

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

NDA # 21,007

NDA # 21,039

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CORRESPONDENCE DATE : 07/13/00
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SPONSOR: Glaxo Smith Kline
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SUBMISSION REVIEWED: Original NDA

DRUG CATEGORY: Antiviral

INDICATION: Treatment of HIV-1 infection

DOSAGE FORM: Oral Capsule

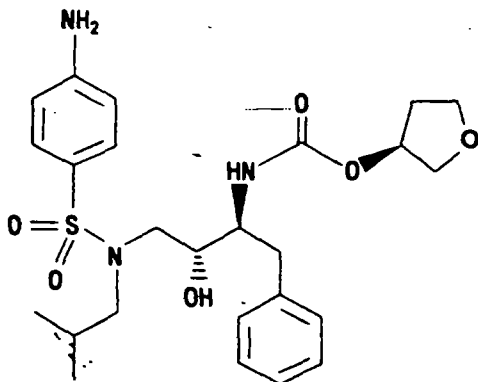
PRODUCT NAMES:

a. PROPRIETARY: Agenerase™

b. NON-PROPRIETARY: Amprenavir

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

STRUCTURAL FORMULA



MOLECULAR FORMULA: C₂₅H₃₅N₃O₆S

MOLECULAR WEIGHT: 505.64

SUPPORTING DOCUMENTS: IND [DMF # [DMF [DMF #]]]
 [DMF # [DMF # [DMF # [DMF [DMF #]]]]]

BACKGROUND:

Amprenavir (APV) is a synthetic inhibitor of HIV-1 protease. APV preferentially inhibits recombinant HIV-1 protease with a K_i value of 0.6 nM and does not substantially inhibit cellular aspartic proteinases pepsin, cathepsin D, and renin. APV 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. APV has been demonstrated to exhibit anti-HIV-1 activity both in vitro and in vivo. The anti-HIV-1 activity of APV varied with cell types, multiplicity of infection and assay conditions used. The IC_{50} values of APV against HIV-1 IIIB ranged from 0.012 to 0.41 μ M. The IC_{50} value of APV against HIV-1 clinical isolates (n=9) ranged from 0.0008 to 0.0380 μ M. In cell culture studies, APV exhibited synergistic anti-HIV-1 activity in combination with zidovudine (ZDV), didanosine (ddI), abacavir, or saquinavir (SQV), and additive anti-HIV-1 activity in combination with indinavir (IDV), nelfinavir (NFV), or ritonavir (RTV).

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

HIV-1 isolates with reduced susceptibility to APV were also obtained from patients treated with APV. Clinical isolates from APV treated patients contained either single or different combinations of V32I, M46I/L, I47V, I50V, I54L/M and I84V mutations in the protease gene. Most of these mutations were also detected in APV resistant HIV-1 variants selected in vitro. A number of accessory mutations in the HIV-1 protease gene, e.g. L10I, K20R, M36I, M46I, L63P, A71T and V77 I exist as natural polymorphisms (Kozol et al., 1996). These accessory mutations alone do not change significantly the protease inhibitor (PI) sensitivity of the wild type virus, but may reduce PI susceptibility of HIV-1 isolates in the presence of a key mutation.

Glaxo Smith Kline (GSK) has filed supplemental NDAs, 21-007 & 21-039 to provide results from clinical studies in order to update the package insert. Amprenavir was first approved on April 15, 1999 under accelerated approval regulations. In the current application, GSK has submitted results of two studies: PROA/B3001 (a 48 week study comparing the safety, efficacy and durability of APV in combination with ZDV and

lamivudine (3TC) versus ZDV and 3TC) and PROAB 3006 (comparing the safety and efficacy of APV with IDV in combination with nucleoside reverse transcriptase inhibitor (NRTIs) in protease inhibitor (PI) naïve, NRTI-experienced HIV-infected patients)

Microbiology data for the study PROAB 3001 and PROAB3006 pertaining to genotypic and phenotypic resistance are reviewed here.

SUMMARY

PROAB 3001: Genotypic and phenotypic analysis of HIV-1 protease and reverse transcriptase from subjects participating in PROAB 3001, a phase III study to evaluate the safety, efficacy and durability of APV in combination with ZDV and 3TC, compared with ZDV and 3TC alone: 48 Week Report (Report RM 1999/00147/02).

Objectives

Primary

1. To assess the association of baseline genotypic and phenotypic information with virological success or failure by week 48 for subjects randomized to receive APV/3TC/ZDV
2. To assess the association of on-therapy APV, ZDV, and 3TC susceptibility with virological success or failure by week 48 for subjects randomized to receive APV/3TC/ZDV.
3. To compare the treatment effect of on-therapy genotypic and phenotypic changes.

Study Design

PROAB3001 was designed as a double-blind, placebo-controlled phase III trial to evaluate the safety, antiviral efficacy and durability of antiviral response of APV in combination with ZDV and 3TC versus the double combination of ZDV and 3TC alone, in PI-naïve subjects with plasma HIV RNA >10,000 copies/mL and no more than four weeks experience with any NRTI or NNRTI. Approximately 290 subjects were randomized to receive either regimen 1 (APV 1200 mg BID + ZDV 300 mg BID + 3TC 150 mg BID) or regimen 2 (ZDV 300 mg BID + 3TC 150 mg BID + placebo BID) for a minimum of 48 weeks.

Virological failure was defined as plasma HIV-1 RNA levels of ≥ 400 copies/mL (at two consecutive time points from 16 weeks of randomized therapy, or at the last time point for which data was available, or subject prematurely discontinued the randomized phase due to virological failure). Plasma samples were collected at baseline, and weeks 2, 8, 16, 24, 32 and 48. Genotypic and phenotypic analyses of plasma samples obtained at different times were carried out for HIV-1 protease, (codon 1-99), p7/p1 and p1/p6 Gag cleavage sites and reverse transcriptase (codons 41, 67, 70, 151, 184, 210, 215, and 219). Plasma HIV-1 RNA was quantified using the assay.

I. Pre-and Post-therapy Viral Load

A total of 66 subjects were either randomly selected or identified as additional failures from the PROA 3001 study. Forty-seven out of the 66 completed 48 weeks of randomization treatment or discontinued the randomized phase of the study due to virological failure. The other 19 subjects discontinued the randomized phase of the study for reasons other than virological failure. The 47 subjects included in the analyses were 25 subjects receiving APV/ZDV/3TC and 22 receiving ZDV/3TC. Table 1 summarizes the virological response for the 25 subjects receiving APV/ZDV/3TC.

Table 1: Virological response by week 48 for subjects receiving APV/ZDV/3TC (n=25)

<u>Virological response</u>	<u>APV/ZDV/3TC (n=25)</u>
<400 copies/mL	11
≥400 copies/mL	14

The median baseline plasma HIV-1 RNA ranged from 3.99 to 5.63 log₁₀ copies/mL for patients with virologic failure in the APV/ZDV/3TC arm (n= 14). At the time of last genotypic analysis (week 16-48), the median plasma HIV-1 RNA ranged 2.6 to 6.01 log₁₀ copies/mL for patients (n =14) failing APV/ZDV/3TC therapy (sNDA 21007, vol 2, pages 70-72, Table 4).

II. Genotypic Analysis

APV/ZDV/3TC arm

II (a). Protease Mutations

Protease genotypes of baseline and on-therapy HIV-1 isolates from patients who failed 16 to 48 weeks of therapy are shown in Table 2. Data for baseline isolates from 2 patients (1086 & 1238) and for on-therapy isolates from 3 patients (1087, 1197 and 1693) were not available. Data presented in Table 2 are derived from (sNDA 21007, vol 2, pages 68-72, Table 4) and APPENDIX 5 (sNDA 21007, vol 2, pages 87-116). Only mutations at selected amino acid codons (key PI mutations or accessory mutations) are shown.

Although, mutations in more than 25 amino acid codons were observed; the significance of most of these mutations is not known.

Table 2: Protease genotypes of HIV-1 isolates from patients failing APV/ZDV/3TC therapy

	L101V	M36I	I50V	L63P	V77I
Baseline (n=12)	2/12	0/12	0/12	8/12	3/12
On-therapy (n=11)	3/11	0/11	1/11	8/11	2/11

Table 2 shows that the PI-accessory mutations L10I, L63P and V77I were detected in the protease gene of HIV-1 isolates obtained at baseline and on-therapy. These mutations (M36I, L63P, V77I) were also detected in baseline isolates from patients experiencing virologic success on APV/3TC/ZDV therapy (sNDA 21007, vol 2, pages 68-72, Table 4). However, the APV resistance associated- mutation I50V was detected in isolates from only one patient failing APV/ZDV/3TC therapy.

II (b). RT Mutations

The RT genotypes of on-therapy HIV-1 isolates from patients failing APV/ZDV/3TC therapy are shown in Table 3. Baseline RT genotype was available for isolates from 10 patients who failed APV/ZDV/3TC therapy (sNDA 21007, vol 2, pages 68-72, Table 4). None of the baseline isolates contained the 3TC resistance-associated M184V mutation. Similarly baseline isolates from 8 of these patients (n=10) did not contain any mutation associated with ZDV resistance. However, baseline isolates from 2 patients contained D67A mutations in the RT gene. D67A mutations have not been associated with ZDV resistance. Data for RT genotypes of on-therapy isolates were available from 11 patients failing APV/ZDV/3TC therapy (Table 3).

Table 3: RT Genotypes of on-therapy HIV-1 isolates from patients failing APV/ZDV/3TC therapy (plasma HIV-1 RNA \geq 400 copies/mL)

	WT	M184V	M41L, D67N, K70R L210W, K219Q
APV/ZDV/3TC (n = 11)	3/11	8/11	2/11

Table 3 shows that M184V was the major RT mutation detected in HIV-1 isolates from 8 patients receiving APV/ZDV/3TC therapy for 16 to 48 weeks. Two of these isolates also contained ZDV-resistance associated mutations.

III. Phenotypic Analysis

III (a). Phenotypic analysis of baseline isolates from patients failing APV/ZDV/3TC therapy

Drug-susceptibility data were available for HIV-1 isolates from 14 patients who failed APV/ZDV/3TC therapy (NDA 21-007, vol 2, pages 70-72, Table 4). Baseline isolates from all 14 patients were susceptible to the PIs tested (IDV, NFV, RTV, SQV, and APV) except that baseline isolate from one patient was resistant to NFV, and data for one patient each for APV and RTV susceptibility were not available. Baseline isolates from 12 patients were susceptible to 3TC. However, isolates from 2 other patients showed a 5- to 16-fold reduction in susceptibility to 3TC. These isolates did not harbor 3TC resistance-associated mutations M184V. Similarly, baseline isolates from 11 patients

were susceptible to ZDV, but isolates from 3 patients showed a 5- to 9-fold reduction in susceptibility to ZDV.

III (b). Phenotypic analysis of on-therapy isolates from patients failing APV/ZDV/3TC therapy

Phenotypic analysis data for PI susceptibility were available from 9 of 14 patients failing APV/ZDV/3TC therapy (NDA 21007, vol 2, pages 70-72, Table 4). On-therapy isolates from these 9 patients were susceptible to most PIs tested (IDV, NFV, RTV, and SQV) except that IDV susceptibility data were not available for isolates from one patient. Similarly, on-therapy isolates from 6 patients were susceptible to APV. However, an isolate from one patient exhibited a 7-fold reduction in susceptibility to APV in vitro. Data for APV susceptibility were not available for isolates from the remaining two patients. The genotypic analysis of the HIV-1 isolate that exhibited a 7-fold reduction in APV susceptibility was not available.

3TC susceptibility data were not available for isolates from 7 of 14 patients failing APV/ZDV/3TC therapy (NDA 21007, vol 2, pages 70-72, Table 4). On-therapy isolates from 3 patients were susceptible to 3TC. However, isolates from 4 patients showed a 52- to 68-fold reduction in susceptibility to 3TC. Similarly, isolates from one patient exhibited a 22-fold reduction in susceptibility to ZDV. On therapy-isolates from 8 patients were susceptible to ZDV. Phenotypic analysis data for ZDV susceptibility were not available for HIV-1 isolates from the remaining 5 patients. Isolates from one patient that showed a 52-fold reduction in susceptibility to 3TC harbored an M184V mutation. Genotypes were not available for isolates from other 3 patients that exhibited a reduction in susceptibility to 3TC. Similarly, genotypes were not available for isolates that exhibited a 22-fold reduction in susceptibility to ZDV. Therefore, a correlation of genotype to phenotype could not be established for most isolates exhibiting reduction in drug susceptibility due to lack of data.

PROAB3006

Objective: The objective of this clinical trial was to compare the safety and efficacy of APV with IDV in combination with nucleoside reverse transcriptase inhibitors (NRTIs) in protease inhibitor (PI) naïve, NRTI-experienced HIV-infected patients. An additional objective was to analyze the genotypes and phenotypes of protease and RT resistant HIV-1 variants from selected patients participating in PROAB3006 study.

Subjects were randomized to receive either APV (1200 mg BID) or IDV (800 mg TID) in addition to the concurrent NRTI therapy for a minimum of 48 weeks. Subjects who discontinued the randomized phase for reasons other than virologic failure (i.e., adverse experience, lost-to-follow-up, etc) were not included.

For the interpretation of safety and efficacy data, please see the Medical Officer's review. The pertinent genotypic and phenotypic analysis data from patients failing APV plus NRTI therapy are presented here.

Virological failure was defined as plasma HIV-1 RNA levels of ≥ 400 copies/mL (at two consecutive time points at week 8 or beyond or at the last time point within the randomized phase), or premature discontinuation of the randomized phase prior to Week 48 due to virologic failure.

IV. Pre and Post-therapy Viral Load

The median baseline viral load for subjects enrolled in PROAB3006 was $4.4 \log_{10}$ copies/mL. Table 4 summarizes the virological response for selected subjects receiving APV and NRTI therapy.

Table 4: Virological response by 48 week for selected subjects enrolled in PROAB3006

	APV
N	69
<400 copies/mL	6
> 400 copies/mL	37
Premature discontinuation	26

V. Genotypic Analysis of HIV-1 isolates from patients failing APV plus NRTI therapy

V (a). Protease Mutations:

Genotypic analysis of HIV-1 isolates from 48 patients failing APV plus NRTI therapy showed that HIV-1 isolates from 31 patients developed APV resistance-associated mutations during therapy. Genotypic and phenotypic analyses of the protease gene for matched baseline and on-therapy isolates from these 31 subjects are presented.

Baseline Genotype

Genotypic analysis showed that none of the baseline isolates contained APV resistance-associated mutations in the protease gene. However, baseline isolates harbored accessory mutations L10I, M36I, L63P, A71T/V, V77I, and V82I. The mutation L63P was most prevalent (>50%) in baseline isolates. The mutation L63P was detected either alone, or in combination with other accessory mutations; L10I/V, M36I, A71T/V, V77I, and V82I (NDA 21007, vol 3, Page 92, Table 20, listings 11.1 to 11.5, pages 323-397, and listing 12, pages 398-413).

On-therapy Genotype

The key PI mutations that emerged during APV plus NRTI therapy are shown in Tables 5-9. These tables also contain APV and RTV phenotypic susceptibility data (summarized in section VII) for isolates from the respective patients.

Genotypic analysis showed that HIV-1 isolates from patients failing APV therapy contained either M46I/L, I50V, I54L/M, I84V mutations alone, or in different combinations with L10I, V32I, L33F, M46I/L, I47V, I50V, I54L/M, L63P/V, A71T/V, V77L, and I84V (Table 25, NDA 21007, vol 3.0, page 104). Five distinct patterns of mutations associated with APV resistance emerged during APV therapy, and these are shown in descending order; I54L/M (n=10), I50V (n=9), V32I+I47V (n=7), M46L (n=5) and I84V (n=3). It should be noted that isolates from some patients (n=3) developed two different mutational patterns during therapy and these patients are included in both mutational patterns (please see Tables-5-9).

(1): I54L/M mutation in combination with other APV-resistance associated mutations (n=10)

HIV-1 isolates from ten of the 31 subjects developed I54L/M mutation alone, or in combination with other APV resistance-associated mutations (Table 5). Six isolates had isoleucine to leucine amino acid substitution at position 54 and four were isoleucine to methionine.

Table 5: Genotypic and phenotypic analysis of HIV- 1 isolates that developed I54L/M mutation during APV plus NRTI therapy

Subject	Visit week	Genotypic changes from baseline		Susceptibility (FR)*	
		Protease	Cleavage sites	APV	RTV
2456	48	L33F, I54L	None	ND	ND
2460	48	M46M/L, I54L	None	ND	ND
2504	48	I54M	None	3.25	1.93
2728	48	M46L, I54L	None	7.66	4.12
2808	48	I54M	None	ND	ND
2866	48	M46I, I54I/M	None	4.06	4.57
3136	48	M46I/M, I54I/L,84I/V	P7/p1AP2V P1/p6 Lp1'P	5.62	2.67
3710	48	M46L,I54L	None	ND	2.6
2442	24	I54M	None	5.73	1.27
2438	-16	I54L	None	ND	ND

*Fold resistance compared to reference standard HIV-1 HXB-2

Comment: HIV-1 isolates from patient 2438 also contained V32I + I47V mutations. This patient is also included in Table 7.

2. I50V mutation in combination with other APV resistance-associated mutations (n=9)

HIV-1 isolates from nine of 31 subjects who failed APV plus NRTI therapy developed I50V mutation alone, or in combination with other mutations associated with APV-resistance (Table 6). Additionally, on therapy HIV-1 isolates from five of these 9 patients also developed the Gag p1/p6 cleavage site substitution L to F at position p1'. The mutation I50V alone (see Table 10, patient 2459) or in combination with M46I/L (Table 6) manifested APV resistance.

Table 6: Genotypic and phenotypic analysis of HIV-1 isolates that developed I50V mutation during APV plus NRTI therapy

Subject	Visit (week)	Genotypic changes from baseline		Susceptibility (FR)	
		Protease	Cleavage sites	APV	RTV
2459	16	I50I/V	None	ND	ND
2812	48	M46I, I50V, A71A/V	None	ND	ND
2851	48	M46I, I50V, V82I	p1/p6, Lp1'F	26.6	89.2
2891	36	I50V, A71V	p1/p6, Lp1'F	ND	ND
3567	48	I50V, V77I V82I	p1/p6 Lp1'F	40.9	39.5
3573	36	M46L, I50V L63R	p1/p6 Lp1'F	20.9	87.7
3721	48	M46I, I50V	p1/p6 Lp1'F	15	3.55
4357	48	M46I, I50V, A71A/V	None	6.66	ND
4359	48	M46L, I50V	None	6.17	2.77

ND = No data

3. V32I + I47V mutation in combination with other APV resistance-associated mutations (n=7)

HIV-1 isolates from seven of the 31 subjects developed both V32I and I47V mutations in combination with other mutations associated with APV resistance (Table 7). One of the seven isolates also had an A to V change at position p2 of the p7/p1 Gag cleavage site.

Table 7: Genotypic and phenotypic analysis of HIV-1 isolates that developed V32I + I47V mutation during APV plus NRTI therapy

Subject	Visit (week)	Genotypic changes from baseline		Susceptibility (FR)	
		Protease	Cleavages sites	APV	RTV
3068	24	V32I, I47V,	None	2.89	1.43
3254	48	L10I, V32I, M46I, I47V	None	4.47	4.3
3573	24	V32I, I47V	None	4.36	1.14
4090	48	V32I, I47V	None	24.3	7.67
4119	48	L10F, V32V/I, M46M/L/I I47I/V	p7/p1 AP2A/V	4.88	11.3
3549	24	V32I, I47V	None	5.31	5.85
2438	24	V32I, I47V	None	ND	ND

Comment: HIV-1 isolates from subject 3573 also contained an I50V mutation. This patient is also included in Table 6.

(4). M46L mutation in combination with other mutation associated with APV resistance (n=5)

HIV-1 isolates from five of 31 subjects developed an M46L mutation either alone or in combination with accessory mutations associated with APV resistance (Table 8).

Table 8: Genotypic and phenotypic analysis of HIV-1 isolates that developed M46L mutation during APV/NRTI therapy

Subject	Visit week	Genotypic changes from baseline		Susceptibility (FR)	
		Protease	Cleavage sites	APV	RTV
2871	48	L33F, M46L, A71V	P7/p1 AP2A/V	4.77	0.98
2853	48	M46L	None	0.14	1.35
3469	48	M46M/L, V77I	None	1.95	2.18
3570	16	L10F, M46L	None	ND	ND
3110	44	M46L, V82I	None	6.2	2.9

(5): I84V mutation in combination with other APV-resistance associated mutations (n=3)

HIV-1 isolates from three of 31 subjects developed an I84V mutation alone, or in combination with other APV resistance associated mutations (Table 9). The I84V amino acid substitution was accompanied by the p1/p7 cleavage site mutation A to V at position P2 in all three isolates.

Table 9: Genotypic and phenotypic analysis of HIV-1 isolates that developed I84V mutation during APV plus NRTI therapy

Subject	Visit (week)	Genotypic changes from baseline		Susceptibility (FR)	
		Protease	Cleavage sites	APV	RTV
3345	48	M46L, L63A, I84V	P7/p1 AP2A/V	3.44	3.54
3571	48	I84V	P7/p1 AP2A/V	20.4	8.35
3136	48	M46L, I54I/L, I84I/V	P7/p1 AP2V, P1/p6 Lp1'P	5.62	2.67

Comment: HIV-1 isolates from patient 3136 also contained an I54I/L mutation. This patient is also included in Table 5.

V (b). RT Mutations

Most of the APV resistant isolates contained ZDV resistance-associated mutations (M41L, D67N, K70R, T215Y/F, K219Q/E) in combination with the 3TC resistance-associated M184V and/or NNRTI resistance-associated mutations (K103N, Y181C) at baseline (NDA 21007, vol 3, Listing 14, pages 422-426). Some baseline isolates contained only the M184V mutation.

Most of the APV resistant isolates (23/31) contained ZDV resistance-associated mutations in combination with the 3TC resistance associated mutation M184V and/or NNRTI associated mutation (A98S, K103N, Y181C) during APV/plus NRTI therapy. However, some on-therapy isolates contained only ZDV resistance-associated mutations (3/31) or the mutation M184V (3/31) (NDA 21007, vol 3, pages 466-467). Isolates from one patient contained an A98S mutation only, and the RT genotype was not available for on therapy isolates from another patient (NDA 21007, vol 3, pages 466-467).

VI. Phenotypic Analysis of HIV-1 isolates failing APV plus NRTIs therapy

VI. Phenotypic analysis of baseline and on-therapy isolates

Baseline phenotypic analysis data were available for HIV-1 isolates from 22/31 patients failing APV/NRTI therapy. Most of the baseline isolates (21/31) had reduced susceptibility to ZDV and/or 3TC, or nevirapine (NVP). However, these isolates were susceptible to APV in vitro (NDA 21007, vol 3, Listing 16, pages 450-458)

Phenotypic analysis of on therapy HIV-1 isolates from 24 NRTI experienced, PI-naïve patients treated with APV plus NRTIs for 16 to 48 weeks showed that isolates from 19 patients exhibited a 6- to 41-fold decrease in susceptibility to APV in vitro compared to wild-type virus (Tables 5-9). In addition to PI susceptibility, on-therapy isolates were tested for their susceptibility to NRTIs and NNRTIs. Phenotypic analysis showed that most of the on-therapy isolates (22/24) exhibited a 4- to 62-fold decrease in susceptibility to 3TC in vitro as compared to wild-type virus (NDA 21007, vol 3, appendix 3, page 466). Of these, 11 isolates exhibited a 9- to 510-fold decrease in susceptibility to ZDV, and a 4- to 1364-fold decrease in susceptibility to NVP. Additionally, one isolate showed a 489-fold decrease in susceptibility only to NVP.

VII. Cross-resistance of APV resistant isolates to IDV, NFV, RTV, SQV and lopinavir (LPV)

The sponsor had earlier submitted data on cross-resistance of the APV resistant isolates to different PIs. These data were generated using an old version of the assay. Phenotypic susceptibility data of APV resistant isolates to RTV are shown in Tables 5-9. Data on the susceptibility of APV resistant isolates to IDV or SQV were also provided by the sponsor and reviewed (data not shown). Results of this study showed that 10 of the 18 APV resistant isolates exhibited a 4- to 80-fold decrease in susceptibility to RTV.

However, all APV resistant isolates for which data were available (18/19) were susceptible to IDV or SQV.

Recently, the sponsor submitted a second data set on the cross-resistance of the APV resistant isolates to different PIs. These data were obtained using a new version of the assay and are presented in Table 10. Table 10 (page 13) shows that on-therapy isolates from 15 of 21 patients exhibited a 4- to 17-fold decrease in susceptibility to APV. Five of the 15 APV resistant isolates exhibited a 4-to 8-fold reduction in susceptibility to RTV. Similarly, 4 of the 15 APV resistant isolates also exhibited a 4- to 6-fold reduction in susceptibility to LPV. Additionally, 1 of the 15 APV resistant isolate showed a 6-fold reduction in susceptibility to NFV. However, APV resistant isolates were susceptible to either IDV or SQV (data not shown).

CONCLUSIONS

Genotypic analysis showed that most of the HIV-1 isolates from patients failing APV/3TC/ZDV therapy (PROAB3001) harbored an M184V mutation. The APV resistance associated mutation I50V was detected in on-therapy isolates from one of 11 patients failing APV/3TC/ZDV therapy. Accessory mutations (L10I, M36I, L63P, A71T/V, and V77I) were detected in baseline and on-therapy isolates. As stated previously, these mutations by themselves do not confer PI resistance. Phenotypic analysis showed that most of the on-therapy isolates were susceptible to APV and the other PIs tested (IDV, NFV, RTV, SQV). An on-therapy isolate from one of 9 patients failing APV/ZDV/3TC therapy exhibited a 7-fold decrease in APV susceptibility. Limited data was available on susceptibility of on-therapy isolates to 3TC and ZDV. Isolates from 4 of 7 patients exhibited a 52- to 68-fold decrease in susceptibility to 3TC. Similarly, isolates from 1 of 9 patients exhibited a 22-fold decrease in susceptibility to ZDV. A correlation between genotypes and phenotypes of isolates with reduced susceptibility to 3TC and ZDV could not be established.

Genotypic and phenotypic analyses were performed on HIV-1 isolates from a subset of patients enrolled in clinical trial PROAB3006. In this trial, the safety and efficacy of APV was compared with IDV in combination with NRTI therapy in NRTI experienced PI naïve HIV-1 infected patients. Genotypic analysis of HIV-1 isolates from 48 patients failing APV and NRTIs therapy showed that isolates from 31 patients harbored either single or different combinations of protease mutations V32I, M46I/L, I47V, I50V, I54L/M and I84V. Most of these mutations were also observed in APV resistant isolates selected in vitro.

Phenotypic analysis of HIV-1 isolates from 21 patients treated with APV in combination with NRTIs for 16 to 48 weeks identified isolates from 15 patients that exhibited a 4- to 17-fold decrease in susceptibility to APV in vitro compared to wild-type virus.

A correlation of genotypes to phenotypes indicates that some mutations either alone, e.g. I50V, I54L/M, or I84V, or in combination with other PI mutations, e.g., M46I plus I50V, V32I plus I47V, M46L plus I54L, M46L, L63A plus I84V, M46L plus V82I were sufficient to cause APV resistance.

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Cross-resistance to other PIs was observed among APV-resistant isolates. Five of fifteen APV-resistant isolates exhibited a 4- to 8-fold decrease in susceptibility to RTV. However, APV-resistant isolates were susceptible to either IDV or SQV. Similar results were obtained from *in vitro* studies. The potential for cross-resistance of amprenavir resistant HIV-1 isolates to other PIs, i.e. NFV or LPV has not been fully evaluated.

Besides mutations in the protease gene, some APV resistant isolates contained mutations in the Gag cleavage sites p7/p1 and p1/p6 Gag cleavage sites. Cleavage site mutations provide growth advantage to the mutant virus in the presence of drug (Zhang *et al.*, 1997)

Table 10: Phenotypic susceptibility of HIV-1 isolates failing APV plus NRTI therapy (fold resistance relative to control)

Subject	Visit	APV	NFV	RTV	LPV
2438	16	ND	ND	ND	ND
2438	24	5.90	3.20	4.40	0.80
2442	24	4.90	2.10	0.80	0.80
2456	48	7.60	5.90	1.80	1.80
2459	16	16.0	1.40	5.0	2.40
2460	48	0.70	0.50	0.70	0.70
2504	48	4.0	2.40	0.90	1.20
2728	48	2.0	6.20	2.0	1.80
2808	48	3.40	2.30	2.60	1.40
2812	48	ND	ND	ND	ND
2851	48	ND	ND	ND	ND
2866	48	2.0	1.50	3.90	0.90
2891	36	5.90	3.30	3.80	2.90
3068	24	5.40	2.30	1.80	2.80
3136	48	17.40	2.70	4.70	1.70
3254	48	ND	1.10	0.50	0.40
3345	48	4.50	0.80	1.60	4.50
3549	24	7.0	2.0	2.0	4.50
3567	48	9.60	1.40	8.30	2.0
3571	48	10.30	1.30	4.60	3.20
3573	24	2.60	1.10	0.70	2.90
3573	36	5.90	2.10	1.30	3.40
3710	48	ND	ND	ND	ND
3721	48	5.0	3.70	3.60	5.70
4090	48	ND	ND	ND	ND
4119	48	3.60	2.10	4.80	6.60
4357	48	5.30	1.0	2.50	4.0
4359	48	3.30	0.70	1.50	0.90

AMPRENAVIR LABEL

MICROBIOLOGY:

Mechanism of Action: Amprenavir is an inhibitor of the 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* antiviral activity of amprenavir was evaluated in HIV-1_{IIIB} infected lymphoblastic cell lines (MT-4, H9) and in peripheral blood lymphocytes. The 50% inhibitory concentration (IC₅₀) of amprenavir ranged from 0.08 to 0.41 μM (1 μM = 0.50 $\mu\text{g/ml}$), depending upon the cells employed. Amprenavir exhibited synergistic anti-HIV-1 activity in combination with either abacavir, zidovudine, didanosine or saquinavir, and additive anti-HIV-1 activity in combination with either indinavir, nelfinavir, or 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 decreased susceptibility to amprenavir have been selected *in vitro* and 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 V32I, M46I/L, I47V, I50V, I54L/M, and I84V, as well as mutations in the p7/p1 and p1/p6 Gag cleavage sites. Phenotypic analysis of HIV-1 isolates from 21 nucleoside reverse transcriptase inhibitor (NRTI) experienced, protease inhibitor naïve patients treated with amprenavir in combination with NRTIs for 16 to 48 weeks identified isolates from 15 patients that exhibited a 4- to 17-fold decrease in susceptibility to amprenavir *in vitro* compared to wild-type virus. Clinical isolates that exhibited a decrease in amprenavir susceptibility harbored one or more amprenavir-associated mutations. The clinical relevance of genotypic and phenotypic changes associated with amprenavir therapy is under evaluation.

Cross-Resistance: Varying degrees of HIV-1 cross-resistance among protease inhibitors have been observed. Five of 15 amprenavir-resistant isolates exhibited a 4-to 8-fold decrease in susceptibility to ritonavir. However, amprenavir-resistant isolates were susceptible to either indinavir or saquinavir.

METHODOLOGY:

Methodology for Genotypic Analysis:

Plasma RNA samples were used for sequence determinations. A portion of the HIV-1 genome encoding the protease and the Gag cleavage sites (883 bp) was amplified by nested PCR using outer primers CS-1 and Comb-3, and inner primers CS-2 and RVP-3.

A 659 bp fragment containing the coding region for amino acids 15 to 235 of RT was generated using outer primers A-35 and NE-1 and inner primers Comb-2 and Comb-3. Primer sequences are listed below.

CS-1 (5' TAGAAGAAATGATGACAGCATGTCAGGG-3')
 CS-2 (5'-GAGGACCCGGCCATAAAGCAAGAGTTTTGGC-3')
 A-35 (5'-TTGGTTGCACTTTAAATTTTCCCATTAGCCCTATT-3')
 NE-1 (5'-CCACTAACTTCTGTATGTCATTGACAGTCCAGCT-3')
 Comb-2 (5'-CTGTACCAGTAAAATTAAGCCAGG-3)
 Comb-3 (5'- ATAGGCTGTACTGTCCATTTATCAGG-3')
 Rvp-3 (5'-GGCAAATACTGGAGTATTGTATGG-3')
 CP-1 (5'-GAAGGCACATAGCCAGAAATTGCAGGG-3')
 3'Inprot (5'-CCTGGCTTTAATTTTACTGG-3')
 RH14 (5'-TACTGCATTTACCATACCTAGTATAAAC-3')
 BR (5'-GGTGATCCTTTCCATCC-3')

The resultant DNA was sequenced using the .

with internal primers from the 3' (3'Inprot), and the 5' (CP-1) ends of the protease and 3' (RH14) and 5' (BR) ends of RT. Sequencing reactions were resolved on an sequencer according to the manufacturer's instructions .

Data were analyzed using the .

Mutant : wild type ratios greater than 80% were designated as the mutant.

Methodology for Phenotypic Analysis

Phenotypic analysis was performed using a recombinant virus assay .

This is an experimental assay, and its accuracy, sensitivity, and reproducibility has not been validated. The assay is performed as follows:

DNA fragments were co-transfected with the linearized plasmid, by into MT4 cells, to produce recombinant virus by DNA recombination and subsequent expression. This plasmid has a deletion of the protease coding region and p1/p6 and p7/p1 Gag cleavage sites, and allows the generation of recombinant viruses containing both the subject plasma virus CS (cleavage sites) and protease regions. Cultures were monitored for virus cytopathic effect (CPE), and virus supernatants and cell pellets prepared and when the CPE was complete.

Assays for recombinant virus infectivity (TCID₅₀) and susceptibility were performed using the dye uptake method with the vital dye 3-(4,5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT) to quantify cell viability. For each assay, 10 concentrations of each compound were used over a 2-fold dilution range with each dilution set up in quadruple. The concentration of compound which protected 50% of the cells from virus killing (IC₅₀) was determined from regression analysis of the plot of percentage of cell death against drug concentrations. The relative susceptibility compared with the HIV-1 reference strain HXB-2 analyzed in the same assay was

calculated. The inherent variability of the phenotypic assay was estimated to be 4-fold.

Methodology for HIV RNA copy number determination:

The approved 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. 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 400 to 750,000 copies/mL.

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RECOMMENDATION:

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

/s/ _____ 5/22/01
Microbiologist

CONCURRENCES:

HFD-530/Dep Dir _____ Signature _____ Date _____
HFD-530/S micro _____ Signature _____ Date _____
CC:

- HFD-530/Original sNDA (21-007)
- HFD-530/ Division File
- HFD-530/S Micro
- HFD-530/Review Micro
- HFD-530/CSO, Truffa, M.