

**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)**

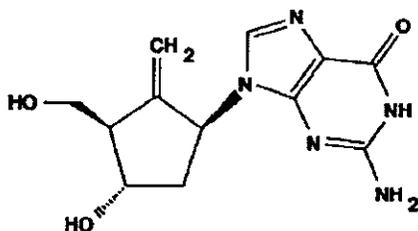
**Proprietary:**

**Non-Proprietary/USAN:** entecavir

**Code Name/Number:** BMS-20475

**Chemical Name:** {[1*S*-(1 $\alpha$ ,3 $\alpha$ ,4 $\beta$ )]-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

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**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; CC<sub>50</sub>, 50% cytotoxic concentration; DHBV, duck hepatitis B virus; HBeAg, Hepatitis B e antigen; HBV, hepatitis B virus; HBIg, hepatitis B immunoglobulin; IC<sub>50</sub>, 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;

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*Executive Summary*

Entecavir (ETV), a nucleoside analog, has demonstrated inhibitory activity against hepatitis B virus (HBV) replication with an average IC<sub>50</sub> value of 3.75 nM in the HBV stably-transfected human liver HepG2 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,

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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.**

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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-

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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 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<sub>RF</sub> and 47  $\mu$ 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  $\mu$ M for ETV, providing a selectivity index of >8,000 ( $CC_{50}$  value/ $IC_{50}$  value). DNA polymerases  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\epsilon$  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  $\gamma$  in vitro occurred at concentrations up to 300  $\mu$ M ETV-TP indicating no significant recognition by polymerase  $\gamma$ . 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.

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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-naïve 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-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.

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 log<sub>10</sub> 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.

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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)

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[Lisa K. Naeger, Ph.D.]

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**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

45 Page(s) Withheld

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

§ 552(b)(5) Deliberative Process

§ 552(b)(5) Draft Labeling

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/s/

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