CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-977
20-978

MICROBIOLOGY REVIEW(S)

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

NDA # 20-977

REVIEWER	: LALJI MISHRA, Ph.D.
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SUBMISSION REVIEWED: 000

DRUG CATEGORY: Antiviral

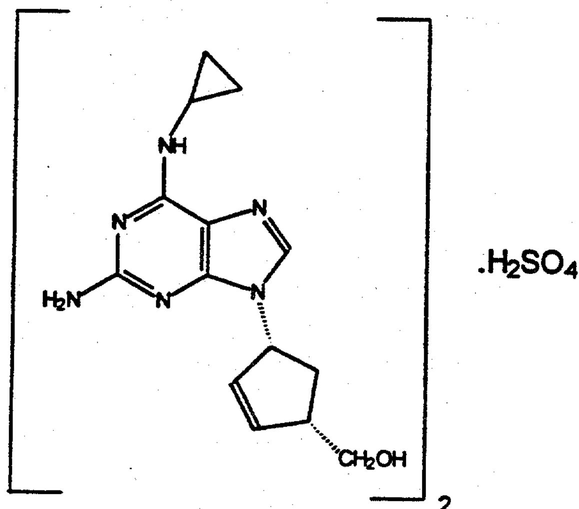
INDICATION: Treatment of HIV-1 infection

DRUG FORM: Tablet

PRODUCT NAME:

a. PROPRIETY: Ziagen ™
b. NON-PROPRIETY: Abacavir sulfate
c. CHEMICAL: [(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl)-2-cyclopenetene-1-methanol

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS:

BACKGROUND:

Glaxo Wellcome Inc. has developed a new antiretroviral drug abacavir sulfate (new chemical entity), and seeks marketing approval (NDA # 20-977) for use of abacavir sulfate in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection in adults and pediatric (≥3 months) patients. Abacavir sulfate ([(1S,cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl)-2-cyclopenetene-1-methanol sulfate (salt) (2:1) is a synthetic carbocyclic nucleoside analogue, and inhibits HIV-1 reverse transcriptase activity. Like other approved nucleoside analogue reverse transcriptase inhibitors (NRTIs), abacavir sulfate has been demonstrated to exhibit anti-HIV-1 activity both in vitro and in vivo. The sponsor has conducted numerous phase II/III clinical trials, and used reductions in viral load and increases in CD4 cells as surrogate markers for efficacy.

Three classes of drugs have been approved by the FDA for the treatment of HIV-1 infection. These are NRTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and HIV-1 protease inhibitors (PIs). Both NRTIs and NNRTIs inhibit HIV-1 reverse transcriptase. Reverse transcriptase plays a pivotal role in the early stages of HIV-1 replication in that it is essential for the synthesis of viral DNA. By inhibiting HIV-1 reverse transcriptase activity, both NRTIs and NNRTIs block the synthesis of HIV-1 viral DNA, thereby inhibiting HIV-1 replication.

The FDA approved NRTIs are didanosine (ddI), lamivudine (3TC), stavudine (d4T), zalcitabine (ddC), and zidovudine (ZDV). Delavirdine (DLV), nevirapine (NVP) and efavirenz (EFV) are the FDA approved NNRTIs. The FDA approved PIs are indinavir (IDV), ritonavir (RTV), nelfinavir (NLV) and saquinavir (SQV). HIV-1 protease activity is needed for proteolytic processing of the gag (Pr 55 ^{gag}) and gag-pol polyprotein precursors (Pr 160 $^{gag\text{-}pol}$). By inhibiting HIV-1 protease activity, PIs interefere with processing of the immature HIV-1 virion to mature infectious virus. Consequently, non-infectious HIV-1 particles are produced.

HIV-1 is a causative etiologic agent of acquired immune deficiency syndrome (AIDS) in humans. HIV-1 has a diploid RNA genome of 9.7 Kilobase (kb). The HIV-1 genome encodes for viral proteins; Gag, Pol, Env, Vif, Vpu, Vpr, Tat, Rev, and Nef. The target cells for HIV-1 infection include T-lymphocytes, monocytes/macrophages, glial cells, langerhans cells, and bone marrow progenitor cells. CD4 is the primary cell-surface receptor for HIV-1, facilitating cellular infection via endocytosis, and is found on T-lymphocytes, monocytes/macrophages and microglia cells. In addition to the CD4 receptor, several co-receptors for HIV-1 cellular infection have been recently identified (Deng et al., 1996; Chan and Kim, 1998; Litman, 1998). These co-receptors are characterized as chemokine receptors.

The major co-receptors used by monocyte/macrophage tropic HIV-1 are CCR3 and CCR5. The co-receptor CXCR4 is used by T-cell tropic HIV-1. The infection of cells with HIV-1 begins with the attachment of envelope protein gp120 to CD4 receptors. After binding of gp120 to CD4, the V3 loop of gp120 interacts with the appropriate co-receptors (CCR3, CCR5 or CXCR4). Subsequently, the HIV-1 envelope protein gp41 undergoes a conformational change and consequently mediates the fusion of the viral envelope with the host cell membrane. After entry into the cell, HIV-1 virion is uncoated, and viral replication begins. First, viral genomic RNA is copied into first strand complimentary DNA (c-DNA) by the viral reverse transcriptase. Ribonuclease H coded by the HIV-1 Pol gene, partially degrades the original RNA template bound to the first strand DNA. The synthesis of second strand DNA is also carried out by the viral reverse transcriptase. The double strand viral DNA is translocated to the host cell nucleus and is inserted into the host chromosome by viral integrase. After integration into the cell genome, the viral DNA (proviral DNA) perpetuates like cellular genes through mitotic activity, and is maintained in progeny cells after cell division. Cellular RNA polymerase II transcribes proviral DNA to produce genomic and subgenomic viral mRNAs. HIV-1 viral mRNAs are grouped as early and late mRNAs based on their temporal expression during the HIV-1 replication cycle. The early viral mRNAs consist of the multiply spliced ~2 kb mRNA species that encode the viral regulatory proteins Tat, Nef, and Rev (Cullen 1991; Haseltine, 1991). The late viral mRNAs consist of the unspliced (~9 kb) and singly spliced (~4kb) transcripts that encode the virion structural proteins, Gag-Pol, and Env, respectively. Genetic analyses have shown that Rev gene product is absolutely required for the production of unspliced HIV-1 RNA species. In the absence of functional Rev protein, only the fully spliced class of HIV-1 mRNAs are found (Cullen, 1991; Haseltine, 1991). The HIV-1 mutants lacking the Rev gene fail to properly express the Gag-Pol structural genes and are thus replication defective (Cullen, 1991).

The major virion structural proteins Gag, Pol (virion enzyme), and Env (virion envelope protein) are initially translated into large polyprotein precursors Pr 55 ^{gag}, Pr 160 $^{gag\text{-}pol}$, and gp 160 env from a polycistronic mRNA (Debouck and Metcalf, 1990; Huff 1991; Meek 1992). These polyprotein precursors are subsequently proteolytically processed to yield the mature structural proteins and enzymes of the virion. The precursor polyprotein Pr 55 ^{gag} is processed to yield the viral structural proteins p17, p24, p7, and p6. The gag-pol fusion polyprotein, Pr 160 $^{gag\text{-}pol}$ is processed to yield gag structural proteins followed by the protease (PR), reverse transcriptase, (RT), ribonuclease H (RNaseH) and integrase (IN). The processing of Pr 55 ^{gag}, and Pr 160 $^{gag\text{-}pol}$ is carried out by the viral protease. The HIV-1 protease is generated by self cleavage of the gag-pol polyprotein precursor. The gp 160 envelope polyprotein is processed into gp120 and gp 41 transmembrane envelope protein by trypsin like cellular proteases.

Abacavir sulfate is also known as 1592U89. Most of the virologic preclinical studies refer to abacavir as 1592U89. Therefore, in this review the name 1592U89 will be used interchangeably with abacavir when referring to the study drug. Abacavir sulfate (1592U89 hemisulfate), the (-) enantiomer but not the (+) enantiomer of 1592U89, has

anti-HIV-1 activity. The (+) enantiomer of 1592U89 was inactive at 200 uM (Daluge et al., 1997). Abacavir is enantiomerically pure with the 1S, 4R absolute configuration on the cyclopentene ring.

SUMMARY:

The sponsor has provided data on the metabolism and mechanism of action of abacavir, effects of abacavir on HIV-1 reverse transcriptase and cellular DNA polymerase activities, and antiviral activity of abacavir in vitro. Additionally, effects of abacavir on cellular growth and cellular DNA synthesis, on bone marrow progenitor cells, and telomerase activity and telomere length were also studied. Abacavir's ability to select for HIV-1 variants that are less susceptible to the drug activity was also evaluated in in vitro and in clinical studies. The microbiologic relevant data from these pre-clinical studies are presented and reviewed below.

I. **Metabolism of abacavir (1592U89):**

Abacavir (1592U89) is a carbocyclic nucleoside analogue. In order for a nucleoside analogue to exhibit antiviral activity, the compound must first be phosphorylated. In CEM cells, 1592U89 enters by non-facilitated diffusion (Faletto et al., 1997). Cell culture studies on the metabolism and phosphorylation of 1592U89 are described below.

I (a). **Intracellular metabolism of 1592U89 in CEM cells:**

The anabolism of 1592U89 was studied in CEM cells and was compared to that of its guanine analogue 1144U88 (1R, 4S)-9-[4-(hydroxymethyl)-2-cyclopenten-1-yl] guanine (carbovir [CBV]). CEM cells were treated with 10 uM [^{3}H] 1592U89 or [^{3}H] CBV for 3 to 72 hours. Cellular extracts were prepared at designated time points and analyzed for mono-, di- and triphosphates of respective compounds by reversed phase high performance liquid chromatography (RP-HPLC) as described by Faletto et al., 1997. The amount of intracellular metabolites produced in CEM cells treated for 24 hours with either 10 uM [^{3}H] 1592U89 or [^{3}H] CBV is shown in Table 1.

Results from Table 1 show that both 1592U89 and CBV produced carbovir 5'-monophosphate (CBV-MP), carbovir 5'-diphosphate (CBV-DP), and carbovir 5'-triphosphate (CBV-TP). CBV-TP was the predominant phosphorylated form. 1592U89-MP was only detected in 1592U89-treated cells. However, neither 1592U89-DP nor 1592U89-TP was detected in cells treated with 1592U89. The amount of 1592U89-MP was eight-fold higher than the levels of CBV-MP detected in 1592U89 treated CEM cells. In addition, amino CBV and its MP (amino CBV-MP) were also detected in CEM cells following incubation with 1592U89.

Table 1: Levels of intracellular anabolites in CEM cells incubated with 10 uM [^{3}H] 1592U89 or [^{3}H] CBV for 24 hours

Compound	Mean amt (pmol/10^6 cells) ± SEM from	
	1592U89	CBV
1592U89	17 ± 2	ND
Nucleobase [(a)]	ND	ND
1592U89-MP	0.073 ± 0.008	ND
1592U89-DP	ND	ND
1592U89-TP	ND	ND
AminoCBV	0.18 ± 0.02	ND
AminoCBV-MP	0.10 ± 0.03	ND
CBV	0.22 ± 0.04	18 ± 2
CBV-MP	0.009 ± 0.003	0.053 ± 0.008
CBV-DP	0.083 ± 0.026	0.13 ± 0.03
CBV-TP	0.16 ± 0.02	0.33 ± 0.06

ND = not detected
(a) = 2-Amino-6-(cyclopropylamino)purine from 1592U89 or guanine from CBV

These data provide evidence that 1592U89 is phosphorylated to CBV-TP, similar to triphosphate metabolites of other nucleoside analogues. However, cellular enzymes involved in the phosphorylation of 1592U89 are different than those used for the phosphorylation of other known nucleoside analogues (ZDV, ddI, 3TC, ddC, d4T). 1592U89 is phosphorylated to its 5'-monophosphate by the cellular enzyme adenosine phosphotransferase. Subsequently, 1592U89-MP is deaminated to 1144U88 5'-monophosphate (CBV-MP) by a cytosolic deaminase. CBV-MP is then further converted to di- and triphosphate forms by cellular kinases (Faletto et al., 1997). Additional studies with the metabolic inhibitor dCF (adenosine/adenylate deaminase inhibitor) or EHNA (adenosine deaminase inhibitor) demonstrated that 1592U89-MP is deaminated prior to conversion to CBV-MP (Faletto et al., 1997). dCF completely inhibited the formation of CBV-TP from 1592U89 whereas EHNA did not inhibit CBV-TP formation from 1592U89.

I (b). **Intracellular half-life of carbovir -5'-triphosphate**: (Faletto, 1994, NDA20-977; vol 4.1:180-197. Glaxo-Wellcome Report # TEIN/94/0009).

The purpose of this experiment was to determine the intracellular half-life of CBV-TP. CEM cells incubated with 10 uM of ^{3}H-1592U89 or ^{3}H-CBV for 48 hours accumulated CBV-TP levels of 0.22 and 0.30 pmol/10^6 cells, respectively. After 48-hours incubation, cells were washed, resuspended in drug-free medium, and harvested at various times following the washout. The intracellular levels of CBV-TP were determined by strong anion-exchange (SAX) HPLC of cellular extracts. The intracellular half-life of CBV-TP produced from both CBV and 1592U89 was estimated to be 3.3 hours in CEM cells.

I (c). **Metabolism of 1592U89 in animals**:

Abacavir is rapidly absorbed in mice and monkeys following oral administration with an absolute oral bioavailability of greater than 76% (NDA 20-977; vol l : 94). The absolute oral bioavailability in humans was 83%. Similarly, the absolute bioavailability for the tablet was 86% (NDA 20-977; vol l: 131). The metabolism of abacavir is similar in mice, monkeys and humans. Abacavir is metabolized in liver by alcohol dehydrogenase and UDP-glucuronyltransferase. The major metabolites are 5'-carboxylate and 5'-glucuronide (NDA 20-977; vol l : 134). In humans, a total of 83% of a dose is recovered in the urine (<2% as unchanged abacavir, 30% as 5'-carboxylate [2269W93] and 36% as 5'-glucuronide [361W94]). The remaining 15% of the dose found in the urine is minor metabolite. In addition, 16% of the dose was eliminated in feces (NDA 20-977; vol l : 134).

In humans at therapeutic dosages (300 mg twice daily given in tablet form), the steady-state peak plasma concentration (C_{max}) achieved was 3.0 ug/mL (10 uM). The AUC_{τ} was 6.02 ug.h/mL (NDA 20-977; vol l : 137). Single dose pharmacokinetic studies have shown that abacavir is rapidly absorbed after oral administration. The time to peak concentration (T_{max}) occurs 1 to 2 hours following dosing of abacavir tablets over a wide dose range, and within 0.5 to 1 hour following dosing with abacavir solution.

II. **Mechanism of action**:

The anti-HIV-1 activity of 1592U89 is mediated through a guanine analogue, 1144U88-TP (CBV-TP). CBV-TP inhibits the activity of HIV-1 reverse transcriptase both by competing with the natural substrate, deoxyguanosine 5'-triphospahte (dGTP), and by its incorporation into viral DNA. The lack of the 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation and, therefore, the viral DNA synthesis is terminated.

II (a). **Substrate and Inhibitor kinetics of CBV-TP (1144U88-triphosphate) with HIV-1 Reverse Transcriptase**:

The purpose of this experiment was to determine the K_m and K_i values of dGTP and carbovir triphosphate for purified HIV-1 reverse transcriptase using several template-primers (Daluge et al., 1997). Reaction mixtures, assay conditions, and methods of data analysis for determination of K_m and K_i values with different template primers were described previously (Reardon, 1993). To determine K_i values, the substrate concentration was held fixed and the inhibitor concentration was varied. Apparent K_i values were determined by nonlinear least squares fitting of the data. Results of these studies are shown in Table 2.

Table 2: Inhibition of HIV-RT by CBV-TP (1144U88-triphosphate)

Template-primer	Substrate	Inhibitor	K_i (uM)	K_m (uM)	K_i/K_m
r44:d22	dGTP	CBV-TP	0.54 ± 0.08	0.15	3.6
poly (rC)-oligo(dG)	dGTP	CBV-TP	0.04 ± 0.01	12	0.0033
Calf thymus DNA	dGTP	CBV-TP	0.021 ± 0.003	0.26	0.083

In another study, Parker et al. (1991) determined the effect of CBV-TP and 2', 3'-dideoxyguanosine 5'-triphosphate (ddGTP) on the rate of incorporation of deoxynucleotides by HIV-1 reverse transcriptase using 16 S ribosomal RNA (rRNA) from E. coli as a template. Results showed that the rate of incorporation of deoxynucleotides by HIV-1 reverse transcriptase in the presence of inhibitory concentrations of CBV-TP or ddGTP was linear and that CBV-TP was a potent inhibitor of HIV reverse transcriptase using the rRNA template. The K_i/K_m ratios for CBV-TP and ddGTP were less than 1, indicating strong binding of the inhibitors to HIV-1 reverse transcriptase in the presence of substrate.

III. Effect of CBV-TP (1144U88-TP) on Human DNA polymerases α, β, γ and ε:

Human polymerases α, β and ε play an important role in the replication and repair of cellular DNA. DNA polymerase γ is involved in the synthesis of mitochondrial DNA. The objective of this study (Reardon, 1994) was to determine the effect of CBV-TP on the activities of cellular polymerases. Polymerases α, β, γ, and ε were purified from HeLa cells by a modification of published procedures (Parker et al., 1991). The K_m value for each nucleotide substrate was determined with the other three nucleotide substrates at 50 uM. Similarly, K_i values for nucleotide analogues were determined with the non-competing nucleotide substrates at 50 uM. To determine K_i values, the substrate concentration was held fixed and the inhibitor concentration was varied. Apparent K_i values were determined by non-linear least squares fitting of the data. K_i values for CBV-TP and ddGTP for cellular polymerases are shown in Table 3.

Based on the K_i/K_m ratio (Table 3) it appears that CBV-TP was a weak inhibitor of human cellular DNA polymerase α. However, it did not inhibit polymerases β, γ and ε. In contrast to CBV-TP, ddGTP was a strong inhibitor of DNA polymerases β and γ. DNA polymerase γ is involved in the synthesis of mitochondrial DNA. Compounds which inhibit mitochondrial DNA synthesis have been implicated to cause peripheral neuropathy.

Table 3: K_i values for CBV-TP and ddGTP

Enzyme	Inhibitor	K_i (uM)	K_i/K_m
Pol α	CBV-TP	6.9 ± 0.9	8
	ddGTP	27 ± 6	30
Pol β	CBV-TP	340 ± 30	240
	ddGTP	1.7 ± 0.3	1.2
Pol γ	CBV-TP	14 ± 2	100
	ddGTP	0.016 ± 0.002	0.12
Pol ε	CBV-TP	410 ± 80	164
	ddGTP	67 ± 9	27

The K_m values (uM) for dGTP for cellular polymerases were: $\alpha = 0.90 \pm 0.05$, $\beta = 1.4 \pm 0.1$, $\gamma = 0.14 \pm 0.01$, and $\varepsilon = 2.5 \pm 0.3$

Comments:

1. It appears that CBV-TP would not be cytotoxic since the K_i values of CBV-TP for cellular DNA polymerases are several fold higher than the respective K_m values. Although, the K_i/K_m ratio for cellular DNA polymerase α for CBV-TP suggested low level polymerase inhibition (Table 3), results from another study showed that cellular DNA polymerase α was not inhibited by CBV-TP (Parker et al., 1991).

2. The data from Table 2 and 3 show that K_i values of CBV-TP for HIV-1 reverse transcriptase and cellular DNA polymerase α were 0.021 uM and 6.9 uM, respectively using calf-thymus DNA as template-primer. Thus, the Ki value of CBV-TP for HIV-1 reverse transcriptase was 328-fold less than the K_i value for cellular DNA polymerase α. This observation suggests that carbovir triphosphate specifically inhibits HIV-1 reverse transcriptase.

IV. **Anti-HIV-1 activity of abacavir:**

IV (a). **Anti-HIV-1 activity of abacavir (1592U89) against HIV-1 III B and clinical isolates in vitro:**

The anti-HIV-1 activity of 1592U89 was assessed against a laboratory strain HIV-1 IIIB and several clinical isolates in PHA-stimulated peripheral blood lymphocytes (PBLs). PBLs from seronegative donors were infected with either HIV-1 IIIB at multiplicities of infection (m.o.i.) of 10 x tissue culture infective dose ($TCID_{50}$), or 100 x $TCID_{50}$, or with clinical isolates. Infected cells were treated with different concentrations of 1592U89 and

incubated for 7 days at 37°C. The anti-HIV-1 activity was determined by measuring the inhibition of supernatant reverse transcriptase activity. Results showed that the mean 50% inhibitory concentrations (IC50) values of 1592U89 against HIV-III B and eight clinical isolates were 3.7 ± 2.6 uM, and 0.26 ± 0.18 uM, respectively.

The anti-HIV-1 activity of 1592U9 against HIV-1 III B was also assessed in CD4+ CEM cells by measuring the inhibition of p24 antigen production, and in CD4+ HeLa cells by a plaque reduction assay. The IC 50 values of 1592U89 against HIV-1 IIIB in CD4+CEM cells and CD4+ HeLa cells were 3.8 uM and 5.8 uM, respectively.

IV (b). **Anti-HIV-1 activity of 1592U89 in MT-4 cells:**

MT-4 cells (cells transformed with human T cell lymphotropic virus type-1) were infected with HIV-1 IIIB at a m.o.i. of 10 x $TCID_{50}$. HIV-1 infected cells were incubated for 5 days in the presence of various concentrations of 1592U89. The anti-HIV-1 activity of 1592U89 was measured by the inhibition of HIV-1 induced cytopathic effect (CPE). The IC50 value of 1592U89 against HIV-1 IIIB was 4.0 ± 1.6 uM in MT-4 cells (NDA 20-977;vol 4.1, page 8, Table 3). The sponsor stated that 1592U89 was approximately 2-fold more potent when MT-4 cells were infected with HIV-1 at 10 x $TCID_{50}$ (IC50 = 4.6 uM) than when MT-4 cells were infected with 100 x $TCID_{50}$ (IC50 = 12 uM).

IV (c). **Anti-HIV-1 activity of 1592U89 against monocyte/macrophage tropic HIV-1:**

The IC50 of 1592U89 against a monocyte/macrophage tropic HIV-1 BaL strain varied from 0.07 to 1.0 uM using a p24 antigen inhibition assay (NDA 20-977; vol 4.1, page 17).

IV (d). **Antiviral activity of 1592U89 against drug -resistant HIV-1 strains:**

The antiviral activity of 1592U89 against ZDV, ddI, 3TC, ddC and NNRTI resistant HIV-1 was determined in CD4+ HeLa cells by a plaque reduction assay (inhibition of syncytia formation). These drug-resistant strains of HIV-1 were generated by a recombinant method (Daluge et a l., 1997) and their susceptibility (IC50) to NRTIs or NNRTIs has been previously determined (Table 4). Results on the susceptibility of drug-resistant HIV-1 to 1592U89 are shown in Table 4.

These results show that recombinant HIV-1 (cloned) viruses resistant to ZDV and NNRTIs are susceptible to 1592U89 in vitro. However, ddI and ddC resistant recombinant HIV-1 (HIV-1 74V) was cross-resistant to 1592U89. An HIV-1 recombinant virus carrying the M18V RT mutation was susceptible to 1592U89 in vitro. This mutation confers high level resistance to 3TC, and some degree of cross- resistance to ddI and ddC in vitro.

Table 4: Sensitivity of drug-resistant strains of HIV-1 to 1592U89

Virus clone	Known resistance's (fold IC50 increase)[a]	Mean 1592U89 IC50 ± SD (uM)	Fold change
HIV-1 HXB2	None (wild type)	5.8 ± 3.1	1.0
HIV-1 RTMN (41L/215Y)	ZDV (60-70)	11 ± 4	1.9
HIV-1 RTMC (67N/70R/215F/219Q)	ZDV (120)	13 ± 4	2.1
HIV-1 74V	ddI (5-10), ddC (16)	21 ± 8	3.6
HIV-1 181C	NNRTIs (>100)	9.6 ± 0.02	1.7
HIV-1 184V	3TC (>500), ddI & ddC (2-10)	13 ± 6	2.2

[a] = compared with wild type

IV (e). **Anti-HIV-1 activity of 1592U89 in combination with other anti-HIV-1 compounds**:

The anti-HIV-1 activity of 1592U89 in combination with other NRTIs (ZDV, ddI, ddC, 3TC, and d4T), and a NNRTI (nevirapine), and a protease inhibitor (141W94) was determined in MT-4 cells infected with HIV-1 IIIB using an MTT assay or the propidium iodide assay. Antiviral activities were determined for each compound alone, and in combination with 1592U89 and these data were used to determine fractional inhibitory concentrations (FIC). The FIC plot (not shown) revealed that 1592U89 in combinations with ZDV, nevirapine or 141W94 exhibited synergistic anti-HIV-1 activity. However, combinations of 1592U89 with ddI, ddC, 3TC or d4T exhibited additive anti-HIV activity in vitro (Daluge et al., 1997).

V. **Anti-HIV-1 activity of 1592U89 in SCID-hu mouse model**:

The in vivo anti-HIV-1 activity of 1592U89 was assessed in the SCID-hu mouse-model. In this model, human lymph nodes are surgically transplanted into the immunodeficient C.B-17 scid/scid mouse (NDA 20-977; vol 4.1 : 89). SCID-hu mice infected with HIV-1 develop viremia. SCID-hu mice were treated with 1592U89 at a dose of 300 or 150 mg/kg/day. The test compound 1592U89 was administered twice daily by intraperitoneal (IP) injection beginning 48 hours before HIV-1 infection and continued for sixteen days. SCID-hu mice were infected with 120,000 $TCID_{50}$ units of HIV-1 (JRCSF) 1 hour after dosing (150 mg/kg/dose or 75 mg/kg/dose). For comparison, ZDV was tested in parallel. ZDV was administered IP twice daily to give a total daily dose of 150 or 75 mg/kg/day. Viremia was assessed by polymerase chain reaction (PCR) amplification of viral RNA from plasma on day 14 post-infection. The results of this study showed that 1592U89 at a dose of 300 mg/kg/day prevented plasma viremia in 3/7 mice infected with HIV-1 (Rabin 1992, NDA 20-977, vol 4.1, pages 88-102). However, at a dose of 150 mg/kg/day, 1592U89 was not effective in preventing infection. Of those receiving

1592U89 at a dose of 150 mg/kg/day, only 1/7 mice was not viremic. Under similar experimental conditions, ZDV at doses of 150 mg/kg/day and 75 mg/kg/day suppressed HIV-1 infection in 6/7 and 4/7 mice, respectively. The equivalent daily doses in mice are estimated to be 12 times higher, on a mg/kg basis than those used in humans. These results suggest that in comparison to ZDV, 1592U89 exhibited lower antiviral activity in the SCID-hu mouse model.

VI. **In vitro cytotoxicity of abacavir (1592U89)**:

VI (a). **Effect of abacavir (1592U89) on cell growth**: (Daluge et al., 1997).

The purpose of this study was to determine the in vitro cytotoxicity of 1592U89 in various cell lines, and compare it with the cytotoxicity of ZDV, ddI and ddC. Three cell lines exhibiting T-cell markers (Molt-4, CEM, CD4+ CEM), and a cell line exhibiting B-cell markers (IM-9) were tested. Cells ($4x10^4$ cells/mL) were incubated in the presence of various concentrations of 1592U89, or ZDV, ddI or ddC for 4 days. The growth of each cell line in the absence, and presence of drug was measured after four days of incubation using the propidium iodide method. The concentrations of 1592U89 which inhibited growth by 50% (TC50 values) are shown in Table 5.

Table 5: Drug concentrations causing 50% growth inhibition (TC50) of human leukemic cells in vitro

Cell Line	Mean TC 50 + SE (uM) of			
	1592U89	ZDV	ddI	ddC
IM-9	110 ± 40	400 ± 10	600 ± 60	110 ± 10
CEM	160 ± 20	40 ± 20	700 ± 500	9.0 ± 1.0
CD4+ CEM	140± 30	800 ± 200	1600 ±200	10 ± 1
Molt-4	20 ± 10	20 ± 10	1500 ± 300	7.5 ± 0.4

Results presented in Table 5 show that except for the Molt-4 cells, the TC50 of 1592U89 ranged from 110-160 uM. For the Molt-4 cells, the TC50 was 20 uM. The TC50 values of ZDV, ddI and ddC are also shown in Table 5. The compound 1592U89 was less cytotoxic than ddC, but more cytotoxic than ddI. The cytotoxic profile of 1592U89 for Molt-4 cells was similar to that of ZDV. However, ZDV was 4-fold more cytotoxic to CEM cells than 1592U89. A therapeutic index (TI) for 1592U89 was estimated to be 37 based on its TC 50 value of 140 uM for CD4 + CEM cells, and the IC 50 value of 3.8 uM against HIV-1 IIIB in CD4+ CEM cells,

VI (b). **Effect of 1592U89 on cellular DNA synthesis**: (Faletto, M.B. 1994. Glaxo Wellcome report TEIN/94/0038).

The purpose of this study was to determine the effect of 1592U89 on cellular DNA synthesis. Human CD4+CEM cells in logarithmic growth were incubated with 1, 10 and 100 uM of 1592U89 over a 48-hour time course. The incorporation of ^{3}H-thymidine into intracellular TCA-insoluble material was used as a quantitative measure of DNA synthesis. 1592U89 at concentrations of 1 and 10 uM had no effect on the incorporation of ^{3}H-thymidine into DNA at 4, 24 and 48 hours. However, CEM cells treated with 100 uM of 1592U89 for 4 hours incorporated 32% less ^{3}H-thymidine than untreated control cells. On the other hand, CEM cells treated with 100 uM of 1592U89 for 24 and 48 hours incorporated ^{3}H- thymidine at levels similar to the untreated control cells. This result suggests that 1592U89 at 100 uM was not inhibitory to cellular DNA synthesis.

VII. **In vitro cytotoxicity of abacavir (1592U89) to human bone marrow progenitor cells**: (Averett, D. 1993. Glaxo Wellcome report TEZA/93/0067-00 (NDA 20-977, vol 4.1: 63-68), Daluge et al., 1997).

The objective of this study was to determine the effect of 1592U89 on colony formation by human bone marrow progenitor cells in vitro. The IC50 values were determined using in vitro human bone marrow progenitor cell assays (Averett, 1993; Daluge et al., 1997). The average IC50 values of 1592U89 for granulocyte-macrophage CFU (CFU-GM) and erythroid burst-forming unit (BFU-E) were 140 ±16, and 100 ± 9 uM, respectively. The IC50 values of CBV for CFU-(GM) and BFU (E) were 50 ± 8, and 82 ± 15 uM, respectively. Therefore, CBV was 2-3 fold more cytotoxic to CFU-GM than abacavir (1592U89).

VIII. **Selection of abacavir (1592U89) resistant HIV-1 in vitro:**

The development of HIV-1 variants that are less susceptible (resistant) to 1592U89 in vitro was demonstrated by serial passage of HIV-1 (HXB2) and ZDV-resistant HIV-1 (RTMC) in MT-4 cells in the presence of increasing concentrations of 1592U89. MT-4 cells ($2x10^6$ cells/culture) were infected with cell-free HIV-1 supernatants at a low m.o.i. (<0.1 plaque forming units/mL). Infected cells were treated with 1592U89 at concentration 1-2 x of IC50 value (4.5 uM). Cultures were incubated at 37^0 C until an extensive CPE was observed. The culture supernatants were harvested and used for further passage. The concentration of 1592U89 was doubled at each passage during 10 passages in MT-4 cells. The susceptibility of passaged virus isolates to 1592U89 was determined by a plaque reduction assay in CD4+HeLa cells. Phenotypic analysis showed that for HIV-1 (HXB2), no significant change in susceptibility to 1592U89 was observed until passage 8 (Tisdale et al., 1997). At passage 8, an approximately 3-fold increase in IC50 was observed in vitro compared to baseline IC50 value for HIV-1 HXB2. By passage 10, the IC50 had increased to 10-fold higher than the baseline IC 50 for HIV-1

HXB2. For ZDV resistant HIV-1 (RTMC), a four to five-fold increase in IC50 was observed by passages 8 to 10 compared to baseline IC 50 value.

VIII (a). **Genotypic analysis of the RT gene of abacavir (1592U89) resistant HIV-1 variants selected in vitro:**

For genotypic analysis of the RT gene of HIV-1 variants, DNA extracts were prepared from passages 1 to 10 of HIV-1 HXB2 and HIV-1 RTMC infected MT-4 cells passaged in the presence of increasing concentrations of 1592U89. Selective PCR was used to detect mutations in the reverse transcriptase gene at codon positions 65, 74, and 184 from virus isolates and infected cell DNA extracts from various passages. The results of selective PCR analysis to determine the time and order of appearances of these mutations in the passaged virus are summarized in Table 6. Table 6 shows that a mutation at codon 184 was the first to develop at passages 4 to 5. However, phenotypic analysis showed that the susceptibility of HIV1 HXB2 to 1592U89 at passage 5 did not change (Tisadle et al., 1997). The IC50 of 1592U89 for HIV-1 HXB2 at passage 5 was similar to that of baseline value (Tisdale et al., 1997). By passages 6 to 10, the HIV-1 variant had mutations at codons 74 and 65. A 6 to 8-fold increase in IC50 value of 1592U89 (IC50 =75 uM) was observed for HIV-1 variants at passages 8-10 (Tisdale et al., 1997). The increase in IC50 values of 1592U89 for passage 8 to 10 viruses coincided with the emergence of additional mutations at codons 74 and 65 (Table 6). To confirm the pattern of mutations induced by 1592U89, HIV-1 HXB2 was passaged in a similar study as described above. The genotypes of the HIV-1 variant at different passages were determined (Tisdale et al., 1997). Besides mutations at codons 184 (M184V) and 74 (L74V), an additional mutation at codon 115 (Y115F) was detected.

The ZDV resistant HIV-1 strain (RTMC) developed a 4 to 5-fold increase in IC50 value for 1592U89 by passages 8 - 10 with the simultaneous development of a mutation at codon 184. For the HIV-1 RTMC variant (p 8 - 10), M184V was the only mutation detected. As discussed above, the RT mutation M184V in the HXB2 strain by itself did not confer a significant IC50 value shift to 1592U89. Thus, in the case of HIV-1 RTMC variant (p 8 - 10), RT mutations 67N, 70R, 215F, and 219Q were in concert with the newly developed M184V that contributed to the decrease in abacavir susceptibility in vitro.

VIII (b). **Correlation of abacavir resistant HIV-1 genotypes with phenotypes:**

To determine the contribution of different RT mutations to the abacavir resistance phenotypes, infectious clones were prepared with single, double and triple combinations of the mutations observed in infected cell passages (section VIII a). RT mutant viruses were prepared by the site directed mutagenesis of the RT gene from an HIV-1 HXB2 strain and of other established laboratory HIV-1 variants ($HXB2_{74V}$, $HXB2_{184V}$, $RTMC_{184V}$). Recombinant viruses were generated using the procedure described by Kellam and Larder (1994). Recombinant viruses were sequenced within the RT region to

confirm each genotype, and viral titers were determined using a HeLa-CD4+ plaque assay. Recombinant viruses containing mutations within the RT gene were analyzed for susceptibility to 1592U89 by using a HeLa-CD4+ plaque assay. The IC50 values of abacavir (1592U89) for recombinant viruses with single, double or triple mutation combinations are shown in Table 7. In addition, the susceptibility of these recombinant viruses to other NRTIs was also tested and the respective IC50 values are also shown in Table 7.

Table 6: Mutation (s) in the RT gene in HIV-1 isolates selected for resistance to abacavir (1592U89)

HIV-1 isolate and passages	codon	Sequence Change	Amino acid change
HXB2			
4-5 (1st series)	184	ATG → GTG	M → V
6-10 (1st series)	184	ATG → GTG	M → V
	74	TTA → GTA	L → V
	65	AAA → AGA	K → R
4-5 (2nd series)	184	ATG → GTG	M → V
6-10 (2nd series)	184	ATG → GTG	M → V
	74	TTA → GTA	L → V
	115	TAT → TTT	Y → F
RTMC, 8-10	184	ATG → GTG	M → V

Table 7: Susceptibility of recombinant viruses containing abacavir-associated RT mutations to abacavir and other nucleoside analogue RT inhibitors:

Recombinant virus (genotype)	IC50 (uM)			
	1592U89	ddI	ddC	3TC
HXB2 (wild type				
65R				
74V				
115F				
184V				
65R/74V				
65R/184V				
65R/74V/184V				
74V/184V				
74V/115F				
115F/184V				
74V/115F/184V				

The following conclusions are drawn from the results presented in Table 7 and Tisdale et al., 1997).

1. Recombinant viruses containing single mutations were less (2 to 4 -fold) susceptible to 1592U89 than the wild type HIV-1 HXB2.
2. Recombinant viruses with double mutations containing the 184V mutation were more abacavir-resistant than those with the single mutation. These viruses exhibited six to nine-fold reductions in susceptibility to 1592U89 compared to wild type.
3. Recombinant viruses with triple mutations (65R/74V/184V or 74V/115F/184V) displayed the highest degree of resistance with approximately a 10-fold decrease in susceptibility to abacavir compared to wild type.
4. Recombinant viruses with single, double or triple mutations (65R/74V/115F or 74V/115F/184V) were susceptible to d4T and ZDV (data not shown, Tisdale et al., 1997).
5. As expected, recombinant viruses containing 65R and L74V mutations exhibited a decrease in susceptibility to ddI, ddC and 3TC (approximately 3-fold decrease for ddI, ddC and 12-fold for 3TC compared to baseline). Similarly, recombinant viruses containing 65R and 184V mutations exhibited a decrease in susceptibility to ddI, ddC and 3TC (approximately 5 to 7-fold for ddI, ddC and >70 fold for 3TC). Furthermore, recombinant viruses containing triple mutations (65R/74V/184V or 74V/115F/184V) exhibited decreases in susceptibility to ddI, ddC, and 3TC (approximately 5 to 6-fold for ddI, ddC and >70-fold for 3TC).
6. Recombinant viruses containing L74V mutation alone, or in double combination with 184V, or in triple combination with 115F and 184V (74V/115F/184V) exhibited decreased susceptibility to 1592U89, ddI, ddC and 3TC in vitro.
7. As expected, all recombinant viruses containing 184V mutation exhibited a high degree of resistance (>50 fold) to 3TC in vitro compared to baseline.

IX. Effect of abacavir on telomerase activity and telomere length in human lymphocytes and bone marrow stem cells:

Telomeres are unique DNA-protein structures found at the ends of eukaryotic chromosomes. Telomeres protect genomic DNA from degradation and recombination events such as end-to-end fusion, rearrangements, chromosomal translocations, and chromosomal loss. Telomeric DNA is characterized by an array of tandemly repeated hexameric sequences (TTAGGG in mammals) which are typically 8-20 kilobases in length. In normal somatic cells, DNA polymerases are unable to replicate the extreme ends of telomeric DNA, therefore, at every replication cycle a small region at the end of chromosome is left uncopied. This leads to progressive shortening of the telomeres (20-200 bp per cell division). The decrease in telomere length would cause genetic instability and eventual cell death. To compensate this loss, telomeres are lengthened by the attachment of telomeric subunits synthesized by an enzyme called telomerase (For complete review, see Greider and Blackburn (1996).

Telomerase consists of mainly protein and a single molecule of RNA which contains the critical nucleotide template for telomeric subunits. Telomerase catalyzes the addition of TTAGGG repeats to the ends of chromosomes using a complementary sequence of its own RNA as a template. Telomerase is a mammalian reverse transcriptase. The catalytic subunit of telomerase has structural and enzymatic similarities to viral reverse transcriptases (Lundblad, 1998). Since telomerase functions as a reverse transcriptase, it is of interest to examine if abacavir could inhibit telomerase activity. Results showed that CBV-TP (0.01 to 5 uM) inhibits telomere elongation by telomerase in vitro in a dose dependent fashion (Madison et al., 1998).

X (a). **Effect of abacavir on growth of CD4+ and CD8+, and CD34+ cells:**

CD4+ and CD8+ T cells were isolated by incubation of mononuclear cells with magnetized polystyrene beads coated with a primary monoclonal antibody specific for CD4 or CD8 membrane antigen. Cells bound to beads were washed, detached, and resuspended with beads coated with antibodies to CD3 and CD28. CD4+ and CD8+ T lymphocytes were cultured for approximately five weeks with and without the addition of 10 uM abacavir to culture media. The results showed that abacavir had no detrimental effect on growth of CD4+ cell culture. However, data on the effect of abacavir on CD8+ cell culture were not provided. The CD34+ bone marrow stem cells were isolated from human bone marrow mononuclear cells as described elsewhere (Glaxo Wellcome report RR1998/00029/00). CD34+ cells were cultured in a media supplemented with cytokines (20 ng/mL rh IL-3, 50 ng/mL rh IL-6, 50 ng/mL rhSCF, and 25 ng/mL rhFlt-3 ligand) with or without the addition of either 0 uM, 2 uM, 10 uM, or 20 uM abacavir. The results showed that abacavir at concentrations of 2-10 uM had no effect on population doubling of CD34+ cells. However, 20 uM of abacavir inhibited population doubling by 57% of CD34+ cells by day 20.

X (b). **Effect of abacavir on telomere length in CD4+ and CD8+ and CD34+ cells:**

Telomere length was estimated by measuring the mean terminal restriction fragment (TRF) size of genomic DNA. In brief, genomic DNA was isolated from CD4+ T cells and digested with Hinf I and Rsa I restriction enzymes. Cleaved DNA was separated on 0.6% agarose gel and transferred to a nylon membrane by Southern blotting. The Southern blot was then hybridized with a probe specific for telomeric repeats. Detection of the position of the hybridized probe was accomplished using a Streptavidin - conjugated Horseradish Peroxidase system. The results (data not shown) revealed that culture with no drug treatment showed a decrease of 2.5 kb over 46.5 mean population doublings (MPD) in telomere length. Similarly, cultures treated with 10 uM drug showed a decrease of 2.8 kb over 44 doublings. The normal rate of PBL telomere loss is 50-100 bp/MPD. This result showed that CD4+ T cells exhibited progressive telomere shortening as a function of cell division in vitro, but telomere length was not affected by 10 uM abacavir. Abacavir at concenetrations of 2-10 uM did not affect telomere length of CD34+ stem cells. The telomere length in CD34+cell treated with 20 uM abacavir could not be estimated (Glaxo Wellcome report RR1998/00029/00).

XI. Clinical Virology (Abacavir Resistance):

CNAA2001:

Title of Study: Resistance profile of HIV-1 RT inhibitor 1592U89 after monotherapy and combination therapy (Report RM1998/00100/00).

Objective: This trial examined the safety and antiviral effect of abacavir alone and in combination with ZDV in 79 HIV-1 infected patients including antiretroviral naïve patients and those patients with <12 weeks of prior ZDV therapy. After 12 weeks of therapy, HIV-1 specific nucleic acid sequences could be obtained from only 30 study patients on monotherapy and 23 study patients on combination therapy. Plasma virus isolates RT regions (amino acids 20 through 260) were amplified for sequence analysis. Phenotypic resistance to abacavir, ZDV and 3TC was determined by an experimental recombinant virus assay (Hertogs et al., 1998).

Genotypic analyses:

After abacavir monotherapy, isolates from 18/30 patients (60%) had RT-specific mutations resulting in the following amino acid substitutions: K65R, L74V, M184V.

- M184V: 14/30 patients (47%)
- L74V: 10/30 (33%)
- K65R: 6/30 (20%)
- M184V and L74V: 8/30 (27%)
- K65R and M184V: 4/30 (13%)

After abacavir + ZDV combination therapy, isolates from 4/23 patients (17%) had developed abacavir resistance associated mutations: K65R, L74V and M184V.

Phenotypic analyses:

Of the 18 treatment naïve patients on abacavir monotherapy who acquired RT mutations, 17 patients' isolates showed a 3- fold decrease in susceptibility to abacavir in vitro compared to baseline. In patients on abacavir/ZDV combination therapy, all isolates harboring abacavir resistance RT mutations showed a 2-fold decrease in susceptibility to abacavir compared to baseline

CNAA B3003:

Title of Study: Genotypic and phenotypic analysis of HIV-1 reverse transcriptase in antiretroviral drug naïve HIV-1 infected individuals following 16 weeks of ABC/3TC/ZDV versus 3TC/ZDV (Report RM1997/00702/00).

Objective: This phase III trial was designed to compare the antiretroviral activity, safety, and tolerance of 3TC/ZDV versus 3TC/ZDV/ABC in antiretroviral naïve subjects following 16 weeks of treatment.

The genotype and phenotype of patients' viral isolates at baseline and following 16 weeks of treatment was analyzed. All subjects with a week 16 plasma viral load higher than 2.6 $\log_{10}$ (>400) HIV-1 RNA copies/mL, as well as a random set of subjects were initially selected for analysis.

Sixty-six subjects from the 3TC/ZDV arm and 72 from the ABC/3TC/ZDV arm completed 16 weeks of study therapy. However, for many samples genotypic analysis was not possible because of negative RT-PCR results. The inability to acquire HIV-1 nucleic acid material from plasma sample could be due to low viral loads. Paired baseline and week 16 genotypes were obtained for 34 of 66 subjects from the 3TC/ZDV arm and from 7 of 72 subjects from the 3TC/ZDV/ABC arm.

Genotypic analysis:

Genotypic analysis showed that the M184V RT mutation was the only mutation detected in HIV-1 isolates from 33/34 patients on 3TC/ZDV arm, and likewise in 3/7 patients on ABC/3TC/ZDV arm after 16 week of combination therapy. Other abacavir-associated mutations (K65R, L74V and Y115F) were not detected in patients on ABC/3TC/ZDV regimen after 16 weeks of therapy. All these patient's isolates harbored the wild-type HIV-1 RT gene sequence at baseline.

Phenotypic analysis:

Of the 3 treatment naïve patients who received ABC/3TC/ZDV and developed the M184V mutation, HIV-1 isolates showed a 1 to 3-fold and a 33 to 47-fold decrease in susceptibility (IC50) to abacavir and 3TC, respectively. Abacavir specific susceptibility changes observed here were not considered significant because of assay variability.

CNAA 2003

Title of study: Resistance, Cross-resistance and Viral Load Response to Addition of Abacavir (1592, ABC) to Current Therapy in Nucleoside Reverse Transcriptase Inhibitor-Experienced Subjects (Report RM1997/00609/00).

Objective: This trial was designed to evaluate the safety and efficacy of abacavir in combination with other NRTIs in NRTI-experienced subjects. This 24 week, four arm, open-label study enrolled subjects with >12 months experience on ZDV, >6 months experience on 3TC (plus >12 months ZDV), >6 months experience on ddI (+/- any ZDV experience) or >6 months experience on d4T. Plasma virus from each subject was quantified by the .
HIV-1 RT mutations genotype and susceptibility to ZDV, 3TC, ddI, d4T, ddC, and abacavir were evaluated using a recombinant virus assay.

<u>Genotypic and phenotypic analyses of HIV-1 variants from abacavir treated patients with prior ZDV/3TC experience</u>:

Seven patients with >12 months experience with ZDV plus >9 months experience with 3TC were treated with abacavir in combination with ZDV/3TC for 24 weeks. HIV-1 isolates obtained prior to abacavir therapy from 6/7 experienced patients harbored multiple ZDV resistance associated mutations (M41L, D67N, K70R, T215Yor F, K219Q). HIV-1 isolates from 4/7 of these ZDV/3TC experienced patients had the 3TC resistance associated mutation M184V at baseline. Genotypic analysis showed that HIV-1 isolates from 6/7 patients had maintained multiple ZDV mutations in the RT gene after 24 weeks of abacavir/3TC/ZDV combination treatment. HIV-1 isolates from 6/7 patients had the RT M184V mutation after 4-24 weeks of combination therapy,

HIV-1 isolates from 7/7 of these 3TC/ZDV experienced patients exhibited a 2 to10-fold decrease in susceptibility (IC50) to abacavir compared to wild type virus prior to abacavir/3TC/ZDV combination therapy. Phenotypic analysis of HIV-1 isolates from 6/7 patients after 24 weeks of combination therapy showed that susceptibility to abacavir did not appreciably change compared to that of pre-abacavir treatment isolates.

The viral load for 4/7 patients receiving abacavir in combination with ZDV/3TC for 24 weeks changed very little from the baseline value (- 0.36 to 0.55 $\log_{10}$ copies/mL). These patients had 4.54 to 5.84 $\log_{10}$ copies/mL RNA at baseline. Viral load data for 2/7 of these patients for the week 24 was not available. However, after 4 weeks of abacavir/ZDV/3TC combination therapy, the viral load change from baseline for these patients was negligible (-0.38 to 0.14 $\log_{10}$ copies/mL). A reduction of 1.51 $\log_{10}$ copies/mL from the baseline was observed for one patient (1/7) at weeks 4 and 24 after abacavir/ZDV/3TC combination therapy.

<u>Genotypic and phenotypic analyses of HIV-1 variants from abacavir treated patients with prior ZDV/ddI experience</u>:

Three patients with prior ZDV (0-21 months) and ddI (13-64 months) experience were treated with abacavir in combination with ZDV and ddI for 24 weeks. Genotypic analysis showed that HIV-1 isolates from 2/3 patients had 2 or more ZDV associated mutations (M41L, D67N, K70R, T215Y) at baseline. In addition, HIV-1 isolates from one of these patients also contained ddI and abacavir associated mutations (L74V and Y115F). After 4- 24 weeks of combination therapy with abacavir, HIV-1 isolates from 2 of these patients had three ZDV associated mutations (M41L, D67N, T215Y). HIV-1 isolates from the third patient receiving abacavir/ZDV/ddI combination therapy harbored NNRTI associated mutations at codon positions K101E and K103N in the RT gene. These mutations were detected both at baseline and after 4 weeks of therapy. These unexpected baseline mutations could have resulted from transmission of resistant virus, or failure to accurately report prior therapy.

Phenotypic analysis of HIV-1 isolates from the abacavir/ZDV/ddI cohort at baseline showed 1 to 5, 5 to 9, and 1 to 30-fold resistance to abacavir, 3TC, and ZDV, respectively using a recombinant virus assay. Phenotypic analysis data for isolates for the week 4 or 24 were not available.

After 24 weeks of abacavir/ZDV/ddI combination therapy these patients (n = 3) showed a reduction of 0.31 to 0.64 $\log_{10}$ copies/mL RNA from baseline (4.54-5.84 $\log_{10}$ copies/mL). Failure to inhibit viral replication could be due to the presence of multiple ZDV resistance conferring mutations in these patients' isolates. Thus, with respect to viral load changes from baseline these patients did not benefit from the addition of abacavir to the existing ZDV/ddI combination therapy.

<u>Genotypic and phenotypic analyses of HIV-1 variants from abacavir treated patients with prior ZDV experience</u>:

Eight patients with prior ZDV experience (13-38 months) received abacavir/ZDV combination therapy. Genotypic analysis showed that HIV-1 isolates from 4/8 patients had wild type genotype after 4-24 weeks of combination therapy. Two of these patients showed plasma viral load reduction of 2 $\log_{10}$ copies/mL after 24 weeks of combination therapy. Plasma viral load data for the other 2/4 patients were not available for week 24. Viral load for one of these patients harboring wild-type HIV-1 isolate did not decrease after 4 weeks of combination therapy. HIV-1 isolates from the remaining 4/8 patients contained either L74V, M184V mutations (n=1), or M41L, D67N, K70R, L74V, M184V, K219 Q (n = 2) or M184V mutation alone (n=1) after 4-24 weeks of combination therapy. Three of these patients showed a >1 $\log_{10}$ copies/mL viral load reduction from baseline after 24 weeks of abacavir combination therapy. However, viral load for one of these patients harboring HIV-1 isolate with M41L, L74V, M184V, and T215Y mutations did not change after 24 weeks of combination therapy. These mutations were also detected in baseline HIV-1 isolates from this patient.

CNAA 3006

Title of study: Comparison of viral resistance and viral load response in therapy experienced pediatric subjects treated with the combination abacavir (ABC) lamivudine (3TC)/zidovudine (ZDV) vs the combination 3TC/ZDV (Report RM1997/00749/00).

Objective: This clinical trial was designed to evaluate the safety and efficacy of abacavir/3TC/ZDV versus 3TC/ZDV in antiretroviral therapy experienced pediatric patients. The primary endpoint of this study was a decrease in HIV-1 RNA to below 10, 000 copies/mL as measured by the Amplicor HIV-1 Monitor™ test.

This study enrolled 205 subjects age 90 days to 13 years of age with documented prior antiretroviral experience greater than 12 weeks prior to screening.

Genotype analyses:

Genotypic analyses were only provided for codon mutations known to be involved in phenotypic resistance to ddC, d4T, ddI, 3TC, ZDV, and abacavir. Specifically mutations at RT codons 41, 65, 67, 69, 70, 74, 75, 77, 115, 151, 184, 210, 215, and 219 were monitored. Baseline genotypic analysis data were provided for 96/102 subjects in the abacavir/3TC/ZDV arm. Week 16 on-therapy genotypic data were provided for 89/102 subjects in the abacavir /3TC/ZDV arm. The baseline and on-therapy genotypes for HIV-1 isolates from pediatric patients with prior ZDV/3TC experience and receiving abacavir/3TC/ZDV combination therapy are shown in Tables 8 and 9, respectively.

Table 8: Baseline genotype of HIV-1 isolates from pediatric patients (CNAA3006)

Prior NRTI experience	Number of patients	Baseline RT genotype
ZDV+3TC	n = 86	M41L, D67N, K70R, 210W, T215Y (n = 2)
		M41L, D67N, K70R, 210W, T215Y, M184V (n = 57)
		M184V alone (n = 26)
		Wild-type (n=1)
ZDV+3TC+ddI	n = 9	M41L, D67N, K70R, 210W, T215Y, M184V (n = 5)
		M184V (n=1)
		M41L, D67N, 210W, T215Y (n=1)
		Wild type (n=2)
ZDV+3TC+d4T +ddC	n = 1	M41L,D67N, K70R,210W,T215F

Table 9: On-therapy (week 16) genotype of HIV-1 isolates from pediatric patients (CNAA 3006)

Prior NRTI Expereince	Number of patients	RT Genotype
ZDV+3TC	n=81	M41L, D67N, K70R, M184V, 210W, T215F, K219Q (n = 62) M184V alone (n = 16) Y115F, Q151M (n = 1) M41L, Q151M (n =1) Wild-type (n =1)
ZDV+3TC+ ddI	n =8	M41L, D67N, K70R, M184V, 210W, 215Y (n = 6) wild-type (n = 2)

<u>**Genotype and viral load in abacavir/ZDV/3TC treated patients**</u>:

Nine of 81 (11%) patients with prior ZDV/3TC treatment and receiving abacavir/3TC/ZDV combination therapy had 1, or >1 $\log_{10}$ reduction in plasma HIV-1RNA copies/mL from baseline by week 16. HIV-1 isolates from 5/9 of these patients harbored only the M184V RT mutation at week 16. HIV-1 isolates from the remaining 4 patients had M184V RT mutation plus 2 or more ZDV-associated mutations (M41L, D67N, K70R, 210W, T215Y, K219Q) at week 16. At baseline, HIV-1 isolates from 6/9 of these patients harbored the M184V mutation only. HIV-1 isolates from 2/9 of these patients had M184V and ZDV-associated mutations (M41L, K70R, T215Y/F, K 219 Q/E), and isolate from 1/9 of these patients contained only ZDV associated mutations at baseline.

Twelve of 81 (15%) patients with prior ZDV/3TC treatment and receiving abacavir/3TC/ZDV combination therapy had >0.5 but <1.0 $\log_{10}$ reduction in HIV RNA copies/mL from baseline by week 16. HIV-1 isolates from 4/12 of these patients contained only M184V mutation, and HIV-1 isolates from 7/12 of these patients contained M184V mutation in combination with 2 or more ZDV-associated mutations at week 16. Isolates from 1/12 of these patients developed abacavir-associated RT mutation Y115F and multi-drug resistance-conferring mutation Q151M along with the M184V mutation during therapy. At baseline, HIV-1 isolates from 4/12 of these patients contained only the M184V mutation. HIV-1 isolates from 7/12 of these patients had M184V mutation in combination with either ddI- resistance conferring mutation L74V (n =1), or 1 (n =1) or ≥ 4 (n = 5) ZDV-associated mutations (M41L, D67N, K70R, T215Y/F, K219 Q) at baseline. An isolate from 1/12 of these patients contained ZDV mutations (M41L, T215Y) at baseline.

Of those patients with prior ZDV/3TC/ddI treatment experience and receiving abacavir/3TC/ZDV combination therapy (n=9), one patient showed viral load reduction of 1 $\log_{10}$ copies/mL in plasma RNA from baseline. HIV- 1 isolates from this patient contained RT mutations L74V, and M184V at baseline and mutations L74V, M184V and T 215Y after combination therapy. Two patients showed plasma HIV RNA reduction of 0.51 to 0.58 $\log_{10}$ copies/mL from baseline by 16 weeks of therapy. Isolates from 1/2 of these patients contained RT mutations, M184V, T215Y at baseline and wild type genotype after abacavir combination therapy. HIV-1 isolates from the other patient had wild type genotype at the baseline, and developed RT mutations K70R and M184V during therapy. Viral load reductions for the remaining six patients (6/9) after 16 weeks of combination therapy was <0.5 $\log_{10}$ copies/mL from the baseline.

Thirty-four of 81 (42%) patients with prior ZDV/3TC treatment and receiving abacavir/3TC/ZDV combination therapy showed < 0.5 (- 0.5 to - 0.01) $\log_{10}$ copies/mL RNA reduction from baseline after 16 weeks of combination therapy. HIV-1 isolates from 24/34 of these patients harbored 2 or more ZDV-associated mutations (M41L, D67N, K70R, M184V, 210W, T215Y/F, K219Q/E), and the 3TC resistance-conferring mutation M184V at week 16. HIV-1 isolates from 8/34 of these patients had M184V mutation, and one of the ZDV–associated mutations (M41L or K70R or T215Y) at week 16 of therapy. HIV-1 isolates from 1/34 of these patients contained the M184V mutation only, and isolate from another patient (1/34) had wild-type genotype after 16 weeks of combination therapy. At baseline, HIV-1 isolates from 7/34 of these patients had the M184V mutation only. HIV-1 isolates from 5/34 of these patients had M184V mutation and one of the ZDV- associated mutations (M41L or K70R) at baseline. HIV-1 isolates from 20/34 of these patients had M184V mutation and 2 or more ZDV-associated mutations (M41L, D67N, K70R, T215Y/F, K 219Q/E) at baseline. HIV-1 isolates from one of these patients (1/34) contained the M184V mutation, ZDV-associated mutation M41L, and multi-drug resistance conferring mutation Q151M at baseline. Genotype of HIV-1 isolate from 1/34 of these patients could not be determined due to negative PCR results.

CNAB 3009

Title: Intensification with Abacavir (1592, ABC) Reduces Viral Load in 3TC/ZDV Pretreated Patients with the 184V mutation (Report Number: RR1998/00084/00).

Objective: This phase III trail was designed to evaluate the efficacy of abacavir in combination with ZDV/3TC in patients previously treated for a period greater than or equal to 12 weeks with ZDV (600 mg/day) and 3TC (300 mg/day) (NUCB 3027 study).

All enrolled subjects received the following treatment: combivir at 150 mg 3TC/300 mg ZDV BID plus abacavir 300 mg BID for 48 weeks. Subjects were assessed at baseline, week 4, week 8, and every 8 weeks thereafter for HIV RNA (Amplicor HIV-1 Monitor™ test). The assay limit is 400 copies/mL (2.6 $\log_{10}$ copies/mL). However, this lower

HIV-1 RNA limit was not achieved by many of the study participants during treatment, so a higher HIV- RNA limit at 500 copies/mL (2.7 $\log_{10}$ copies/ mL) was selected as an endpoint.

Fifty-three patients were enrolled in this study, and fifty-two subjects have completed 16 weeks of therapy. At baseline the median HIV RNA was 2.9 $\log_{10}$ copies/mL.

HIV RT genotype was determined for 22 study participants at baseline and for 12 patients at week 8.

Results:

Baseline viral RNA $\log_{10}$ copies/mL

Patients with >500 copies/mL (>2.7 $\log_{10}$ copies/mL) = 32/53 (60%).
Patients with < 500 copies/mL (<2.7 $\log_{10}$ copies/mL) = 21/53 (40%).

On-therapy (Week 8) viral RNA $\log_{10}$ copies /mL

Patients with >500 copies/mL (>2.7 $\log_{10}$ copies/mL) = 12/50 (24%).
Patients with < 500 copies/mL (<2.7 $\log_{10}$ copies/mL) = 38/50 (76%).

On-therapy (Week 16) viral RNA $\log_{10}$ copies /mL

Patients with >500 copies/mL (>2.7 $\log_{10}$ copies/mL) = 11/34 (32%).
Patients with <500 copies/mL (<2.7 $\log_{10}$ copies/mL) = 23/34 (68%).

Baseline Genotypic analysis (n = 22):

The following mutations were detected in baseline HIV-1 isolates from 22 patients enrolled in CNAB 3009.

M184V mutation only = 11/22 (50%).
M184V and ZDV mutations = 5/22 (23%).
ZDV mutation only = 2/22 (9%)/
Wild-type genotype = 2/22 (9%).
M184V and 69P mutations = 1/22 (5%)
ZDV mutations and 69N mutation = 1/22 (5%)

On-therapy (week 8) Genotypic analysis (n = 12):

The following mutations were detected in 12 patients' isolates receiving abacavir and combivir combination therapy.

M184V mutation only = 7/12 (58%)

M184V and ZDV mutations = 3/12 (25%)).
ZDV mutations only = 2/11 (8 %).

Viral load and genotype at week 8:

1. Five of the 7 patients (71%) with the M184V mutation alone had viral load $>2.7 \log_{10}$ copies/mL after 8 weeks of abacavir combination therapy.
2. Two of the 7 patients (29%) with the M184V mutation alone had viral load $<2.7 \log_{10}$ copies/mL after 8 weeks of abacavir combination therapy.
3. Three of the 3 patients (100%) with M184V and ZDV mutations had viral load $<2.7 \log_{10}$ copies/mL after 8 weeks of abacavir combination therapy.

The viral load was $>2.7 \log_{10}$ RNA copies/mL in five patients harboring HIV-1 isolates with the M184V mutation after 8 weeks of abacavir/3TV/ZDV combination therapy. Although, these 5 patients appeared to be failing therapy, 3 of these 5 patients showed a 0.98 to 1 $\log_{10}$ reduction in plasma RNA copies/mL from baseline after 8 weeks of combination therapy. This preliminary observation suggests that some patients may benefit from abacavir therapy even in the presence of the M184V mutation. Conversely, 2/5 patients harboring the M184V mutation at baseline did not respond to abacavir/3TC/ZDV combination therapy. There was a very little change (- 0.44 to 0.31 log 10 copies/mL) in plasma RNA for these two patients after 8 weeks of combination therapy.

METHODOLOGY:

Methodology for Genotypic Analysis:

The HIV-1 RT coding region was amplified by the outer primer pair A-35/OUT 3.1 and the inner primer pair comb2/IN 3.1 using conditions as described by Larder et al., 1995. Some samples were amplified using A35/NE1 as outer primers and comb2/comb3 as inner primers.
A-35 (5'-TTGGTTGCACTTTAAATTTTCCCATTTAGTCCTATT-3')
NE1-35 (5'-CCCACTAACTTCTGTATGTCATTGACAGTCCAGCT-3')
Comb-2 (5'-CTGTACCAGTAAAATTAAAGCCAGG-3)
Comb-4 (5'- ATAGGCTGTACTGTCCATTTATCAGG-3')
OUT 3 (5'-CATTGCTCTCCAATTACTGTGATATTTCTCATG-3')
IN-3 (5'-TCTATTCCATCTAAAAATAGTACTTTCCTGATTCC-3')
Sequencing reactions were performed using 1 ul of the nested PCR product as template for the Prism FS dye terminator cycle sequencing kit (Applied Biosystems) and resolved on an ABI 373 DNA sequencer according to the manufacturers instructions. Data were analyzed with the
Larder et al., 1993) and aligned with
Where mixed viral populations were present, a ratio of mutant to wild type electropherogram peak size greater than 80% was designated as mutant.

Methodology for Phenotypic Analysis (Antivirogram™):

1. Synthesis of PR- and RT-cDNA:

Plasma samples were obtained from HIV-1 infected individuals and stored at –70°C until use. Viral RNA was isolated from 200 uL of plasma using QIAamp Viral RNA extraction Kit (Qiagen, Hilden, Germany) according to the procedure described by the manufacturer. Complimentary DNA (cDNA) encoding PR and RT fragments of the isolated viral RNA were made with the ____________

____________, as described by Hertogs et al., 1998. A 2.2 kb PR-RT-coding sequence was amplified from cDNA by nested PCR. The first round of PCR used primers PRTO-5 (5-' GCCCCTAGGAAAAAGGGCTGTTGG-3') and OUT3 (5'-CATTGCTCTCCAATTACTGTGATATTTCTCATG-3'). The primers for second round PCR were PRTI-5 (5'- TGAAAGATTGTACTGAGAGACAGG-3') and IN3 (5'-TCTATTCCATCTAAAAATAGTACTTTCCTGATTCC-3').
For amplification of RT-only coding sequences (1515 nt), PCR was performed with primers IN5 and IN3 as described by Kellam and Larder, 1994.

2. Construction of the proviral clone pGEMT3ΔPRT:

The proviral molecular clone ____________ from which the RT sequence was deleted (Kellam and Larder, 1994) served as the starting material for the construction of a PR-RT deleted proviral clone. PR- and RT-coding sequences were deleted from the HIV proviral genome starting from the Ahd I cleavage site (nucleotide position 2280; amino acid 9 of the PR-gene) to nucleotide position 4115 (amino acid 483 of the RT gene) as described by Hertogs et al., (1998).

3. Co-transfection of PR-RT coding sequences with pGEMT3ΔPRT:

MT-4 cells were electroporated in the presence of 10 ug of Bst E II -linearized pGEMT3ΔPRT and approximately 5 ug of purified PR-RT PCR product as described by Hertog et al., (1998). Cell culture supernatants containing recombinant virus were harvested 8 to 10 days after transfection and stored at -70°C for subsequent titration and susceptibility determinations.

4. Drug susceptibility assays:

HIV-1 drug susceptibility was determined by an MT-4 cell -3-(4,5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT)(MT-4/MTT)- based on a CPE protection assay. MT-4 cells were infected with 200 50% cell culture infective doses (200 $TCID_{50}$) of recombinant viruses in the presence of five-fold dilutions of different antiretroviral drugs. Four replicate determinations were performed in duplicate plates for each concentration of antiretroviral drug. Four wild -type recombinant viruses derived from HIV-1 III B/LAV RNA were generated and tested in parallel with clinical samples for each assay.

Fold-resistance were calculated by dividing the mean 50% inhibitory concentration (IC_{50}) for a recombinant virus from a patient by the mean IC_{50} for recombinant wild-type viruses. The inherent variability of the phenotypic assay (antivirogram) was estimated to be 2 to 3-fold.

Methodology for HIV RNA copy number determination:

The ;st was used to determine HIV-1 copy number in all study participants plasma samples. The procedure is fully described in the test kit package insert (NDA 20-977; vol 5: pages 224-251). The quantitation of HIV-1 viral RNA is performed using a Quantitation Standard (QS). The QS is a non-infectious RNA transcript that contains the identical primer binding sites as the HIV-1 target and a unique probe binding region that allows QS amplicon to be distinguished from HIV-1 amplicon. The QS is incorporated into each individual specimen at known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target. HIV-1 RNA levels in the test specimens are determined by comparing the absorbance of the specimen to the absorbance obtained for the Quantitation Standard. test can quantitate plasma associated HIV-1 RNA at concentrations in the range $\geq$ 400 to 750,000 copies/mL.

ABACAVIR LABEL (as of 11/25/98)

MICROBIOLOGY

Mechanism of Action: Abacavir is a carbocyclic synthetic nucleoside analogue. Intracellularly, abacavir is converted by cellular enzymes to the active metabolite carbovir triphosphate. Carbivir triphosphate is an analogue of deoxyguanosine-5'-triphosphate (dGTP). Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation and, therefore, the viral DNA growth is terminated.

Antiviral Activity In Vitro: The in vitro anti-HIV-1 activity of abacavir was evaluated against a T-cell tropic laboratory strain HIV-1 IIIB, a monocyte/macrophage tropic laboratory strain HIV-1BaL and clinical isolates in lymphoblastic cell lines, primary monocytes/macrophages, and peripheral blood mononuclear cells, respectively. The concentration of drug necessary to inhibit viral replication by 50 percent (IC_{50}) ranged from 3.7 to 5.8 uM against HIV-1 IIIB, and was 0.26 $\pm$ 0.18 uM (1 uM = 0.28 mcg/mL) against eight clinical isolates. The IC_{50} of abacavir against HIV-1 BaL varied from 0.07 to 1.0 uM. Abacavir had synergistic activity in combination with amprenavir, nevirapine, and zidovudine, and additive activity in combination with didanosine, lamivudine, stavudine, and zalcitabine in vitro. These drug combinations have not been adequately

studied in vivo. The relationship between in vitro susceptibility of HIV to abacavir and the inhibition of HIV replication in humans has not been established.

Drug Resistance: HIV-1 isolates with reduced sensitivity to abacavir have been selected in vitro and were also obtained from patients treated with abacavir. Genetic analysis of isolates from abacavir- treated patients showed point mutations in the RT gene that resulted in amino acid substitutions at positions K65R, L74V, Y115F, and M184V. Mutations M184V and L74V were most frequently observed in clinical isolates. Phenotypic analysis of HIV-1 isolates that harbored abacavir - associated mutations from 17 patients after 12 weeks of abacavir monotherapy exhibited a 3-fold decrease in susceptibility to abacavir in vitro. The clinical relevance of genotypic and phenotypic changes associated with abacavir therapy has not been established.

Cross-Resistance: Recombinant laboratory strains of HIV-1 (HXB2) containing multiple RT mutations conferring abacavir resistance exhibited cross-resistance to lamivudine, didanosine and zalcitabine in vitro. Cross-resistance between abacavir and HIV protease inhibitors is unlikely because of the different enzyme targets involved. Cross-resistance between abacavir and non-nucleoside reverse transcriptase inhibitors is unlikely because of different binding sites on the RT.

Precaution: Patients with prolonged prior zidovudine and lamivudine combination therapy, and harboring HIV-1 isolates that contained multiple nucleoside reverse transcriptase inhibitor resistance - conferring mutations benefited minimally from the addition of abacavir to their existing therapy.

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CONCLUSIONS:

Glaxo Wellcome Inc. has developed a new antiretroviral drug abacavir sulfate (new chemical entity), and seeks marketing approval (NDA # 20-977) for use of abacavir sulfate in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric (≥3 monthts) patients. With respect to microbiology, this NDA is approvable.

Abacavir sulfate ([(1S,cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl)-2-cyclopenetene-1-methanol sulfate (salt) (2:1) is a synthetic carbocyclic nucleoside analogue. In support of this NDA, with respect to microbiology, the sponsor has provided data on the metabolism and mechanism of action of abacavir, effects of abacavir on HIV-1 reverse transcriptase and cellular DNA polymerase activities, and antiviral activity of abacavir <u>in vitro</u>. Additionally, effects of abacavir on cellular growth and

cellular DNA synthesis, on bone marrow progenitor cells, and telomerase activity and telomere length were also studied. Abacavir's ability to select for HIV-1 variants that are less susceptible to the drug activity was also evaluated in in vitro and in clinical studies.

Abacavir is taken up by CEM- cells by non-facilitated diffusion. Abacavir is initially phosphorylated to 5'- abacavir monophosphate by cellular enzyme adenosine phosphotransferase, and subsequently converted to carbovir monophosphate by a cytosolic deaminase. Carbovir monophosphate is ultimately converted to carbivir triphosphate (CBV-TP) by cellular kinases. CBV-TP is the active metabolite through which abacavir exerts its antiviral activity. The intracellular half-life of CBV-TP was estimated to be 3.3 hr in CEM cells. CBV-TP inhibits HIV-1 replication by two known mechanisms: 1) it inhibits HIV reverse transcriptase by competing with the natural substrate deoxyguanosine 5'- triphosphate (K_i = 0.021 uM); 2) it inhibits viral DNA synthesis by causing DNA chain termination because CBV-TP lacks the 3'-hydroxyl group necessary for DNA elongation.

CBV-TP is a weak inhibitor of human cellular polymerase α (K_i = 6.9 uM). However, it did not inhibit polymerases β, γ, and ε. Human polymerases α, β, ε play an important role in the replication and repair of cellular DNA. DNA polymerase γ is involved in the synthesis of mitochondrial DNA.

The in vitro anti-HIV-1 activity of abacavir was evaluated against a T-cell tropic laboratory strain HIV-1 IIIB, a monocyte/macrophage tropic laboratory strain HIV-1BaL and clinical isolates in lymphoblastic cell lines (CEM, MT-4), primary monocytes/macrophages, and peripheral blood mononuclear cells, respectively. The anti-HIV-1 activity was determined by measuring either the inhibition of HIV-1 induced cytopathic effect (PE), supernatant reverse transcriptase activity, or supernatant p24 antigen production. The concentration of drug necessary to inhibit viral replication by 50 percent (IC_{50}) ranged from 3.7 to 5.8 uM against HIV-1 IIIB, and was 0.26 ± 0.18 uM (1 uM = 0.28 mcg/mL) against eight clinical isolates. The IC_{50} of abacavir against HIV-1 BaL varied from 0.07 to 1.0 uM. Abacavir had synergistic activity in combination with 141W94 (amprenavir), NVP, and ZDV, and additive activity in combination with ddI, 3TC, d4T, and ddC in vitro.

The anti-HIV activity of abacavir was also assessed in a SCID-hu mouse model. Abacavir at a dose of 300 mg/kg/day prevented plasma viremia in 3/7 mice infected with HIV-1. Under similar experimental conditions, zidovudine at doses of 150 mg/kg/day and 75 mg/kg/day suppressed HIV-1 infection 6/7 and 4/7 mice, respectively. Thus, abacavir in comparison to ZDV exhibited lower antiviral activity in the SCID-hu mouse model. The relationship between in vivo suppression of HIV-1 replication in SCID –hu mouse model and the inhibition of HIV-1 replication in humans has not been established.

Pharmacokinetic studies in humans showed that an abacavir plasma concentration of 3.0 ug/ml (10.7 uM) was achieved following administration of 300 mg tablet twice daily. The

plasma concentration of abacavir achieved in vivo at the recommended dosage (300 mg BID) is 2-3 times higher than the IC50 value of abacavir against laboratory strains of HIV-1 observed in vitro.

The in vitro cytotoxicity of abacavir was determined for T- and B-cell lines (CEM, Molt-4, IM-9) using cell growth inhibition assays. The concentration of abacavir that inhibited cell growth by 50% (TC50) ranged from 110-160 uM for IM-9 and CEM cells. However, for the Molt-4 cells, the TC50 was 20 uM. Abacavir at concentrations 1-100 uM did not inhibit cellular DNA synthesis as measured by incorporation of ^{3}-H thymidine. The in vitro cytotoxicity of abacavir to human bone marrow progenitor cells was determined using the colony formation inhibition assay. The IC50 values of abacavir for granulocyte-macrophage CFU (CFU-GM) and erythroid burst-forming unit (BFU-E) were 140 ± 16, and 100 ± 9 uM, respectively. The therapeutic index (TI) for abacavir was estimated to be 37 in CEM cells.

Abacavir at a concentration of 10 uM did not affect the growth of CD4+, and CD34+ cells. Similarly, abacavir at a concentration of 10 uM did not affect telomer length in CD4+, and CD34+ cells. The effect of higher concentrations of abacavir (>10 uM) on telomere length of CD4+ and CD34+ cells is not known. Abacavir inhibited telomerase activity in a dose dependent fashion at concentrations of 0.01-5 uM.

Abacavir resistant HIV-1 strains were selected by passaging HIV-1 HXB2 and HIV-1 RTMC in the presence of increasing concentrations of abacavir. Abacavir resistant viruses (>3-fold increase in IC50 compared to HIV-1 HXB2) were selected for by 8-10 passages. Genotypic analysis showed that abacavir resistant isolates selected in vitro had mutations in the reverse transcriptase gene resulting in amino acid substitutions at positions K65R, L74V, Y115F, and M184V. M184V mutation was the first to develop after 4-5 passages. To delineate the role of individual mutations in conferring abacavir susceptibility decreases, recombinant HIV-1 HXB2 viruses containing single, double or triple mutations were tested for their susceptibility to abacavir in vitro. Phenotypic analysis showed that recombinant viruses with triple mutations (K65R/L74V/M184V or L74V/Y115F/M184V) exhibited a 9-10 fold decrease in susceptibility to abacavir in vitro. Recombinat viruses containing only one mutation (K65R, L74V, 115F, or 184V) were slightly less susceptible (<4-fold increase in IC50 from parent virus) to abacavir in vitro. Recombinant viruses containing mutations K65R and L74V, or a combination of L74V and Y115F mutations also exhibited a 4-fold decrease in susceptibility to abacavir. However, recombinant viruses containing a combination of either K65R/M184V, L74V/M184V, or Y115F/M184V were 7 to 9- fold less susceptible to abacavir as compared to wild type HIV-1. Thus, a combination of mutations (K65R, L74V, Y115F, and M184V) appears to be responsible for significant shift in HIV-1 susceptibility to abacavir in vitro. Recombinant viruses containing double mutations (K65R/L74V or K65R/M184V, L74V/M184V, Y115F/M184V) conferred cross-resistance to ddI, 3TC, and ddC in vitro. Similarly, recombinant viruses containing triple combinations

(K65R/L74V/M184V or L74V/Y115F/M184V) were cross-resistant to ddI, 3TC and ddC in vitro.

HIV-1 isolates with reduced sensitivity to abacavir were also obtained from patients treated with abacavir. Mutations in the RT gene of HIV-1 isolates from abacavir treated patients were similar to those detected in abacavir resistant HIV-1 isolates selected in vitro. For patients enrolled in clinical trial CNAA 2001, HIV-1 isolates from 18/30 evaluable patients (60%) had mutations in the reverse transcriptase gene resulting in amino acid substitutions at position K65R, L74V, M184V in the reverse transcriptase after abacavir monotherapy. Similarly, HIV-1 isolates from 4/23 patients (17%) had developed abacavir resistance-associated mutations K65R, L74V and M184V after abacavir/ZDV combination therapy.

Paired baseline and week 16 genotypes of HIV-1 isolates were analysed from 34 of 66 evaluable patients from the 3TC/ZDV arm and from 7 of 72 evaluable patients from the 3TC/ZDV/ABC arm enrolled in CNAAB3003. All these patients were antiretroviral therapy naïve and their HIV-1 isolates harbored the wild-type RT gene sequence at baseline. Genotypic analysis of HIV-1 isolates after 16 weeks of combination therapy with abacavir/ZDV/3TC showed that isolates from 3/7 patients contained only M184V mutation. The other abacavir- associated mutations were not detected. HIV-1 isolates from 33/34 patients from 3TC/ZDV arm contained only M184V mutation.

Genotypic analysis of HIV-1 isolates from patients with prolonged exposure to ZDV plus 3TC and receiving abacavir combination therapy for 24 weeks showed that isolates from most of these patients (6/7) contained multiple ZDV resistance-conferring mutations (M41L, D67N, K70R, T215Y, K219Q) and 3TC resistance-conferring mutation M184V after 24 weeks of therapy. Baseline isolates from 6/7 of these experienced patients contained multiple ZDV resistance conferring-mutations, and from 4/7 contained the M184V mutation. Viral load for 4/7 of these ZDV/3TC experienced patients receiving abacavir in combination with ZDV/3TC changed very little from the baseline.

The mutation M184V and multiple ZDV resistance-conferring mutations were also observed at baseline in isolates from pediatric patients (62/96) with at least two years of ZDV plus 3TC treatment experience and receiving abacavir/3TC/ZDV combination therapy for 16 weeks. Additionally, only the M184V mutation was detected in isolates from 27/96 pediatric patients with prior ZDV/3TC experience at baseline. After 16 weeks of abacavir/3TC/ZDV therapy, HIV-1 isolates from 68/89 pediatric patients contained M184V and ZDV mutations, and from 16/89 patients contained only the M184V mutation.

Thus, the mutation M184V and multiple ZDV resistance-conferring mutations were predominant in HIV-1 isolates from adult and pediatric patients with prolonged prior NRTI experience receiving abacavir/3TC/ZDV combination therapy. With respect to viral load change, patients with prolonged prior exposure to ZDV/3TC and harboring multiple NRTI resistance-conferring mutations benefited minimally from the addition of

abacavir to their existing therapy. The multiple ZDV resistance-conferring mutations in concert with the M184V mutation may contribute to the loss of abacavir efficacy. However, the role of M184V mutation alone with respect to the loss of abacavir efficacy is not fully understood.

RECOMMENDATION:

With respect to microbiology, this NDA on abacavir is approvable.

Phase IV. Commitment: The sponsor is requested to continue to provide data, as they become available, on viral resistance analyses from all on-going clinical trials.

/S/
Microbiologist

CONCURRENCES:

HFD-530/Dep Dir /S/ Signature 12/8/98 Date
HFD-530/S micro /S/ Signature 4 Dec 98 Date

CC:

HFD-530/Original NDA
HFD-530/ Division File
HFD-530/MO
HFD-530/Pharm
HFD/530/Chem
HFD-530/S Micro
HFD-530/Review Micro
HFD-530/CSO, Truffa, M.