CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21.007
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MICROBIOLOGY REVIEW(S)

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

NDA # 21007

REVIEWER	: LALJI MISHRA, Ph.D.
CORRESPONDENCE DATE	: 06/24/98
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SPONSOR: Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

SUBMISSION REVIEWED: N-000

DRUG CATEGORY: Antiviral

INDICATION: Treatment of HIV-1 infection

DOSAGE FORM: Capsule

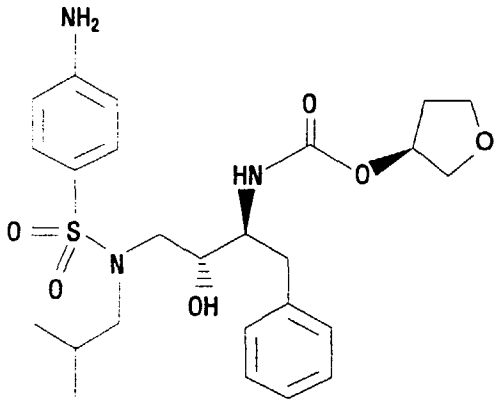
PRODUCT NAMES:

a. PROPRIETY: Agenerase ™

b. NON-PROPRIETY: Amprenavir

c. CHEMICAL: (3S)-tetrahydro-3-furyl N-[(1S,2R)-3-(4-amino-N-isobutylbenzenesulfonamido)-1-benzyl-2-hydroxypropyl]carbamate

STRUCTURAL FORMULA



MOLECULAR FORMUA: $C_{25}H_{35}N_3O_6S$

MOLECULAR WEIGHT: 505.64

SUPPORTING DOCUMENTS: IF #

BACKGROUND:

Glaxo Wellcome, Inc. seeks marketing approval for Agenerase (amprenavir) in combination with other antiretroviral agents for the treatment of HIV-1 infection. Amprenavir is a synthetic inhibitor of HIV-1 protease. Like other approved protease inhibitors (PIs), amprenavir has been demonstrated to exhibit anti-HIV-1 activity both in vitro and in vivo.

HIV-1 has a diploid RNA genome of 9.7 Kilobase (kb). The major HIV-1 structural proteins Gag, Pol, and Env (virion envelope protein) are initially translated into large polyprotein precursors Pr 55 ^{gag}, Pr 160 $^{gag\text{-}pol}$, and gp160 env from a polycistronic mRNA (Debouck and Metcalf, 1990; Huff 1991; Meek 1992). These polyprotein precursors are subsequently proteolytically cleaved and 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 cleavage 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 precursors.

The HIV-1 protease is a homodimer consisting of two identical 99 amino acid polypeptide chains and belongs to the family of aspartic proteases. It shares a highly conserved active site sequence Asp-Thr- (ser)-Gly with other aspartic proteases. HIV-1 encoded protease plays an obligatory role in the maturation and infectivity of viral particles. Specifically, the protease activity is needed for proteolytic cleavage of the gag (Pr 55 ^{gag}) and gag-pol polyprotein precursors (Pr 160 $^{gag\text{-}pol}$). By inhibiting HIV-1 protease activity, amprenavir interferes with the processing of gag-pol polyprotein precursors into functional proteins. Consequently, non-infectious immature virus particles are produced.

Amprenavir is also known as 141W94. Most of the virological preclinical studies refer to amprenavir as 141W94. Therefore, in this review the name 141W94 will be used interchangeably with amprenavir when referring to the study drug.

SUMMARY:

The sponsor has provided data on the antiviral activity and mechanism of action of amprenavir. Additional studies on the cellular uptake of amprenavir and its effects on cellular growth and on bone marrow progenitor cells were also provided. Amprenavir's ability to select for HIV-1 variants that are less susceptible to its antiviral activity was

also evaluated in in vitro and in clinical studies. The relevant microbiologic data from these pre-clinical studies are presented and reviewed below.

I. **Antiviral activity of 141W94 in vitro**:

The anti-HIV-1 activity of 141W94 was evaluated in acutely and chronically infected lymphoblastoid cell lines and in peripheral blood lymphocytes (PBLs), as measured by inhibition of HIV-1 induced cytopathic effects (CPE) or by inhibition of supernatant reverse transcriptase (RT) activity. The anti-HIV-1 activity of 141W94 varied depending on the virus strain, host cell type and assay used. The relevant studies are summarized below.

I (a). **Antiviral activity of 141W94 in acutely infected MT-4 cells and PBLs**:

MT-4 cells (a cell line transformed by human T-cell lymphotropic virus type I) were infected with HIV-1 IIIB at 100 x tissue culture infectious doses ($TCID_{50}$) and the antiviral activity was determined by examining changes in CPE.
Phytohemagglutinin A (PHA)-stimulated PBLs were infected with HIV-1 IIIB for 3 hours and then treated with various concentrations of 141W94 for 7 days. The anti-HIV-1 activity of 141W94 in PBLs was determined by measuring the inhibition of reverse transcriptase (RT) activity. The IC_{50} values of 141W94 against HIV-1 IIIB in MT-4 and PBLs were 0.084 ± 0.012 μM, and 0.08 ± 0.005 μM, respectively. 141W94 at concentrations of up to 100 μM was not cytotoxic to MT-4 cells in cell culture studies. The therapeutic index for 141W94 in T-cells lines is estimated to be 988. No data were provided on the cytotoxicity of 141W94 to PBLs.

I (b).**Antiviral activity of 141W94 in a chronically infected cell line**:

H9 III-B, a chronically infected HIV-1 IIIb producing cell line, was treated with various concentrations of 141W94 (concentration range not provided) for 5 days. On day 5, a 1:10 dilution of cell free supernatants was used to infect MT-4 cells and the infectivity of cell free supernatant was determined by assaying for CPE. The mean IC_{50} value for 141W94 was 0.41 ± 0.08 μM in H9 III-B cells.

I (c). **Antiviral activity of 141W94 against clinical isolates**.

The anti-HIV-1 activity of 141W94 was evaluated against nine different HIV-1 clinical isolates in PBLs. PHA-stimulated PBLs were infected with 200 $TCID_{50}$ of zidovudine (ZDV)–resistant (n=3) and ZDV-sensitive (n=6) clinically derived HIV-1 isolates. Infected cells were treated with various concentrations of 141W94 for 7 days, and anti-HIV-1 activity was assessed by inhibition of RT activity. The mean IC_{50} for 141W94 against three ZDV-resistant isolates was 0.019 μM (range ———— μM), and the mean IC_{50} for 141W94 against six ZDV-sensitive HIV-1 isolates was 0.012 μM (——
—— μM). Thus, both ZDV-sensitive and ZDV-resistant HIV-1 isolates were susceptible to 141W94.

I (d). **In vitro cytotoxicity of 141W94**:

The effects of 141W94 on growth inhibition of T- cell lines (Molt-4, CEM, CD4+CEM) and a B-cell line (IM9) were determined. Cell lines were incubated in the presence of or absense of various concentrations (250 uM – 4uM) of 141W94, and cell growth was evaluated by the addition of a fluorescent stain for DNA. The concentration of 141W94 that inhibited growth by 50% (TC_{50} values) are shown in Table 2. The TC_{50} values of 141W94 ranged from 57uM to 150 μM for T-cell lines and was 90 μM for a B-cell line, IM9.

Table 2: Fifty-percent inhibitory concentration of 141W94

Cell Line	Molt-4	CEM	CD4+CEM	IM9
TC50 (μM)	83 ± 8	57 ± 3	150 ± 23	90 ± 5

I (e). **Antiviral activity of 141W94 in combination with nucleoside reverse transcriptase inhibitors (NRTIs) and PIs:**

The anti-HIV-1 activity of 141W94 in combination with NRTIs zidovudine (ZDV), didanosine (ddI), or abacavir or in combination with PIs saquinavir (SQV), indinavir (IDV), ritonavir (RTV), or nelfinavir (NFV) was determined in MT-4 cells acutely infected with HIV-1 IIIB. In these in vitro studies, 141W94 exhibited synergistic anti-HIV-1 activity in combination with ZDV, ddI, abacavir or SQV, and additive anti-HIV-1 activity in combination with IDV, NFV or RTV.

I (f). Effect of human plasma and serum on anti-HIV-1 activity of 141W94:

141W94 binds approximately 90% to a-acid glycoprotein (AGP) fraction in human serum. The purpose of this study was to determine the effect of protein binding on the antiviral activity of 141W94 (Livingston, et al., 1995). CEM-CCRF cells (a T cell line) were infected with HIV-1 and cultured in media containing 141W94 and either 15% fetal bovine serum, 45% human serum, 45% human plasma, 45% human plasma + 0.8 mg/ml of a-AGP, or 45% human plasma + 1.2 mg/ml a-AGP. IC50 values of 141W94 are summarized below:

15% fetal bovine serum	12nM
45% human serum	24nM
45% human plasma	24nM
45% human plasma + 0.8 mg/ml a-AGP	64nM
45% human plasma + 1.2 mg/ml a-AGP	39nM

There was a 2 fold reduction in IC50 values of 141G94 in the presence of human serum or plasma, and a 3-5 fold reduction in the presence of human plasma containing a-AGP.

II. **Transport of 141W94 into CD4+ CEM cells:**

The purpose of this study was to determine the cellular uptake and binding of 141W94. Cellular association of 34 μM [^{14}C] –141W94 was measured as described by Domin et, al, 1994, and showed that 141W94 rapidly associated with CD4+/CEM cells in a concentration dependent manner. In the membrane/cytosol binding assay, a concentrated cell suspension was equilibrated with [^{14}C] 141W94 and sonicated. Aliquots of the sonicates were counted and the suspension was centrifuged. The supernatant was counted and ultrafiltrated. The ultrafiltrate was then counted. Results showed that 141W94 was partly membrane bound (~ 40%) and partly cytosolic protein bound (~20%). The intracellular levels of 141W94 was 4-fold higher than the extracellular levels. This study showed that 141W94 enters the cell cytoplasm; the site of action of the HIV-1 protease.

III. **Mechanism of action**:

Amprenavir is an inhibitor of HIV-1 protease. It binds to the active site of HIV-1 protease and thereby prevents the processing of viral gag and gag-pol polyprotein precursors, resulting in the formation of immature non-infectious viral particles.

III(a). **Inhibition of HIV-1 protease activity**:

Amprenavir was tested for its specificity of protease inhibition. Ki values of amprenavir for HIV-1, HIV-2 proteases and cellular aspartic proteinases were determined in a standard assay using 1 nM enzyme and different concentrations of substrate and inhibitor. The reaction product was quantified by high pressure liquid chromatography analysis. The inhibitory constant K_i was determined using the — software. Results are shown in Table 1.

Table 1: Inhibition of HIV-1, HIV-2 and cellular proteases by 141W94

Inhibitor	141W94				
Protease	HIV-1	HIV-2	Pepsin	Cathepsin D	Renin
Ki (nM)	0.6	19	3200	>10000	1750

Amprenavir inhibited HIV-1 protease with a Ki of 0.6 nM, suggesting a strong inhibition of protease activity. HIV-2 protease was inhibited to a lesser degree. Amprenavir was a minimal inhibitor of cellular aspartic proteinases, pepsin, cathepsin D, and renin. The K_i values of amprenavir for pepsin and renin were 5000, and 3000-fold higher than the K_i vlaue for HIV-1 protease. These results demonstrate that amprenavir preferentially inhibits HIV-1 protease.

III (b). **Effect of 141W94 on HIV-1 protease function (processing of gag polyprotein):**

The effect of 141W94 on the activity of HIV-1 protease was demonstrated in Cos cells transfected with a plasmid containing gag-pol gene. Cos cells transfected with HIV- gag pol gene produce non-infectious core particles. These core particles comprise the correctly processed viral structural proteins (p24, p17, p7, p6), protease, reverse transcriptase and integrase. This cell line is a model system for investigating the effect of a protease inhibitor on the processing of the gag-pol polyprotein mediated by HIV-1 protease. Cos cells were treated with various concentrations of 141W94 (0.3-100 nM) and then labeled with [^{35}S] Met. The labeled viral proteins were immunoprecipitated and resolved on ——————————. The appearance of unprocessed and partially processed gag protein (p55, p40, and p35) and the disappearance of fully processed capsid protein (p24) in 141W94 treated cells occurred in a dose-dependent fashion. The IC_{50} of 141W94 in this experimental cell system was 2.3 nM. This study provides evidence that 141W94 inhibits protease mediated processing of HIV-1 gag-pol polyprotein into functional proteins.

IV. **Effect of 141W94 on human bone marrow progenitor cells in vitro**:
The cytotoxicity of 141W94 to human bone marrow progenitor cells was evaluated using an in vitro human bone marrow progenitor cell colony formation inhibition assay. The IC_{50} values of 141W94 for CFU–GM (colony forming unit-granulocyte-macrophage) and BFU-E (burst-forming unit-erythroid) were 110 ± 29, and 100 ± 22 μM, respectively (this NDA; 2.26:89.). These results suggest that 141W94 was not cytotoxic to human bone marrow progenitor cells in vitro.

V. **Selection of 141W94 resistant HIV-1 isolates in vitro :**

The development of HIV-1 variants that are less susceptible (resistant) to amprenavir in vitro was demonstrated by serial passage of HIV-1 (HXB2) in MT-4 cells in the presence of increasing concentrations of amprenavir. MT-4 cells were infected with HIV-1HXB2 at a low m.o.i. (<0.1 pfu/cell). Infected cells were treated with amprenavir at concentrations 1-2 x of IC_{50} value (85 nM). Cultures were incubated at 37^{0}C until an extensive CPE was observed. The concentration of amprenavir was maintained or doubled at each passage during 6 passage in MT-4 cells. The susceptibility of passage 4 and 6 virus was determined in comparison with the original virus (Table 3).
DNA was extracted from HIV-1 HXB2 infected MT-4 cells of different passages and protease coding region was amplified by PCR and sequenced.

Table 3: In vitro passage of HXB2 in presence of increasing concentrations of 141W94

Passage number	Conc(nM)	Mutation	IC50 (nM)
0			85
1	200	ND	ND
2	400	I50V	ND
3	800	I50V	ND
4	800	I50V	413.2
5	1600	I50V,M46L	ND
6	1600	I50V,M46L,I84V	745.9

ND= not done

Phenotypic analysis showed that for HIV-1 HXB2 at passage 4, approximately 5-fold reduction in susceptibility to 141W94 was observed in vitro compared to baseline IC_{50} value. By passage 6, the IC_{50} had increased approximately 10 fold higher than the baseline IC_{50} .

Genetic analysis of the protease gene from these passaged viruses showed that three mutations in the protease at amino acid positions 46 (M46L), 50 (I50V), and 84 (I84V) developed during 2-6 passages in the presence of amprenavir. I50V mutation appeared first at passage 2. By passage 5, mutations I50V was maintained and a second mutation M46L appeared. By passage 6, additional mutation I84V developed.
An additional mutation at amino acid position 47 (I47V) was detected in HIV-1 variants selected in vitro with either amprenavir or an analogue of amprenavir. (Boone and Cheng, 1994, Tisdale et al., 1998).

To determine the contribution of above mutations to amprenavir resistance, infectious clones were prepared with a single, double and triple combination of mutations. Recombinant viruses containing mutations within the protease gene were analyzed for susceptibility to amprenavir. Results of this study showed that recombinant viruses containing a single mutation, I50V showed 2.5 fold increase in IC_{50} value compared to wild type HIV HXB2. Similarly, recombinant viruses containing either I84Vor I47V mutation showed >1 but < 2-fold increase in IC_{50} value of amprenavir. However, recombinant viruses containing triple mutations I50V + M46I +I47V showed a 15-fold increase in IC_{50} values.

VI. **Cross-resistance analysis of HIV Protease inhibitor Resistant Strains**:

The objective of this study was to evaluate the suseptibility of 141W94-resistant HIV mutants to five different protease inhibitors. HIV-1 mutants were prepared by site-directed mutagenesis (Kellam and Larder 1994, Maschera et al., 1995, Partaledis et al., 1995) and harbored one or more protease mutations ((I15V, M46L) that have been associated with 141W94 pressure in vitro. Additionally, HIV-1 mutants were prepared that contained protease gene mutations that have previously been associated

with IDV, RTV, or SQV resistance(Condra et al., 1995, Jacobson et al., 1996). These mutants were then tested for their susceptibility to five different protease inhibitors.

Infectivity ($TCID_{50}$) and drug susceptibility of the mutants were evaluated. The concentration of amprenavir that protected 50% of the cells from killing (IC_{50}) was determined using a regression analysis of the plot of percentage of cell death against drug concentrations. Shifts in virus susceptibility (fold increase or decrease), as compared to wild-type HXB2, were determined in parallel assays and are shown in Table 4. True shifts in susceptibility (IC50) were considered to be changes greater than 4-fold.

Table 4: Cross-resistance analysis of HIV-1 protease resistant variants

Virus variant	Shift in susceptibility relative to wild-type (HXB2) (IC50)				
	SAQ	IDV	RTV	NFV	APV
HXB2	1	1	1	1	1
50V	-2.3	-2.0	+4.4	-2.2	+3.2
46I/50V	-2.8	-2	+2.4	-2.4	+7.1
46L/50V	-4.7	-4	+1.7	+2	+3
46I/47V/ 50V	-1.3	-1.2	+5.6	+2.3	+11.3
48V/90M	+40	+2	+2.1	1.2	1
10R/46I/63P /82T/84V	+3	+11	+24	+8	+3
46I/63P/7 /82T/84V	+3.2	+10	+16	+5.5	+2

+ = fold increase in IC50 from baseline
- = fold decrease in IC50 from baseline

Cross resistance to other protease inhibitors was observed among amprenavir (APV)-resistant mutants. In this study, amprenavir (APV)- resistant HIV-1 variants were susceptible to SAQ, IDV, but cross- resistant to RTV. The evaluated HIV-1 mutants that were initially resistance to IDV or RTV remained susceptible to APV.

CLINICAL VIROLOGY (Amprenavir Resistance):

PROA 1002:

In study PROA 1002, changes in the phenotype and genotype of the protease gene in HIV-1 variants isolated from patients who had failed APV/ZDV/3TC combination therapy were analyzed. In this trial, patients previously treated with amprenavir (Phase A study) for 4 weeks were allowed to receive ZDV/3TC combination therapy for 30-56 weeks (Phase B). Patients were then switched to amprenavir (1200 mg BID), ZDV 300 mg BID and 3TC 150 mg BID (Phase C).

Genotypic analysis:

HIV-1 isolates from 4 patients failing 12-24 weeks of combination therapy were analyzed for genotypic changes in the protease gene. Baseline HIV-1 isolates from these patients did not harbor APV-associated resistance mutation in the protease gene. However, HIV-1 isolates from 2/4 patients developed mutations in the protease gene at weeks 12-24 of combination therapy. HIV-1 isolates from one patient contained mutations L10I, L33F, I54V and isolates from another patient contained mutations T12V, M46L, I50i/v (this NDA; 11.5: 32, Table 2). Additionally, HIV-1 isolates from all 4 patients had ZDV and 3TC resistance -associated mutations.

Phenotypic analysis:

Baseline HIV-1 isolates from all 4 patients were susceptible to amprenavir. Phenotypic analysis at week 24 of combination therapy showed that HIV-1 isolates harboring I54V mutation exhibited a 6-fold reduction in susceptibility to amprenavir. Similarly, HIV-1 isolates harboring I50v + M46L mutations showed a 5-fold reduction in susceptibility to amprenavir . HIV-1 isolates exhibiting amprenavir resistance were also resistant to ZDV and 3TC.

PROA 2002

This trial evaluated the safety and antiviral activity of multiple amprenavir doses in combination with ZDV/3TC in PI naïve patients. Patients (n=80) were assigned to one of four APV or placebo treatment arms: (Group A: 900 mg BID, Group B: 1050 mg BID, Group C: 1200 mg BID, Group D: APV-placebo matched to 1050 BID). Patients also received 300 mg BID ZDV, and 150 mg BID 3TC. After Week 12, patients in the control group (Group D) received active 1050 mg BID APV instead of APV-placebo. Patients enrolled in study PROA 2002 were either antiretroviral therapy naïve or with less than 1 year NRTI experience. Patients with prior experience with 3TC or any protease inhibitor were excluded.

Genotypic analysis:

Genotyping analysis of HIV-1 isolates from 19 patients who had failed therapy was completed. These patients either failed to respond to combination therapy (decrease of <0.7 $\log_{10}$ copies/ml from baseline in plasma HIV-1 RNA), or following an initial response to <400 copies/mL showed a rebound in plasma HIV-1 RNA to >400 copies/mL at two consecutive time points.

Baseline genotype:

HIV-1 isolates from 12 of the 19 patients contained natural polymorphisms (K20R, M36I, L63P, A71T, V77 I) in the protease gene at baseline. Baseline HIV-1 isolates from 17/19 patients did not harbor any PI- resistance associated mutations. However, baseline HIV-1 isolates from 2/19 patients harbored PI- resistance associated mutations

M46I, I54V, A71V, V82T, and L90 M mutations. These mutations are associated with resistance to IDV, NFV, RTV and SQV.

Baseline HIV-1 isolates from five of 19 patients had ZDV mutations only (M41L, D67N, K70R, T215F/Y, K 219Q). Baseline HIV-1 isolates from another patient contained both the ZDV resistance-associated mutations and 3TC-resistance mutation M184V. Baseline HIV-1 isolates from nine of 19 patients did not harbor any mutation associated with NRTIs-resistance. RT genotypes could not be determined for the remaining 4 patients' isolates,

On-Therapy genotype:

HIV-1 isolates from 4/19 patients developed the APV resistance-associated I50V mutation after 24-36 weeks combination therapy (Table 5). Two of the patients had also acquired 3TC resistance-associated mutation at week 24 (data not shown). HIV-1 isolates from another patient developed the I50V mutation at week 24 and 3TC associated mutation M184V and ZDV mutations by week 24-36. HIV-1 isolates from the fourth patient had ZDV and PI resistance-associated mutations at baseline, and developed the I50V mutation at week 24, and the M46I mutation at week 44.

The L63P polymorphism was present during amprenavir therapy in HIV-1 isolates from all four patients. HIV-1 isolates from two patients developed the I84V mutation and isolates from another patient developed the I54V mutation. The mutation I84V has been associated with resistance to ritonavir, and indinavir and the I54V mutation associated with resistance to ritonavir.

Table 5: Genotypic and phenotypic analysis of APV resistant HIV-1 isolates from patients enrolled in PROA 2002 study

Patient ID	Cohort	Time point	Viral Load RNA $\log_{10}$ copies/ml	Key PI mutation	Fold resistance To APV
600	A	Day 1	5.2	None	1
		Wk 24	4.5	I50V	1
		Wk 36	4.6	None	1
466	B	Day1	6	I54V, A71V, V82T, L90M	1
		Wk 24	5.1	I50V,I54V,A71V,V82A,L90M	ND
		Wk 36	5.7	I50i/v, I54V,A71V, V82T,L90M	11
		Wk 44	5.6	M46I, I50V,I54V,A71V,V82T,L90M	ND
468	D	Day 1	4.9	None	1
		Wk 24	ND	None	1
		Wk 36	3.7	I50V	5
		Wk 44	3.9	I50V	5
564	D	Day 1	4.8	None	1

		Wk 24 Wk 36 Wk 44	3.7 4.2 3.7	None I50V I50V	7 6 7

ND= No data

HIV-1 isolates from 16 of 19 patients contained RT mutations associated with resistance to ZDV, 3TC or both. HIV-1 isolates from two patients neither contained any ZDV or 3TC associated resistance mutations, nor any PI resistance- associated mutations. Genotype of HIV-1 isolates from one patient on APV/ZDV/3TC combination therapy could not be determined.

Phenotypic analysis:

Baseline phenotype:

Phenotypic analysis was performed for baseline HIV-1 isolates from 18/19 patients. These baseline HIV-1 isolates were susceptible to APV in vitro. Of these, baseline HIV-1 isolates from one patient were resistant to other PIs (IDV, NFV, RTV, SQV)

On-therapy phenotype:

Matched baseline and week 24-36 susceptibility data were available for HIV-1 isolates from 15 patients. HIV-1 isolates from three patients (466, 468, 564, Table 5) exhibited a 5 to11-fold decrease in susceptibility to APV after 24 to 36 weeks of combination therapy.

HIV-1 isolates from 10/19 evaluable patients (patients isolates for whom genotypic and/or phenotypic data available) exhibited phenotypic resistance to ZDV, 3TC or both (2 to >68 fold for ZDV, 2 to >31-fold for 3TC) after 24 to 44 weeks of amprenavir/ZDV/3TC combination therapy. HIV-1 isolates from 3/19 patients did not show any reduction in susceptibility to ZDV or 3TC. On therapy RT phenotypic data were not available for HIV-1 isolates from 5 patients enrolled in this study. HIV-1 isolate from 1/19 patients had neither genotypic nor phenotypic evidence of ZDV/3TC resistance.

Correlation of genotype with phenotype:

Phenotypic analysis showed that HIV-1 isolates (matched baseline and on-therapy) from 3/15 patients exhibited a 5 to 11-fold decrease in susceptibility to amprenavir. APV-resistant isolates contained either I50V mutation alone or mutations I50V and M46L in the protease gene. However, HIV-1 isolates from one patient harboring I50V mutation did not show any decrease in susceptibility to APV at week 24. Similarly,HIV-1 isolates harboring I84V mutations (n=2) and I54V mutation (n=1) did not show any reduction in

susceptibility to amprenavir. Additionally, the p1/p6 cleavage site mutation L-F was also detected in 2/3 amprenavir resistant isolates.

Cross-resistance:

Two of the 3 amprenavir resistant clinical HIV-1 isolates exhibited a 3-5 fold decrease in susceptibility to RTV. Cross- resistance of amprenvir resistant isolates to RTV has also been observed in vitro.

PROA/B 3006

Objective: The objective of this clinical trial was to compare the safety and efficacy of APV with IDV in combination with NRTI in NRTI experienced, PI naïve HIV-infected patients. An additional objective was to identify mutations in the protease and RT genes which emerged during PI –NRTI combination therapy.

Approximately 486 NRTI experienced patients (≥12 weeks prior NRTI therapy) were enrolled in this phase III study. Patients were randomized to receive either APV (1200 mg BID) or IDV (800 mg TID) in addition to concurrent NRTI therapy for a minimum of 48 weeks. Genotypic and phenotypic analyses of HIV-1 isolates were performed on a subset of patients (n=60).

Genotypic analysis:

Genotypic analysis was performed for samples obtained at day 1 and Week 16. Summary of the protease genotyping results for HIV-1 isolates from patients in the APV and IDV arms are summarized below..

Baseline genotype

APV arms:

- HIV-1 isolates from 2/30 patients had A71V, L63P, and V77I mutations.
- HIV-1 isolates from 1/30 patients had L10I, M46I, I54V, I84V, L90M, and L63P mutations.
- Baseline HIV-1 isolates from 27/30 patients did not harbor any PI-resistance associated mutation. However, some of these isolates harbored accessory mutations L10V, K20R, M36I, L63P, and V77I.
- HIV-1 isolates from 22/30 patients had one or more NRTIs resistance associated mutations.
- HIV-1 isolates from 5/30 patients did not harbor any NRTI associated mutations.
- RT genotypes of HIV-1 isolates from 3/30 patients were not available.

IDV arm:

- HIV-1 isolates from 3/30 patients had A71V mutation in the protease gene. The natural polymorphism V77I and/or 63P were also observed in the PR gene of these isolates.
- HIV-1 isolates from 27/30 patients did not harbor any PI- resistance associated mutations. However, some of these isolates contained accessory mutations, L10V, K20R, M36I, L63P, and V77I.
- HIV-1 isolates from 24/30 patients contained 1 or more NRTI resistance associated mutations.
- HIV-1 isolates from 3/30 patients did not harbor any NRTI or NNRTI resistance mutations.
- Genotypes of HIV-1 isolates from the remaining 3/30 patients were not available.

On-therapy (week16) genotype:

APV arms:

- HIV-1 isolate from 1/30 patients harbored the A71V mutation.
- HV-1 isolate from1/30 patients developed PI- accessory mutations L10I, V32I, and I47V and L63P.
- Genotypes of HIV-1 isolates from 28/30 patients were not available because of low plasma HIV-1 RNA levels and technical difficulty in RT-PCR.

IDV arm:

- Genotypes for HIV isolates from 30/30 patients were not available. Plasma HIV-1 RNA levels for 24/30 patients at week was 2.6-2.8 $\log_{10}$ copies/mL.

Phenotypic analysis:

IC_{50} values were determined for APV, IDV, NFV, RTV, SQV, 3TC, ZDV, d4T, ddC, ddI and NVP, and sensitivity relative to the HXB2 control expressed as fold resistance (FR) was calculated.

Phenotypic analysis of baseline HIV-1 isolates:

APV arm:

- HIV-1 isolates from 1/30 patients showed 8-fold reduction in susceptibility to NFV but were susceptible to APV.
- HIV-1 isolates from another patient (1/30) showed 6-fold reduced susceptibility to APV. However, APV resistance associated-mutations were not detected in the baseline HIV-1 isolates from this patient.
- HIV-1 isolates from 19/30 patients were susceptible to all PIs tested (APV, IDV, NFV, RTV, and SQV).

- Phenotypic analysis data on the susceptibility of HIV-1 isolates from the remaining 9/30 patient's isolates were not available.
- HIV-1 isolates from 18/30 patients showed reduced susceptibility to one or more NRTIs. HIV-1 isolates from 2/30 patients were susceptible to all NRTIs tested (ZDV, 3TC, d4T, ddC, ddI) and NNRTI (NVP).
- RT phenotypic analysis data for HIV-1 isolates from 10/30 patients were not available.

IDV arm:

- HIV-1 isolates from 15/30 patients were susceptible to all protease inhibitors tested (APV, IDV, NFV, RTV, or SQV).
- Phenotypic analysis data on the susceptibility to protease inhibitors for HIV-1 isolates from the remaining 15/30 patients were not available.
- RT phenotypic analysis showed that HIV-1 isolates from 5/30 patients were susceptible to all the NRTIs/NNRTIs tested (3TC, ZDV, D4T, ddC, NVP).
- HIV-1 isolates from 11/30 patients showed reduced susceptibility to one or more of the NRTIs tested (3TC, ZDV, d4T, ddC, ddI). Of these, HIV-1 isolates from 3 patients showed a reduced susceptibility to NNRTI (NVP).
- RT phenotypic analysis for HIV-1 isolates from 14/30 patients were not available.

On-therapy (week 16) phenotypic analysis data :

APV arms

- HIV-1 isolates from 2/30 patients tested were susceptible to amprenavir and other PIs (IDV, NFV, RTV, or SQV).
- HIV-1 isolate from 1/30 patients were 6-fold resistant to ritonavir and susceptible to APV, and other PIs.
- Phenotypic analysis data for HIV-1 isolates from 27/30 patients were not available.
- Plasma HIV-1 RNA levels for some of these patients (14/30) were 2.6-2.9 $\log_{10}$ copies/mL
- RT phenotypes for HIV-1 isolates from 2/30 patients tested showed reduced susceptibility to 3TC and ZDV.

IDV arm:

- Phenotypic analysis data were not available for HIV-1 isolates from any a patients. Most of the patients (24/30) had plasma HIV-1 RNA levels of 2.6-2.8 $\log_{10}$ copies/ml.

Additional Failure Group:

HIV-1 isolates from 29 patients failing virologically (plasma HIV-1 RNA $\geq$ 400 copies/mL) at week 16 of combination therapy were included for genotypic and

phenotypic analysis (Additional Failures group). Of these, 17 patients had been randomized to the APV treatment arm, and 12 to the IDV treatment arm.
Genotypic and phenotypic analysis of HIV-1 isolates from patients obtained at baseline and week 16 are summarized below.

Baseline genotype:

APV arm:

- HIV-1 isolate from 1/17 patients harbored A71T, L63P, and V77I mutations (this NDA; 11.5: 208, Table 4).
- HIV-1 isolates from the 15/17 patients did not contain any PI resistance-associated mutations. However, the accessory mutations M36I, L63P and V77I were present in some isolates. Genotypic analysis data was not available for isolates from 1/17 patients.
- HIV-1 isolates from 13/17 contained one or more NRTI resistance mutations.
- RT genotypic analysis data for HIV-1 isolates from 4/17 patients were not available.

IDV arm:

- HIV-1 isolates from 11/17 did not contain any PI associated-mutations. However, accessory mutations L63P, M36I and V77I were observed in some isolates.
- Genotypic analysis data for HIV-1 isolates from 1/12 patients were not available.
- HIV-1 isolates from 8/12 patients contained one or more NRTI resistance associated mutations. HIV-1 isolates from one of these NRTI resistant mutants also contained multi-drug resistance mutation Q151M.
- RT genotypic analysis data for HIV-1 isolates from the remaining 4/12 patients were not available.

On-therapy (week 16) genotypes:

APV arm:

- HIV-1 isolates from 4/17 patients harbored one of the PI resistance- associated mutations, I50i/v, I54M, A71T or I54L.
- HIV-1 isolates from 13/17 patients did not contain any PI associated mutations. However, accessory mutations M36I, L63P and V77I were observed in most of these isolates.
- HIV-1 isolates from 15/17 patients contained one or more NRTI resistance associated - mutations.
- RT genotypic analysis data for the remaining 2/17 patients were not avaialble.

IDV arm:

- HIV-1 isolate from 1/12 patients developed V82t/a mutation.

- HIV-1 isolates from 11/12 patients did not contain any PI- resistance mutation. However, HIV-1 isolates from most of these patients contained the accessory mutations M36I, L63P and V77I.
- HIV-1 isolates from 8/12 patients contained one or more NRTI resistance associated - mutations.
- HIV-1 isolates from 1/12 patients did not contain any mutation associated with NRTI resistance and RT genotypic analysis data for HhIV-1 isolates from 3/12 patients were not available.

Phenotypic analysis:

Baseline:

APV arm:

- HIV-1 isolates from 12/17 patients tested were susceptible to all PIs tested (APV, IDV, NFV, RTV, or SQV). Phenotypic analysis data for HIV-1 isolates from the remaining 5 patients were not available.
- HIV-1 isolates from 11/17 exhibited reduced susceptibility to 3TC and/or ZDV. HIV-1 isolates from 3 of these (11) patients were also resistant to NNRTI (NVP). HIV-1 isolates from 1/17 patients were susceptible to all the NRTIs and NNRTIs tested (3TC, ZDV, d4T, ddC, ddI, NVP).
- RT phenotypic analysis data from the remaining 5/17 patients were not available

IDV arms:

- HIV-1 isolates from 9/12 patients were susceptible to all PIs tested (APV, IDV, NFV, RTV, SQV).
- Phenotypic analysis data (susceptibility to PIs) for HIV-1 isolates from 3/12 patients were not available.
- HIV-1 isolates from 6/12 patients exhibited reduced susceptibility to one or more NRTIs tested (3TC, ZDV, d4T or ddI).
- HIV-1 isolates from 3/12 patients were sensitive to all NRTIs tested.
- RT phenotypic analysis data for HIV-1 isolates from the remaining 3/12 patients were not available..

On-therapy (wk 16) phenotype analysis:

APV arm:

- HIV-1 isolates from 1/17 patient showed a 5-fold reduction in susceptibility to APV. However, HIV-1 isolates from this patients were susceptible to NFV and SQV. Isolates from this patient contained the I54L mutation.
- HIV-1 isolates from 10/17 patients were susceptible to most PIs tested (APV, IDV, NFV, RTV, SQV). Isolates from one of these patients showed a 5-fold reduced susceptibility to IDV.

- Phenotypic analysis data (susceptibility to PIs) for HIV-1 isolates from 6/17 patients were not available including for isolates that developed the I50i/v mutation
- RT phenotypic analysis showed that HIV-1 isolates from 10/17 patients were resistant to one or more NRTIs tested. HIV-1 isolates from 4 of these patients were resistant to NVP.
- HIV-1 isolates from 1/17 patients were susceptible to all NRTIs and NVP.
- Phenotypic analysis data (NRTI/NNRTI resistance) for HIV-1 isolates from 6/17 patients were not available.

IDV arm:

- HIV-1 isolates from 6/12 patients were susceptible to most PIs tested (APV, IDV, NFV, RTV, or SQV).
- HIV-1 isolates from 1/12 patients were resistant to IDV and NFV, and susceptible to other PIs..
- Phenotypic analysis data (susceptibility to PIs) were not available for HIV-1 isolates from 5/12 patients including for the isolates with the V82t/a mutation.
- RT phenotypic analysis showed that HIV-1 isolates from 2/12 patients were resistant to 3TC only. HIV-1 isolates from 2/12 patients were resistant to ZDV only, and HIV-1 isolates from 1/12 patients were resistant to both 3TC and ZDV.
- HIV-1 isolates from remaining 3/12 patients were susceptible to all NRTIs tested and also to NVP.
- RT phenotypic analysis data for the remaining 4/12 patients were not available.

In summary, HIV-1 isolates from 1/17 patients failing APV/3TC/ZDV combination therapy exhibited a 5-fold reduction in susceptibility to amprenavir. Plasma HIV-1 RNA levels for this patient at week 16 was 4.18 $\log_{10}$ copies/mL.

CNAA2007

Objective: The objective of this phase II study was to analyze the HIV-1 RT and gag CS/Pro (cleavage sites/protease) genotypes and phenotypes of baseline HIV-1 isolates from PI and NRTI experienced patients and correlate the baseline genotypes and phenotypes with the viral load response to abacavir (ABC), APV and efavirenz (EFV) in patients completing 16 weeks of combination treatment.

Baseline plasma samples were obtained from all subjects participating in this trial (n=99). Reverse transcriptase and cleavage sites/protease coding region genotypes were obtained for 98 and 97 subjects, respectively. The "virology sub-population" has been defined as all patients (n= 65) who continued all 3 study drugs up to week 16. All patients had prior experience with NRTIs and PIs.

Baseline genotypes:

The frequency of specific individual mutations in the protease and gag cleavage sites of HIV-1 isolates from for all patients and from virology sub-population are summarized in table 6.

Results from Table 6 show that key mutations including D30N, V32I, G48V were present in 3%, 4%, and 12% of patients' isolates. Another key mutation, the I84V was present in 27% of patients' isolates. At baseline, the majority of the patients viral isolates harbored 5 to 8 PI resistance-associated mutations (this NDA; 11.5: 300, Table 2).
Like the protease, the RT region of study patients showed several resistance associated-mutations (this NDA; 11.5: 301, Table 3) in accordance with prior retroviral treatment experience. Majority of the patients' baseline isolates (55%) contained >1 ZDV mutation + M184V/I mutation. The T69D and >1 ZDV mutation without the M184V/I were the second and third most common NRTIs-associated resistance mutational pattern observed in 27% and 26% of the isolates, respectively. Only 3/98 isolates had wild type RT genotypes.

Baseline phenotype:

Baseline phenotypic resistance data for APV were obtained for HIV-1 isolates from 55 patients participating in APV, ABC, EFV combination therapy (virology sub-population). Baseline HIV-1 isolates were also tested for susceptibility to other PIs.

A total of 45% of patient's HIV-1 isolates exhibited decrease in susceptibility to APV. Most of the baseline HIV-1 isolates from PI experienced patients exhibited marked decrease in susceptibility to SAQ, IDV, NFV and RTV.

Table 6: Summary of baseline cs/Pro genotypes of HIV-1 isolates from all patients and from Virology sub-population

Mutations	Frequency All Subjects	Frequency Virology Sub-population
Protease mutation	**(n=97)**	**(n=65)**
Auxillary Mutations		
L10I/v/f/r	72%	72%
K20R	13%	14%
M36I	32%	32%
M46L/I	54%	57%
P/L63P	84%	86%
A71V/T	61%	64%
V77I	38%	37%
Key mutations		
D30N	3%	0%
V32I	4%	5%
G48V	12%	8%

I50V	1% mixture	0%
I54V	36%	35%
V82A/F/T	42%	43%
I84V	27%	24%
L90M	63%	72%
Cleavage mutations	**(n=96)**	**(n=63)**
P7/p1 Q to R (p3)	0%	0%
P7/p1 A to V (p2)	34%	44%
P1/p6 L to F (p1')	8%	9%
P1/p6 L to P or V (p1')	7%	6%

Table 7: Frequency of Baseline HIV-1 isolates with reduced susceptibility to PIs tested

Patients (%) with baseline isolates ≥ 4 fold reduced susceptibility to PIs tested				
Amprenavir	Saquinavir	Indinavir	Nelfinavir	Ritonavir
25/55 (45%)	41/57(72%)	44/56 (79%)	46/57 (81%)	46/55 (84%)

Relationship between genotype and phenotype:

Amprenavir :

The frequency of PI associated-mutations in baseline HIV-1 isolates exhibiting ≤4-fold (sensitive) and >4-fold decreased susceptibility to APV (resistant) is summarized here. Mutations K20R, M36I, M46L/I, I54V, A71V/T, V77I, V82A/T/f were prevalent to the same extent in both APV-sensitive and APV-resistant HIV-1 isolates, (this NDA ; 11.5:305, Table 6). These mutations individually, do not appreciably affect APV susceptibility of HIV-1 isolates from PI-experienced subjects. In contrast, the mutations L10I/v/f/r, L63P, I84V, and L90M were more prevalent in APV resistant HIV-1 isolates. The mutation I84V was detected in 68% of the APV-resistant HIV-1 isolates, compared to 10 % in APV-sensitive HIV-1 isolates. The I84V mutation was linked with theL90M mutation. The L90M without I84Vmutation was more prevalent in APV-sensitive HIV-1 isolates (43%) than APV-resistant HIV-1 isolates (24%). Thus, L90 M alone did not contribute to APV-resistance. Similarly, mutations L10I/v/f+ L90M in combination with the I84V mutation also showed high prevalence (68%) in APV-resistant HIV-1 isolates. The L90M +L10I/v/f/r mutations without I84 pattern were equally present in the APV-resistant and APV-sensitive HIV-1 isolates. This study showed that the presence of mutation I84V in baseline HIV-1 isolates correlated with the APV- resistance phenotype. All of the APV resistant baseline HIV isolates (n=25) from PI-experienced patients were resistant to IDV, NFV, RTV, and SQV (this NDA; 11.5, 335-336, appendix 6).

Correlation between baseline genotypes to plasma HIV-1 RNA response:

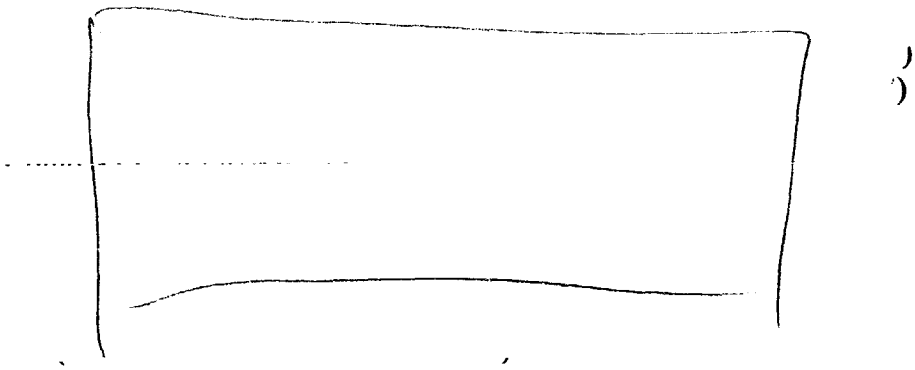
The baseline genotypes of HIV-1 isolates from patients receiving APV, ABC and EFV combination therapy were evaluated for their effect on viral load response at week 16. Patients showing a decrease in plasma HIV-RNA ≥1 $\log_{10}$ copies/mL by week 16 were

classified as responder and those with plasma HIV-1 RNA decrease of <1 $\log_{10}$ copies/mL were non-responders. The I54Vwas predominant mutation observed in non-responders (44%) than the responders (23%). Since patients were receiving combination therapy of APV, ABC and EFV, contribution of baseline APV-associated mutations alone to the viral load response at wk 16 is difficult to assess. The other combination drugs ABC and EFV were also exhibiting their anti-HIV-1 activity. Similarly, mutations associated with ABC and EFV therapy would affect plasma HIV-1 RNA levels. Therefore, the mutation I54V, identified as a predominant baseline mutation in non-responders possibly increased the viral load. However, direct correlation of viral load response to combination therapy for week 16 with baseline genotypes of patient's isolates has not been established.

METHODOLOGY:

Methodology for Genotypic Analysis:

Plasma RNA samples were used for sequence determinations. The HIV-1 protease coding region and cleavage sites was amplified by , and A 659 fragment containing the coding region for amino acids 15 to 235 of the RT was generated using and Primer sequences are listed below.



Sequencing reactions were performed using according to the manufacturer's instructions. Data were analyzed using the software. Mutant: wild type mixtures with electropherogram peak sizes 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 -70^0C until use. Viral RNA was isolated from 200 µL of plasma using ( ) 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 Expand Reverse Transcriptase ( ) as described by Hertogs et al., 1998. A 2.2 kb PR-RT-coding sequence was amplified from cDNA by 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 pHIVΔRTBstEII 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 -70^0C 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 Amplicor HIV-1 Monitor™ test 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 (this NDA; 11.6: 3-20). 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. The Roche amplicor HIV-1 Monitor test can quantitate plasma associated HIV-1 RNA at concentrations in the range $\geq$ 400 to 750,000 copies/mL.

Amprenavir Label (Draft)

MICROBIOLOGY:

Mechanism of Action: Amprenavir is an inhibitor of HIV-1 protease. Amprenavir binds to the active site of HIV-1 protease and thereby prevents the processing of viral gag and gag-pol polyprotein precursors, resulting in the formation of immature non-infectious viral particles.

Antiviral Activity in Vitro: The in vitro anti-HIV-1 activity of amprenavir was evaluated in HIV-1IIIB infected lymphoblastic cell lines (MT-4, H9) and in peripheral blood lymphocytes. The 50% inhibitory concentration (IC_{50}) of amprenavir ranged from _____ Amprenavir exhibited synergistic anti-HIV-1 activity in combination with abacavir, zidovudine, didanosine or saquinavir, and additive anti-HIV-1 activity in combination with indinavir, nelfinavir and ritonavir in vitro. These drug combinations have not been adequately studied in humans. The relationship between in vitro anti-HIV-1 activity of amprenavir and the inhibition of HIV-1 replication in humans has not been defined.

Resistance: HIV-1 isolates with a decrease in susceptibility to amprenavir have been selected in vitro and were also obtained from patients treated with amprenavir. Genotypic analysis of isolates from amprenavir treated patients showed mutations in the HIV-1 protease gene resulting in amino acid substitutions primarily at positions M46I/L, I47V, I50V, I54L, I54V and I84V, as well as mutations in the viral protease p1/p6 cleavage site. Phenotypic analysis of HIV-1 isolates from some patients on amprenavir monotherapy for – to 12 weeks showed a 5 to 10-fold decrease in susceptibility to amprenavir in vitro compared to baseline. Phenotypic analysis of HIV-1 isolates from 28 patients treated with amprenavir in combination with zidovudine and lamivudine for 16-36 weeks identified isolates from 6 patients that exhibited a 5 to 11-fold decrease in susceptibility to amprenavir in vitro compared to wild type virus. Clinical isolates that exhibited a decrease in amprenavir susceptibility harbored _____ amprenavir

associated mutations. The clinical relevance of genotypic and phenotypic changes associated with amprenavir therapy has not been established.

Cross-Resistance: HIV-1 cross-resistance to all protease inhibitors has been observed. The potential for protease inhibitor cross–resistance in HIV-1 isolates from amprenavir treated patients has not been fully evaluated.

CONCLUSIONS:

With respect to microbiology, NDA 21-007 for the marketing of Argenase (amprenavir) in combination with other antiretroviral agents as a treatment for HIV infection is recommended for approval.

1. The sponsor has provided data on the antiviral activity and mechanism of action of amprenavir. Additional data were provided on the selection of drug resistant mutants and clinical isolates following drug treatment or pressure and on the effects of amprenavir on cellular growth and on bone marrow progenitor cells.

2. Amprenavir preferentially inhibited HIV-1 protease with a K_I value of 0.6 nM and did not substantially inhibit cellular aspartic proteinases pepsin, cathepsin D, and renin.

3. The anti-HIV-1 activity of amprenavir varied with cell types, multiplicity of infection, and assay conditions used experimentally. The IC_{50} values of amprenavir against HIV-1 IIIB ranged from ——— uM. The IC_{50} values of amprenavir against HIV-1 clinical isolates ranged from ——— μM. In cell culture studies, amprenavir exhibited synergistic anti-HIV-1 activity in combination with ZDV, ddI, abacavir, or SQV, and additive anti-HIV-1 activity in combination with IDV, NFV, or RTV.

4. Amprenavir resistant HIV-1 variants were selected by passaging HIV-1 HXB2 in the presence of increasing concentrations of amprenavir. Genotypic analysis showed that amprenavir resistant isolates selected in vitro had mutations in the protease gene resulting in amino acid substitutions at positions M46L, I50V, I84V, and I47V. Phenotypic analysis demonstrated recombinant viruses containing a single mutation I50V showed <3- fold decrease in susceptibility to amprenavir in vitro. In contrast, viruses which contained triple mutations (I50V+ M46I + I47V) exhibited a 15-fold decrease in susceptibility.

5. HIV-1 isolates with reduced sensitivity to amprenavir were also obtained from patients treated with amprenavir. Clinical isolates from amprenavir treated patients contained M46I/L, I47V, I50, I54V, I54L and I84V mutations in the protease gene. Most of these mutations were also detected in amprenavir resistant HIV-1 variants selected in vitro.

6. HIV-1 isolates from 4/15 evaluable patients on amprenavir monotherapy for 4 to 12 weeks exhibited a 5 to 10-fold decrease in susceptibility to amprenavir in vitro.

These isolates contained either I50V mutation alone or additional mutations M46I/L and I47V.

7. HIV-1 isolates from 19 PI naïve and NRTI experienced patients treated with amprenavir in combination with zidovudine and lamivuidine for 24-36 weeks (Phase I/II study, PROA1002C, PROA2002) were evaluated for phenotypic and genotypic changes. HIV-1 isolates from 5/19 patients exhibited a 5-11-fold decrease in susceptibility to amprenavir in vitro compared to wild type. Four of these 5 amprenavir resistant HIV-1 isolates contained the I50V mutation and the other isolate developed I54V mutation in the protease gene. Additionally, HIV-1 isolates from most patients contained ZDV and 3TC resistance conferring RT mutations after therapy and plasma HIV-1 RNA in all patients (n=23) rebounded. Therefore, it appears that ZDV and/or 3TC resistance conferring RT mutations and APV resistance mutations impart growth advantage to HIV-1 in patients treated with APV/ZDV/3TC combinations and thereby increase virus replication and decrease the efficacy of amprenavir combination therapy.

8. Genotypic and phenotypic analyses were performed on HIV-1 isolates from a subset of patients (n=30) enrolled in clinical trial PROA/B3006. In this trial, the safety and efficacy of amprenavir was compared with indinavir in combination with NRTI therapy in NRTI experienced PI naïve HIV-1 infected patients. Genotypic analysis of baseline HIV-1 isolates from 30 patients randomized to receive APV/ZDV/3TC combination therapy for 16 weeks showed that HIV-1 isolates from 27/30 patients did not harbor any PI resistance-associated mutation. However, baseline HIV-1 isolates from 2 patients from APV treatment group harbored A71V mutation, and another isolate contained M46L, I54V, I84V, L 90M mutations. These mutations are associated with resistance to IDV, RTV and SQV. Mutations M46L, I54V, and I84V are also associated with APV resistance. It appears that these two patients had prior treatment experience with some protease inhibitors. Genotypes of HIV-1 isolates from 28/30 patients receiving APV combination therapy were not characterized at week 16 possibly due to low plasma levels of HIV-1 RNA, and inability to generate PCR products. HIV-1 isolates from the remaining 2 patients were analyzed. HIV-1 isolate from one patient continued to maintain A71V mutation at week 16 of APV/ZDV/3TC combination therapy. HIV-isolate from the second patient did not contain any major PI resistance mutation at week 16. However, the isolate from this patient developed several accessory mutations during therapy (L10I, V32I, I47V).

9. HIV-1 isolates from additional 17 patients from APV treatment virologic failure group were evaluated for genotypic and phenotypic changes. HIV-1 isolates from one of the 17 patients had A71T mutation at baseline. Baseline HIV-1 isolates from 15 patients tested did not harbor any detectable PI mutations. However, HIV-1 isolates from 4/17 patients developed PI resistance associated mutations at week 16 of APV combination therapy. HIV-1 isolates from these patients (4/17) contained either I50V, I54M, A71T mutations or the I54L mutation. HIV1 isolates from the remaining 13/17 patients did not contain any PI mutation. HIV-1 isolates harboring I54M (n=1) and A71T (n=1) mutations were susceptible to amprenavir. However, HIV-1 isolate containing I54L (n=1) was 5-fold less susceptible to amprenavir compared to wild type. Data on the

amprenavir sensitivity of HIV-1 isolate harboring I50V mutation was not available. Although many patient isolates did not have any PI mutation but they were still failing combination therapy with ZDV/3TC. This is because most patients had prior NRTI experience and isolates from majority of these patients contained ZDV- and/or 3TC resistance conferring mutations. These mutations were causing drug failure.

10. Baseline genotypes and phenotypes of HIV-1 isolates were analyzed from patients (n=99) with prior PI and NRTI treatment experience participating in CNAA 2007. The mutations D30N, V32I, G48V, I50V, I54V, I54V, V82A/F/T, I84V and L90M were detected in baseline HIV-1 isolates from these patients. Mutations I54V, V82A/F/T, I84V and L90 M were more frequent in baseline isolates. Baseline isolates from most patients harbored 5 to 8 PI–resistance associated mutations. Phenotypic analysis showed that baseline HIV-1 isolates from patients with prior PI experience were resistant to APV, SAQ, IDV, NFV, and RTV. The frequency of baseline isolates exhibiting reduced susceptibility to APV, SAQ, IDV, NFV, and RTV were 45%, 72%, 79%, 81% and 84% respectively. For patients (n=55) participating in combination therapy study with APV, abacavir and efavirenz, mutations in the baseline HIV-1 isolates were correlated with a reduced susceptibility to APV. Baseline HIV-1 isolates exhibiting >4-fold reduced ssuceptibility to APV (n=25) contained I84V mutation linked with L90M in the protease gene. Baseline genotype was also correlated with viral load response at week 16. The mutation I54V was most frequent in non-responders (patients with plasma HIV-1 RNA decrease of <0.5 $\log_{10}$ copies/mL). However, in combination therapy, the role of a mutation associated with a single drug to viral load response was difficult to assess since other two drugs used in the combination also had anti-HIV-1 activity. Additional mutations associated with combination drugs develop during therapy and the baseline mutation alone can not predict the efficacy of drug combination therapy. It is likely that baseline mutations persisted during therapy and additional mutations associated with amprenavir, abacavir and efavirenz resistance developed during therapy. Genotypic analysis data for week 16 were not available.

11. Besides mutations in the protease gene, mutations in the Gag cleavage sites p1/p6 were identified in some HIV-1 isolates exhibiting phenotypic resistance to amprenavir. The Gag p1/p6 cleavage site mutations L-F developed in HIV-1 isolates containing I50V mutation. Cleavage site mutations provide growth advantage to the mutant virus in the presence of drug (Zhang et al., 1997).

12. Data in the current NDA are limited with respect to the development of cross resistance among PIs following amprenavir therapy. Cross- resistance to HIV-1 isolates has been observed among protease inhibitors. In vitro studies showed that amprenavir resistant HIV-1 variants were susceptible to indinavir, or saquinavir. However, amprenvir resistant HIV-1 variants were found to be resistant to ritonavir. Similarly, some of the amprenavir resistant clinical isolates were found to be susceptible to indinavir, nelfinavir and saquianvir, and cross-resistant to ritonavir. Clinical isolates resistant to nelfinavir were susceptible to amprenavir. The potential for protease inhibitor cross-resistance in HIV-1 isolates from amprenavir treated pateints has not been fully evaluated.

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RECOMMENDATION:

With respect to microbiology, this application is recommended for approval.

Phase IV Considerations: Data provided in this NDA on the development of resistance to amprenavir following therapy are limited, both in the number of patient isolates evaluated and in the characterization of the development of cross resistance to other protease inhibitors. Therefore, the sponsor is requested to evaluate the development of resistance and cross resistance of sequential HIV-1 isolates from patients enrolled in ongoing clinical trials for amprenavir. Specifically, these studies should include:

1. Phenotypic susceptibility of the paired isolates to test agents (amprenavir and other protease inhibitors).
2. Genotypic basis for the changes in susceptibility, including mutations in both viral target genes and extra-genic sites such as the protease cleavage sites
3. Cross resistance of amprenavir-resistant variants to protease inhibitors and vice versa.

/S/

Microbiologist

CONCURRENCES:

HFD-530/Dep Dir _____ Signature _____ Date
HFD-530/S Micro _____ Signature _____ Date

CC:

HFD-530/Original NDA
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HFD-530/Chem
HFD-530/S Micro
HFD-530/Review Micro
HFD-530/CSO, Truffa, M.