

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-797

21-798

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 21797, 21798

Serial #: 000

Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Applicant's Name and Address:

Bristol-Myers Squibb Company
5 Research Parkway
P.O. Box 5100
Wallingford, CT 06492

Submission Dates:

Correspondence Date: September 29, 2004

CDER Receipt Date: September 30, 2004

Assigned Date: September 29, 2004

Antiviral Advisory Committee Meeting: March 11, 2005

DAVDP Action Date: March 18, 2004

PDUFA Date: March 29, 2004

Review Complete Date: March 14, 2005

Amendments:

Related/Supporting Documents: IND52196

Product Name(s)

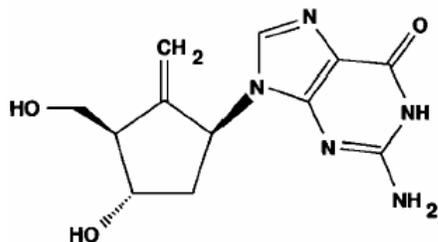
Proprietary:

Non-Proprietary/USAN: entecavir

Code Name/Number: BMS-20475

Chemical Name: {[1*S*-(1 α ,3 α ,4 β)]-2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6*H*-purin-6-one monohydrate }

Structural Formula:



Dosage Form(s): 0.5 and 1mg tablets; 0.05 mg/mL solution

Route(s) of Administration: Oral

Indication(s): Treatment of HBV infection

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Recommended Dosage: 0.5 mg once daily; For lamivudine-refractory patients, 1 mg once daily

Dispensed: Rx OTC _____

Abbreviations: ADV, adefovir dipivoxil; ALT, alanine aminotransferase; CC₅₀, 50% cytotoxic concentration; DHBV, duck hepatitis B virus; HBeAg, Hepatitis B e antigen; HBV, hepatitis B virus; HBIg, hepatitis B immunoglobulin; IC₅₀, 50% inhibitory concentration; LLOQ, lower limit of quantification; LAM, lamivudine; OLT, orthotopic liver transplant; PCR, polymerase chain reaction; RT, reverse transcriptase; TI, therapeutic index; ULOQ, upper limit of quantification; WHV, woodchuck hepatitis virus;

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Executive Summary

Entecavir (ETV), a nucleoside analog, has demonstrated inhibitory activity against hepatitis B virus (HBV) replication with an average IC_{50} value of 3.75 nM in the HBV stably-transfected human liver HepG2.2.15 cell line. The active intracellular moiety of ETV, ETV-triphosphate, is a competitive inhibitor of dGTP and functions as a non-obligate chain terminator. Cell culture studies have shown that viruses with the lamivudine (LAM) resistance-associated amino acid substitutions rtM204V/I and rtL180M in the HBV polymerase (RT) display cross-resistance to ETV, having approximately 5- to 30-fold reduced susceptibility in vitro. Resistance analyses from early Phase II studies provided evidence that substitutions at positions rtT184, rtS202 and/or rtM250 are associated with ETV resistance, but developed only when LAM resistance mutations were present. The addition of substitutions at rtT184, rtS202 and/or rtM250 together with the LAM-resistance mutations, rtL180M and rtM204V, in recombinant viruses resulted in 38- to 2,000-fold reduced susceptibility to ETV in vitro. HBV clones engineered to contain the adefovir (ADV) resistance substitutions rtN236T or rtA181V remained susceptible to ETV (0.3- and 1.1-fold change over WT, respectively).

The efficacy of ETV was examined in both nucleoside treatment-naïve and LAM-experienced patient populations. In nucleoside treatment-naïve studies 022 (HBeAg positive subjects) and 027 (HBeAg negative subjects), 83% (541/653) of patients on 0.5 mg QD ETV treatment were suppressed with serum HBV DNA <400 copies/mL as quantified by the COBAS Amplicor HBV Monitor PCR assay at week 48 compared to 59% (363/619) of patients on 100 mg QD LAM treatment. Genotypic and phenotypic analyses of paired clinical isolates obtained at study entry and Week 48 were performed to monitor baseline and emerging amino acid substitutions and to determine their impact on virologic response to ETV. In treatment-naïve studies 022 and 027, no ETV-associated resistant substitutions at rtI169, rtT184, rtS202, and/or rtM250 were detected in any isolate on ETV therapy by 48 weeks. Two treatment-naïve subjects experienced virologic rebound on ETV treatment but had no detectable amino acid changes emerge on treatment and no change in phenotypic susceptibility to ETV, ADV or LAM.

Clinical studies 014 and 026 examined the efficacy of 1 mg QD ETV compared to 100 mg QD LAM in patients with LAM-refractory HBV with prior LAM experience. A lower proportion of LAM-refractory subjects with chronic HBV infection achieved serum HBV DNA levels <400 copies/mL at week 48 on ETV treatment compared to nucleoside-naïve subjects (21% vs. 83%). In these studies, LAM-resistant substitutions rtL180M and rtM204V/I were detected in >80% of baseline isolates from both the ETV and LAM arms and these substitutions were maintained during the study, presumably because of the selective advantage in the presence of LAM and ETV. In study 014, genotypic analyses of paired clinical isolates determined that LAM-resistance substitutions rtL80V, rtL180M, rtM204V or I emerged in the HBV of 17% (7/42) of patients on ETV by week 48. These substitutions often arose in the context of mixtures at these sites at baseline and other LAM-resistance mutations at baseline. Despite the emergence of LAM-resistance substitutions,

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

the viral load in 4 of 7 patients was suppressed below 300 copies/mL (LLOQ) and the other 3 subjects experienced $>2 \log_{10}$ reductions in viral load at the time the isolate developed the LAM-resistant mutations. ETV-associated resistance substitutions at rtT184 developed on 1 mg ETV therapy in 5 (12%) patients after week 48 in study 014 and coincided with rebounds in viral load.

In study 026, substitutions at RT residues rtI169, rtT184, rtS202 and/or rtM250 emerged on therapy in 9% (12/134) of ETV subjects with week 48 data. In all cases, the ETV-resistant substitutions emerged when pre-existing LAM-resistant changes were present. One isolate from a patient who experienced virologic rebound by week 48 in study 026 developed the rtT184A/S substitution on ETV treatment. In addition, in supportive study 015, virologic rebound occurred in one patient by week 48 and five more patients on ETV after 48 weeks. Isolates from these patients in study 015 showed the development of ETV-resistance substitutions rtT184A/S/G, rtS202G, and/or rtM250V which were linked to LAM-resistant changes rtL180M and rtM204V and coincided with virologic rebound.

Overall, while no genotypic or phenotypic evidence of ETV resistance was detected in two studies of ETV treated treatment-naïve subjects at 48 weeks, 7.4% (14/189) of LAM-refractory subjects treated with ETV in studies 014, 015 and 026 had evidence of emerging ETV-resistance substitutions by week 48. The ETV-associated resistance substitutions at rtI169, rtT184, rtS202 and/or rtM250 emerged in the presence of pre-existing LAM-resistant substitutions in all cases. ETV-associated resistance substitutions were associated with virologic rebound in 3 of 14 subjects at week 48 and in additional subjects (10/14) after 48 weeks. Furthermore, these substitutions were associated with phenotypic ETV resistance. The median fold change from reference of ETV susceptibility was 48-fold for the ETV isolates that developed ETV-resistance substitutions in studies 015 and 026 at 48 weeks (n = 14). The ETV-resistant clinical isolates were susceptible to ADV, but remained resistant to LAM. Post 48-week follow-up data of ETV-treated patients is needed to obtain long term resistance data and to determine the ETV resistance pathway in nucleoside treatment-naïve subjects

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for entecavir is approvable with respect to microbiology for the treatment of chronic HBV.

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

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

1. Determine the in vitro susceptibility to ETV and ADV of substitutions at rtI169 alone and in the context of LAM and ETV-associated resistance mutations.
2. Determine the in vitro susceptibility to ETV of tenofovir-associated resistance substitutions at rtA194 to ETV in a lamivudine-resistant background.
3. Follow ETV-treated subjects to obtain long-term (144 week) resistance data. Continue to perform genotypic and phenotypic analyses of HBV DNA from patients receiving long-term entecavir therapy in ongoing clinical trials 022, 027, 026, 038, 048, and 901. Provide 96-, 144-, and 240-week data on the genotypic and phenotypic analyses of isolates from entecavir-treated patients with chronic HBV who experienced virologic rebound in serum HBV DNA levels in both the nucleoside-naïve and lamivudine-refractory studies.
Protocol submissions: studies ongoing
Report submissions: Summary reports of overall consecutive resistance analyses submitted annually.
4. Conduct and submit a final study report to evaluate the safety, efficacy, and resistance profile of entecavir used in combination with another oral anti-HBV therapy in treatment-naïve or treatment-experienced patients with chronic HBV to determine if there is any added benefit of combination therapy. Study suggestion: ETV in combination with ADV vs. ETV in combination with IFN.
Protocol submission: December, 2005
Final report submission: 2009

2. Summary of OND Microbiology Assessments

2.1. Brief Overview of the Microbiological Program

Complete non-clinical virology reports on studies of mechanism of action, cytotoxicity, antiviral activity in cell culture and animals, in vitro combination activity assessments, and in vitro phenotypic analyses were submitted with this NDA application. Resistance data from pivotal studies 014, 026, 022, and 027 as well as the supportive trial 015 have been submitted in the requested HBV resistance template format. Baseline and post-baseline genotypes of 1790 isolates from 808 patients as well as 62 phenotypes from 25 patients have been submitted.

2.1.1. Non-clinical Summary

ETV-TP is effective against HBV replication and the three distinct enzymatic activities of the viral polymerase: priming, reverse transcription responsible for first-

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

strand DNA synthesis, and DNA-dependent DNA polymerization which performs second-strand DNA synthesis. ETV-TP is a competitive inhibitor of dGTP and functions as a non-obligate chain terminator for cellular polymerases resulting in chain termination after 1 to 5 bases. The K_i of ETV-TP ranges from 1.2 to 2.6 nM with K_m values ranging from 8.4 to 13.3 nM for dGTP giving K_i/K_m ratios of 0.1. The low (<1) K_i/K_m ratios substantiate that the HBV polymerase favors ETV-TP compared to the natural substrate and corroborate that ETV-TP is an effective inhibitor of HBV polymerase.

Entecavir demonstrated antiviral activity against HBV with an average IC_{50} value of 3.75 nM in the HepG2.2.15 HBV stably-transfected human liver cell line. In transiently transfected Huh7 liver cells and HepG2 cells, ETV had IC_{50} values of 0.36 nM and 3.6 nM, respectively. ETV demonstrated specificity for HBV showing little antiviral activity against Herpes simplex virus (HSV-1), varicella zoster virus (VZV), human cytomegalovirus (HCMV), HIV-1 and influenza with IC_{50} values ranging from 890 nM for HIV-1_{RF} and 47 μ M for VZV.

ETV demonstrated activity against chronically WHV-infected woodchucks and DHBV DNA Replication in DHBV-infected ducklings. ETV dosages of 0.02 to 0.5 mg/kg once-daily for 4 weeks in woodchucks resulted in 2- to 3 \log_{10} reductions in serum WHV DNA levels. In a subsequent 12-week study, monitored by PCR assay, the serum WHV DNA was reduced to undetectable levels (<200 copies/mL) in all woodchucks dosed orally at the 0.1 mg/kg ETV daily regimen. Treatment of ducklings with 1 mg/kg/day ETV by oral gavage for 21 days resulted in reduction in serum DHBV DNA in all 6 treated ducklings to undetectable levels using a PCR based assay.

Cytotoxicity assays yielded CC_{50} values of 30 to 114 μ M for ETV, providing a selectivity index of $>8,000$ (CC_{50} value/ IC_{50} value). DNA polymerases α , β , γ , and ϵ incorporated ETV-TP into DNA in in vitro reactions but at a much lower rate than dGTP and were weakly inhibited by ETV-TP at concentrations approximately 10,000-fold higher than those required to inhibit the HBV polymerase. No inhibition of mitochondrial DNA polymerase γ in vitro occurred at concentrations up to 300 μ M ETV-TP indicating no significant recognition by polymerase γ . These results suggest that ETV-TP is unlikely to significantly inhibit cellular polymerases at clinically relevant concentrations.

In HBV replication assays, the antiviral activity of ETV was not affected by the presence of the HIV NRTIs abacavir, didanosine, lamivudine, stavudine, tenofovir, or zidovudine. ETV had no inhibitory effect on the anti-HIV activity of any of these six HIV NRTIs tested. These results indicate that co-administration of ETV with HIV NRTIs will not reduce the antiviral activity against either HBV or HIV.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Cell culture studies have shown that viruses with LAM-resistant substitutions rtM204V/I, rtL180M, rtL80V/I and rtV173L have approximately 5- to 30-fold cross resistance to ETV in cell culture studies. The addition of substitutions at positions rtT184, rtS202 and rtM250 to the LAM-resistance substitutions rtL180M and rtM204V resulted in 38- to 2,000-fold reduced susceptibility to ETV. HBV clones engineered to contain the adefovir-resistance substitutions rtN236T or rtA181V remained susceptible to ETV (0.3- and 1.1-fold change over WT, respectively).

2.1.2. Clinical Microbiology Summary

Studies 022 and 027 examined the activity of 0.5 mg ETV QD compared to 100 mg LAM QD in nucleoside treatment-naive subjects and studies 014, 015, and 026 examined the efficacy of 1 mg ETV QD compared to 100 mg LAM QD in patients with prior LAM experience and LAM-refractory HBV. Genotypic and phenotypic analyses of paired clinical isolates obtained at baseline and post-baseline were performed to monitor baseline and emerging amino acid substitutions in the HBV polymerase (RT) and to determine their impact on virologic response to ETV.

In nucleoside treatment-naïve studies 022 (HBeAg positive subjects) and 027 (HBeAg negative subjects), 83% (541/653) of patients on ETV treatment were suppressed with serum HBV DNA <400 copies/mL as quantified by the COBAS Amplicor HBV Monitor PCR assay at week 48 compared to 59% (363/619) of patients on LAM treatment. No ETV-resistant substitutions at residues rtT184S/A/I, rtS202G, and/or rtM250L were detected in any isolate on ETV therapy by 48 weeks in study 022 and 027. Two treatment-naive subjects experienced virologic rebound on ETV treatment but had no detectable amino acid changes emerge on treatment and no change in phenotypic susceptibility to ETV, ADV or LAM.

In clinical studies 014 and 026, 21% (36/174) of patients on ETV were suppressed to below 400 copies/mL HBV DNA as quantified by the COBAS Amplicor HBV Monitor PCR assay at week 48 compared to 1% of patients on LAM. In these studies, LAM-resistant substitutions rtL180M and rtM204V/I were detected in >80% of baseline isolates from both the ETV and LAM arms and these substitutions were maintained during the study, presumably because of the selective advantage in the presence of LAM and ETV. In study 014, genotypic analyses determined that LAM-resistance substitutions rtL80V, rtL180M, rtM204V or I emerged in the HBV of 17% (7/42) of patients on ETV by week 48. These substitutions often arose in the context of mixtures at these sites at baseline and other LAM-resistance mutations at baseline. Despite the emergence of LAM-resistance substitutions, the viral load in 4 of 7 patients was suppressed below 300 copies/mL (LLOQ) and the other 3 subjects experienced >2 log₁₀ reductions in viral load at the time the isolate developed the LAM-resistant mutations. ETV-associated resistance substitutions at rtT184 developed on 1 mg ETV therapy in 5 (12%) patients after week 48 in study 014 and coincided with rebounds in viral load.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

In study 026, substitutions at HBV polymerase residues rtI169, rtT184, rtS202 and/or rtM250 emerged on therapy in 9% (12/134) of ETV subjects with week 48 data. In all cases, the ETV-resistant substitutions emerged when pre-existing LAM-resistant changes were present. Two of the 12 ETV subjects that developed ETV resistance substitutions in their HBV experienced virologic rebound by week 48 and additional subjects (10/14) had virologic rebound after 48 weeks of ETV treatment.

The supportive study 015 examined the antiviral activity of open label ETV 1 mg QD in OLT (orthotopic liver transplant) recipients (n = 9) who were > 100 days post-transplant and had recurrent HBV infection despite prophylaxis with anti-HBV antibody. In this study, virologic rebound occurred in 6 out of 8 patients - one in the first year therapy, one in the second year, and four in the third year while 2 patients maintained HBV DNA suppression with no rebound out to 127 and 131 weeks of therapy. Genotypic data showed the development of ETV-resistance substitutions in seven of eight patient isolates at rtS202G or I (n=5), rtT184S/I/A/L/F (n=4) or rtM250V (n=1), and these substitutions were linked to LAM-resistant changes rtL180M and rtM204V.

Phenotypic data provided for isolates from studies 015 and 026 showed that the ETV-associated resistance substitutions were associated with phenotypic ETV resistance. The median change from reference of ETV susceptibility was 48-fold (range 4.2 to 6971) for the isolates that developed ETV-resistance substitutions at 48 weeks in studies 015 and 026 (n = 14). The ETV-resistant clinical isolates were susceptible to ADV, but remained resistant to LAM. Cross-resistance to ETV was not observed with ADV-resistant HBV.

In summary, a higher proportion of nucleoside-naïve subjects with chronic HBV infection achieved serum HBV DNA levels <400 copies/mL on ETV treatment compared to LAM-refractory subjects (83% vs. 21%). Genotypic or phenotypic evidence of ETV resistance has not been detected at 48 weeks of ETV treatment in two studies of nucleoside treatment-naïve patients. However, by week 48, 7.4% (14/189) of LAM-refractory subjects treated with 1.0 mg ETV in studies 014 and 026 had evidence of emerging ETV-associated resistance substitutions at rtI169, rtT184, rtS202, and/or rtM250. These ETV resistance substitutions emerged when pre-existing LAM-resistant changes were already present and were associated with virologic rebound.

3. Administrative

3.1. Reviewer's Signature(s)

[Lisa K. Naeger, Ph.D.]

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Senior Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Signatory Authority _____ Signature _____ Date

HFD-530/Micro TL _____ Signature _____ Date

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

OND Microbiology Review

1. Introduction and Background

1.1. Important Milestones in Product Development

The applicant has conducted an extensive global development program for ETV in the treatment of chronic HBV infection. The NDA package for ETV contains study reports and datasets for 4 pivotal and 7 supportive clinical studies conducted at multiple sites, both within the U.S. and at international sites. At the time these studies were initiated, lamivudine (LAM) was the only approved oral treatment for chronic HBV infection and was chosen as the appropriate comparator for the Phase 3 studies.

1.2 Methodology

Genotypic Analysis



The HBV polymerase (RT) contains 344 amino acid residues. A general numbering system has been proposed by Stuyver *et al.* to provide uniformity in the numbering of the amino acid residues for the HBV RT from the eight different viral genotypes. Wild

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

type (WT) HBV RT sequences (n = 250) of known genotype were retrieved from GenBank National Center for Biotechnology Information URL: www.ncbi.nlm.nih.gov using their respective accession numbers and analyzed to identify residues which are conserved and polymorphic. The 250 amino acid sequences were aligned using the Clustal W alignment method in MegAlign (DNASTAR, Inc.) and the sequence heterogeneity at each of 344 amino acids of the HBV RT was determined. Conserved positions were defined as residues that have the same amino acid among 99% (≥ 247 of the 250) WT RT sequences. Polymorphic positions were defined as residues that vary $> 1\%$ (≥ 4 times) among the 250 WT RT sequences.

Comparison of this RT sequence database with the sequence derived from clinical isolates allowed the identification of emergent changes on therapy. Emergent substitutions were identified by comparison of the baseline (study entry, pre-treatment) isolate sequence to that of isolates obtained while on study. Substitutions of RT residues that occurred on study were considered noteworthy if they occurred at any conserved residue position or if they involved substitution of a polymorphic residue with an amino acid not found among the aligned WT HBV sequences. When isolates contained multiple viruses with different substitutions, a mixture of three or more residues was reported as an X. If a mixture consists of two residues, both were listed.

Subject HBV isolates were assigned to one of the eight HBV genotypes through phylogenetic comparison of their RT sequences with those of reference HBV genotypes. Nucleotide sequence corresponding to amino acids 1 to 344 of the HBV RT of the subject isolates and genotype reference sequences were aligned and compared using the Clustal W method of the MegAlign software. Ninety HBV sequences from GenBank representing eight HBV genotypes (A through H) were used as reference sequences with 15 genotype A sequences, 13 genotype B sequences, 17 genotype C sequences, 14 genotype D sequences, 4 genotype E sequences, 14 genotype F sequences, 10 genotype G sequences, and 3 genotype H sequences. This phylogenetic analysis resulted in the clustering of reference sequences into distinct HBV genome genotype groups. Subject HBV isolates were assigned to one of the eight HBV genotypes based on clustering within a particular reference genotype group.

Ultrasensitive Method to Detect Rare HBV Variants with Population Quasispecies

Patients with chronic HBV infections often harbor complex mixtures of virus populations, referred to as quasispecies. Viruses with a selective growth advantage often predominate within the mixtures. While antiviral therapy selects for viruses with resistance mutations, removal of therapy will usually cause re-emergence of WT virus and genotypic reversion of the viral population to primarily a WT genotype. However, drug resistant viruses can be present at reduced, often undetectable levels within the patient quasispecies and can re-emerge upon the re-initiation of antiviral therapy. These viruses are often below levels of detection by conventional methods such as population nucleotide sequencing or specific hybridization by line-probe assays. A discriminatory real-time PCR method was established to detect rare virus variants that contain unique

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

single nucleotide polymorphisms (SNP) at a given nucleotide position. Standardizing each sample by parallel amplification with nondiscriminatory primers was found to significantly reduce false positive and negative results. In addition, novel primer design strategies were utilized to detect SNPs that were unattainable with prior methodology. The limit of detection ranged from 0.1 to 0.001% depending on the specific SNP measured. This methodology was used to detect LAM-resistant HBV viruses within a predominantly WT HBV population. It should be noted that this assay can detect polymorphisms present in the virus population at concentrations consistent with the likely background mutation rate of the viral polymerase.

Phenotypic Analyses

Phenotypic drug susceptibility assays for HBV are limited by a lack of complete HBV multi-cycle replication in cell culture and the need to measure replication through levels of progeny viral DNA rather than cytopathology or protein expression. Since recombinant HBV that express exogenous reporter genes do not replicate, transient assays using transfection of recombinant plasmids that express the HBV genome have been established. HepG2 cells are transfected with recombinant plasmids that direct expression of high levels of HBV pregenomic RNA and viral proteins. The HBV polymerase directs replication of the DNA genome through reverse transcription and DNA-dependent DNA synthesis. The encapsidated progeny genomes are released into the media as complete HBV virions, but they are unable to complete subsequent rounds of cell infection. This system was used for determining the activity of ETV in vitro and the phenotypes of HBV isolates resistant to replication inhibitors in cell culture, including LAM and ADV.

[REDACTED] (b) (4)

Multiple independent phenotypic measurements of an individual WT reference clone and a collection of clinical WT reference populations led to the establishment of a WT reference ETV IC₅₀ value of 5.4 nM for individual clones and 1.5 nM for isolate populations. Reference values were used for the standardization of ETV susceptibility IC₅₀ values for all subject clones and populations.

Serum HBV DNA

Serum HBV DNA levels were quantified by the investigational Roche COBAS Amplicor PCR method [upper limit of quantification 2.0x10⁵ copies/ml; lower limit of quantification (LLOQ) 300 copies/mL. Diluted samples were assayed when the HBV DNA level exceeded 2.0 x10³ copies/mL. In some instances, as noted, HBV DNA levels were determined using the Chiron Quantiplex bDNA method [LLOQ 7.0 x10⁵ copies/ml, 2.5 pg/mL or 0.7 megagenome equivalents (MEq/mL)]. Note that at the time

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

these ETV studies were started, the Roche COBAS Amplicor PCR assay had an LLOQ of 200 copies/mL. Upon review of the assay data in Europe, Roche changed the LLOQ for the COBAS assay to 300 copies/mL. The applicant used serum HBV DNA < 400 copies/mL for efficacy endpoints. The LLOQ for the Roche Amplicor PCR assay, which is done manually, is 1000 copies/mL and differs from the COBAS Amplicor assay which is an automated assay.

Roche COBAS Amplicor Performance Characteristics

The limit of detection, linear range and precision of the COBAS AMPLICOR HBV MONITOR Test were determined by analysis of serial dilutions of a well characterized stock of HBV virus in both HBV-negative EDTA human plasma and HBV-negative serum. The virus stock was obtained from the (b) (4)

The genotype of the virus (Genotype A) and the concentration of the stock virus was determined by the vendor. Preparation of the viral panels and analysis with the COBAS AMPLICOR HBV MONITOR Test were performed in-house.

The Limit of Detection was determined by analysis of three independent dilutions of the HBV virus stock in both HBV-negative EDTA human plasma and HBV-negative serum using two lots of the COBAS AMPLICOR HBV MONITOR Test. The study demonstrates that the assay can detect virions at concentrations as low as 300 copies/mL HBV DNA with a positivity rate greater than 95% in both EDTA-plasma and serum (Table A; COBAS Amplicor HBV Monitor package insert) provided that the OD of the selected D-cup is within the specified OD range (0.15-2.00).

Table A. Limit of Detection of the COBAS AMPLICOR HBV MONITOR Test

Nominal Input (HBV DNA copies/mL)	EDTA Plasma			Serum		
	No. Replicates	No. Positives	Positivity Rate	No. Replicates	No. Positives	Positivity Rate
1,000	106	106	100%	108	108	100%
800	107	107	100%	108	108	100%
600	108	108	100%	108	108	100%
500	107	107	100%	108	107	99.1%
400	108	108	100%	108	107	99.1%
300	107	106	99.1%	108	106	98.1%
200	108	100	92.6%	108	90	83.3%

The linear range was determined by analysis of serial dilutions of the HBV virus stock in both HBV negative EDTA human plasma and HBV-negative serum using three lots of the COBAS AMPLICOR HBV MONITOR Test. The test was found to give a linear response from 300 (LLOQ, log₁₀ = 2.48) HBV DNA copies/mL to at least 200,000 (ULOQ, log₁₀ = 5.30) HBV DNA copies/mL in both EDTA-plasma and serum.

Precision was evaluated using SAS's PROC MIXED (method=REML) analysis method. This method permits the determination of the assay precision through analysis of the

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

source of variance of the assay. A run, consisting of 2 replicates of each sample, was performed daily for 4 days by each of 2 operators. Each sample was taken through the entire COMAS AMPLICOR HBV MONITOR Test procedure including specimen preparation, amplification and detection. Therefore, the precision reported here represents all aspects of the test procedure. The results from this precision study are shown below (Table B; COBAS Amplicor HBV Monitor package insert).

The performance of the COBAS AMPLICOR HBV MONITOR Test on HBV genotypes was evaluated by analysis of 12 purified, linearized and quantitated plasmid DNAs containing representative sequence inserts from HBV genotypes A through F. Each plasmid was diluted to nominal input concentrations of 1500, 15,000 and 150,000 HBV DNA copies/mL. Each dilution was tested a total of twelve times using two kit lots, of the COBAS AMPLICOR HBV MONITOR Test. The COBAS AMPLICOR HBV MONITOR Test gave equivalent results for hepatitis B plasmid DNAs from genotypes A through F. However, both of the genotype F plasmid DNAs yielded significantly lower results than the nominal input and did not detect the isolate at input concentrations less than 150,000 HBV DNA copies/mL. Sequence analysis of the genotype F plasmid DNAs and other genotype F sequences indicated that there are several nucleotide mismatches between genotype F isolates and the sequence of one of the primers used in the COBAS AMPLICOR HBV MONITOR Test. Therefore, genotype F will not be amplified with the same efficiency as the other genotypes and will result in a lower viral load result.

Genotype G was not tested in the plasmid DNA study as isolates were not available. However, sequence information from this genotype indicate that this genotype has a 36 nucleotide insertion within the viral sequence that is captured by the detection probe utilized in the COBAS AMPLICOR HBV MONITOR Test. Therefore, it is expected that the Test will not accurately detect genotype G. Additional studies were performed with sixty-eight HBV-positive clinical specimens of known genotypes. At least ten individual specimens from genotypes A through F and one single specimen of genotype G were included in the study. Genotypes A through E were equivalently amplified and detected by the COBAS AMPLICOR HBV MONITOR Test. Results from the genotype F specimens indicated that this genotype was not equivalently amplified and detected by this test. The single HBV genotype G clinical specimen yielded results comparable to those obtained with genotypes A through E. However, test results from a single clinical specimen are not predictive of assay performance with HBV genotype G.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table B. Precision of the COBAS AMPLICOR HBV MONITOR Test

Copies/mL Nominal	Log copies/mL Nominal	Result	Sources of Variation	SD(log Titer)	95% CI for SD		%CV (Titer)	95% CI for % CV		% Var. Attrib
					Lower	Upper		Lower	Upper	
300	2.5	2.2	Lot	0.0000	--	--	0.0	--	--	0.0
			Operator	0.1106	0.042	104.338	25.9	10	Inf	13.6
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0000	--	--	0.0	--	--	0.0
			Residual	0.2784	0.222	0.374	71.3	55	105	86.4
			Total	0.2996	0.225	0.450	78.1	55	139	100.0
500	2.7	2.4	Lot	0.1440	0.059	23.133	34.1	14	--	23.8
			Operator	0.0000	--	--	0.0	--	--	0.0
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0689	0.021	Inf	16.0	5	Inf	5.4
			Residual	0.2484	0.185	0.378	62.2	45	106	70.8
			Total	0.2953	0.206	0.519	76.7	50	178	100.0
2500	3.4	3.2	Lot	0.0366	0.014	17.085	8.5	3	Inf	15.7
			Operator	0.0000	--	--	0.0	--	--	0.0
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0000	--	--	0.0	--	--	0.0
			Residual	0.0849	0.068	0.114	19.7	16	27	84.3
			Total	0.0925	0.069	0.142	21.5	16	34	100.0
12500	4.1	4.0	Lot	0.0230	0.009	14.422	5.3	2	Inf	15.3
			Operator	0.0000	--	--	0.0	--	--	0.0
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0124	0.004	Inf	2.9	1	Inf	4.4
			Residual	0.0528	0.039	0.080	12.2	9	19	80.3
			Total	0.0589	0.044	0.090	13.6	10	21	100.0
65000	4.8	4.7	Lot	0.0000	--	--	0.0	--	--	0.0
			Operator	0.0050	--	--	1.1	--	--	0.4
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0429	0.023	0.209	9.9	5	51	30.0
			Residual	0.0655	0.049	0.100	15.2	11	23	69.6
			Total	0.0785	0.062	0.107	18.2	14	25	100.0
200000	5.3	5.1	Lot	0.0000	--	--	0.0	--	--	0.0
			Operator	0.0000	--	--	0.0	--	--	0.0
			Operator* Lot	0.0000	--	--	0.0	--	--	0.0
			Run (Operator)	0.0365	0.022	0.110	8.4	5	26	37.7
			Residual	0.0468	0.055	0.071	10.8	8	17	62.3
			Total	0.0594	0.047	0.081	13.7	11	19	100.0

The clinical specificity of the COBAS AMPLICOR HBV MONITOR Test was determined by analysis of HBV-negative human serum and EDTA-plasma blood donors. A total of 216 specimens (108 serum specimens and 108 EDTA-plasma specimens) that were non-reactive for one or more of the following markers (HBsAg, HBeAg, anti-HBs, anti-HBe, HBc IgG or HBc IgM) were analyzed. All specimens were negative for HBV DNA. Based on these results, the clinical specificity of the COBAS AMPLICOR HBV MONITOR Test is 100%.

The analytical specificity of the COBAS AMPLICOR HBV MONITOR Test was evaluated by adding cultured cells, cultured virus, or purified nucleic acid from the following organisms and viruses into HBV-negative human EDTA plasma or analyzing specimens from infected patients (Table C; COBAS Amplicor HBV Monitor package insert). None of the non-HBV organisms, viruses or purified nucleic acids tested were positive for HBV DNA.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table C. Analytical Specificity Specimens

Adenovirus, Human Type 2	HIV-1, Subtype C
Adenovirus, Human Type 3	HIV-1, Subtype D
Adenovirus, Human Type 7	HIV-1, Subtype E
<i>Chlamydia trachomatis</i>	HIV-1, Subtype F
Coxsackievirus B1	HIV-1, Subtype G
Cytomegalovirus AD-169	HCV, genotype 1a
Cytomegalovirus Davis	HCV, genotype 2b
Cytomegalovirus Towne	HCV, genotype 2a/2c
Echovirus 1	HCV, genotype 3a
Epstein-Barr Virus	HCV, genotype 3c
Hepatitis A	HCV, genotype 4c/4d
Herpes simplex type 1F	Human Papilloma Virus Type 16
Herpes simplex type 1HF	Human Papilloma Virus Type 18
Herpes simplex type 1 MacIntyre	Human Papilloma Virus Type 6a
Herpes simplex type II	<i>Propionibacterium acnes</i>
Human Herpes virus 6B	<i>Staphylococcus aureus</i>
HIV-1, Subtype A	Varicella-zoster virus Ellen
HIV-1, Subtype B	Varicella-zoster virus Oka

1.3 Prior FDA Microbiological Reviews

Submission: IND52196 SN 106

Reviewer: Jules O'Rear, Ph.D.

Date: 7/13/01

The reviewer asked the applicant to provide a detailed protocol for evaluating genotypes in individuals failing treatment (non-responders, partial responders) and those undergoing rebound.

BMS Response: Most currently available commercial assays for HBV resistance testing detect only a selected number of mutations in the YMDD domain of the HBV DNA polymerase. We are in the process of selecting an alternative methodology for genotyping which will assess a broader range of mutations and are currently working with our central laboratory to assure we can implement the testing and obtain quality results. As soon as we have accomplished this, we will provide the Agency with a detailed protocol.

The reviewer recommended that the applicant quantify HBV DNA levels on all samples using both the Chiron Quantiplex™ and Roche PCR assays and use as an endpoint a specific drop in viral load rather than a lack of detection with one assay.

BMS Response: All subjects enrolled in the AI463-026 study will be viremic on lamivudine prior to entry into the study and approximately 50% of these subjects will be assigned to receive entecavir and the other 50% will continue lamivudine during the study. We anticipate that a substantial proportion of subjects assigned to entecavir, but not those assigned to lamivudine, will achieve undetectable levels of HBV DNA by the Chiron Quantiplex™ assay on study. The PCR assay is more sensitive and provides a better understanding of viral load decline, particularly in samples that are undetectable by the Quantiplex™ assay, but it may provide little additional information in samples that are still detectable by the Quantiplex™ assay. Therefore, we will add a PCR assay (Roche Amplicor™) on all samples at Week 24 that are undetectable by the Quantiplex™ assay. As currently stated in the protocol, we will obtain PCR assay at baseline, at Week 48 and at post-dosing Week 24. We will provide the performance characteristics for each assay if they are not approved by the time of submission of our entecavir NDA.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

In our first Phase 2 trial (AI463-004), we had selected a 2-log decrease in viral load as an endpoint. However, we now know from the results of our subsequent Phase 2 trial (AI463-005) that in treatment naive subjects, entecavir at a dose of 0.5 mg achieved undetectable levels in 84% of the subjects by the Quantiplex™ assay and in 26% of the subjects by the PCR assay by Week 22. Although the proportion of subjects who achieve undetectable levels by either assay may be lower in the AI463-026 study, as the population in this study may be more difficult to treat, demonstrating that subjects achieve undetectable levels, is clinically more relevant than that they achieve a 2-log decrease. In addition, we believe that to make treatment decisions in the clinical arena, it is more important to know if and when HBV DNA becomes undetectable in the serum. We have chosen Chiron Quantiplex assay to accomplish this, as it is a more readily available assay and the results can be compared to other clinical trials with nucleoside analog that have used the Quantiplex™ assay. Therefore, we would prefer to retain lack of detection by the Quantiplex™ assay, as a component of our Composite Endpoint.

The reviewer requested a description of the assay for HBV cccDNA and assay performance characteristics.

BMS Response: Measuring HBV cccDNA in liver specimens is currently still a research tool available at only a few research laboratories. We are in the process of selecting the methodology for cccDNA and will work with our central laboratory to assure we can obtain quality results. As soon as we have accomplished this, we will provide the Agency with a detailed protocol.

Submission: IND52196 SN 144

Reviewer: Jules O’Rear, Ph.D.

Date: 9/27/02

There is a concern that ETV could antagonize the anti-HIV activity of NRTIs and conversely that NRTIs could antagonize the anti-HBV activity of ETV. The reviewer requested that the applicant determine the in vitro combination activity relationships against HIV for ETV with all approved and investigational NRTIs, and conversely, the combination activity relationships against HBV of all approved and investigational NRTIs with ETV. The applicant responded that combination studies in cell culture between ETV and antiretroviral NRTIs were ongoing and that initial results indicated no evidence of antagonism.

Submission: IND52196 SN 225

Reviewer: Jules O’Rear, Ph.D.

Date: 2/9/04

The reviewer advised BMS to submit the resistance data used to support NDA approval in the HBV resistance template format. All amino acid positions spanning the regions subjected to DNA sequence analysis need to be provided. The DAVDP HBV template format utilizes blanks to indicate identity with the WT standard sequence to provide an acceptable “signal to noise” ratio which facilitates the identification of important residues. The applicant may utilize a hyphen (-) to indicate regions for which nucleotide sequence data are missing. Viral load and outcome should be included for sorting of the data. BMS was also advised to include an outcome analysis (based on virologic failures $>10^4$ copies/mL) to identify any specific polymerase polymorphisms that may affect entecavir activity in vivo, i.e., identify any polymorphisms present at baseline associated with responders and any associated with non-responders.

Submission: IND52196 SN 260

Reviewer: Jules O’Rear, Ph.D.

Date: 7/8/04

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

The reviewer conveyed the following to the applicant. A well-developed phenotypic assay may be the best means to determine the most effective regimen for an HBV-infected individual. Ideally, a phenotypic assay should identify non-responders (*i.e.*, shift in susceptibility relative to WT for baseline samples), responders who rebound (*i.e.*, shift in susceptibility of the failure isolate relative to the baseline isolate and WT), and responders (*i.e.*, little to no shift in susceptibility of end of study isolates relative to baseline/WT). The reviewer requested a sub-study containing a random sampling of patient samples with adequate numbers to determine the accuracy of the assay with respect to patient phenotype. In addition, the reviewer stated that cross-resistance should be evaluated with the same phenotypic assay. The antiviral activity of entecavir to HBV isolates containing all reported lamivudine and adefovir resistance-associated mutations should be determined, and the antiviral activity of lamivudine and adefovir against entecavir resistant isolates should be evaluated.

BMS Response: The BMS phenotypic assay that was developed will include validation as indicated by the agency to the extent that such samples exist. **Non-responders:** There are few available subjects that are true non-responders. A selection of these will be tested for clinically relevant baseline resistance to ETV. **Responders who rebound:** All are currently included in our phenotypic analysis. **Responders:** We will select a random sample of 20 - 25 subjects who responded, but still have detectable virus, for evaluation from studies 022 and 027.

All ETV resistant isolates are assayed against lamivudine, adefovir and entecavir. It has been previously reported that adefovir-resistant isolates are susceptible to entecavir. We have much data on the ETV susceptibility of lamivudine resistant isolates, but do not have the appropriate adefovir-resistant clinical isolates to assay against ETV.

The reviewer advised the applicant to evaluate cross-resistance for adefovir-resistant clinical isolates as they become available.

1.4 Major microbiological issues that arose during product development

1. It was recommended that the applicant quantify HBV DNA levels on all samples using both the Chiron Quantiplex™ and Roche PCR assays and use as an endpoint a specific drop in viral load rather than a lack of detection with one assay. The applicant chose Chiron Quantiplex assay, as it is a more readily available assay and the results can be compared to other clinical trials with nucleoside analogs that have used the Quantiplex™ assay. Therefore, they retained lack of detection by the Quantiplex™ assay, as a component of our Composite Endpoint. They agreed to add a PCR assay (Roche Amplicor™) on all samples at Week 24 that are undetectable by the Quantiplex™ assay and as currently stated in the protocol, obtain PCR assay at baseline, at Week 48 and at post-dosing Week 24.
2. BMS was advised to submit the resistance data used to support NDA approval in the HBV resistance template format with all amino acid positions spanning the regions subjected to DNA sequence analysis provided, blanks indicating identity with the WT standard sequence and hypens indicating regions of missing nucleotide sequence.
3. At the time the ETV studies were started, the Roche COBAS Amplicor PCR assay had an LLOQ of 200 copies/mL. Upon review of the assay data in Europe, Roche changed the LLOQ for the COBAS assay to 300 copies/mL. With respect to BMS

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

study conduct and datasets, this change in the LLOQ was implemented by the Phase 3 laboratory ((b) (4)) in early 2004. The applicant used serum HBV DNA <400 copies/mL for efficacy endpoints. The LLOQ for the Roche Amplicor PCR assay, which is done manually, is 1000 copies/mL and differs from the COBAS Amplicor assay which is an automated assay.

1.5 State of antimicrobials used for the indication (s) sought

The currently available drugs for the treatment of chronic HBV infection are recombinant α interferons (α -IFNs) and two approved oral therapies lamivudine (LAM) and adefovir dipivoxil (ADV). Recombinant α interferons (α -IFNs) act primarily as endogenous immunomodulatory agents and have secondary intrinsic antiviral effects. The anti-HBV nucleoside analogs ADV and LAM directly inhibit viral replication of HBV and both also inhibit HIV. Each of these anti-HBV drugs has characteristics that limit its clinical value in patients. α -IFN is administered by injection, is frequently associated with fever, flu-like symptoms, neutropenia, and depression, and is contraindicated in patients with decompensated liver function. LAM is well tolerated and is available worldwide, but its long-term efficacy is limited by the development of resistance. Resistance occurs in 24% of patients after one year of therapy and 70% of patients after four years. The mutations rtM204V or I in the YMDD motif in the C domain of the HBV polymerase confer resistance to lamivudine. The rtL80V, rtL180M and rtV173L mutations in the B domain of HBV polymerase are also frequently observed in conjunction with the YMDD mutations and appear to enhance replication fitness of the YMDD mutant HBV. Adefovir has a favorable resistance profile, but resistance-associated mutations have been identified in long-term resistance surveillance of ADV clinical studies. Mutation rtN236T was detected in 1.7% (4/238) of patients after 96 weeks of ADV therapy. A second conserved site mutation rtA181V was also observed in 0.8% of patients (2/238) after 96 weeks of ADV. The incidence of these ADV resistance-associated mutations was 0% (0/629) for 0-48 weeks, 2% (6/293) for 49-96 weeks and 1.8% (3/163) for 97-144 weeks with a cumulative probability of 3.9% in developing ADV resistance at year 3. The ADV dose has been limited to 10 mg because of nephrotoxicity. The treatment of chronic HBV infection continues to present a clinical challenge and there is a need for effective and durable HBV treatment strategies and options.

2. Non-clinical Microbiology

Mechanism of Action Studies

Like other nucleoside analogs, ETV is phosphorylated to its mono-, di-, and tri-phosphate forms by cellular kinases. ETV was efficiently phosphorylated to its active triphosphate form at low nM concentrations. When HepG2 cells were labeled with 50 nM ETV (10-fold the IC₅₀ value), ETV-TP was detectable as early as 1 hour after drug administration and

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**MICROBIOLOGY DRAFT REVIEW**

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

accumulated during the first 20 hours with a plateau at approximately 250 nM after 12 hours. Kinetics of accumulation were slower at lower nM concentrations despite more efficient phosphorylation. Two cytosolic kinases (thymidine kinase TK-1 and deoxycytosine kinase dCK) were found to primarily be responsible for ETV phosphorylation. The purified mitochondrial enzyme, deoxyguanosine kinase dGK, was also capable of phosphorylating ETV. The mono- and di-phosphate forms do not accumulate to a substantial extent. The intracellular half-life of entecavir-triphosphate (ETV-TP) is 14 to 15 hr in both normal and HBV-infected liver cells.

The replication of HBV involves three distinct enzymatic activities of the viral polymerase: a discrete priming activity, a reverse transcriptase activity responsible for first-strand DNA synthesis, and a DNA-dependent DNA polymerase activity which performs second-strand DNA synthesis. Entecavir-triphosphate (ETV-TP) and lamivudine-triphosphate (3TC-TP) were tested against the distinct hepadnaviral polymerase activities and expressed as the ratios of ETV-TP:dGTP substrate to allow comparison among assays that utilize different concentrations of dGTP substrate. ETV-TP was effective against all three activities of the hepadnaviral replication, while 3TC-TP inhibited the reverse transcription and DNA synthesis reactions but not the priming reaction (Table 1; Report 930007620, page 12). The low inhibition:substrate ratios of ETV-TP indicate that the hepadnaviral polymerases use ETV-TP more effectively than natural substrate. This is further confirmed by the K_i and K_m analyses.

Table 1. In Vitro Inhibition of HBV Polymerase Activities by Nucleoside Analog Triphosphates

Analog	IC ₅₀ [(Analog-TP)/(dNTP)] ^a		
	DHBV Priming	HBV Endogenous RT	WHV Endogenous
ETV-TP	0.30	0.32	0.18
LVD-TP	>400	>4.70	7.44

^a The IC₅₀ values are expressed as the ratios of the nucleoside analog triphosphate to natural substrate triphosphate (dCTP for LAM, dGTP for ETV) that yielded 50% inhibition of control activity levels in each assay system. Values represent the mean from 2 or more experiments.

An analysis of kinetic parameters for the HBV polymerase by reciprocal plots demonstrated that ETV-TP is a competitive inhibitor of dGTP. The K_i of ETV-TP ranges from 1.2 to 2.6 nM with K_m values ranging from 8.4 to 13.3 nM for dGTP giving a K_i/K_m ratio of 0.1. The low (<1) K_i/K_m ratio substantiates that the polymerase has a higher affinity for ETV-TP than natural substrate and corroborate that ETV-TP is an effective inhibitor of HBV polymerase.

The mechanism by which ETV-TP inhibits the HBV polymerase was tested by endogenous sequencing. Purified baculovirus-expressed HBV nucleocapsids were subjected to *in vitro* DNA synthesis reactions in the presence of the 4 ³²P-labeled deoxynucleotides and ETV-TP or one of the 4 ddNTPs and the resultant products were analyzed on a polyacrylamide

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**MICROBIOLOGY DRAFT REVIEW****NDA:** 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05**Microbiology Reviewer:** Lisa K. Naeger, Ph.D.

sequencing gel. ETV-TP caused chain-termination at 2 or 3 nucleotides downstream from dG positions in the sequence primarily following dG doublets. The results indicate that ETV-TP is a non-obligate chain-terminator of HBV polymerase.

DNA polymerases α , β , γ , and ϵ incorporate ETV-TP into DNA but at much lower rates than dGTP and require higher concentrations. ETV-TP is not efficiently recognized by the cellular DNA polymerases with K_i/K_m ratios (ETV-TP/dGTP) ranging from 2.5 – 22 (See Table 4).

***In Vitro* Anti-HBV Activity**

The concentration required to reduce HBV replication by 50% (IC_{50}) for ETV was determined in the HepG2.2.15 cell line, which is a human liver cell line that harbors integrated HBV genomes and constitutively produces HBV virion particles. Anti-HBV activity was determined by measuring the reduction in secreted extracellular virion HBV DNA with DNA dot blot hybridization after treatment with drug. Entecavir demonstrated activity against HBV in 9 experiments with an average IC_{50} value of 3.75 nM in HepG2.2.15 cells (Report 51975, page 8). Measurement of the extracellular HBV genome and the intracellular single-strand DNA species gave the same IC_{50} values for ETV (3-4 nM) and LAM (116-140 nM), but lower IC_{50} values (0.015 nM for ETV and 3 nM for LAM) for the intracellular relaxed circular DNA.

In HBV DNA transfected Huh7 liver cells and HepG2 cells, ETV had IC_{50} values of 0.36 nM and 3.6 nM, respectively. In comparison, lamivudine, adefovir, and LdT were 30- to >100-fold less active than ETV. Penciclovir, the active metabolite of famciclovir (FCV) and ganciclovir (GCV) were inactive against HBV (IC_{50} value $\geq 100 \mu M$). ETV demonstrated little antiviral activity against Herpes simplex virus (HSV-1), varicella zoster virus (VZV), human cytomegalovirus (HCMV), HIV-1 and influenza with IC_{50} values ranging from 890 nM for HIV-1^{RF} and 47 μM for VZV (Table 2; Report 930007620, page 11).

Table 2. Antiviral Activity and Cytotoxicity Evaluation

Virus	Cell Line	Tissue Origin	EC_{50} (μM)^a	CC_{50} (μM)^a
HBV	HepG2.2.15	human liver	0.00375	30
- ^b	Huh7	human liver	-	114
HIV	CEM-SS	human T cell leukemia	>10	21
-	MT-2	human T cell leukemia	-	17
-	MT-4	human T cell leukemia	-	3.6
Influenza virus	MDBK	bovine kidney	>80	75
HCMV	HFF	human foreskin fibroblasts	15	-
HSV-1	WI-38	human embryonic lung	32	>90
VZV	WI-38	human embryonic lung	30-60	120

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

In Vivo Anti-HBV Activity

HBV infects only humans and other higher primates making it difficult to study drugs that act against HBV. Therefore, related viruses that infect woodchucks, ground squirrels and domestic ducks are used as surrogates to evaluate the effects of anti-HBV drugs. The eastern woodchuck, *Marmota monax*, can be chronically-infected with woodchuck hepatitis virus (WHV), and is a widely accepted animal model for studies on the pathogenesis and therapy of human HBV infection. Initial studies in woodchucks showed that ETV demonstrated antiviral activity when administered either intraperitoneally or orally. Oral doses of ETV ranging from 0.02 to 0.5 mg/kg once-daily for 4 weeks in woodchucks resulted in 2- to 3 log₁₀ reductions in serum WHV DNA levels. In a subsequent 12-week study, monitored by a semi-quantitative polymerase chain reaction (PCR) assay (lower limit of detection = 200 to 2,000 copies/mL), the serum WHV DNA was reduced to undetectable levels in all woodchucks dosed orally at the 0.1 mg/kg ETV daily regimen. This represented a decrease in WHV titers of 7 logs. Four of 6 woodchucks receiving 0.02 mg/kg/day ETV also demonstrated a decline in serum viral DNA to undetectable levels; the viral DNA levels in the other 2 animals declined by up to 99%.

The activity of ETV in woodchucks was further analyzed in a long-term maintenance therapy study. Woodchucks chronically-infected with WHV were given oral ETV 0.5 mg/kg daily for 8 weeks followed by 0.5 mg/kg once weekly for an additional 12 (designated 14-month treatment group, 5 animals) or 34 months (designated 36-month treatment group, 5 animals). The 0.5 mg/kg daily dosing gave serum exposure levels equivalent to the human 1 mg dose. Serum viral DNA, monitored by quantitative PCR (lower limit of detection = 200 copies/ml), viral core antigen, and cccDNA became undetectable in these animals during the treatment period. Furthermore, a sustained response, defined as an inability to detect virus during the 2-year post-treatment period, was observed in 2 out of 5 woodchucks in the 14-month treatment group. Survival rates for animals treated for 14 or 36 months were 40 and 80% at the end of the 3-year study period, respectively, compared to historical controls analyzed where nearly all the infected animals (96%) die with hepatocellular carcinoma (HCC) within 3-4 years after infection. Two surviving woodchucks in the 14-month treatment group that maintained viral DNA below detectable limits for more than 2 years after withdrawal of therapy had no evidence of HCC at necropsy. Three of the 4 surviving animals in the 36-month treatment group had maintained undetectable viral DNA levels, and one animal returned to pretreatment viral DNA levels 12 weeks after withdrawal of therapy. None of the 4 surviving woodchucks had evidence of HCC at necropsy. In addition, there was no evidence of ETV resistance in the WHV polymerase after up to 3 years of treatment. Therefore, long-term ETV therapy in WHV chronically-infected woodchucks extended the life span of the treated animals and delayed the onset of HCC. Despite the long treatment period, there was no evidence of the emergence of resistant viral variants as confirmed by sequencing the polymerase domain of WHV where no mutations were detected.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

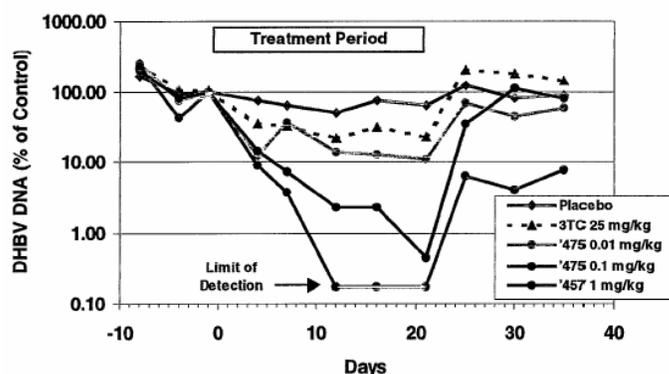
MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

DHBV was also used as a model of HBV for replication and antiviral studies in the ETV development program. The duck hepatitis B virus (DHBV) resembles HBV less closely structurally and genetically than the woodchuck and squirrel viruses, but there are essentially no noted differences in the replication cycles of DHBV and HBV. Entecavir demonstrated antiviral activity in ducklings infected with DHBV (Figure 1; Report 910066432, page 9). Treatment of ducklings with 1 mg/kg/day ETV by oral gavage for 21 days resulted in reduction in serum DHBV DNA in all 6 treated ducklings to undetectable levels using a PCR based assay (lower limit of detection = 10 DHBV copies/mL, reduction >2 log₁₀). Treatment with 0.1 mg/kg/day ETV resulted in 5 of 6 treated ducklings achieving decreases in viral DNA to undetectable levels, with an average viral load decrease of >99%. Reducing the ETV dose to 0.01 mg/kg/day resulted in an average viral load decrease of 90%. None of the lamivudine-treated ducklings achieved undetectable levels of viral DNA. ETV was also effective in decreasing the DHBV DNA levels in duck livers following 21-days of treatment, showing average reductions of 45%, 83%, and 96% at doses of 0.01, 0.1, and 1.0 mg/kg/day, respectively, compared to a 12% reduction for 25 mg/kg/day lamivudine (3TC) treatment.

Figure 1. Inhibition of DHBV DNA Replication in ETV-Treated Ducklings



Cytotoxicity

Cytotoxicity assays using MTT and XTT dye reduction assays and [³H]-thymidine incorporation in liver cells yielded CC₅₀ (concentration resulting in cytotoxicity in 50% of cells) values of 30 to 114 μM for ETV (Table 2 above), providing a selectivity index of >8,000 (CC₅₀ value/IC₅₀ value). Cytotoxicity in other cell types ranged from 3.6 to 120 μM, with MT-4 cells being the most sensitive. Consistent with the lack of activity against mitochondrial DNA polymerase γ, ETV did not inhibit cellular mitochondrial DNA synthesis.

Entecavir has the potential to be incorporated into cellular DNA and to inhibit cellular DNA polymerases. The ribose isostere on ETV possesses a 3'-hydroxyl group making it possible for ETV to be incorporated into elongating DNA chains. In vitro biochemical assays were used to measure the activity of ETV-TP against cellular polymerases that perform priming

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

(DNA polymerase α), processive replication (δ and ϵ), DNA repair (β), and mitochondrial DNA replication (γ). DNA polymerases α , β , δ and ϵ were weakly inhibited by ETV-TP at concentrations approximately 10,000-fold higher than those required to inhibit the HBV polymerase (Table 3; Report 930007620, page 15). No inhibition of mitochondrial DNA polymerase γ occurred at concentrations up to 300 μ M ETV-TP (K_i/K_m ratio > 1300) indicating no significant recognition by polymerase γ . In addition, high exposure levels of ETV had no appreciable adverse effects on mitochondrial DNA synthesis in HepG2 hepatoma cells treated for 4 days and did not affect oxidative metabolism.

ETV-TP functioned as a non-obligate chain terminator for cellular polymerases resulting in chain termination after 1 to 5 bases. In addition, the 3' - 5' exonuclease activity of replicative pol δ and pol ϵ were capable of excising terminal ETV from primer templates. The ratios of K_i to K_m indicated that even at equivalent concentrations, cellular polymerases prefer dGTP to ETV, while HBV polymerase favors ETV compared to the natural base with a K_i/K_m ratio less than 1 (Table 3). These results suggest that ETV is unlikely to significantly inhibit cellular polymerases at clinically relevant concentrations.

Table 3. Effects of ETV on Cellular Polymerases

DNA Polymerase	dGTP K_m (μM)	ETV K_i (μM)	K_i/K_m
HBV polymerase	0.012	0.0032	0.27
DNA Polymerase α	12.4	31	2.5
DNA Polymerase β	2.0	40	20
DNA Polymerase γ	0.12	> 160	> 1,300
DNA Polymerase δ	0.81	18	22
DNA Polymerase ϵ	8.2	40	4.9

In carcinogenicity studies, ETV caused an increased incidence of species, gender and tissue-specific adenomas in mouse lungs. The mechanism(s) by which ETV induces these mouse-specific adenomas is under investigation. While the results from the genotoxicity assays suggested that a genotoxic mechanism was unlikely, studies were undertaken to further investigate an earlier observation of low level incorporation into cellular DNA. The potential for low level incorporation of ETV into the DNA of cells exposed to clinically relevant concentrations of ETV was examined and compared with those of the deoxyguanosine analog penciclovir (PCV), which also carries a 3'-OH moiety and acts as a "de facto" chain terminator *in vitro*. PCV is the active form of the FDA approved oral valine ester prodrug, famciclovir, which is given daily for the suppression of genital herpes. The incorporation of ETV and PCV into cellular DNA in different cell types was evaluated in conjunction with their intracellular triphosphate levels. Both ETV and PCV were incorporated into HepG2 hepatoma cellular DNA at very low levels following extracellular exposure levels ranging from 0.2-fold their C_{ave} to 5-fold their C_{max} . Levels of incorporation were found to correlate with intracellular concentrations of ETV and PCV triphosphate.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

PCV incorporation into primary human fibroblast cell DNA occurred at 11- and 17-fold higher levels than ETV at C_{ave} and C_{max} levels, respectively, despite similar intracellular levels of triphosphates. Furthermore, the size of HepG2 cellular DNA containing incorporated ETV was found by HPLC gel filtration to be truncated relative to thymidine-labeled DNA, consistent with the DNA chain-termination activity of ETV in vitro. Taken together with the finding that replicative proofreading polymerases are able to excise ETV from the DNA in vitro, these results suggest that any ETV incorporated into DNA is likely to cause a termination event and/or be excised prior to subsequent elongation of the DNA chain. In summary, ETV-TP incorporation into HepG2 cellular DNA is low, similar to penciclovir-TP incorporation into cellular DNA, correlates with intracellular concentrations, and results in chain-termination.

Epithelial alveolar type II cells are the primary cell type implicated for the increase incidence of lung adenomas observed in rodent carcinogenicity studies with ETV. There were no observed differences in the levels of ETV-TP in a panel of alveolar type II cell lines of mouse, rat and human origins indicating that differential phosphorylation was not a likely cause of the mouse-specific lung tumors.

In Vitro Antiviral Drug Combination Relationships

Unlike LAM and ADV, which have activity against both HBV and HIV-1, ETV only has activity against HBV. With approximately 10% of HIV-1 infected individuals also co-infected with HBV, it is expected that ETV will be co-administered with antiretroviral therapies that include HIV NRTIs. Since these NRTIs use the same cellular enzymes for phosphorylation to their active forms, the possibility existed that antagonistic effects would be observed upon co-administration. In vitro drug combination studies were performed to determine if the inhibition of HBV or HIV is adversely affected when ETV is combined with HIV NRTIs using concentrations that meet or exceed the maximum concentration found in the blood of patients treated with these drugs. Antiviral activity against HBV was measured in a human hepatoma cell line (HepG2) transfected with plasmid DNA that drives the expression of a terminally redundant HBV pregenomic RNA. The production of extracellular HBV DNA was measured five days after transfection by dot blot hybridization. In the HBV replication assays, the antiviral activity of ETV was not affected by the presence of the HIV NRTIs abacavir, didanosine, stavudine, or zidovudine, at either the C_{max} or 5X C_{max} for each HIV NRTI (Table 4; Report 930002741, page 13).

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table 4. The Anti-HBV activity of ETV in the presence of HIV NRTIs

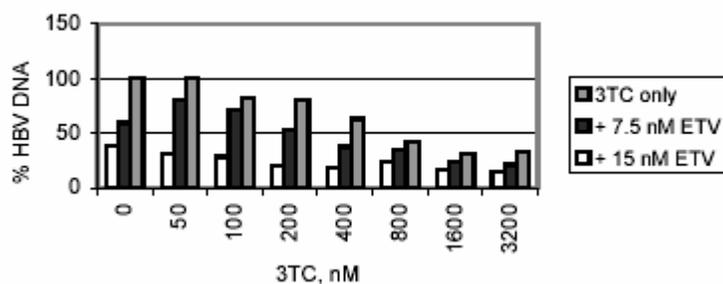
NRTI ^b	ETV EC ₅₀ ^a	ETV EC ₅₀ NRTI ^a	
		1X C _{max}	5X C _{max}
ABV	14.4 +/- 4.8	12.5 +/- 5.9	7.4 +/- 0.9
AZT	13.8 +/- 2.5	17.3 +/- 8.2	15.4 +/- 8.1
ddI	12.0 +/- 0.4	11.1 +/- 1.2	12.7 +/- 1.4
d4T	17.9 +/- 6.0	17.9 +/- 1.8	19.0 +/- 3.9

^a Average of 2 independent transfections, each in triplicate; assay measurements vary 2 - 3 fold.

^b EC₅₀ values were determined for ETV alone, ETV + 1X C_{max}, and ETV + 5X C_{max} for each NRTI tested.

LAM and TFV have antiviral activity against both HBV and HIV-1. The antiviral efficacy of a single concentration of ETV was measured in the presence of increasing concentrations of LAM and TFV to determine whether co-administration of either compound could cause a reduction in the inhibition of HBV replication. Dose-response measurements were carried out in the presence of 7.5 and 15 nM ETV, concentrations that yielded an average of 27% and 54% inhibition of HBV replication, respectively. The introduction of increasing concentrations of either LAM (Fig. 2; Report 930002741, page 14) or TFV (Fig. 3; Report 930002741, page 14) did not affect the inhibition of HBV replication by ETV. At concentrations of the HIV NRTIs greater than the IC₅₀ values, the dose-response relationship in the presence of ETV paralleled that seen in the absence of ETV. These results demonstrated that suboptimal concentrations of LAM and TFV had no impact on the antiviral efficacy of ETV while higher concentrations augmented the inhibition of HBV replication.

Figure 2. The Anti-HBV Activity of LAM with ETV



HBV replication was measured in HepG2 cells transfected with pCMVHBV. Transfected cells were treated with LAM only at the concentrations indicated, LAM + 7.5 nM ETV, or LAM + 15 nM ETV. Values represent the level of extracellular HBV DNA as a percentage of untreated controls.

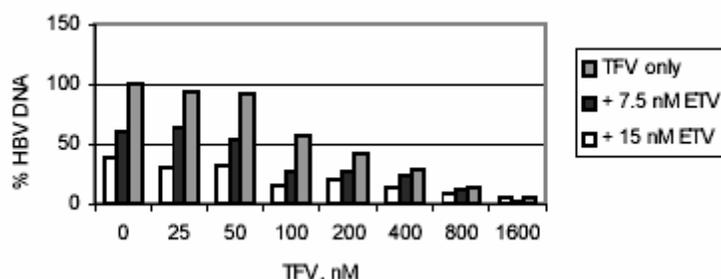
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Figure 3. The Anti-HBV Activity of TFV with ETV



HBV replication was measured in HepG2 cells transfected with pCMVHBV. Transfected cells were treated with TFV only at the concentrations indicated, TFV + 7.5 nM ETV, or TFV + 15 nM ETV. Values represent the level of extracellular HBV DNA as a percentage of untreated controls.

The anti-HIV activity of the combination of NRTIs with ETV was measured using cells infected with three different HIV strains (RF, LAI, NL4-3) using a reverse transcriptase assay. ETV does not exhibit antiviral activity against HIV, so to determine the effect of ETV co-administration on the activity of HIV NRTIs, IC₅₀ values were determined for each of the six HIV NRTIs in the absence of ETV and in the presence of 25 nM (0.8X C_{max}) and 125 nM (4.2X C_{max}) ETV. The IC₅₀ values of the HIV NRTIs in the presence of ETV at up to 4X the C_{max} of the 1 milligram dose were within 2-fold (assay variation is 2- to 4-fold) of the IC₅₀ values for the NRTIs in the absence of ETV (Table 5; Report 930002741, page 15) indicating that ETV had no inhibitory effect on the anti-HIV activity of any of the six HIV NRTIs tested. These results indicate that co-administration of ETV with HIV NRTIs will not reduce the antiviral activity against either HBV or HIV.

Table 5. The Anti-HIV Activity of NRTIs in Combination with ETV

HIV-1 Strain	ETV		NRTI EC ₅₀ (nM) ^a				
	(nM)	d4T	DDI	AZT	3TC	ABV	TFV
RF	0	80 ± 29	660 ± 379	0.86 ± 0.39	31.0 ± 14.5	520 ± 170	NA
	25	84 ± 29	970 ± 210	0.64 ± 0.38	28.4 ± 7.9	500 ± 180	NA
	125	130 ± 4	920 ± 270	0.98 ± 0.46	21.0 ± 10.4	770 ± 320	NA
LAI	0	920 ± 90	2010 ± 230	9.06 ± 2.54	112 ± 2	480 ± 10	18.0 ± 2.0
	25	840 ± 70	2610 ± 110	10.7 ± 1.5	209 ± 108	690 ± 70	17.0 ± 4/0
	125	820 ± 30	1950 ± 200	8.48 ± 1.50	241 ± 17	730 ± 20	28.0 ± 18.0
NL4-3	0	1440 ± 410	5150 ± 1700	10.2 ± 3.6	233 ± 120	2420 ± 200	60.0 ± 6.0
	25	1410 ± 620	6470 ± 1360	15.6 ± 8.9	594 ± 170	2720 ± 40	68.0 ± 21.0
	125	1450 ± 400	5850 ± 1590	23.5 ± 16.5	656 ± 352	2780 ± 1150	48.0 ± 5.0

^a Average of 2 independent determinations, each in triplicate. Assay measurements vary 2 - 4 fold. NA = not available.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Resistance/Cross-Resistance

HBV RT substitutions rtM204V/I, rtL180M, rtL80V/I and rtV173L have been found in breakthrough virus in patients chronically infected with HBV who were treated with LAM. Cell culture studies have shown that viruses with LAM-resistant substitutions have approximately 5- to 30-fold cross resistance to ETV in cell culture studies. An HBV expression clone containing the HBV genotype D serotype *ayw* genome was used to create the pCMV-HBV 180B3 reference HBV WT clone and serve as the backbone for all RT recombinants in the transient phenotype assay. The RT domain was derived by engineering the specific substitution by site-directed mutagenesis into the laboratory reference sequence or by cloning it in its entirety from clinical isolates enrolled in ETV clinical trials. The ETV susceptibility of laboratory and clinical isolate clones with the rtL180M and rtM204V LAM-resistance substitutions decreased 8-fold (Table 6; Report 930007377, page 20). In a laboratory RT background, the addition of rtT184G to the rtL180M and rtM204V substitutions decreased ETV susceptibility 38-fold, while the same substitutions decreased susceptibility 73-fold in a clinical isolate background. Susceptibility to ETV was significantly decreased by >200-fold in clones that contained the substitution rtM250V in addition to rtL180M and rtM204V substitutions in both lab and clinical isolates. Moreover, the addition of substitutions rtT184G and rtS202I to rtL180M and rtM204V in laboratory and clinical isolates resulted in substantial decreases of >2000-fold in ETV susceptibility.

Lab clones engineered to contain the ADV resistance substitutions rtA181V or rtN236T were tested for ETV cross-resistance in the phenotypic assay. The rtN236T or rtA181V substitutions resulted in a 2.6- and 1.4-fold decrease in the ADV IC₅₀ values, respectively and remained susceptible to ETV (0.3- and 1.1-fold change over WT, respectively).

The genotype of clinical isolates affects their phenotypic susceptibility to ETV (Fig. 4; Report 930007377, page 28). The average WT ETV susceptibility index was 1 (median 0.8). The average phenotypic susceptibility decreased 22-fold with a median decrease of 18-fold for populations with lamivudine-resistant genotypes and 611-fold with a median decrease of 83-fold for populations with ETV-resistance genotypes.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table 6. Phenotypic Susceptibility of WT, LAM-resistant and ETV-resistant HBV

RT Genotype	RT Backbone	ETV EC ₅₀ (nM)	Std. Dev.	Fold Change ^a	n ^b
WT ^c	Lab	5.4	2.7	-	81
rtL180M, rtM204V (LVD ^k)	Lab	41	22	8	51
rtL180M, rtM204V, rtT184G (ETV ^h)	Lab	206	118	38	7
rtL180M, rtM204V, rtM250V ^d (ETV ^h)	Lab	1,306	628	242	4
rtL180M, rtM204V, rtT184G, rtS202I (ETV ^h)	Lab	>15,000	-	>2,778	4
WT ^e	Clinical Isolate	4.3	2.7	-	16
rtL180M, rtM204V ^f (LVD ^k)	Clinical Isolate	34	27	8	17
rtL180M, rtM204V, rtT184G ^g (ETV ^h)	Clinical Isolate	315	194	73	22
rtL180M, rtM204V, rtM250V ^{d,h} (ETV ^h)	Clinical Isolate	2,873	2,776	668	12
rtL180M, rtM204V, rtT184G, rtS202I ⁱ (ETV ^h)	Clinical Isolate	>15,000	-	>3,488	12

Source: Notebooks # 52709, 53270, 55372, 55573, 55574, 55579, 55589, 56492, 57569, 58029, 58069, 58634, 58661, 58693, 59212, 59305, 59306, 59307, 59338, 59339, 59362, 59364, 59372, 59373.

^a Fold-change, ETV IC₅₀ value of test clone/ WT IC₅₀ value of either the lab or clinical isolate WT clone.

^b n, number of independent experiments.

^c WT plasmid clone was pCMV-HBV 180B3.10

^d One of three clinical isolate clones and the lab clone contained a rtV173L substitution that has no effect on LAM or ETV resistance.

^e Data averaged from 4 clones derived from subjects AI463015-5-2001 (genotype D), AI463901-20-6098 (genotype D), and AI463901-46-6196 (genotype B).

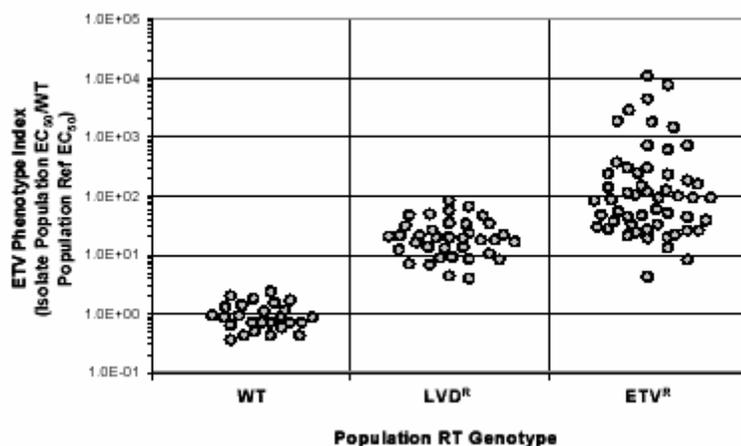
^f Data averaged from 5 clones derived from subjects AI463015-5-2001 (genotype D), AI463901-15-6001 (genotype A), AI463901-21-6015 (genotype C), AI463901-1-6206 (genotype C), and AI463901-18-6257 (genotype A).

^g Data averaged from 3 clones derived from subjects AI463015-5-2001 (genotype D) and AI463901-42-6215 (genotype B).

^h Data averaged from 3 clones derived from subjects AI463015-5-2001 (genotype D) and AI463901-21-6015 (genotype C).

ⁱ Data averaged from 2 clones derived from subject AI463015-5-2001 (genotype D).

Figure 4. ETV Phenotypic Susceptibility of WT, LAM- and ETV-Resistant Clinical Isolates.



WT isolates (n = 27) ; LAM-resistant isolates (LAM^R ; n = 37) ; ETV-resistant isolates (n = 56)

3. Clinical Studies

Safety

Adverse events (AEs) were reported frequently in the nucleoside-naïve patients although there were few differences in the pattern of AEs reported by ETV-treated patients compared to LAM-treated patients. On treatment AEs reported in > 5% of patients in either arm in the nucleoside-naïve studies included: headache, upper respiratory infection, nasopharyngitis, cough, pyrexia, abdominal pain, diarrhea, fatigue, arthralgia, dizziness, nausea, influenza, sore throat, rhinorrhea, dyspepsia, ALT increased, blood amylase increased, back pain, and myalgia. Most of the reported events were mild and not considered related to study treatment. The proportions of patients with reported AEs considered by the investigators to be possibly or probably related to blinded study drug were similar in the 2 treatment groups (ETV 37%, LAM 38%). The pattern of commonly reported AEs was very similar in the LAM-refractory patients. The number of patients who developed SAEs (death, hospitalization, cancer, congenital anomaly, life-threatening condition, or other medically significant event) while on study was small. Similarly, the number of patients discontinuing their assigned study drug because of an AE or SAE was low, 1% for ETV-treated patients and 4% for LAM-treated patients. The most commonly observed hematologic/coagulation abnormalities in the nucleoside-naïve patients were prolonged prothrombin time (PT) and an increased international normalization ratio (INR). There were few significant abnormalities in serum biochemical tests identified in either the nucleoside-naïve or LAM-refractory patient populations. Elevations of pancreatic enzymes, increased creatinine, and abnormalities in electrolytes occurred rarely and with similar prevalence across the treatment groups. The most commonly observed biochemical abnormalities were elevations in liver transaminases. There were a total of 15 deaths during treatment with study drugs during all of the reported ETV trials. In the nucleoside-naïve studies there were 6 deaths among the 1347 subjects (0.4%) while in the LAM-refractory studies there were 6 deaths in the 373 (2%) patients receiving study drug. Three additional deaths were reported from the Phase 2 supportive studies. None of the deaths were considered by the investigators to be related to study drugs but one death was thought to be related to withdrawal of study drug.

Evaluation of malignancies was of special interest during the review process because chronic HBV is known to be a strong risk factor for development of hepatocellular carcinoma (HCC) and because the results of the rodent carcinogenicity studies suggested that ETV might itself be a potential carcinogen. Early pre-clinical

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

studies using a woodchuck model suggested that administration of ETV to HBV-infected woodchucks might decrease the occurrence of HCC in animals that were maintained on the drug long-term.

As of the cut-off date reported in the NDA, a total of 27 malignancies had been identified in 26 patients (17 ETV patients and 9 LAM patients). No malignancies were diagnosed among the 108 patients who originally received placebo in early clinical trials. In addition, there were 5 patients (3 ETV and 2 LAM) who were reported to have lesions that were categorized as pre-malignant or unclassifiable.

The applicant calculated the rate of malignancies over time for patients receiving ETV or LAM in the clinical trials. They note that the overall rate of malignant neoplasms was 8.5 per 1000 patient years of observation for patients receiving ETV and 7.8 per 1000 patient years for patients receiving LAM. This compares to rates of 9.7 per 1000 patient years for all cancers in patients with chronic HBV and 3.8 per 1000 patients years in patients without evidence of HBV calculated from a U.S. cohort study commissioned by BMS. For HCC, the most commonly reported malignancy, the rate was 3.5 per 1000 patient years for ETV patients and 3.4 per 1000 patient years for LAM patients. These rates compare to rates of 4.6 per 1000 patient years in patients with chronic HBV and 0.02 per 1000 patient years in the non-HBV comparator group calculated from the U.S. cohort stud. Addition of the new cases reported in the IND Safety Update brings the total number of patients with identified malignancies in the ETV development program to 37. Of these patients 28 were in the randomized populations: 19/1497 ETV patients (1.3%) and 9/899 LAM patients (1%). Nine patients were in special study populations (decompensated, HIV/HBV co-infected, or receiving dual therapy): 3 receiving ETV alone, 2 receiving ADV alone, and 4 receiving combination therapy with ETV+LAM. Please refer to the clinical review by Medical Officer Linda Lewis for a detailed analysis of the safety of ETV.

Efficacy

The primary efficacy endpoints for the Phase 3 studies were based on the change in liver histology over the initial 48 week study period when patients received blinded study drug. Histologic improvement was defined as ≥ 2 point decrease in the Knodell necroinflammatory score with no worsening in the fibrosis score. All liver biopsy specimens were evaluated by a single reader who was blinded to treatment group and biopsy order and assigned the histologic scores by both the Knodell and Ishak criteria. The FDA statistical analysis confirmed the applicant's analysis of the primary efficacy endpoint demonstrating that patients receiving ETV experienced superior overall histologic improvement compared to LAM in all 3 studies (P values ≤ 0.02 in all studies).

Treatment effects of ETV compared to LAM were also assessed for a number of secondary endpoints using virologic, serologic and biochemical measurements. Although the Phase 3 studies were originally designed using the HBV bDNA assay for treatment management decisions, discussions with the FDA prior to submitting the NDA identified the HBV PCR assay as the assay that provided better discrimination in virologic endpoints. FDA analyses confirmed the applicant's results for the proportion of patients with HBV DNA below LLOQ by PCR at Week 48, change from baseline in HBV DNA by PCR at Week 48, ALT normalization, and HBeAg seroconversion (Table 7; Linda Lewis' Medical Officer review).

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table 7. Virologic, Serologic, and Biochemical Endpoints at Week 48 in Studies 022, 027, and 026

	Study 022		Study 027		Study 026	
	ETV 0.5 mg	LAM 100 mg	ETV 0.5 mg	LAM 100 mg	ETV 1 mg	LAM 100 mg
HBV DNA PCR < LLOQ*	72% [#]	42%	95% [#]	77%	22% [#]	1%
Log ₁₀ HBV DNA by PCR – mean change from baseline*	-7.0 [#]	-5.5	-5.2 [#]	-4.7	-5.1 [#]	-0.5
HBeAg seroconversion	21%	18%	NA	NA	8%	3%
ALT Normalization (< 1 x ULN)	69%	61%	78%	71%	65%	17%

NA: not applicable

*LLOQ: Lower limit of quantification calculated as < 400 copies/mL. Values reported as < 400 assigned value of 399 copies/mL.

[#]ETV significantly better than LAM, all P values < 0.01.

The efficacy of ETV was examined in both nucleoside treatment-naïve and LAM-experienced patient populations. In nucleoside treatment-naïve studies 022 (HBeAg positive subjects) and 027 (HBeAg negative subjects), 83% (541/653) of patients on 0.5 mg QD ETV treatment were suppressed with HBV DNA < 400 copies/mL as quantified by the COBAS Amplicor HBV Monitor PCR assay at week 48 compared to 59% (363/619) of patients on 100 mg QD LAM treatment.

Clinical studies 014 and 026 examined the efficacy of 1 mg QD ETV compared to 100 mg QD LAM in patients with LAM-refractory HBV with prior LAM experience. In these two studies, 21% (36/174) of patients on ETV were suppressed to below 400 copies/mL HBV DNA as quantified by the COBAS Amplicor HBV Monitor PCR assay at week 48 compared to 1% of patients on LAM. Please refer to the reviews by Medical Officer Linda Lewis and Statistician Tom Hammerstrom for a detailed analysis of the efficacy of ETV.

4. Clinical Microbiology

Clinical Resistance Analyses

LAMIVUDINE-REFRACTORY TRIALS

Resistance data in patients with LAM-refractory HBV from pivotal studies 014 (n = 181) and 026 (n = 286) and supplemental study 015 (n = 9) were submitted in this NDA application.

Study 014

Study 014 was designed to determine the antiviral activity and safety of three doses of ETV (0.1, 0.5 and 1.0 mg) in patients with LAM-refractory HBV with a primary endpoint of proportion of subjects who achieved HBV DNA below the lower limit of quantification (LLOQ = 0.7 MEq/mL) by the bDNA assay at week 24. HBV DNA levels were also measured by the more sensitive Cobas Amplicor PCR assay (LLOQ = 300 copies/mL) over 48 weeks. Genotypic analysis monitored the maintenance of LAM-resistance substitutions

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

and the emergence of ETV-associated substitutions. At study entry, 157 (87%) of the 181 patients had evidence of LAM-resistance mutations rtM204V or I and/or rtL180M or mixtures at these sites. The LAM-resistant mutations were maintained on ETV therapy with >90% of genotyped patient isolates still containing LAM-resistant substitutions at 24 and 48 weeks. No patients in 1 mg ETV arm experienced viral rebound up to 48 weeks ($\geq 1 \log_{10}$ rise in viral DNA levels while on therapy), compared to 5 in the LAM arm.

Baseline Analysis

Ten patient isolates in the 1.0 mg ETV (n = 5) and 100 mg LAM (n = 6) arms had no detectable LAM-resistance substitutions at baseline. Three of these patient isolates (37-06068, 37-06114 and 37-06086) in the 100 mg LAM arm developed both L180M and M204V substitutions on treatment by week 24. Two patient isolates from the 1.0 mg ETV arm, (30-06156 and 37-06115; only 30-06156 in the database) developed mixtures at amino acids rtL180 and rtM204 by week 24 and mutations rtL180M and rtM204I by week 48. Viral loads of these two patients on 1.0 mg ETV were suppressed below quantification levels (PCR < 300 copies/mL and bDNA = 0.699) by week 48. The applicant argues that since enrollment into this study was contingent on patients failing prior LAM therapy, the patients without detectable LAM-resistant substitutions at baseline may have harbored these substitutions below the 25% limit of detection. Regardless, it is apparent that ETV inhibited and could suppress some viruses to below detection limits even with the outgrowth of LAM-resistant virus.

Thirty-six isolates in the 1 mg ETV arm of study 014 had detectable LAM-resistant mutations at baseline. Twenty-two percent (8/36) of the isolates were suppressed to below 300 copies/mL as measured by the Cobas Amplicor PCR assay and 64% (23/36) were suppressed to ≤ 0.699 by the bDNA assay. In comparison, of the 42 isolates in the LAM arm which had detectable LAM-resistant mutations at baseline, 0% (0/42) of the isolates were suppressed to below 300 copies/mL as measured by the PCR assay and 5% (2/42) were suppressed to ≤ 0.699 by the bDNA assay.

ETV-Treatment Emergent Substitutions in HBV RT

When paired baseline and on-treatment samples were compared, amino acid substitutions emerged in the HBV of 7 patients (30-06143, 30-06156, 37-06113, 42-06194, 50-06028, 50-06180, 37-06115) on 1 mg ETV by week 48 (Appendix 1). The LAM-resistance substitutions rtL80V, rtL180M, rtM204V or I arising in the context of mixtures at these sites at baseline and other LAM-resistant mutations at baseline were found in the viruses of all seven patients. In addition, the substitution rtL229L/W emerged on 1 mg ETV treatment in the week 24 isolate of patient 30-06143 together with the rtL180M and rtM204V substitutions arising from mixtures at these sites at baseline. Four of the seven patients were suppressed with viral loads below detection (300 copies/mL) and the other three experienced $> 2 \log_{10}$ reductions in viral load at the time the isolate developed the LAM-resistant mutations. These 3 patients experienced further reductions in viral load after the emergence of these substitutions. The reductions in viral load that coincide with the emergence of substitutions rtL180M, rtM204V or I, rtL80V or I, and rtL229W/L suggest

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

that these substitutions do not contribute significantly to reduced ETV susceptibility. The applicant indicated in their virology report that the week 24 isolate from patient 50-6082 developed the conserved site substitution rtL157L/M and the week 48 isolate from patient 37-6115 developed the rtR110G substitution. The dataset submitted with the NDA contained no genotypic information for these isolates. The development of rtL157L/M and rtR110G in these patients did not appear to reduce ETV susceptibility or affect viral load reduction.

The Emergence of T184 Substitutions Coincide with Viral Load Rebounds

Substitutions at rtT184 developed on 1 mg ETV therapy in 5 patients (1-06206, 9-06130, 30-06095, 50-06028, 50-06249) after week 48. The development of substitutions at rtT184 coincided with rebounds in viral load. Substitutions at rtT184 arose prior to week 48 in one patient (29-6016) on 0.1 mg ETV suggesting a lower resistance barrier and supporting the use of the higher dose of 1 mg ETV in the LAM-experienced HBV-infected patient population. Additional substitutions that developed on 1 mg ETV treatment after week 48 included substitutions at rtV207I and rtL229V.

Study 026

Study 026 (n = 286) was a double-blind comparative study of the efficacy of entecavir (ETV) 1.0 mg once daily (QD), compared with lamivudine (LAM) 100 mg, in subjects with chronic hepatitis B virus (HBV) infection who had previously demonstrated an incomplete response to LAM and were viremic while on LAM. There were co-primary endpoints at week 48: histologic improvement and proportion of patients with both undetectable HBV DNA by bDNA assay (<0.7 MEq/mL) and normalization of ALT (<1.25 X ULN). Genotypic and phenotypic analyses of paired clinical isolates obtained at study entry and Week 48 were performed to monitor baseline and emerging amino acid substitutions in the HBV reverse transcriptase and to determine their impact on virologic response to ETV. All treated subjects with available paired baseline and on treatment samples available were evaluated from both the ETV and LAM study arms. In addition, isolates from all subjects displaying virologic rebound, even beyond 48 weeks of therapy, were evaluated.

Study 026 Baseline Mutations

LAM-resistant substitutions at rtL180M and rtM204V/I in the RT domain were detected by population sequence analysis in 84% (231/276) of baseline isolates from both the ETV and LAM arms [82% (114/139) in the ETV arm and 85% (117/137) in the LAM arm]. The LAM-resistant substitutions were maintained during the study, presumably because of the selective advantage in the presence of LAM and ETV

Study 026 Mutations Emerging on ETV Treatment

The substitution rtT184S/A/I developed in 5 patient isolates in the ETV arm and 1 in the LAM arm by week 48 in the context of lamivudine-resistance mutations rtL180M and rtM204V or I (Table 8). The mutation rtS202G or rtS202G/S developed in 6 patient isolates in the context of lamivudine-resistance mutations rtL180M and rtM204V or I in the ETV arm. No changes developed at rtS202 in the LAM arm, but three isolates had the rtS202G/S

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

substitution at baseline in this arm. One patient isolate in the ETV arm had the rtM250M/L substitution develop on treatment in the context of the M204I/M mutation. In total, eleven patient isolates developed the ETV-resistant mutations rtT184S/A/I, rtS202G/S, or rtM250L/M by week 48 out of 139 (8%) isolates in the ETV arm all in the context of lamivudine-resistant mutations rtM204V or I and rtL180M. Five of these 11 (45%) patients did not achieve ≤ 0.7 MEq/mL by the bDNA assay and were virologic failures. Interestingly, 5/6 of the responders had the rtM204I mutation or a mixture of rtM204I/V instead of the rtM204V mutation.

Table 8. Mutations Developing on 1.0 mg ETV Treatment in Study 026

SUBJ	PID	TRT	WK	PCR	bDNA	HBV polymerase Mutations*	<0.7	ETV BL	ETV ref
80256	AI463026-73-80256	ETV 1.0 mg	48	8700000	5.1	L80I/L L180M T184S/A M204V L229L/V C332S	F	19	58
80069	AI463026-52-80069	ETV 1.0 mg	48	3.81E+08	85	L180M T184T/A S202S/G M204V L229L/V	F	18	753
80204	AI463026-76-80204	ETV 1.0 mg	48	77300	0.699999	L80I/L L180L/M T184T/I M204I	R	1.2	24
80252	AI463026-12-80252	ETV 1.0 mg	47	2530	0.699999	L80I/L R110G/R I169L/I L180L/M A181S/A T184T/I M204I I269I/L	R	2.5	23
80393	AI463026-67-80393	ETV 1.0 mg	49	20500	0.699999	L80I/L L180M T184T/S M204I/V	R	2	25
80016	AI463026-36-80016	ETV 1.0 mg	47	199	0.70	M204I/V L229L/F M250M/L C332S	R	.2	4.2
80041	AI463026-131-80041	ETV 1.0 mg	46	5190000	4.20	L180M S202G M204V	F	18	343
80113	AI463026-98-80113	ETV 1.0 mg	50	116000	0.70	L80L/V V173V/L L180M S202S/G M204I/V	R	1.1	12
80130	AI463026-135-80130	ETV 1.0 mg	48	9000000	6.20	L80V/L L180M S202S/G M204V	F	1	20
80174	AI463026-135-80174	ETV 1.0 mg	48	104000	0.70	L180M S202S/G M204V L229L/M C332S	R	2.9	121
80194	AI463026-15-80194	ETV 1.0 mg	49	1.42E+09	495.70	L180M S202S/G M204V M250M/L C332S	F	11	2139
80249	AI463026-138-80249	ETV 1.0 mg	49	992	0.7	I169M/I A181T/A M204I M250I/M	R	2.3	38

*Mutations that Develop on Treatment are bolded.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Four subjects (80194, 80069, 80256 and 80041) who were viral DNA failures (HBV DNA > 0.7 mEq/mL at week 48) had a >3-fold reduction in ETV susceptibility from baseline ranging from 11- to 19-fold. Patient isolate 80130 was a virologic failure with 9×10^6 copies/mL HBV DNA by PCR and 6.2 mEq/mL by bDNA at week 48, but had only a 1-fold change in ETV susceptibility from baseline. The remaining 6 patient isolates were virologic responders achieving < 0.7 MEq/mL by bDNA assay and had < 3-fold changes in ETV susceptibility from baseline. The median fold change in ETV susceptibility from reference for the 5 ETV failure isolates that developed ETV-resistance substitutions was 343 (range 20-2139) compared to 24 (range 4.2 – 121) for the 7 responder isolates. The median fold change from baseline in ETV susceptibility of the ETV failure isolates that developed ETV-resistance substitutions was 18 compared to 1.6 for responder isolates. Overall, from the phenotypic data available in this application, the median fold change in ETV susceptibility from reference was 48 for ETV isolates that developed ETV resistance substitutions in studies 015 and 026 (n = 14).

Data after 48 weeks will be needed to determine the effect of the emergence of the ETV-associated substitutions on long term response, since the HBV DNA data suggests that several of these subjects have viral loads that are no longer declining or are increasing at week 48 (Fig. 5; Report 930007365, page 29). Analysis of individual clones of the isolates that had two substitutions at rtT184 and rtS202 or at rtM250 and rtS202 showed that the substitutions were not genetically linked and represented two distinct virus populations. There was no apparent correlation between clade and the development of substitutions at sites rtT184, rtM250, or rtS202. The isolates that developed these substitutions were from clades A, B, C, D and F.

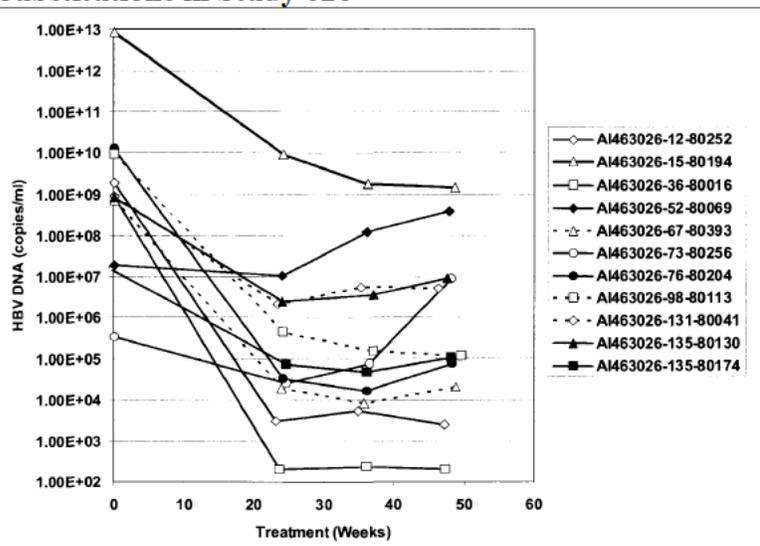
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Figure 5. HBV DNA Levels of ETV-Treated Patients with Emerging ETV-Resistance Substitutions in Study 026



Cross-resistance

In study 026, all isolates with emerging ETV-resistance substitutions showed maximum 4-fold changes (range 0.6 to 4.3; median 2.2) in ADV susceptibility relative to WT reference but were highly resistance to lamivudine (IC₅₀ values >100 μM). Clinical isolates containing lamivudine-resistant substitutions showed a median decrease of 20-fold in ETV susceptibility, which is consistent with previous data.

Study 015

Study 015 examined the antiviral activity of open label ETV 1 mg QD in OLT (orthotopic liver transplant) recipients who were > 100 days post-transplant, receiving a stable regimen of immunosuppressive agents and had recurrent HBV infection (both HBeAg positive and negative) despite prophylaxis with anti-HBV agents (i.e. HBIg, LAM, FCV, ADV, emtricitabine, ganciclovir). Nine subjects were treated with ETV and had serum samples collected for analysis. There was a mix-up in samples from patient 2010 and so results from this patient have been omitted. The genotypic and viral load changes during therapy were monitored for eight subjects (Table 9).

Table 9. Emerging ETV Genotypic and Phenotypic Resistance in Study 015

PID	Week	HBV DNA (c/mL)	BL mutations	HBV polymerase Mutations Developing on TRT	ETV fold-change from BL	ETV fold-change from ref
AI463015-4-2002	0	5X10 ⁹	L180M/I, M204M/V		.	33
	100	500000		I169S/I L180M	21	679

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

				M204V S202S/G N238N/H V207I/V		
AI463015-4-2003	0	280000000 0	M204I		.	21
	79	800000		L180M/L M204I/V	.	.
	150	510000000		L180M M204V T184T/F/L/A S202S/G N238N/H	11	219
AI463015-5-2001	0	240000000	L180M M204V T184T/S		.	32
	48	300000			2.6	85
	68	1200000		T184G S202I N238N/D	217	6971
	92	500000000 1		I169T/I	311	10022
AI463015-15-2004	0	500000000 1	L180M M204V C332R/S		.	.
	48	500			.	.
	131	99			.	.
AI463015-15-2005	0	350000000	L180M/V M204I/V C332S		.	52
	47	500000			0.6	33
	127	5300		S202S/G	0.5	25
AI463015-15-2006	0	380000000	L180M M204V		.	31
	47	850000			0.7	22
	110	270000		I169T/I M250M/V	3.4	106
	129	6700000		S202S/G	54	1683
AI463015-15-2008	0	340000000	L180M M204I		.	8.2
	108	79000		T184T/S S202S/G M204I/V N238H/N	83	676
	124	40000000		M204V S202G	211	1717
AI463015-16-2007	0	430000000 0	L180M M204V		.	29
	36	6400000		T184T/A	3.2	91
	95	2X10 ⁹			5.9	168

Virologic rebound in six patients in study 015 occurred subsequent to the detection of genotypic ETV resistance. One subject (2007) rebounded in the first year of therapy, one rebounded in the second year of treatment (2001), four rebounded in the third year (2002, 2003, 2006, 2008) and two maintained HBV DNA suppression with no rebound out to 127 and 131 weeks of therapy (2004, 2005). Seven of the eight patients showed the development of ETV-resistance substitutions at rtS202G or I (n=5), rtT184 (n=4) or rtM250V (n=1). These substitutions were linked to LAM-resistant changes rtL180M and rtM204V.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

There was a correlation between virologic rebound and decreased ETV susceptibility in vitro with IC₅₀ values \geq 100 nM. All these isolates retained adefovir susceptibility.

META-ANALYSIS

Resistance datasets from studies 014, 015 and 026 in lamivudine-experienced patients were combined for a meta-analysis. Failures (n = 188) and responders (n = 431) were separated based upon a viral load cutoff of 1000 copies/ml using the last available patient sample (usually 48 weeks). The 1000 copies/ml cutoff was chosen because this level was previously used for the resistance analysis in the adefovir NDA. Amino acid substitutions occurring between baseline and failure at each reverse transcriptase position were quantified using MS Excel and the macro "JulesMacro2.xls!Macro1." Specific substitutions that occurred 2 times or more were further analyzed. Candidate resistance-associated positions were further screened for polymorphisms. Polymorphisms were not considered, with one exception, as these could result from linkage to another resistance mutation.

Twelve subjects were identified with an rtS202G/I amino acid substitution in a background of LAM resistance-associated substitutions at rtM204I/V and other positions (AI463015-4-2002, AI463015-4-2003, AI463015-5-2001, AI463015-15-2005, AI463015-15-2006, AI463015-15-2008, AI3026-15-80194, AI3026-52-80069, AI463026-98-80113, AI463026-131-80041, AI463026-135-80130, and AI463026-135-80174). Subjects AI463014-37-6113, AI463014-50-6028, AI463015-4-2002, AI463015-5-2001, AI463015-15-2006, and AI463026-12-80252 were found to have an rtI169T/S/L substitution in a LAM resistance-associated background. Likewise, rtT184L in a LAM-resistance background was associated with failure as found in subjects AI463014-1-6206, AI463014-9-6130, and AI463015-4-2003. The polymorphism rtT184S occurring at a entecavir resistance-associated position may confer reduced susceptibility to entecavir as observed in subjects #AI463014-49-6027, AI463015-5-2001, and AI463026-24-80256.

Through this meta-analysis, it was revealed that substitutions at rtI169 developed in 7 isolates on 1 mg ETV treatment in the context of the LAM-resistant mutations rtL180M and rtM204V or I and ETV-associated substitutions at rtT184, rtM202 and rtM250 (Table 10). The rtI169 substitutions were not highlighted by the applicant. Therefore, data examining the effect of this substitution on ETV susceptibility in phenotypic testing of site-directed clones is not currently available. Since the rtI169 substitutions occur in the context of LAM and ETV-associated resistance substitutions, it is difficult to determine the contribution of the rtI169 substitutions to decreased ETV susceptibility from the limited clinical data from the 7 patient isolates where the substitution developed on ETV treatment. One isolate 26-53-80228 in the 100 mg LAM arm also developed the rtI169M/I substitution.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Table 10. Isolates with an Emergent Substitution at I169

PID	TRT	Week	HBV DNA (c/mL)	BL mutations In HBV polymerase	HBV polymerase Mutations Developing on TRT	ETV fold-change from BL	ETV fold-change from ref
14-37-6113	ETV 1 mg	195	3100000	L180M/L M204M/V	L180M M204V I169I/T T184X S219A/S	.	.
14-50-6028	ETV 1 mg	102	3310000	L80I/L L180M M204V/I L217R/L V253I/V Q271H/Q C332C/S	M204V I169I/T T184X L217R V253I Q271H C332S	.	.
15-4-2002	ETV 1 mg	151	9.5 X10 ⁸	L180M/I, M204M/V	I169S/I L180M M204V S202S/G N238N/H V207I/V	17	556
15-5-2001	ETV 1 mg	92	5X10 ⁹	L180M M204V T184T/S	T184G S202I N238N/D I169T/I	311	10022
15-15-2006	ETV 1 mg	110	270000	L180M M204V	I169T/I M250M/V	3.4	106
26-12-80252	ETV 1 mg	47	2530	L80I L180M M204I	L80I/L R110G/R I169I/L L180M/L A181S/A T184I/T I269I/L	2.5	23
26-138-80249	ETV 1 mg	49	992	V84V/M A181A/T M204I M250I/M	I169I/M	2.3	38
26-53-80228	LAM 100mg	48	1.51X10 ⁹	L180M/L M204V/M	I169I/M L180M M204V L229L/V	.	.

TREATMENT-NAÏVE TRIALS

Resistance data from pivotal studies 022 (n = 709) and 027 (n = 638) in nucleoside treatment-naïve patients were submitted in this NDA application.

Study 022

Study AI463022 was a double-blind comparison of once daily 0.5 mg ETV versus 100 mg lamivudine in the treatment of nucleoside treatment-naïve, HBeAg positive subjects with chronic HBV infection. Genotyping of paired samples (223 baseline samples and 138 week 48 samples) from 223 randomly selected subjects in the ETV arm was performed to monitor for the appearance of emergent changes within the HBV polymerase RT domain. Isolates with novel substitutions were cloned and assayed for phenotypic resistance. Samples from

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

all subjects experiencing virologic rebound were analyzed for genotypic and phenotypic changes. Only two subjects from this study exhibited a virologic rebound while on ETV (0.56%, 2/354) with no evidence of emerging substitutions or phenotypic resistance to ETV while on therapy. In contrast, 21 subjects (6% [21/355]) experienced a virologic rebound by week 48 while on lamivudine therapy. An additional 34 subjects on the lamivudine arm experienced virologic rebound during the extended blinded dosing period beyond week 52. Isolates from 46 of these 55 (84%) subjects had detectable lamivudine-resistance substitutions (rtL80, rtL180, rtM204) preceding virologic failure.

No ETV-resistant substitutions (rtT184S/A/I, rtS202G, rtM250L) emerged in any isolate on ETV therapy by 48 weeks in study 022. The two subjects who experienced virologic rebound on ETV treatment (22-191-10441 and 22-208-10912) had no detectable amino acid changes emerge on treatment and no change in phenotypic susceptibility to ETV, ADV or LAM. Novel emerging RT substitutions at conserved and polymorphic sites appeared by week 48 in 32 patients in the ETV arm, but none of these substitutions was associated with virologic rebound or decreased ETV susceptibility. In isolates from four ETV-treated patients, LAM-resistant substitutions were detected at week 48 and not at baseline by population-based sequence analysis. The emergence of these substitutions was not associated with virologic rebound. Using a more sensitive single nucleotide polymorphism (SNP) PCR assay, the baseline samples from these four patients were analyzed for lamivudine substitutions that may have been below the limit of the population-based sequence assay (25%) at baseline. The LAM-resistant substitution rtM204V or I were detected with the SNP PCR assay in the baseline samples from all four subjects. As noted above, the SNP PCR assay may detect mutations present at the background level due to the error rate of the viral polymerase.

Study 027

Study AI463027 was a double-blind comparison of once daily 0.5 mg ETV versus 100 mg LAM in the treatment of nucleoside treatment-naïve, HBeAg negative and HBeAb positive subjects with chronic HBV infection. Genotyping of paired samples from 211 randomly selected subjects in the ETV arm was performed to monitor for the appearance of emergent changes within the HBV polymerase RT domain. The polymerase regions of HBV isolates with novel substitutions were cloned into recombinant DNA clones and assayed for phenotypic resistance. Samples from all subjects experiencing virologic rebound were analyzed for genotypic and phenotypic changes. No subjects exhibited a virologic rebound while on ETV therapy in this study, while 8 subjects experienced a virologic rebound while on LAM therapy which all had detectable LAM-resistance substitutions (rtL80, rtL180, rtM204).

No ETV-resistant substitutions (rtT184S/A/I, rtS202G, rtM250L) emerged in any isolate on ETV therapy by 48 weeks in study 027. No subjects experienced virologic rebound on ETV treatment by week 48 in this study. Novel emerging RT substitutions at conserved and polymorphic sites appeared by week 48 in 18 patients in the ETV arm, but none of these substitutions was associated with virologic rebound or decreased ETV susceptibility. In

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

isolates from two ETV-treated patients, LAM-resistant substitutions (rtL80I, rtL180M, rtM204I) were detected at week 48 but not at baseline by population-based sequence analysis. Using the SNP PCR assay, the LAM-resistant substitution rtM204V or I were detected in the baseline samples from both subjects. Three subjects in the ETV arm had LAM-resistant mutations at baseline (one isolate had rtM204I, rtL180M/L, rtL80V and the other two isolates had rtL80V/I) but achieved <200 copies/mL by PCR assay at week 48.

BASELINE ANALYSES

Response by Baseline Genotype in Study 026

LAM-resistant substitutions at rtL180M and rtM204V/I in the RT domain were detected by population-based sequence analysis at baseline in 82% (114/139) in the ETV arm and 85% (117/137) in the LAM arm. Fifty-six to fifty-nine percent of the isolates that had the LAM-resistance substitutions rtL80I or V, rtM204 I or V, or rtL180M at week 48 were from patients who were ETV treatment responders as defined by achieving <0.7 MEq/mL by bDNA assay compared to 70% responders in the ETV arm overall (Table 11). Eighty-one percent of isolates with a mixture of rtM204I/V at week 48 were responders.

Table 11. Percentage of Isolates with Lamivudine-Resistance Substitutions that Responded to ETV Treatment

Substitution at Week 48	# Responders (%) <0.7 MEq/mL	n
Total	95 (70%)	135
L80I or V	27 (57%)	47
M204I	20 (56%)	36
M204V	27 (57%)	47
M204I/V	13 (81%)	16
L180M	47 (59%)	79

The ETV-resistance substitutions in the ETV-treated subjects at baseline in study 026 are shown in Table 12. In this study, substitutions rtT184S/A/I were present at baseline along with LAM-resistance substitutions at rtL80, rtV173, rtL180, and rtM204 in 3 patient isolates in the ETV arm (80251, 80122, 80245) and 9 isolates in the LAM arm. All three isolates in the ETV arm were responders by bDNA assay and all 9 isolates in the LAM arm were failures. Two patient isolates (80194, 80297) on ETV treatment did not achieve <0.7 mEq/mL HBV bDNA and contained LAM-associated resistance mutations and ETV-associated resistant substitutions rtM250M/L and/or rtS202S/G. However, two other isolates in the ETV arm (80282, 80249) contained substitution rtM250M/L at baseline and 2 (50%) responded to below 0.7 MEq/mL by bDNA assay. At this time, there is not enough data on baseline genotypic mutational patterns to predict a lack of response to ETV.

Table 12. ETV-Treated Subjects with ETV-Resistance Substitutions at Baseline in Study 026

PID	TRT	WK	PCR	bDNA	HBV polymerase Mutations*	≤0.7	ETV BL	ETV ref
------------	------------	-----------	------------	-------------	--------------------------------------	-------------	-------------------	--------------------

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

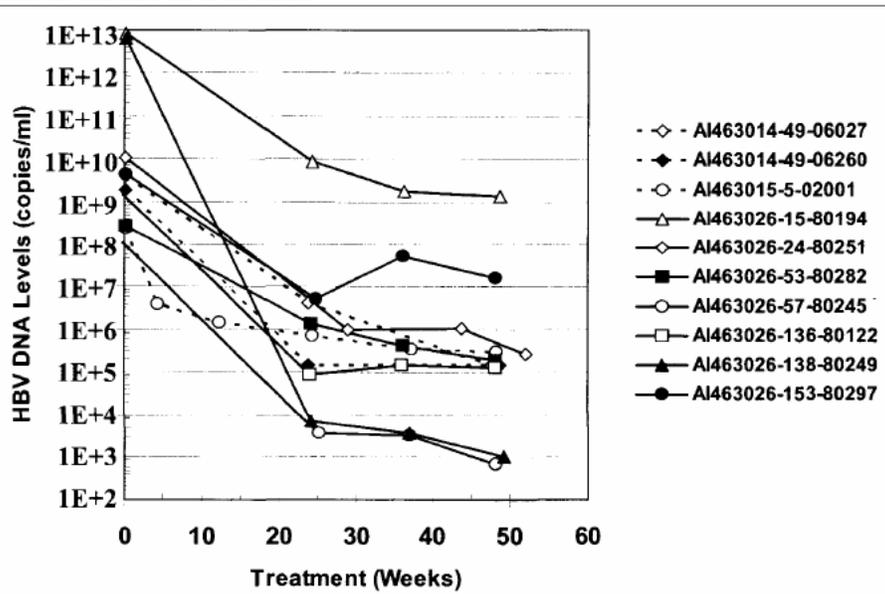
AI463026-15-80194	ETV 1.0 mg	49	1.42x10 ⁹	495.70	L180M S202S/G M204V M250M/L C332S	F	11	2139
AI463026-153-80297	ETV 1.0 mg	48	1.58x10 ⁷	12.1	V173V/L L180M/L M204V/I L229L/V M250M/L	F	1.3	36
AI463026-24-80251	ETV 1.0 mg	52	267000	0.699999	L80I/L V173V/L L180M T184S/T M204V	R	1.6	47
AI463026-53-80282	ETV 1.0 mg	48	189000	0.7	L80I/L L180L/M M204V/I L229L/F M250M/L	R	4.5	108
AI463026-136-80122	ETV 1.0 mg	48	128000	0.699999	L80L/V L180M A181A/V T184T/I M204V	R	4.3	30
AI463026-138-80249	ETV 1.0 mg	49	992	0.7	I169M/I A181T/A M204I M250I/M L80L/I Y124Y/H L180M T184S/T M204V L229L/V	R	2.3	38
AI463026-57-80245	ETV 1.0 mg	48	691	0.699999	C332C/R	R	.	.

*Mutations that Develop on Treatment are bolded.

The efficacy in the treatment-naïve clinical trials versus the lamivudine treatment-experienced trials shows that the presence of LAM-associated resistance mutations at baseline moderates the response to ETV treatment. In the treatment-naïve trials 022 and 027, 83% of ETV-treated subjects achieved viral DNA reductions to below 400 copies/mL by the Cobas Amplicor PCR assay at 48 weeks compared to only 54% for LAM-treated subjects. In contrast, in treatment-experienced studies 014 and 026, 21% of ETV-treated subjects achieved a reduction in HBV DNA levels to below 400 copies/mL by PCR assay by week 48 compared to 1% of subjects receiving LAM.

Ten patient isolates in the ETV arms and 13 in the LAM arms of studies 014, 015 and 026 had ETV-associated resistance substitutions at rtT184, rtS202, and/or rtM250 at baseline. Even with the presence of these mutations in ≥25% of their HBV at baseline, the patients responded to ETV treatment as shown in Fig. 6 (Report 930007375, page 30), although none of the patients achieved less than 300 copies/mL by PCR assay at week 48. Subject 80194 and subject 80297 showed a reduction in viral DNA to only 1.4x10⁹ and 1.6x10⁷, respectively, at week 48. Subject 80194 had a 194-fold change in ETV susceptibility from reference at baseline while subject 80297 had a 24-fold change. These results indicate that the presence of ETV-associated resistance mutations at baseline does not predict a lack of response to ETV treatment or the emergence of ETV resistance and viral rebound during 48 weeks of ETV treatment.

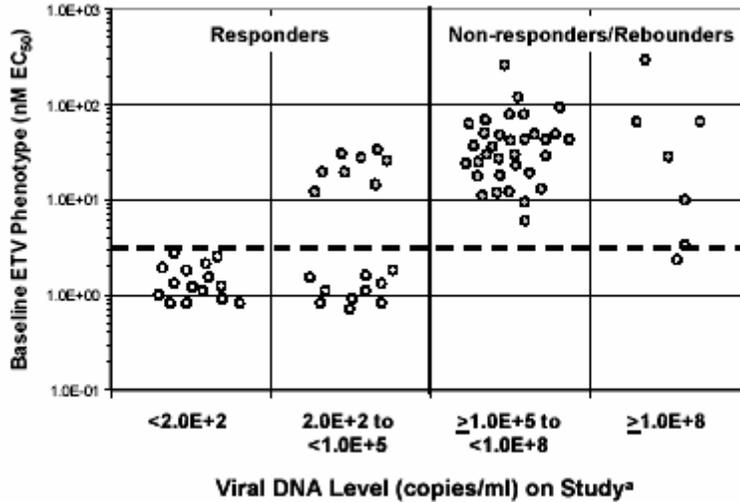
Figure 6. HBV DNA Levels of ETV-treated Subjects with ETV-Resistance Substitutions at Baseline



Response by Baseline Phenotypic

The correlation between in vitro phenotype and virologic response in experimental subjects was analyzed and submitted by the applicant. Baseline population phenotype susceptibility data were plotted against HBV DNA levels, measured between weeks 48 to 120 of ETV therapy (Figure 7; Report 930007375, page 41). The subjects chosen for this analysis included those having isolates with WT genotypes (primarily from study AI463022), isolates from subjects in various studies with baseline or emergent LAM- or ETV-resistance substitutions, and those with virologic rebound. Isolates from subjects with the greatest response to therapy (on-study viral DNA levels of <200 copies/ml) all had a baseline population susceptibility phenotype IC_{50} value of <3 nM (average = 1.4 nM) (Figure 7). Overall, 96% (25/26) of the subjects tested with baseline IC_{50} values of <3 nM had 1.0×10^5 copies/ml at Week 48, and all were genotypically WT. Of the subjects with on-study viral DNA levels between 200 and IC_{50} values of <3 nM. Overall, 76% of the ETV responders had baseline population phenotype IC_{50} values of <3 nM. In contrast, 98% of subjects with the least suppression in viral DNA levels (non-responders/rebounders; IC_{50} values of >3 nM. Subject baseline isolates with an IC_{50} value <3 nM were genotypically WT. In contrast, 98% (46/47) of subject baseline populations with phenotype IC_{50} values >3 nM had detectable LAM- and/or ETV-resistance substitutions. However, 34 subjects in studies AI463014 and AI463026 with LAM-resistant HBV showed reductions in HBV DNA levels to <200 copies/ml on ETV.

Figure 7. Correlation between Baseline Population Phenotype and Virologic Response



Additionally, the relationship between phenotypic susceptibility, genotype and virologic response was characterized using samples from subjects in study AI463015 who displayed a viral rebound while on ETV therapy. These data show correlation of HBV DNA levels, genotype, and population phenotype because subjects were studied longitudinally over an extended period of time. In six of the seven subjects (AI463015-5-2001, AI463015-4-2002, AI463015-4-2003, AI463015-15-2006, AI463015-16-2007, and AI463015-15-2008) an increase in the ETV IC₅₀ value was observed just prior to or coincident with an increase in viral DNA levels (rebound). In addition, isolates from the same six subjects displayed reduced ETV susceptibility just prior to or coincident with the detection of an ETV-resistant genotype. The only exception was subject AI463015-15-2005, who did not display a rebound in HBV DNA levels on therapy or a decrease in ETV susceptibility, despite an rtS202S/G ETV-resistant substitution detected at week 127.

From the 62 phenotypes from 25 patients submitted with the application, there was not enough data to determine a correlation between baseline phenotype and response. Consistent with the >100 phenotypic cutoff suggested by the applicant, patient 80194 from study 026 did not respond to ETV therapy by week 48 and had a baseline ETV phenotype of 193-fold from reference, while patient 2005 from study 015 achieved HBV suppression by week 48 with <0.7 mEq/mL HBV DNA by the bDNA assay and had a 52-fold change in ETV susceptibility from reference - the next highest baseline phenotype in the database. Both of these isolates had LAM-resistance mutations rtL80I, rtL180L/M, rtM204V/I at baseline. Isolate 80194 contained a rtM250M/L mixture at baseline and developed a rtS202G/S on treatment. Isolate 2005 also developed a rtS202G/S substitution on ETV treatment by week 127.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**MICROBIOLOGY DRAFT REVIEW****NDA:** 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05**Microbiology Reviewer:** Lisa K. Naeger, Ph.D.**Response to Treatment by HBV Subtype**

The distribution of HBV subtypes submitted in the resistance dataset is shown in Table 13. There were similar proportions of subtypes A, B, C, and D in the studies overall and between ETV and LAM arms. There were minimal isolates (<2%) that were subtypes E, F, G, and H in both arms. The response to ETV at week 48 by HBV subtype is shown in Table 14. The response ≤ 0.7 mEq/mL by bDNA assay ranged from 69% to 100% for all subtypes. The response ≤ 300 copies/mL by PCR assay ranged from 32% to 52% for subtypes A, B, C, and D. These limited data suggest that the response to ETV is not remarkably affected by subtype.

Table 13. Distribution of HBV Subtypes

Subtype	# in ETV arm (n = 623)	# in LAM arm (n = 180)
A	137 (22%)	51 (28%)
B	101 (16%)	20 (11%)
C	159 (26%)	38 (21%)
D	205 (33%)	69 (38%)
E	3	0
F	15	1
G	2	0
H	1	1

Table 14. ETV Response by HBV Subtype at Week 48

Subtype	N at week 48	≤ 0.7 MEq bDNA	<300 copies/mL PCR
A	71	54 (76%)	24 (34%)
B	67	58 (87%)	35 (52%)
C	81	73 (90%)	41 (51%)
D	77	53 (69%)	25 (32%)
E	2	2 (100%)	0 (0%)
F	9	9 (100%)	1 (11%)
G	2	2 (100%)	1 (50%)

Baseline Polymorphism Analysis

In addition to a paired analysis to identify resistance-associated mutations in the meta-analysis above, disproportionate representation of baseline polymorphisms in the failure or responder populations was evaluated to identify genotypes which may be unusually sensitive or resistant to entecavir. Several positions were found to be disproportionately represented but these did not identify a specific genotype. No substitutions at any amino acid appeared to notably affect response.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

SUMMARY

Overall, the following conclusions can be summarized from the ETV resistance data submitted from studies 014, 015, 022, 026, and 027.

- Greater proportions of nucleoside-naïve subjects (83%) with chronic HBV infection achieved HBV DNA levels < 400 copies/mL on ETV treatment compared to LAM-refractory subjects (21%).
- Genotypic or phenotypic evidence of resistance to ETV in nucleoside treatment-naïve patients chronically infected with HBV (n = 430) has not been observed up to 48 weeks of 0.5 mg QD ETV treatment in studies 022 (HBeAg-positive patients) and 027 (HBeAg-negative patients), including 2 subjects in 022 who experienced a confirmed virologic rebound.
- 7.4% (14/189) of LAM-refractory subjects treated with 1.0 mg ETV had evidence of emerging ETV-resistance substitutions by week 48.
- ETV-associated resistance substitutions at rtI169, rtT184, rtS202, and/or rtM250 emerged when LAM-resistant mutations at rtL180 and/or rtM204 were present and were associated with virologic rebound upon prolonged ETV therapy
- Overall, five ETV treated subjects exhibited a confirmed rebound in their HBV DNA levels of $\geq 1 \log_{10}$ from nadir by week 48
 - Two isolates from study 022 with no detected ETV-resistant substitutions or other genotypic changes at week 48
 - One isolate from study 015 which developed an rtT184A
 - Two isolates from study 026 which developed rtT184A/S substitutions in the context of rtL180M/L and rtM204V/I mutations
- 10/14 isolates that developed ETV resistance substitutions by week 48 had virologic rebound after 48 weeks of ETV treatment
- LAM-resistance substitutions rtL80V, rtL180M, rtM204V or I can emerge in the HBV of patients on 1 mg ETV by week 48. These substitutions often arise in the context of mixtures at these sites at baseline and other LAM-resistant mutations at baseline.
- Even when LAM-resistant mutations emerged in HBV on ETV therapy, ETV can suppress HBV DNA levels to below detection limits.
- > 2 \log_{10} reductions in viral load and viral load suppression below 400 copies/mL HBV DNA can occur in subjects with LAM-resistance in their HBV at baseline when treated with 1 mg ETV
- Cross-resistance to ETV was not observed with adefovir-resistant HBV in vitro.
- Clinically, HBV developing ETV resistance-associated substitutions in addition to LAM-associated resistance substitutions were susceptible to adefovir but remained resistant to lamivudine.

CONCLUSION

This NDA is approvable with respect to microbiology for the treatment of chronic HBV. Studies evaluating treatment responses to ETV and resistance to ETV are continuing to be monitored post 48 weeks. Post 48-week follow-up data of ETV-treated patients is needed to obtain long term resistance data and to determine the ETV resistance pathway in nucleoside treatment-naïve subjects. In addition, the *in vitro* phenotypic susceptibility of substitutions at rtI169 alone and in the context of LAM and ETV-associated resistance mutations to ETV and adefovir are needed.

5. Package Insert

Mechanism of Action

Entecavir, a guanosine nucleoside analogue with activity against HBV polymerase, is efficiently phosphorylated to the active triphosphate form, which has an intracellular half-life of 15 hours. By competing with the natural substrate deoxyguanosine triphosphate, entecavir triphosphate functionally inhibits all three activities of the HBV polymerase (reverse transcriptase, rt): (1) base priming, (2) reverse transcription of the negative strand from the pregenomic messenger RNA, and (3) synthesis of the positive strand of HBV DNA. Entecavir triphosphate has an inhibition constant (K_i) for HBV DNA polymerase of 0.0012 μM . Entecavir triphosphate is a weak inhibitor of cellular DNA polymerases α , β , and δ and mitochondrial DNA polymerase γ with K_i values ranging from 18 to $>160 \mu\text{M}$.

Antiviral Activity

Entecavir inhibited HBV DNA synthesis (50% reduction, EC_{50}) at a concentration of 0.004 μM in human HepG2 cells transfected with wild-type HBV. The median EC_{50} value for entecavir against lamivudine-resistant HBV (rtL180M, rtM204V) was 0.026 μM (range 0.010-0.059 μM). In contrast, no clinically relevant activity was noted against human immunodeficiency virus (HIV) type 1 (EC_{50} value $>10 \mu\text{M}$) grown in cell culture.

Daily or weekly entecavir treatment significantly reduced viral DNA levels (4 to 8 \log_{10}) in two relevant animal models, woodchucks chronically infected with woodchuck hepatitis virus (WHV) and ducks infected with duck HBV. Long-term studies in woodchucks

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

demonstrated that oral weekly dosing of 0.5 mg/kg entecavir (equivalent to the 1-mg human dose) maintained viral DNA levels at undetectable levels (<200 copies/mL by PCR) for up to 3 years in 3 of 5 woodchucks. No entecavir resistance changes were detected in the HBV polymerase in any of the treated animals for up to 3 years of treatment.

The coadministration of HIV nucleoside reverse transcriptase inhibitors (NRTIs) with BARACLUDE is unlikely to reduce the antiviral efficacy of BARACLUDE against HBV or of any of these agents against HIV. In HBV combination assays *in vitro*, abacavir, didanosine, lamivudine, stavudine, tenofovir, or zidovudine were not antagonistic to the anti-HBV activity of entecavir over a wide range of concentrations. In HIV antiviral assays, entecavir was not antagonistic to the *in vitro* anti-HIV activity of these six NRTIs at >4 times the C_{max} of entecavir.

Resistance

In Vitro

In cell-based assays, 8- to 30-fold reductions in entecavir phenotypic susceptibility were observed for lamivudine-resistant strains. Further reductions (>70-fold) in entecavir phenotypic susceptibility required the presence of primary lamivudine resistance amino acid substitutions (rtL180M and/or rtM204V/I) along with additional substitutions at residues rtT184, rtS202, or rtM250, or a combination of these substitutions with or without an rtI169 substitution in the HBV polymerase.

Clinical Studies

- *Nucleoside-naïve patients:* Eighty-one percent of HBV chronically infected nucleoside-naïve patients receiving entecavir 0.5 mg once daily achieved a reduction in viral load to <300 copies/mL at 48 weeks. Genotypic analysis of serum HBV DNA from nucleoside-naïve HBeAg-positive (Study AI463022; n=219) or HBeAg-negative (Study AI463027; n=211) patients detected no genotypic changes in the HBV polymerase associated with phenotypic resistance to entecavir at Week 48. No genotypic or phenotypic evidence of entecavir resistance was detected in the 2 patients who experienced a confirmed virologic rebound (≥1 log increase from nadir) in Study AI463022.
- *Lamivudine-refractory patients:* Twenty-two percent of lamivudine-refractory patients with chronic HBV infection achieved HBV DNA levels <300 copies/mL at Week 48 on

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

entecavir 1 mg once daily. Genotypic analysis of clinical samples from those patients with detectable viral DNA identified 7% (13/189) with evidence of emerging entecavir resistance-associated substitutions at rtI169, rtT184, rtS202, and/or rtM250 by Week 48 when pre-existing lamivudine resistance mutations rtL180M and/or rtM204V/I were present. Of the 13 patients with genotypic resistance, 3 experienced virologic rebound (≥ 1 log increase from nadir) by Week 48, with the majority of these 13 patients experiencing virologic rebound beyond Week 48.

Cross-resistance

Cross-resistance has been observed among HBV nucleoside analogues. In cell-based assays, entecavir had 8- to 30-fold less inhibition of replication of HBV containing lamivudine resistance mutations rtL180M and/or rtM204V/I than of wild-type virus. Recombinant HBV genomes encoding adefovir resistance-associated substitutions at either rtN236T or rtA181V remained susceptible to entecavir. HBV isolates from lamivudine-refractory patients failing entecavir therapy were susceptible *in vitro* to adefovir but retained resistance to lamivudine.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Appendices

APPENDIX 1.

Substitutions that Emerge on 1.0 mg ETV Therapy in Study 014

PID	Week	Viral Load	HBV Polymerase Baseline substitutions	HBV Polymerase Substitutions Developing on ETV
50-6082	Baseline	1.6X10 ⁹	L180M, M204V	
	24	1000	No genotype	
	48	199	No genotype	
50-6180	Baseline	468000	L180M M204I	
	24	199	No genotype	
	48	199		L80V
30-06143	Baseline	2.85X10 ⁹	L180M/L M204V/M	
	24	5.7X10 ⁶		L180M M204V L229L/W
	133	477000		
30- 06156	Baseline	5.27X10 ⁸		
	25	360		
	48	199		L80I L180M M204I
	173	199		
37- 06113	Baseline	1.09X10 ¹⁰	L180M/L M204M/V	
	24	9080000		L180M M204V I169T/I
42- 06194	Baseline	16X10 ⁸	M204I	
	24	2200		L180M/L V207X Q334Q/P
	48	215		
25-06236	Baseline	2.93X10 ⁸	L180M/L, M204V/I, V207X L229V/L	
	24	11100		
	48	1890		
	76	203		M204I V207I L229V
30- 06163	Baseline	2.59X10 ⁸	S53N/T L80V/L D134N V173L/V L180M/L M204V/I	
	25	22200		
	48	32500		
	108	7.6X10 ⁷		S53N L80V D134N/S I163I/V L180M T222A/T
	120	958		
	156	199		
37- 06071	Baseline	8.45 X10 ⁸	N139H/N I163I/V L180M/L M204I/V C332S/C L336M/L	
	24	392000		
	48	357		
	74	199		
	154	199		

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 **SN:** 000 **DATE REVIEWED:** 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

	174	26600		N139Q I163V L180M M204V V207V/L N238N/T C332S L336M
	186	199		
	198	299		
37-06115	0	7.2X10 ⁹		
	25	3.3X10 ²		L180M M204V/M
	49	199		L80V S106T R110G N123K N124L L180M M204I S256G V278C
50- 06028	Baseline	6.43 X10 ⁹	L80I/L L180M M204V/I L217R/L V253I/V Q271H/Q C332C/S	
	24	4.86 X10 ⁷		M204V
	48	2660000		
	86	462000		
	103	3310000		I169I/T T184X L217R V253I Q271H C332S
1-06206	Baseline	1.6X10 ⁹	L180M, M204V	
	24	974000		
	48	120000		
	53	360000		T184X
	64	1120000		T184I/L
9- 06130	Baseline	1.12 X10 ⁹	L180M, M204V	
	24	107000		
	48	195000		
	154	47600		
	166	144000		T184X
	178	1220000		T184L
30-06095	Baseline	9.87 X10 ⁹	D134N/S L180M M204V L229L/V	
	24	3200000		
	48	1050000		
	76	1700000		
	156	4080		
	168	2950		H94R/H T118H D134S T184L L229V W243R/W
	180	1670		
50- 06249	Baseline	8.3 X10 ⁸	L80I/L V173L/V L180M M204V	
	24	44400		
	48	2250		
	72	1.2 X10 ⁸		
	94	1.2 X10 ⁶		L80I
	122	1.31 X10 ⁸		T184F

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

APPENDIX 2.

Isolates with evidence of emerging ETV-resistance substitutions in LAM-refractory subjects on 1.0 mg ETV (n= 189)

Subject	Treatment	Week	I169	T184	S202	M250
AI463015-16-2001	ETV 1.0 mg	68		G		
AI463015-16-2007	ETV 1.0 mg	36.1428571		A/T		
AI463026-131-80041	ETV 1.0 mg	46.4285714			G	
AI463026-12-80252	ETV 1.0 mg	47.1428571	I/L	T/I		
AI463026-36-80016	ETV 1.0 mg	47.2857143				M/L
AI463026-135-80130	ETV 1.0 mg	47.5714286			S/G	
AI463026-52-80069	ETV 1.0 mg	47.8571429		T/A	S/G	
AI463026-135-80174	ETV 1.0 mg	48.1428571			S/G	
AI463026-73-80256	ETV 1.0 mg	48.1428571		S/A		
AI463026-76-80204	ETV 1.0 mg	48.2857143		T/I		
AI463026-15-80194	ETV 1.0 mg	48.7142857			S/G	M/L
AI463026-67-80393	ETV 1.0 mg	48.8571429		T/S		
AI463026-138-80249	ETV 1.0 mg	49.1428571	I/M			M/I
AI463026-98-80113	ETV 1.0 mg	49.7142857			S/G	
AI463014-1-6206	ETV 1.0 mg	77	I/L			
AI463015-5-2001	ETV 1.0 mg	84.4285714	I/T	G	I	
AI463014-50-6028	ETV 1.0 mg	102.857143	I/T	X		
AI463015-15-2008	ETV 1.0 mg	108.142857		S/T	G/S	
AI463014-50-6249	ETV 1.0 mg	121.857143		F		
AI463015-15-2005	ETV 1.0 mg	127		G/S		
AI463015-15-2006	ETV 1.0 mg	129.142857	I/T		G/S	M/V
AI463015-4-2003	ETV 1.0 mg	149.571429		F/L/ A/TG/S		
AI463015-4-2002	ETV 1.0 mg	151.285714	I/S		G/S	
AI463014-30-6095	ETV 1.0 mg	168.428571		L		
AI463014-9-6130	ETV 1.0 mg	178.428571		L		
AI463014-37-6113	ETV 1.0 mg	195	I/T	X		

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY DRAFT REVIEW

NDA: 21797 and 21798 SN: 000 DATE REVIEWED: 03/14/05

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

APPENDIX 3.

List of Rebounders

PID	Week	HBV Polymerase Mutations Developing on TRT	ETV fold from BL	ETV fold from ref
15-4-2002	151	I169S/I L180M M204V S202S/G N238N/H V207I/V	21	679
15-4-2003	150	L180M M204V T184T/F/L/A S202S/G N238N/H	11	219
15-16-2007	36	T184T/A	3.2	91
15-5-2001	92	T184G S202I N238N/D I169T/I	311	10022
15-15-2006	129	I169T/I 250M/V S202S/G	54	1683
15-15-2008	108	T184T/S S202S/G M204I/V N238H/N	83	676
26-52-80069	48	T184T/A S202S/G	18	753
26-73-80256	48	T184S/A L229L/V	19	58
26-98-80113	50	S202S/G	1.1	12
26-131-80041	46 (>48)	S202G M204V	18	343
26-135-80174	48 (>48)	S202S/G L229L/M	2.9	121
26-136-80122	48 (>48)	M204V	4.3	30
22-191-10441	48	None	1.1	2.3
22-208-10912	48	None	1.1	1.7
14-1-06206	53	T184I/L	-	-
14-9-06130	166	T184L	-	-
14-30-06095	168	T184L	-	-
14-50-06028	103	I169I/T, T184X	-	-
14-50-06249	72	L80I, T184F	-	-

APPENDIX 4.

Phenotype of Post-Baseline Samples

Fold-change from Ref	>100	<100	>50	<50	>30	<30	>10	<10
Rebound	5 (45%)		5 (42%)		5 (31%)		5 (23%)	
Non-Responders	4 (36%)	5 (36%)	5 (42%)	4 (31%)	6 (38%)	3 (33%)	7 (32%)	2 (67%)
Responders	2 (18%)	9 (64%)	2 (17%)	9 (69%)	5 (31%)	6 (67%)	10 (45%)	1 (33%)
n	11	14	12	13	16	9	22	3

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

/s/

Lisa Naeger
3/29/05 09:12:06 AM
MICROBIOLOGIST
microbiology review

Julian O Rear
3/29/05 09:48:38 AM
MICROBIOLOGIST

James Farrelly
3/29/05 09:56:38 AM
PHARMACOLOGIST