.
- The mean half-life of BMS-200475 in healthy Japanese subjects ranged from 20 to 53 hours. Reported half lives study AI463021 was shorter than previous estimates in healthy subjects. Pharmacokinetics sampling in this study was likely not adequate to characterize the terminal elimination of entecavir (sampling occurred up to 48 hours). Thus, half life and AUC(INF) may be underestimated.
- Elimination of entecavir was predominantly through renal excretion as unchanged drug, through both glomerular filtration and tubular secretion.

4.1.2.3. Placebo-Controlled, Ascending Multiple-Dose to Evaluate the Safety and Pharmacokinetics of Entecavir (BMS-200475) in Japanese Healthy Male Subjects (Protocol AI463029).

Objectives:

- Primary: to assess the safety and tolerability of multiple oral doses of BMS-200475 in Japanese healthy male subjects.
- Secondary: to determine the pharmacokinetics of BMS-200475 following the multiple oral doses in Japanese male subjects.

Study Design:

This was a randomized, double-blinded, placebo-controlled, sequential, ascending multiple-dose study. Eight subjects were assigned to each of 3 sequential panels: 0.1, 0.5, and 1.0 mg or matched placebo. For each dose level all subjects received once daily oral doses of either BMS-200475 (N=6) or placebo (N=2) administered as a capsule formulation for 14 days in the fasted state. Blood and urine samples were obtained from each subject on Days 1, 7, and 14 for pharmacokinetic evaluation.

Pharmacokinetic Measurements:

Blood (plasma) and urine samples were collected at specified times for the measurement of concentrations of BMS-200475.

- Venous blood samples for measurement of plasma BMS-200475 were obtained as follows:
  - Days 1 and 7: pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours post-dose;
  - Day 14: pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, 72, 96, and 168 hours post-dose.
- Urine samples were collected for intervals of 0-3, 3-6, 6-12, 12-24, and 24-48 hours on Days 1, 7, and 14.

Pharmacokinetic/Statistical Analysis:

Entecavir pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Summary statistics were tabulated for each of the pharmacokinetic parameters.

Pharmacokinetic Results:
Pharmacokinetic results are summarized in the table and figures below.

Figure 1 Mean Concentrations of BMS-200475 in Plasma Following Administration of Multiple Oral Daily Doses of 0.1, 0.5 or 1 mg BMS-200475 in Healthy Japanese Subjects. (n=6 per dose group)

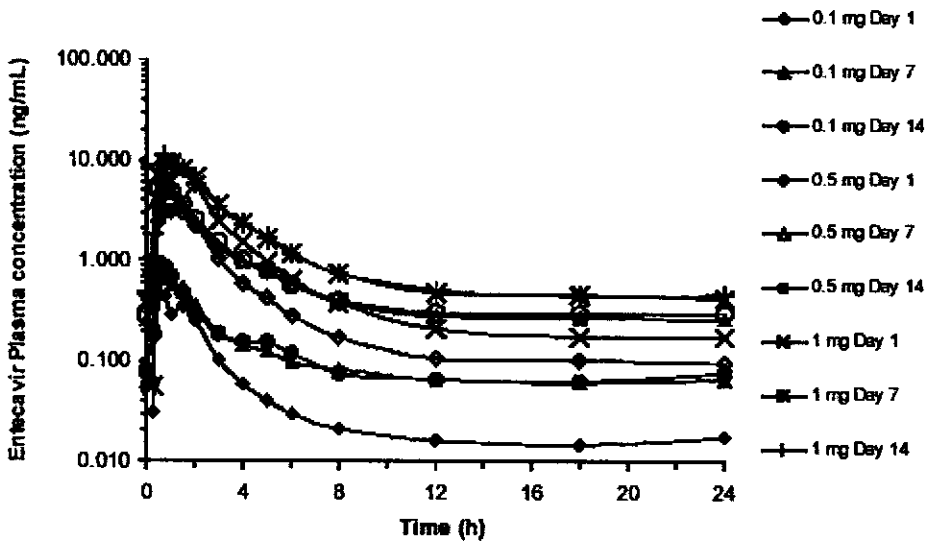
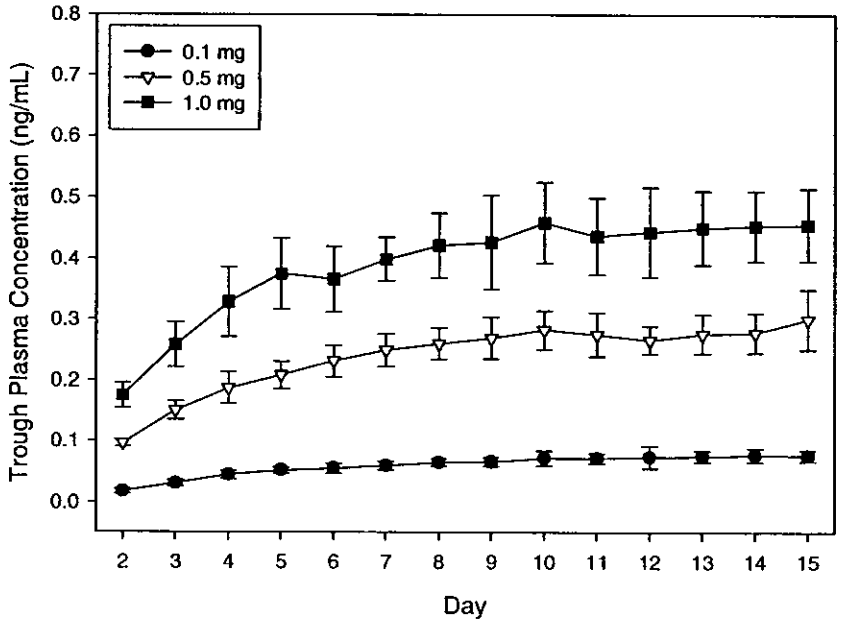


Figure 2 Mean (SD) Trough Concentrations of BMS-20475 in Plasma Following Administration of Multiple Oral Daily of 0.1, 0.5 or 1 mg BMS-200475 for 14 Days in Healthy Japanese Subjects. (n=6 per dose group*)



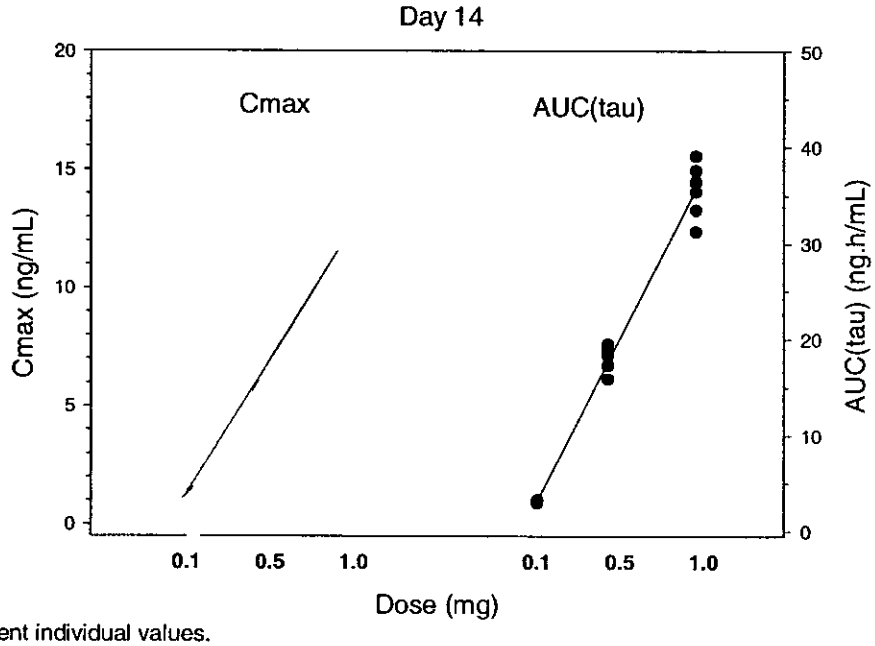
* Study Days 2 - 10, 1.0 mg dose group n=7 due to one replacement subject. Study Days 8 – 15, 0.1 mg dose group n=5 due to one discontinuation.

Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 0.1, 0.5, and 1.0 mg Entecavir in Healthy Japanese Subjects. (n=6 per dose group)

Day	Dose (mg)	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC(TAU) (ng•h/mL)	T-HALF[b] (hr)	CLT/F (mL/min)	CLR (mL/min)	UR[b] (%)
1	0.1	0.56 (39.2)	1.13 —	1.17 (22.7)	NA	NA	347.09 (18.1)	24.42 (4.33)
	0.5	4.49 (21.1)	1.13 —	10.07 (11.5)	NA	NA	318.50 (19.0)	39.00 (6.71)
	1.0[c]	10.14 (22.2)	1.00 —	24.09 (12.1)	NA	NA	365.66 (5.9)	53.06 (5.2)
7	0.1[d]	0.78[e] (20.9)	1.25[e] ~	2.73 (9.4)	NA	610.98 (9.4)	380.73 (15.5)	63.95[e] (5.44)
	0.5	6.49 (22.9)	0.75 ~	16.97 (8.8)	NA	491.27 (8.8)	364.84 (14.6)	74.45 (5.72)
	1.0[c]	12.15 (24.7)	1.50 —	34.57 (8.6)	NA	488.14 (11.0)	382.22[f] (11.0)	78.32[f] (11.02)
14	0.1[d]	0.95 (29.8)	0.75 —	2.98 (4.7)	91.54[g] (27.37)	559.23 (4.7)	396.45 (9.3)	71.05 (5.27)
	0.5	6.43 (34.8)	0.63 ~	17.78 (7.4)	96.60 (20.26)	468.67 (7.4)	372.05 (17.1)	79.80 (8.62)
	1.0	11.64 (19.7)	0.75 —	35.42 (8.1)	83.34 (19.02)	470.48 (8.1)	366.35 (8.8)	77.95 (3.81)

Data presented as geometric mean (CV%) unless otherwise specified.
NA Not applicable
[a] Data presented as median (minimum, maximum).
[b] Data presented as arithmetic mean (SD).
[c] n=7 (including the data for a subject discontinued on Day 10)
[d] n=5 (one subject discontinued on Day 7)
[e] n=6 (including the data from a discontinued subject until 18 hours after dosing on Day 7)
[f] n=6 (UR of a discontinued subject was not calculated)
[g] n=4 (half life for one subjects was not calculated because plasma concentrations of terminal phase did not decline in a log-linear manner)

Figure 3 Plots of Cmax and AUC(TAU) Versus Dose Following Administration of Multiple Oral Daily Doses of 0.1, 0.5, and 1.0 mg BMS-200475 for 14 Days in Healthy Japanese Subjects. (n=6 per dose group*)



Symbols represent individual values.

Assessment/Conclusion:

- Tmax for BMS-200475 was approximately 1 hour following oral administration in healthy male Japanese subjects.
- The data suggests that Cmax and AUC increased approximately dose proportionally in the dose range of 0.5 to 1.0 mg. The increase in Cmax and AUC was slightly more than dose proportional in the dose range of 0.1 to 0.5 mg. Based on a comparison of AUCtau following 14 days of daily administration, exposure to BMS-200475 was greater in Japanese subjects versus US subjects, with mean AUCtau values of 35.42 and 26.38 ng·h/mL, respectively.
- The terminal plasma half-life of BMS-200475 on Day 14 was approximately 90 hours. Steady state was reached 8-10 days after daily single doses of BMS-200475, with approximately 2-fold accumulation of BMS-200475 exposure upon multiple dosing. These findings were similar to those with US healthy subjects.
- Elimination of entecavir was predominantly through renal excretion as unchanged drug, which involved both glomerular filtration and tubular secretion.

4.1.2.4. The Safety, Tolerability and Pharmacokinetic Study of Entecavir in Healthy Male Volunteers in China (Protocol AI463018).

Objectives:

- Part A: To assess the safety and tolerability of a single 0.05, 0.1, 0.5, or 1.0 mg dose of oral entecavir in healthy male Chinese subjects.
- Part B:
  - Primary: To assess the pharmacokinetics (PK, as measured in plasma) of a single 0.05, 0.1, 0.5, or 1.0 mg dose of oral entecavir in healthy male Chinese subjects.
  - Secondary: To assess the safety and tolerability of a single 0.05, 0.1, 0.5, or 1.0 mg dose of oral entecavir in healthy male Chinese subjects.
- Part C:
  - Primary: To assess the safety and tolerability of multiple once-daily oral doses of 0.05, 0.1, 0.5, or 1.0 mg entecavir in healthy male Chinese subjects.
  - Secondary: To assess the PK of a single oral dose followed by multiple once-daily oral doses of entecavir in healthy male Chinese subjects at doses of 0.05, 0.1, 0.5, or 1.0 mg.

Study Design:

Part A:

Part A was a randomized, double-blind, placebo-controlled, single ascending dose study in healthy male Chinese subjects. Subjects were randomized to one of 4 dose groups of 8 subjects each, to receive in an ascending fashion, single doses of 0.05, 0.1, 0.5, or 1.0 mg of entecavir or matched placebo (randomized 3:1). Escalation to the next dose panel occurred following a review of the clinical adverse events and clinical laboratory data collected from a given dose level by the Principal Investigator in consultation with the BMS Medical Monitor. If a dose was found to be safe and well tolerated, then the succeeding panel of 8 subjects was randomized to the next higher dose of entecavir (N = 6) or placebo (N = 2). Subjects were discharged from the study center on Day 3 and from the study after follow up on Day 9 ± 1.

Part B:

Part B was a randomized, open-label, 4-treatment, 4-period, crossover study in healthy male Chinese subjects. Sixteen (16) subjects received four doses (0.05, 0.1, 0.5, and 1.0 mg) of entecavir according to the randomization schedule generated prior to the start of the study. One

dose was studied during each period. No placebo was administered. A washout period of at least 7 days separated each dose. Entecavir was administered on the morning of Day 1 with approximately 200 mL of water. Each subject was observed in the clinical study unit for 72 hours after dosing, at which point they were furloughed from the unit on Day 4. Subjects returned to the unit on Day 7 to begin the next period of the study, and to receive the next dose to which they were randomized. After administration of the last dose, they returned on Day 10 ± 1 for follow up. Blood was collected in each period for plasma PK analysis.

Part C:
Part C was a randomized, double-blind, placebo-controlled, multiple-dose study. Twenty (20) subjects were randomized in a 4:4:2 ratio to receive either entecavir 0.1 mg, entecavir 0.5 mg, or placebo, respectively, as a single dose on Day 1 followed by a 72-hour washout period. On Day 4, subjects commenced dosing once daily for 9 days to reach steady-state, and were furloughed from the unit on Day 15, 72 hours after administration of the last dose. Subjects returned on Day 19 for follow up evaluations. Blood for plasma PK analysis was collected.

Formulations:
Entecavir and placebo for oral administration were supplied as — , capsules, as follows:

Drug	Strength	Batch Number	Label Batch	BMS List No.	Expiration Date
Placebo	—	N99060	N99060	200475-R000-003-0	31-May-2004
Entecavir	0.05 mg	N99110	N99110	200475-RX05-019-0	30-Sep-2001
Entecavir	0.1 mg	N98071	N98071	200475-R0X1-011-0	31-May-2003
Entecavir	0.5 mg	N99049	N99049	200475-R0X5-014-0	30-Nov-2003

Pharmacokinetic Measurements:
Blood samples were collected at specified times for the measurement of plasma entecavir concentrations, as follows:

- Part B: pre-dose and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48 and 72 hours post-dose of each period;
- Part C:
  - Day 1: pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours post-dose;
  - Days 6 to 11: pre-dose and 1.5 hours after dosing (trough and peak)
  - Day 12: pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48 and 72 hours post-dose.

Pharmacokinetic/Statistical Analysis:
Entecavir PK parameters were derived from plasma concentration versus time data via noncompartmental methods. The distributions of the pharmacokinetic variables were summarized by dose for Part B, and study day and dose for Part C. Simple summary statistics were calculated and reported for the PK parameter values.

Study Population Results:
Part A:

- Thirty-two (32) subjects were enrolled and completed the study. Twenty-four (24) received entecavir (6 subjects in each dose group) and 8 subjects received placebo. All subjects were Chinese males.

Part B:

- Sixteen (16) subjects were enrolled and 15 completed all periods of this study. One (1) subject discontinued due to an adverse event, a moderate elevation in ALT, after receiving 2 doses of entecavir. The other 15 subjects received 4 doses of entecavir (0.05, 0.1, 0.5, and 1.0 mg) in a crossover fashion according to the pre-specified randomization sequence. All subjects were Chinese males.

Part C:

- Twenty (20) subjects were enrolled and completed the study. Eight (8) subjects each were randomized to receive either 0.1 or 0.5 mg entecavir, and 4 to receive placebo. All subjects were Chinese males.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463018*			
Demographic	Part A	Part B	Part C
Age (yr)	25 (18 – 44)	24 (20 – 36)	24 (20 – 37)
Weight (kg)	60.4 (51 – 81)	64.5 (52.5 – 77)	67.4 (59 – 80)
Height (cm)	171 (157 – 185)	171.9 (160 – 182)	173.6 (158 – 182)
BMI (kg/m^2)	20.63 (18.21 – 25.56)	21.97 (19.96 – 24.58)	22.34 (19.71 – 24.90)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following administration of single oral doses of 0.05, 0.1, 0.5, or 1.0 mg entecavir in healthy male Chinese subjects in Part B of the study are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of single oral doses of 0.05, 0.1, 0.5, or 1.0 mg entecavir in healthy male Chinese subjects in Part B of the study are summarized in Table 1. Mean concentration-time profiles for entecavir in plasma following administration of single and multiple oral doses of 0.1 and 0.5 mg entecavir in healthy male Chinese subjects in Part C of the study are presented in Figure 2. Mean trough concentrations of entecavir in plasma following administration of multiple oral daily doses of 0.1 and 0.5 mg entecavir in healthy male chinese subjects in Figure 3. Entecavir plasma pharmacokinetic parameters following administration of single and multiple oral doses of 0.1 and 0.5 mg entecavir in healthy male Chinese subjects in Part C of the study are summarized in Table 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Single Oral Daily Doses of 0.05, 0.1, 0.5, or 1.0 mg Entecavir in Healthy Male Chinese Subjects. (n=16 per dose group)

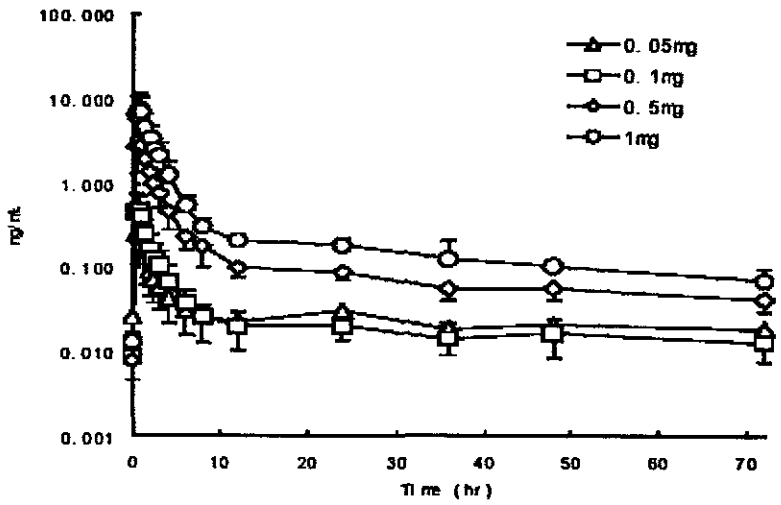


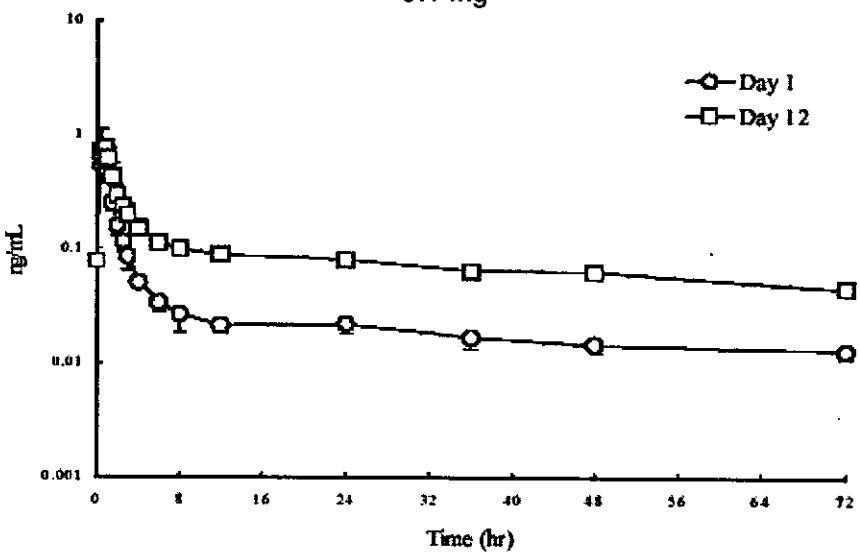
Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Single Oral Daily Doses of 0.05, 0.1, 0.5, or 1.0 mg Entecavir in Healthy Male Chinese Subjects. (n=16 per dose group)

Pharmacokinetic Variable	0.05 mg mean (± SD) (N=16)	0.1 mg mean (± SD) (N=16)	0.5 mg mean (± SD) (N=16)	1.0 mg mean (± SD) (N=16)
Cmax (ng/ml)	0.292 ±0.114	0.607 ±0.214	3.592 ±0.969	9.161 ±2.658
Tmax[a] (h)	0.63	0.75	0.75	0.75
AUC(0-24) (ng•h/ml)	0.997 ±0.389	1.312 ±0.373	8.469 ±1.863	20.054 ±3.896
AUC(0-T)[b] (ng•h/ml)	1.891 ±0.983	2.049 ±0.649	11.189 ±2.299	25.291 ±4.173
T-half (h)	157.47 ±115.29	91.56 ±52.74	52.52 ±22.26	39.29 ±11.55

[a] Median (minimum, maximum)
[b] T = 72 hours

Figure 2 Mean Concentrations of Entecavir in Plasma Following Administration of Single and Multiple Oral Daily Doses of 0.1 and 0.5 mg Entecavir in Healthy Male Chinese Subjects. (n=8 per dose group)

0.1 mg



0.5 mg

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ON ORIGINAL

Figure 3 Mean Trough Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily of 0.1 and 0.5 mg Entecavir in Healthy Male Chinese Subjects. (n=8 per dose group)

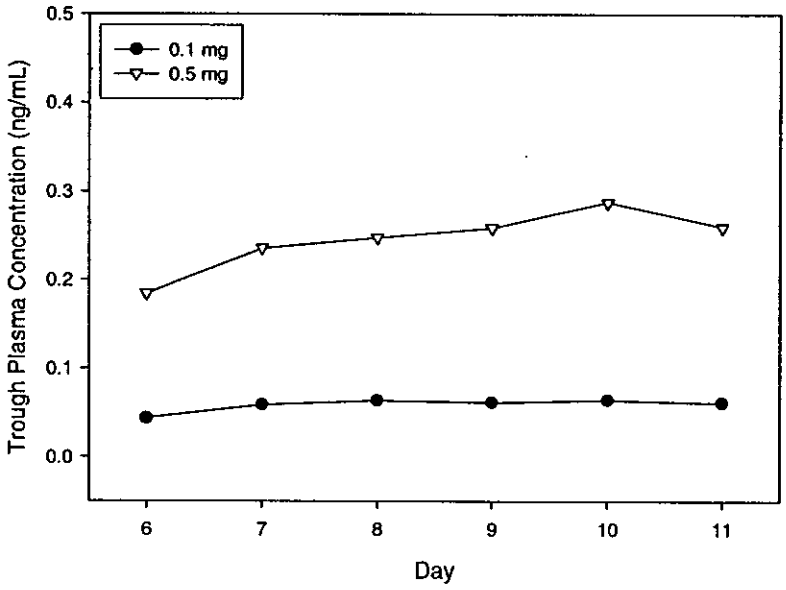


Table 2 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Single and Multiple Oral Daily Doses of 0.1 and 0.5 mg Entecavir in Healthy Male Chinese Subjects. (n=8 per dose group)

	0.1 mg (N=8)		0.5 mg (N=8)	
	Day 1	Day 12	Day 1	Day 12
Cmax (ng/ml)	0.629 ±0.088	0.883 ±0.208	3.627 ±1.026	6.359 ±1.426
Tmax[a](h)	0.75	0.75	1.00	0.75
AUC(TAU)[b] (ng.hr/ml)	1.374 ±0.153	3.269 ±0.379	9.791 ±1 822	17.422 ±2.826
T-half (h)	80.62 ±25.04	70.31 ±33.42	42.98 ±14.42	53.77 ±13 80
AI[c]	-	2 39 ±0.24	-	1 80 ±0.214

Data presented as arithmetic mean ±SD unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] TAU = 24 hours.
[c] AI = accumulation index; i.e. AUC(TAU) on Day 12/AUC(TAU) on Day 1

Assessment/Conclusion:

- Tmax for entecavir was approximately 1 hour following oral administration in healthy male Chinese subjects.
- Following single doses of 0.05, 0.1, 0.5, or 1.0 mg entecavir in healthy male chinese subjects, Cmax, AUC(0-T) and AUC(0-24) appeared to increase somewhat more than proportionally with dose, but regression analysis showed a linear relationship with dose for all three parameters. Based on a comparison of AUCtau following multiple dose administration of 0.5 mg, exposure to BMS-200475 was greater in Chinese subjects versus US subjects, with mean AUCtau values of 17.42 and 14.78 ng·h/mL, respectively. Similar exposures were observed in Chinese and Japanese subjects.
- The data suggests that Cmax and AUC increased approximately dose proportionally upon multiple dosing in the dose range of 0.5 to 1.0 mg.
- Following daily oral doses of entecavir of 0.1 mg and 0.5 mg, steady state was achieved by about 5 - 6 days. Entecavir accumulation at steady state was approximately 2-fold. Both time to steady state and accumulation index are consistent with an effective half-life of approximately 24 hours.
- The terminal plasma half-life of BMS-200475 on Day 14 was approximately 90 hours. Half-life was long relative to the duration of the sample collection (72 hours) and carry-over was observed following multiple periods of single dosing. The half-life of entecavir was most likely not well estimated in this study and should be interpreted with caution.

4.1.2.5. Single Dose Pharmacokinetics and Safety of Entecavir in Subjects with Renal Function Impairment (Protocol AI463011).

Objectives:

- Primary: to assess the effects of selected degrees of renal impairment on the single dose pharmacokinetics (PK) of entecavir.
- Secondary: (1) to assess the safety and tolerability of a single, oral dose of entecavir in subjects with renal impairment; (2) to assess the pharmacokinetics of entecavir in subjects with renal impairment undergoing hemodialysis or continuous ambulatory peritoneal dialysis (CAPD); and (3) to determine the amount of entecavir removed by hemodialysis or CAPD.

Study Design:

This trial was an open-label, parallel group, single-dose study in 36 evaluable subjects. Six (6) subjects were to be assigned to each of 6 groups based on underlying renal function, as outlined below:

1. Group A: normal renal function [creatinine clearance (CLcr) > 80 mL/min]
2. Group B: mild renal function impairment (CLcr > 50 to ≤ 80 mL/min)
3. Group C: moderate renal function impairment (CLcr ≥ 30 to 50 mL/min)
4. Group D: severe renal function impairment (CLcr < 30 mL/min) not requiring dialysis
5. Group E: severe renal function impairment managed with hemodialysis
6. Group F: severe renal function impairment managed with CAPD.

Healthy subjects in Group A were to be representative of the renal-impaired groups (Groups B, C, and D) regarding age (± 5 years), weight (± 20%), and gender. Target demographic parameter values for the healthy control subjects were to be determined when a total of at least 12 subjects from Groups B, C, or D had been enrolled. CLcr was determined for subjects up to 4 weeks prior to study enrollment and was documented on two occasions at least 5 days apart. Each subject's CLcr was calculated based on total urinary creatinine excretion over 24 hours and serum creatinine. A third determination of CLcr was performed if the difference between

the two values exceeded 25% for Group A or varied by more than 15 mL/min for subjects in Groups B, C, or D.

Subjects in Groups A, B, C, D, and F received a single oral dose of 1.0 mg entecavir. Blood and urine samples were collected for pharmacokinetic analyses up to 336 hours postdose. Subjects in Groups A, B, C, D, and F were discharged from the study on Day 15.

For subjects requiring hemodialysis (Group E), a single 1.0 mg oral dose was administered on two occasions. In Period 1, a single 1.0 mg dose was administered 2 hours prior to the hemodialysis session with blood samples collected predose, during the hemodialysis session, and up to 336 hours postdose. Following a washout period of at least 28 days, subjects entered Period 2 and were administered a second 1.0 mg oral dose immediately after a hemodialysis session, with blood samples collected predose and continuing up to 336 hours post dose. Samples of dialysate fluid were collected during hemodialysis sessions to assess the role of hemodialysis on entecavir pharmacokinetics. Subjects in Group E were discharged from the study on Day 15 of Period 2 following safety evaluations and pharmacokinetic sampling.

For subjects requiring CAPD (Group F), a single 1.0 mg dose was given immediately prior to the first exchange of the day, with blood samples collected during peritoneal dialysis and for up to 336 hours after dosing.

Formulations:
Entecavir tablets were supplied as 0.5-mg — , white — , tablets. The product batch number for entecavir tablets was N01030 and the label batch number was 2F59620. The expiry date of the tablets was 29-Feb-2004.

Pharmacokinetic Measurements:
Blood (plasma), urine, and dialysate samples were collected at specified times for the measurement of concentrations of BMS-200475.

1. Blood Sample Collection
   - For subjects in Groups A, B, C, D, and F, serial blood samples were collected at predose and at 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, 12, 18, 24, 36, 48, 72, 96, 120, 144, 168, and 336 hours post dose.
   - For subjects in Group E in Period 1, serial blood samples were collected at predose and at 15, 30, and 45 minutes and 1, 2, 3, 4 (2 samples), 5, 6, 7, 12, 24, 36, 48, 72, 168, and 336 hours post dose. At 4 hours post dose, one sample designated as "4 hours" was the blood sample entering the dialyzer, another sample designated as "4 hours 1 minute" was the blood sample leaving the dialyer. For subjects in Group E in Period 2, serial blood samples were collected at predose and at 15, 30, and 45 minutes and 1, 2, 3, 4, 5, 6, 7, 12, 24, 36, 48, 72, 168, and 336 hours post dose.
2. Urine Sample Collection (Groups A to D)
   - For subjects in Groups A, B, C, and D, urine was collected in periods of 0-3, 3-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours for the analysis of entecavir. A predose urine sample was also collected on Day 1.
3. Dialysate Sample Collection (Groups E and F)
   - For subjects in Group E, the volume of the dialysate recovered during blood sample collection period was collected and recorded each hour throughout the 4-hour dialysis session.
   - For subjects in Group F, the total volume of the dialysate recovered over each 24 hour time period was collected for 168 hours postdose.

Pharmacokinetic/Statistical Analysis:
Single-dose PK of orally administered entecavir were derived from plasma concentration versus time and urinary excretion data. Because the protein binding of entecavir is low (approximately 13%), only total plasma concentrations were determined and all derived pharmacokinetic parameters are based on total plasma concentrations. Extraction ratio of the dialysis process (E), hemodialysis clearance (CL_{HD}), and dialysis clearance for CAPD patients (CL_{CAPD}) were calculated. Summary statistics for each of the pharmacokinetic variables were tabulated by group. The association between CLcr and entecavir AUC was assessed by linear regression of log(AUC) on log(CLcr).

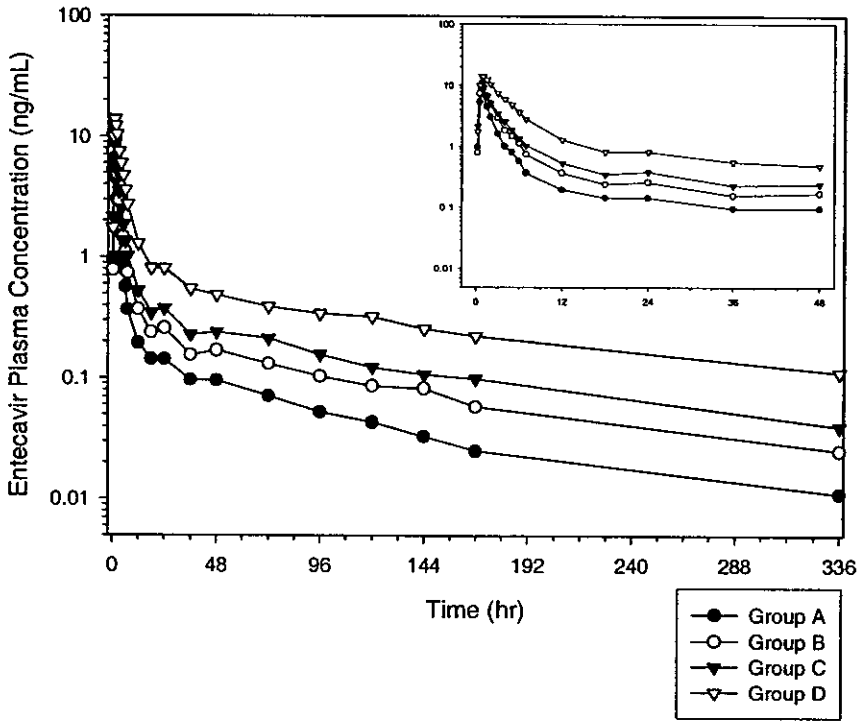
Study Population Results:
Six (6) subjects were enrolled into each of Groups A to E, and 4 subjects were enrolled into Group F due to difficulty with recruitment. All 34 enrolled subjects completed treatment

Demographic and Baseline Characteristics:

Demographic and Baseline Characteristics – Study AI463011							
Demographic	Renal Impairment/Dialysis Group						All Subjects (N=34)
	A (N=6)	B (N=6)	C (N=6)	D (N=6)	E (N=6)	F (N=6)	
Age							
Mean	53	59	62	48	52	58	55
SD	4	12	15	13	7	11	11
Range	46-59	36-69	41-80	34-66	41-60	43-68	34-80
Gender, n (%)							
Male	4 (67)	5 (83)	4 (67)	4 (67)	5 (83)	1 (17)	23 (68)
Female	2 (33)	1 (17)	2 (33)	2 (33)	1 (17)	3 (33)	11 (32)
Race, n (%)							
White	5 (83)	5 (83)	5 (83)	5 (83)	-	2 (33)	22 (65)
Black	1 (17)	1 (17)	1 (17)	-	5 (83)	1 (17)	9 (26)
Native Am.	-	-	-	1 (17)	1 (17)	-	2 (6)
Asian/PI	-	-	-	-	-	1 (17)	1 (3)
Weight, kg							
Mean	83.0	81.6	90.9	74.5	77.3	75.8	80.8
SD	11.7	17.4	15.9	13.9	18.0	6.7	14.8
Range	66-98	65-113	71-109	50-86	54-98	67-83	50-113
Height, cm							
Mean	173.3	176.5	170.6	166.7	170.1	159.4	170.0
SD	3.5	7.5	7.5	5.6	8.4	4.4	7.8
Range	168-177	163-185	158-178	163-178	155-180	156-165	155-185
BMI, kg/m^2							
Mean	27.8	26.2	31.3	27.0	26.5	29.9	28.0
SD	3.3	4.9	4.1	5.1	4.6	4.2	4.5
Range	23.32	22.35	24-35	18-32	21-31	25-34	18-35

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 1.0 mg in subjects with varying degrees of renal impairment (Groups A through D) are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 1.0 mg in subjects with renal impairment are summarized in Table 1. A summary of the regression analyses of log(AUC(INF)) and log(Cmax) on log(CLcr) and CLT/F and CLR on CLcr is presented in Table 2, and a regression plot of CLT/F versus CLcr is presented in Figure 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 1.0 mg in Subjects with Varying Degrees of Renal Impairment. (n=6 per group)



Group A: normal renal function [creatinine clearance (CLcr) > 80 mL/min]
Group B: mild renal function impairment (CLcr > 50 ≤ 80 mL/min)
Group C: moderate renal function impairment (CLcr 30 to 50 mL/min)
Group D: severe renal function impairment (CLcr < 30 mL/min) not requiring dialysis

Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 1.0 mg in Subjects with Renal Impairment.

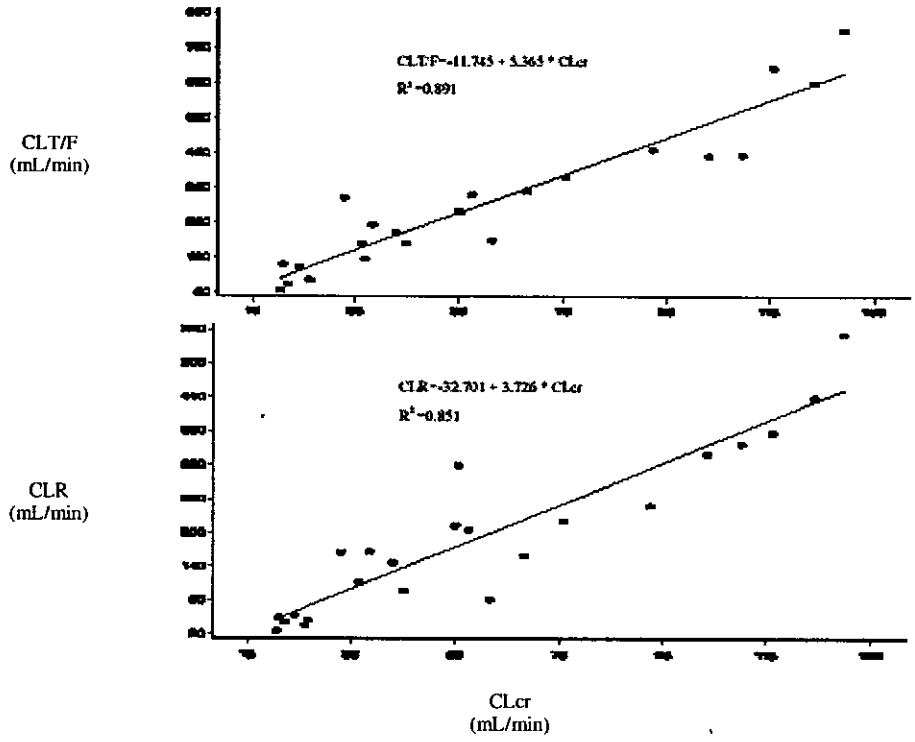
Parameter	A (N=6)	B (N=6)	C (N=6)	D (N=6)	E1 (N=6)	E2 (N=6)	F (N=4)
Cmax (ng/mL)	8.06 (30.70)	10.43 (37.2)	10.53 (22.7)	15.3 (33.8)	12.12 (41.1)	15.37 (56.4)	16.56 (29.7)
AUC(INF) (ng•h/mL)	29.15 (25.0)	54.94 (23.80)	75.77 (25.3)	171.65 (29.2)	153.61 (21.0)	346.14 (40.8)	558.32 (92.5)
AUC(0-T) (ng•h/mL)	27.90 (25.6)	51.46 (22.8)	69.49 (22.7)	145.66 (31.5)	127.10 (20.2)	233.91 (28.4)	221.80 (11.6)
Tmax[a] (hr)	0.75	0.88	0.63	0.88	0.88	0.75	1.00
$t_{½}$[b] (hr)	77.39 (29.88)	113.21 (13.67)	130.65 (25.74)	162.34 (33.49)	155.55 (33.16)	276.34 (143.40)	802.16 (872.37)
CLT/F[b] (mL/min)	588.11 (153.73)	309.18 (62.61)	226.26 (60.11)	100.58 (29.05)	110.69 (24.64)	50.61 (16.54)	35.66 (19.58)
UR[b] (%)	59.60 (10.11)	52.74 (22.75)	43.31[c] (7.16)	28.36 (7.02)	NA	NA	NA
CLR[b] (mL/min)	383.18 (101.80)	197.90 (78.11)	135.57[c] (31.55)	40.27 (10.11)	NA	NA	NA
CLcr[b] (mL/min)	112.58 (13.57)	62.17 (8.12)	38.83 (4.43)	23.33 (2.56)	NA	NA	NA
CL_{HD}[b] (mL/min)	NA	NA	NA	NA	168.86 (43.50)	NA	NA
E[b] (%)	NA	NA	NA	NA	31.82 (15.27)	NA	NA
CL_{CAPD}[b] (mL/min)	NA	NA	NA	NA	NA	NA	0.65

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
[c] N=5
Group A: Subjects with normal renal function (CLcr > 80 mL/min)
Group B: Subjects with mild renal function impairment (CLcr > 50 ≤ 80 mL/min)
Group C: Subjects with moderate renal function impairment (CLcr 30-50 mL/min)
Group D: Subjects with severe renal function impairment (CLcr < 30 mL/min)
Group E1: Subjects with severe renal function impairment managed with hemodialysis in period 1
Group E2: Subjects with severe renal function impairment managed with hemodialysis in period 2
Group F: Subjects with severe renal function impairment managed with CAPD

Table 2 Results of Regression Analyses for Entecavir Cmax, AUC(INF), CLT/F, and CLR

Exposure Parameter (Y)	Est. Regression on Log Scale $Ln(Y)=\alpha_0+\alpha_1 ln(CLcr)$		Est. Regression on Original Scale $Y=\beta_0 CLcr^{\beta 1}$	
	α_0 (95%CI)	α_1 (95%CI)	β_0 (95%CI)	β_1 (95%CI)
Cmax (ng/mL)	3.81 (2.93, 4.69)	-0.37 (-0.59, -0.14)	45.07 (18.68, 108.77)	-0.37 (-0.59, -0.14)
AUC(INF) (ng•h/mL)	8.40 (7.65, 9.15)	-1.07 (-1.26, -0.88)	4447.49 (2106.39, 9390.53)	-1.07 (-1.26, -0.88)
Clearance Parameter (Y)	**Est. Regression on Original Scale $Y=\alpha_0+\alpha_1*CLcr$**			
	α_0 (95%CI)		α_1 (95%CI)	
CLT/F (mL/min)	-11.745 (-68.789, 45.300)		5.365 (4.534, 6.197)	
CLR (mL/min)	-32.701 (-81.978, 16.576)		3.726 (3.018, 4.433)	

Figure 2 Regression of CLT/F and CLR versus CLcr for Entecavir.



Assessment/Conclusion:

- The elimination of entecavir in humans is predominantly through urinary excretion which includes both glomerular filtration and active tubular secretion. It was therefore anticipated that renal impairment would have a significant impact on the elimination of entecavir, resulting in an increase in entecavir exposure with increasing severity of renal impairment. In the current study, as the severity of renal insufficiency increased, the systemic exposure of entecavir increased, accompanied with a decrease in urinary recovery. There were ~ 0.3-1.1 fold increases in Cmax and ~ 0.9-18 fold increases in the exposure (AUC(INF)) for subjects with mild to severe renal impairment, including those requiring dialysis, compared to subjects with normal renal function. As anticipated, based on the higher drug exposure, mean apparent total body clearance (CLT/F) and renal clearance (CLR) was decreased and T-HALF was increased in subjects with renal impairment compared with healthy subjects.
- The relationship between CLcr and both CLR and CLT/F was linear over the CLcr range of 20 to > 80 mL/min. Thus, the extent of renal function impairment is predictive of the renal elimination of entecavir (as measured by CLR) as well as the total body clearance of entecavir. In addition, CLR is approximately 3.7-fold greater than CLcr (based on a slope of 3.7) across the range of CLcr studied (20 to 130 mL/min). This finding indicates that net tubular secretion of entecavir is maintained even in the presence of progressive renal impairment.
- Hemodialysis played a significant role in elimination of entecavir in subjects with severe renal impairment managed with hemodialysis. Entecavir exposure (based on mean AUC(INF)) was reduced by 56% compared to the exposure in the absence of hemodialysis on the day of dosing. The results also indicated that CAPD played a minimal role in elimination of entecavir in subjects with severe renal impairment managed with CAPD since recovery in the dialysate over a period of 7 days post entecavir dose was negligible (mean value = 0.003 mg), compared to a recovery of 0.13 mg in the dialysate of 4-hour hemodialysis session.
- Dosage adjustment of entecavir is warranted when administered to patients with renal impairment. Please refer to the Pharmacometrics review of the population analysis and simulation report 930007867 and the Clinical Pharmacology and Biopharmaceutics review for proposed labeling and specific recommendations.

4.1.2.6. Single Dose Pharmacokinetics and Safety of Entecavir in Subjects with Hepatic Impairment (Protocol AI463032).

Objectives:

- Primary: to compare the pharmacokinetics of a single 1.0 mg oral dose of entecavir in subjects with hepatic impairment to the pharmacokinetics (PK) of a single 1.0 mg oral dose of entecavir in healthy control subjects.
- Secondary: to assess the safety and tolerability of single, oral doses of entecavir in subjects with hepatic impairment and in healthy control subjects.

Study Design:

This was an open-label, single-dose, non-randomized study in a minimum of 24 subjects with or without hepatic impairment. At least 12 subjects with Grade B or Grade C hepatic impairment as defined by Child-Pugh classification and 12 healthy subjects with normal hepatic function were to be enrolled. Healthy control subjects were matched (1:1) to the hepatically impaired subjects with regard to age (± 5 years), weight (±15%), and sex. Each healthy control subject was enrolled after the matched hepatically impaired subject had completed at least Day 8 of the

study. Each subject received a single 1.0 mg oral dose of entecavir in the fasted state on Day 1 and remained in the clinical study unit through Day 5. Blood and urine samples were obtained from each subject for up to 14 days after dosing for pharmacokinetic evaluation.

Formulations:
Entecavir tablets were supplied as 0.5-mg — , white — tablets. The product batch number for entecavir tablets was N01030 and the label batch number was 2F59620. The expiry date of the tablets was 28-Feb-2004.

Pharmacokinetic Measurements:
Blood (plasma) and urine samples were collected at specified times for the measurement of concentrations of BMS-200475.

- Serial blood samples (5 mL) for measurement of plasma BMS-200475 were obtained at pre-dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 12, 18, 24, 36, 48, 72, 96, 168 and 336 hours post-dose.
- Urine samples were collected at pre-dose on Day 1 and at the following intervals post-dose: 0-6, 6-12, 12-24, 24-48, 48-72, and 72-96 hours.

Pharmacokinetic/Statistical Analysis:
Single-dose PK of orally administered entecavir were derived from plasma concentration versus time and urinary excretion data. Because the protein binding of entecavir is low (approximately 13%), only total plasma concentrations were determined and all derived pharmacokinetic parameters are based on total plasma concentrations. Absence of a clinically significant effect of hepatic impairment on entecavir PK was concluded if the 90% confidence intervals (CIs) for the ratios of the geometric means for hepatically impaired and healthy subjects were contained within 67% to 150% for Cmax and AUC(INF). The CIs were constructed from the results of analyses of variance (ANOVA) on log(Cmax) and log[AUC(INF)]. Summary statistics for each of the pharmacokinetic variables were tabulated by group.

Reviewer Comment: The Applicant defined "absence of a clinically significant effect" as the 90% CIs for the ratios of geometric means falling between 67-150%. This range was based on clinical data and PK/PD modeling which suggested that a 33% decrease or 50% increase in entecavir exposure is not expected to impact clinical safety or efficacy. Per FDA guidance, if a wider boundary than the standard 80-125% can be supported clinically, the Agency may recognize this delineation of no effect boundaries as acceptable.

Study Population Results:
Thirty-two (32) subjects (16 with hepatic impairment and 16 healthy subjects) were enrolled in this study and completed treatment.

Demographic and Baseline Characteristics:
Twenty (20) subjects were male, and 12 subjects were female. Twenty-one (21) subjects were white, 5 were black, 5 were Hispanic/Latino, and 1 was Asian/Pacific Islander. The ages ranged from 38 to 66 years, with a mean of 51 years. The weight ranged from 55.5 to 129.1 kg, witha mean of 89.5 kg. The height ranged from 149.9 to 188.5 cm, with a mean of 172.5 cm. The body mass index (BMI) ranged from 19.2 to 37.9 kg/m^2, with a mean of 30.0 kg/m^2. The demographic characteristics of the two subject groups were similar.

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 1.0 mg in healthy subjects and subjects with hepatic impairment are presented in Figure

1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 1.0 mg in healthy subjects and subjects with hepatic impairment are summarized in Table 1. Box plots of entecavir Cmax and AUC(INF) are presented in Figure 2. The geometric means, ratios of geometric means, and their 90% confidence intervals for Cmax and AUC(INF) are summarized in Table 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects and Subjects with Hepatic Impairment. (n=16 per group)

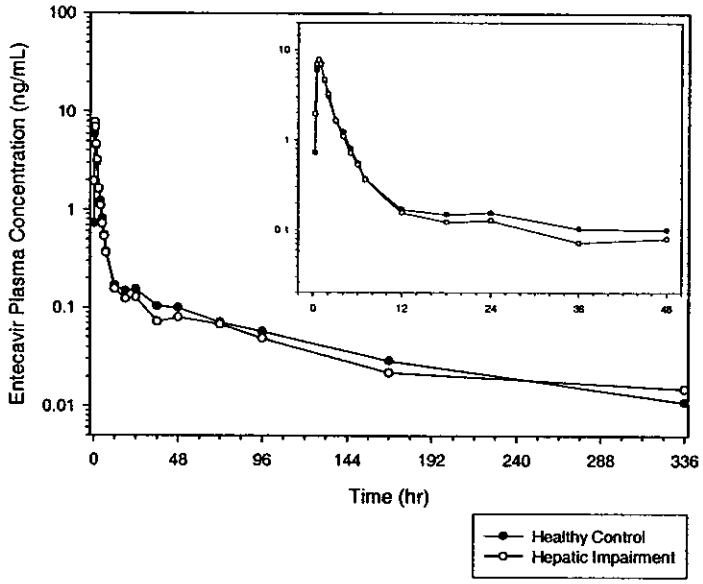
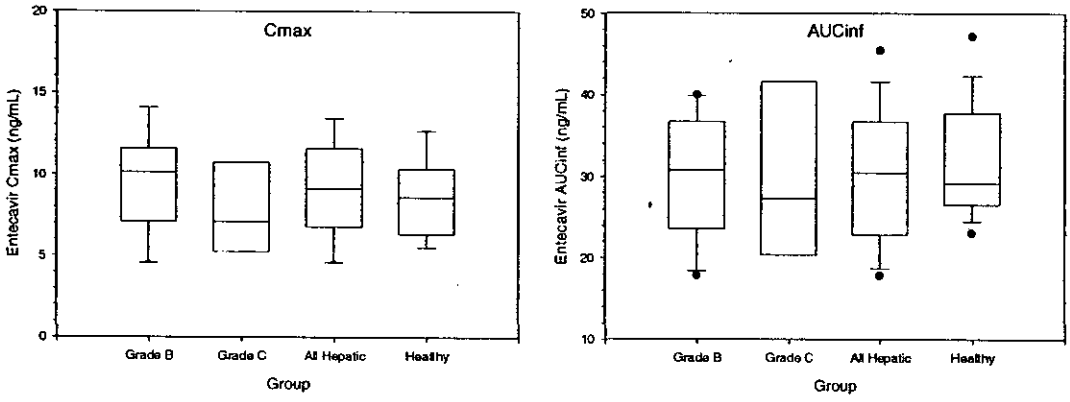


Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects and Subjects with Hepatic Impairment.

Parameter	Child-Pugh Grade B (N=12)	Child-Pugh Grade C (N=4)	Hepatic Impairment (Grades B and C) (N=16)	Healthy (N=16)
Cmax (ng/mL)	8.99 (32.7)	7.25 (39.6)	8.52 (34.3)	8.22 (29.9)
AUC(INF) (ng•h/mL)	29.33 (24.3)	28.17 (38.4)	29.03 (26.9)	31.28 (21.6)
AUC(0-T) (ng•h/mL)	27.22 (25.4)	25.66 (31.1)	26.82 (25.9)	30.07 (22.5)
Tmax[a] (hr)	0.75	0.88	0.75	0.75
$t_{½}$[b] (hr)	87.1 (41.0)	109.9 (70.6)	92.8 (48.3)	92.1 (14.5)
CLT/F[b] (mL/min)	588.0 (169.1)	621.0 (214.3)	596.3 (174.2)	543.5 (108.8)
UR[b] (%)	44.5[c] (25.9)	47.2 (13.3)	45.2[d] (22.8)	52.8 (15.2)
CLR[b] (mL/min)	309.3[c] (143.5)	383.1 (200.6)	329.0[d] (156.4)	374.2 (133.2)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
[c] N=11
[d] N=15

Figure 2 Entecavir Cmax and AUC(INF) Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects and Subjects with Hepatic Impairment. (n=16 per group)



Symbols: Line, median value; box, 25th and 75 percentiles; whiskers, 10th and 90th percentiles; dot, outlier.

Table 2 Comparison of Geometric Mean Ratios and Confidence Intervals for Entecavir Cmax and AUC(INF) Following Administration of a Single Oral Dose of 1.0 mg in Healthy Subjects and Subjects with Hepatic Impairment.

Parameter	Group	Geometric Mean	Ratio (90%CI)
Cmax (ng/mL)	Hepatic Impairment	8.52	1.036 (0.942, 1.139)
	Healthy	8.22	
AUC(INF) (ng•h/mL)	Hepatic Impairment	29.03	0.928 (0.813, 1.059)
	Healthy	31.28	

Treatment comparison: hepatic impairment vs. healthy.

Assessment/Conclusion:

- Entecavir exposure was not statistically significantly altered in subjects with moderate to severe hepatic impairment when compared to subjects with normal hepatic function. The 90% confidence intervals for the hepatic to healthy Cmax and AUC(INF) ratios were well within the pre-specified no-effect interval of 0.67 to 1.50, as well as within the standard interval of 0.80 to 1.25. Half-lives, clearance values, and urinary excretion rates were comparable between the two groups.
- Entecavir exposure between Child-Pugh Class Grades B and C within the hepatic impairment group was similar, indicating that the degree of hepatic impairment does not affect entecavir elimination.
- As entecavir is predominantly eliminated via urinary excretion (both glomerular filtration and tubular secretion) and no phase I metabolites have been identified in humans, the influence of hepatic impairment on the pharmacokinetics of entecavir was expected to be minor, which is supported by the current findings in this hepatic impairment study. Therefore, entecavir may be administered to patients with mild, moderate, or severe hepatic impairment without dose adjustment.

4.1.2.7. A Pilot Study of the Safety, Pharmacokinetics and Antiviral Activity of Open-Label Entecavir in Liver Transplant Recipients Re-Infected with Hepatitis B Virus (Protocol AI463015).

Objectives:

- Primary: (1) To assess the safety of entecavir, as measured by the incidence of adverse events and laboratory abnormalities, and (2) To assess the first-dose (Day 1) and steady-state (Day 14) pharmacokinetic (PK) parameters of entecavir when given with a stable dose of tacrolimus or cyclosporine.
- Secondary: (1) To determine the proportion of subjects who achieve undetectable hepatitis B virus deoxyribonucleic acid (HBV DNA) levels. (2) To determine the proportion of subjects who achieve ≥1 log10 reduction and the proportion who achieve ≥ 2 log10 reduction in HBV DNA levels at Week 24 and who then maintain the reduction in HBV DNA levels at Week 48; (3) To determine the proportion of subjects with undetectable serum hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) at Weeks 24 and 48; (4) To determine the proportion of subjects who achieve seroconversion at Week 24 and who then maintain seroconversion at Week 48; (5) To determine the proportion of subjects with normalization or improvement (≥ 50% decrease from baseline) in serum alanine aminotransferase (ALT) level at Weeks 24 and 48; (6) To determine the proportion of subjects demonstrating an improvement in histologic activity, a decrease in HBV core antigen and/or covalently closed circular DNA (cccDNA) and safety of entecavir on liver biopsy at Weeks 24 and 48; (7) To

assess genotypic alterations in HBV. (8) To assess blood concentrations of entecavir by random sampling during dosing.

Study Design:
This was a multinational, multicenter, open-label study in ten (10) orthotopic liver transplant (OLT) recipients given oral entecavir 1.0 mg once daily in an outpatient setting. Clinically stable OLT recipients > 100 days post-transplant with recurrent chronic HBV despite anti-HBV prophylaxis were enrolled. On Day 1, eligible subjects discontinued their current nucleoside analogue therapy, but could continue hepatitis B immunoglobulin (HBIG). Response to entecavir therapy was assessed at Weeks 12 and 24. Subjects with undetectable HBV DNA by the Chiron assay at Week 24 continued entecavir therapy until Week 48. At Week 48, subjects could elect to continue entecavir therapy until Week 104 or until a separate open-label oral entecavir protocol became available. At Week 104, HBV DNA results from the Week 96 visit were reviewed by the investigator, and if the results were undetectable by Chiron assay, or if there was a ≥ 1 $\log_{10}$ drop in HBV DNA by PCR, then the subject could continue to receive entecavir until an open-label extended-treatment protocol became available. PK parameters for entecavir were assessed using blood samples drawn on Day 1 (first dose) and Day 14 (steady-state), and random PK samples for entecavir were obtained at Weeks 4, 12, 24, 36, and 48.

Formulations:
- Entecavir, 1.0-mg capsule administered orally, once daily, Batch Numbers N00003 and N01071.
- Entecavir 0.5-mg capsule administered orally, 2 capsules once daily, Batch Number N99049.
- Entecavir, 0.5-mg tablet administered orally, 2 tablets once daily, Batch Number 8MEE156.

Pharmacokinetic Measurements:
Blood (plasma) samples were collected at specified times for the measurement of concentrations of BMS-200475.
- On Days 1 and 14, serial blood samples (5 mL) for measurement of plasma BMS-200475 were obtained at pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 hours post-dose.
- Random PK sampling for entecavir was obtained > 1 hour after dosing at Weeks 4, 12, 24, 36, and 48.

Pharmacokinetic/Statistical Analysis:
Single and multiple dose PK of orally administered entecavir were derived from plasma concentration versus time data. Because there was only one treatment arm in the study, all analyses are descriptive. Parameters represented by continuous variables were summarized using the mean, median, standard error, standard deviation, and minimum and maximum values. Geometric means and coefficients of variation were reported for Cmax, AUC(TAU), and accumulation index (AI). Medians and ranges were presented for Tmax.

Study Population Results:
Ten (10) subjects were enrolled in the study, of which 9 were treated with open-label medication. All 9 subjects completed treatment, and 7 subjects went on to continue treatment in another study (ie, Study AI463900 [3 subjects] and Study AI463901 [4 subjects]).

Demographic and Baseline Characteristics:
The demographic and baseline characteristics of the study population were as follows:

Demographics and Baseline Characteristics – Study AI463015	
Age* (yr)	53 (43 – 64)
Gender N(%)	8 (89%) Male 1 (11%) Female
Race N(%)	6 (67%) White 2 (22%) Asian/Pacific Islander 1 (11%) Other (Lebanese)
Region N(%)	6 (67%) North American 2 (22%) European 1 (11%) Asia
Country N(%)	4 (44%) US 2 (22%) Canada 2 (22%) Germany 1 (11%) Australia
Weight* (kg)	76 (65 – 102)
Height* (cm)	171 (165 – 178)
BMI* (kg/m^2)	26.0 (22.7 – 33.2)
Immunosuppressive Agent N(%)	5 (56%) cyclosporine 4 (44%) tacrolimus

* Data presented as mean (range).

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following administration of single and multiple oral doses of 1.0 mg QD in OLT recipients are presented by day in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of single and multiple oral doses of 1.0 mg QD in OLT recipients are presented by day and co-administered immunosuppressive agent in Table 1. A scatter and regression plot of Day 14 CLT/F versus CLcr is presented in Figure 2. A scatter plot of entecavir plasma concentrations obtained from random sampling (overlaying the mean [SD] entecavir plasma concentration-time profile on Day 14) in Orthoptic Liver Transplant Recipients is displayed in Figure 3.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Single and Multiple Oral Doses of 1.0 mg QD in Orthoptic Liver Transplant Recipients (N=9)

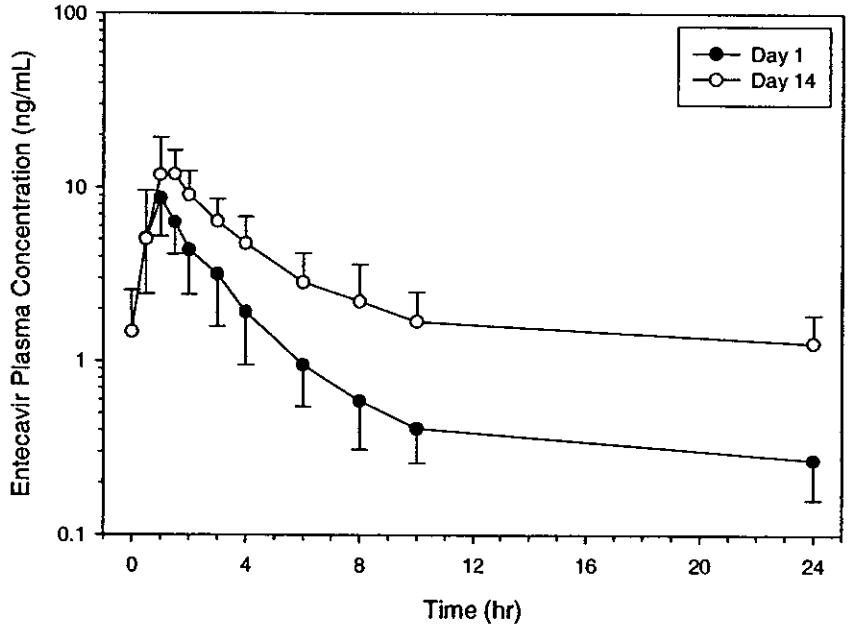


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Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Single and Multiple Oral Doses of 1.0 mg QD in Orthoptic Liver Transplant Recipients (N=9)

Pharmacokinetic Parameter	Cyclosporine (n = 5)		Tacrolimus (n = 4)		All (n = 9)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Cmax (ng/mL)						
Geometric Mean	8.08	14.03	8.50	12.52	8.26	13.34
(CV%)	(20)	(37)	(50)	(45)	(37)	(38)
AUC(TAU) (ng•h/mL)						
Geometric Mean	25.90	70.24	25.01	55.98	25.50	63.50
(CV%)	(27)	(29)	(46)	(52)	(34)	(37)
Tmax (h)						
Median	1.00	1.50	1.00	1.00	1.00	1.00
min			—			
max			—			)
AI						
Geometric Mean	NA	2.71	NA	2.24	NA	2.49
(CV%)		(9)		(10)		(13)
CLcr (mL/min)						
Mean	47.05	48.46	92.70	83.52	67.34	64.04
(SD)	(2.75)	(4.16)	(25.55)	(31.31)	(28.77)	(26.79)
CLT/F (mL/min)						
Mean	NA	246.53	NA	331.63	NA	284.35
(SD)		(77.70)		(177.54)		(129.81)

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Figure 2 Regression of Entecavir CLT/F versus CLcr in Orthoptic Liver Transplant Recipients.

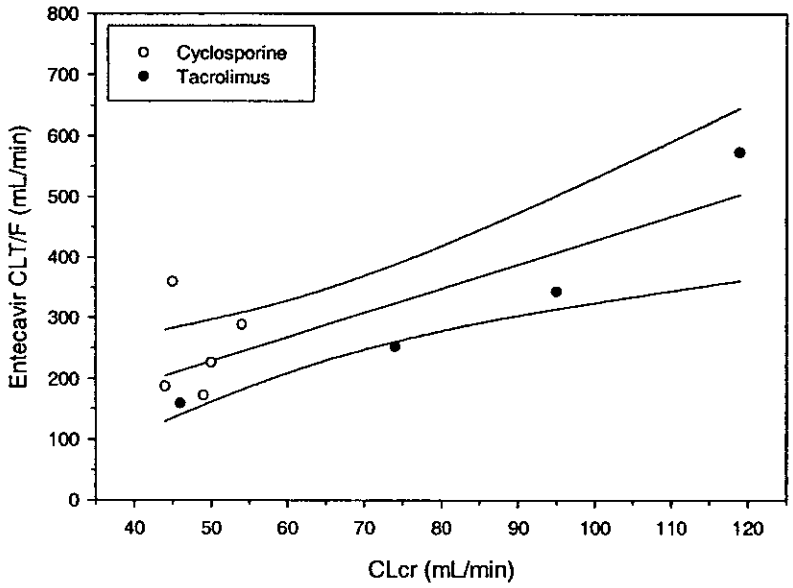
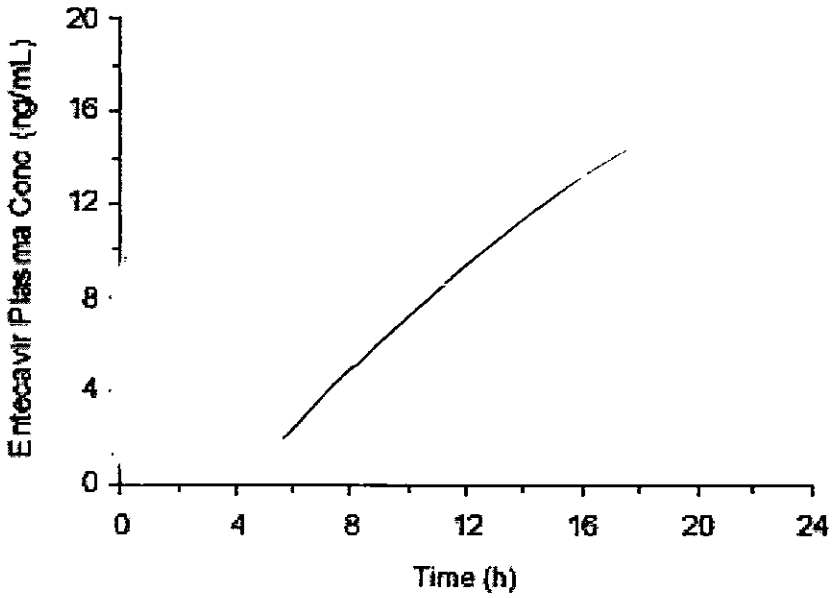


Figure 3 Scatter Plot of Entecavir Plasma Concentrations Obtained From Random Sampling in Orthoptic Liver Transplant Recipients.



Line represents mean (SD) entecavir plasma concentration-time profile on Day 14.

Assessment/Conclusion:

- Upon cross-study comparison with data from healthy subjects administered entecavir 1.0 mg QD for 14 days (AI463033), overall exposure to entecavir in OLT patients was somewhat greater than exposures observed in healthy subjects. In patients, for Day 1, entecavir geometric mean Cmax and AUC(TAU) values were 8.3 ng/mL and 25.5 ng•h/mL, respectively, and on Day 14 entecavir Cmax and AUC(TAU) were 13.3 ng/mL and 63.5 ng•h/mL, respectively. In comparison in healthy subjects, for Day 1 Cmax and AUC(TAU) values of 6.8 ng/mL and 16.4 ng•h/mL, respectively, and Day 14 entecavir Cmax and AUC(TAU) values of 8.2 ng/mL and 26.4 ng•h/mL, respectively, were observed. Thus, the exposure to entecavir was approximately 1.6- and 2.4-fold greater [based on AUC(TAU)] in the subjects in this study compared to healthy subjects on Days 1 and 14, respectively, following daily 1.0 mg entecavir doses.
- The subjects in the current study were > 100 days post-transplant, and on a stable dose of tacrolimus or cyclosporine, drugs that are known to have acute and chronic effects on renal function, and renal function impairment was shown to affect the PK of entecavir in Study AI463011. A review of the subjects' renal function in this study revealed that their estimated creatinine clearance (CLcr) ranged from 44 to 119 mL/min; thus, the subjects in this study would be classified as having renal function ranging from moderately impaired (CLcr 30 to 50 mL/min) to normal (CLcr > 80 mL/min). The majority of the subjects (5 of 9) had moderate renal function impairment on Day 14 of the study. Regression of CLT/F on CLcr resulted in a linear relationship between the 2 parameters ($R^2 = 0.670$), congruent to previous study findings that entecavir total body clearance is correlated with renal function. Of note, the subjects that tended to have the greater degree of renal function impairment were on concomitant cyclosporine therapy, consistent with the known greater potential for renal toxicity with cyclosporine compared to tacrolimus. Also of note, individual accumulation index (AI) values do not appear to vary widely with differences in renal function. This suggests that even with moderate renal impairment, there is a predictable accumulation of entecavir following daily dosing to steady state. The AI range observed in this study (2.0 - 3.1) is comparable to that observed in healthy subjects (1.6 - 2.7) in AI463033.
- Individual entecavir plasma concentrations from random sampling in Weeks 4, 12, 24, 36, and 48 were all within the range of entecavir plasma concentrations observed on Day 14 (presumed steady state) in each subject. This finding suggests that after the first 2 weeks of dosing there were no marked changes in entecavir exposure over the subsequent 46-week dosing period.
- The PK results from this current study are consistent with the anticipated results based on the renal function of the subjects. Therefore, it appears that the greater entecavir exposures in the subjects in this study (compared to healthy subjects), is unlikely due to a drug-drug interaction between entecavir and the concomitant immunosuppressant agent (cyclosporine or tacrolimus), but rather is secondary to renal impairment that may have resulted from the immunosuppressive therapy. Dosage adjustment for entecavir, when used in subjects following liver transplantation and on concomitant cyclosporine or tacrolimus immunosuppressive therapy, should be considered on the basis of renal function as appropriate.

4.1.3. Extrinsic Factors

4.1.3.1. Open-Label, Sequential Design, Drug Interaction Study of Entecavir and Lamivudine in Healthy Subjects (Protocol AI463058).

Objectives:

- Primary: to assess the effect of lamivudine on the pharmacokinetics of entecavir and to assess the effect of entecavir on the pharmacokinetics of lamivudine.
- Secondary: to assess the safety of entecavir and lamivudine when administered alone or in combination.

Study Design:

This was an open-label, multiple-dose, sequential design study in 30 healthy subjects. Subjects received 150 mg lamivudine every 12 hours (q12 h) on Days 1 to 4, 1 mg entecavir once daily (QD) on Days 5 to 14, and 1 mg entecavir QD plus 150 mg lamivudine q12 h on Days 15 to 24 in the fasted state. Blood samples for pharmacokinetic analysis were collected up to Day 25. Twenty-four- (24-) hour urine samples for pharmacokinetic analysis were collected on Days 4, 14, and 24. Subjects were discharged from the study upon completion of study procedures on Day 25.

Formulations:

Entecavir tablets were supplied by the Sponsor as 0.5-mg [redacted], white [redacted], film coated tablets packaged in bottles of 25 tablets/bottle. The label batch number for entecavir tablets was 3B67994, and the product batch number was 8MDE141, with an expiration date of 30-Apr-2004. Lamivudine (Epivir®) tablets were supplied by the investigator as white, modified diamond-shaped, film-coated tablets packaged in bottles of 60 tablets/bottle with an expiration date of February 2008 (lot 32P0587). Each lamivudine tablet contained 150 mg of lamivudine.

Pharmacokinetic Measurements:

Blood (plasma) and urine samples were collected at specified times for the analyses of concentrations of entecavir and/or lamivudine.

- Blood samples for plasma entecavir were obtained as follows:
  - Days 14 and 24: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 6 to 13 and 15, 17, 19, 21, and 23: predose (trough) sampling.
- Blood samples for plasma lamivudine were obtained as follows:
  - Day 1: predose sample
  - Days 4 and 24: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.25, 12.5, 12.75, 13, 13.5, 14, 15, 16, 18, 20, and 24 hours following dosing;
  - Days 15, 17, 19, 21, and 23: predose (trough) sampling.
- Urine samples were collected predose on Day 1 and up to 24 hours post-dose on Days 4 and 24 for lamivudine and Days 14 and 24 for entecavir over the following intervals relative to drug administration:
  - Prior to dosing and 0-12 and 12-24 hours following dosing.

Pharmacokinetic/Statistical Analysis:

Entecavir and lamivudine pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Absence of an effect of co-administration of lamivudine on entecavir Cmax and AUC(TAU) was concluded if the 90% confidence intervals (CIs) for the ratios of the geometric means with and

without concomitant lamivudine were contained within 80% to 125%. Similarly, absence of an effect of co-administration of entecavir on lamivudine Cmax and AUC(TAU) was concluded if the 90% CIs for the ratios of the geometric means with and without concomitant entecavir were contained within 80% to 125%. These CIs were constructed from the results of analyses of variance on log(Cmax) and log(AUC(TAU)) of entecavir and lamivudine. Summary statistics were tabulated for all pharmacokinetic parameters.

Study Population Results:
Thirty (30) subjects were enrolled and 29 (97%) completed treatment. One (1) subject withdrew consent for personal reasons and discontinued from the study on Day 4 after having received 4 doses of lamivudine alone.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463058*	
Age (yr)	30 (18 – 43)
Gender N (%)	21 Males (70%) 9 Females (30%)
Weight (kg)	76.8 (58.1 – 100.7)
Height (cm)	176.8 (157.5 – 189.2)
BMI (kg/m^2)	24.6 (18.0 – 29.7)
Race N (%)	25 White (83%) 4 Black/African American (13%) 1 Asian (3%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of lamivudine in healthy subjects are presented in Figure 1. Mean trough concentrations of entecavir in plasma following multiple oral doses of 1.0 mg entecavir QD both with and without multiple doses of lamivudine in healthy subjects are presented in Figure 2. Entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of lamivudine in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of lamivudine in healthy subjects is presented in Table 2.

Mean concentration-time profiles for lamivudine in plasma following multiple oral doses of 150 mg lamivudine q12h both with and without multiple doses of entecavir in healthy subjects are presented in Figure 3. Mean trough concentrations of lamivudine in plasma following multiple oral doses of 150 mg lamivudine q12h both with and without multiple doses of entecavir in healthy subjects are presented in Figure 4. Lamivudine plasma pharmacokinetic parameters following multiple oral doses of 150 mg lamivudine q12h both with and without multiple doses of entecavir in healthy subjects are summarized in Table 3. A summary of statistical analysis of lamivudine plasma pharmacokinetic parameters following multiple oral doses of 150 mg lamivudine q12h both with and without multiple doses of entecavir in healthy subjects is presented in Table 4.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Lamivudine in Healthy Subjects.

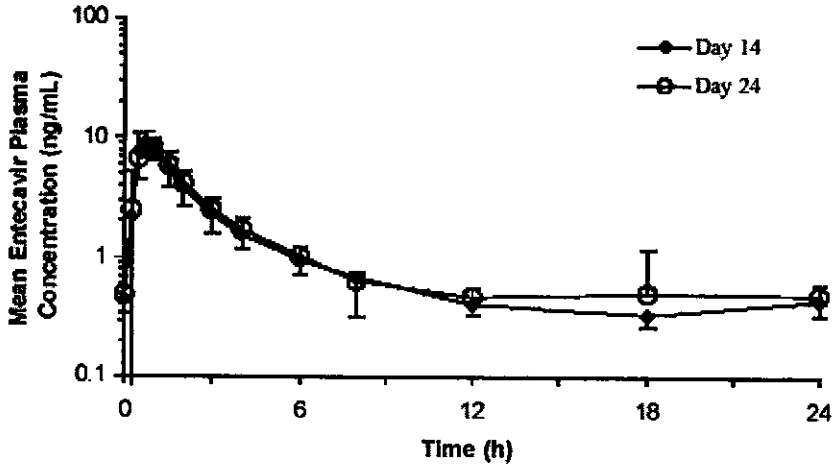
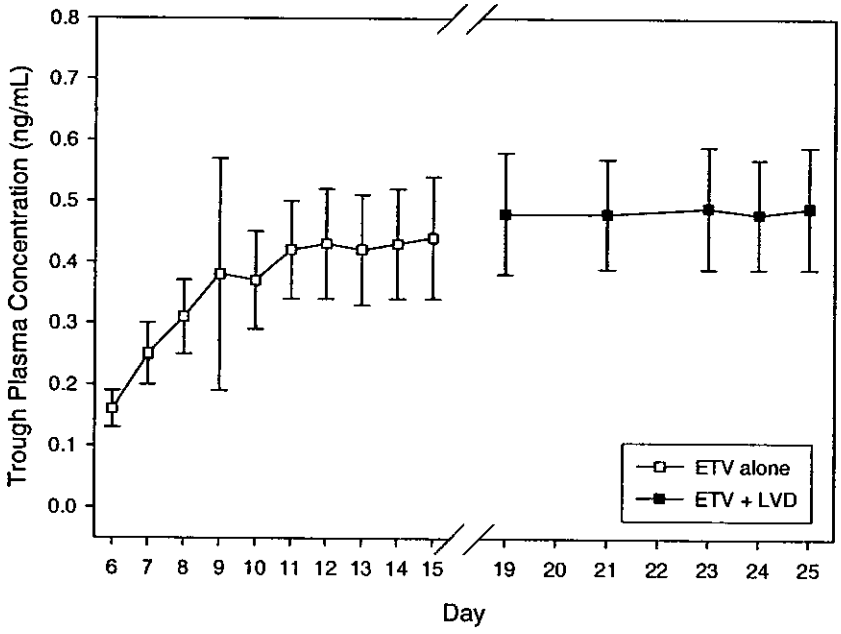


Figure 2 Mean (SD) Trough Concentrations of Entecavir in Plasma Following Multiple Oral Doses of 1.0 mg Entecavir QD with and without Multiple Doses of Lamivudine in Healthy Subjects.



ETV entecavir
LVD lamivudine

Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Lamivudine in Healthy Subjects.

Parameter	Entecavir Alone (Day 14)	Entecavir + Lamivudine (Day 24)
Cmax (ng/mL)	10.05 (20)	9.84 (23)
AUC(TAU) (ng•h/mL)	26.86 (17)	28.66 (22)
Cmin (ng/mL)	0.42 (24)	0.48 (20)
Tmax[a] (hr)	0.75	0.75
UR[b] (%)	63.85 (10.07)	64.61 (13.74)
CLR[b] (mL/min)	399.09 (74.07)	379.75 (91.26)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 14: 1 mg entecavir QD (reference)
Day 24: 150 mg lamivudine q12h and 1 mg entecavir QD (test)

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Lamivudine in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means Point Estimate (90% CI) (Day 24 vs. Day 14)
	Entecavir Alone (Day 14)	Entecavir + Lamivudine (Day 24)	
Cmax (ng/mL)	10.05	9.84	0.979 (0.902, 1.063)
AUC(TAU) (ng•h/mL)	26.86	28.66	1.067 (1.026, 1.109)
Cmin (ng/mL)	0.42	0.48	1.134 (1.078, 1.193)

Day 14: 1 mg entecavir QD (reference)
Day 24: 150 mg lamivudine q12h and 1 mg entecavir QD (test)

Figure 3 Mean Concentrations of Lamivudine in Plasma Following Administration of Multiple Oral Doses of 150 mg Lamivudine Q12H with (Day 24) and without (Day 4) Entecavir in Healthy Subjects.

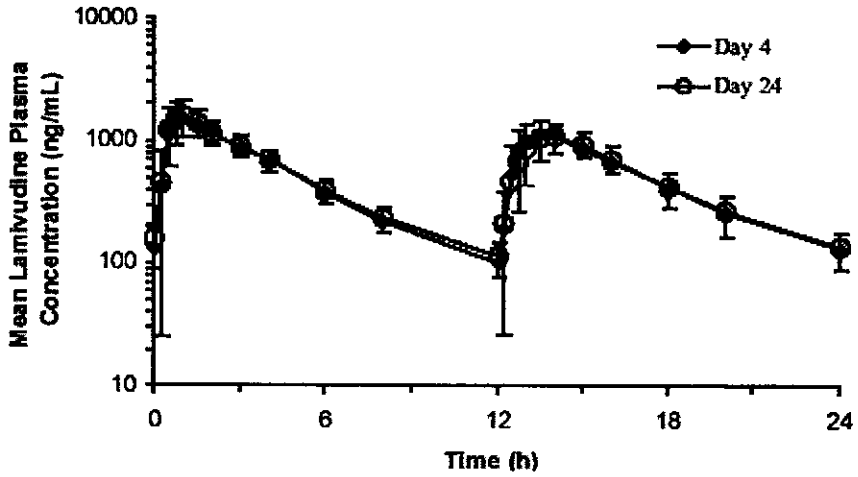
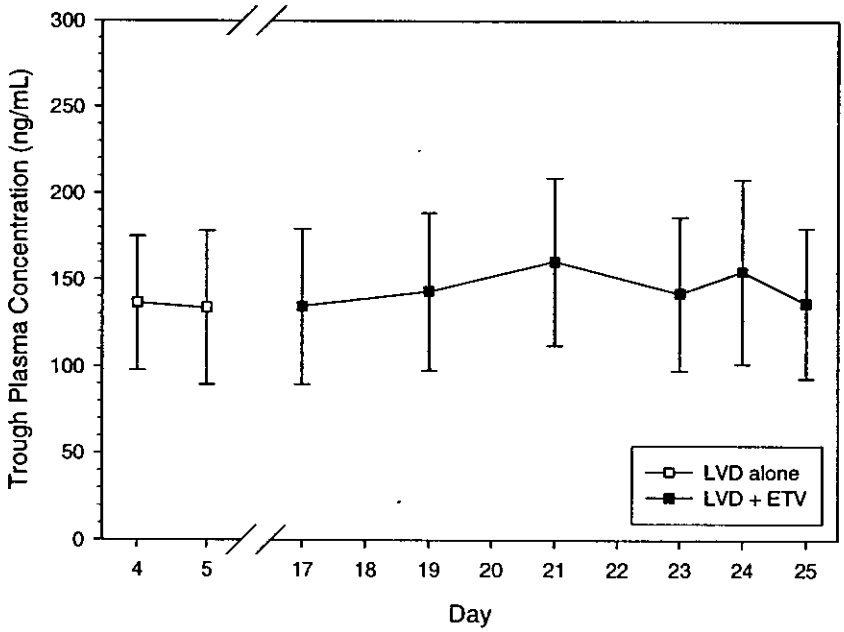


Figure 4 Mean (SD) Trough Concentrations of Lamivudine in Plasma Following Multiple Oral Doses of 150 mg Lamivudine Q12H with and without Multiple Doses of Entecavir in Healthy Subjects.



ETV entecavir
LVD lamivudine

Table 3 Summary of Lamivudine Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 150 mg Lamivudine Q12H with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	Lamivudine Alone (Day 4)	Lamivudine + Entecavir (Day 24)
Cmax (ng/mL)	1689.90 (23)	1701.99 (27)
AUC(TAU) (ng•h/mL)	6389.38 (16)	6558.58 (16)
Cmin (ng/mL)	99.28 (24)	113.35 (26)
Tmax[a] (hr)	0.75 —	1.00 —
UR[b] (%)	69.52 (11.42)	64.33 (15.12)
CLR[b] (mL/min)	274.39 (46.57)	246.29 (57.75)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 4: 150 mg lamivudine q12h (reference)
Day 24: 150 mg lamivudine q12h and 1 mg entecavir QD (test)

Table 4 Summary of Statistical Analysis of Lamivudine Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 150 mg Lamivudine Q12H with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means Point Estimate (90% CI) (Day 24 vs. Day 4)
	Lamivudine Alone (Day 4)	Lamivudine + Entecavir (Day 24)	
Cmax (ng/mL)	1689.90	1701.99	1.007 (0.928, 1.093)
AUC(TAU) (ng•h/mL)	6389.39	6558.58	1.027 (0.985, 1.069)
Cmin (ng/mL)	99.28	113.35	1.142 (1.093, 1.192)

Day 4: 150 mg lamivudine q12h (reference)
Day 24: 150 mg lamivudine q12h and 1 mg entecavir QD (test)

Assessment/Conclusion:

- Both lamivudine and entecavir are eliminated predominantly unchanged in urine by a process that includes net tubular secretion. As renal tubular secretion and reabsorption may involve saturable and/or competitive processes and co-administration of lamivudine and entecavir in the HBV population is foreseeable, the current study was undertaken to investigate potential pharmacokinetic interactions of the two drugs at steady state after multiple doses. This study used the highest anticipated daily dose of entecavir (1 mg) for subjects who have failed lamivudine treatment. The selected dosage of lamivudine for this study was its highest labeled daily dose of 300 mg, administered as 150 mg q12 h.
- A previous single-dose interaction study of entecavir and lamivudine (Study AI463010) showed an indeterminate pharmacokinetic interaction of entecavir on lamivudine, manifested by a decrease of 20% and 23% in lamivudine Cmax and AUC, respectively. The current study was conducted using a multiple-dose study design to further assess the magnitude of this interaction between entecavir and lamivudine at steady-state. Since both drugs will be administered chronically, this study is representative of the potential impact of an interaction during combination antiviral therapy.
- No effect of co-administration of lamivudine on entecavir pharmacokinetics was observed. The adjusted geometric means for entecavir AUC(TAU), Cmax, and Cmin when co-administered with lamivudine were similar to those values for entecavir alone, and the corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Individual entecavir concentration-time profiles both with and without lamivudine were practically super-imposable, indicative of a lack of pharmacokinetic interaction. Absorption (Tmax) and renal excretion of entecavir (%UR and CLR) are also comparable on Days 14 and 24. Based on the Cmin values, entecavir appeared to reach its steady-state by 7 days of 1 mg QD dosing (Day 11).
- No effect of co-administration of entecavir on lamivudine pharmacokinetics was observed. The adjusted geometric means for lamivudine AUC(TAU), Cmax, and Cmin when co-administered with entecavir were similar to those values for lamivudine alone for both the dosing interval of 0-12 hours and 12-24 hours, and the corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Minimal differences were observed between individual lamivudine concentration-time profiles both with and without entecavir, supporting a lack of pharmacokinetic interaction. Absorption (Tmax) and renal excretion of lamivudine (%UR and CLR) were also comparable on Days 4 and 24 for both the dosing intervals of 0-12 hours and 12-24 hours. Based on the Cmin values, lamivudine appeared to reach steady-state by Day 4 following 150 mg q 12 h dosing.
- Overall, the statistical analyses of entecavir and lamivudine pharmacokinetic parameters indicated a lack of effect of one drug on the steady-state pharmacokinetics of the other; therefore, entecavir and lamivudine may be co-administered for HBV infection or HBV/HIV co-infection without the need for dose modification.

4.1.3.2. Open-Label, Sequential Design, Drug Interaction Study of Entecavir and Adefovir in Healthy Subjects (Protocol AI463063).

Objectives:

- Primary: to assess the effect of adefovir on the pharmacokinetics of entecavir and to assess the effect of entecavir on the pharmacokinetics of adefovir.
- Secondary: to assess the safety of entecavir and adefovir when administered alone or in combination.

Study Design:
This was an open-label, multiple-dose, sequential design study in 26 healthy subjects. Subjects received 10 mg adefovir once daily (QD) on Days 1 to 4, 1 mg entecavir QD on Days 5 to 14, and 1 mg entecavir QD plus 10 mg adefovir on Days 15 to 24 in the fasted state. Blood samples for pharmacokinetic analysis were collected up to Day 25. Twenty-four- (24-) hour urine samples for pharmacokinetic analysis were collected on Days 4, 14, and 24. Subjects were discharged from the study upon completion of study procedures on Day 25.

Formulations:
Entecavir tablets were supplied by the Sponsor as 0.5-mg — ., white — , film coated tablets packaged in bottles of 25 tablets/bottle. The batch number for entecavir tablets was 8MDE141, with an expiration date of 30-Apr-2004. Adefovir ( ) tablets were supplied by the investigator as white tablets packaged in bottles of 30 tablets/bottle. The lot numbers for adefovir were TDJ004 with an expiration date of January 2005 and TDJ002 with an expiration date of July 2004. Each tablet contained 10 mg of adefovir.

Pharmacokinetic Measurements:
Blood (plasma) and urine samples were collected at specified times for the analyses of concentrations of entecavir and/or adefovir.

- Blood samples for plasma entecavir were obtained as follows:
  - Days 14 and 24: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 6 to 13 and 15: predose (trough) sampling.
- Blood samples for plasma adefovir were obtained as follows:
  - Day 1: predose sample
  - Days 4 and 24: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following dosing.
- Urine samples were collected predose on Day 1 and up to 24 hours post-dose on Days 4 and 24 for adefovir and Days 14 and 24 for entecavir over the following intervals relative to drug administration:
  - Prior to dosing and 0-12 and 12-24 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Entecavir and adefovir pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Absence of an effect of co-administration of 10 mg adefovir on entecavir Cmax and AUC(TAU) was concluded if the 90% confidence intervals (CIs) for the ratios of the geometric means with and without concomitant adefovir were contained within 80% to 125%. Similarly, absence of an effect of co-administration of 1 mg entecavir on adefovir Cmax and AUC(TAU) was concluded if the 90% CIs for the ratios of the geometric means with and without concomitant entecavir were contained within 80% to 125%. These CIs were constructed from the results of analyses of variance on log(Cmax) and log(AUC(TAU)) of entecavir and adefovir. Summary statistics were tabulated for all pharmacokinetic parameters.

Study Population Results:
Twenty-six (26) subjects were enrolled and 22 (85%) completed treatment. One (1) subject discontinued during the second treatment period due to a death in the family. Two (2) subjects were discharged during the second treatment period because they were argumentative and disrupting the other subjects. A fourth subject exhibited increased anger after losing his wallet

and required local police to escort him out of the clinic after being discharged from the study; he was discharged during the third treatment period, after receiving 6 doses of entecavir co-administered with adefovir. The disruptive behavior exhibited by the three subjects was in the opinion of the investigator not related to study drug.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463063*	
Age (yr)	26 (18 – 43)
Gender N (%)	18 Males (69%) 8 Females (31%)
Weight (kg)	75.1 (51.5 – 95.3)
Height (cm)	172.4 (158.0 – 186.7)
BMI (kg/m^2)	25.3 (19.6 – 29.1)
Race N (%)	15 White (58%) 10 Black (38%) 1 Hispanic/Latino (4%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of adefovir in healthy subjects are presented in Figure 1. Mean trough concentrations of entecavir in plasma following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of adefovir in healthy subjects are presented in Figure 2. Entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of adefovir in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of adefovir in healthy subjects is presented in Table 2.

Mean concentration-time profiles for adefovir in plasma following multiple oral doses of 10 mg adefovir QD both with and without multiple doses of entecavir in healthy subjects are presented in Figure 3. Adefovir plasma pharmacokinetic parameters following multiple oral doses of 10 mg adefovir QD both with and without multiple doses of entecavir in healthy subjects are summarized in Table 3. A summary of statistical analysis of adefovir plasma pharmacokinetic parameters following multiple oral doses of 10 mg adefovir QD both with and without multiple doses of entecavir in healthy subjects is presented in Table 4.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Adefovir in Healthy Subjects.

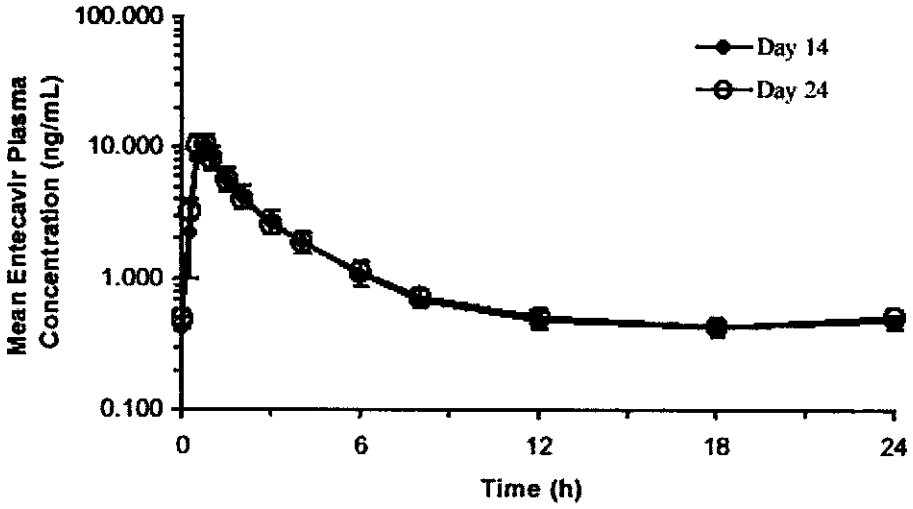
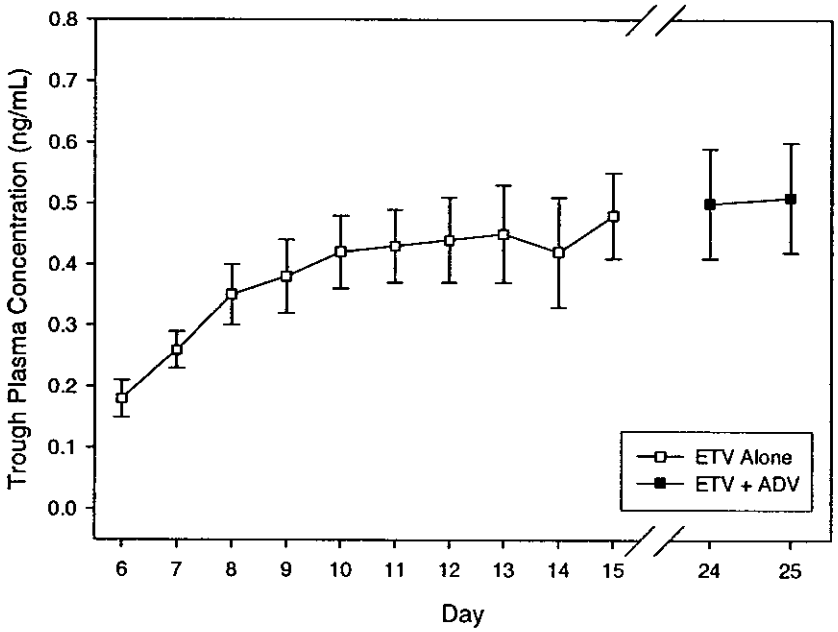


Figure 2 Mean (SD) Trough Concentrations of Entecavir in Plasma Following Multiple Oral Doses of 1 mg Entecavir QD with and without Multiple Doses of Adefovir in Healthy Subjects.



ETV entecavir
ADV adefovir

Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Adefovir in Healthy Subjects.

Parameter	Entecavir Alone (Day 14) N=22	Entecavir + Adefovir (Day 24) N=22
Cmax (ng/mL)	10.76 (18)	11.48 (14)
AUC(TAU) (ng•h/mL)	30.52 (13)	31.39 (12)
Cmin (ng/mL)	0.47 (14)	0.51 (17)
Tmax[a] (hr)	0.75	0.50
UR[b] (%)	58.82 (19.74)	59.70 (22.61)
CLR[b] (mL/min)	323.86 (116.21)	312.89 (112.59)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 14: 1 mg entecavir QD (reference)
Day 24: 10 mg adefovir QD and 1 mg entecavir QD (test)

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 24) and without (Day 14) Adefovir in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means Point Estimate (90% CI) (Day 24 vs. Day 14)
	Entecavir Alone (Day 14) N=22	Entecavir + Adefovir (Day 24) N=22	
Cmax (ng/mL)	10.76	11.48	1.067 (0.998, 1.141)
AUC(TAU) (ng•h/mL)	30.52	31.39	1.029 (1.005, 1.053)
Cmin (ng/mL)	0.47	0.51	1.077 (1.019, 1.139)

Day 14: 1 mg entecavir QD (reference)
Day 24: 10 mg adefovir QD and 1 mg entecavir QD (test)

Figure 3 Mean Concentrations of Adefovir in Plasma Following Administration of Multiple Oral Doses of 10 mg Adefovir QD with (Day 24) and without (Day 4) Entecavir in Healthy Subjects.

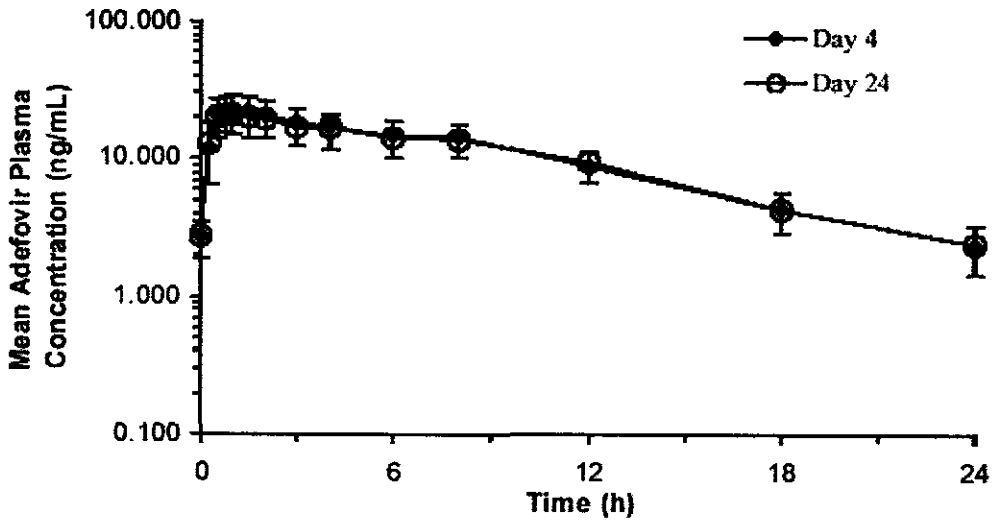


Table 3 Summary of Adefovir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 10 mg Adefovir QD with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	**Adefovir Alone (Day 4) N=22**	**Adefovir + Entecavir (Day 24) N=22**
Cmax (ng/mL)	25.65 (24)	21.94 (24)
AUC(TAU) (ng•h/mL)	236.71 (24)	228.74 (26)
Cmin (ng/mL)	2.12 (41)	2.22 (40)
Tmax[a] (hr)	1.00 —	0.75 —
UR[b] (%)	19.31 (6.39)	21.52 (7.24)
CLR[b] (mL/min)	136.16 (46.62)	154.66 (45.32)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 4: 10 mg adefovir QD (reference)
Day 24: 10 mg adefovir QD and 1 mg entecavir QD (test)

Table 4 Summary of Statistical Analysis of Adefovir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 10 mg Adefovir QD with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means Point Estimate (90% CI) (Day 24 vs. Day 4)
	Adefovir Alone (Day 4)	Adefovir + Entecavir (Day 24)	
Cmax (ng/mL)	25.65	21.95	0.856 (0.803, 0.912)
AUC(TAU) (ng•h/mL)	236.71	228.74	0.966 (0.897, 1.041)
Cmin (ng/mL)	2.13	2.23	1.047 (0.916, 1.197)

Day 4: 10 mg adefovir QD (reference)
Day 24: 10 mg adefovir QD and 1 mg entecavir QD (test)

Assessment/Conclusion:

- Both adefovir and entecavir are eliminated predominantly unchanged in urine by a process that includes net tubular secretion. As renal tubular secretion and reabsorption may involve saturable and/or competitive processes and co-administration of adefovir and entecavir in the HBV population is foreseeable, the current study was undertaken to investigate potential pharmacokinetic interactions of the two drugs at steady state after multiple doses. This study used the highest anticipated daily dose of entecavir (1 mg), and the selected dosage of adefovir for this study was its labeled daily dose of 10 mg QD.
- No effect of co-administration of adefovir on entecavir pharmacokinetics was observed. The adjusted geometric means for entecavir AUC(TAU), Cmax, and Cmin when co-administered with adefovir were similar to those values for entecavir alone, and the corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Individual entecavir concentration-time profiles both with and without adefovir were practically super-imposable, indicative of a lack of pharmacokinetic interaction. Absorption (Tmax) and renal excretion of entecavir (%UR and CLR) were also comparable between treatments. Based on the Cmin values, entecavir appeared to reach steady-state following 5-10 days of 1 mg QD dosing.
- No effect of co-administration of entecavir on adefovir pharmacokinetics was observed. The adjusted geometric means for adefovir AUC(TAU), Cmax, and Cmin when co-administered with entecavir were similar to those values for adefovir alone, and the corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Minimal differences were observed between individual adefovir concentration-time profiles both with and without entecavir, supporting a lack of pharmacokinetic interaction. Absorption (Tmax) and renal excretion of adefovir (%UR and CLR) were also comparable between treatments.
- Overall, the statistical analyses of entecavir and adefovir pharmacokinetic parameters indicated a lack of pharmacokinetic interaction upon multiple dosing; therefore, entecavir and adefovir may be co-administered without the need for dose modification.

4.1.3.3. Open-Label, Sequential Design, Drug Interaction Study of Entecavir and Tenofovir in Healthy Subjects (Protocol AI463066).

Objectives:

- Primary: to assess the effect of tenofovir on the pharmacokinetics of entecavir and to assess the effect of entecavir on the pharmacokinetics of tenofovir.
- Secondary: 1) to assess the safety of entecavir and tenofovir when administered alone or in combination, and 2) to assess the effect of entecavir on cytochrome P450 (CYP) 3A4 enzyme induction as measured by urinary 6β-hydroxycortisol (6β-OHC)-to-cortisol (COR) ratios.

Study Design:

This was an open-label, multiple-dose, sequential design study in 34 healthy subjects. Subjects received 300 mg tenofovir once daily (QD) on Days 1 to 5, 1 mg entecavir QD on Days 6 to 15, and 1 mg entecavir QD plus 300 mg tenofovir on Days 16 to 25 in the fasted state. Blood samples for pharmacokinetic analysis were collected up to Day 25. Twenty-four- (24-) hour urine samples for pharmacokinetic analysis were collected on Days 5, 15, and 25. Subjects were discharged from the study upon completion of study procedures on Day 26.

For assessment of CYP3A4 induction potential, a 24-hour urine sample was obtained on Days -1, 5, 10, and 15 for determination of 6β-OHC to COR ratios. On Days 5 and 15, aliquots from the 24-hour urine collection were used for both the pharmacokinetics of the administered drug and the assessment of CYP3A4 induction potential.

Formulations:

Entecavir tablets were supplied by the Sponsor as 0.5-mg — white — , film-coated tablets packaged in bottles of 25 tablets/bottle. The product label batch number for entecavir tablets was 8MDE141 and the identification number 200475-K0X5-039, with an expiration date of 30-Apr-2005. Tenofovir (Viread®) was provided by the investigator. Each tenofovir tablet contained 300 mg tenofovir disoproxil fumarate. The lot number for tenofovir was FBK091 with an expiration date of Feb-2005.

Pharmacokinetic Measurements:

Blood (plasma) and urine samples were collected at specified times for the analyses of concentrations of entecavir and/or tenofovir.

- Blood samples for plasma entecavir were obtained as follows:
  - Days 15 and 25: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 6, 8, 10, 12 to 14, 16, 18, 20, and 22 to 26: predose (trough) sampling.
- Blood samples for plasma tenofovir were obtained as follows:
  - Days 5 and 25: prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following dosing;
  - Days 1 to 6, 16, 18, 20, and 22 to 26: predose (trough) sampling.
- Urine samples were collected predose on Day 1 and up to 24 hours post-dose on Days 5 and 25 for tenofovir and Days 15 and 25 for entecavir over the following intervals relative to drug administration:
  - Prior to dosing and 0-24 hours following dosing.
- 24-hour urine samples were collected on Days -1, 5, 10, and 15 for determination of 6β-OHC to COR ratios.

Pharmacokinetic/Statistical Analysis:
Entecavir and tenofovir pharmacokinetic parameter values were calculated by noncompartmental methods, and total urinary recovery over the 24-hour interval was assessed. Absence of an effect of co-administration of tenofovir on entecavir Cmax and AUC(TAU) was concluded if the 90% confidence intervals (CIs) for the ratios of the geometric means with and without concomitant tenofovir were contained within 80% to 125%. Similarly, absence of an effect of co-administration of entecavir on tenofovir Cmax and AUC(TAU) was concluded if the 90% CIs for the ratios of the geometric means with and without concomitant entecavir were contained within 80% to 125%. These CIs were constructed from the results of analyses of variance on log(Cmax) and log(AUC(TAU)) of entecavir and tenofovir. Summary statistics were tabulated for all pharmacokinetic parameters.

Study Population Results:
Thirty-four (34) subjects were enrolled and 28 (82%) completed the study. Six subjects discontinued the trial early. Two subjects discontinued on Day 7 and Day 8, respectively, due to personal reasons. Two subjects discontinued on Day 9 and on Day 7, respectively, due to withdrawal of consent. Two subjects were discontinued on Day 12 due to the termination of the study by BMS at the request of the FDA to stop all multiple dose studies of entecavir in healthy subjects.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463066*	
Age (yr)	30 (18 – 45)
Gender N (%)	32 Males (94%) 2 Females (6%)
Weight (kg)	78.8 (60.8 – 106.6)
Height (cm)	177.5 (160.0 – 198.1)
BMI (kg/m^2)	25.0 (20.2 – 29.4)
Race N (%)	28 White (82%) 6 Black (18%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of tenofovir in healthy subjects are presented in Figure 1. Mean trough concentrations of entecavir in plasma following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of tenofovir in healthy subjects are presented in Figure 2. Entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of tenofovir in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following multiple oral doses of 1 mg entecavir QD both with and without multiple doses of tenofovir in healthy subjects is presented in Table 2.

Mean concentration-time profiles for tenofovir in plasma following multiple oral doses of 10 mg tenofovir QD both with and without multiple doses of entecavir in healthy subjects are presented in Figure 3. Mean trough concentrations of tenofovir in plasma following multiple oral doses of 300 mg tenofovir QD both with and without multiple doses of entecavir in healthy subjects are

presented in Figure 4. Tenofovir plasma pharmacokinetic parameters following multiple oral doses of 300 mg tenofovir QD both with and without multiple doses of entecavir in healthy subjects are summarized in Table 3. A summary of statistical analysis of tenofovir plasma pharmacokinetic parameters following multiple oral doses of 300 mg tenofovir QD both with and without multiple doses of entecavir in healthy subjects is presented in Table 4.

Summary statistics for urinary 6β-OHC, COR, and 6β-OHC/COR ratios are summarized in Table 5. A summary of statistical analysis of urinary 6β-OHC/COR ratios is presented in Table 6.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 25) and without (Day 15) Tenofovir in Healthy Subjects.

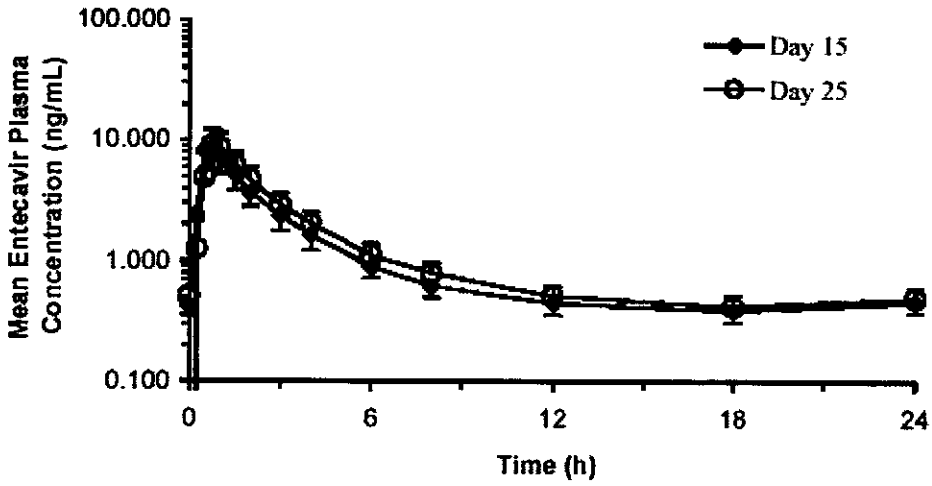
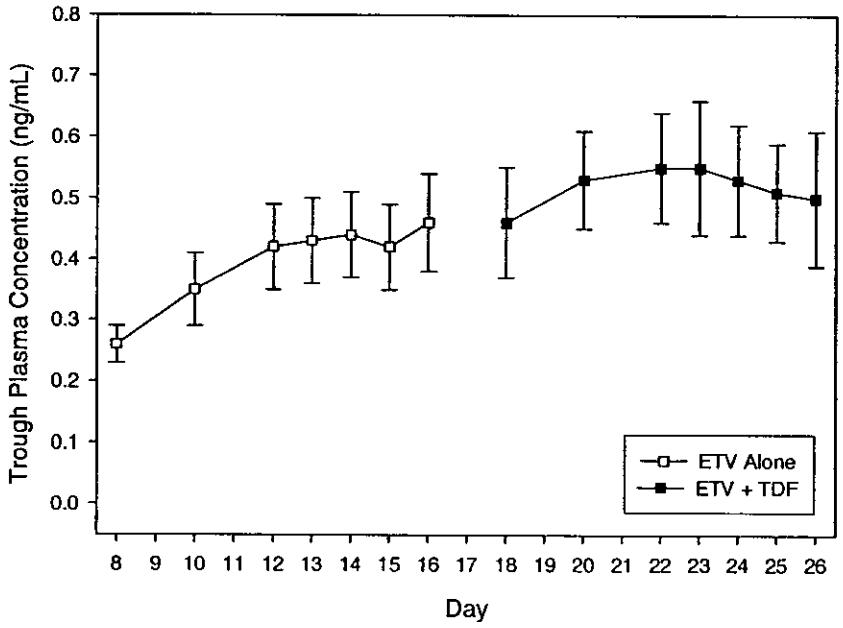


Figure 2 Mean (SD) Trough Concentrations of Entecavir in Plasma Following Multiple Oral Doses of 1 mg Entecavir QD with and without Multiple Doses of Tenofovir in Healthy Subjects.



ETV entecavir
TDF tenofovir

Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 25) and without (Day 15) Tenofovir in Healthy Subjects.

Parameter	Entecavir Alone (Day 15)	Entecavir + Tenofovir (Day 25)
Cmax (ng/mL)	9.69 (22)	9.55 (26)
AUC(TAU) (ng•h/mL)	27.02 (15)	30.45 (16)
Cmin (ng/mL)	0.45 (18)	0.48 (22)
Tmax[a] (hr)	0.63 —	0.75 —
UR[b] (%)	61.42 (14.50)	60.61 (15.80)
CLR[b] (mL/min)	384.72 (119.70)	335.35 (109.13)

N = 28
Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 15: 1 mg entecavir QD (reference)
Day 25: 300 mg tenofovir QD and 1 mg entecavir QD (test)

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Daily Doses of 1 mg Entecavir with (Day 25) and without (Day 15) Tenofovir in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means Point Estimate (90% CI) (Day 25 vs. Day 15)
	Entecavir Alone (Day 15)	Entecavir + Tenofovir (Day 25)	
Cmax (ng/mL)	9.69	9.55	0.985 (0.905, 1.073)
AUC(TAU) (ng•h/mL)	27.02	30.45	1.127 (1.106, 1.149)
Cmin (ng/mL)	0.45	0.48	1.071 (1.014, 1.132)

Day 15: 1 mg entecavir QD (reference)
Day 25: 300 mg tenofovir QD and 1 mg entecavir QD (test)

Figure 3 Mean Concentrations of Tenofovir in Plasma Following Administration of Multiple Oral Doses of 300 mg Tenofovir QD with (Day 25) and without (Day 5) Entecavir in Healthy Subjects.

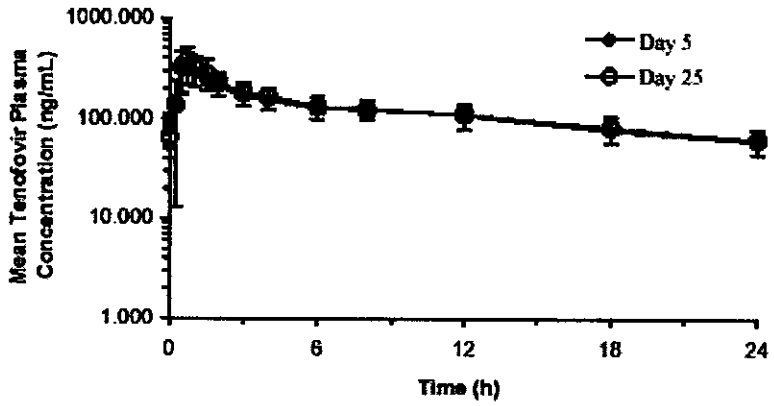
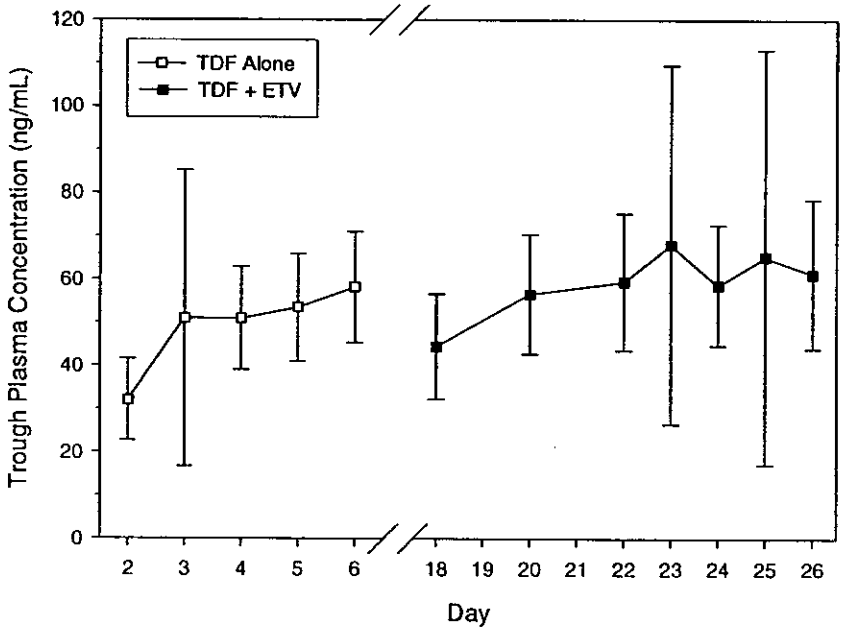


Figure 4 Mean (SD) Trough Concentrations of Tenofovir in Plasma Following Multiple Oral Doses of 300 mg Tenofovir QD with and without Multiple Doses of Entecavir in Healthy Subjects.



ETV entecavir
TDF tenofovir

Table 3 Summary of Tenofovir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 300 mg Tenofovir QD with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	Tenofovir Alone (Day 5)	Tenofovir + Entecavir (Day 25)
Cmax (ng/mL)	390.59 (31)	393.66 (33)
AUC(TAU) (ng•h/mL)	2728.18 (19)	2863.49 (22)
Cmin (ng/mL)	56.78 (22)	59.00 (28)
Tmax[a] (hr)	0.63	0.75
UR[b] (%)	11.05 (3.78)	11.25 (4.31)
CLR[b] (mL/min)	202.88 (73.87)	197.19 (82.64)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).
Day 5: 300 mg tenofovir QD (reference)
Day 25: 300 mg tenofovir QD and 1 mg entecavir QD (test)

Table 4 Summary of Statistical Analysis of Tenofovir Plasma Pharmacokinetic Parameters Following Administration of Multiple Oral Doses of 300 mg Tenofovir QD with and without Multiple Doses of Entecavir in Healthy Subjects.

Parameter	Adjusted Geometric Means		Ratios of Adjusted Geometric Means: Point Estimate (90% CI) (Day 25 vs. Day 5)
	Tenofovir Alone (Day 5)	Tenofovir + Entecavir (Day 25)	
Cmax (ng/mL)	390.60	393.66	1.008 (0.934, 1.087)
AUC(TAU) (ng•h/mL)	2728.18	2863.49	1.050 (1.000, 1.102)
Cmin (ng/mL)	56.78	59.00	1.039 (0.980, 1.102)

Day 5: 300 mg tenofovir QD (reference)
Day 25: 300 mg tenofovir QD and 1 mg entecavir QD (test)

Table 5 Summary Statistics for Urinary 6β-OHC, COR, and 6β-OHC/COR Ratios.

Parameter	Day -1 (N=34)	Day 5 (N=34)	Day 10 (N=31)	Day 15 (N=27)
6β-OHC	124.21 (62)	91.50 (74)	90.46 (76)	74.56 (89)
COR	16.33 (57)	12.96 (86)	11.61 (64)	10.01 (59)
6β-OHC/COR	7.61 (35)	7.06 (41)	7.79 (44)	7.45 (38)

Data presented as geometric mean (CV%).
Day 5: 5 days after tenofovir 300 mg QD dosing
Day 10: 5 days after entecavir 1 mg QD dosing
Day 15: 10 days after entecavir 1 mg QD dosing

Table 6 Summary of Statistical Analysis of Urinary 6β-OHC/COR Ratios.

Day	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means: Point Estimate (90% CI)
Day -1 (N=34)	7.608	-	-
Day 5 (N=34)	7.058	Day 5 vs. Day -1	0.928 (0.841, 1.022)
Day 10 (N=31)	7.976	Day 10 vs. Day -1	1.048 (0.947, 1.160)
Day 15 (N=27)	7.553	Day 15 vs. Day -1	0.993 (0.895, 1.102)

Day 5: 5 days after tenofovir 300 mg QD dosing
Day 10: 5 days after entecavir 1 mg QD dosing
Day 15: 10 days after entecavir 1 mg QD dosing

Assessment/Conclusion:

- Both tenofovir and entecavir are primarily eliminated by the kidneys, a combination of glomerular filtration and active tubular secretion. As renal tubular secretion and reabsorption may involve saturable and/or competitive processes and co-administration of tenofovir and entecavir in the HBV population is foreseeable, the current study was undertaken to investigate potential pharmacokinetic interactions of the two drugs after multiple doses. This study used the highest anticipated daily dose of entecavir (1 mg), and the selected dosage of tenofovir for this study was its labeled daily dose of 300 mg QD.
- No effect of co-administration of tenofovir on entecavir pharmacokinetics was observed. The adjusted geometric means for entecavir AUC(TAU), Cmax, and Cmin when co-administered with tenofovir were similar to those values for entecavir alone, and the corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Minimal differences were observed between individual entecavir concentration-time profiles both with and without tenofovir, supporting a lack of pharmacokinetic interaction. Absorption (Tmax) and renal excretion of entecavir (%UR and CLR) were also comparable between treatments. Based on the Cmin values, entecavir appeared to reach steady-state following 6-10 days of 1 mg QD dosing.
- No effect of co-administration of entecavir on tenofovir pharmacokinetics was observed. The adjusted geometric means for tenofovir AUC(TAU), Cmax, and Cmin when co-administered with entecavir were similar to those values for tenofovir alone, and the

corresponding 90% CIs satisfied the pre-specified criteria for lack of effect. Absorption (Tmax) and renal excretion of tenofovir (%UR and CLR) were also comparable between treatments.

- Neither tenofovir nor entecavir had an apparent induction effect on CYP3A4 following 10 days of 1 mg/day dosing and 5 days of 300 mg/day dosing, respectively, based on urinary 6β-OHC/COR ratios. These findings are consistent with the results from an in vitro entecavir CYP induction study using freshly isolated primary cultured human hepatocytes showing that entecavir does not induce CYP1A2, 2B6, 2C9, 2C19, and 3A4/5.
- Overall, the statistical analyses of entecavir and tenofovir pharmacokinetic parameters indicated a lack of pharmacokinetic interaction upon multiple dosing, therefore entecavir and tenofovir may be co-administered without the need for dose modification.

4.1.4. General Biopharmaceutics

4.1.4.1. Effect of a High Fat Meal and a Light Meal on the Pharmacokinetics of Entecavir in Healthy Subjects (Protocol AI463016).

Objectives:

- Primary: to assess the effect of a light meal or high-fat meal on the pharmacokinetics of entecavir in healthy subjects.
- Secondary: to assess the safety of entecavir.

Study Design:

This was an open-label, randomized, three-period, three-treatment, crossover study balanced for carryover effects in healthy subjects. A total of 42 subjects were randomized in Period 1 to one of six sequences to receive a single oral dose of 0.5 mg entecavir in one of three treatments; in a fasted condition (Treatment A), within 5 minutes after consuming a light meal (Treatment B; approximately 20% of total caloric content of the meal is fat), or within 5 minutes after consuming a standard high-fat breakfast (Treatment C; approximately 52% of total caloric content of the meal is fat). A description of the high-fat breakfast and the light breakfast is presented in Appendix 1 and 2. The alternate treatments were administered in Periods 2 and 3. A washout period of at least 7 days separated each dose. For each treatment period, subjects were admitted to the clinical facility the evening prior to dosing (Day -1) and were confined until 72 hours post-dose. Blood samples were collected for pharmacokinetic analysis up to 72 hours post-dose. Subjects were discharged from the study on Day 4 of Period 3.

Formulations:

Entecavir tablets were supplied as 0.5 mg — , white — tablets packaged in bottles of 25 tablets/bottle. The batch number for entecavir tablets was N01024, with an expiration date of 31-May-2002. Each subject received 0.5 mg entecavir as a single oral dose for each period.

Pharmacokinetic Measurements:

Blood (plasma) samples were collected at the following specified times during each period for the analyses of concentrations of entecavir:

- Prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, and 72 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Entecavir pharmacokinetic parameter values were calculated by noncompartmental methods. "Absence of a food effect" on AUC(0-T) or Cmax was concluded if the corresponding 90% confidence interval (CI) for the ratio of fed to fasted population geometric means was contained within an equivalence interval from 80% to 125%. "Presence of a food effect" was concluded if the corresponding 90% CI was entirely outside the equivalence interval. The CIs were constructed from the results of analyses
of variance on log(AUC(0-T)) and log(Cmax). Summary statistics were tabulated by treatment for other pharmacokinetic parameters.

Study Population Results:
Forty-two (42) subjects were enrolled and randomized to treatment in this study. Of these 42 subjects, 37 (88%) completed treatment and 5 (12%) discontinued from the study early. Two (2) subjects withdrew consent during Period 1 (one subject had received Treatment A, and the other had received Treatment B), two subjects discontinued to go into active military duty (during Period 3 after receiving Treatment C), and one subject discontinued during Period 2 due to noncompliance (inability to ingest the high-fat diet; he had not received entecavir during Period 2 but received Treatment B in Period 1).

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463016*	
Age (yr)	31 (18 – 45)
Gender N (%)	35 Males (83%) 7 Females (17%)
Weight (kg)	76.3 (54.5 – 97.3)
Height (cm)	176.6 (160.0 – 189.2)
BMI (kg/m^2)	24.5 (18.0 – 29.9)
Race N (%)	34 White (81%) 7 Black (17%) 1 Asian/Pacific Islander (2%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 0.5 mg entecavir in the fasted and fed (high fat and light meals) states in healthy subjects are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg entecavir in the fasted and fed (high fat and light meals) states in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg entecavir in the fasted and fed (high fat and light meals) states in healthy subjects is presented in Table 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 0.5 mg Entecavir in the Fasted and Fed (High Fat and Light Meals) States in Healthy Subjects.

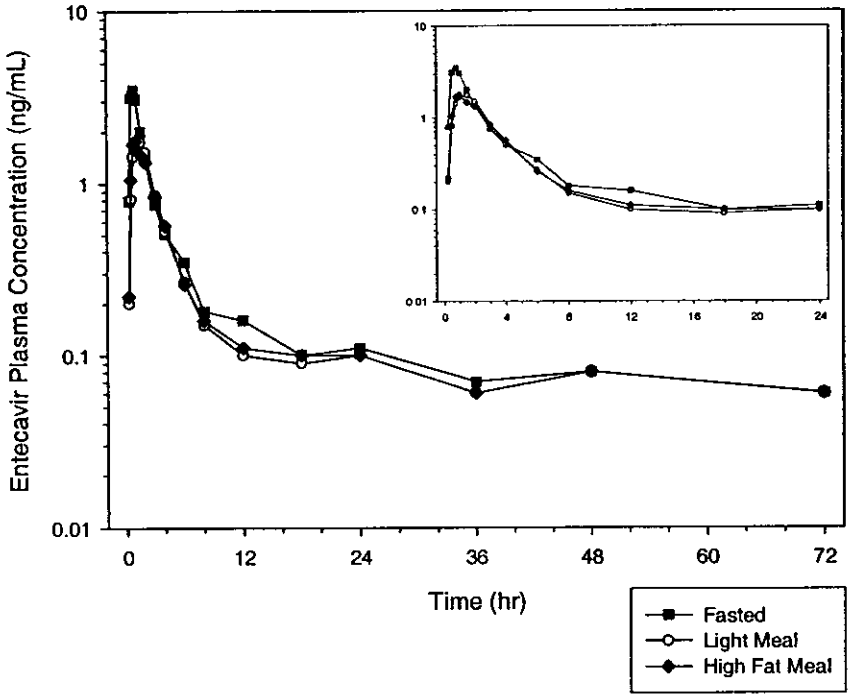


Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg Entecavir in the Fasted and Fed (High Fat and Light Meals) States in Healthy Subjects.

Parameter	Fasted (N=39)	Light Meal (N=39)	High-Fat Meal (N=37)
Cmax (ng/mL)	3.9 (34)	2.2 (36)	2.1 (41)
AUC(0-T) (ng•h/mL)	12.7 (20)	10.2 (22)	10.3 (23)
Tmax[a] (hr)	0.75	1.5	1.0

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg in the Fasted and Fed (High Fat and Light Meals) States Entecavir in Healthy Subjects.

Parameter	Treatment	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means Point Estimate (90% CI)
Cmax (ng/mL)	A	3.873	-	-
	B	2.188	B vs. A	0.5649 (0.5085, 0.6277)
	C	2.091	C vs. A	0.5397 (0.4850, 0.6007)
AUC(0-T) (ng•h/mL)	A	12.692	-	-
	B	10.141	B vs. A	0.7990 (0.7693, 0.8298)
	C	10.382	C vs. A	0.8180 (0.7870, 0.8502)

Treatment A: Fasted
Treatment B: Light Meal
Treatment C: High-Fat Meal

Assessment/Conclusion:

- In a previous study (AI463003), a food effect on entecavir pharmacokinetics was demonstrated when a single dose of 20 mg entecavir was administered as capsules with a high-fat meal. To assess the effect of food on entecavir pharmacokinetics when entecavir is given at a projected therapeutic dose (0.5 mg) as a tablet, the current study was undertaken with either a high-fat or a light meal in two separate periods. Per FDA guidance, in general, the highest strength of a drug product intended to be marketed should be tested in a food effect study. The highest intended clinical dose of entecavir is 1 mg in lamivudine-refractory patients, and no safety concerns exist that would preclude its use in a food effect study. Entecavir demonstrates linear pharmacokinetics at doses up to 20 mg and consistent variability in exposure across doses, therefore the results from the current study utilizing 0.5 mg entecavir are applicable to the highest clinical dose of 1 mg.
- Similar to the reported results in Study AI463003, food decreased the rate and the extent of absorption of entecavir. For both the light and high-fat meals, the 90% CI of the ratio of the geometric means for Cmax fell entirely below the established criteria of 0.80 to 1.25, while only the lower bound of 90% CI fell below the range of 0.80 to 1.25 for AUC. The light and high-fat meals reduced Cmax by 44% and 46%, and AUC by 20% and 18%, respectively, compared to the fasted treatment. In conclusion, both the light and high-fat meals had a clear effect on the pharmacokinetic parameter Cmax. The Applicant states the food effect on AUC was indeterminate. Per FDA guidance, an absence of food effect is not established if the 90% CI for the ratio of population geometric means is not contained in the equivalence limits of 80-125%, therefore it should also be concluded both the light and high-fat meals had an effect on the pharmacokinetic parameter AUC(0-T). The study results also demonstrated that there is no apparent difference in such food effect between high-fat and light meals.
- The Applicant states the clinical significance of this food effect on the systemic exposure of entecavir is unknown. The change in log HBV DNA over time has been shown to be exposure dependent with entecavir (Study AI463017). In addition, in order to achieve a pharmacodynamic response comparable to that observed in nucleoside-naïve patients following 0.5 mg dosing, a higher dose of entecavir (1 mg) is required. Therefore, the clinical significance of an approximate 45% decrease in entecavir Cmax and 20% decrease in AUC could be a diminished response to entecavir, especially in lamivudine refractory patients. As stated in the proposed labeling, entecavir should be administered on an empty stomach, at least 2 hours before and at least 2 hours after a meal.

Appendix 1 Content of High-Fat Breakfast

Food Item	Calories[a] (kcal)	Fat[a] (g)	Carbohydrates[a] (g)	Protein[a] (g)
2 eggs fried in butter	184	13.8	1.2	12.4
2 slices white bread toasted	134	1.8	24.8	4.1
1 tablespoon butter	102	11.5	trace	0.1
1 tablespoon jelly	52	trace	13.5	0.1
2 strips bacon	72	6.2	trace	3.8
4 ounces hash brown potatoes	244	12.4	31.5	3.6
8 ounces whole milk	157	8.9	11.4	8.0
Total	945	54.6	82.4	32.1
Calories		487 kcal	330 kcal	128 kcal
% of Total Calories		51.5%	34.9%	13.6%

[a] US Department of Agriculture Nutrient Database for Standard Reference, Release 12 (March 1998)

Appendix 2 Content of Light Breakfast

Food Item	Calories	Fat (g)	Carbohydrates (g)	Protein (g)
2 slices white bread toasted	134	1.8	23.4	4.2
1 teaspoonful low fat margarine	50	6.0	trace	0
1 tablespoon jelly	55	trace	14.1	trace
5 oz orange juice	70	0.1	16.4	1.1
5 oz of skim milk	70	0.25	7.4	5.3
Total	379	8.2	61.3	10.6
% Total Calories	100	20	68	12

4.1.4.2. Bioequivalence Study of Entecavir Tablets Relative to Entecavir Capsules in Healthy Subjects (Protocol AI463034).

Objectives:

- Primary: to demonstrate the bioequivalence of entecavir tablet with entecavir capsule.
- Secondary: to assess the safety of entecavir when administered as a single oral dose.

Rationale:

Entecavir was initially manufactured as capsules for use in Phase I/II clinical trials. A film-coated tablet formulation was developed in strengths of 0.1, 0.5, and 1 mg using a [redacted], for later Phase I and Phase II studies, and all the Phase III clinical studies. The tablet formulation intended for marketing differs only in shape and color. The purpose of this study was to demonstrate the bioequivalence of the tablets with the current capsules.

Study Design:
This was an open-label, randomized, two-period, two-treatment, crossover study in healthy subjects. Forty (40) subjects were randomized to receive a single oral dose of 0.5 mg entecavir capsule (Treatment A) and a single oral dose of 0.5 mg entecavir tablet (Treatment B) in randomized order. For each treatment period, subjects were admitted to the clinical facility the evening prior to dosing (Day -1) and were confined until 72 hours post-dose. Blood samples were collected for pharmacokinetic analysis up to 72 hours post-dose. Subjects were discharged from the study on Day 4 of Period 2. There was a 7-day washout period between each dose. Each subject fasted for at least 8 hours prior to oral administration of entecavir.

Study Site:

Formulations:
- Test Product: Entecavir tablets were supplied as 0.5 mg — white — tablets packaged in bottles of 25 tablets/bottle. The batch number for entecavir tablets was N01024, with an expiration date of 31-May-2002.
- Reference Therapy: Entecavir capsules were supplied as 0.5 mg — capsules packaged in bottles of 25 capsules/bottle. The batch number for entecavir capsules was N00242 with an expiration date of 31-Jan-2003.

Pharmacokinetic Measurements:
Blood (plasma) samples were collected at the following specified times during each Period for the analyses of concentrations of entecavir:
- Prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, and 72 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Single dose entecavir pharmacokinetic parameter values were calculated by noncompartmental methods. Bioequivalence was concluded if the 90% CIs for the ratios of population geometric means of the tablet to the capsule were contained within 80% to 125% for Cmax and AUC(0-T). The confidence intervals were constructed from the results of analyses of variance on log(Cmax) and log(AUC(0-T)). Medians, minima, and maxima were reported for Tmax by formulation.

Study Population Results:
Forty (40) subjects were enrolled and randomized to treatment in this study. Of these 40 subjects, 37 (92.5%) completed treatment and 3 (7.5%) discontinued from the study early. One subject discontinued for personal reasons after receiving Treatment A, one subject for personal reasons after Treatment B, and one subject due to an adverse event after receiving Treatment B (upper respiratory tract infection; considered by Investigator to be unrelated to study drug).

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463034*	
Age (yr)	26 (19 – 43)
Gender N (%)	23 Males (57.5%) 17 Females (42.5%)
Weight (kg)	70.6 (50.9 – 111.6)
Height (cm)	173.7 (154.9 – 198.1)
BMI (kg/m^2)	23.5 (18.7 – 28.8)
Race N (%)	33 White (82.5%) 7 Black (17.5%)

*Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 0.5 mg tablet and capsule formulations of entecavir in healthy subjects are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg tablet and capsule formulations of entecavir in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg tablet and capsule formulations of entecavir in healthy subjects is presented in Table 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 0.5 mg Tablet and Capsule Formulations of Entecavir in Healthy Subjects.

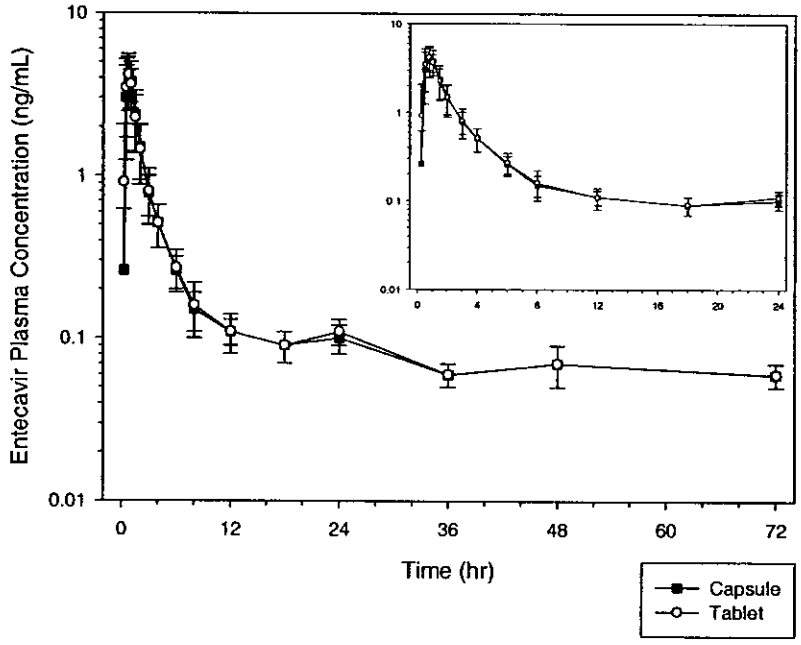


Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg Tablet and Capsule Formulations of Entecavir in Healthy Subjects.

Parameter	Capsule (N=37)	Tablet (N=37)
Cmax (ng/mL)	4.38 (32)	4.47 (26)
AUC(0-T) (ng•h/mL)	12.57 (21)	12.84 (21)
Tmax[a] (hr)	0.75 —	0.75 —

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg Tablet and Capsule Formulations of Entecavir in Healthy Subjects.

Parameter	Treatment	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means Point Estimate (90% CI)
Cmax (ng/mL)	Capsule	4.38	Tablet vs. Capsule	1.0186 (0.9535, 1.0881)
	Tablet	4.46		
AUC(0-T) (ng•h/mL)	Capsule	12.59	Tablet vs. Capsule	1.0177 (0.9999, 1.0358)
	Tablet	12.81		

Assessment/Conclusion:

- The tablet formulation is currently being used in all Phase III clinical trials and will be the formulation intended for marketing. The results from this study have demonstrated that 0.5 mg entecavir tablet formulation is bioequivalent to 0.5 mg entecavir capsule formulation.
- The projected dose regimens of entecavir are 0.5 mg and 1 mg QD for nucleoside naïve patients and those with viremia on lamivudine, respectively. The manufacture of entecavir 0.5 and 1 mg strength formulation is scalable for both tablet and capsule in that their excipient composition and the ratio are identical for both strengths.

4.1.4.3. Bioequivalence Study of Entecavir Oral Solution Relative to Entecavir Tablet in Healthy Subjects (Protocol AI463035).

Objectives:

- Primary: to demonstrate the bioequivalence of entecavir oral solution relative to entecavir tablet with respect to area under the plasma concentration-time curve (AUC) from time zero to the time of last quantifiable concentration [AUC(0-T)].
- Secondary: to assess the safety of entecavir when administered as an oral solution or tablet.

Rationale:

Entecavir 0.5 mg tablet is the formulation used in Phase III clinical trials. An oral solution formulation of entecavir was developed for use in special populations such as subjects who need dose modification (eg, pediatric subjects and subjects with renal impairment) and subjects who have difficulty with tablet administration (eg subjects on enteral tubes). The purpose of this

study was to demonstrate the bioequivalence of the oral solution of entecavir with the current tablet formulation.

Study Design:
This was an open-label, randomized, two-period, two-treatment, crossover study in 24 healthy subjects. Subjects were to receive the following 2 treatments in one of two randomly assigned treatment sequences:

- 0.5 mg entecavir tablet formulation (administered as one 0.5 mg tablet)
- 0.5 mg entecavir solution formulation (administered as a 10 mL dose at a concentration of 0.05 mg/mL)

Subjects were admitted to the clinical facility the evening prior to dosing for each period (Day -1). Treatments were to be administered on Day 1 of each period according to the randomization schedule. For each period, subjects remained in the clinical facility for 72 hours after dosing. Subjects returned the clinical unit on Days 6, 8, 10, and 12 for collection of pharmacokinetic samples. A washout period of at least 14 days separated each dose. Subjects were discharged from the study on Day 12 of Period 2. Blood samples for pharmacokinetic assessment of entecavir were collected from pre-dose to 264 hours post-dose. Each subject fasted for 10 hours prior until 4 hours after study drug administration.

Study Site:

Formulations:

- Test Product: Entecavir oral solution was supplied as an orange-flavored, clear, colorless to pale yellow, ready-to-use solution in bottles of 120 mL at a concentration of 0.05 mg/mL. Ten (10) mL of entecavir oral solution were administered using dosing syringes that were supplied with the study drug. The label batch, product batch, and product numbers were 2M52826, 8MHE234, and 200475-JX05-058, respectively, with an expiration date of 30-Sep-2003.
- Reference Therapy: Entecavir oral tablets were supplied as 0.5 mg white — film-coated tablets. The label batch, product batch, and product numbers were 2F59620, N01030, and 200475-K0X5-039, respectively, with an expiration date of 29-Feb-2004.

Pharmacokinetic Measurements:
Blood (plasma) samples were collected at the following specified times during each Period for the analyses of concentrations of entecavir:

- Prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 120, 168, 216, and 264 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Single dose entecavir pharmacokinetic parameter values were calculated by noncompartmental methods. Bioequivalence was concluded with respect to AUC(0-T) if the 90% confidence interval for the ratio of population geometric means of 0.5 mg oral solution and 0.5 mg tablet was contained within 80% to 125% for AUC(0-T). In addition, analyses of variance were performed on log(Cmax) and log(AUC(INF)).

Study Population Results:
Twenty-four (24) subjects were enrolled and randomized to treatment in this study. Of these 24 subjects, 22 (91.7%) completed treatment and 2 (8.3%) discontinued from the study after Period 1. One subject never returned for Period 2 and was lost to follow-up, and one subject discontinued due to a death in the family. Both subjects only received the tablet formulation.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463035*	
Age (yr)	31 (20 – 44)
Gender N (%)	23 Males (95.8%) 1 Female (4.2%)
Weight (kg)	79.9 (58.6 – 102.9)
Height (cm)	176.3 (160.0 – 192.0)
BMI (kg/m^2)	25.7 (18.0 – 29.6)
Race N (%)	16 Black (66.7%) 7 White (29.2%) 1 Asian/Pacific Islander (4.2%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:
Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of 0.5 mg as solution and tablet formulations of entecavir in healthy subjects are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg as solution and tablet formulations of entecavir in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following administration of a single oral dose of 0.5 mg as solution and tablet formulations of entecavir in healthy subjects is presented in Table 2.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of 0.5 mg as Solution and Tablet Formulations of Entecavir in Healthy Subjects.

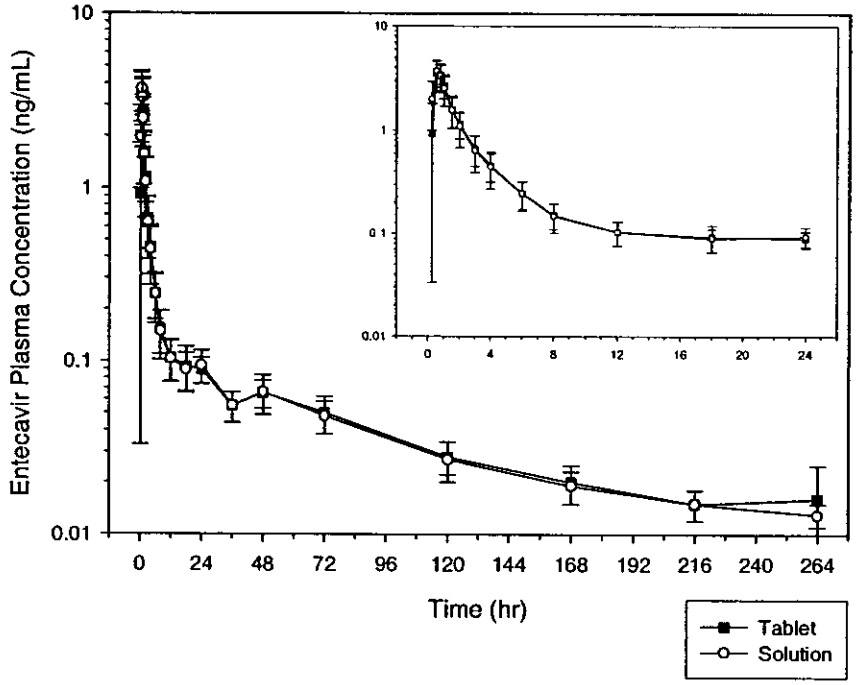


Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg as Solution and Tablet Formulations of Entecavir in Healthy Subjects.

Parameter	Tablet (N=21)	Solution (N=21)
Cmax (ng/mL)	3.79 (23)	3.66 (27)
AUC(INF) (ng•h/mL)	16.52 (22)	16.44 (23)
AUC(0-T) (ng•h/mL)	14.72 (19)	14.29 (26)
Tmax[a] (hr)	0.50 —	0.50 —
T-half[b] (h)	86.14 (40.23)	93.63 (59.88)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of 0.5 mg as solution and Tablet Formulations of Entecavir in Healthy Subjects.

Parameter	Treatment	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means Point Estimate (90% CI)
Cmax (ng/mL)	Tablet	3.803	Solution vs. Tablet	0.974 (0.920, 1.031)
	Solution	3.680		
AUC(0-T) (ng•h/mL)	Tablet	14.707	Solution vs. Tablet	1.001 (0.936, 1.070)
	Solution	14.319		
AUC(INF) (ng•h/mL)	Tablet	16.490	Solution vs. Tablet	0.968 (0.918, 1.021)
	Solution	16.503		

Entecavir 0.5 mg Tablet = Reference
Entecavir 0.5 mg Oral Solution = Test

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Assessment/Conclusion:

- For one subject, none of the plasma samples obtained following the 0.5 mg tablet treatment (Period 2) contained quantifiable concentrations of entecavir. This subject had the expected exposure to entecavir during the first treatment period following administration of the 0.5 mg entecavir solution. Because this subject had no entecavir exposure during one treatment period, this subject's data was removed from the statistical analysis. In addition, all aspects of the study conduct and analysis were reviewed, including subject compliance, bioanalysis of the plasma samples, and release data for the formulations. A review of study conduct did not suggest non-compliance with study procedures. Results of a repeat bioanalytical analysis of samples for this subject were comparable to the original analysis results. Drug product performance was consistent for the tablet formulation and deemed unlikely to be the cause of the anomalous results for the study outlier.
- In conclusion, the lack of exposure observed following administration of the 0.5 mg tablet in Period 2 in the outlier subject suggests that the subject did not swallow the 0.5 mg entecavir tablet. Exclusion of this subject from the statistical analysis for the bioequivalence assessment demonstrates that the entecavir oral solution is bioequivalent, with respect to both AUC and Cmax, to the entecavir tablet formulation. This finding indicates that the solution and tablet formulations may be used interchangeably. The relative bioavailability of entecavir tablet is essentially 100%; thus, entecavir bioavailability is not dissolution rate limited.
- —

4.1.4.4. Bioequivalence Study of a Single Entecavir 1.0 mg Tablet Relative to Two Entecavir 0.5 mg Tablets in Healthy Subjects (Protocol AI463065).

Objectives:

- Primary: to demonstrate bioequivalence of a single entecavir 1.0 mg oral tablet relative to 2 entecavir 0.5 mg tablets with respect to maximum observed plasma concentration (Cmax) and area under the plasma concentration-time curve (AUC) from time zero to the time of last quantifiable concentration [AUC(0-T)].
- Secondary: to assess the safety of a 1.0 mg dose of entecavir when administered as a 1.0 mg tablet or as 2 x 0.5 mg tablets.

Rationale:

Entecavir is manufactured as a 0.5 mg tablet, the formulation used in Phase III clinical trials and the proposed clinical dose in treatment naïve HBV patients. The proposed dose for lamivudine-refractory patients is 1.0 mg, and in the Phase III clinical trials, 2 x 0.5 mg tablets were used to provide the 1.0 mg dose. A 1.0 mg tablet is a more desirable formulation because it provides lamivudine-refractory patients with a lower pill burden and may facilitate treatment adherence. The 1.0 mg tablet is compositionally proportional to the 0.5 mg tablet. The purpose of this study was to demonstrate the bioequivalence of the 1.0 mg tablet relative to 2 x 0.5 mg reference tablets.

Study Design:

This was an open-label, randomized, two-period, two-treatment, crossover study in 30 healthy subjects. Subjects were to receive the following 2 treatments in one of two randomly assigned treatment sequences:

- Entecavir 2 x 0.5 mg tablets
- Entecavir 1 x 1.0 mg tablet.

Subjects were admitted to the clinical facility the evening prior to dosing for each period (Day -1). Treatments were to be administered on Day 1 of each period according to the randomization schedule. For each period, subjects remained in the clinical facility for 72 hours after dosing. Subjects returned the clinical facility on Days 6, 8, 10, and 12 for collection of pharmacokinetic samples. A washout period of at least 14 days separated each dose. Subjects were discharged from the study on Day 12 of Period 2. Blood samples for pharmacokinetic assessment of entecavir were collected from pre-dose to 264 hours post-dose. Each subject fasted for 10 hours prior until 4 hours after study drug administration.

Study Site:

Formulations:

- Test Product: Entecavir tablets were supplied as 1.0 mg triangular, pink, film-coated tablets, with "BMS" and — engraved on one side and no engravings on the other side. The entecavir 1.0 mg tablets were packaged in bottles of 25 tablets/bottle. The label batch, product batch, and product numbers were 3F70522, 8MEE101, and 200475-K001-049, respectively, with an expiration date of 31-May-2004.
- Reference Therapy: Entecavir oral tablets were supplied as 0.5 mg white — film-coated tablets. The label batch, product batch, and product numbers were 2F59620, N01030, and 200475-K0X5-039, respectively, with an expiration date of 29-Feb-2004.

Pharmacokinetic Measurements:
Blood (plasma) samples were collected at the following specified times during each Period for the analyses of concentrations of entecavir:

- Prior to dosing and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 120, 168, 216, and 264 hours following dosing.

Pharmacokinetic/Statistical Analysis:
Single dose entecavir pharmacokinetic parameter values were calculated by noncompartmental methods. Bioequivalence was concluded with respect to Cmax and AUC(0-T) if the 90% confidence intervals (CIs) for the ratios of population geometric means of the entecavir 1.0 mg tablet to entecavir 2 x 0.5 mg tablets were contained within 80% to 125%. The 90% CIs were constructed from the results of analyses of variance on log(Cmax) and log[AUC(0-T)]. Similarly, 90% CIs
were also calculated for log[AUC(INF)]. Descriptive statistics for all pharmacokinetic parameters were provided by formulation.

Study Population Results:
All thirty (30) randomized subjects completed the study.

Demographic and Baseline Characteristics:

Demographics and Baseline Characteristics – Study AI463065*	
Age (yr)	28 (18 – 43)
Gender N (%)	25 Males (83%) 5 Female (17%)
Weight (kg)	69.7 (53.1 – 87.1)
Height (cm)	173.6 (154.9 – 195.6)
BMI (kg/m^2)	23.4 (19.3 – 29.3)
Race N (%)	26 Black (87%) 3 White (10%) 1 Asian/Pacific Islander (3%)

* Data presented as mean (range) unless otherwise specified.

Pharmacokinetic Results:

Mean concentration-time profiles for entecavir in plasma following administration of a single oral dose of entecavir 1.0 mg oral tablet and two entecavir 0.5 mg tablets in healthy subjects are presented in Figure 1. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of entecavir 1.0 mg oral tablet and two entecavir 0.5 mg tablets in healthy subjects are summarized in Table 1. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following administration of a single oral dose of entecavir 1.0 mg oral tablet and two entecavir 0.5 mg tablets in healthy subjects is presented in Table 2.

Upon review of the subject data, it was noted that entecavir plasma concentrations obtained for one subject indicated at least 20-fold lower exposure to the drug during the second period (Treatment B, 1.0 mg tablet) compared to exposure during the first period (Treatment A, 2 x 0.5 mg tablets) for the same subject. Entecavir plasma concentrations during the first period (Treatment A, 2 x 0.5 mg tablets) for the subject indicated exposure comparable to the other subjects in the same dosing period. Consequently, the statistical analysis was repeated with this subject excluded. Entecavir plasma pharmacokinetic parameters following administration of a single oral dose of entecavir 1.0 mg oral tablet and two entecavir 0.5 mg tablets in healthy subjects excluding the outlier are summarized in Table 3. A summary of statistical analysis of entecavir plasma pharmacokinetic parameters following administration of a single oral dose of entecavir 1.0 mg oral tablet and two entecavir 0.5 mg tablets in healthy subjects excluding the outlier is presented in Table 4.

Figure 1 Mean Concentrations of Entecavir in Plasma Following Administration of a Single Oral Dose of Entecavir 1.0 mg Oral Tablet and Two Entecavir 0.5 mg Tablets in Healthy Subjects.

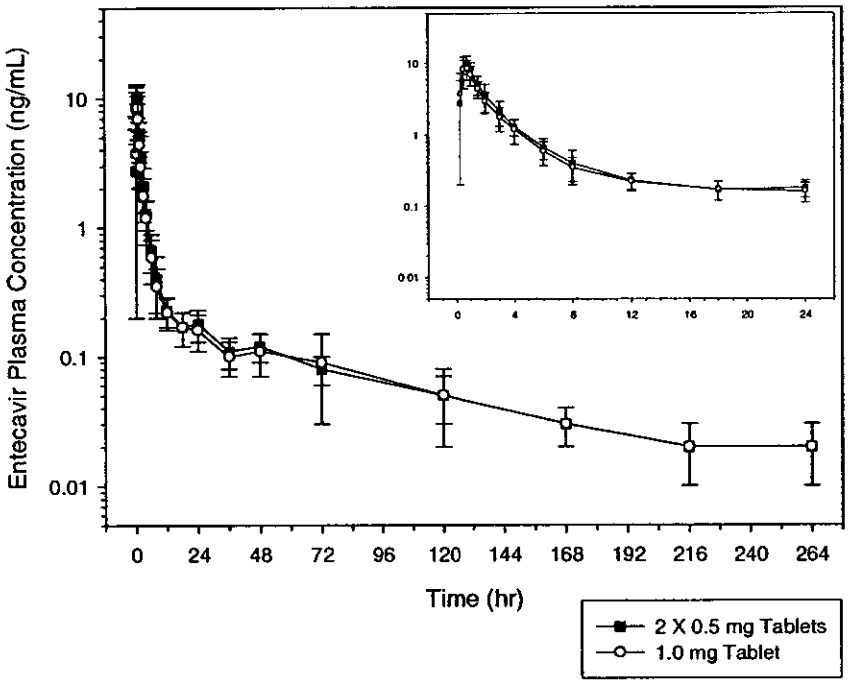

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Table 1 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of Entecavir 1.0 mg Oral Tablet and Two Entecavir 0.5 mg Tablets in Healthy Subjects.

Parameter	2 x 0.5 mg Tablet (N=30)	1.0 mg Tablet (N=30)
Cmax (ng/mL)	10.71 (22)	8.51 (32)
AUC(INF) (ng•h/mL)	37.83 (22)	32.99 (24)
AUC(0-T) (ng•h/mL)	35.04 (20)	30.13 (25)
Tmax[a] (hr)	0.75	0.50
T-half[b] (h)	109.45 (63.3)	98.65 (45.8)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Table 2 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of a Single Oral Dose of Entecavir 1.0 mg Oral Tablet and Two Entecavir 0.5 mg Tablets in Healthy Subjects.

Parameter	Treatment	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means Point Estimate (90% CI)
Cmax (ng/mL)	2 x 0.5 mg Tablet	10.71	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.794 (0.626, 1.007)
	1.0 mg Tablet	8.51		
AUC(0-T) (ng•h/mL)	2 x 0.5 mg Tablet	35.04	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.860 (0.704, 1.051)
	1.0 mg Tablet	30.13		
AUC(INF) (ng•h/mL)	2 x 0.5 mg Tablet	37.83	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.872 (0.746, 1.019)
	1.0 mg Tablet	32.99		

Entecavir 2 x 0.5 mg Tablet = Reference
Entecavir 1.0 mg Tablet = Test

Table 3 Summary of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Single Oral Dose of Entecavir 1.0 mg Oral Tablet and Two Entecavir 0.5 mg Tablets in Healthy Subjects (Excluding the Outlier Subject).

Parameter	2 x 0.5 mg Tablet (N=29)	1.0 mg Tablet (N=29)
Cmax (ng/mL)	10.65 (23)	9.66 (25)
AUC(INF) (ng•h/mL)	37.71 (22)	35.88 (16)
AUC(0-T) (ng•h/mL)	34.89 (21)	33.64 (17)
Tmax[a] (hr)	0.75 —	0.50 —
T-half[b] (h)	110.45 (64.2)	98.58 (46.6)

Data presented as geometric mean (CV%) unless otherwise specified.
[a] Data presented as median (minimum, maximum).
[b] Data presented as mean (SD).

Table 4 Summary of Statistical Analysis of Entecavir Plasma Pharmacokinetic Parameters Following Administration of Single Oral Dose of Entecavir 1.0 mg Oral Tablet and Two Entecavir 0.5 mg Tablets in Healthy Subjects (Excluding the Outlier Subject).

Parameter	Treatment	Adjusted Geometric Mean	Contrast	Ratios of Adjusted Geometric Means Point Estimate (90% CI)
Cmax (ng/mL)	2 x 0.5 mg Tablet	10.64	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.906 (0.837, 0.981)
	1.0 mg Tablet	9.64		
AUC(0-T) (ng•h/mL)	2 x 0.5 mg Tablet	34.81	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.966 (0.933, 1.000)
	1.0 mg Tablet	33.62		
AUC(INF) (ng•h/mL)	2 x 0.5 mg Tablet	37.63	1.0 mg Tablet vs. 2 x 0.5 mg Tablet	0.953 (0.918, 0.990)
	1.0 mg Tablet	35.87		

Entecavir 2 x 0.5 mg Tablet = Reference
Entecavir 1.0 mg Tablet = Test

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Assessment/Conclusion:

- Entecavir 0.5 mg tablet formulation is currently being investigated in Phase III clinical trials. Since the recommended dose for lamivudine-refractory patients is 1.0 mg, 2 x 0.5 mg tablets were used in clinical trials for these patients, and a 1.0 mg tablet formulation has been developed. The 1.0 mg tablet is compositionally proportional to the 0.5 mg tablet. Comparative dissolution profiles for the 0.5 and 1.0 mg tablets are comparable and indicate greater than [redacted] dissolution in 10 minutes for both formulations (for further assessment of dissolution, please refer to the Clinical Pharmacology review for NDA #21797). This study was conducted to demonstrate the bioequivalence of the 1.0 mg tablet relative to the 2 x 0.5 mg reference tablets.
- One subject (an African-American female) had approximately 20-fold lower plasma entecavir concentrations consistently throughout the concentration-time profile following dosing with the 1.0 mg tablet (Test, Period 2), compared to the plasma-concentration time data of the other subjects following dosing with the 1.0 mg tablet. This subject had the expected exposure to entecavir during the first treatment period following administration of the 2 x 0.5 mg tablet (Reference, Period 1). Because of this unexpected finding all aspects of the study conduct and analysis were reviewed, including subject compliance, bioanalysis of the plasma samples, and release data for the formulations. A review of study conduct did not suggest non-compliance with study procedures. Results of a repeat bioanalytical analysis of samples for this subject were comparable to the original analysis results. Drug product performance was consistent for the tablet formulation and deemed unlikely to be the cause of the anomalous results for the study outlier. A review of pharmacokinetic data (Cmax and AUC) from 15 Phase I clinical pharmacology studies for entecavir revealed no other subjects with similar magnitudes of exposure, regardless of formulation and demography.
- When all subjects were included in the statistical analyses, the point estimate of the adjusted geometric means ratios for AUC(0-T) and Cmax indicated that these parameters for the 1.0 mg tablet were approximately 14 and 21% lower, respectively, when compared to 2 x 0.5 mg tablets. The corresponding 90% CIs were not within the pre-specified bioequivalence interval (0.80-1.25). However, when the outlier subject was excluded from the statistical analyses, bioequivalence criterion was met for AUC(0-T), AUC(INF) and Cmax. In addition, the Applicant included a bioequivalance analysis using a nonparametric method, with the rationale that the outlier violated the assumption of normality for parametric methods. Although the results from the nonparametric analysis of both the full and reduced data sets indicate that the 1.0 mg tablet is bioequivalent to the 2 x 0.5 mg tablet, and are consistent with the conclusion based on the parametric analysis after data from the outlier subject was excluded, these results are not included in the review.
- In conclusion, the results obtained following administration of the 1.0 mg tablet in the outlier subject appear to be anomalous due unexplainable reason(s). Exclusion of this subject from the statistical analysis for the bioequivalence assessment results in the 1.0 mg tablet being bioequivalent to the 2 x 0.5 mg tablets with respect to Cmax, AUC(0-T), and AUC(INF).
- [redacted]

4.1.5. In vitro Studies

4.1.5.1. Inhibition of the Catalytic Activities of cDNA-expressed Human Cytochrome P4501A2, Cytochrome P4502B6, Cytochrome P4502C9, Cytochrome P4502C19, Cytochrome P4502D6, Cytochrome P4502E1, and Cytochrome P4503A4 by the Test Substance Entecavir (Study 930004689).

Objectives:

- To determine whether the test substance entecavir inhibits human cytochrome P450 (CYP) catalytic activity (specifically CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4).

Methods:

- Preparations of cDNA-expressed enzyme protein in a mixture containing 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and each of the following probe substrates were incubated at 37ºC: phenacetin (50 μM), [^{14}C]-*S*-mephenytoin (50 μM), diclofenac (6 μM), bufuralol (10μM), p-nitrophenol (100 μM), and testosterone (120 μM). The following positive controls were used for each enzyme: CYP1A2, 7,8-benzoflavone (0.3 μM); CYP2B6, tranylcypromine (100 μM); CYP2C9, sulfaphenazone (3 μM), CYP2C19, tranylcypromine (100 μM); CYP2D6, quinidine (1 μM); CYP2E1, 4-methylpyrazole (50 μM); and ketoconazole (1μM). Final entecavir test concentrations studied were 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM.
- Catalytic activities for CYP1A2, CYP2C9, CYP2D6, and CYP3A4 were calculated using standard curves. Catalytic activity for CYP2E1 was determined using absorbance. Catalytic activities for CYP2B6 and CYP2C19 were determined radiometrically. For each CYP450 isoform, the IC_{50} was calculated by linear interpolation. The linear interpolation used the mean percent inhibition for each entecavir concentration.

Results:

A summary of IC50 results for entecavir using cDNA-expressed cytochromes P450 as an enzyme source is presented in Table 1.

Table 1 Effects of Treating cDNA-Expressed Cytochromes P450 with Entecavir

Isoform of Cytochrome P450	IC_{50} (μM)
CYP1A2	> 300
CYP2B6	> 300
CYP2C9	> 300
CYP2C19	> 300
CYP2D6	> 300
CYP2E1	> 300
CYP3A4	> 300

Assessment/Conclusion:

- The catalytic activities of CYP1A2 (phenacetin hydroxylase), CYP2B6 [(*S*)-mephenytoin N-demethylase], CYP2C9 (diclofenac 4′-hydroxylase), CYP2C19 [(*S*-mephenytoin 4′-hydroxylase], CYP2D6 (bufuralol 1′-hydroxylase), CYP2E1 (p-nitrophenol hydroxylase), and CYP3A4 (testosterone 6β-hydroxylase) were either not inhibited or inhibited by less than 50% at concentrations of up to 300 μM entecavir, the highest concentration examined.
- At clinically relevant concentrations (approximately < 0.1 μM or 30 ng/mL), entecavir inhibited catalytic activities no greater than 9% for all enzymes studied.

4.1.5.2. A Study to Assess the Potential for Inhibition of Cytochrome P4502D6-Catalyzed Bufuralol 1′-Hydroxylase Activity by BMS-200475 (Study 910060540).

Objectives:

- To determine whether the test substance BMS-200475 inhibits the polymorphic human cytochrome P4502D6.

Methods:

- Inhibition of CYP2D6 was measured using the model substrate bufuralol and cDNA-derived CYP2D6 in microsomes prepared from a human lymphoblastoid cell line. The inhibition study consisted of two parts, a range finding analysis followed by a more detailed study to determine apparent K_i. In the range finding study, a single bufuralol concentration (10 μM) and eleven entecavir concentrations (200, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM) were tested in duplicate. A low microsome concentration was used because of the rapid metabolism of bufuralol. Preparations of cDNA-expressed enzyme protein in a mixture containing 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride, and (±)-bufuralol (10 μM). Catalytic activity for CYP2D6 was quantitated via HPLC, comparing to a standard curve of product 1′-hydroxybufuralol.

Results:

A summary of inhibition results for entecavir using cDNA-expressed cytochrome P4502D6 is presented in Table 1.

Table 1 Effects of Treating cDNA-Expressed Cytochrome P4502D6 with Entecavir

Concentration	Pmol per Incubation		% Inhibition	
	Replicate #1	Replicate #2	Replicate #1	Replicate #2
0	463	445	-	-
0.01	477	458	-5	-1
0.03	465	444	-2	2
0.1	459	432	-1	5
0.3	444	474	2	-5
1	448	449	2	1
3	453	442	0	3
10	434	442	4	3
30	435	436	4	4
100	419	403	8	1
300	364	368	20	19

Assessment/Conclusion:

- BMS-200475 did not substantially inhibit CYP2D6-catalyzed (±)-bufuralol 1´-hydroxylase activity. Entecavir inhibited less than 50% at concentrations of up to 300 μM, the highest concentration examined (IC_{50} value could not be calculated). The apparent K_i could not be determined.
- It appears the apparent K_i and IC_{50} values cited for CYP2D6 inhibitors in the Applicant's study report are from historical data. The lack of positive controls in this study limits the applicability of study results.

4.1.5.3. Inhibition of Cytochrome P4503A4-Catalyzed Testosterone 6β-Hydroxylase Activity by the Test Substance BMS-200475 (Study 910060541).

Objectives:

- To determine whether the test substance BMS-200475 inhibits human cytochrome P4503A4 (CYP3A4).

Methods:

- Inhibition of CYP3A4 was measured using the model substrate testosterone and cDNA-derived CYP3A4 in microsomes prepared from a human lymphoblastoid cell line. The inhibition study consisted of two parts, a range finding analysis followed by a more detailed study to determine apparent K_i. In the range finding study, a single testosterone concentration (120 μM) and eleven entecavir concentrations (200, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM) were tested in duplicate. Preparations of cDNA-expressed enzyme protein in a mixture containing 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and testosterone (120 μM). Testosterone metabolism was assessed by the production of the 6β-hydroxytestosterone metabolite assayed via HPLC. Catalytic activity for CYP3A4 was calculated using the absorbance of a standard curve.

Results:

A summary of inhibition results for entecavir using cDNA-expressed cytochrome P4503A4 is presented in Table 1.

Table 1 Effects of Treating cDNA-Expressed Cytochrome P4503A4 with Entecavir

Concentration	Pmol per Incubation		% Inhibition	
	Replicate #1	Replicate #2	Replicate #1	Replicate #2
0	2690	2686	-	-
0.01	2603	2616	3	3
0.03	1744	2565	35	5
0.1	2551	2564	5	5
0.3	2573	2606	4	3
1	2519	2470	6	8
3	2494	2481	7	8
10	2667	2581	1	4
30	2692	2443	0	9
100	2418	2426	10	10
300	2260	2247	16	16

Assessment/Conclusion:

- BMS-200475 did not substantially inhibit CYP3A4-catalyzed testosterone 6β-hydroxylase activity. Entecavir inhibited less than 50% at concentrations of up to 300 μM, the highest concentration examined. The apparent K_i could not be determined.
- It appears the apparent K_i and IC_{50} values cited for CYP3A4 inhibitors in the Applicant's study report are from historical data. The lack of positive controls in this study limits the applicability of study results.

4.1.5.4. In vitro evaluation of Entecavir as an Inducer of Cytochrome P450 Expression in cultured Human Hepatocytes (Study XT033016).

Objectives:

- To investigate the effect of entecavir on the expression of cytochrome P450 enzymes 1A2, 2B6, 2C9, 2C19 and 3A4/5, in primary cultures of human hepatocytes.

Methods:

- Preparations of cultured human hepatocytes from three separate human livers (seeded approximately 1.2-1.5 x 106 viable hepatocytes/mL; 3 mL per dish) were treated with 0.1% DMSO (vehicle), β-naphthoflavone (33 μM), phenobarbital (750 μM), rifampin (20 μM), and entecavir (0.1, 1 and 10 μM) once daily for three consecutive days. The following probe substrates were added: 7-Ethoxyresorufin was added as a solution (4.0 μL, 2.5 mM) in DMSO (0.4% v/v, final DMSO concentration); bupropion was added as a solution (25 μL, 10 mM) in water; diclofenac was added as a solution (10 μL, 10 mM) in water; S-mephenytoin was added as a solution (2.29 μL, 35 mM) in methanol (1.2% v/v, final methanol concentration); and testosterone was added as a solution (10 μL, 12.5 mM) in methanol (2% v/v, final methanol concentration). Hepatocytes were harvested 24 hours after the last treatment. Microsomes were prepared from the hepatocytes and used to measure the activity and fold-increases in CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4/5. *O*-dealkylation of 7-ethoxyresorufin was measured by the fluorimetric method; hydroxylation of bupropion by [redacted] HPLC; 4′-hydroxydiclofenac by [redacted] HPLC; 4′-hydroxylation of *S*-mephenytoin by [redacted] HPLC; and 6β-hydroxylation of testosterone by [redacted] HPLC. Also, immunoreactive protein levels of the human CYPs -1A2, 2B6, 2C9, and 3A4/5 in the microsomes from all the treatment groups were determined by western blotting.
- Individual rates of reaction from like treatment groups were averaged (n = 3) and standard deviations were determined. Fold increase (rounded to one significant figure) was presented either as fraction of control or as fold increase over control, where the control refers to the corresponding vehicle treated samples. To determine significant differences between group means, first, equal variance and normality tests were conducted to determine if the data were parametrically distributed. A t-test was performed to compare enzyme activity (expressed as mean fold induction) between each treatment group and the vehicle control ($p < 0.05$ or 5% level of significance).

Results:

The effects of treating cultured human hepatocytes with entecavir or prototypical inducers on the expression of cytochrome P450 enzymes are summarized in Tables 1 and 2 by enzyme activity and fold increase over control, respectively.

Table 1 Effects of Treating Cultured Human Hepatocytes with Entecavir or Prototypical Inducers on the Expression of Cytochrome P450 Enzymes

Treatment	Conc.	Enzymatic Activity (pmol/mg protein/min)				
		CYP1A2[a]	CYP2B6[b]	CYP2C9[c]	CYP2C19[d]	CYP3A4/5[e]
Dimethyl sulfoxide	0.1%	6.38 ± 1.05	21.3 ± 9.0	1120 ± 330	16.8 ± 9.3	3010 ± 1510
Entecavir	0.1μM	6.59 ± 1.47	19.7 ± 10.0	1090 ± 260	21.4 ± 7.3	3190 ± 1690
Entecavir	1.0μM	6.35 ± 1.19	21.7 ± 10.6	1110 ± 340	17.6 ± 9.8	3160 ± 1540
Entecavir	10μM	6.70 ± 1.42	21.3 ± 9.8	1170 ± 350	22.5 ± 9.1	3270 ± 1470
β-Naphthoflavone	33μM	40.5 ± 3.4	96.8 ± 55.2	1330 ± 360	48.0 ± 11.1	1560 ± 1100
Phenobarbital	750μM	19.1 ± 7.5	233 ± 112	1920 ± 560	63.6 ± 27.0	14000 ± 800
Rifampin	20μM	15.9 ± 6.6	100 ± 73	2530 ± 620	118 ± 56	16700 ± 1600

Values presented are the means ± SD of three human hepatocyte preparations.
Conc. = concentration
Rates expressed as pmol/mg protein/min, as measured by the following:
[a] 7-Ethoxyresorufin O-dealkylation (EROD)
[b] Bupropion hydroxylation
[c] Diclofenac 4′-hydroxylation
[d] S-mephenytoin 4′-hydroxylation
[e] Testosterone 6β-hydroxylation

Table 2 Effects of Treating Cultured Human Hepatocytes with Entecavir or Prototypical Inducers on the Expression of Cytochrome P450 Enzymes Expressed as Fold-Increase

Treatment	Conc.	Fold Induction				
		CYP1A2[a]	CYP2B6[b]	CYP2C9[c]	CYP2C19[d]	CYP3A4/5[e]
Dimethyl sulfoxide	0.1%	1.00 ± 0.17	1.00 ± 0.42	1.00 ± 0.29	1.00 ± 0.56	1.00 ± 0.50
Entecavir	0.1μM	1.03 ± 0.09	0.911 ± 0.175	0.988 ± 0.086	1.39 ± 0.33	1.04 ± 0.07
Entecavir	1.0μM	0.992 ± 0.027	0.989 ± 0.113	0.988 ± 0.056	1.05 ± 0.06	1.06 ± 0.03§
Entecavir	10μM	1.04 ± 0.07	0.984 ± 0.068	1.05 ± 0.02§	1.44 ± 0.33	1.12 ± 0.10
β-Naphthoflavone	33μM	6.53 ± 1.68§	4.60 ± 1.52§	1.20 ± 0.13	3.61 ± 2.47	0.456 ± 0.191§
Phenobarbital	750μM	2.98 ± 0.97§	10.8 ± 1.6†	1.72 ± 0.15*	4.01 ± 0.66*	5.98 ± 3.96
Rifampin	20μM	2.46 ± 0.80§	4.73 ± 2.24§	2.29 ± 0.20†	7.20 ± 0.91†	7.51 ± 5.89

Values presented are the means ± CV of three human hepatocyte preparations.
Conc. = concentration
Rates expressed as pmol/mg protein/min, as measured by the following:
[a] 7-Ethoxyresorufin O-dealkylation (EROD)
[b] Bupropion hydroxylation
[c] Diclofenac 4′-hydroxylation
[d] S-mephenytoin 4′-hydroxylation
[e] Testosterone 6β-hydroxylation
* $p < 0.005$
§ $p < 0.05$
† $p < 0.001$

Assessment/Conclusion:

- Treatment of cultured human hepatocytes with entecavir did not cause an increase in EROD activity compared with hepatocytes treated with vehicle (0.1% DMSO).
- Treatment of cultured human hepatocytes with entecavir did not cause an increase in bupropion hydroxylase (CYP2B6) activity.
- Treatment of cultured human hepatocytes with entecavir did not cause an increase in diclofenac 4′-hydroxylase (CYP2C9) activity at 0.1 and 1 μM. At 10 μM, the fold-induction was statistically significant but the magnitude of the increase was only 5% when compared with hepatocytes treated with the vehicle (0.1% DMSO).
- In all the three hepatocyte preparations tested, treatment of cultured human hepatocytes with entecavir did not cause an increase in S-mephenytoin 4′-hydroxylase (CYP2C19) activity.
- Treatment of cultured human hepatocytes with entecavir did not cause an increase in testosterone 6β-hydroxylase activity (CYP3A4) activity at 0.1 and 10 μM. At 1 μM, the fold-induction was statistically significant but the magnitude of the increase was only 6% when compared with hepatocytes treated with the vehicle (0.1% DMSO).
- Western immunoblotting data showed that treatment of cultured human hepatocytes with entecavir did not cause an increase in protein levels for all the CYP enzymes examined.
- In conclusion, under conditions where the positive controls, β-naphthoflavone (CYP1A2), phenobarbital (CYP2B6), and rifampin (CYP2C9, CYP2C19 and CYP3A4/5) caused appropriate increases in enzyme activity and immunoreactive protein levels, entecavir, in general, did not cause an increase in the activity of any of the enzymes, suggesting that entecavir is not an inducer of any of the CYP enzymes examined.

4.1.5.5. In Vitro Determination of Mouse, Rat, Dog, Monkey, and Human Serum Protein Binding and/or Red Blood Cell Distribution of [14C]-BMS-200475

Objectives:

- To determine the in vitro protein binding of [^{14}C]-BMS-200475 to mouse, rat, dog, monkey, and human serum proteins and the in vitro distribution of [^{14}C]-BMS-200475 in rat, dog, monkey, and human red blood cells.

Methods:

- In vitro protein binding and red blood cell (RBC) distribution of [^{14}C]-BMS-200475 were determined by . of spiked serum samples and incubation of spiked whole blood samples, respectively.
- For protein binding, serum from fresh blood samples obtained following an overnight fast of at least 10 hr was separated and spiked with [^{14}C]-BMS-200475 to obtain concentrations of 50, 500, and 5000 ng/mL. Triplicate serum samples were centrifuged and the radioactivity in 600μL aliquots of serum prior to and pooled samples (200 μL/replicate) was determined by . Phosphate buffered saline (PBS, 0.134 M, pH 7.4) solutions at concentrations of 50, 500, and 5000 ng/mL were used for non-specific binding determination. Serum protein binding was determined by with a
- To obtain the extent of protein binding for [^{14}C]-BMS-200475, the radioactivity (DPM) in the 600 μL of the serum (DPM_{Serum}) and the pooled , were determined and the percentages of free and protein-bound drug were calculated as follows:

$$\text{Protein Bound Drug } (\%) = \frac{\text{mean DPM}_{\text{Serum}} - \text{DPM}}{\text{mean DPM}_{\text{Serum}}} \times 100$$

- For RBC distribution, whole blood was spiked with [^{14}C]-BMS-200475 at concentrations of 50, 500, and 5000 ng/mL. Triplicate whole blood samples were incubated at ambient temperature for 1 hr with shaking. Plasma was separated after incubation, and radioactivity was determined by —. Aliquots of PBS solutions at concentrations of 50, 500, and 5000 ng/mL were assayed by — and represented the initial radioactive concentrations of the drug in whole blood. Hematocrit values were determined in triplicate at each concentration.
- The extent of RBC distribution of [^{14}C]-BMS-200475 was determined using the following equation:

$$\text{RBC Distribution } (\%) = \left[1 - \frac{C_{\text{Plasma}} \times (1 - H)}{C_{\text{Blood}}}\right] \times 100$$

where, C_{Blood} and C_{Plasma} are the radioactivity in whole blood and plasma, respectively, and H is the mean hematocrit value.

- Human subjects did not consume any medication (including over-the-counter medications) within 24 hr of blood collection.

Results:

The non-specific binding of [^{14}C]-BMS-200475 to the — was ≤ 1.3% at concentrations ranging from 50 to 5000 ng/mL. In general, protein binding of [^{14}C]-BMS-200475 is low, approximately 13%, and independent of concentration. [^{14}C]-BMS-200475 uniformly distributes between plasma and red blood cells (RBCs) in whole human blood. Serum protein binding and RBC distribution of [^{14}C]-BMS-200475 in human subjects over a range of concentrations is presented in Table 1 below.

Table 1 In Vitro Protein Binding and RBC Distribution of [^{14}C]-BMS-200475 Proteins

Concentration (ng/mL)	Protein Binding (%)	Mean (SD) RBC Distribution (%) N=3
50	13.8	56.5 (0.30)
500	13.9	52.1 (0.07)
5000	11.9	47.5 (0.15)
Overall Mean	13.2	52.0

Assessment/Conclusion:

In general, protein binding of [^{14}C]-BMS-200475 is low, approximately 13%, and independent of concentration. [^{14}C]-BMS-200475 uniformly distributes between plasma and red blood cells (RBCs) in whole human blood.

Reviewer Comment: Protein binding assessments were not performed over a clinically relevant concentration range of BMS-200475, due to available clinical data at the time of assay and the lack of sensitivity of — at concentrations below 50 ng/mL. In addition, no reference compounds covering a range of protein binding were included in this assay.

4.1.5.6. BMS-200475 is not a Substrate of Human P-Glycoprotein (Study P-GP).

Objectives:

- To evaluate BMS-200475 as a substrate of human P-glycoprotein (P-gp) using Caco-2 cells.

Methods:

- Caco-2 cells (passage #17) were obtained from the [redacted]. Caco-2 cells were seeded onto a [redacted] membrane at a density of 80,000 cells/cm2. The cells were grown in culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 100 U/mL penicillin-G, and 100 μg/mL streptomycin. The culture medium was replaced every two days and the cells were maintained at 37°C, 95% relative humidity, and 5% CO2.
- Permeability studies were conducted with the monolayers cultured for approximately 21 days, and the cell passage numbers were between 50 and 80. Both the apical-to-basolateral transport (absorptive direction) as well as the basolateral-to-apical transport (secretory direction) of BMS-200475 was measured (n=3). The transport medium was Hank's balanced salt solution, pH 7.4, on both apical and basolateral sides. The initial concentration of BMS-200475 used was 35 μM. Studies were initiated by adding an appropriate volume of buffer containing BMS-200475 to either the apical or basolateral side of the monolayer. The monolayers were then incubated for 2 hours at 37°C. Samples were taken from both compartments at the end of the 2-hour incubation period.
- The concentrations of BMS-200475 were analyzed by a specific HPLC-UV assay.
- For each transport experiment, the permeability coefficient (Pc) was calculated according to the following equation: Pc = dA/(dt·S·C0), where dA/dt is the flux of BMS-200475 across the monolayer (nmole/sec), S is the surface area of the cell monolayer (0.33 cm^2), and C0 is the initial concentration (μM) in the donor compartment. The permeability coefficient values are expressed as nm/sec.

Results:

The permeability results of BMS-200475 in the Caco-2 cell model are summarized in Table 1.

Table 1 Permeability (nm/sec) of BMS-200475 in Caco-2 Cells

Replicate	Apical-to-Basolateral (Absorptive)	Apical-to-Basolateral (Secretory)
#1	< 15	< 15
#2	< 15	< 15
#3	< 15	< 15

Permeability coefficient is estimated to be less than 15 nm/sec based on the lowest limit of quantitation [redacted]

Assessment/Conclusion:

- Both the apical-to-basolateral permeability (absorptive direction) and basolateral-to-apical permeability (secretory direction) of BMS-200475 was very low (<15 nm/sec), indicating BMS-200475 is a poor P-gp substrate.

4.1.6. Pharmacokinetic/Pharmacodynamic Studies

4.1.6.1. Retrospective Analysis of Electrocardiograms Collected in Phase I Studies Following Oral Administration of Entecavir (Protocol AI463041).

Objectives:

- Primary: To assess the effect of entecavir on the QT interval corrected for heart rate using Bazett's formula (QTcB).
- Secondary: (1) To assess the effect of entecavir on the QT interval corrected for heart rate using Fridericia's formula (QTcF). (2) To assess the effect of entecavir on the PR interval.
- Tertiary: (1) To assess the effect of entecavir on the relationship between the QT interval and RR interval. (2) To assess the effect of entecavir on the RR interval. (3) To assess the effect of entecavir on the QRS interval. (4) To assess the effect of entecavir on the absolute QT interval. (5) To assess the effect of entecavir on the heart rate (HR).

Study Design:

Studies AI463001, AI463002, AI463010, AI463033 and AI463034 were selected for the retrospective analysis since these were studies for which pre-dose and post-dose ECGs were obtained during administration of entecavir. These studies evaluated single and multiple doses of entecavir at 0.1 mg, the therapeutic dose range (0.5 and 1.0 mg), as well as doses significantly higher (up to 40 mg) than therapeutic doses. Entecavir was dosed once-daily in all multiple-dose studies. All studies were randomized studies in healthy adult subjects. In the individual study reports, ECG intervals were provided as automated measurements. The ECG data used in this retrospective analysis were based on manual readings of the ECGs by a cardiologist. A summary of individual Phase I studies included in the analysis is presented in the following table.

Study Number	Dosing Schedule	Doses Studied	Planned Number of Subjects	ECG and PK Sample Schedule
AI463001	Sequential single dose	1, 2.5, 5, 10, 20, 40 mg or placebo	6 in each dose panel; 12 placebo	Day 1: predose and 2 h
AI463002	Sequential-multiple dose for 14 days	2.5, 5, 10, 20 mg or placebo	6 in each dose panel; 8 placebo	Days 1, 7 and 14: predose and 2 h
AI463010	Three-period, three-treatment, crossover study with at least a 7-day washout period between each dose	1 mg	36	Periods 1, 2, and 3, Day 1: predose and 1 h
AI463033	Sequential multiple dose for 14 days	0.1, 0.5, 1.0 mg or placebo	6 in each dose panel; 6 placebo	Days 1, 7, and 14: predose and 1 h
AI463034	Two single doses of 0.5 mg entecavir with at least a 7-day washout period between each dose	0.5 mg tablet or 0.5 mg capsule	40	Periods 1 and 2, Day 1: predose and 1 h

Formulations:

In general, entecavir was administered orally as either a capsule or tablet formulation at doses ranging from 0.1 to 40 mg.

Pharmacokinetic Measurements:
The actual entecavir plasma concentration measured at the time of the ECG (CECG), either 1 or 2 hours after dosing, was used to assess the effect of entecavir on the QTcB, QTcF, PR, RR, absolute QT, QRS, HR, and QT versus RR intervals.

Pharmacokinetic/Statistical Analysis:
The effect of entecavir on the QTcB, QTcF, or PR intervals was assessed by counts of borderline and prolonged intervals and by linear regressions of CECG versus change from baseline of QTc or PR interval. The effect of entecavir on the QT interval was also assessed based on the results from linear mixed effects regression analyses of QT on RR carried out separately for each study day. Summary statistics for the QTcB, QTcF, PR, RR, absolute QT, QRS intervals, and HR recorded at selected times and corresponding changes from baseline were summarized by treatment, study day, and timepoint.

Study Population Results:
- AI463001: 48 subjects randomized; 48 subjects completed
- AI463002: 33 subjects randomized; 31 (94%) subjects completed; 2 (6%) discontinued
- AI463010: 41 subjects randomized; 36 (88%) subjects completed; 5 (12%) discontinued
- AI463033: 26 subjects randomized; 24 (92.3%) subjects completed; 2 (7.7%) discontinued
- AI463034; 40 subjects randomized; 37 (92.5%) completed; 3 (7.5%) discontinued

There were no discontinuations related to issues of cardiac safety. Additional details are presented in the individual clinical study reports.

Demographic and Baseline Characteristics:
The demographic and baseline characteristics of the individual study populations are presented in the individual clinical study reports. A frequency distribution of sex by treatment is presented in the following table.

Frequency Distribution of Sex – Study AI463041*		
Treatment[a]	Sex	
	Male	Female
Placebo (N=27)	22 (81%)	5 (19%)
0.1 mg (N=7)	7 (100%)	0 (0%)
0.5 mg (N=44)	29 (66%)	15 (34%)
0.5 mg tablet** (N=39)	22 (56%)	17 (44%)
1 mg (N=52)	38 (73%)	14 (27%)
2.5 mg (N=13)	12 (92%)	1 (8%)
5 mg (N=12)	12 (100%)	0 (0%)
10 mg (N=12)	11 (92%)	1 (8%)
20 mg (N=12)	12 (100%)	0 (0%)
40 mg (N=6)	5 (83%)	1 (17%)

Frequency Distribution of Sex – Study AI463041*		
Treatment[a]	Sex	
	Male	Female
All entecavir doses[b] (N=160)	126 (79%)	34 (21%)

* Data presented as N (%).
** 0.5 mg was administered as both capsule and tablet formulations (remaining doses were capsules)
[a] Unless specified, all treatments were capsule formulation.
[b] The subjects from Study AI463034 who received a 0.5 mg tablet and capsule are counted only once.

Pharmacokinetic Results:
A summary of entecavir CECG, the actual entecavir plasma concentration measured at the time of the ECG either 1 or 2 hours after dosing (anticipated time of Cmax), following administration of single and multiple oral doses of placebo or entecavir in healthy subjects is presented in Table 1.

Table 1 Summary of Entecavir CECG Following Administration of Single and Multiple Oral Doses of Entecavir in Healthy Subjects

Dose (mg)	Day					
	1		7		14	
	1 Hour	2 Hour	1 Hour	2 Hour	1 Hour	2 Hour
0.1	0.34[a] (26)	-	0.47[a] (21)	-	0.45[b] (30)	-
0.5	3.44[c] (34)	-	3.96[b] (14)	-	3.83[b] (20)	-
0.5*	3.55[d] (23)	-	-	-	-	-
1.0	5.51[e] (36)	2.60[b] (17)	4.89[b] (45)	-	7.06[b] (27)	-
2.5	-	7.57[f] (21)	-	9.73[b] (14)	-	11.02[b] (19)
5.0	-	18.35[f] (22)	-	29.17[b] (28)	-	23.55[b] (31)
10	-	47.23[f] (23)	-	44.68[b] (12)	-	48.12[b] (19)
20	-	95.43[f] (31)	-	94.15[b] (31)	-	99.22[g] (21)
40	-	277.48[b] (33)	-	-	-	-

Data presented as geometric mean (CV%).
- Not applicable
* Tablet formulation
[a] N=7; [b] N=6; [c] N=43; [d] N=39; [e] N=46; [f] N=12; [g] N=5

Pharmacodynamic Results:
A summary of QTcB and change from baseline in QTcB following administration of single and multiple oral doses of placebo or entecavir in healthy subjects is presented in Table 2. A categorical assessment of change from baseline in QTcB following administration of single and multiple oral doses of placebo or entecavir in healthy subjects is presented in Table 3.

Table 2 Summary of QTcB and Change From Baseline in QTcB Following Administration of Single and Multiple Oral Doses of Placebo or Entecavir in Healthy Subjects

Dose (mg)	Day					
	Predose		1		14	
	1 Hour	2 Hour	1 Hour	2 Hour	1 Hour	2 Hour
QTcB (msec)						
PL	380[a] (17)	380[b] (16)	375[a] (17)	370[b] (20)	383[c] (12)	376[c] (12)
0.1	389[a] (15)	-	375[a] (25)	-	380[d] (20)	-
0.5	387[e] (22)	-	387[e] (22)	-	378[d] (25)	-
0.5*	390[f] (19)	-	386[f] (24)	-	-	-
1.0	386[g] (20)	384[d] (28)	382[g] (15)	380[d] (20)	376[d] (15)	-
2.5	-	368[h] (18)	-	365[h] (18)	-	366[d] (10)
5.0	-	383[i] (29)	-	374[i] (23)	-	372[d] (23)
10	-	374[i] (19)	-	368[i] (11)	-	371[d] (22)
20	-	367[i] (17)	-	363[i] (16)	-	353[i] (22)
40	-	362[d] (18)	-	359[d] (17)	-	-
Δ QTcB (msec)						
PL	-	-	-5.7[a] (13.7)	-9.9[b] (15.0)	3.7[d] (20.5)	-7.8[c] (19.9)
0.1	-	-	-14.4[a] (19.8)	-	-8.2[d] (12.1)	-
0.5	-	-	-0.4[e] (19.0)	-	-6.5[d] (13.4)	-
0.5*	-	-	-4.1[f] (17.4)	-	-	-
1.0	-	-	-3.4[g] (17.6)	-3.8[d] (10.3)	2.5[d] (12.8)	-
2.5	-	-	-	-3.8[h] (19.8)	-	-12.0[d] (7.6)
5.0	-	-	-	-8.8[i] (23.8)	-	-14.8[d] (29.9)
10	-	-	-	-6.8[i] (15.8)	-	-10.7[d] (19.6)
20	-	-	-	-4.3[i] (9.1)	-	-17.2[i] (15.0)
40	-	-	-	-3.0[d] (17.8)	-	-

Data presented as geometric mean (CV%).
PL Placebo
- Not applicable
* Tablet formulation
[a] N=7; [b] N=20; [c] N=8; [d] N=6; [e] N=44; [f] N=39; [g] N=46; [h] N=13; [i] N=12; [j] N=5

Table 3 Categorical Assessment of Change From Baseline in QTcB Following Administration of Single and Multiple Oral Doses of Placebo or Entecavir in Healthy Subjects

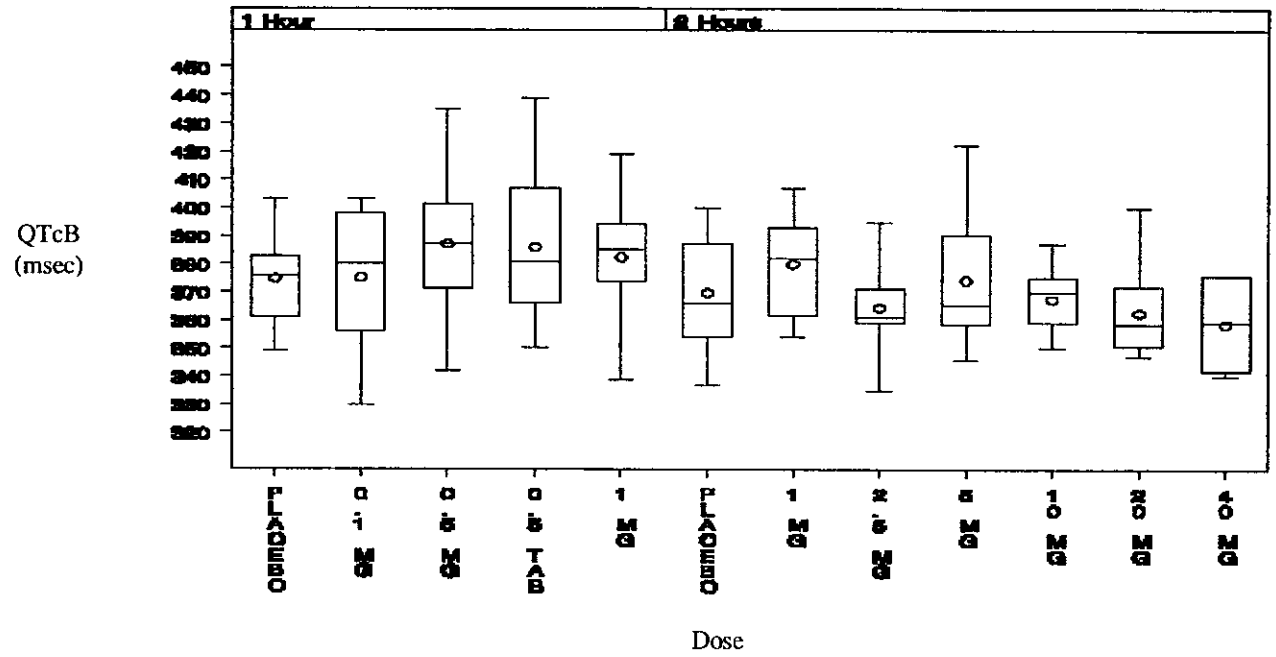
Dose (mg)	Δ QTcB (msec)					
	Male N (%)		Female N (%)		All N (%)	
	30 to 60	> 60	30 to 60	> 60	30 to 60	> 60
PL (N=27, 22M, 5F)	2 (9%)	0 (0%)	0 (0%)	0 (0%)	2 (7%)	0 (0%)
0.1 (N=7, 7M, 0F)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
0.5 (N=44, 29M, 15F)	2 (7%)	0 (0%)	0 (0%)	0 (0%)	2 (5%)	0 (0%)
0.5* (N=39, 22M, 17F)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)
1.0 (N=52, 38M, 14F)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
2.5 (N=13, 12M, 1F)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	2 (15%)	0 (0%)
5.0 (N=12, 12M, 0F)	2 (17%)	0 (0%)	-	-	2 (17%)	0 (0%)
10 (N=12, 11M, 1F)	1 (9%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)
20 (N=12, 12M, 0F)	2 (17%)	0 (0%)	-	-	2 (17%)	0 (0%)
40 (N=6, 5M, 1F)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
All Entecavir Doses	11 (9%)	0 (0%)	0 (0%)	0 (0%)	11 (7%)	0 (0%)

PL Placebo
- Not applicable
* Tablet formulation
[a] N=7; [b] N=20; [c] N=8; [d] N=6; [e] N=44; [f] N=39; [g] N=46; [h] N=13; [i] N=12; [j] N=5

Pharmacokinetic/Pharmacodynamic Results:
Box plots of QTcB and change from baseline in QTcB versus dose following administration of single and multiple oral doses of placebo or entecavir are presented in Figures 1 and 2, respectively. Scatter plots and regression analysis of change from baseline in QTcB and CECG following administration of single and multiple oral doses of placebo or entecavir are presented in Figure 3 and Table 4.

Figure 1 Box Plots of QTcB Versus Dose Following Administration of Single (Day 1) and Multiple (Day 14) Oral Doses of Placebo or Entecavir

Day 1



Day 14

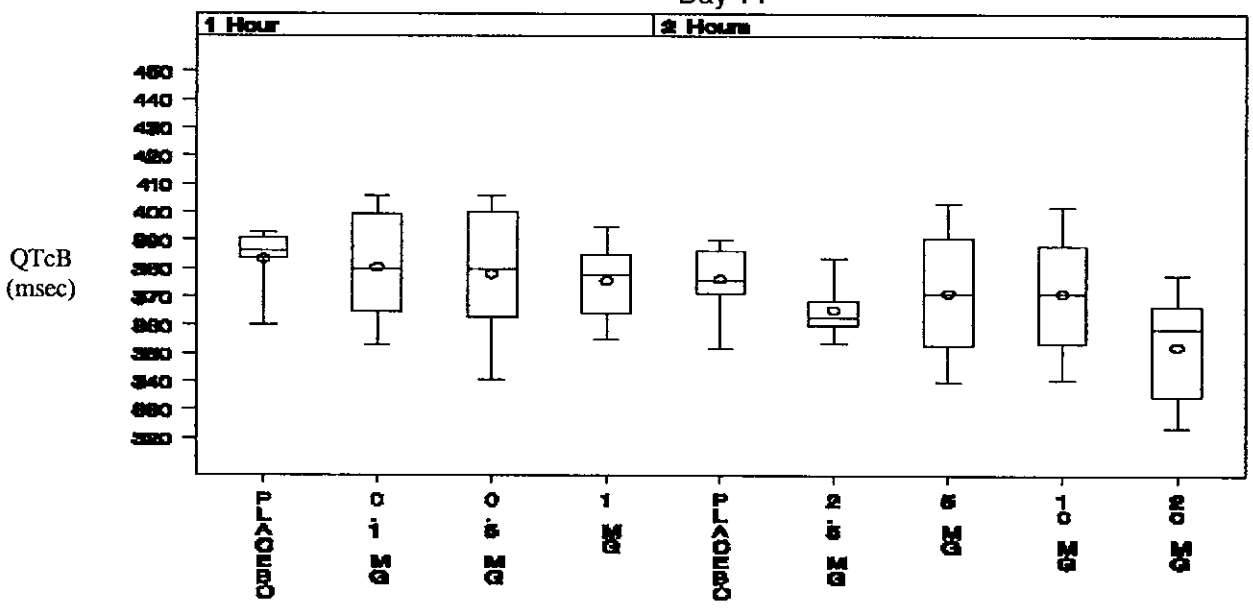


Figure 2 Box Plots of Change From Baseline in QTcB Versus Dose Following Administration of Single (Day 1) and Multiple (Day 14) Oral Doses of Placebo or Entecavir

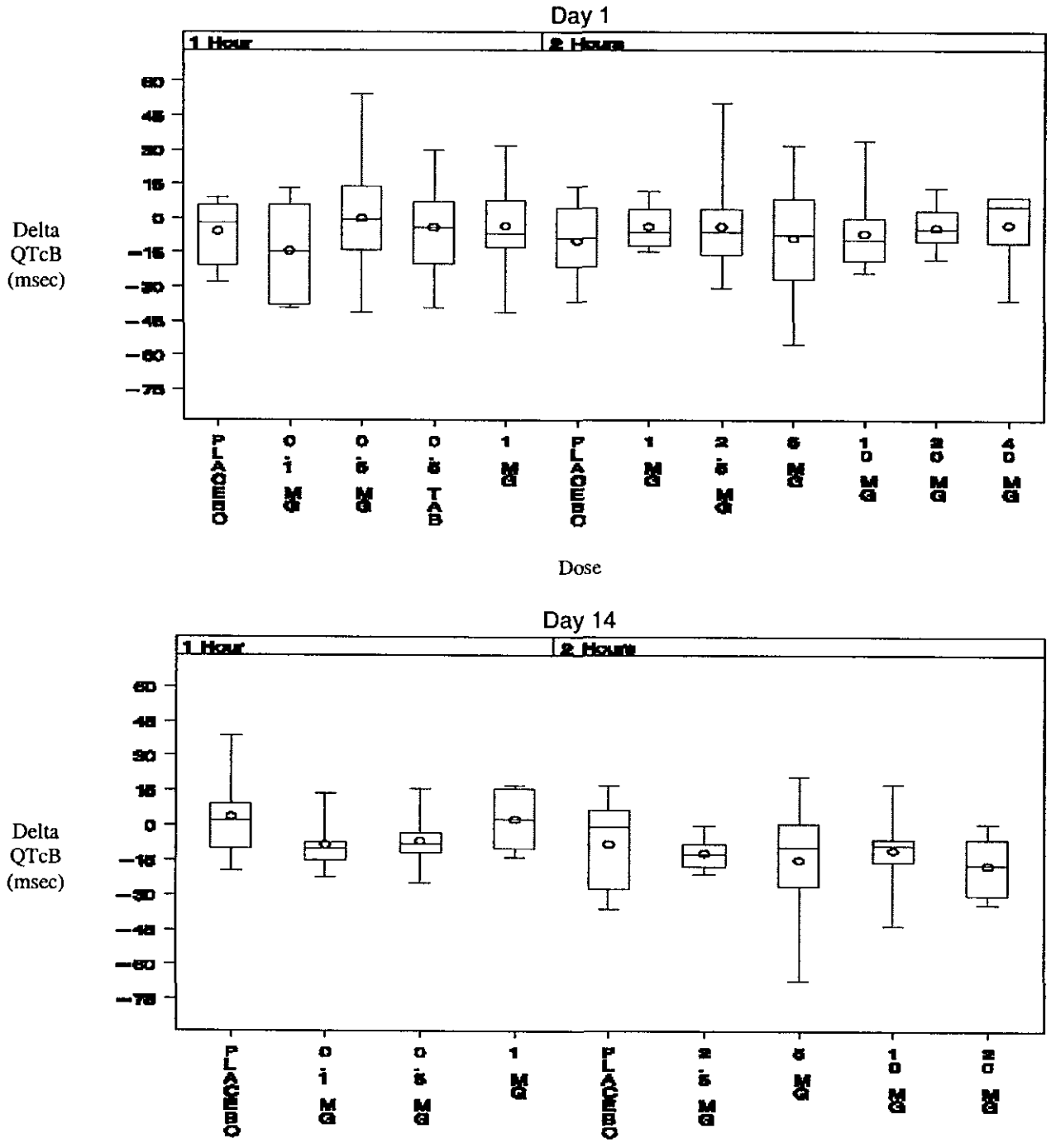
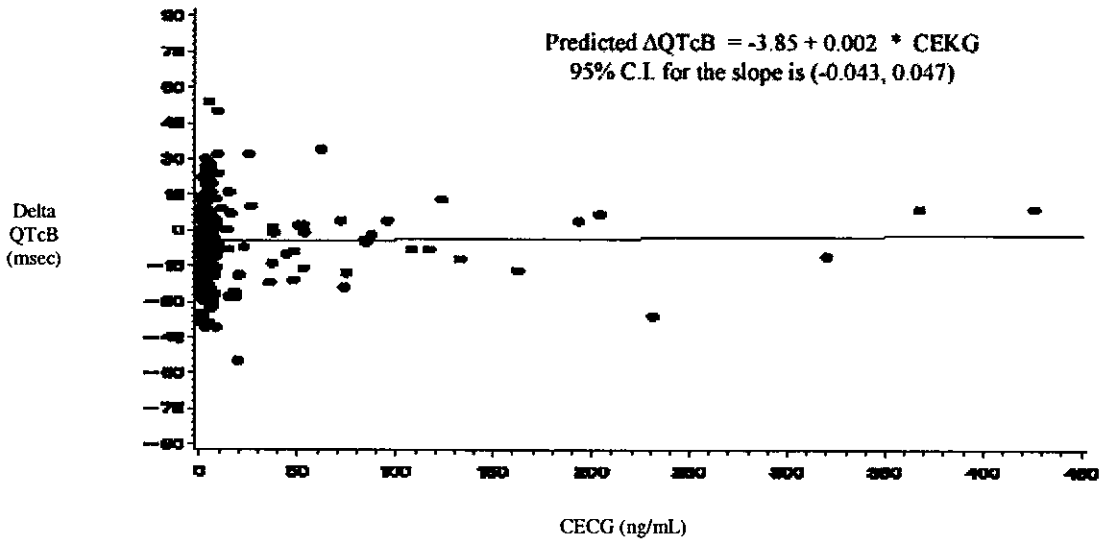


Figure 3 Scatter Plots and Regression Analysis of CECG and Change From Baseline in QTcB Following Administration of Single and Multiple Oral Doses of Placebo or Entecavir

Day 1



Day 14

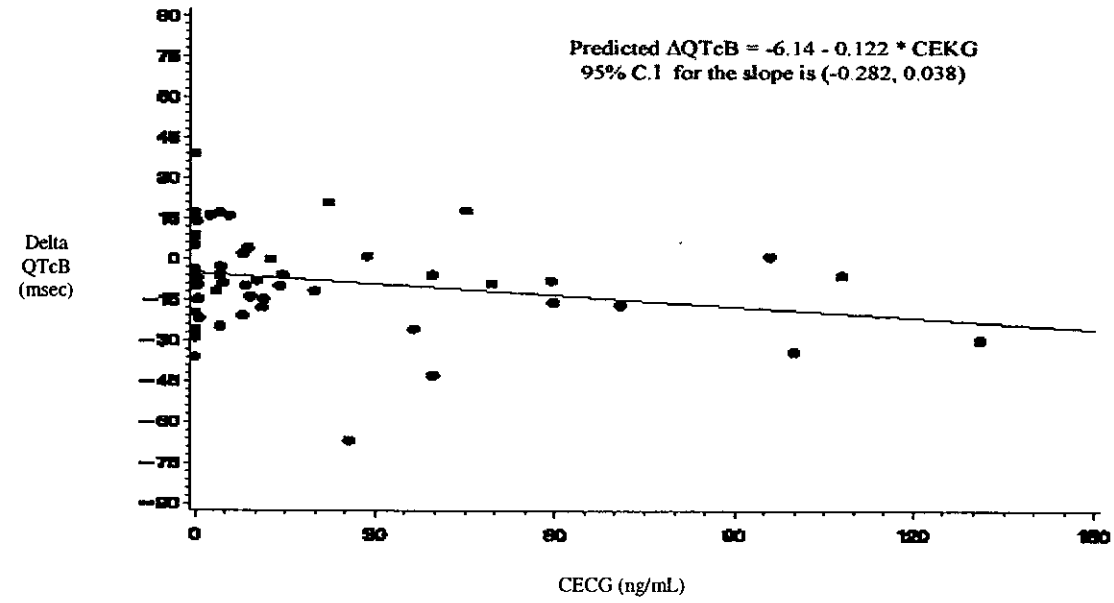


Table 4 Linear Regression of Change From Baseline in QTcB and CECG Following Administration of Single and Multiple Oral Doses of Placebo or Entecavir in Healthy Subjects

Study Day	Intercept		Slope	
	Point Estimate	95% CI	Point Estimate	95% CI
1 (N=195)	-3.85	(-6.57, -1.13)	0.002	(-0.043, 0.047)
14 (N=41)	-6.14	(-12.89, 0.62)	-0.122	(-0.282, 0.038)

Assessment/Conclusion:

- In general, no dose- or day of administration-related patterns in mean QTcB or change in QTcB following entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg were observed. The mean QTcB and change in QTcB values for entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg were comparable to placebo on Days 1, 7, and 14 (mean change in QTcB values ranged from -9.9 to 3.7 msec for placebo and -17.2 to 7.3 msec for entecavir). Based on regression analysis, for each additional 10 ng/mL of entecavir CECG, the estimated increase in change in QTcB ranged between –1.22 and 0.02 msec. The estimated slope of the linear regression on Day 1 was slightly greater than zero, whereas the estimated slope on Days 7 and 14 were negative. On all the days, the 95% confidence intervals for slope contained zero, indicating that the regressions on CECG were not statistically significant.
- A secondary outcome measure for this retrospective analysis was the QT corrected for heart rate by Fridericia's formula (QTcF), as Bazett's formula tends to under and over correct at extremes of heart rate. Similar to observations with Bazett's correction, no dose- or day of administration-related patterns in QTcF following entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg were apparent. The mean QTcF and change in QTcF values for entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg were comparable to placebo (mean change in QTcF values ranged from -6.5 to 8.0 msec for placebo and from -13.4 to 7.7 msec for all entecavir doses). Based on regression analysis, for each additional 10 ng/mL of entecavir CECG, the estimated change in QTcF ranged between -0.92 and 0.03 msec. All 95% confidence intervals for the linear regressions of change in QTcF on CECG include zero except for the intercept on Day 1 (-5.46, -0.99).
- In this retrospective analysis, no subject had any QTcB or QTcF > 500 msec or prolonged QTcB or QTcF (> 470 msec for females; > 450 msec for males), nor did any subject have a prolonged change in QTcB or QTcF (> 60 msec). The overall incidence of borderline and prolonged change in QTcB or QTcF were comparable between placebo and entecavir doses up to 20 mg for up to 14 days or as a single dose of 40 mg. All of the cases of borderline changes in QTcB and QTcF occurred in males, and none occurred at the highest dose studied (40 mg). Corresponding CECG values for the borderline and prolonged changes in QTcB and QTcF ranged from 0 to 62.02 ng/mL, and no concentration-response relationship was discernable. *This assessment of categorical changes in this retrospective analysis underscores the limitations of study AI463041. Although no QTcB or QTcF measurements > 500 msec, instances of prolonged QTcB or QTcF, or prolonged changes in QTcB or QTcF were observed in this retrospective dataset, instances of QTc and changes in QTc greater than the established thresholds can be found in the study populations included in this analysis. For example, in study AI463010 two male subjects on entecavir had QTc values > 450 msec and changes in QTc > 60 msec 48 hours following a single 1 mg dose (Subject*

30: QTc 467 msec, change in QTc 67 msec; Subject 33: QTc 501 msec, change in QTc 79 msec). These 48-hour measurements were not reflected in this retrospective analysis. In general, the selection criteria for inclusion of data in retrospective safety analyses are critical for identification of outliers and safety signals (specifically QT prolongation).

- An additional secondary outcome measure for this retrospective analysis was the PR interval. The reported PR prolongations (< 250 msec) were mild, asymptomatic, not associated with second- or third-degree AV block, and are unlikely to be clinically significant. The regression analyses produced 95% confidence intervals for the slopes that included zero on Days 1 and 7 but not on Day 14. Entecavir may have mild concentration-dependent effects on PR interval on Day 14 at doses up to 20 mg; however, at the maximum clinically relevant dose of 1 mg, mean change in PR obtained on Day 14 was 1.7 msec, which is not expected to be a clinically relevant change. *Three cases of first-degree AV block were encountered in other phase 1 studies for entecavir not included in this retrospective analysis. Two subjects who had received entecavir 1 mg in study AI463065 presented with mild but treatment-emergent first-degree AV block at discharge deemed not related to study drug by the investigator. In addition, one subject in Japanese study AI463029 was recorded as having first-degree AV block at multiple times during the study while receiving multiple daily doses of entecavir 1 mg. This subject's highest recorded PR value was 356 msec predose on Day 8 of entecavir treatment, at which time the subject was discontinued from study without being dosed. The clinical significance (or lack of) of PR prolongation caused by entecavir requires further assessment in the patient population, specifically in the phase 2/3 studies under review in the application.*
- Analyses performed on the QT interval normalized to a heart rate of 60 bpm, heart rate, absolute QT interval, RR interval, and QRS interval indicate there is no apparent effect on these parameters following entecavir administration for up to 14 days at doses up to 20 mg or as a single dose up to 40 mg in healthy adult subjects.
- Data from this retrospective analysis further supports nonclinical data from in vitro [rabbit Purkinje fibers or potassium channel currents (hERG)] and in vivo (3-month oral toxicity in dogs and a one-year oral toxicity in monkeys) studies which indicate that entecavir has a low potential for prolongation of QT intervals.
- In addition, in vitro studies have demonstrated that entecavir is neither an inhibitor nor an inducer of cytochrome (CYP) P450 enzymes, so the potential for CYP P450-mediated interactions is low between entecavir and P450 substrates that have demonstrated arrhythmogenic potential.
- Although the Day 1 slope was positive for the linear regressions, these analyses produced 95% confidence intervals for the slopes that included zero in all cases for Days 1, 7, and 14. Entecavir CECG plasma concentration ranged from [redacted] ng/mL in these analyses. The mean CECG for the 1 mg dose, the highest clinically relevant dose, ranged from 1.00 to 9.15 ng/mL in these analyses. Therefore, the Day 1 positive slope finding is not considered to be clinically relevant.
- In conclusion, the selection criteria for inclusion of data in the Sponsor's retrospective analysis of ECGs collected in Phase 1 studies was not sufficient for identifying outliers for cardiac safety in the entecavir Phase 1 population. In addition, data used for describing the concentration-response relationships in this analysis do not include these outlier observations; therefore, the quantitative descriptions of concentration-response should be interpreted with caution. A definitive QT study was not conducted per agreement with the Sponsor. Despite these limitations, the results of the retrospective analysis are supportive of the in vitro cardiovascular safety studies and the cardiac adverse event profiles obtained in the Phase 3 program. Upon discussion of these findings with the Medical Officer, it is

concluded that entecavir is not expected to result in any clinically relevant effects on cardiac safety.

4.2. Consult Reviews

4.2.1. Population Pharmacokinetics/Pharmacodynamics

4.2.1.1. Population Pharmacokinetics and Pharmacodynamics of Entecavir in Adults with Chronic Hepatitis B Virus Infection (Report AI463017)

EXECUTIVE SUMMERY: This study evaluated the pharmacokinetics of entecavir in patients with chronic hepatitis B virus infection, and showed that the pharmacokinetics of entecavir is similar between patients and healthy subjects with the similar creatinine clearance and body weight. This information will be included in the label. The pharmcodynamic analysis confirmed previous finding that prior treatment with lamivudine resulted in a diminished response to ETV; thereby, requiring a higher dose in lamivudine refractory subjects to achieve a response comparable to the response in nucleoside naïve subjects.

OBJECTIVES:

- To describe the pharmacokinetics of entecavir in subjects with HBV with a population pharmacokinetic model;
- To identify and quantify covariate effects on the pharmacokinetics of entecavir;
- To characterize relationships between measures of entecavir exposure and change in hepatitis B virus (HBV) DNA with a population PK/PD model;
- To explore relationships between measures of entecavir exposure and the severity of selected adverse events.

STUDY DESIGN: The data used in the present analysis come from 3 Phase II clinical studies, AI463004, AI463005, and AI463014. The following summarizes the study design for these 3 studies.

<u>AI463004</u>
Study AI463004 was a pilot study in adults with chronic hepatitis B virus (HBV) infection (both treatment naïve and lamivudine/interferon pretreated) who had well compensated liver disease. It was a randomized, double-blind, placebo-controlled; dose escalating trial (doses of 0.05, 0.1, 0.5, and 1.0 mg once daily) in which the treatment period was 28 days and the post-dosing follow-up period was 6 months. HBV DNA data were collected at baseline, Week 4 and Week 8. Adverse event data were collected at all study visits. Subjects had trough blood samples drawn on Days 1, 7, 14, 21, and 28, prior to the daily dose, for analysis of the pharmacokinetics of ETV.

<u>AI463005</u>
Study AI463005 was a randomized, double-blind study of 3 doses of ETV (0.01, 0.1 and 0.5 mg QD) given for 24 weeks compared to lamivudine (100 mg QD) in adults with chronic hepatitis B virus (HBV) infection who were nucleoside-naïve while on lamivudine therapy. Randomization was stratified by HBeAg status. HBV DNA data were collected at baseline, Week 4, Week 12, Week 22, and Week 36. Adverse event data were collected at all study visits. Pharmacokinetic sampling for ETV was performed at 1.5, 3, 6, and 10 hours post-dose on Day 1 and at least 1 hour post-dose at Weeks 4, 12 and 22. Pharmacokinetic samples were also obtained prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after dosing on Days 1 and 14 for Site 22.

AI463014

Study AI463014 was a multinational, randomized, double-blind study of ETV (0.1, 0.5, and 1.0 mg QD) compared with continued lamivudine therapy (100 mg QD) in subjects with chronic hepatitis B infection with viremia while on lamivudine therapy. Study drug dosing was continued for up to 48 weeks. HBV DNA data were collected at baseline, Week 24, and Week 48. Adverse event data were collected at all study visits. Pharmacokinetic sampling for ETV was performed at 1.5, 3, and 6 hours post-dose on Day 1 (strongly recommended but not required), and at least 1 hour post-dose at Weeks 4, 12, 24, 36, and 48 (strongly recommended but not required).

DATA

Pharmacokinetics

The pharmacokinetic data used in the population pharmacokinetic analysis represent all available concentration data collected in studies AI463004, AI463005, and AI463014, with the exception of 169out of a total of 1282 samples (13.2%) for study AI463005, and 132 out of a total of 794 samples (16.6%) for study AI463014. These samples were either analyzed or matched to the appropriate subject after the final pharmacokinetic database was created.

Model Building and Validation Databases

Initially, pharmacokinetic data were available from 265 subjects. During the database creation, 41 subjects did not have recoverable pharmacokinetic data, leaving a total of 224 subjects with at least one merged pharmacokinetic observation. Approximately 20 percent of the 224 subjects (45 subjects) were removed from the full database for the creation of the internal validation database. During preliminary model building, an additional 11 observations were removed due to high weighted residuals (absolute value of weighted residuals greater than 5).

Summary statistics of subject demographics for pharmacokinetic model development are provided in the following Tables 2 and 3.

Table 2: Summary of Baseline Demographics for the Pharmacokinetic Model Building Database (Studies AI463004, AI463005, and AI463014; n=177)

Baseline Characteristic (Units)[a]	Mean (SD)	Median	Range
Age (y)	38.8 (13.8)	38.0	16-75
Height (cm)	169 (8.47)	170	145-190
Weight (kg)	69.8 (12.7)	70.0	45-107
Ideal Body Weight (kg)	64.3 (8.95)	65.5	38.8-84
Body Mass Index (kg/m^2)	24.2 (3.58)	23.9	17-35.4
Creatinine Clearance[b] (mL/min)	103 (27.0)	98.9	48.5-150
Creatinine Clearance using IBW (mL/min)[b]	96.0 (25.0)	93.1	40.2 150
Bilirubin (mg/dL)	0.832 (0.437)	0.800	0.200-2.4
Alk Phos (IU/L)	103 (68.0)	82.0	11.0-413
Albumin (g/dL)	4.23 (0.391)	4.2	3-5.4
ALT (IU/L)	106 (90.3)	78.0	10-564
AST (IU/L)	62.8 (47.5)	48.0	11-295
Amylase (IU/L)	80.5 (39.0)	74.0	19-246
Sex	Male =136 (76.8%); Female = 41 (23.2%)		
Race[c]	Caucasian=88 (49.7%); Non-Caucasian=89 (50.3%)		
Study	Study AI463004 N = 30	Study AI463005 N = 93	Study AI463014 N = 54

[a] These are baseline demographic values only. Changes of patient weight, BMI, lab values etc. over time were recorded in the database

[b] Creatinine Clearance was calculated using Cockcroft and Gault and was capped at 150 mL/min as a reasonable upper limit.

[c] For a complete listing of patient numbers by race, please see Table 3.

Table 3: Number of Subjects per Racial Category in Model Building Dataset (n=177)

Racial Category	Racial Characteristics	Number of Patients in Model Building Dataset
1	Caucasian	88
2	African American	5
3	Asian / Pacific Islander	72
4	Hispanic	5
5	Other	7

The validation data set was created using only subjects enrolled in Study AI463005 and Study AI463014, since preliminary model based evaluation had been conducted using data from Study AI463004 and some of the subject data from Study AI463005. Subjects enrolled in Study AI463005 whose data had been used for the preliminary evaluation were not included in the validation database. The remaining subjects were randomly selected from Studies AI463005 and AI463014 so that 20% of the total number of subjects with pharmacokinetic data was included in the validation database. The number of subjects was balanced so that the percent inclusion in the validation database reflected the number of subjects from each study in the model-building database. Therefore, 27 subjects from Study AI463005 and 18 subjects from Study AI463014 were included in the validation database.

The validation database initially consisted of 304 observations from 45 subjects. During data assembly, 60 records were removed because the concentration value was missing or not reported; therefore, the final validation database consisted of 244 observations. Exclusion of these pharmacokinetic observations did not result in the complete exclusion of any subjects. A summary of baseline demographics for the pharmacokinetic validation database are provided in Table 4.

Table 4: Summary of Baseline Demographics for the Pharmacokinetic Validation Database (Studies AI463005 and AI463014; n=45)

Baseline Characteristic (Units)[a]	Mean (SD)	Median	Range
Age (y)	38.5 (13.3)	36.0	19-66
Height (cm)	167 (8.29)	168	151-182
Weight (kg)	67.9 (16.1)	64.0	40-106
Ideal Body Weight (kg)	61.6 (9.08)	64.1	44.2-76.8
Body Mass Index (kg/m^2)	24.2 (4.78)	22.9	17.3-38.5
Creatinine Clearance[b] (mL/min)	93.0 (28.6)	90.3	10.2-150
Creatinine Clearance using IBW (mL/min)[b]	84.9 (24.1)	83.3	8.53-126
Bilirubin (mg/dL)	0.833 (0.405)	0.700	0.300-2.60
Alk Phos (IU/L)	85.1 (51.2)	71.0	14-228
Albumin (g/dL)	4.32 (0.418)	4.33	3.6-5.26
ALT (IU/L)	86.6 (77.6)	59.0	16-357
AST (IU/L)	55.1 (35.7)	47.0	7-189
Amylase (IU/L)	95.8 (61.5)	77.0	12-265
Sex	Male =32 (71.1%); Female = 13 (28.9%)		
Race	Caucasian=13 (28.9%); Non Caucasian=32 (71.1%)		
Study	Study AI463004[c] N = 0	Study AI463005 N = 27	Study AI463014 N = 18

[a] These are baseline demographic values only. Changes of patient weight, BMI, lab values etc. over time were recorded in the database

[b] Creatinine Clearance was calculated using Cockcroft and Gault and was capped at 150 mL/min as a reasonable upper limit.

[c] AI463004 data excluded since preliminary model-based evaluation was conducted with this data.

Pharmacodynamics

A total of 1004 viral load observations from 313 patients enrolled in Studies AI463004, AI463005, and AI463014 were initially available for inclusion in the pharmacodynamic database. During database assembly, a total of 242 observations from 84 patients were excluded because there were no derived pharmacokinetic parameters for those patients. There was no other reason for data removal. The final database used for the initial model building consisted of 762 observations from 229 subjects (75.9% of the total observations and 73.2% of the subjects with data originally available for analysis). This analysis was exploratory in nature and therefore no validation data set was created. A summary of baseline demographics for the viral load database is listed in Table 6.

Table 6: Summary of Baseline Demographics for the Viral Load Database (Studies AI463004, AI463005, and AI463014; n=229)

Baseline Characteristic (Units)[a]	Mean (SD)	Median	Range
Age (y)	38.7 (13.7)	37.0	16.0-75.0
Height (cm)	169 (8 54)	170	145-190
Weight (kg)	69.4 (13.5)	69.0	40.0-107
Ideal Body Weight (kg)	63.9 (9.08)	65.0	38.8-84.0
Body Mass Index (kg/m^2)	24.2 (3.84)	23.8	17.0-28.5
Creatinine Clearance[b] (mL/min)	101 (24.6)	98.3	48.5-150
Creatinine Clearance using IBW (mL/min)[b]	94.4 (23.9)	91.8	40 2-150
Bilirubin (mg/dL)	0.83 (0.43)	0.75	0.20-2.6
Alk Phos (IU/L)	100 (66.3)	81.0	11 3-413
Albumin (g/dL)	4.25 (0.39)	4.20	3.00-5.40
ALT (IU/L)	107 (113)	73.0	14.0-1154
AST (IU/L)	63.5 (54.7)	49 0	7.00-520
Amylase (IU/L)	83 7 (45.1)	75	12 0-265
Baseline HBV DNA (Log copies/mL)	8.42 (1 02)	8.51	4.57-11.0
AUCss (ug/L*h)	15.6 (20.7)	5 99	0-150
Css,max (ug/L)	2.82 (3 40)	0.912	0-20.6
Css,min (ug/L)	0.39 (0 59)	0.15	0-5.02
Sex	Males =176 (76.9%); Females = 53 (23.1%)		
Race	Caucasian=97 (42 4%); Non-Caucasian=132 (57.6%)		
Study	Study AI463004 N = 38	Study AI463005 N = 119	Study AI463014 N = 72
Dose	Placebo= 8; 0.01 mg=47; 0.050 mg=4; 0.1 mg=66; 0.5 mg=75; 1.0 mg=29		
Prior Lamivudivine	Yes=85; No=144		
Prior Interferon	Yes=74; No=155		

[a] These are baseline demographic values only. Changes of patient weight, BMI, lab values etc. over time were recorded in the database

[b] Creatinine Clearance was calculated using Cockcroft and Gault and was capped at 150 mL/min as a reasonable upper limit.

MODELS: — conducted all the PK/PD modeling of entecavir, using NONMEM Version V level 1.1 (Globomax LLC Hanover, MD). Appendix AI463017_1 and Appendix AI463017_2 show the PK and PD model-building processes, respectively.

Structural Models

The pharmacokinetics of entecavir had been described previously using a three-compartment model with first order input and linear clearance. This model was evaluated initially, but did not converge successfully. Consequently, various one and two and three compartment structural models with first order elimination and either first or zero order input were evaluated. Because the data were obtained at steady state, an absorption lag time was not evaluated. There also

was no information describing a third compartment in the sparse data obtained at steady state. Once a simpler structural model was identified, the model was modified to mimic the performance of the Phase I model by adding a covariate function describing the effect of dose on inter-compartmental clearance.

A direct effect inhibitory Emax model was used to describe the change in viral load (HBV DNA) over time using a user written PRED routine. The model was parameterized for the maximum reduction in viral load (RESPmax) and the time to half maximal reduction (TDAY50).

The final basic structural model was selected on the basis of goodness-of-fit as judged by the likelihood ratio test and the Akaike Information Criteria. Other model assessment criteria included primarily checking for visual improvements in the agreement between the observed and predicted observations, reduction of the weighted residuals for the predicted values, and reductions in inter-individual variability (IIV) or random residual variability (RRV).

Covariate Models

The covariate models used in this analysis for both the pharmacokinetic and pharmacodynamic assessment were defined to represent covariate influences as shifts in the parameter of interest from the parameter value observed in a hypothetical reference subject. The reference subject used in this analysis was a subject with demographic factors that were either approximately equal to the mean or median (e.g. weight, creatinine clearance) or were scaled to a reasonable value.

Continuous covariates such as age or weight were modeled using a general slope function:

$$TVP = P_{pop} \cdot \prod_{i=1}^{n} cov_i^{\theta_i}$$

as well as an intercept slope function:

$$TVP = P_{pop} + \sum_{i=1}^{n} \mathrm{cov}_i \cdot \theta_i$$

In these equations, TVP represents the typical predicted PK/PD parameter for the "reference" individual with covariate value(s) cov_i, P_{pop} represents the population central tendency for the PK/PD parameter TVP (first equation) or the intercept value for the PK/PD parameter TVP (second equation), cov_i represents the individual value for that covariate normalized by the approximate median value for the subject population, and θ_i represents a scale factor.

Because body weight is correlated with other predictors, it was desirable to fix this component of the covariate-parameter relationships to a known (theoretically and empirically) allometric relationship to evaluate the effect of body size. This model was only tested in the pharmacokinetic portion of this evaluation.

$$TVP_{Clearance} = P_{pop} * \left(\frac{Wt}{Median} \right)^{0.75}$$

$$TVP_{Volume} = P_{pop} * \left(\frac{Wt}{Median} \right)$$

Categorical covariates (e.g., sex and race) were modeled using the general equation:

$$TVP = P_{pop} \cdot (1 + \mathrm{cov}_i \cdot \theta_i)$$

In this equation, cov_i is either 0 (for the standard or reference subject), or 1 for the comparative subject.

Covariates were first examined for potential effect on structural parameters by graphical assessment, followed by an evaluation using a generalized additive modeling (GAM) approach as implemented in XPOSE 3. The GAM is analogous to the stepwise multiple linear regression but is not restricted to linear model shapes. The Xpose program enables a linear or non-linear stepwise search of selected covariate influence on a parameter to be carried out according to a defined hierarchy. The relative impact of these covariates on the PK/PD of entecavir was ultimately assessed by the associated decrease in objective function together with the magnitude of the covariate effects and any associated reduction in inter-individual variability. Initial covariate selection was conducted using the base model. All covariates were initially modeled individually on each structural model parameter and were then combined on parameters based on the results of the likelihood ratio test (forward addition). The First Order Conditional Estimation (FOCE) method was used for all covariate assessments because the change in objective function has been found to more reliably follow a chi square distribution. Only those covariates that individually influenced the structural parameters were added in descending order of magnitude ("forward addition" method). The covariate was retained in the final model if it resulted in at least 10 points reduction in the objective function from the previous model.

In the evaluation of alternate models, the goodness-of-fit criteria included significant reductions in the objective function ($\geq$ 10 points), improvement in the prediction of the observed concentrations, reduction of the standard errors with respect to the parameter estimates, improvement in the random scatter of weighted residuals around the line of identity in plots of weighted residuals versus predicted concentrations, minimization of the inter-individual variances and an improvement in their prediction, and a reduction in the magnitude of the residual variability.

Pharmacostatistical Model

The pharmacostatistical model component includes the inter-individual, and intra-individual error models and random residual models. Inter-individual and intraindividual error models describe the residual variation in pharmacokinetic parameters after correction for fixed effects, and the residual error model describes the underlying distribution of the error in the measured pharmacokinetic observations. Inter-individual variability was described using the equation $P_j = TVP \bullet e^{\eta j}$. In this equation P_j is the individual value for the pharmacokinetic parameter in the jth individual and η_j is an independent random variable with a mean of zero and variance ω_{p2}. Inter-occasion variability was evaluated in the pharmacokinetic model but was not identifiable. Inter-occasion variability was not evaluated in the viral load pharmacodynamic model. The residual variability for the pharmacokinetic model was described using a combined additive and constant coefficient of variation (CCV) model.

Model Validation:

Validation of the final pharmacokinetic model was carried out by generating the maximum *a posteriori* (MAP) Bayesian estimates for the validation data set using the final model parameter estimates. In this evaluation, the final model, together with the final population parameters from the model-building dataset, was applied to the validation dataset to obtain estimates of entecavir concentrations for the typical subject as well as the individual Bayesian estimates using MAXEVALS=0 (maximum *a posteriori* Bayesian assessment). These predictions were

graphically compared to the actual observed concentrations. Agreement between the predicted and observed concentrations was evaluated to verify model predictive reliability. Finally, because the pharmacokinetic evaluations required assessment of derived parameters (e.g. AUCss), a limited check was conducted comparing data derived noncompartmental evaluations of AUC(INF) with model derived calculation of AUCss by linear regression.

The pharmacodynamic model that evaluated the change in HBV DNA over time was an exploratory evaluation and therefore did not undergo the same extent of qualification as the pharmacokinetic model.

RESULTS AND DISCUSSION:

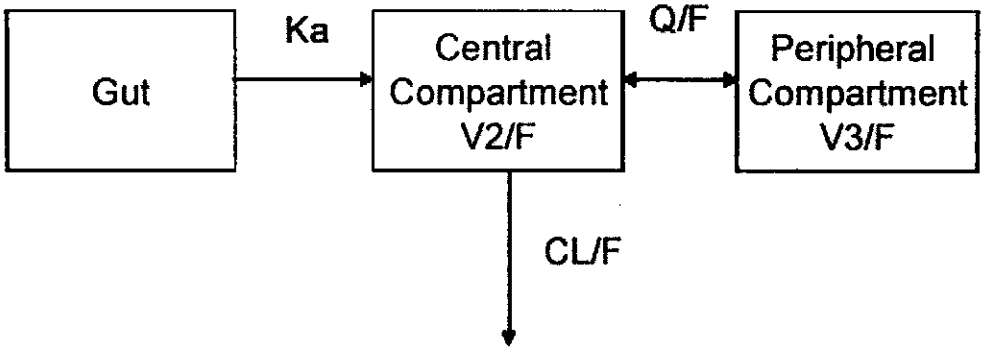
The study designs of the three Phase II trials are adequate for the population PK/PD analysis. The data integrity and model building process (Appendix AI463017_1 and Appendix AI463017_2) are acceptable.

Pharmacokinetics

Final Base Model

The final pharmacokinetic structural model was a two-compartment model with linear clearance. This model (Model 95, Appendix AI463017_1) was parameterized in terms of apparent clearance (CL/F), the volume of distribution of the central (V2/F) and peripheral (V3/F) compartments, inter-compartmental clearance (Q/F) and the absorption rate constant (Ka) using ADVAN4 and TRANS2. The absorption rate constant was fixed to a value of 6 based on the results of a sensitivity analysis. The model that was developed in the present analysis was a simplification of the model defined in the Phase I evaluation, which was a three-compartment model with first order input and output and concentration dependent distribution into a peripheral compartment. In the present analysis, dose power was used as a covariate on inter-compartmental clearance as a means of mimicking the behavior of the Phase I model. The residual error model was a combined constant coefficient of variation (CCV) and additive model. Inter-individual variability was described for CL/F, V2/F, Q/F, and V3/F with terms describing the correlation between CL/F and V2/F, and between V2/F and V3/F. Inter-occasion variability was investigated, but was not found to be identifiable. The FOCE method with Interaction and SLOW option was used to obtain the base pharmacokinetic parameter estimates. Terms describing the correlation between apparent clearance and the central volume of distribution and between the central and peripheral volumes of distribution were also included. The general schematic diagram for this model is given in Figure 1.

Figure 1: **General Schematic Diagram of Base Pharmacokinetic Model**



A plot of observed versus typical predicted concentrations is presented in Figure 3. The plot of observed versus typical predicted concentration shows that the data are generally uniformly scattered about the line of unity, with the previously mentioned underestimation of some of the peak concentrations. The plot of weighted residuals versus time (Figure 4) shows generally uniform scatter about the line of identity, which suggests that there is no systematic bias in the model. The range of weighted residuals is generally acceptable with the majority of observations having an associated weighted residual value of ±4 (2.3% have weighted residuals that exceed this value), although some observations have weighted residuals that are greater than 10.

Figure 3 **Observed versus Typical Predicted Concentrations - Base Pharmacokinetic Model**

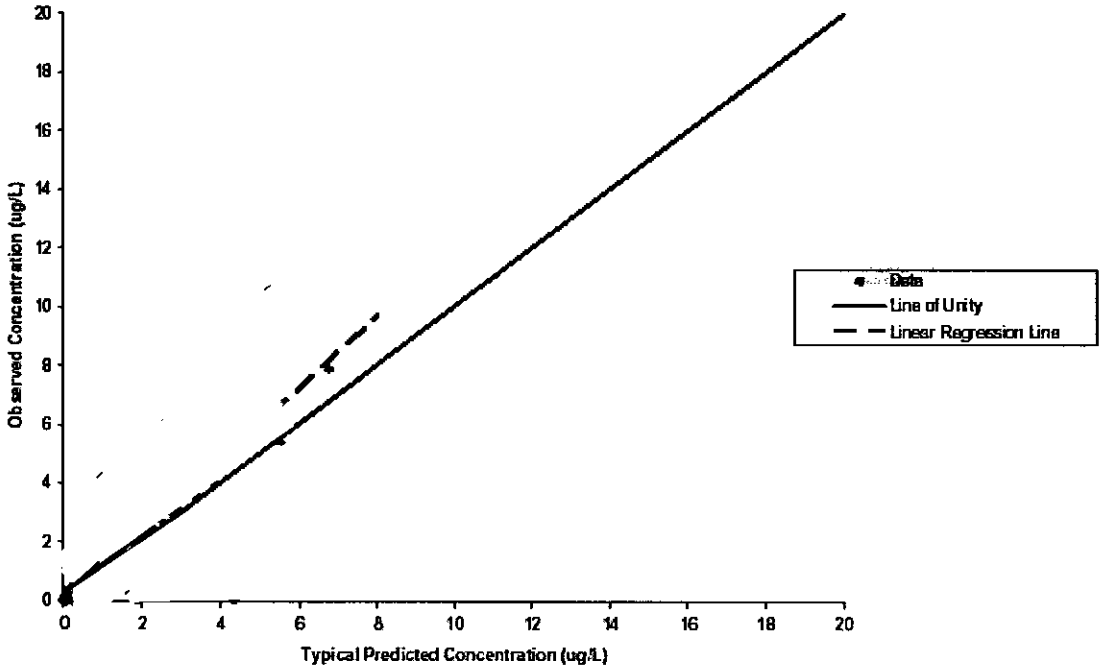
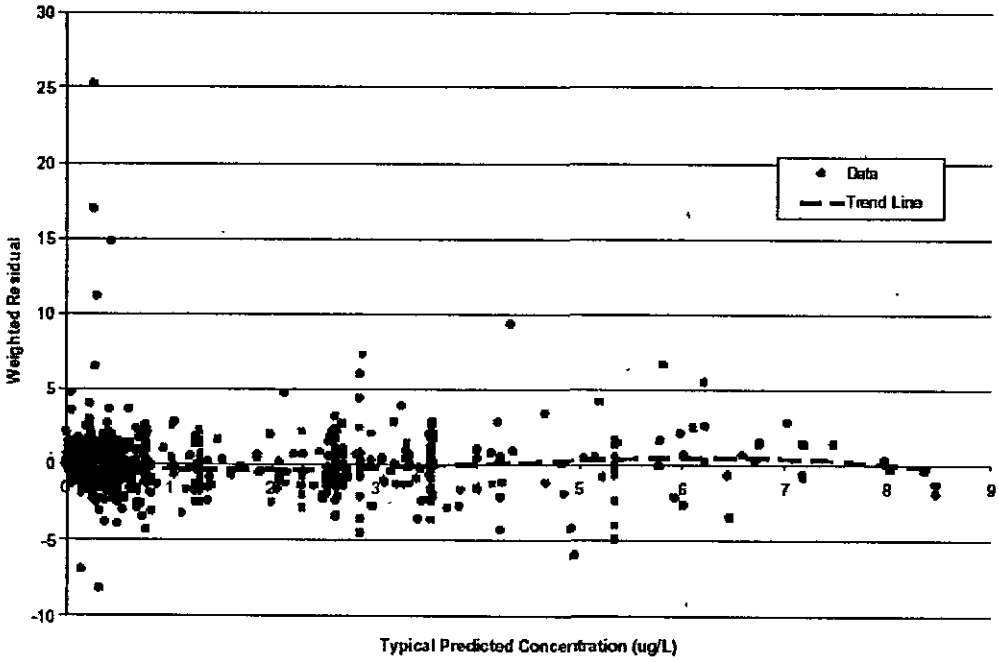


Figure 4 **Weighted Residuals versus Typical Predicted Concentrations - Base Pharmacokinetic Model**



Final Model

Creatinine clearance, as calculated using ideal body weight, was the only covariate identified in the pharmacokinetic analysis. The effect of renal function on the pharmacokinetics of ETV was evaluated using two measures: calculated creatinine clearance using actual body weight and calculated creatinine clearance using ideal body weight. Both of these values were found to significantly contribute to the inter-individual variability. There is a strong correlation between decreasing renal function and decreasing clearance. The use of creatinine clearance calculated with ideal body weight was ultimately selected due to its better ability to explain inter-individual variance. However, both measures of renal function were found to be statistically significant. The objective function was reduced by 35 points by the addition of this covariate factor. The addition of the covariate factors substantially reduced the inter-individual variance terms for all parameters although the residual variability was unchanged.

The following covariate effects were found to be insignificant; age, gender, race, weight, ideal body weight, height, body mass index, and hepatic function parameters (ALT, AST, albumin, alkaline phosphatase, amylase, and bilirubin). The effect of body size on the pharmacokinetics of ETV was evaluated using several measures: weight, ideal body weight, height, and body mass index. While there was a tendency for subjects with increasing body size to have higher clearances, this tendency was not statistically significant when tested as a covariate. None of these measures were found to significantly contribute to the inter-individual variability.

The equations for the final pharmacokinetic model are given below.

$$\frac{CL}{F} = \Theta_1 + \Theta_7 \bullet \left(\frac{ICrCL}{100}\right) \bullet \exp(\eta_1)$$

$$\frac{V2}{F} = \Theta_2 \bullet \exp(\eta_2)$$

$$\frac{Q}{F} = \Theta_3 \bullet \left(DOSE^{\Theta_4}\right) \bullet \exp(\eta_3)$$

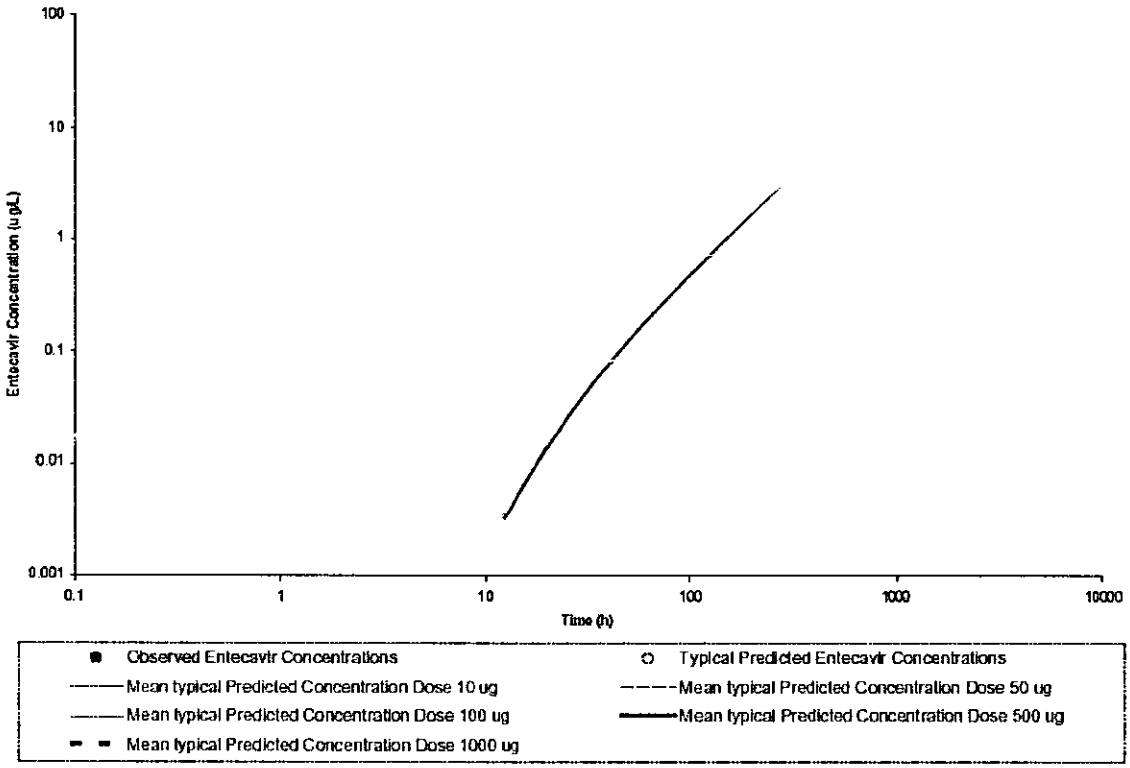
$$\frac{V3}{F} = \Theta_5 \bullet \exp(\eta_4)$$

$$Ka = \Theta_6$$

The control stream for the final model is presented in Appendix AI463017_3.

A plot of observed entecavir concentrations versus time overlaid with the typical predicted concentrations is shown in Figure 21. The agreement is reasonable, although as with the base model, the highest concentrations are still not well described by the typical predicted values.

Figure 21 Observed Log-Transformed Concentrations versus Log-Transformed Time Overlaid With Log-Transformed Typical Predicted Concentrations – Final Pharmacokinetic Model



A plot of the observed versus typical predicted concentrations for the final pharmacokinetic model is provided in Figure 22. The plot demonstrates that the data are generally uniformly scattered about the line of unity. Although the underestimation of some of the peak

concentrations still exists, the plot of weighted residuals versus typical predicted concentrations (Figure 23) shows a marginal improvement in the weighted residuals, with an apparent increase in the number of observations with weighted residuals less than ±4 as compared to the base model (only 1.5% have weighted residuals that exceed this value), and the extreme values are somewhat lower. These diagnostic plots demonstrate that the model performance is generally acceptable.

Figure 22 Observed versus Typical Predicted Concentrations - Final Pharmacokinetic Model

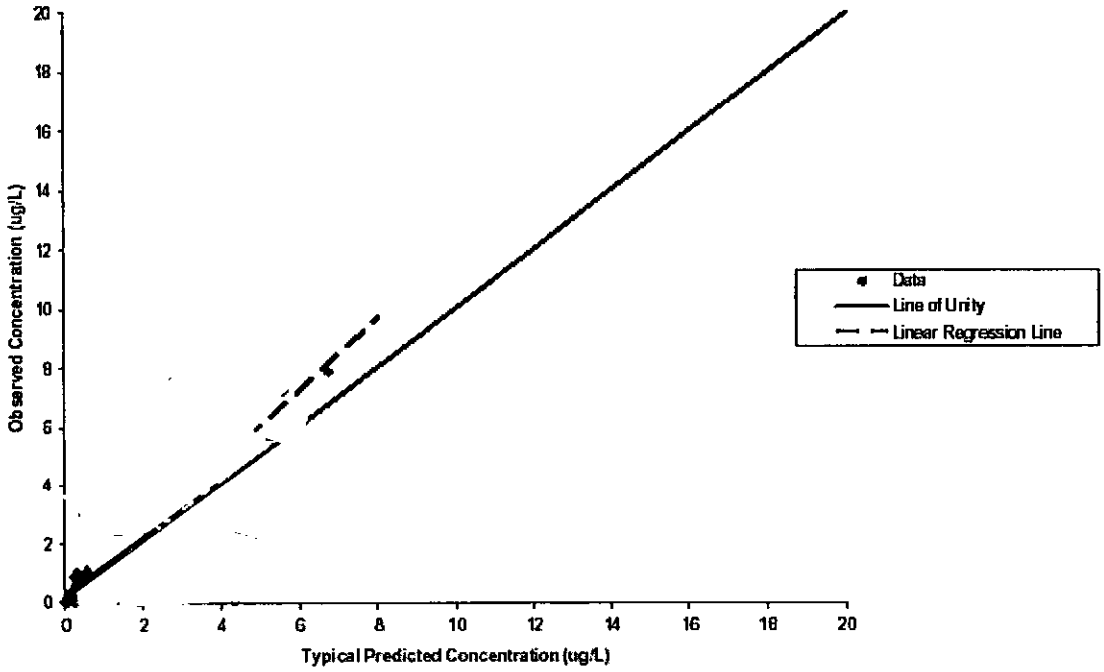
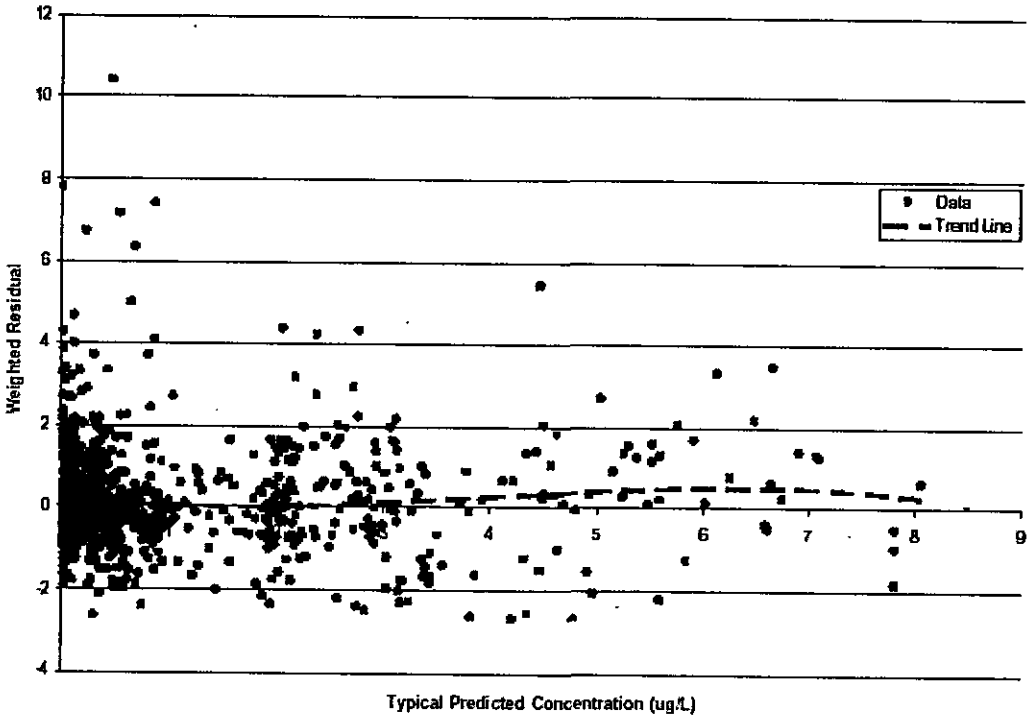


Figure 23 **Weighted Residuals versus Typical Predicted Concentrations - Final Pharmacokinetic Model**



An assessment of the maximum a posteriori Bayesian evaluation of the internal validation database was conducted. A plot of observed versus typical predicted concentrations from this evaluation is given in Figure 24.The performance of the final model on the internal validation data set was judged to be acceptable.

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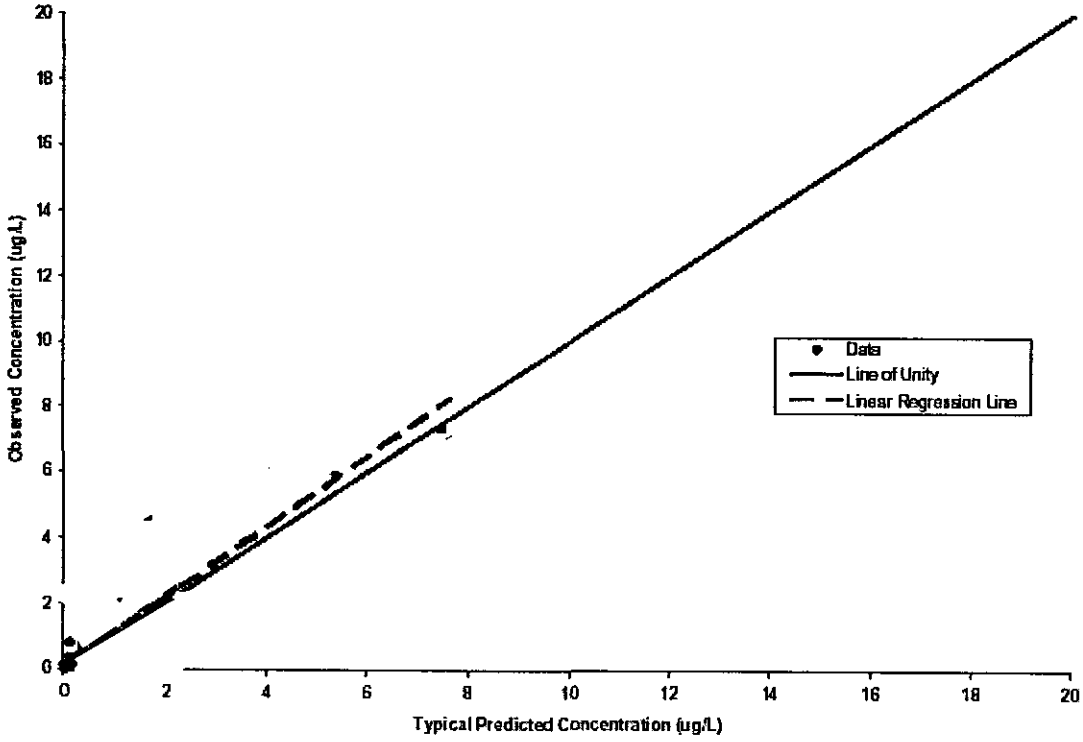
Figure 24 **Observed versus Typical Predicted Concentrations – Final Pharmacokinetic Model Using Validation Database**



A listing of the final parameter estimates and their associated standard errors is given in Table 8.

Table 8: **Parameter Estimates and Associated Standard Errors for Final Pharmacokinetic Model**

Parameter (Units)		Population Mean (SE[a])	%CV Inter-Individual Variance (SE*)
CL/F (L/h)	Θ_1	9.91 (40.1)	40.2 (24.6)
Effect of CrCL	Θ_7	18.3 (22.3)	
V2/F (L)	Θ_2	113 (7.3)	32.8 (33.2)
Q/F (L/h)	Θ_3	324 (19.4)	31.9 (25.4)
Effect of Dose	Θ_4	-0.334 (13.7)	
V3/F (L)	Θ_5	1840 (11.4)	52.9 (52.9)
KA (h-1)	Θ_6	6 FIX	NE
CCV Residual Error (as %CV)			36.2 (8.7)
Additive Residual Error (ng/mL)			0.002 (139)

[a] - SE given as %CV
NE – Not Estimated

In general, the parameter estimates are in good agreement with parameters reported previously. For a subject with a creatinine clearance of 100 mL/min, the model estimated CL/F

was 28.21 L/h. The parameters and their symmetric, asymptotic associated 95% confidence intervals are listed in Table 9. These intervals are narrow, suggesting that the parameters are well defined. However, as mentioned previously, the confidence intervals for the additive portion of the residual error do contain 0, suggesting that the residual error model could be simplified to a constant coefficient of variation model.

Table 9: Median and 95 Percent Confidence Intervals of Final Pharmacokinetic Model

		Summary Statistics			
Parameter		Units	Median	Lower 95% CI	Upper 95% CI
CL	Θ_1	L/h	9.91	2.12	17.70
Effect of Creatinine Clearance	Θ_7		18.3	10.30	26.30
V2/F	Θ_2	L	113	96.83	129.17
Q/F	Θ_3	L/h	324	200.80	447.20
Effect of Dose	Θ_4		-0.334	-0.24	-0.42
V3/F	Θ_5	L	1840	1428.87	2251.13
Inter-individual Variance CL/F	η_1	%CV	40.2	20.82	59.58
Inter-individual Variance V2/F	η_2	%CV	32.8	11.46	54.14
Inter-individual Variance Q/F	η_3	%CV	31.9	16.02	47.78
Inter-individual Variance V3/F	η_4	%CV	52.9	-1.95	107.75
Constant Coefficient of Variation Residual Error	ε_1	%CV	36.2	30.03	42.37
Additive Residual Error	ε_2	ug/L	0.002	0.00	0.01

Pharmacodynamics

The best pharmacodynamic model identified in the present analysis was a direct effect inhibitory Emax model (Model 54, Appendix AI463017_4). The model was parameterized for the maximum reduction in HBV DNA (RESPmax) and the time to half maximal reduction (TDAY50). Inter-individual variability was described for all parameters. A term describing the correlation between RESPmax and TDAY50 was also included. There were no terms describing inter-occasion variability. The model utilized an additive residual error model. The FOCE method was used to obtain the base pharmacodynamic parameter estimates. The equations for the final pharmacodynamic model parameters are given below.

$$RESP\max = \Theta_1 \bullet \left(\frac{Dose}{1000}\right)^{\Theta_3} \bullet (1 + LVD \bullet \Theta_4) \bullet \exp(\eta_1)$$

$$TDAY50 = \Theta_2 \bullet \left(\frac{Dose}{1000}\right)^{\Theta_3} \bullet \exp(\eta_2)$$

$$Effect = 0 - \frac{RESP\max \bullet Day}{TDAY50 + Day}$$

Results indicated the maximum reduction in HBV DNA was dependent on the administered dose of entecavir, and if the subject had been previously treated with lamivudine. The time to half maximal reduction in HBV DNA was affected by administered entecavir dose.

A plot of observed change in HBV DNA versus time overlaid with typical predicted change in HBV DNA is shown in Figure 60. The plot of observed versus predicted change in HBV DNA for the final model and the plot of weighted residuals versus time are shown in Figures 61 and 62. These diagnostic plots indicate that the final model performance is acceptable.

Figure 60 **Observed Change in HBV DNA versus Time Overlaid With Typical Predicted Change in HBV DNA – Final Pharmacokinetic Model**

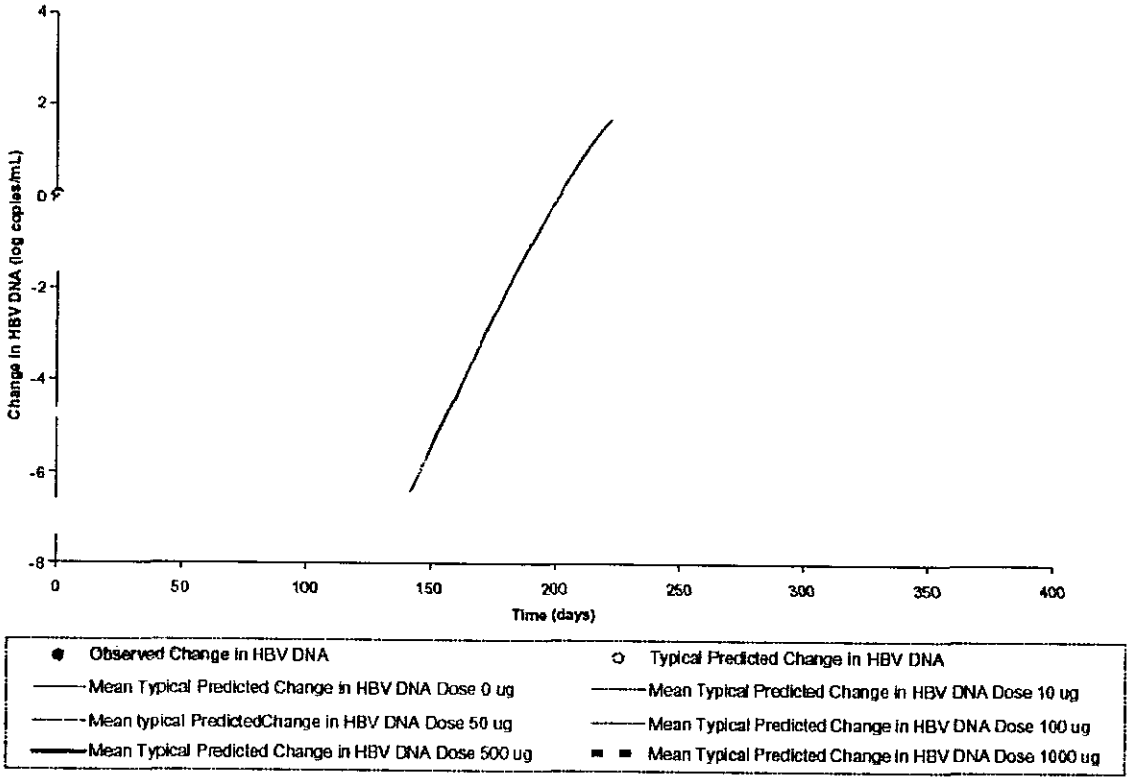


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Figure 61 Observed versus Typical Predicted Change in HBV DNA – Final Pharmacodynamic Model

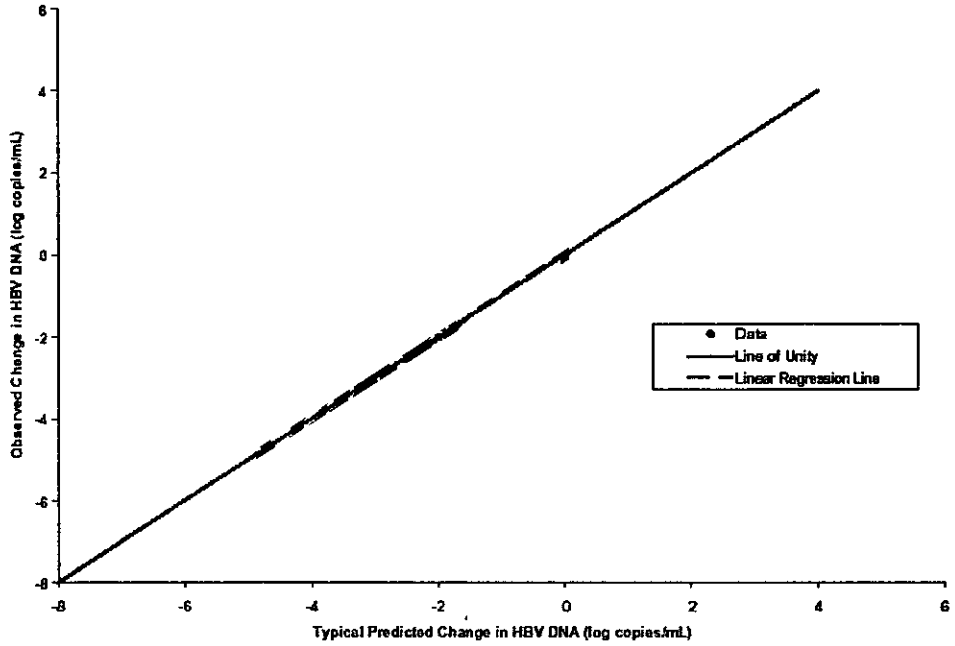
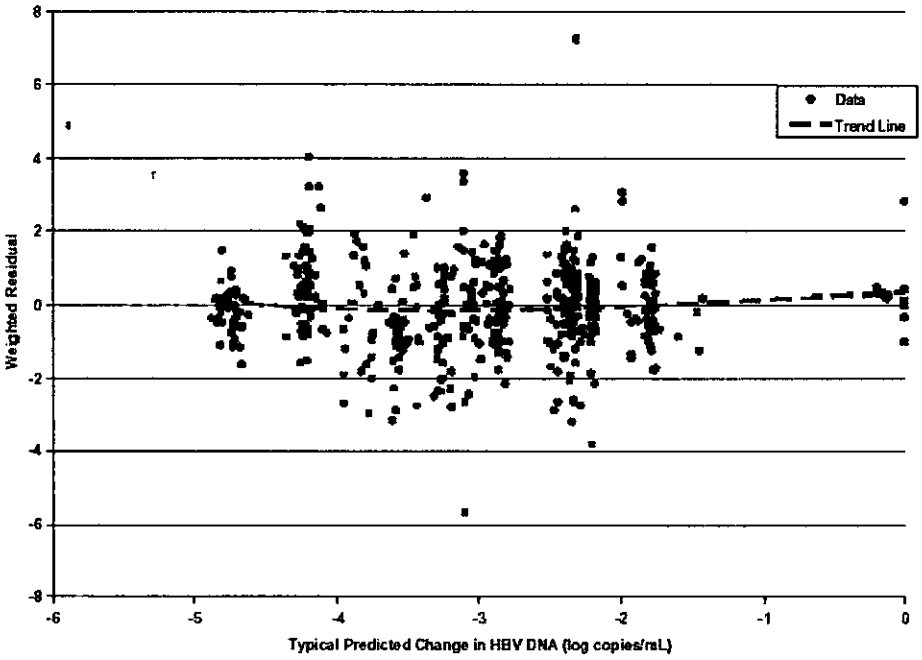


Figure 62 Weighted Residuals versus Typical Predicted Change in HBV DNA – Final Pharmacodynamic Model



The parameters for the final model are given Table 10.

Table 10: Parameter Estimates and Associated Standard Errors for Final Base Pharmacodynamic Model

Parameter (Units)		Population Mean (SE[a])	%CV Inter-Individual Variance (SE*)
RESPmax (Change in log HBV DNA)	Θ_1	6.36 (5.1)	51.5 (33.4)
Effect of Dose	Θ_3	0.209 (8.9)	
Effect of LVD	Θ_4	-0.261 (16.3)	
TDAY50 (Days)	Θ_2	33.1 (12.4)	37 7 (14.9)
Effect of Dose	Θ_5	0 266 (16 7)	
Additive Residual Error (log copies/mL)			0.54 (17.6)

[a] - SE given as %CV
NE – Not Estimated

The impact of covariates on the time course of HBV DNA reduction was explored using nonstochastic simulation. The results are shown graphically in Figures 2 and 3. Increasing doses of ETV in lamivudine naïve subjects (Figure 80) result in both a faster reduction and a greater maximum reduction in HBV DNA. A similar trend was seen with subjects who were refractory to lamivudine (Figure 81). However subjects who are lamivudine refractory have a decreased maximal reduction in HBV DNA at a given dose compared to treatment naïve subjects.

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Figure 80 **Effect of Entecavir Dose on the Time Course of HBV DNA Reduction for Lamivudine Naïve Subjects**

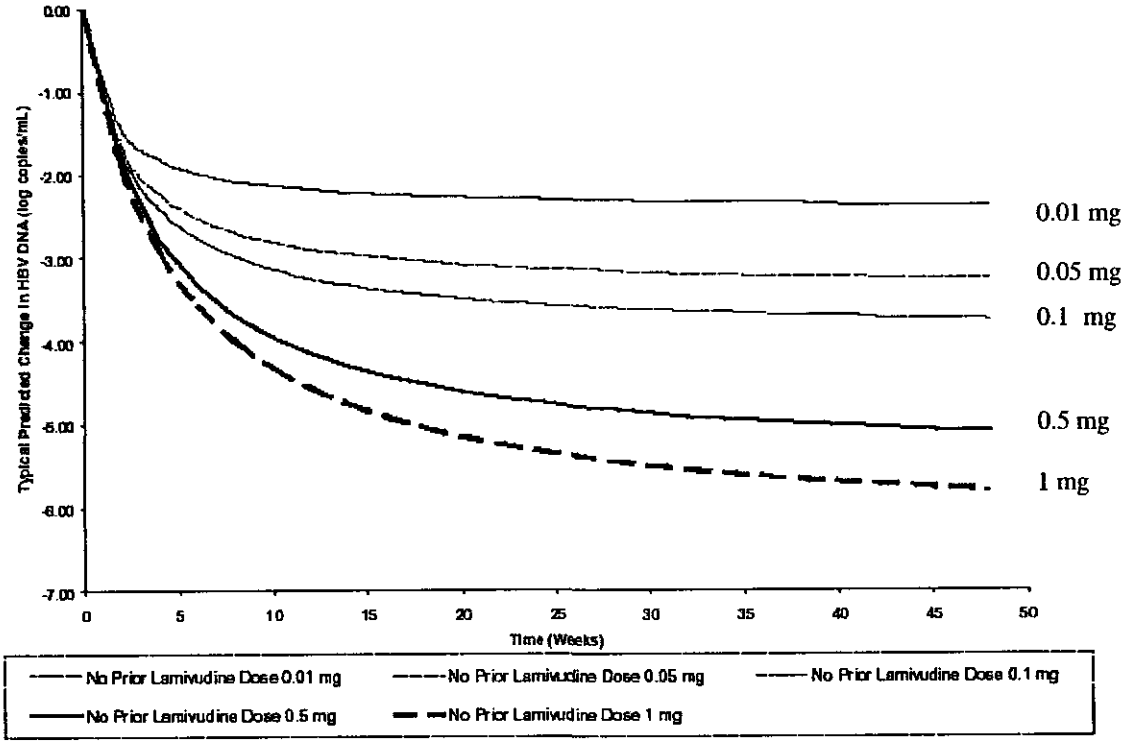
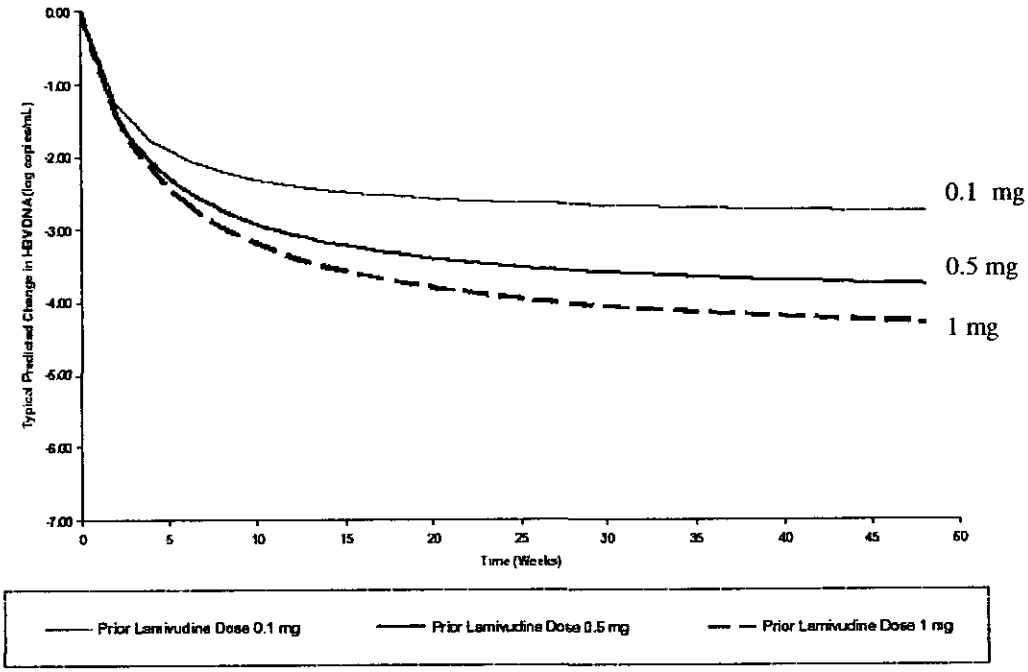
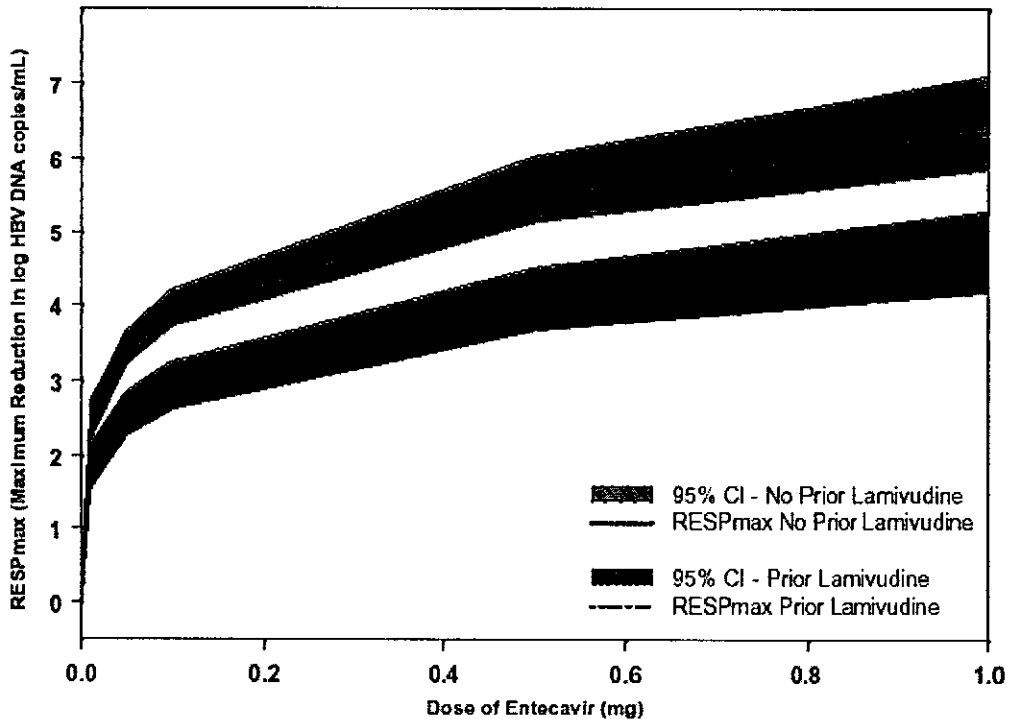


Figure 81 **Effect of Entecavir Dose on the Time Course of HBV DNA Reduction for Lamivudine Refractory Subjects**



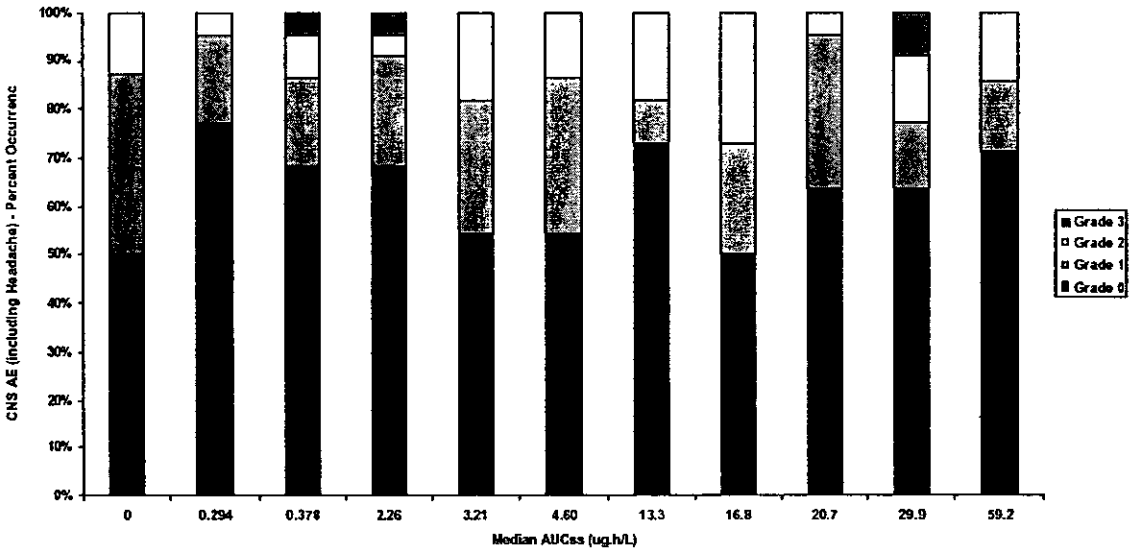
The effect of increasing the dose of ETV on the predicted maximum reduction in HBV DNA (RESPmax) for treatment naïve subjects and subjects with previous exposure to lamivudine is shown in Figure 82. The maximum value of RESPmax for lamivudine naïve subjects treated with ETV 0.5 mg QD was 5.5 (bootstrapped 95% confidence intervals 5.2 to 6.0). This value was used as a target value to compare the RESPmax that could be achieved by subjects who were refractory to lamivudine (dashed horizontal line). In order to achieve a predicted maximum reduction in HBV DNA (RESPmax) that is comparable to the predicted maximum reduction in HBV DNA achieved by 0.5 mg dose in lamivudine naïve subjects, subjects who have had prior treatment with lamivudine require an increased dose (1.0 mg of ETV). In this evaluation, the RESPmax value calculated for lamivudine refractory subjects receiving 1.0 mg ETV was 4.7 (bootstrapped 95% confidence intervals 4.2 to 5.3); thus, the maximum response in lamivudine refractory subjects given a 1.0 mg dose was within 15% of the maximum response in nucleoside naïve subjects given a 0.5 mg dose. It must be noted that RESPmax is a model parameter and that an increase in the value of this parameter will result in a correspondingly greater decrease in HBV DNA. Consequently, these plots appear to be inverted when compared to the HBV DNA plots shown elsewhere.

Figure 82 Relationship Between Maximum Reduction in HBV DNA (RESPmax) and Dose of Entecavir



There was no obvious relationship between exposure (AUC, Cmax, and Dose) and severity of AEs (headache, pooled CNS AEs [headache, photophobia, blurred vision, somnolence, lethargy, and dizziness], and pooled GI AEs [nausea, vomiting, and dyspepsia]) based on visual inspection of the plotted data. Therefore, no formal logistic analysis was conducted. The lack of a relationship between any of these parameters is illustrated in Figure 72 which shows the relationship between AUC and severity of CNS AEs. Plots of Cmax or Dose versus CNS AEs gave a similar result. The absence of a relationship between AE severity and ETV exposure was also true for headache and pooled GI AEs.

Figure 72 Relationship Between AUCss and CNS Adverse Events



The lack of any demonstrable relationship between increased ETV exposure and AE severity, as modeled over a range up to 1.0 mg of ETV, suggests that this proven therapeutic range falls below any toxicity-defined dose-limit for ETV based on observed, short-term clinical events.

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CONCLUSIONS

- The pharmacokinetics of ETV in HBV-infected patients was described by a two-compartment open model with first order absorption and first order elimination from the central compartment.
- The typical value for clearance for a reference patient with a creatinine clearance of 100 mL min was 28.21 L/hr, which is in agreement with previously reported values for healthy subjects.
- Creatinine clearance was the only covariate found to significantly contribute to inter-individual variability in clearance in this evaluation.
- There was no significant effect of age, sex, or race on ETV pharmacokinetics and no tested markers of hepatic function (albumin, ALT, AST, bilirubin, alkaline phosphatase, and amylase) contributed to inter-individual variability in ETV pharmacokinetics.
- The change in log HBV DNA over time was exposure dependent and was described by a direct effect inhibitory Emax model.
- Prior treatment with lamivudine resulted in a diminished response to ETV; thereby, requiring a higher dose in lamivudine refractory subjects to achieve a comparable response to nucleoside naïve subjects.
- Over the range of doses tested in these studies, there was no relationship between exposure and severity of AEs (headache, GI, and CNS).

33 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

____ § 552(b)(5) Deliberative Process

____ § 552(b)(5) Draft Labeling

4.2.1.2. Population Pharmacokinetics of Entecavir after a Single 1.0 Mg Oral Dose Administration in Subjects with Selected Degrees of Renal Impairment (Report 930007867)

STUDY RATIONALE: The sponsor decided to use population PK methods to analyze the pharmacokinetics of entecavir in subjects with renal impairment (Study AI463011) based on the following factors:

- Population pharmacokinetic methods had been used to characterize entecavir pharmacokinetics in Hepatitis B infected subjects (Report AI463017). Therefore, this model could be further evaluated in the present Phase I study in subjects with renal impairment.
- Traditional Phase I renal impairment study designs involve single dose treatment. However, there was a need to determine the range of steady state concentrations expected in renally impaired subjects who may be administered this drug. Characterization by population pharmacokinetic methods resulted in the development of a model that was used to simulate the expected steady state concentration time profiles for renally impaired subjects at both 0.5 and 1 mg doses given once daily. The model also provided a tool to use simulation to explore alternative dose regimens in subjects with selected degrees of renal failure.

Reviewer's Note: In earlier single dose escalation studies, entecavir Cmax and AUC increased in a greater than dose proportional manner over the dose range of 0.05 to 40 mg. This finding was likely driven in part by the inability to accurately define the terminal phase of the concentration-time profile because blood samples were not collected long enough (typically 48 hours) to accurately determine half-life (130 hr). In multiple dose escalation studies, entecavir's Cmax and AUC increased in an approximate dose-proportional manner in the dose range of 0.5 mg - 20 mg. In this population PK analysis, linear PK of entecavir was assumed. The assumption is reasonable, because AUC_{inf} for subjects with normal renal impairment in this study (with blood sampling up to 336 hrs) is comparable to the steady-state AUC for this population in other multiple dose studies. In addition, because entecavir has linear PK, single dose PK should be able to predict steady-state PK. Therefore, the conclusion from this analysis should be consistent with that from single dose analysis.

OBJECTIVES:

- To develop a population pharmacokinetic model for orally administered entecavir using data collected during a Phase I trial in subjects with selected degrees of renal impairment.
- To characterize the pharmacokinetics and pharmacokinetic variability of entecavir in subjects with selected degrees of renal impairment.
- To identify and quantify covariate effects on pharmacokinetics and pharmacokinetic variability of entecavir.
- To use the model developed in the present analysis to simulate expected concentration time profiles for alternative dose regimens and to support dose selection for subjects with selected degrees of renal impairment.

STUDY DESIGN:

This was a population pharmacokinetic analysis using data from a Phase I study, AI463011. AI463011 was designed as an open-label, parallel group, single-dose study.

Population pharmacokinetic models were developed and evaluated using plasma concentration-time data from 34 subjects with selected degrees of renal impairment that received a single 1.0 mg oral dose of entecavir. The 34 subjects were allocated into 6 groups of 4 to 6 subjects based on creatinine clearance (CLcr) as defined below. Note that the hemodialysis group, listed as Group "5, 6," represents one group of subjects that received a single 1.0 mg dose on 2 separate occasions (once 2h prior to hemodialysis and once immediately following dialysis). Thus, there are 7 groups listed, representing the 6 renal function groups studied (Table 1).

Table 1: Subject Group

Subject Group[a]	Description	Creatinine Clearance (CLcr)
1 (Subjects 1 - 6)	Normal renal function	> 80 mL/min
2 (Subjects 7 - 12)	Mild renal function impairment	>50 ≤ 80 mL/min
3 (Subjects 13 - 18)	Moderate renal function impairment	30 -50 mL/min
4 (Subjects 19 - 24)	Severe renal function impairment[b]	< 30 mL/min
5,6 (Subjects 25 - 30)	Severe renal function impairment managed with hemodialysis subjects	Not determined
7 (Subjects 31 - 34)	Severe renal function impairment managed with continuous ambulatory peritoneal dialysis (CAPD)	Not determined

[a] Groups 1 to 4 in this evaluation are equivalent to Groups A to D in the Clinical Study Report for Study AI463011. Groups 5 and 6 are equivalent to Group E1 (subjects given a single 1.0 mg dose administered 2 hours prior to hemodialysis), and Group E2 (subjects given a 1.0 mg dose immediately after a hemodialysis session), respectively, and Group 7 in this evaluation is equivalent to Group F in the Clinical Study Report for Study AI463011

[b] not requiring dialysis

A dense pharmacokinetic sampling strategy ("block design") was employed in this study. After dosing, blood samples for evaluation of entecavir levels were collected at the following nominal times after dosing: predose (0), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 12, 18, 24 hours and 1.5, 2, 3, 4, 5, 6, 7, 14 days post dose.

DATA: The pharmacokinetic data used in the population pharmacokinetic analysis represent all available concentration data collected in BMS Study AI463011 with the exception of the concentration time data taken during hemodialysis. Because of the small number of subjects available for inclusion in this dataset, no subjects were removed for the creation of the internal validation database.

It has been shown that hemodialysis removes 13% of the administered dose and represents an alternative mechanism of drug clearance, whereas CAPD removes less than 1% of the administered dose and has no impact on entecavir clearance. In consequence, (i) data from Group 5 (i.e. data from subjects with dose administration prior to the hemodialysis session) were not used in this PK analysis and (ii) data from Group 6 (i.e., data from subjects with dose administration after the hemodialysis session) and Group 7 (i.e., data from subjects on CAPD) were pooled and treated identically. Furthermore, dose adjustment recommendations will indicate that subjects

undergoing hemodialysis should receive their dose of entecavir following a day's hemodialysis session.

Reviewer's Note: The non-compartment analysis (NCA) showed that subjects in Group 7 have similar half-life and AUC_{inf} as compared to that in Group 6, except for one subject (Subject AI 463011-32) in Group 7, who has a very long half-life (2104 hours) and thus high AUC_{inf} (1720 ng.h/mL)). This subject has a relative comparable AUC_{0-366h} values as other CAPD and hemodialysis subjects. This subject is a 57-year-old male, with pre-existing medical conditions, including end stage renal disease managed with CAPD, insulin dependent diabetes mellitus, hypertension, anemia, and coronary artery disease, experienced severe syncope during the follow-up period, 15 days after administration of 1 mg ETV. The subject had several pre-existing medical conditions that could have contributed to the cause of the syncope. He was also on several medications, including the following: insulin, furosemide, aspirin, amlodipine, metorpolol, losartan, sildenafil and rosiglitazone. The investigator considered the SAE to be unrelated to study drug. The medical reviewer indicated that the investigator's judgment is reasonable.

A total of 711 plasma concentrations from 34 subjects (85.56% of the original observations and 100% of the original subjects available for evaluation) was included in the final merged database used for this evaluation. A summary of the data removed during the database creation is given in Table 3.

Table 3: Summary of Pharmacokinetics Observations Excluded from Analysis

Reason for Removal	Number of Points Removed
Measurable pre-dose concentration	5
Apparent error in the sample time	1
No reported concentration	3
Sample taken during dialysis (Group 5)	108

Demographic characteristics for subjects used in this study are summarized in Table 2.

Table 2: Demographic Characteristics

Characteristic	Renal Impairment/Dialysis Group						All
	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)	Group 4 (n = 6)	Group 5,6 (n = 6)	Group 7 (n = 4)	Subjects (n = 34)
Age, years							
Mean	53	59	62	48	52	58	55
SD	4	12	15	13	7	11	11
Range	46-59	36-69	41-80	34-66	41-60	43-68	34-80
Gender, n (%)							
Male	4 (67)	5 (83)	4 (67)	4 (67)	5 (83)	1 (17)	23 (68)
Female	2 (33)	1 (17)	2 (33)	2 (33)	1 (17)	3 (33)	11(32)
Race, n (%)							
White	5 (83)	5 (83)	5 (83)	5 (83)	—	2 (33)	22 (65)
Black	1 (17)	1 (17)	1 (17)	—	5 (83)	1 (17)	9 (26)
Native Am.	—	—	—	1 (17)	1 (17)	—	2 (6)
Asian/PI	—	—	—	—	—	1 (17)	1 (3)
Weight, kg							
Mean	83.0	81.6	90.9	74.5	77.3	75.8	80.8
SD	11.7	17.4	15.9	13.9	18.0	6.7	14.8
Range	66-98	65-113	71-109	50-86	54-98	67-83	50-113
Height, cm							
Mean	173.3	176.5	170.6	166.7	170.1	159.4	170.0
SD	3.5	7.5	7.5	5.6	8.4	4.4	7.8
Range	168-177	163-185	158-178	163-178	155-180	156-165	155-185
BMI, kg/m^2							
Mean	27.8	26.2	31.3	27.0	26.5	29.9	28.0
SD	3.3	4.9	4.1	5.1	4.6	4.2	4.5
Range	23-32	22-35	24-35	18-32	21-31	25-34	18-35
CL_{CR}, mL/min					NA	NA	NA
Mean	112.6	62.2	38.8	23.3			
SD	13.6	8.1	4.4	2.6			
Range	93-130	55-76	33-45	21-27			

Abbreviations: Am. = American, BMI = body mass index, PI = Pacific Islander, SD = standard deviation, and NA = not applicable.

MODELS: , conducted all the PK/PD modeling of entecavir, using NONMEM Version V level 1.1 (Globomax LLC Hanover, MD). The details of the PK model building were described in the review for Report AI463017).

Model Validation

No internal validation was conducted. However, because the data in the present study were for a single dose and the model performance in predicting steady state conditions needed to be proven, the model was used to simulate data from the Phase 2 Studies AI463004, AI463005, and AI463014. The Phase 2 data represented steady state concentration time data from a wide range of doses. In addition, the Phase 2 database contained subjects with normal renal function as well as subjects with mild and moderate

renal impairment. The simulated concentration time data were compared to the observed data for consistency.

In addition, model-predicted area under the curve (AUC) values were compared with AUC values determined by non-compartmental analyses (NCA). A limited predictive check was conducted on 300 replicates to examine the distribution of apparent individual and typical (ie, population mean) clearance values simulated using the final model with those obtained from the original data in the model building data set. Finally, simulated concentration time data were overlaid on a plot of observed concentrations versus time to obtain a visual comparison of the ability of the model to simulate a reasonable concentration time curve for single dose data.

The predictive validity of the model was examined by comparing the model prediction based on typical parameter values. The relative error and the root mean square error (eg, bias and precision) were calculated. Finally, symmetric 95% confidence intervals were provided based on the asymptotic standard errors of the parameter estimates.

Simulation for Dose Adjustment

Dose adjustment was evaluated using the method of inverse prediction based on a linear regression of AUCss versus dose, as entecavir exposure at steady state appears to be linearly related to dose over a wide range of doses. Estimates of 24h AUCss were generated as AUCss=DOSE/CL. Exposures (i.e. AUCss) in subjects with selected degrees of renal impairment were simulated for various candidate doses and compared with a clinically meaningful AUCss target (reference) range. The lower limit of the reference range exposure was selected based on the lower limit of the predicted AUCss values determined in subjects with normal renal function. The upper limit was taken to be 2 times the geometric mean AUCss value in subjects with normal renal function. Based on the efficacy and safety of entecavir, the approach is acceptable. The goal was to manage dose adjustments so that at least 75% of simulated AUCss values fell within the target range for a given degree of renal impairment.

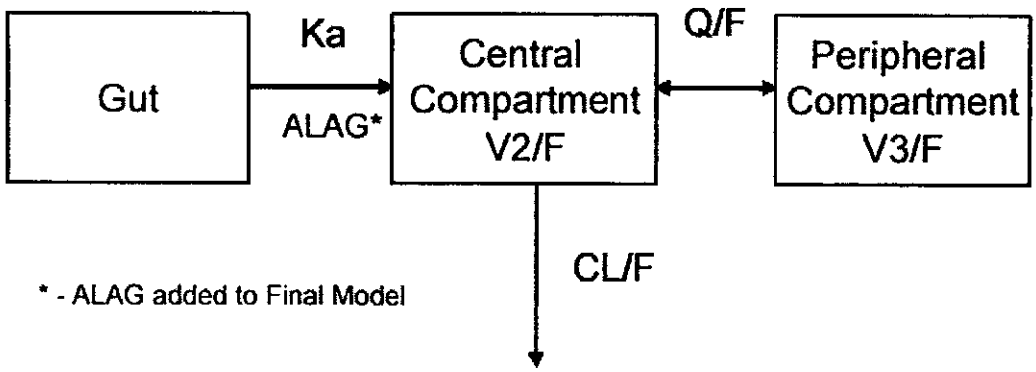
RESULTS AND DISCUSSION:

The study design of the renal impairment study is adequate for the population PK analysis. The model chosen in this population PK analysis is similar to the previous Phase II population PK/PD. In addition, the model was validated externally by the Phase 2 Studies AI463004, AI463005, and AI463014 including subjects with normal renal function as well as subjects with mild and moderate renal impairment. The data integrity and model building process (Appendix 930007867_1) are acceptable.

Final base model

The best pharmacokinetic model identified in the present analysis was a two compartment model with first order input, a fixed lag time, and first order elimination, and is illustrated in Figure 1:

Figure 1: General Schematic Diagram of Pharmacokinetic Model



Because the estimation of lag time results in a discontinuity of the concentration time function that causes numerical instability, the value of ALAG was fixed to its estimate of 0.24 hour in order to allow the model to converge successfully. Since body weight can affect more than one parameter simultaneously, allometric factors on apparent clearance and volume of distribution were fixed to 0.75 and 1.0, respectively. Similar to the Phase 2 population pharmacokinetic model, a dose effect on the apparent inter-compartmental clearance was included in the final base model. Inter-individual variability was described for all parameters with the exception of the absorption rate constant.

A plot of observed versus typical predicted concentrations is presented in Figure 3. A plot of weighted residuals versus the typical predicted concentrations is given in Figure 4. These plots indicate that there are several factors that need to be noted. The observed versus typical predicted plot shows that the data are generally uniformly scattered about the line of unity, although there is a problem with predicted concentrations of 6 ng/mL being over-estimates of the observed values. This trend may be due to misspecification of the input part of the model. The plot of weighted residuals versus typical predicted concentrations shows that the range of weighted residuals is acceptable with the majority of observations falling between –4 and +4, although there appears to be a pattern where the earliest time points generally have negative weighted residuals followed by generally positive values. Again, this appears to be related to model misspecification of the input function. Overall, the base model performance is acceptable.

Figure 3 **Observed versus Typical Predicted Concentrations – Base Model**

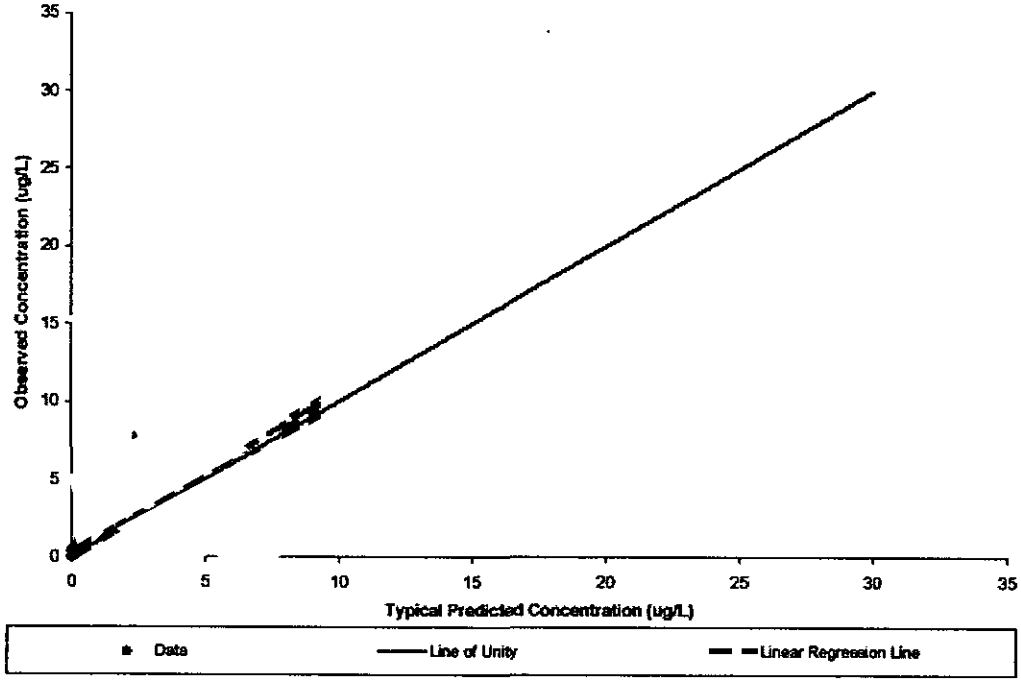
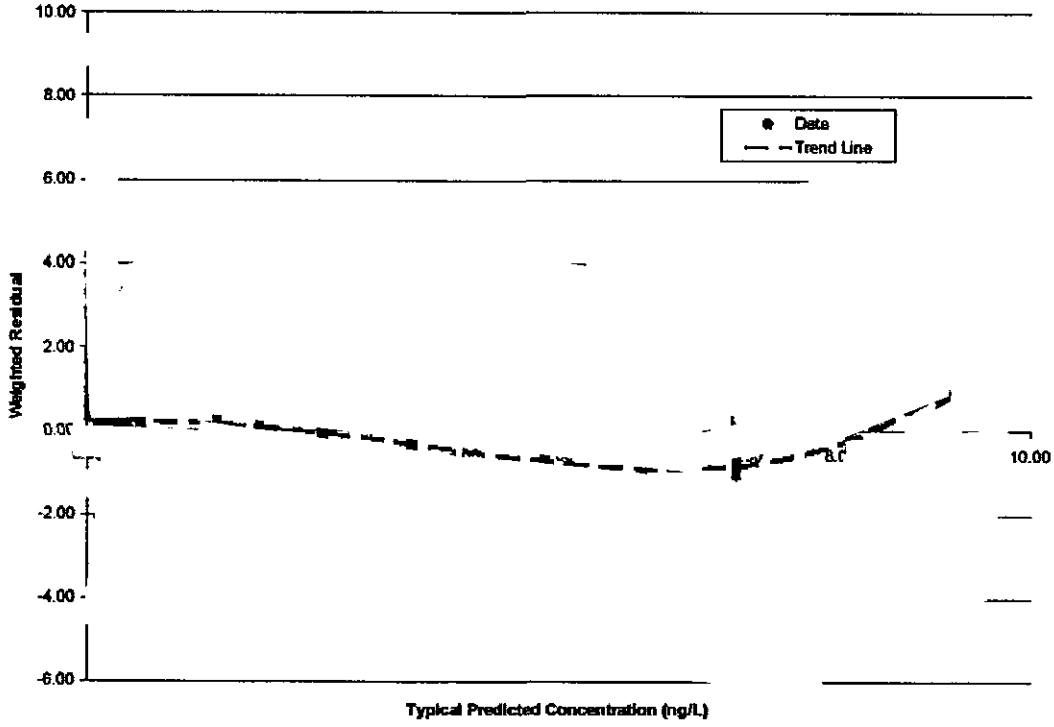


Figure 4 **Weighted Residuals versus Typical Predicted Concentrations – Base Model**



Final model

The best pharmacokinetic model identified in the present analysis (Appendix 1, Model 81) was a two compartment model with first order input, a fixed lag time (ALAG), and first order elimination. Because only one dose was tested in the present study in renally impaired subjects, the effect of dose was fixed to the final value estimated in the Phase II population pharmacokinetics analysis. In addition, because the estimation of lag time results in a discontinuity of the concentration time function that causes numerical instability, this value was fixed to its estimated value of 0.24 h in order to allow the model to converge successfully. The parameters describing the allometric model were fixed to the standard values. The model included terms describing correlation between CL/F, V2/F and Q/F. Because this was a single dose evaluation, inter-occasion variability was not evaluated. The model utilized a combined constant coefficient of variation model with an additive component to describe residual error. The final forms of the equations for the model parameters are given below.

$$\frac{CL}{F} = \Theta_1 \bullet \left(\frac{Weight}{70}\right)^{0.75} + \Theta_7 \bullet \left(\frac{CrCL}{100}\right) \bullet \exp(\eta_1)$$

$$\frac{V2}{F} = \Theta_2 \bullet \left(\frac{Weight}{70}\right) \bullet \exp(\eta_2)$$

$$\frac{Q}{F} = \left(\Theta_3 \bullet \left(DOSE^{\Theta_4}\right)\right) \bullet \left(\frac{Weight}{70}\right)^{0.75} \bullet \exp(\eta_3)$$

$$\frac{V3}{F} = \Theta_5 \bullet \left(\frac{Weight}{70}\right) \bullet \exp(\eta_4)$$

$$Ka = \Theta_6$$

$$Alag = \Theta_8$$

Covariates identified to contribute to inter-individual variability in CL/F included renal function and total body weight. Total body weight was identified in this analysis but not in the previous population PK analysis for Phase II trials. In this analysis, CLcr (CrCL in the equation) was calculated based on 24-hour urine data, while the previous population PK analysis for Phase II trials used the CLcr based on Cockcroft Gault equation where age and body weight were included. A creatinine clearance value of 5 was assigned for Groups 6 and 7 in this study, which is reasonable. The plot of observed versus typical predicted concentrations (Figure 6) for the final model predicts a much higher range of concentrations (15 ng/mL) than does the base model (8 ng/mL). The poor behavior at estimated concentrations of 6 ng/mL appears to have been largely resolved. The plot of weighted residuals versus time (Figure 7) shows a marginal degeneration in the weighted residuals, with an apparent increase in the range of weighted residuals (-4 to +10) as compared to the base model (-4 to +8). The visual pattern of weighted residuals appears to have resolved somewhat although early time points still have an apparent pattern suggesting that the absorption model is still somewhat misspecified.

Figure 6 Observed versus Typical Predicted Concentrations – Final Model

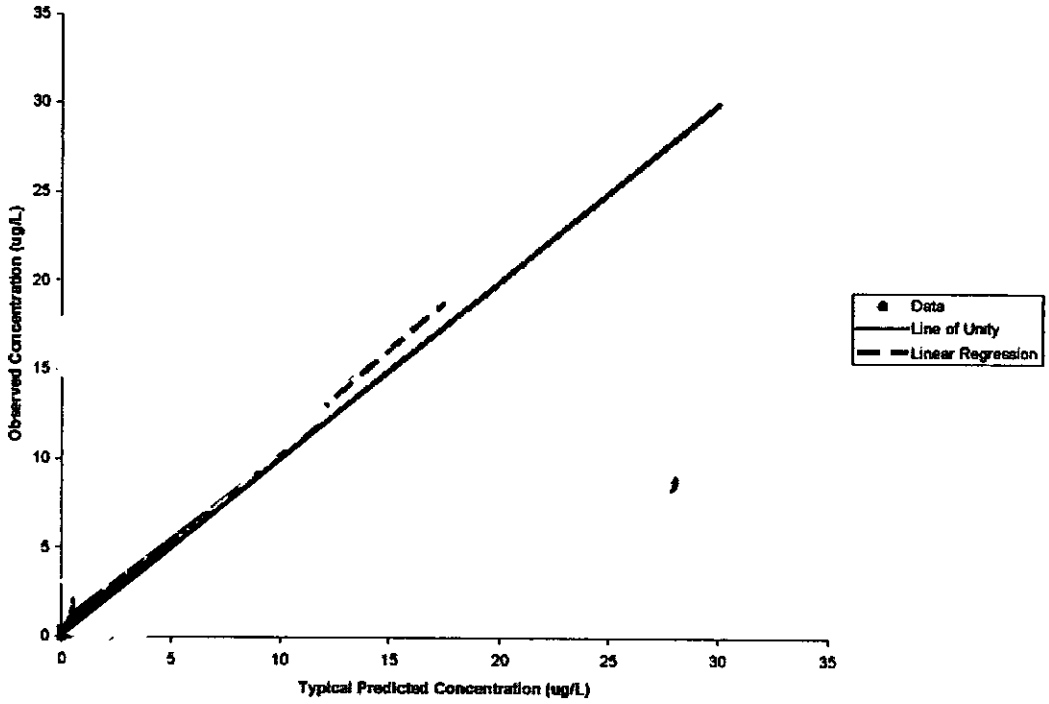


Figure 7 Weighted Residuals versus Typical Predicted Concentrations – Final Model

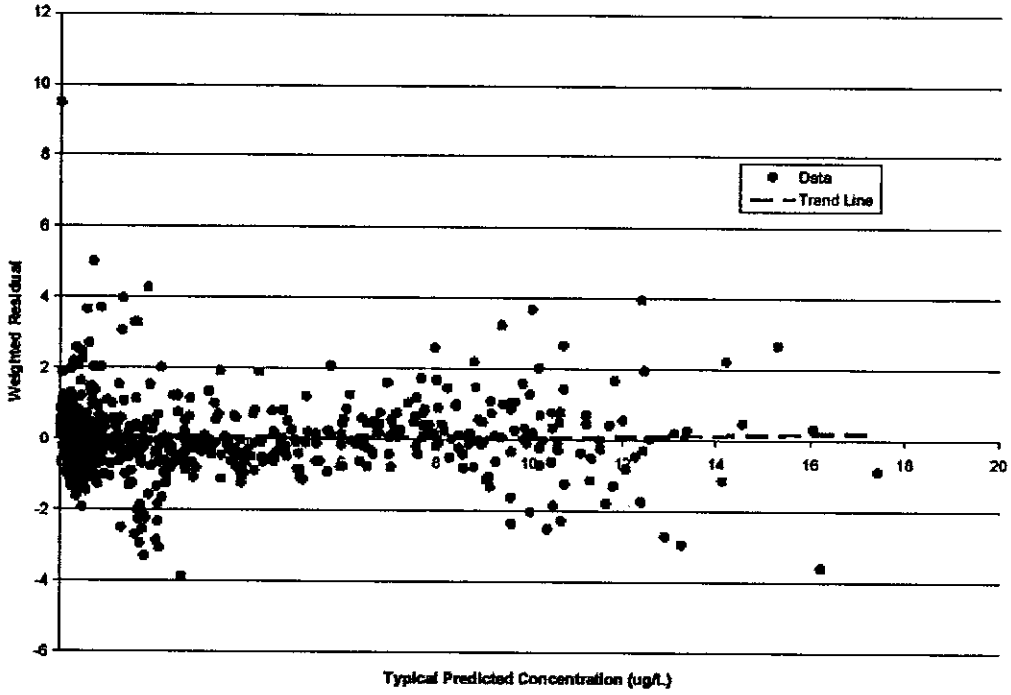
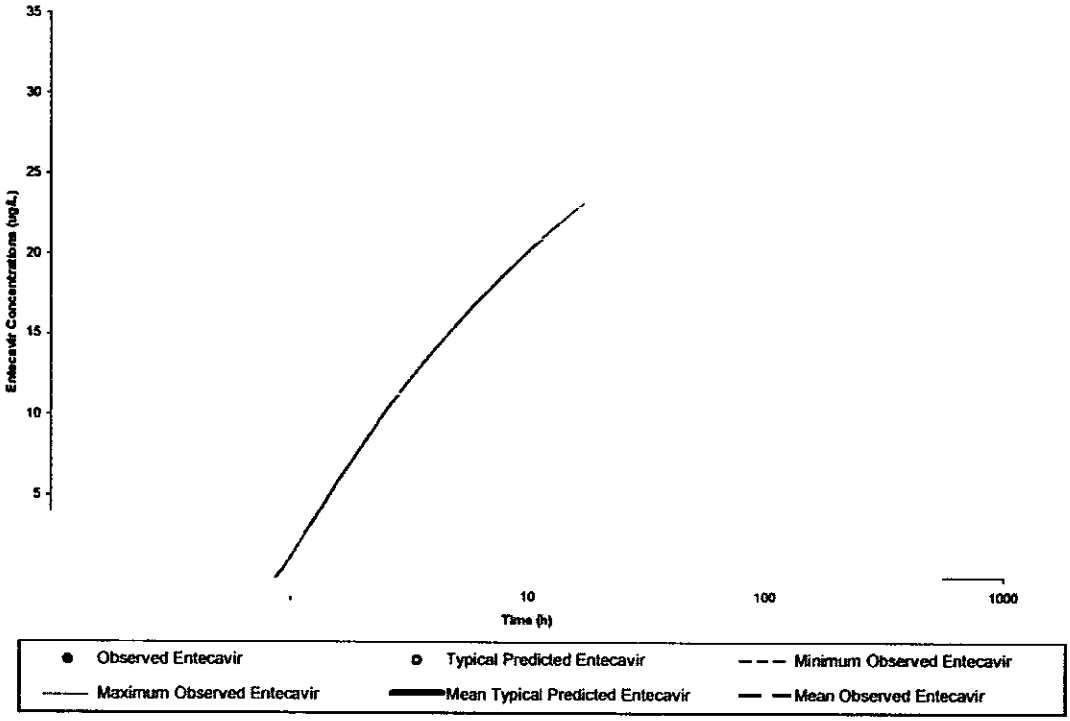


Figure 8: Observed Concentrations Versus Time Overlaid with Typical Predicted Concentrations - Final Model



The objective function was reduced by approximately 306 points by the addition of the two covariate factors, and the estimates of inter-individual variability for all parameters were substantially reduced. In addition, there was a minor reduction in the constant coefficient of variation (CCV) and additive portions of the residual error. Finally, clearance was estimated with slightly smaller standard error in the final model as compared with the base model. The control stream for the final model is shown in Appendix 930007867_2 (Note: the theta numbers in the control stream are different from the theta numbers listed here). A listing of the final parameter estimates is given in Table 4.

Table 4: Parameter Estimates and Associated Standard Errors for Final Pharmacokinetic Model

Parameter (Units)		Population Mean (SE*)	%CV Inter-Individual Variance (SE*)
CL/F (L/h) Effect of CrCL Allometric Factor	Θ_1 Θ_7	2.27 (12.6) 26.6 (8.40) 0.75 FIX	28.3 (30.1)
V2/F (L) Allometric Factor	Θ_2	73.5 (6.60) 1 FIX	30.7 (29.6)
Q/F (L/h) Effect of Dose Allometric Factor	Θ_3 Θ_4	134 (8.10) -0.3 FIX 0.75 FIX	35.6 (32.6)
V3/F (L) Allometric Factor	Θ_5	706 (7.50) 1 FIX	27.6 (28.1)
Ka (h-1)	Θ_6	16.0 (17.1)	NE
ALAG (h)	Θ_8	0.24 FIX	NE
CCV Residual Error (as %CV)			26.7 (12.9)
Additive Residual Error (ng/mL)			0.039 (69.9)

* - SE given as %CV

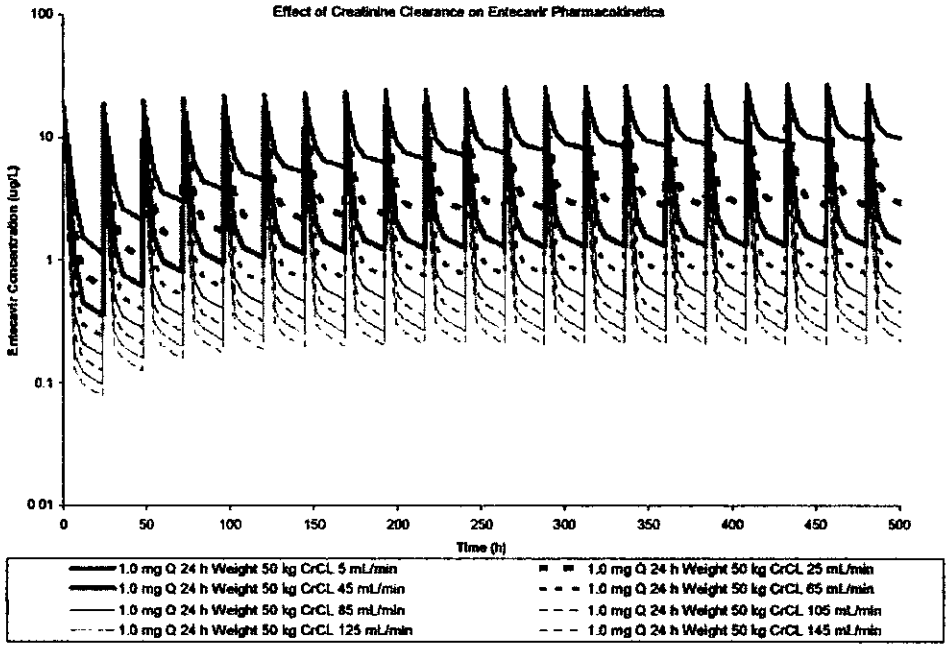
NE – Not Estimated

Fix - Denotes some parameters fixed at specific values in order to facilitate estimating other parameters.

Over the reported weight range of 49.7 to 113 kg and a reported creatinine clearance range of 5 to 130 mL/min, CL/F varies from 2.51 L/h to 48.8 L/h. The control stream for the final model is presented in Appendix III. The population mean estimates for clearance for a reference subject weighing 70 kg and having a creatinine clearance of 100 mL/min was 28.87 L/hr, which is in agreement with previously reported values in subjects with chronic Hepatitis B virus infection (Study AI463017).

The inter-individual variability for the parameters is low, with the highest estimated inter-individual variability on inter-compartmental clearance being approximately 36%. The constant coefficient of variation portion of the residual error (27%) is reasonable, and is also consistent with estimates obtained in both Phase 1 and Phase 2 analyses. The additive portion of the residual error (0.04 ng/mL) is reasonable. Because this is a Phase 1 study of subjects with selected degrees of renal impairment, the subjects included in

Figure 10 Effect of Renal Function on Entecavir Pharmacokinetics



A plot of creatinine clearance versus entecavir clearance is given in Figure 11. As can be seen, there is a linear relationship between CLcr and entecavir clearance.

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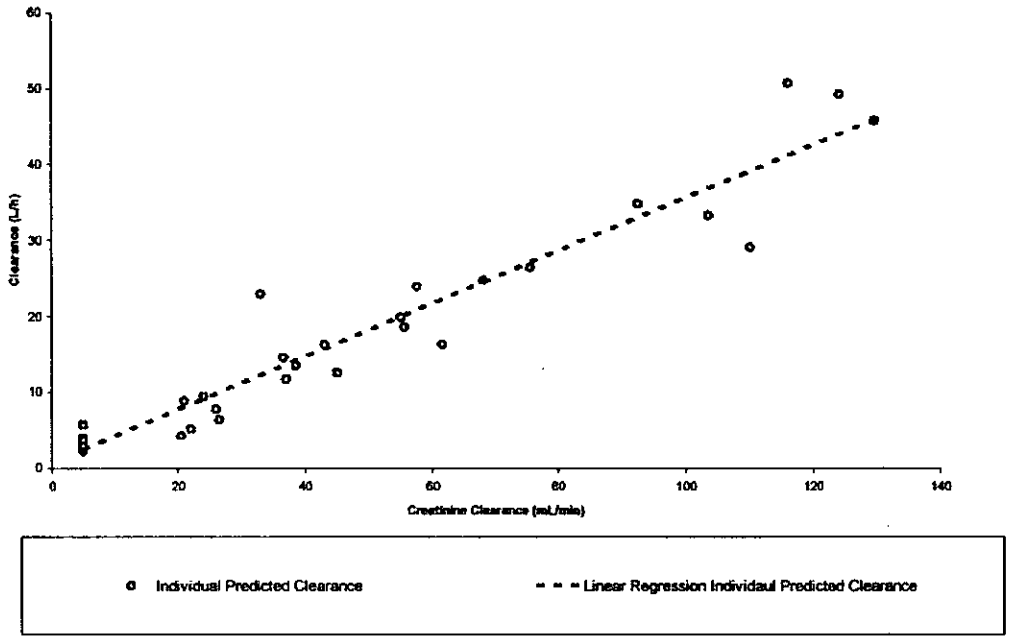
Figure 11 **Relationship Between Observed Creatinine Clearance and Entecavir Clearance (Typical Value of Clearance and Individual Estimates of Clearance)**



Weight has a much less profound effect than creatinine clearance, with subjects whose body weight is high (eg, weight >100 kg) having somewhat lower trough concentrations than subjects with lower body weights (eg, weight < 80 kg). The effect of weight on the pharmacokinetics of entecavir is depicted in Figure 12.

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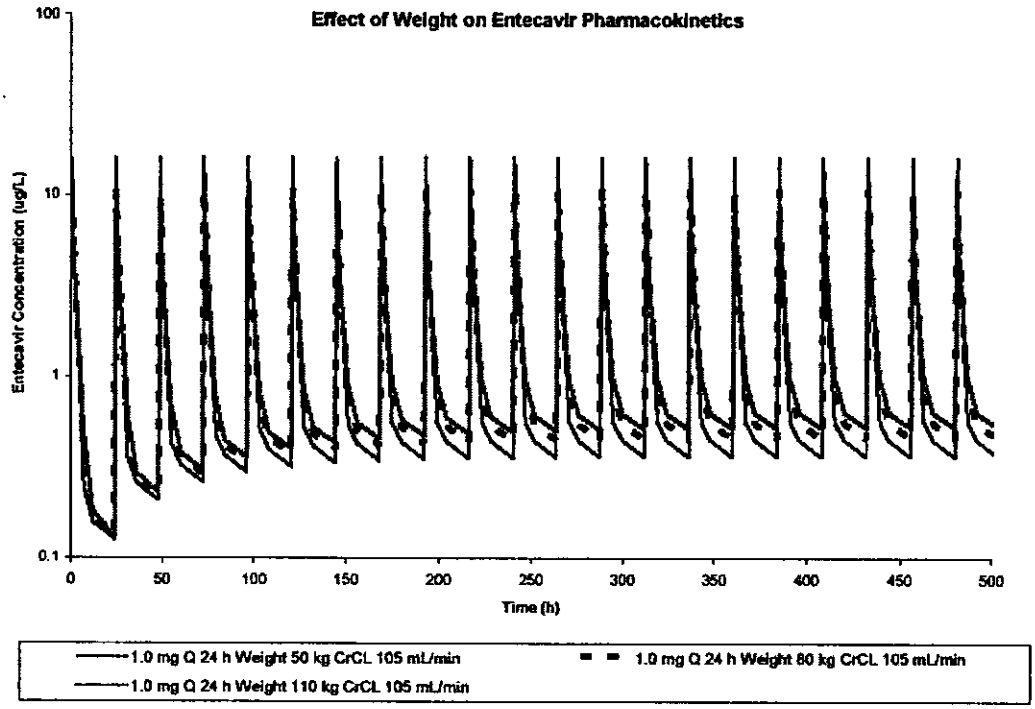
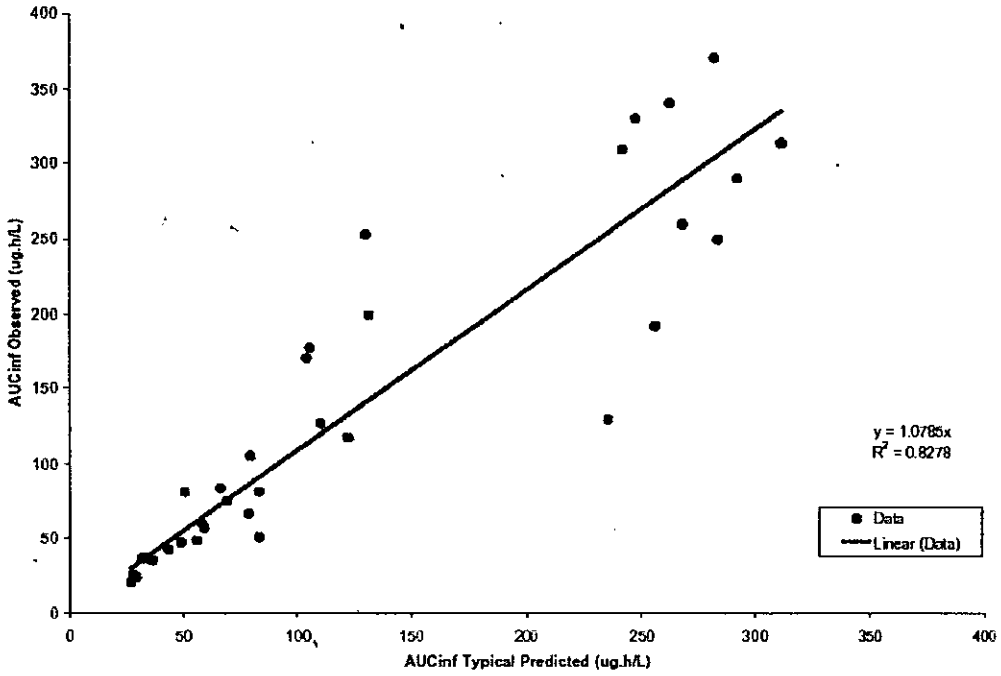
Figure 12 Effect of Weight on Entecavir Pharmacokinetics



Evaluation of Final Model for Simulation

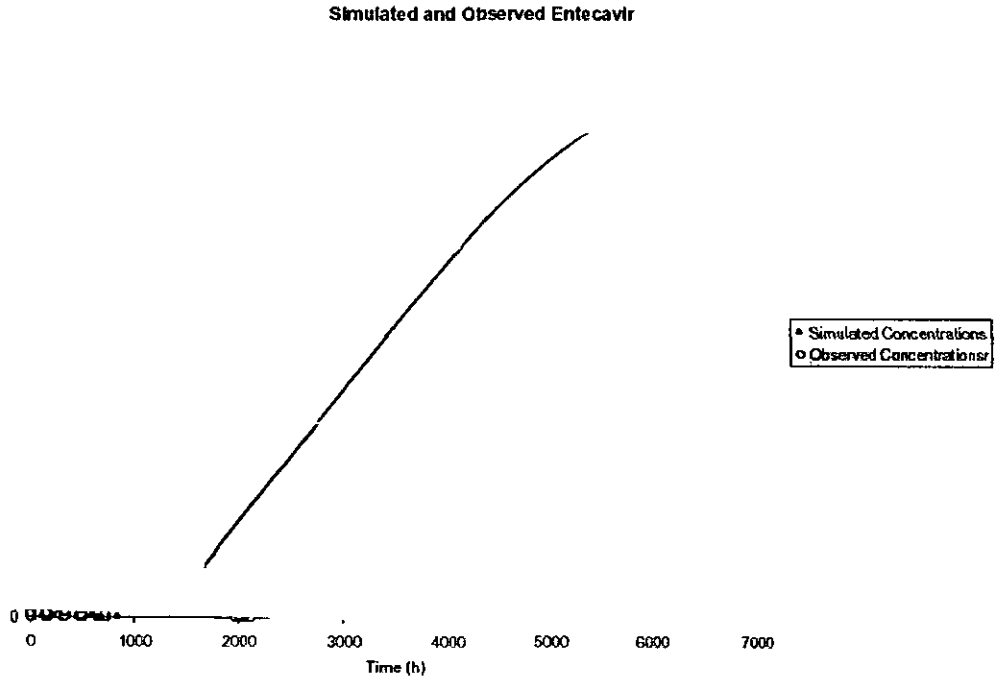
Dose selection for different renal function groups involved evaluations of exposure as measured by AUC. The linear relationship AUC=Dose/CL was used. Therefore, in order to determine the appropriateness of using this formula to calculate AUC, comparisons of the model predicted AUC values and the AUC values determined by non-compartmental analysis (NCA) methods were done. These comparisons showed good agreement for data arising from a single dose of 1 mg entecavir. A representative plot for the typical predicted AUC versus the NCA AUC values is given in Figure 18. These two values representing entecavir exposure appear to be in agreement, suggesting that the typical value of clearance is an acceptable approximation of clearance as determined by non-compartmental methods and that the use of the relationship AUC=DOSE/CL is a reasonable approximation for the noncompartmental analysis derived value of AUC.

Figure 18 **Comparison of NCA AUCinf From Observed Data and AUCinf Generated from Typical Predicted Apparent Clearance**



To test the ability of the pharmacokinetic model to emulate expected concentration time profiles at steady state, the data from the Phase 2 analysis was also simulated using the present pharmacokinetic model. The Phase 2 database contained subjects with normal renal function as well as subjects with mild and moderate renal impairment. The plot of observed data from Studies AI463004, AI463005 and AI463014 overlaid on the simulated data is given in Figure 21. The same trend of somewhat wider ranges of concentrations for the simulation is seen but the general agreement between the simulated and observed profiles is acceptable.

Figure 21 **Simulated and Observed Entecavir Concentrations versus Time for Phase II Studies AI463004, AI463005 and AI463014 (First 50 Replicates)**



Results of Inverse Prediction Analysis

The assumption used in this simulation and inverse prediction analysis was linear pharmacokinetics of entecavir, which is acceptable. A graphical assessment of the simulated AUCss values across several doses for each patient group as compared to the geometric mean simulated AUC value for subjects with normal renal function who are dosed with 1 mg entecavir Q 24 h is given in Figure 23 to Figure 27. In these figures, the horizontal dashed lines represent the lower and upper target response (AUCss) values. The lower limit of the reference range exposure was selected based on the lower limit of the predicted AUCss values determined in subjects with normal renal function. The upper limit was taken to be 2 times the geometric mean AUCss value in subjects with normal renal function. The solid line is the fitted regression line and the dotted lines represent the 95% prediction intervals for the simulated AUCss values for each Group.

Figure 23 Results of Inverse Prediction Analysis for Dose Regimen Adjustment for the 1 mg Q 24h Dose Regimen Group 1

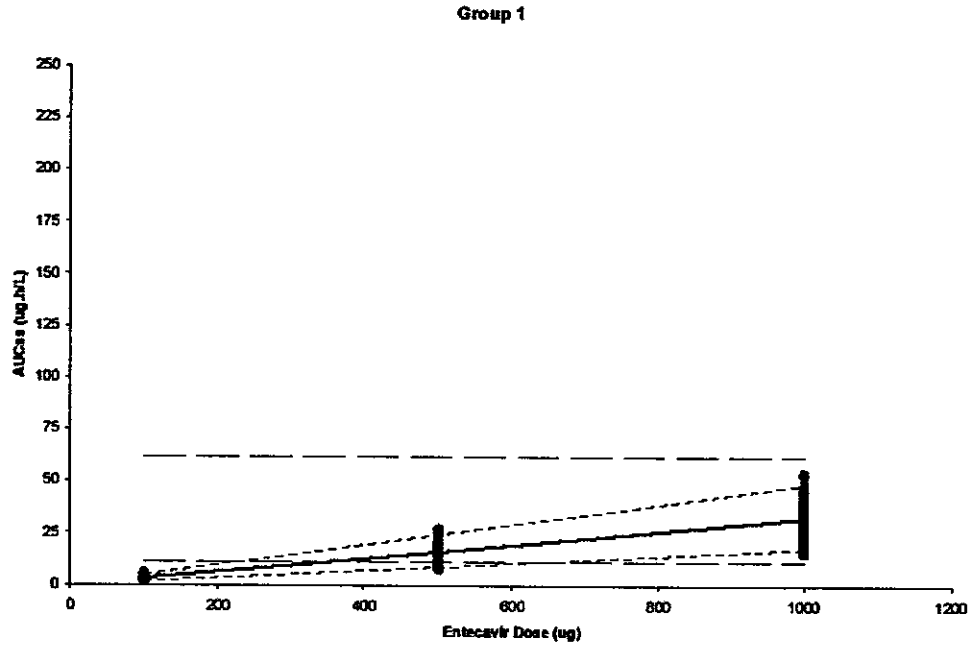


Figure 24 Results of Inverse Prediction Analysis for Dose Regimen Adjustment for the 1 mg Q 24h Dose Regimen Group 2

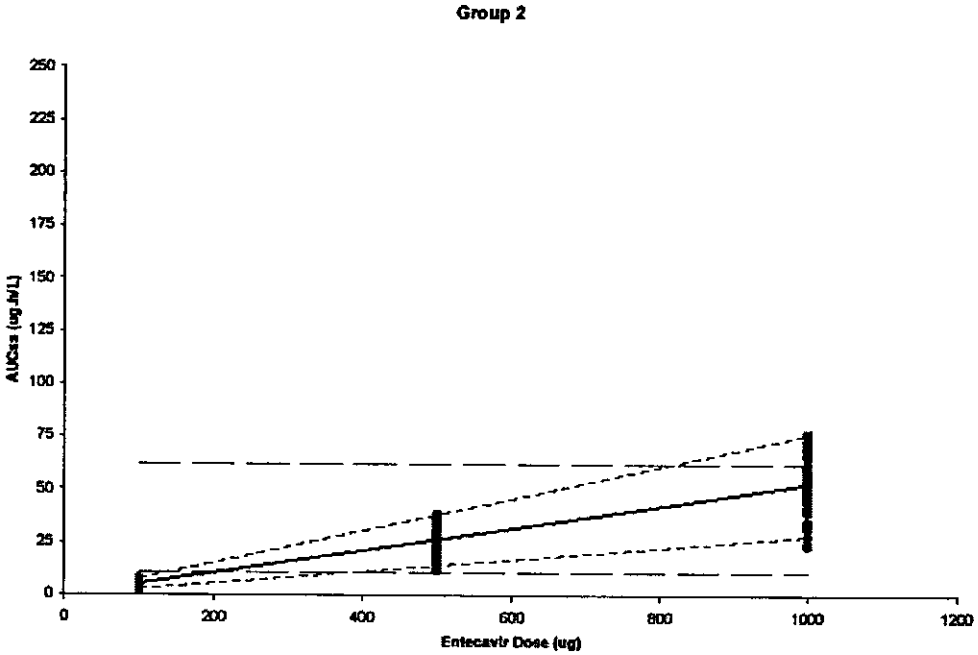


Figure 25 **Results of Inverse Prediction Analysis for Dose Regimen Adjustment for the 1 mg Q 24h Dose Regimen Group 3**

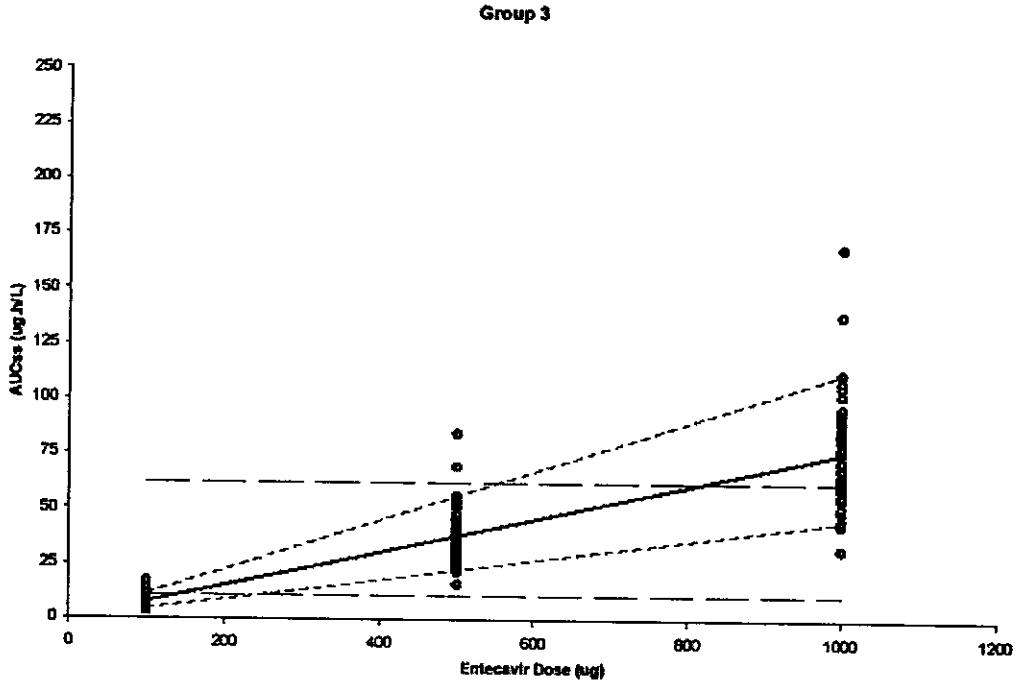


Figure 26 **Results of Inverse Prediction Analysis for Dose Regimen Adjustment for the 1 mg Q 24h Dose Regimen Group 4**

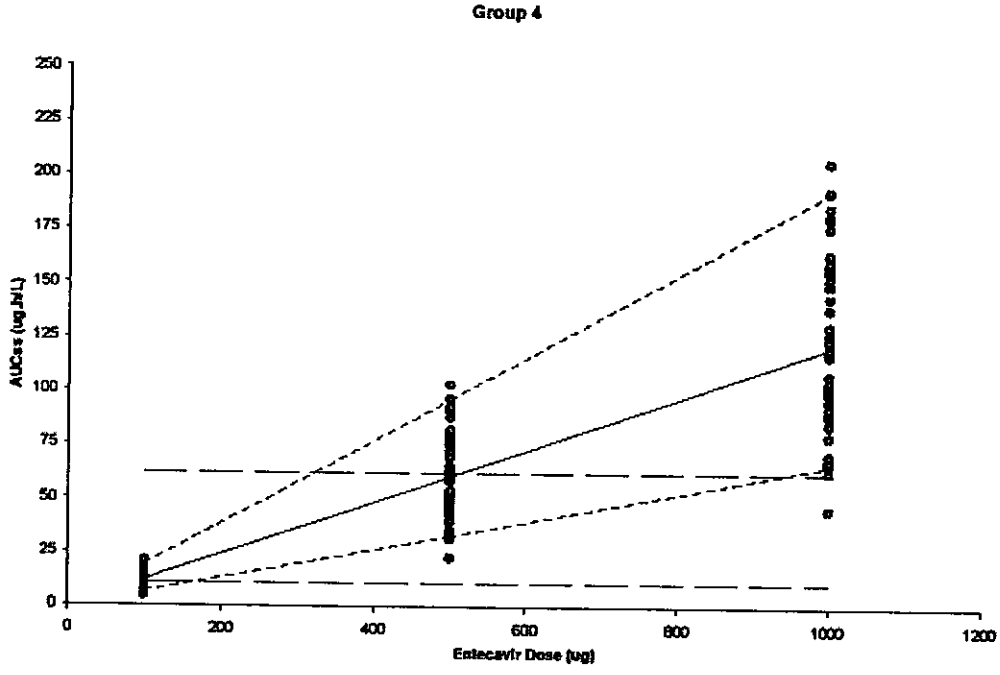
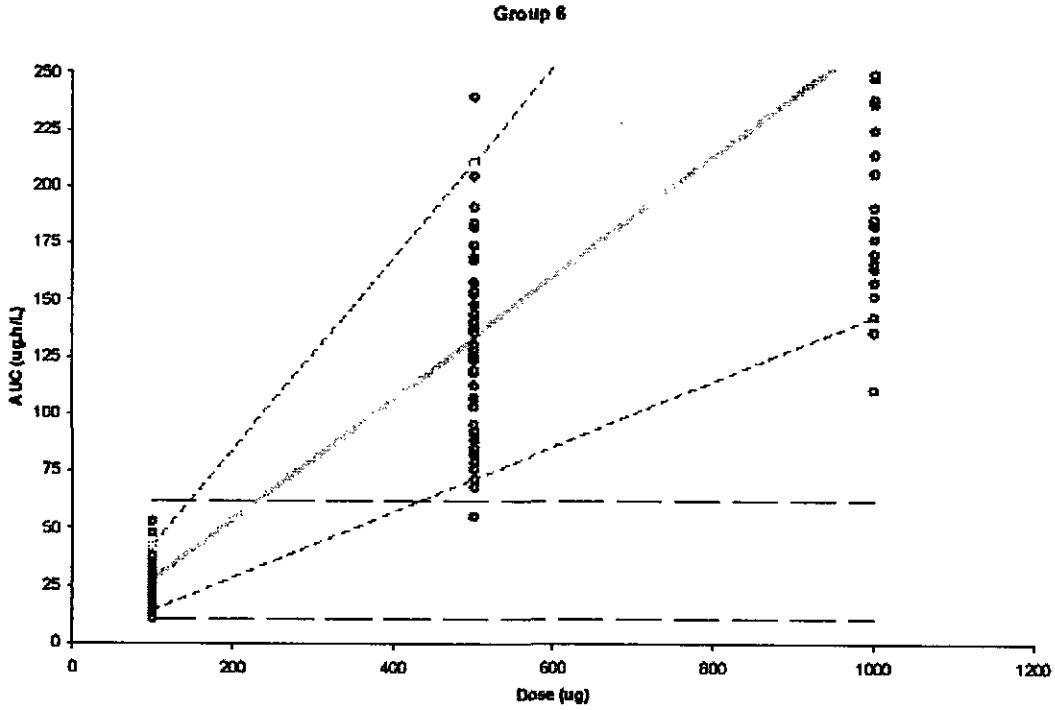


Figure 27 **Results of Inverse Prediction Analysis for Dose Regimen Adjustment for the 1 mg Q 24h Dose Regimen Group 6**



The sponsor recommends the following dose adjustments for renal impaired subject.

Table 12 **Recommended Reduction in Daily Entecavir Dose by Renal Impairment Group**

Renal Impairment Group	Creatinine Clearance Range (mL/min)	Recommended Dose Reduction
Group 1		
Group 2		
Group 3		
Group 4		
Group 6		

* End stage renal disease, a value of 5 was assigned for creatinine clearance.

A box and whisker plot of the expected AUCss values using the final dose recommendations relative to the daily 1 mg dose regimen for each renal impairment Group is given in Figure 30. The heavy dashed line is the upper target (2 times the geometric mean AUCss value from subjects with normal renal function receiving 1 mg entecavir daily. The solid line is the geometric mean AUCss value from subjects with normal renal function receiving 1 mg entecavir daily. A listing of the expected percent of subjects whose AUCss values will fall outside the targeted range of AUCss values is given in Table 13.

Figure 30 **Box and Whisker Plot for AUCss Values By Patient Group for Final Dose Recommendations Based on 1 mg QD Dose**

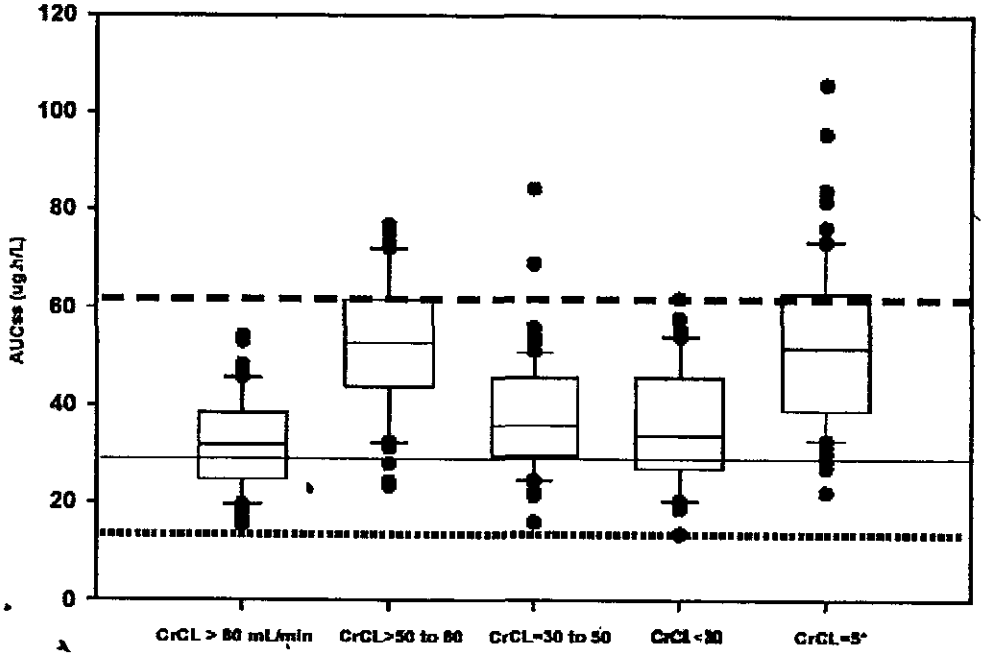


Table 13 **Percent of Simulated AUCss Values Outside the Target Upper and Lower Exposure Limits for Recommended Dose Regimen Based on 1 mg Daily Entecavir Dose**

Patient Group	Recommended Entecavir Dose	Percent Simulated AUCss Values Lower than Lower Target Value	Percent Simulated AUCss Values Higher than Upper Target Value
1	1.0	0	0
2	1.0	0	21.7
3	0.5	0	3.33
4	0.3	1.67	0
6	0.2	0	25.0

The results show that for Group 2 (subjects with mild renal impairment) and Group 6 (renal impaired subjects with dialysis), 21.7% and 25% of AUCss are higher than upper target value, respectively, after dose reductions based on the sponsor's suggestion. For subjects with mild renal impairment, proposed dose was used in the Phase II studies and did not post safety concerns. In addition, using the same dose as recommended for subjects with normal renal function allows this group of the subjects to use tablets. Therefore, no dose adjustment proposed by the sponsor is acceptable. We recommend the sponsor adjust the doses for renal impairment subjects with hemodialysis or CAPD to 10% of the dose required for the subjects with normal renal function, because this

population has not been studied in either Phase II /Phase III clinical trials, and has greater variability on entecavir exposure. Changing the dose to 10% of the recommended dose for patients with normal renal function will provide entecavir exposure more close to that in patients with normal renal function after the full dose, based on the simulated AUCss from population PK analysis and the AUCinf of entecavir from noncompartmental model. The following table shows AUCinf (noncompartental model) fold changes from that in subjects with normal renal function. Hemo group is the group taking entecavir right after hemodialysis. CAPD-1 represents CAPD group without the one subject with extremely long half-life.

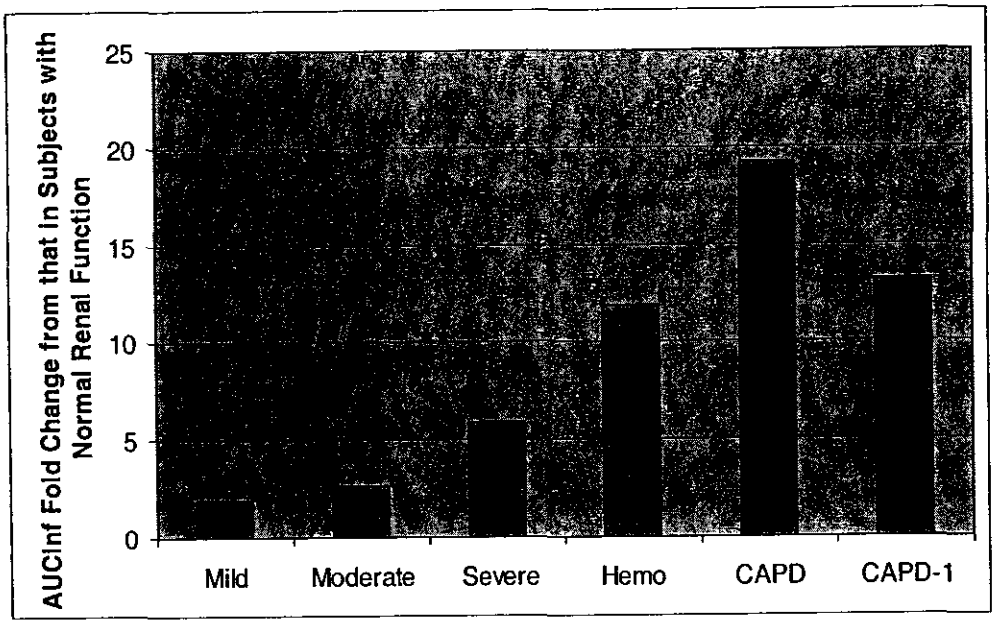


Table 9 shows the recommended dosage in Patients with renal impairment.

Table 9: Recommended Dosage of Entecavir in Patients with Renal Impairment

Creatinine Clearance (mL/min)	Usual Dose (0.5 mg)	Lamivudine Refractory (1 mg)
≥50	0.5 mg once daily	1 mg once daily
30 to <50	0.25 mg once daily	0.5 mg once daily
10 to <30	0.15 mg once daily	0.3 mg once daily
Hemodialysis or CAPD*	0.05 mg once daily	0.1 mg once daily

*Administer after hemodialysis.

CONCLUSIONS:

- The pharmacokinetics of entecavir in subjects with selected degrees of renal impairment were well described by a two-compartment model with first order absorption and first order elimination from the central compartment.
- The typical estimate of clearance for a reference subject weighing 70 kg and having a creatinine clearance of 100 mL/min was in agreement with previously reported values in subjects with chronic Hepatitis B virus infection.
- Creatinine clearance and total body weight were the only covariates found to significantly affect entecavir clearance.
- No dosage adjustment is necessary for subjects with mild renal impairment. In order to manage exposure in subjects with moderate renal impairment, a dose reduction of 50% is recommended. For subjects with severe renal impairment, 30% of the regular dose for subjects with normal renal function is needed to maintain comparable exposure to subjects with normal renal function. In subjects with end stage renal disease managed with hemodialysis or CAPD, 10% of the regular dose (administered after the hemodialysis session) is recommended.

COMMENT TO THE SPONSOR:

Based on the simulated entacavir AUCss from population PK analysis and the AUCinf of entecavir from the non-compartmental model, we recommend you change the dose adjustment for severely renal impaired patients maintained with hemodialysis or CAPD to 10% of the recommended dose for patients with normal renal function. This adjustment will provide entecavir exposure more close to that in patients with normal renal function after the full dose.

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✓ § 552(b)(4) Trade Secret / Confidential

____ § 552(b)(5) Deliberative Process

____ § 552(b)(5) Draft Labeling

4.3. DPEIII Division Director Concurrance on Post-Marketing Commitment

-----Original Message-----
From: Lazor, John A
Sent: Tuesday, March 29, 2005 2:58 PM
To: Bergman, Kimberly; Reynolds, Kellie S
Subject: Entecavir Phase IV

I concur with the following entecavir phase IV commitment:

PMC #7: Conduct and submit a final study report for a study assessing the pharmacokinetics, safety, and efficacy of entecavir in children of age through with chronic HBV. Use entecavir exposure information from pediatric patients to support dose-selection for the efficacy and safety assessments. Pediatric efficacy should be based on the results of a variety of virologic, biochemical, serologic, and composite endpoints over at least 48 weeks of dosing.

4.4. OCPB Filing/Review Form

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Office of Clinical Pharmacology and Biopharmaceutics
New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-797/21-798	Brand Name	(Baraclude™) TBD
OCPB Division (I, II, III)	DPEIII	Generic Name	Entecavir
Medical Division	530	Drug Class	Viral polymerase inhibitor (guanosine analog)
OCPB Reviewer	Kimberly L. Bergman	Indication(s)	Treatment of chronic hepatitis B in adults
OCPB Team Leader	Kellie S. Reynolds	Dosage Form	Tablet: 0.5 and 1 mg entecavir Oral Solution: 0.05 mg entecavir per mL
		Dosing Regimen	0.5 mg QD 1 mg QD for lamivudine-refractory patients
Date of Submission	30SEP2004	Route of Administration	PO
Estimated Due Date of OCPB Review	10FEB2005	Sponsor	Bristol-Myers Squibb
PDUFA Due Date	29MAR2005	Priority Classification	1P
Division Due Date	18MAR2004		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.				
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:	X			
Isozyme characterization:	X			
Blood/plasma ratio:	X			
Plasma protein binding:	X			
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	2		
multiple dose:	X	2		
Patients-				
single dose:	X	1		AI463015: orthoptic liver tx with HepB re-infection
multiple dose:	X	(1)		(AI463015 [see previous])
Dose proportionality -				
fasting / non-fasting single dose:	X			
fasting / non-fasting multiple dose:	X			
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	4		Lamivudine (2), adefovir, tenofovir
In-vivo effects of primary drug:	X	(4)		Lamivudine (2), adefovir, tenofovir
In-vitro:	X			
Subpopulation studies -				
ethnicity:	X	2		Asians (China, Japan)
gender:	X			
pediatrics:				
geriatrics:	X			
renal impairment:	X			
hepatic impairment:	X			

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
PD:				
Phase 1:	X	1		AI463041: retrospective ECG analysis
Phase 2:	X	(1)		(AI463015 [see previous])
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1 (1)		AI463017: population analysis of phase 2 studies 004, 005, and 014 (AI463015 [see previous])
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	X	1		AI463017 (005 and 014)
Data sparse:	X	1 (same report as above)		AI463017 (004)
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	X	3		AI463034: 0.5 mg clinical tablet vs. clinical capsule AI463065: 1.0 mg clinical/commercial tab vs. 2 X 0.5 mg clinical tablet AI463035: 0.5 mg clinical tablet vs. clinical/commercial solution
replicate design; single / multi dose:				
Food-drug interaction studies:	X	2		AI463003: clinical capsule formulation + high fat meal AI463016: clinical tablet formulation + high fat meal or light meal
In-Vitro Dissolution:	X			
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies	19			

Filability and QBR comments				
	"X" if yes	**Comments**		
Application filable ?	**X**	Reasons if the application <u>is not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?		
Comments sent to firm ?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.		
QBR questions (key issues to be considered)	**1. Dose selection** **2. Dosage adjustment recommendations in renal impairment** **3. Bioequivalence findings**			
Other comments or information not included above				
Primary reviewer Signature and Date				
Secondary reviewer Signature and Date				

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

/s/

Kimberly Bergman
3/29/05 03:51:32 PM
BIOPHARMACEUTICS

Jenny H. Zheng
3/29/05 03:56:44 PM
BIOPHARMACEUTICS
Sign off for Pharmacometrix portion

Kellie Reynolds
3/29/05 04:17:55 PM
BIOPHARMACEUTICS