CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-652

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN**: 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 21652 **Serial #:** 000
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
GlaxoSmithKline
PO Box 13398
Five Moore Drive
Research Triangle Park
North Carolina 27709

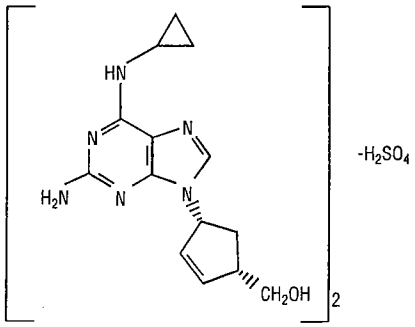
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Initial Submission Dates:
Correspondence Date: October 7, 2003
CDER Receipt Date: October 8, 2003
Assigned Date: October 10, 2003
Review Complete Date: 07/30/04
PDUFA Date: August 8, 2004

Amendments:
Related/Supporting Documents: NDA-20977, NDA-20978

Product Name(s): Epivir/Ziagen (lamivudine/abacavir sulfate)
Proprietary: Epzicom
Non-Proprietary/USAN: lamivudine/abacavir sulfate

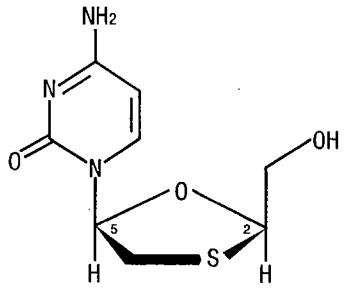
Abacavir sulfate
Chemical Name: *(1S,cis)*-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1)
Molecular Formula: $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$
Molecular Weight: 670.76 daltons
Structural Formula:



Abacavir Sulfate

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN**: 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Lamivudine
Chemical Name: (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one
Molecular Formula: $C_8H_{11}N_3O_3S$
Molecular Weight: 229.3 daltons
Structural Formula:



Lamivudine

Dosage Form(s): tablets
Route(s) of Administration: Oral
Indication(s): Treatment of HIV infection in combination with other antiretroviral agents

Dispensed: Rx X OTC_____

Abbreviations: ABC, abacavir; ABC/LAM, abacavir and lamivudine combination; BID, *bis in die* (twice daily); EFV, efavirenz; HIV-1, human immunodeficiency virus type 1; LAM, lamivudine; LLOQ, lower limit of quantification; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor ; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; PR, protease; OAD, once daily; RT, reverse transcriptase; TLOVR, Time to Loss of Virologic Response; TP, triphosphate; UNG, uracil N-glycosylase ; WT, wild-type

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN**: 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Executive Summary

Abacavir (Ziagen) first received accelerated approval on December 17, 1998 and traditional approval in April 2004. Lamivudine (Epivir) was first approved on November 17, 1995. Both abacavir (ABC) and lamivudine (LAM) are nucleoside analogs that inhibit HIV-1 reverse transcriptase (RT) activity and are members of the nucleoside reverse transcriptase inhibitor (NRTI) class. This submission of NDA-21652 is for approval of the combination of once daily 600 mg ABC plus once daily 300 mg lamivudine for the treatment of HIV in combination with other antiretrovirals.

Abacavir (ABC) is a carbocyclic synthetic nucleoside analog, which is converted intracellularly by cellular enzymes to the active metabolite, carbovir triphosphate, an analog of deoxyguanosine-5′-triphosphate (dGTP). Carbovir triphosphate inhibits the activity of HIV-1 RT in viral DNA synthesis both by competing with the natural substrate dGTP and by its incorporation into viral DNA. Lamivudine (LAM) is a synthetic nucleoside analog, which is phosphorylated intracellularly to its active metabolite lamivudine triphosphate. The principal mode of action of lamivudine triphosphate is inhibition of RT via viral DNA chain termination after incorporation of the nucleoside analog. Abacavir and lamivudine are weak inhibitors of human DNA polymerases-α, β and γ.

The *in vitro* anti–HIV-1 activity of abacavir had IC_{50} (50% inhibitory concentration) values ranging from 3.7 to 5.8 μM against laboratory strains of HIV-1 and had a mean IC_{50} value of 0.26 μM against 8 clinical isolates in monocytes and peripheral blood mononuclear cells. The *in vitro* activity of lamivudine had IC_{50} values ranging from 0.026 μM to 0.148 μM against HIV-1 in monocytes and human peripheral blood lymphocytes. The combination of ABC and LAM (ABC/LAM) has demonstrated antiviral activity against non-subtype B isolates and HIV-2 isolates with equivalent antiviral activity as for subtype B isolates. ABC/LAM had additive to synergistic activity *in vitro* in combination with the NRTIs (emtricitabine, stavudine, tenofovir, zalcitabine, zidovudine), the NNRTIs (delavirdine, efavirenz, nevirapine), the PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir) or the fusion inhibitor, enfuvirtide. The anti-HCV drug, ribavirin, decreased the anti-HIV potency of the combination of ABC/LAM reproducibly by 2- to 6-fold *in vitro*.

HIV-1 isolates with reduced sensitivity to abacavir or lamivudine have been selected *in vitro* and have also been obtained from patients treated with abacavir and lamivudine. The mutations, M184V or I, K65R, L74V and Y115F were selected in the presence of abacavir in combination with lamivudine *in vitro*. Genetic analysis of isolates from abacavir-treated patients demonstrated that mutations K65R, L74V, Y115F, and M184V in HIV-1 reverse transcriptase (RT) contributed to abacavir resistance. Genotypic analysis of isolates recovered from lamivudine-treated patients showed that the resistance was due to the M184V mutation in HIV-1 RT.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN**: 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

The sponsor has submitted Study CNA30021 to support the approval of once daily (OAD) ABC and to support approval of the combination of OAD ABC plus LAM. In Study CNA30021, therapy-naïve adults received Efavirenz (EFV) and 300 mg lamivudine OAD plus ZIAGEN 600 mg OAD or ZIAGEN 300 mg twice daily (BID). The incidence of virologic failure at 48 weeks was similar between the two groups (ABC OAD: 11%; ABC BID: 11%). Genotypic (n = 38) and phenotypic analyses (n = 35) of failure isolates from this study were analyzed. The RT mutations emerging on therapy included M184V/I, L74V, K65R, Y115F and NNRTI-associated mutations (*i.e.*, K103N, K101X, G190S, Y181C). In the treatment failure population, the number of subjects with treatment emergent resistance mutations in OAD ABC arm was 13/18 (72%) compared to 16/20 (80%) in the BID ABC arm. The main resistant mutations were M184V/I (n= 18) and K103N (n = 19). The lamivudine resistant-associated mutation M184V or I developed in 10 isolates (56%) in OAD arm and 8 patient isolates (40%) in the BID arm. The K103N mutation, associated with EFV-resistance, developed in 7 isolates (39%) in the OAD arm and in 12 patient isolates (60%) in the BID arm. NNRTI-resistant mutations including K103N, K101E/N/Q, G190S/A, V179D, Y181C, Y188L/C/F/H, L100I, and/or V106I developed in 11 isolates (61%) in the OAD arm and 16 patient isolates (80%) in the BID arm. Selection of the ABC and LAM-associated resistance mutation M184V or I predominated in failure isolates from therapy with OAD ABC/LAM.

Phenotypic analysis showed that 39% (7/18) of the failure isolates in the ABC OAD arm had a >2.5-fold shift in ABC susceptibility with a median fold shift of 1.3 (range 0.5 – 11) in ABC susceptibility compared to 29% (5/17) of the failure isolates in the BID arm with a >2.5-fold shift in ABC susceptibility with a median fold shift of 0.9 (range 0.7 – 13). Fifty-six percent (10/18) of the failure isolates in the OAD ABC group compared to 41% (7/17) of the failure isolates in the BID ABC group had a >2.5-fold change in lamivudine susceptibility with median fold changes of 81 and 1.1 in the OAD ABC and BID ABC arms, respectively.

Overall, the phenotypic and genotypic data showed similar ABC and LAM phenotypic resistance and a similar number of ABC- and LAM-resistance mutations developing in the OAD and BID ABC arms, supporting the efficacy of OAD ABC/LAM. The phenotypic and genotypic data suggested a slight increase in LAM resistance and the M184V/I mutation in the OAD arm compared to the BID arm, but this difference was not significant. Interestingly, the number of NNRTI-associated resistance mutations that developed in the OAD arm (61%) was less than in the BID arm (80%). These small differences are not explained by the number of NRTI- or NNRTI-associated resistance mutations present in the patient isolates at baseline in the two arms. The small number of isolates in the resistance analysis makes it difficult to make any definitive conclusions, but the similar efficacy at 48 weeks and similar ABC/LAM resistance rates between the two arms supports the approval of OAD ABC/LAM. Long term resistance monitoring > 48 weeks might be needed to demonstrate that OAD ABC/LAM is as durable as BID ABC/LAM.

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN:** 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

2. Summary of OND Microbiology Assessments

2.1. Brief Overview of the Microbiological Program

2.1.1. Non-clinical

Abacavir (ABC) is a synthetic carbocyclic nucleoside analogue that inhibits HIV-1 reverse transcriptase activity (RT) and is a member of the nucleoside reverse transcriptase inhibitor (NRTI) class. Abacavir is phosphorylated to its active metabolite, carbovir triphosphate (CBV-TP), by the cellular enzyme adenosine phosphotransferase. Subsequently, ABC monophosphate is deaminated to carbovir monophosphate (CBV-MP) by a cytosolic deaminase. CBV-MP is then further converted to di- and triphosphate forms by cellular kinases. The intracellular half-life of CBV-TP was 3.3 hours in CEM cells. CBV-TP inhibits the activity of HIV-1 RT both by competing with the natural substrate, deoxyguanosine 5'-triphosphate (dGTP), and by its incorporation into viral DNA resulting in chain termination.

The *in vitro* anti-HIV-1 activity of ABC was evaluated against a T-cell tropic laboratory strain HIV-1_{IIIB}, a monocyte/macrophage tropic laboratory strain HIV-1_{BaL}, and clinical isolates in lymphoblastic cell lines, primary monocytes/macrophages, and PBMCs, and the inhibitory concentration at 50% (IC_{50}) ranged from 3.7 to 5.8 μM against HIV-1_{IIIB}, and was 0.26 ± 0.18 μM against eight clinical isolates. The IC_{50} value of ABC against HIV-1_{BaL} varied from 0.07 to 1.0 μM. ABC had synergistic activity *in vitro* in combination with the NRTI zidovudine (ZDV), the NNRTI nevirapine (NVP), and the PI amprenavir (APV), and, additive activity in combination with the NRTIs didanosine (ddI), LAM, stavudine (d4T), and zalcitabine (ddC). The CC_{50} of ABC ranged from 110-160 μM for IM-9, CEM and $CD4^+$ CEM cells giving a therapeutic index of 423-615 for clinical isolates. CBV-TP is a weak inhibitor of human DNA polymerase α, β and γ.

HIV-1 isolates with reduced susceptibility to ABC have been selected *in vitro*. Phenotypic analyses showed that recombinant viruses containing single or double ABC-resistance-associated mutation (K65R, L74V, Y115F, or M184V) exhibited a 2- to 4-fold reduced susceptibility to ABC compared to wild-type, and recombinant viruses with M184V plus K65R, L74V or Y115F exhibited a 7- to 9-fold reduced susceptibility to ABC compared to wild type. Recombinant viruses containing triple mutations (K65R/L74V/M184V or L74V/Y115F/M184V) exhibited a 10- to 11-fold reduced susceptibility to ABC compared to wild type.

Lamivudine (LAM; 3TC) is a synthetic nucleoside analog. Intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite, lamivudine triphosphate (L-TP). The principal mode of action of L-TP is inhibition of RT via

DNA chain termination after incorporation of the nucleoside analogue. L-TP is a weak inhibitor of human DNA polymerases-α, β and γ.

In vitro activity of LAM against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes). IC_{50} values (50% inhibitory concentrations) for LAM ranged from 0.0026 μM to 15 μM. LAM had anti–HIV-1 activity in all acute virus-cell infections tested.

Viruses containing the ABC and LAM-associated resistance mutations, K65R, L74V, M184V, and Y115F exhibited cross-resistance to didanosine, emtricitabine, tenofovir and zalcitabine *in vitro* and in patients. The combination of ABC/LAM has demonstrated decreased *in vitro* susceptibility to viruses with the mutations K65R with or without the M184V/I mutation, viruses with L74V plus the M184V/I mutation, and viruses with thymidine analog mutations (TAMs: M41L, D67N, K70R, L210W, T215Y/F, K219 E/R/H/Q/N) plus M184V. An increasing number of TAMs is associated with a progressive reduction in ABC susceptibility.

2.1.2. **Clinical Microbiology**

HIV-1 isolates with reduced susceptibility to ABC have been obtained from patients treated with ABC and mutations M184V and L74V were most frequently observed in the clinical isolates. Genotypic analysis of isolates from ABC-treated patients were consistent with resistant virus selected *in vitro*, and showed RT mutations with amino acid substitutions K65R, L74V, Y115F, and M184V. Viruses with ABC-resistance-associated mutations are cross-resistant to NRTIs, ddI, ddC and 3TC. HIV-1 isolates with reduced susceptibility to LAM have been selected *in vitro* and were also obtained from patients treated with lamivudine. Genotypic analysis of isolates selected *in vitro* and recovered from lamivudine-treated patients showed that the resistance was due to RT mutation M184V or I. Cross-resistance to abacavir, didanosine and zalcitabine has been observed in patients harboring lamivudine-resistant HIV-1 isolates.

The sponsor performed phenotypic and genotypic analyses on data from a random sample of isolates and virologic failure isolates collected during the course of clinical study CNA30021. CNA30021 is a phase III, 48-week, randomized, double-blind, multicenter study to evaluate the safety and efficacy of abacavir (ABC) 600 mg once-daily (OAD) versus abacavir 300 mg BID, in combination with lamivudine (LAM) (300 mg OAD) and efavirenz (EFV) (600 mg OAD) in antiretroviral therapy-naïve HIV-1 infected subjects. Both the random sample and virologic failure populations were predominantly infected with group M clade B HIV-1. There were 38 evaluable failure isolates with genotypic and 35 with phenotypic data from study CNA30021. In the ABC OAD regimen, 39% (7/18) of the failure isolates were resistant to ABC and 56% (10/18) were resistant to LAM.

In the ABC BID regimen, 29% (5/17) of the virologic failure isolates were resistant to ABC and 41% (7/17) were resistant to LAM. Resistance is defined as greater than a 2.5-fold change in ABC or LAM susceptibility compared to a reference strain using the [redacted] phenotypic assay. The difference in proportions of ABC and LAM resistant isolates was not significantly different between the ABC OAD and BID arms. The median fold change in ABC susceptibility of the failure isolates was 1.3 and 0.9 in the OAD and BID arms, respectively. The median fold change in LAM susceptibility of the failure isolates was significantly different between arms with values of 81 and 1.1 in the OAD and BID arms, respectively.

In the treatment failure population, the number of subjects with treatment emergent resistance mutations in OAD ABC arm was 13/18 (72%) compared to 16/20 (80%) in the BID ABC arm. The main resistant mutations were M184V/I (n= 18) and the EFV-associated resistance mutation, K103N (n = 19). Eight isolates (21%) had the ABC-resistance mutation, L74V, develop on therapy. Only one isolate developed the K65R mutation and two isolates developed Y115F. ABC-resistant associated mutations, L74V, M184V/I, K65R, and\or Y115F developed in 11 isolates (61%) in the OAD arm and 8 isolates (40%) in the BID arm. LAM-resistant associated mutation M184V or I developed in 10 isolates (56%) in OAD and in 8 patient isolates (40%) in the BID arm. The K103N mutation, associated with EFV-resistance, developed and 7 isolates (39%) in the OAD arm and in 12 isolates (60%) in the BID arm. NNRTI-resistant mutations including K103N, K101E/N/Q, G190S/A, V179D, Y181C, Y188L/C/F/H, L100I, and/or V106I developed in 11 isolates (61%) in the OAD arm and 16 isolates (80%) in the BID arm. Overall, the phenotypic and genotypic data showed similar ABC and LAM phenotypic resistance and a similar number of ABC and LAM resistance mutations developing in the OAD and BID ABC arms, supporting the efficacy of OAD ABC/LAM.

3. Administrative

3.1. Reviewer's Signature(s)

_____
[Lisa K. Naeger, Ph.D.]
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 REVIEW
NDA: 21-652 **SN:** 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

4. OND Microbiology Review

4.1 Introduction and Background

4.1.1 Important Milestones in Product Development

Abacavir first received accelerated approval on December 17, 1998. GlaxoSmithKline (GSK) Inc. filed supplemental new drug applications (sNDAs) # 20-977 and 20-978 to provide results from clinical studies in support of traditional approval of abacavir (Ziagen Tablets and Ziagen Oral Solution) and received traditional approval in April 2004.
The original NDAs for lamivudine (Epivir) were approved on November 17, 1995. Glaxo Smith Kline (GSK) filed two supplemental new drug applications, sNDA 20,564 (SE2-015) and sNDA 20,596 (SE2-016) for marketing approval for a 300 mg once daily (OAD) dosing regimen of Epivir Tablets and Oral solution (lamivudine tablets and oral solution) for the treatment of HIV-1 infection. Epivir Oral Solution was approved on June 24, 2002. Epivir 300 mg once daily (OAD) dosing Tablets and Epivir Solution 300 mg are approved for use in combination therapy for the treatment of HIV-1 infection in adults and adolescents.

4.1.2 Methodology

HIV-1 RNA Analysis

The endpoint of virologic response in clinical study CNA30021was the proportion of subjects with HIV-1 RNA levels below the lower limit of quantification (LLOQ) of 50 copies/mL at week 48. The assay to measure HIV RNA was the Roche Amplicor HIV-1 Monitor™ UltraSensitive — with LLOQ of 50 copies/mL. The Amplicor HIV-1 Monitor™ assays incorporates specimen preparation, reverse transcription of target RNA to generate cDNA, PCR amplification of target cDNA using HIV-1 specific complimentary primes, hybridization of the amplified products to oligonucleotide probes specific to the target, and detection of the probe-bound amplified products by colorimetric determination. [

Genotypic Analyses

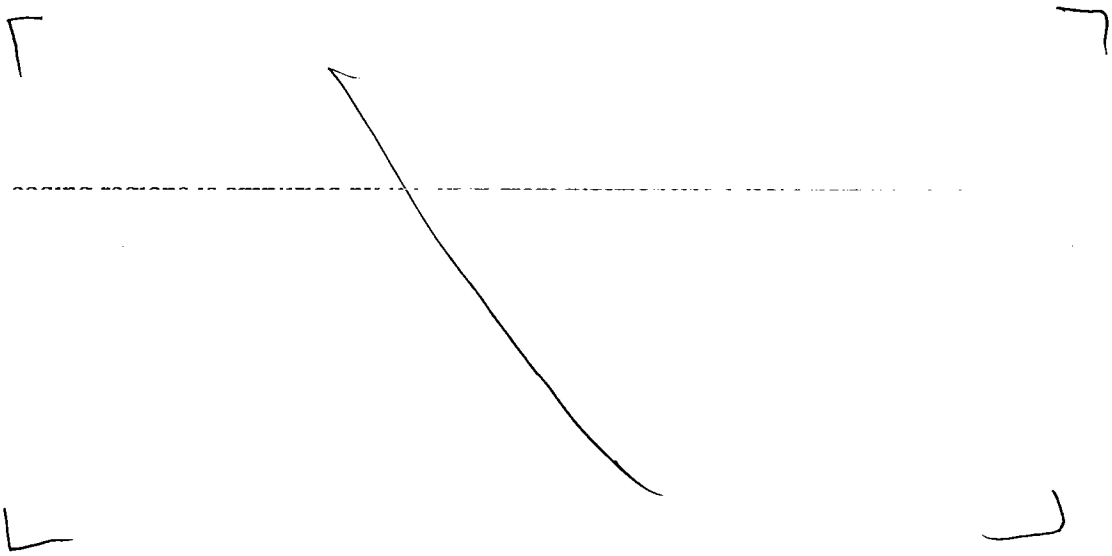
Genotypic analysis of the viral RT was performed in virus present in paired plasma samples obtained at baseline and at either the designated on-therapy timepoint or if plasma HIV-1 RNA was below 500 copies/mL, the nearest timepoint thereafter to 48 weeks when plasma HIV-1 RNA was >500 copies/mL. This analysis was performed in-house using the — genotyping kit.

[redacted]

Phenotypic Analyses

Phenotypic analysis of samples obtained from subjects included in the virologic failure population and the random sample was performed by [redacted] using the single-cycle [redacted] Assay, according to standard procedures. This assay determines the susceptibility of resistance test vectors to licensed antiretroviral drugs, which incorporate the RT derived from the subject's plasma virus. Phenotypic susceptibility to all licensed

nucleoside and non-nucleoside analog RT inhibitors (NRTI and NNRTI) was reported as fold reduction (FR) in the 50% inhibitory concentration (IC_{50}) relative to wild-type control virus



4.1.3 Prior FDA Microbiological Reviews

Reviewer: Lalji Mishra
Review date: 4/8/04

GSK submitted the results of the pivotal clinical trial CNA30024 to support efficacy and safety of abacavir in antiretroviral naïve patients. In addition, GSK submitted the results of studies, CNAB 3002 (use of abacavir in antiretroviral experienced patients), APV 30001 and APV 30002 [use of abacavir in combination with a protease inhibitor (PI), either fosamprenavir or nelfinavir (NFV), and the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV)]. Data on abacavir's metabolism and mechanism of action, *in vitro* anti-HIV-1 activity, *in vitro* combination activity relationships with other antiretroviral agents, selection and characterization of resistant HIV-1 *in vitro* and *in vivo,* and cross-resistance with other NRTIs were previously reviewed (microbiology review of abacavir NDA # 20-977 and NDA # 20-978 dated November 25, 1998, and sNDA # 20-977, and sNDA # 20-978, dated June 6, 2000).

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-652 **SN:** 000 **DATE REVIEWED:** 07/15/04
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Clinical Study CNA 30024: Genotypic and Phenotypic Analysis of HIV-1 Reverse Transcriptase in HIV-1 Infected, Antiretroviral Naïve Adult Subjects over 48 Weeks of Therapy with ABC + 3TC + EFV or ZDV + 3TC + EFV. The genotypes of baseline isolates were available for 99/100 random subjects in both treatment group. The baseline isolates from 2 subjects (# 48678 and # 48703) randomized to the ABC + 3TC + EFV treatment group contained the NRTI resistance-associated mutation V118I alone. These subjects were grouped as responders at week 48. Similarly, baseline isolates from subjects # 49342, # 49453, # 49471, and # 48102 randomized to the ABC + 3TC + EFV treatment group contained the ZDV resistance-associated mutation M41L alone (n=3), or M41L + L210W (n=1). These subjects were also grouped as responders at week 48. Similarly, baseline isolates from subject # 49462 randomized to the ZDV + 3TC + EFV treatment group contained the ZDV resistance-associated mutation M41L. At week 48, this subject was also grouped as a responder.

The baseline isolates from two subjects randomized to the ABC + 3TC + EFV treatment group contained either the NNRTI resistance-associated mutation G190A (# 48031), or, the ZDV resistance-associated mutations D67N + K70R + K219Q (# 48302). These two subjects were grouped as non-responders due to non-virologic reasons at week 48.

The genotypes for baseline isolates were obtained from 31/33 subjects with virologic failure. However, the genotypes of baseline isolates from two subjects (# 48293 from the ABC + 3TC + EFV group and # 48445 from the ZDV + 3TC + EFV group) could not be obtained due to lack of samples. The baseline isolates from 29/31 virologic failure subjects contained wild-type RT genotypes. However, baseline isolates from 1/31 virologic failure subjects contained the NRTI resistance-associated mutation V118I, and from another (1/31) virologic failure subject contained the NNRTI-resistance-associated mutation K103N (Table 7).

Major microbiological issues that arose during product development.

1. The following microbiology requests were sent with the 45-day filing letter in November 2003:

   - Please determine the activity *in vitro* of abacavir and lamivudine for the different clades of HIV-1 and for HIV-2.

   - Sponsor has agreed to examine the *in vitro* drug combination activity analyses for drug interactions of ABC/3TC with all approved anti-HIV agents and submit results in early-mid 2004.

   - Please provide a statement on the combination activity relationship of abacavir and lamivudine to TNV and FTC, and ribavirin (for HCV co-infected patients).

   - The sponsor has agreed to examine the cross-resistance profile of ABC/3TC against primary resistant isolates for each approved NRTI and NNRTI drug and a selected panel of PI resistant isolates. In addition, they will examine the antiviral activity of all approved NRTIs and NNRTIs against isolates containing major mutations observed in clinical trials of ABC/3TC, specifically M184V ± K65R, Y115F, and/or L74V. These results will be submitted in early-mid 2004.

- The sponsor has submitted clinical resistance data from clinical trial CNA30021.

2. On February 23, 2004, the following request was sent to GSK for additional microbiology data.
   - From clinical study CNA30021, please provide the data on the non-clade B isolates in the random sample (n =13) and the virologic failures (n = 5) in the template format. Please indicate the clade of the isolates and if they responded to therapy. Please provide a timeframe for the submission of this requested data.

4.1.4 State of antimicrobials used for the indication (s) sought:

An estimated 40 million people were infected with HIV worldwide and 3 million died from AIDS in 2001. Since HAART regimens have been introduced, the number of AIDS cases decreased dramatically. HAART does not eradicate HIV from patients completely and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that most HIV-infected patients will require antiretroviral therapy throughout their lives.

There are currently nineteen FDA-approved anti-HIV drugs including eight NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), three NNRTIs (delavirdine, efavirenz, nevirapine), seven PIs (atazanavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir), and the fusion inhibitor enfuvirtide (T-20). NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and incorporation into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. PIs work at the late stage of viral replication to prevent infectious virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. Enfuvirtide, also known as T20, is an inhibitor of the gp41 catalyzed fusion of the viral and cellular membranes necessary for entry.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis) and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important

determinant of successful virologic suppression for patients on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in patient compliance and improve clinical outcomes. There is a need for new anti-HIV drugs that are well-tolerated and easy. Furthermore, there is currently demand for co-formulated and co-packaged products that will further reduce pill burden, simplify use, promote adherence and be available for use in undeveloped countries like Africa.

4.2 Non-clinical Microbiology

In Vitro Drug Combination Activity Studies

The interaction of the combined agent ABC/LAM with approved anti-HIV-1 agents was studied using the tetrazolium-based colormetric assay in MT-4 cells. IC_{50} values were calculated and the interaction of each pair of compound combinations as analyzed by the Selleseth *et al.* method (2003), which provides an estimation of the strength of any interaction and of its statistical significance. Synergy and antagonism are defined as deviations from dose-wise additivity. Values for average deviation from additivity in the range of -0.1 to –0.2 indicate weak synergy and values that approach –0.5 indicate strong synergy. Conversely, values of 0.1-0.2 indicate weak antagonism and values around 0.5 indicate strong antagonism. In combination testing, the activity of ABC/LAM was not antagonistic with the activity of the NRTIs - AZT, d4T, ddC, ddI, FTC, or TDF, the NNRTIs - DLV, EFV, or NVP, or the PIs - APV, IDV, LPV, NFV, or RTV (Table 1A).

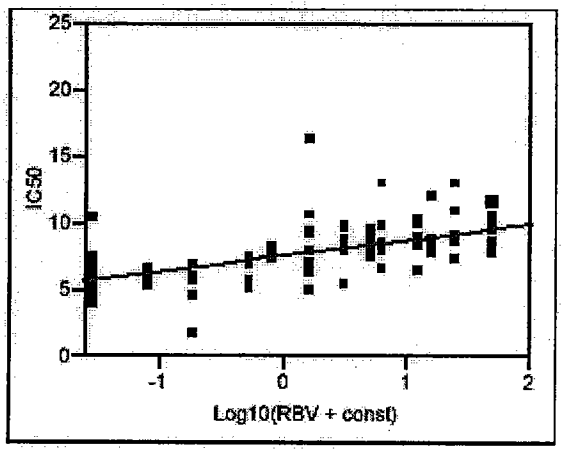
The effect of enfuvirtide (T-20) on the ABC/3TC fixed ratios was also analyzed in CEM-SS cells. The IC_{50} for T-20 in all experiments was 0.016 ± 0.0013 μM. Slight but significant synergy was seen in the combination at the 1:1 ratio, while additivity was seen with the 40:1 ratio. There was no evidence of antagonism or direct cytotoxicity up to the maximum concentration of T-20 tested in CEM-SS cells.

An equimolar mixture of abacavir (ABC) and lamivudine (3TC), individually or in combination, or emtricitabine (FTC) were tested for *in vitro* anti-HIV activity in combination with ribavirin (RBV) at 0.05 to 50 μM. RBV reduced the antiviral potency of both 3TC and FTC by 3.5- or 2.0-fold, respectively as RBV was increased to 50 μM (Table 1B). RBV did not affect the anti-HIV potency of ABC, confirming previous findings (Table 1B). RBV had a reproducible and significant effect on the anti-HIV potency of equimolar mixtures of ABC and 3TC in MT-4 cells, decreasing the potency of the mixture by 1.7-fold as RBV was increased to 50 μM (Figure 1, Table 1B). The effect of RBV on the ABC/3TC mixture probably results from the effect of RBV on 3TC. In CEM-SS cells, the effect of RBV on ABC/3TC antiviral activity is somewhat greater than in MT4 cells. The anti-HIV IC_{50} of the ABC/3TC 1:1 ratio increased 6.0-fold at the 5 μM RBV concentration, and the ABC/3TC 40:1 combination increased 2.4- to 2.7-fold at the 5 μM RBV concentration (Table 1C).

Table 1A. Inhibition of HIV-1_{IIIB} by ABC/LAM in Combination with Other Marketed Anti-HIV Agents in MT-4 cells *In vitro*.

Compound	Deviation from Additivity			Interaction with ABC/3TC
	Average	S.E.	P(t)	
AZT	-0.289	0.026	9.3E-7	Synergy
d4T	0.023	0.042	0.295	Additive
TFV	-0.120	0.031	0.002	Synergy
ddC	-0.039	0.030	0.115	Additive
ddI	-0.090	0.024	0.002	Additive
FTC	-0.097	0.031	0.006	Additive
EFV	-0.132	0.040	0.004	Synergy
NVP	-0.179	0.039	0.0006	Synergy
DLV	-0.059	0.025	0.021	Additive
IDV	-0.175	0.046	0.002	Synergy
LPV	-0.209	0.042	0.0006	Synergy
NFV	-0.228	0.045	0.0002	Synergy
RTV	-0.188	0.032	8.3E-5	Synergy
APV	-0.179	0.041	0.0007	Synergy
SQV	-0.085	0.052	0.068	Additive

Figure 1. Effect of Ribavirin on the Inhibition of HIV-1_{IIIB} by ABC/3TC Mixtures in MT-4 cells *In Vitro*



Bivariate fit of IC_{50} by log_{10}(RBV + const). Pooled results from four experiments.

Table 1B. Regression Analysis of IC_{50} vs log_{10} for Anti-HIV Drugs used in Combination Studies with Ribavirin in MT-4 Cell Assays

	Number of Expts.					IC_{50}, µM at RBV =		Fold Change
Drug		Slope	Std. Error	t Ratio	Prob > t	0 µM	50 µM	
ABC/3TC[2]	4	1.18	0.16	7.4	<0.0001	5.7	9.5	1.7
ABC	3	-0.15	0.28	-0.5	0.6	15.5	15.0	1.0
3TC	3	11.1	1.4	8.1	<0.0001	14.4	50.4	3.5
FTC	2	1.75	0.36	4.8	<0.0001	5.8	11.5	2.0

Pooled results from the indicated number of experiments.
1. The value of the constant was 0.028 µM RBV.
2. ABC/3TC, ABC and 3TC were combined in 1:1 equimolar mixture and the mixture was used as if it were a single drug.

Table 1C. Regression Analysis of IC_{50} vs log_{10} for Anti-HIV Drugs used in Combination Studies with Ribavirin in CEM-SS Cell Assays

					IC_{50} (µM) at RBV =		Fold change
Drug	Slope	S.E.	t Ratio	Prob>|t|	0 µM	5 µM	
ABC/3TC, 1:1 ratio	0.67	0.11	6.24	<0.0001	0.25	1.5	6.0
ABC/3TC, 40:1 ratio, 6/16 expt	3.38	0.60	5.63	<0.0001	4.6	11.1	2.4
ABC/3TC, 40:1 ratio, 6/17 expt	0.34	0.06	5.33	<0.0001	0.43	1.1	2.7

The Antiviral Activity of ABC/LAM against Non-Clade B HIV-1 and HIV-2 Virus Isolates *In vitro*

The antiviral activity of ABC, LAM and zidovudine was analyzed against three virus isolates from each of the seven HIV-1 Group M envelope subtypes A, B, C, D, E, F, and G as well as three Group O (Table 2.) and three HIV-2 isolates in PBMCs. Antiviral activity was measured using a RT activity assay and a p24 assay. Cytotoxicity was measured using MTS staining for PBMC viability. The reported mean IC_{50} value of ABC against eight clinical isolates was 0.26 µM and ranged from 3.7 - 5.8 µM against the laboratory strain HIV-1_{IIIB} *in vitro*. The average ABC IC_{50} values against non-B HIV-1 subtypes ranged from 0.009 to 0.363 µM with the highest antiviral activity against subtype C (Table 2). The IC_{50} values of LAM against HIV-1 ranged from 0.026 to 0.148 µM *in vitro*. The average LAM IC_{50} values against non-B HIV-1 subtypes ranged from 0.021 to 0.05 µM with equivalent antiviral activity against all subtypes (Table 2). ABC and LAM also demonstrated antiviral activity against HIV-2 isolates with average IC_{50} values of 0.228 µM and 0.042 µM, respectively. These results suggest that ABC and LAM should exert antiviral activity against most non-subtype B and HIV-2 isolates equivalent to the antiviral activity it has against subtype B.

Table 2. Antiviral Activity of 3TC, AZT, and Abacavir against HIV-1 Subtypes Isolates in PBMCs

HIV-1	Lamivudine	Abacavir
Subtype	Mean IC_{50} (nM)	Mean IC_{50} (nM)
A	50	345
B	49.5	363
C	46.7	8.8
D	21	262.8
E	30.3	100.6
F	40	84.2
G	34.3	78.5
O	46.7	300.9
HIV-2	41.7	228.1

***In vitro* Selection of Resistant Isolates**

The *in vitro* pattern of HIV selection in the presence of abacavir in combination with lamivudine was analyzed (Harrigan *et al.*). In selection experiments with wild-type HIV-1_{HXB2}, ABC and LAM selected for the mutations M184V/I by passage 3/4, K65R by passage 5, and Y115F by passage 7 similar to selections with ABC alone, which selected for M184V by passage 4/5 and L74V or K65R or Y115F by passage 6-10.

***In Vitro* Resistance and Cross-Resistance**

[redacted] databases of patient samples with genotypic and phenotypic information were analyzed to evaluate the effects of ABC/LAM-associated mutations on resistance phenotypes of other NRTIs, and the NNRTIs. In addition, the effects of other NRTI mutations, NNRTI mutations and PI mutations were evaluated on the susceptibility of ABC and LAM. The total number of samples in the database was >10,000. Resistance cutoffs were biologically and clinically defined. Biological cutoffs were used for zalcitabine, zidovudine, DLV, EFV, NVP, APV, IDV, NFV and SQV, because clinical cutoffs have not been defined. Clinical cutoffs were used for ABC, didanosine, LAM, stavudine, and tenofovir.

Table 3. The Effect of ABC/LAM Mutations and TAMs on ABC and LAM Resistance Phenotypes

	[1] number of samples with					ABC (cutoff: 4.5; max 70)				3TC (cutoff: 3.5; max 200)			
	[2]ZDV	TFV	T215F	T215Y	T215F/Y	mean	med	min - max	SD	mean	med	min - max	SD
ABC/3TC mutations													
65R alone[3]	82	80	0	0	0	2.5	2.5		0.6	9.3	9.3		3.6
65R + 184V alone	54	54	0	0	0	7.2	6.9		1.6	200	200		0.0
74V alone	22	22	0	0	0	1.5	1.6		0.4	1.4	1.4		0.5
74V + 184V alone	74	68	0	0	0	5.6	5.4		1.2	200	200		0.0
115F alone	0	0	0	0	0	na	na		na	na	na		na
184V alone	1720	1521	0	0	0	2.8	2.8		0.7	200	200		8.0
184I alone	27	27	0	0	0	1.6	1.6		0.4	193	200		27.3
Thymidine analogue mutations[4]													
215F/Y - 184V	94	86	26	68	0	1.7	1.7		0.4	1.8	1.7		0.5
215F/Y + 184V	114	100	30	84	0	4.6	4.4		1.2	200	200		0.0
67N,70R - 184V	19	19	0	0	0	1.2	1.1		0.3	1.9	1.8		0.5
67N,70R + 184V	30	18	0	0	0	3.6	3.4		0.9	200	200		0.0
41L, 210W, 215F/Y - 184V	110	101	7	103	0	3.0	2.9		0.9	2.6	2.4		1.0
41L, 210W, 215F/Y + 184V	204	181	6	198	0	6.0	6.1		1.3	199	200		9.4
41L, 215F/Y - 184V	106	92	18	88	0	2.1	2.0		0.7	1.8	1.7		0.6
41L, 215F/Y + 184V	269	245	38	231	0	4.7	4.5		1.1	200	200		7.0
67N, 70R, 215F - 184V	1	1	1	0	0	2.8	2.8		na	7.3	7.3		na
67N, 70R, 215F + 184V	0	0	0	0	0	na	na		na	na	na		na
67N, 70R, 219 - 184V	83	71	0	0	0	2.1	1.8		1.2	2.4	2.1		1.0
67N, 70R, 219 + 184V	181	158	0	0	0	3.9	3.8		0.9	200	200		0.0
67N, 70R, 215F/Y, 219 - 184V	50	45	42	8	0	2.6	2.4		0.8	4.1	3.6		1.9
67N, 70R, 215F/Y, 219 + 184V	72	67	52	20	0	5.4	5.3		1.2	200	200		0.0

1. Subheadings indicate the number of samples with data available for the indicated drug, or containing the indicated mutations.
2. The number under ZDV equals the total number of samples for a given query.
3. Alone means in the absence of any NRTI selected mutation other than those specified.
4. Thymidine analog mutations (TAMs): 41L, 67N, 70R, 210W, 215FY, 219X. Mixtures were excluded for all specified mutations, and groups that specify "T215F/Y" have either F or Y but not both.
5. Red cells indicate values above the cutoff for a given drug. ABC and 3TC cutoffs are clinical cutoffs.
6. na = not applicable

The results indicated that the combination of ABC/LAM will exhibit decrease susceptibility on viruses with the mutations K65R with or without the M184V/I mutation, L74V+ M184V/I, and with TAMs (M41L, D67N, K70R, L210W, T215Y/F, K219X) + M184V (Table 3). One isolate with TAMs (D67N, K70R, T215F) without M184V showed 7.3-fold decreased susceptibility to LAM. ABC/LAM did not show resistance to viruses with NNRTI-associated mutations K103N, G190S/A, Y181C/I or Y188L or to viruses with PI-associated mutations D30N, L90M, I50V, I54X, V82X, I84V, or M46I/L.

In addition to ABC and LAM, the K65R mutation conferred cross-resistance to didanosine, stavudine, tenofovir and zalcitabine, and the L74V+M184V mutations conferred cross-resistance to didanosine and zalcitabine.

4.3. Clinical Studies

Safety

Based on the 48-week data submitted in CNA30021, ABC OAD, in combination with other antiretrovirals, has a safety profile that is acceptable and in general similar to that of ABC BID. The incidences of adverse events (AEs), treatment-emergent AEs, and severe or serious AEs were similar between the ABC OAD + 3TC + EFV and ABC BID + 3TC + EFV treatment groups with the following exception: the OAD arm had significantly more severe ABC hypersusceptibility reaction (HSR) (5% versus 2%) and diarrhea (2% versus

0) AEs than the ABC BID arm. HSR is the most serious of the listed and expected adverse events associated with ABC. In CNA30021, HSR was reported at a slightly higher rate (9% in the OAD arm, 7% in the BID arm) than the labeled rate of 5%. This rate is consistent with rates of 8% observed in each of the two pivotal studies supporting traditional approval of ABC. The safety results demonstrated that both regimens were well tolerated, and safety profiles were comparable over 48 weeks of randomized treatment exposure. For an in depth review of safety, see Dr. James clinical review.

Efficacy
For study CNA30021, the efficacy analyses conducted by the applicant and confirmed by the FDA clinical/statistical review team concluded that overall ABC OAD was non-inferior to ABC BID in treatment-naïve subjects when each treatment was given in combination with 3TC and EFV over a 48 week study period. The two arms had a similar number of virologic responders and virologic failures when evaluating the ITT population, however, the reasons for non-virologic failures differed slightly between the two arms: the ABC OAD arm had slightly more subjects deemed failures due to discontinuations because of adverse events (13% vs 11%), while the ABC BID arm had more subjects deemed treatment failures due to discontinuations for reasons other than adverse events (13% vs 11%). Overall, based on the ITT population and the primary analysis of TLOVR there was no apparent difference in the response rates of the ABC OAD arm versus the ABC BID arm (64% and 65% respectively). No statistically significant difference was seen between the unstratified groups for the primary endpoint of VL < 50 copies/mL in the ITT or As Treated group. For an in depth review of efficacy, see Dr. James clinical review.

4.4 Clinical Microbiology

The sponsor performed phenotypic and genotypic analyses on data collected during the course of clinical study CNA30021. CNA30021 is a phase III, 48-week, randomized, double-blind, multicenter study to evaluate the safety and efficacy of abacavir (ABC) 600 mg OAD versus abacavir 300 mg BID, in combination with lamivudine (LAM) (300 mg OAD) and efavirenz (EFV) (600 mg OAD) in antiretroviral therapy-naïve HIV-1 infected subjects.

A total of 730 subjects (365 subjects per treatment group) with HIV infection were planned for enrollment into this study. A total of 384 subjects received ABC OAD treatment and 386 received ABC BID treatment. A virology substudy included a random sample of 196 subjects and all virologic failures identified by the Time to Loss of Virologic Response (TLOVR) algorithm (Table A). The random sample was selected retrospectively to assess the baseline viral genotype and was stratified by treatment and randomization strata. There were 38 evaluable isolates of virologic failures with baseline and post-baseline genotypes and 35 virologic failure isolates with both genotypic and phenotypic data. For the purposes of genotypic and phenotypic analyses, patients #53385, #51676, and # 52643 in the ABC BID arm were included in the virologic failures group.

Both the random sample and virologic failure populations were predominantly infected with group M clade B HIV-1. The non-B population comprised only 6-7% of the populations in both the random sample (n = 13) and virologic failures (n = 5). The five non-clade B virologic failures were all non-responders and were of different clades including A/AD/AC, 06-CPX/GK/02AG, F1/BF, F1/F2/BF and F1 (Table 4). Other isolates that were in the F1, F1/BF or B/F clades did respond to therapy (Table 4). The sample size of each clade is too small to draw any real conclusions regarding clade and response to treatment.

Table A.

ABC OAD (n=384)	ABC BID (n=386)
Virology substudy (n = 196)	
97	99
Treatment failures (n = 73)	
38	35
Evaluable failures isolates with baseline and post-baseline genotypes (n = 38)	
18	20
Failures with both Genotypic and Phenotypic data (n = 35)	
18	17

Table 4.

Subject	Clade	Outcome	Treatment
52613	06-CPX	Adverse event	ABC BID+3TC+EFV
51029	C	Censored, Responder	ABC BID+3TC+EFV
51119	C	Censored, Responder	ABC BID+3TC+EFV
51878	B/F	Censored, Responder	ABC BID+3TC+EFV
52224	F1	Censored, Responder	ABC BID+3TC+EFV
52784	C/AC	Censored, Responder	ABC BID+3TC+EFV
51128	A/AD/AC	Never Suppressed through week 48	ABC BID+3TC+EFV
52247	F1	Never Suppressed through week 48	ABC BID+3TC+EFV
52269	F1/BF	Never Suppressed through week 48	ABC BID+3TC+EFV
51882	B/F	Adverse event	ABC OAD+3TC+EFV
52846	C/AC	Adverse event	ABC OAD+3TC+EFV
51420	C/AC	Censored, Responder	ABC OAD+3TC+EFV
51908	C/AC	Censored, Responder	ABC OAD+3TC+EFV
52101	F1/BF	Censored, Responder	ABC OAD+3TC+EFV
51538	C/AC	Change of ART	ABC OAD+3TC+EFV
52246	F1/F2/BF	Never Suppressed through week 48	ABC OAD+3TC+EFV
52958	06-CPX/GK/02AG	Never Suppressed through week 48	ABC OAD+3TC+EFV
51392	C/AC	Other	ABC OAD+3TC+EFV

The TLOVR analysis is an intent-to-treat analysis that examines endpoints using the following definitions of treatment failure for patients who have achieved HIV RNA levels below the limit of quantification:

For all subjects with confirmed HIV RNA levels below an assay limit, the time to failure is the earliest time when a specific event had occurred. These events are

- Death
- Permanent discontinuation of the study drug or loss to follow-up
- Confirmed HIV RNA levels above or equal to an assay
- Introduction of a new ARV drug (unless a background drug is changed or reasons of toxicity or intolerance that are clearly attributable to that drug)

Endpoint	N =
Adverse event	33
Change of ART	1
Clinical progression	1
Consent withdrawn	4
Lost to follow-up	8
NON-RESPONSE	76
No PCR available at	2
Other	9
Protocol violation	1
REBOUND	29
RESPONDER	123

The number of patient isolates in the virologic failures group with NRTI- or NNRTI-associated resistance mutations at baseline was not significantly different between arms with eight (8/18, 44%) in the OAD arm and five (5/20, 25%) in the BID arm ($p = 0.21$).

Phenotypic Analysis

There were 35 evaluable treatment failure isolates with both genotypic and phenotypic data (18 in OAD regimen and 17 in BID regimen). In the ABC OAD regimen, 39% (7/18) of the failure isolates were resistant to ABC and 56% (10/18) were resistant to LAM (Table B). In the ABC BID regimen, 29% (5/17) of the failure isolates were resistant to ABC and 41% (7/17) were resistant to LAM. Resistance is defined as greater than a 2.5-fold change in ABC or LAM susceptibility compared to a reference strain using the PhenoSense^{TM} phenotypic assay. The difference in proportions of ABC resistant isolates was not significantly different between the ABC OAD and BID regimen ($p = 0.55$) using normal approximation to the binomial without a continuity correction. The median fold change in ABC susceptibility of the failure isolates was 1.3 and 0.9 in the OAD and BID arms, respectively. There was not significant difference in the proportion of LAM-resistant isolates in the OAD arm (56%) compared to the BID arm (41%) ($p = 0.39$). The

median fold change in LAM susceptibility of the failure isolates was significantly different between arms with values of 81 and 1.1 in the OAD and BID arms, respectively.

Table B. Evaluable Clinical Isolates from Patients on ABC/LAM-Containing Regimens Who Experienced Virologic Failure or Discontinued before Suppression

	ABC OAD		ABC BID
Evaluable isolates	18		17
# ABC >2.5-fold	7 (39%)[1]		5 (29%)[1]
ABC FR Mean	2.7		2.6
ABC FR Median	1.3		0.9
# LAM >2.5-fold	10 (56%)[2]		7 (41%)[2]
LAM FR Mean	55		40.4
LAM FR Median	81		1.1

FR = fold resistance from reference at endpoint
[1]ABC >2.5-fold (P value = 0.55) [Difference in proportions using normal approximation to the Binomial without a continuity correction]
[2]LAM >2.5-fold (P value = 0.39) [Difference in proportions using normal approximation to the Binomial without a continuity correction]

Genotypic Analysis
In the treatment failure population, the number of subjects with treatment emergent resistance mutations in OAD ABC arm was 13/18 (72%) compared to 16/20 (80%) in the BID ABC arm (p = 0.57) (Appendix I and II). The main resistant mutations were M184V/I (n= 18) and K103N (n = 19) (Table C). Eight isolates (21%) had the ABC-resistance mutation, L74V, develop on therapy. Only one isolate developed the K65R mutation and two isolates developed Y115F. ABC-resistant associated mutations, L74V, M184V/I, K65R, and\or Y115F developed in 11 of 18 isolates (61%) in the OAD arm and 8 of 20 isolates (40%) in the BID arm (p = 0.19) (Table D).

Lamivudine-resistant associated mutation M184V or I developed in 10 isolates (56%) in OAD arm and 8 patient isolates (40%) in the BID arm (p = 0.34) (Table D). The K103N mutation, associated with EFV-resistance, developed in 7 patient isolates (39%) in the OAD arm and 12 isolates (60%) in the BID arm (p = 0.19). NNRTI-resistant mutations including K103N, K101E/N/Q, G190S/A, V179D, Y181C, Y188L/C/F/H, L100I, and/or V106I developed in 11 isolates in the OAD arm and 16 patient isolates in the BID arm (Table D).

Overall, the phenotypic and genotypic data suggested a slight increase in ABC and LAM resistance and resistance mutations in the OAD arm compared to the BID arm, but this difference was not significant. Interestingly, the number of NNRTI-associated resistance mutations that developed in the OAD arm (61%) was less than in the BID arm (80%) (p = 0.20). These small differences are not explained by the number of NRTI- or NNRTI-

associated resistance mutations present in the patient isolates at baseline in the two arms. In the OAD arm, 6 of 8 (75%) isolates had baseline NNRTI resistance mutations (*i.e.*, K103N, V108I, V179D, Y188L, and G190A), and in the BID arm, 3 of 5 (60%) patient isolates had baseline NNRTI resistance mutations (i.e., Y188L, V179D). The number of NRTI-associated resistance mutations (M41L, L210F/S/M) at baseline was 4 of 8 (50%) in the OAD arm and 3 of 5 (60%) in the BID arm. The small number of isolates in the resistance analysis makes it difficult to make any definitive conclusions, but the similar efficacy at 48 weeks and similar ABC/LAM resistance rates between the two arms supports the approval of OAD abacavir. Long term resistance monitoring > 48 weeks might be needed to demonstrate that OAD ABC/LAM is as durable as BID ABC/LAM.

Table C. Mutations Developing in the HIV of Patients who Experienced Virologic Failure or Discontinued before Suppression on ABC/LAM Treatment

Mutation	ABC OAD (n = 18)	ABC BID (n = 20)
L74V	5	3
M184V/I	10	8
Y115F	1	1
K101E/N/Q	0	5
K103N/R	7	12
G190A/S	2	1
V179D	3	1
Y181C	0	2
Y188L/C/F/H	1	2
L100I	2	0
V106I	1	1
K65R	1	0
H221Y	2	1
ABC-associated mutations	17	12
LAM-associated mutations	10	8
EFV-associated mutations	16	24

Table D. Number of Virologic Failure Isolates Developing Mutations on ABC/LAM Treatment

# Patient Isolates Developing	ABC OAD (n = 18)	BC BID (n = 20)	p value
ABC Mutations	**11 (61%)**	**8 (40%)**	**0.19**
M184V/I	**10 (56%)**	**8 (40%)**	**0.34**
NNRTI Mutations	**11 (61%)**	**16 (80%)**	**0.20**

Cross-Resistance of Clinical Isolates

All the ABC- and LAM-resistant isolates from the virologic failure subgroup retained susceptibility to stavudine, tenofovir, and zidovudine. There was some cross-resistance to didanosine (ddI) and zalcitabine (ddC). The 10 ABC-resistant isolates from virologic failure group were all resistant to LAM with a median fold change in LAM resistance of >98 and 10% were resistant to ddC and ddI (Table E). Of the 14 LAM-resistant isolates from the virologic failure group, 70% were also resistant to ABC with a median fold change in ABC resistance of 3.9 and 7% were resistant to ddC and ddI (Table F).

Table E. Cross-Resistance of ABC Resistant Isolates (>2.5-fold) from Virologic Failures in Study CNA30021 (N = 10)

	LAM	ddC	ddI	d4T	TDF	ZDV
% of resistant isolates	100%	10%	10%	0%	0%	0%
Mean fold-change in susceptibility	>99	1.9	1.9	0.93	0.5	0.6
Median fold-change in susceptibility	>98.2	1.9	1.7	0.9	0.5	0.3

Mean fold change for ABC = 6; median = 4.8 for ABC-resistant isolates

Table F. Cross-Resistance of LAM Resistant Isolates (>2.5-fold) from Virologic Failures in Study CNA30021 (N = 14)

	ABC	ddC	ddI	d4T	TDF	ZDV
% of resistant isolates	71%	7%	7%	0%	0%	0%
Mean fold-change in susceptibility	4.8	1.8	1.7	0.9	0.5	0.5
Median fold-change in susceptibility	3.9	1.8	1.6	0.8	0.5	0.4

Mean fold change for LAM > 98; median > 99 for LAM-resistant isolates

Response Based on Baseline Genotype

The number of patient isolates in the virologic failures group with NRTI- or NNRTI-associated resistance mutations at baseline was not significantly different between arms with eight (8/18, 42%) in the OAD arm and five (5/20, 25%) in the BID arm (p = 0.21).

There were no isolates that contained the K65R or Y115F mutations at baseline. There was only one isolate with L74I/L at baseline and it was from a non-responder. Two isolates contained the M184V mutation at baseline, one that had a mixture at baseline and responded to therapy. Nine isolates contained the K103N, but interestingly even though EFV was part of the regimen, this mutation did not appear to significantly affect response since six (67%) isolates where from responders (Table G). However, when these nine isolates are divided into dose group, OAD or BID, 100% of the isolates with the K103N mutation at baseline in the BID arm responded compared to 40% in the OAD group (p = 0.07) (Table H). The three isolates with K103N at baseline in the OAD dose group that did not respond to the regimen all developed the L74V mutation and the M184V mutation and one isolate also developed the K65R mutation (Appendix I).

Eighteen isolates contained NAMs (substitutions at M41, D67, K70, L210, T215, K219) at baseline, but there was no difference in the response with 44% of these isolates responding to the therapy and 56% not responding (Table G). Additionally, there was no difference between the dose groups of isolates containing NAMS at baseline with 40% responding in the OAD arm compared to 50% in the BID arm.

Table G. Response Based on Baseline Genotype

Baseline Mutation	Non-responders	Responders	n
K65R	0	0	0
Y115F	0	0	0
L74I/L	1	0	1
M184V	1	1 (V/M)	2
K103N	3 (33%)	6 (67%)	9
NAMs[1]	10 (56%)	8 (44%)	18

[1]NAMS include mutations M41, D67, K70, L210, T215, K219

Table H. Response from Each Dose Group by Baseline Genotype

	OAD		BID	
Baseline Mutation	**Nonresponders**	**Responders**	**Nonresponders**	**Responders**
K103N/R	3 (60%)	2 (40%)	0	4 (100%)
NAMs[1]	6 (60%)	4 (40%)	4 (50%)	4 (50%)

[1]NAMS include mutations at amino acids M41, D67, K70, L210, T215, K219

Given that study CNA30021 was a treatment-naïve study, the numbers of isolates with mutations at baseline are too small to make any conclusions on response rates of ABC/LAM.

Conclusion: The similar efficacy at 48 weeks and the similar ABC/LAM resistance rates between the two arms supports the approval of OAD ABC/LAM (Ezicom). Long term resistance monitoring > 48 weeks might be needed to demonstrate that OAD ABC/LAM is as durable as BID ABC/LAM. This NDA-21652 for the combination of once daily 600 mg ABC/300 mg LAM is approvable with respect to microbiology for the treatment of HIV with other antiretroviral drugs.

6 Page(s) Withheld

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

_____ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Withheld Track Number: Microbiology- 141

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
8/2/04 11:53:54 AM
MICROBIOLOGIST
microbiology review

Julian O Rear
8/2/04 11:59:07 AM
MICROBIOLOGIST

James Farrelly
8/2/04 12:18:02 PM
PHARMACOLOGIST