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## APPROVAL PACKAGE FOR:

## APPLICATION NUMBER
## 21-007/SE7-006
## 21-039/SE7-006

# Microbiology Review(s)

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

NDA # 21,007
NDA # 21,039

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| REVIEWER | : LALJI MISHRA, Ph.D. |
| CORRESPONDENCE DATE | : 07/13/00 |
| CDER RECEIPT DATE | : 07/14/00 |
| REVIEW ASSIGN DATE | : 07/31/00 |
| COMPLETION DATE | : 4/30/01 |

SPONSOR: Glaxo Smith Kline
Five Moore Drive
Research Triangle Park, NC 27709
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SUBMISSION REVIEWED: Original NDA

DRUG CATEGORY: Antiviral

INDICATION: Treatment of HIV-1 infection

DOSAGE FORM: Oral Capsule

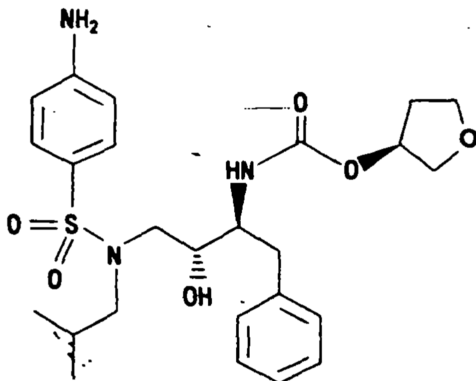
PRODUCT NAMES:

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

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 μM. The $IC_{50}$ value of APV against HIV-1 clinical isolates (n=9) ranged from 0.0008 to 0.0380 μM. 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 $\geq$ 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)**

| Virological response | APV/ZDV/3TC (n=25) |
|---|---|
| <400 copies/mL | 11 |
| $\geq$400 copies/mL | 14 |

The median baseline plasma HIV-1 RNA ranged from 3.99 to 5.63 $\log_{10}$ 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 $\log_{10}$ 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**

| | L10I/V | 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 ≥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, V77I, 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 Protease | Cleavage sites | Susceptibility (FR) 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 + I47Vmutation 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 Protease | Cleavages sites | Susceptibility (FR) 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 | M46I,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 the15 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.

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ON ORIGINAL

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.

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-1IIIB infected lymphoblastic cell lines (MT-4, H9) and in peripheral blood lymphocytes. The 50% inhibitory concentration ($IC_{50}$) of amprenavir ranged from 0.08 to 0.41 μM (1 μM = 0.50 μg/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'-CTGTACCAGTAAAATTAAAGCCAGG-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 ($TCID_{50}$) 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 ($IC_{50}$) 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.

**REFERENCES:**

Averett, D.R. Anti-HIV compound assessment by two novel high-capacity assays. J. Virological Methods. 1989; 23: 263-76.

Averett, D.R., Dark, E.H., and Gallagher, B.J. Growth inhibition of human leukemic cell lines by 141W94UA. Glaxo Wellcome report TEIN/94/0033. 1994. NDA 21-007; 2.26: 96-100.

Boone, L.R., and Cheng, N. In vitro passage of HIV-1 in MT-4 cells treated with 141W94UA. Glaxo Wellcome report TGZZ/94/0045. 1994. NDA 21-007; 2.26: 197-204.

Condra, J.H., Schieif, W.A., Blahy, O.M., Gabryelski, L.J., Graham, D.J., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., Titus, D., Yang, T., Teppler, H., Squires, K.E., Deutsch, P.J., and Emini, E.A. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature. 1995; 374: 569-571.

Hertogs, K., de Bethune, M-P., Miller, V., Ivens,T., Schel, P., Cauwenberge, A.V., Eynde, C.V.D., Gerwen,V.V., Azijn, H., Houtte, M.V., Peeters, F., Staszewski, S., Conant, M., Bloor, S., Kemp, S., Larder, B., and Pauwels, R. A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients treated with antiretroviral drugs. Antimicrob. Agents and Chemother. 1998; 42: 269-276.

Jacobson, H., Hanggi, M., Ott, M., Duncan, I.B., Owen, S., Andreoni, M., Vella, S., and Mous, J. In vivo resistance to a human immunodeficiency virus type 1 proteinase inhibitor: mutations, kinetics, and frequencies. J. Infect. Dis. 1996; 173: 1379-1387.

Kozal, M.J., Shah, N., Shen, N., Yang, R., Fucini, R., Merigan, T.C., Richman, D.D., Morris, D., Hubbell, E., Chee, M., and Gingeras, T.R. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density oligonucleotide arrays. Nature Medicine. 1996; 2: 753-759.

Larder, B.A., Kohli, A., Kellam, P., Kemp, S.D., Kronick, M., and Henfrey, R.D., Quantitative detection of HIV-1 drug resistance mutations by automated DNA sequencing. Nature. 1993; 365: 671-673.

Livingston, D.J., Pazhanisamy, S., Porter, D.J.T., Partaledis, J.A., Tung, R.D., Painter, G.R. Weak binding of VX-478 to human plasma proteins and implications for anti-human immunodeficiency virus therapy. J.Infect. Dis. 1995; 172: 1238-1245.

Maguire, M.F., Klein, A.P., Snowden,W., and Tisdale, S. M. Genotypic and phenotypic analysis of HIV-1 protease and reverse transcriptase from subjects participating in PROAB3006, a phase III study to compare the safety and efficacy of amprenavir with indinavir in combination with nucleoside reverse transcriptase inhibitor (NRTI) therapy in NRTI experienced, protease inhibitor (PI) naïve HIV-1 infected patients; 48 week report. Glaxo Wellcome report no. SR2000/00013/00, sNDA 21007, 3:2-470.

Molla, A., Korneyeva, M., Gao, Q., Vasavanonda, S., Schipper, P.J., Mo, H-M., Markowitz, M., Chernyavskiy, T., Niu, P., Lyons, N., Hsu, A., Granneman, G.R., Ho, D.D., Boucher, C.A.B., Leonard, J.M., Norbeck, D.W., and Kempf, D.J. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nature Medicine. 1996; 2: 760-766

Parker, H., Shortino, D., Tisdale, M., and Snowden,W. Genotypic and phenotypic analysis of HIV-1 protease and reverse transcriptase from subjects participation in PROAB 3001, a phase III study to evaluate the safety, efficacy and durability of amprenavir in combination with ZDV and 3TC, compared with ZDV and 3TC alone: 48 week report. Glaxo Wellcome report RM 1999/00231/00, sNDA 21007, 2; 36-142.

Parry, N.R., Blair, E.D., Wood, J., Myers, R., and Tisdale, M. In vitro evaluation of protease inhibitor combinations against human immunodeficiency virus type-1 (HIV-1). Glaxo Wellcome report SR1998/00004/00. 1998. NDA 21-007; 2.26: 56-67.

Partaledis, J.A., Yamaguchi, K., Tisdale, M., Blair, E.D., Falcione, C., Maschera, B., Myers, R.E., Pazhanisamy, S., Futer, O., Cullinan, A.B., Stuver, C.M., Byrn, R.A., and Livingston, D.J. In vitro selection and characterization of human immunodeficiency virus type 1 (HIV-1) isolates with reduced sensitivity to potent sulfonamide inhibitors of HIV-1 aspartyl protease. J. Virol. 1995; 69: 5228-5235.

Pauwels, R.J., Balzarini, J., Baba, M., Snoek, R., Schols, D., Herdewijn, P., Desmyter, J., and De Clerq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol Methods. 1988; 20: 309-312.

St.Clair, M., Hazen, R.J. Anti-HIV activity of 140W94UB, 141W94UB and 142W94 Glaxo Wellcome report TGZZ/94/0031-01. 1994. NDA 21-007; 2.26: 44-54.

Tisdale, M., Blair, E., and Myers, R. Studies on the development of HIV resistance to 141W94 by in vitro passage in MT-4 cells. Glaxo Wellcome report BBIO/94/0009. 1994. NDA 21-007; 2.26: 165-172.

Tisdale, M., Myers, R.E., Maschera, B., Parry, N.R., Oliver, N.M., and Blair, E.D. Cross-resistance analysis of HIV-1 variants individually selected for resistance to five different protease inhibitors. Antimicrob. Agents Chemother. 1995; 39: 1704-1710.

Zhang, Y-M., Imamichi, H., Imamichi,T., Lane, H.C., Falloon, J., Vasudevachari,M.B., and Salzman, N.P. Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites. J. Virol. 1997; 71: 6662-6670.

**RECOMMENDATION:**

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

/S/ 5/22/01
Microbiologist

CONCURRENCES:

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HFD-530/S micro ______________ Signature ________ Date
CC:

HFD-530/Original sNDA (21-007)
HFD-530/ Division File
HFD-530/S Micro
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