CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-548

MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA 21,548; SN-000 **Review Completed** 09/25/03

Reviewer: LALJI MISHRA, Ph.D.
Date Submitted: 12/19/02
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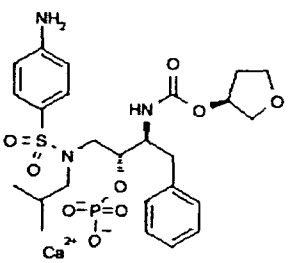
Assigned Date: 01/02/03

Sponsor: GlaxoSmithKline
Five Moore Drive
Research Triangle Park, NC 27709
(919) 483-2100

Product Names:

a. Proprietary: Stelzir™
b. Non-proprietary: Fosamprenavir calcium
c. Chemical: (3S)-tetrahydrofuran-3-yl (1S, 2R)-3-[[(4-aminophenyl) sulphonyl](isobutyl)amino]-1-benzyl-2-(phosphonooxy) propylcarbamate monocalcium salt.

Molecular Formula: $C_{25}H_{34}CaN_3O_9PS$



FOSAMPRENAVIR

Molecular Weight: 623.7
Indication: Treatment of HIV-1 infection in combination with other antiretroviral drugs
Route of Administration/Dosage Form: Oral/Tablet

Additional submissions reviewed:

Supplement #	**Date of Correspondence**	**Date of Receipt**
N21548 BC	01/24/03	02/03/03
N21548 Bl	04/08/03	04/14/03
N21548 BZ	04/02/03	06/17/03
N21548 B2	04/03/03	06/17/03
N21548 Bl	08/25/03	08/25/03
N21548 Bl	08/14/03	08/18/03
N21548 Bl	08/01/03	08/05/03
N21548 BM	07/18/03	07/23/03
N21548 BMI	08/25/03	08/25/03

MICROBIOLOGY REVIEW
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Supporting Documents: IND 47,111, DMF # [redacted] DMF # [redacted] DMF # [redacted]
DMF # [redacted] DMF # [redacted] DMF # [redacted], DMF # [redacted], DMF # [redacted]

BACKGROUND:

Glaxo Smith Kline (GSK) has submitted a new drug application NDA # 21-548 and seeks marketing approval of fosamprenavir (700 mg BID plus 100 mg ritonavir) in combination with other antiretroviral agents for the treatment of HIV-1 infection. Fosamprenavir (GW433908) is a calcium phosphate ester prodrug of amprenavir. Amprenavir (APV) was first approved on April 15, 1999 under accelerated approval regulations for the treatment of HIV-1 infection in adults and children. APV has low bioavailability (30%) and therefore, patients on APV treatment have to take sixteen 150-mg soft gel capsules daily. To optimize adherence and convenience to the APV dosing regimen, GSK developed GW433908. GW433908 is hydrolyzed by cellular alkaline phosphatase to APV and inorganic phosphate in vivo. GW433908 is almost entirely (99%) converted to APV at or near the intestinal epithelium. GW433908 is rapidly converted to APV with minimal GW433908 plasma exposure. APV is primarily the form detected in plasma following an administration of GW433908. APV is an inhibitor of HIV-1 protease.

In the current application, GSK has submitted results of three pivotal studies, APV 30001, APV 30002 and APV 30003 to support the use of GW433908 for the treatment of HIV-1 infection. Resistance (genotypic and phenotypic analyses) data obtained from patients enrolled in APV 30001, APV 30002 and APV 30003 are reviewed here. The sponsor has submitted data on the mechanism of action and in vitro antiviral activity of GW433908 and has referred to NDA # 21-007 for pertinent microbiology data on APV. Data on the mechanism of action of APV, in vitro anti-HIV-1 activity of APV, development of APV resistant HIV-1 mutants in vitro and in vivo, mutations associated with APV-resistance, cross-resistance with other protease inhibitors, and combination activity relationships were previously reviewed (microbiology review of amprenavir NDA #21-007 and sNDA 21-007), and are briefly summarized here.

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 host 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 (Averett, 1989; St.Clair et al., 1994;). In cell culture studies (Parry et al., 1998, Molla et al., 2002)), APV exhibited synergistic anti-HIV-1 activity in combination with the NRTIs abacavir (ABC), didanosine (ddI), and zidovudine (ZDV),

or the PI saquinavir (SQV), and additive anti-HIV-1 activity in combination with PIs indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), or ritonavir (RTV). An analogue of APV in combination with a non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) demonstrated additive anti-HIV-1 activity (NDA 21-548, BZ , GSK letter dated April 2, 2003).

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 I50V mutation 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 (Partledis et al., 1995; Boone and Cheng, 1994; Tisdale et al., 1994,1995; NDA 21-007).

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 (s-NDA 21-007; Maguire et al., 2002). 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. Besides mutations in the protease gene, some APV resistant isolates contained mutations in the p7/p1 and p1/p6 Gag and Gag-Pol polyprotein precursor cleavage sites. Cleavage site mutations provide growth advantage to the mutant virus in the presence of drug (Zhang et al., 1977, Maguire et al. 2002).

Data on the mechanism of action and anti-HIV-1 activity of GW433908 are summarized below. Data on the genotypic and phenotypic analyses of HIV-1 isolates from patients from clinical studies APV 30001, APV 30002 and APV 30003 are reviewed here. For efficacy results of APV 30001, APV 30002 and APV 30003 studies, please see reviews of Medical Officer and Statistician. Clinical studies refer to GW433908 as 908. Therefore, the name 908 will be used interchangeably with GW433908 in this review.

SUMMARY

I. Mechanism of action:

I (a) Inhibition of HIV-1 protease by APV (Data from NDA 21-007):

APV was tested for its specificity of protease inhibition. K_i values of APV for HIV-1 and 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 was initiated by addition of the p-nitophenylalanine substrate His-Lys-Ala-Arg-Val-Leu/p(NO_2) Phe-Glu-Ala-Nle-Ser-NH_2 and incubated at 37^0 C. Reaction product was quantified by [redacted] analysis. The inhibitory constant K_i values were determined using the [redacted] software. Results are shown in Table 1.

APV was a potent inhibitor of HIV-1 protease. However, APV did not inhibit cellular aspartic proteinases, pepsin, cathepsin D, and renin. The K_i values of APV for pepsin and renin were 5000- and 3000-fold higher than the K_i value for HIV-1 protease. These results showed that APV is a specific inhibitor of HIV-1 protease. APV is a less potent inhibitor of HIV-2 protease.

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

Inhibitor	APV				
Protease	HIV-1	HIV-2	Pepsin	Cathepsin D	Renin
K_i (nM)	0.6	19	3200	>10000	1750

I (b) Inhibition of HIV-1 protease by GW433908A — GSK Report; RR1999/00039/00):

The enzymatic activity of HIV-1 and its inhibition by GW4339098A was assayed using a fluorescent substrate, GW280986X [redacted], 1990). The fluorescent substrate, 2 aminobenzoyl-Thr-Ile-Nle-Phe- (p-NO_2)-Gln-Arg-NH_2 (GW280986X) was prepared by [redacted] In this assay, 200 µl of substrate buffer (10 mM MES, pH 5.5, 400 mM NaCl, 0.2% PEG-8000, 10 µM GW280986X) containing a range of concentrations of GW433908A or APV were added to 10 µl of enzyme working solution (a 1:120 dilution of 1.4 µM HIV-1 protease into buffer containing 10 mM MES, pH 5.5, 400 mM NaCl and 0.2% PEG-8000). The fluorescence was monitored every 3 minutes for 36 minutes using a [redacted] multi-well, fluorescence plate reader. The enzymatic activity was determined by estimating the best linear fit to the data.

The apparent dissociation constant (K_{app}) and inhibition constant (K_i) for solutions containing GW433908A were 30 $\pm$ 5 nM and 20 nM, respectively. For APV these values were 0.06 $\pm$ 0.01 nM and 0.04 nM, respectively. The calculated protein concentration in this assay was 0.56 nM. The data for APV estimated an enzyme concentration in the assay of 0.46 nM. These data suggested a stoichiometry of 1.2 or approximately one site on the enzyme for binding one inhibitor molecule. The data for GW433908A estimated a protein concentration of 123 nM. This result suggested either 270 sites on the enzyme for binding one inhibitor molecule, or, more likely, there was an over estimation of the concentration of inhibitor in the assay.

Comment:

The sponsor stated that most of the inhibitory activity in the assay for GW433908A was due to the presence of another molecule at 0.37% of the concentration of GW433908A. A correct estimate of the K_{app} value for the other molecule was 0.37% of the K_{app} value for GW433908A, i.e. 30 $\pm$ 5 nM, or 0.11 $\pm$ 0.02 nM. This K_{app} value, 0.11 $\pm$ 0.02 nM, was not experimentally different from the K_{app} value for APV, 0.06 $\pm$ 0.01 nM. Thus, the observed inhibition of HIV-1 protease by GW433908A could be accounted for by the presence of 0.37% APV or another similarly potent inhibitor present in the sample. It was therefore concluded that GW 433908 by itself has very little inhibitory activity.

II. <u>Anti-HIV-1 activity of GW433908 in vitro</u>: — . GSK Report # RR 1999/00038/00):

The objective of this study was to determine the in vitro anti-HIV-1 activity of GW433908 (sodium salt) and compare it to that of APV. The anti-HIV-1 activity of GW433908 was determined by measuring cell protection from the HIV-1 induced cytopathic effect using MT-4 cells and a propidium iodide dye stain. The propidium iodide dye binds to intracellular DNA and thus, the DNA content of the cell is estimated by quantification of fluorescence emission — 1989). Aliquots of GW433908 (concentration range and volume not provided) were serially diluted in medium (RPMI 1640, 20% fetal calf serum (FCS) and gentamycin) into a 96-well tissue culture plate. Exponentially growing MT-4 cells (5 x 10^5 cells/mL) were infected with HIV-1_{IIIB} at a multiplicity of infection (m.o.i.) of 100 x $TCID_{50}$ and incubated at 37^0C for 1 hour. Mock-infected control cells were also prepared. HIV-1 infected cell suspensions were subsequently diluted 6-fold with fresh medium, and 125 µl of the cell suspension (approximately 10^4 cells) were added to each well of the tissue culture plate containing the prediluted GW433908. Plates were incubated at 37^0C for 5 days. At the end of the incubation period, 27 µl of 5% Nonidet P-40 were added to each well of the plate. After thorough mixing, 60 µl of the mixture were transferred to filter–bottomed 96-well plates. The plates were analyzed in ar — fluorescence reader ( ) and the DNA content of each well was estimated. Results showed that the 50% inhibitory concentration (IC_{50}) for GW433908 was 1,250 $\pm$ 381 nM (n=12). Under similar assay conditions, the IC_{50} value of APV was 88 $\pm$ 10 nM (n=11).

Comments:

1. The IC_{50} of GW433908 was approximately 14-fold higher than that of APV. The sponsor suggested that the anti-HIV-1 activity exhibited by GW433908 in MT-4 cells was due to its hydrolysis to APV.
2. The sponsor did not provide any data on the <u>in vitro</u> cytotoxicity of GW433908 in the the GSK report cited above. However, in response to this reviewer's query [NDA 21-548, N-000 (BZ)], data on the in vitro cytotoxicity of APV (the hydrolyzed product of GW433908) were cited from studies cross referenced to NDA 21-007. No cell cytotoxicity was observed with APV at the maximum concentration tested, 100 µM.

The calculated therapeutic indices (TIs) for GW433908 and APV were 80 and 1136, respectively.

3. In response to a query on the intracellular concentrations of GW433908 and APV in MT-4 cells [NDA 21-548, N-000 (BZ)], the sponsor suggested that the intracellular concentrations of GW433908 and APV could not be measured in the GW433908 treated MT-4 cells due to technical difficulties, physical properties of GW433908 and lack of cell penetration of phosphate esters in general. The sponsor suggested that it is very unlikely that significant amounts of the intact GW433908 would be taken up by MT-4 cells.

Clinical Study

Study APV 30001:

Title: Analyses of HIV genotyping and phenotyping data collected during the course of a clinical study APV 30001: A randomized, parallel, open-label study to compare the efficacy, safety and tolerability of GW433908 (1400 mg BID) with nelfinavir (1250 mg BID) over 48 weeks in antiretroviral therapy naïve HIV-1 infected adults.

Objectives:

1. To assess the development of viral resistance arising during therapy with GW433908 BID and NFV BID in antiretroviral therapy naïve HIV-1 infected adults.
2. To compare the incidence of NRTI and PI resistance between the GW433908 BID and NFV BID arms at virological failure.

Study Design

Two hundred forty-nine antiretroviral therapy naïve subjects were randomized in a 2:1 ratio to the following treatment groups:
Group1: 908 1400 mg BID + ABC 300 mg BID + lamivudine (3TC) 150 mg BID, (n=166)
Group 2: NFV 1250 mg + ABC 300 mg BID + 3TC 150 mg BID, (n=83)
Subjects were stratified at randomization according to their plasma HIV-1 RNA levels at screening (≥ 5,000-10,000 copies/mL, 10,001-100,000 copies/ml, or >100,000 copies/mL).

Genotypic and phenotypic analyses of HIV-1 isolates from patients with virological failure/ongoing replication were performed. Patients who achieved a plasma HIV-1 RNA level <400 copies/mL and subsequently had two or more consecutive samples with HIV-1 plasma RNA ≥1000 copies/mL at Week 12 or beyond (up to Week 48), and patients who remained on randomized treatment until at least Week 12 and failed to achieve a plasma HIV-1 RNA <400 copies/mL were also considered virologic failure. The virological failure/on going replication population constituted 30 subjects from the 908 treatment group (30/166) and 27 from the NFV treatment group (27/83). Thus, the

proportion of patients with virological failure markedly differed in the two treatment groups: 18% for the 908 group and 33% for the NFV group.

III. Genotypes of baseline and on-therapy HIV-1 isolates from patients receiving 908 + ABC + 3TC or NFV + ABC + 3TC with virological failure:

Matched baseline and on-therapy genotypic data (RT, PR, Gag-Pol cleavage site) were available for 29 patients receiving 908 + ABC + 3TC, and 26 patients treated with NFV + ABC + 3TC. The mean baseline plasma HIV-1 RNA levels were similar for both treatment groups with the virological failure: 5.17 $\log_{10}$ copies/mL for the 908 group and 5.22 $\log_{10}$ copies/mL for the NFV group (NDA 21-548, Vol 1, Table 8.5.2, Page 78). The mean plasma HIV-1 RNA levels at Weeks 12, 16, 20, and 24 for patients with virological failure in the 908 group were 3.55, 3.54, 3.47, and 3.49 $\log_{10}$ copies/mL, and 3.07, 2.79, 3.35, and 3.54 $\log_{10}$ copies/mL for patients in the NFV group (NDA 21-548, Vol 1, Table 8.9.2, Pages 91-92).

III (a) Baseline protease and RT mutations:

None of the baseline HIV-1 isolates from patients with virologic failure (n=30) contained mutations associated with APV resistance or other protease inhibitors (PIs). However, baseline HIV-1 isolates from some patients in the 908 and NFV groups with virologic failure contained accessory mutations in the protease gene (Table 2) and Gag-Pol cleavage site mutations (Table 3).

Table 2: Summary of accessory PI mutations in baseline HIV-1 isolates of patients with virologic failure (Source: NDA 21-548; Vol 1, Table 8.12.1; Pages 217-228)

Mutation	908 (N=30)	NFV (N=27)
L10I	4	4
K20R	0	1
M36I	5	6
L63P	16	16
A71T	5	6

Table 3: Summary of Gag-Pol cleavage site mutations in baseline HIV-1 isolates from patients with virologic failure (Source: NDA 21-548; Vol 1, Table 8.13.2; Pages 249-251)

Mutation	908	NFV
p7/p1 A431V	0	0
p1/p6 L449F	0	0
p1/p6 P453L	1	2

Baseline HIV-1 isolates from one virologic failure patient (#2960) randomized to receive 908 contained the M41L, E44D and D67N mutations in the RT gene. Baseline HIV-1

isolates from this patient showed a 3.1-fold reduced susceptibility to 3TC. On-therapy HIV-1 isolates from this patient continued to harbor these mutations. Since these mutations were present in baseline HIV-1 isolates from patient (#2960), they are not considered to be treatment emergent mutations.

III (b) Protease and RT genotypes of on-therapy HIV-1 isolates:

Table 4 shows that on-therapy HIV-1 isolates from 5 of 29 patients with virologic failure on 908 therapy developed APV resistance-associated mutations I54L/M (n=2), I54L + L33F (n=1), V32I + I47V (n=1) and M46I + I47V (n=1). Other APV resistance-associated mutations, e.g., I50V and I84V, were not observed. HIV-1 isolates from the NFV treated patients with virological failure contained the NFV resistance-associated mutations D30N (n=3), L90M (n=3), D30N + L90M (n=1), and N88D (n=1). Mutations D30N and N88D, either alone or in combination with L90M, confer resistance to NFV. Naturally occurring polymorphisms (L10F/I, K20R, A71V/T) were detected in both treatment groups. On-therapy HIV-1 isolates from one patient (1/26) with virologic failure in the NFV treatment group had the Gag-Pol cleavage site mutation p1/p6 P453L (NDA 21-548; Vol 2, Table 8.26. 2; Page 434). On the other hand, none of the on-therapy isolates from 29 patients with virologic failure in the 908 group had any Gag-Pol cleavage site mutations.

Table 4: Summary of treatment emergent RT and PR mutations observed in on-therapy HIV-1 isolates from patients with virological failure (APV 30001) [NDA 21-548, Vol 1, Table 8.21.2, Pages 317-343 and Vol 2, Table 8.24.2, Pages 402-412]

Treatment-Emergent Mutation	908 (n=30)	NFV (n=27)
RT Mutations	N=29	N=26
D67N	0	1
L74V	2	0
M184I	2	4
M184V	14	16
PR Mutations	N=29	N=26
L10F*	1	1
L10I*	3	0
K20R*	2	0
D30N	0	4
V32I	1	0
L33F	1	0
M46I	1	2
I47V	2	0
I54L	2	0
I54M	1	0
A71T*	1	0
A71V*	0	1

N88D	0	1
L90M	0	4

*=naturally occurring polymorphisms

On-therapy HIV-1 isolates from 16/29 patients with virologic failure in 908 group contained the ABC and 3TC resistance-associated mutations M184I/V in the RT gene. In addition, the L74V mutation was present in HIV-1 isolates from 2 patients with virologic failure (Table 4). The mutation L74V is associated with resistance to ABC and ddI. Similarly, M184V and M184I were the most frequently observed mutations in the RT gene of HIV-1 isolates from patients in the NFV treatment group. Both the M184I and the M184V mutations are associated with resistance to 3TC. In addition, the mutation M184V is also associated with resistance to ABC and ddI.

IV. Phenotypic analysis of HIV-1 isolates from patients with virologic failure from 908 and NFV treatment group (Study APV 30001):

IV (a) Phenotypic analysis of baseline HIV-1 isolates from patients with virologic failure:

Baseline HIV-1 isolates from 1 of 29 patients randomized to the 908 treatment arm were resistant to 3TC (NDA 21, 548, Vol 2, Table 8.36.2, Pages 508-510). Baseline HIV-1 isolates from all patients (n=29) were susceptible to the NRTIs ABC, ddI, and ZDV, and the PIs APV, IDV, LPV, NFV, RTV, and SQV. Similarly, baseline HIV-1 isolates from all patients (n=27) randomized to the NFV treatment arms were susceptible to the NRTIs ABC, ddI, and ZDV, and the PIs APV, IDV, LPV, and SQV. However, baseline HIV-1 isolates from 2 of 27 patients, and 1 of 27 patients in the NFV treatment group were resistant to NFV and RTV, respectively.

IV (b) Phenotypic analysis of on-therapy HIV-1 isolates from patients with virologic failure:

Phenotypic analysis of on-therapy HIV-1 isolates from 17 of 28 patients in 908 treatment group with virologic failure were resistant to 3TC. Of these, isolates from 9 patients were resistant to ABC. It should be noted that baseline HIV-1 isolates from one of these 17 patients exhibited resistance to 3TC. On-therapy HIV-1 isolates from all patient (n=28) were susceptible to ddI and ZDV. On-therapy HIV-1 isolates from 3 of 28 patients exhibited a 2.9- to 7.2-fold reduced susceptibility to APV. APV resistant HIV-1 isolates from one patient exhibited a 3.6- to 4.4-fold reduced susceptibility to NFV and RTV, respectively. Similarly, APV resistant isolates from another patient exhibited a 3.0- to 4.8-fold reduced susceptibility to the PIs IDV, LPV, NFV, or RTV. However, these APV resistant isolates were susceptible to the PI SQV.

On-therapy HIV-1 isolates from 20 of 25 patients randomized to NFV with virologic failure exhibited reduced susceptibility to 3TC (NDA 21-548, Vol 2, Table 8.37.2, Pages 511-514). Of these, isolates from 10 patients also exhibited reduced susceptibility to

ABC. On-therapy HIV-1 isolates from all patients (n=25) were susceptible to the PIs APV, IDV, LPV, and SQV. However, on-therapy HIV-1 isolates from 8 of 25 patients were resistant to NFV and isolates from 1 of these 25 patients also exhibited reduced susceptibility to RTV.

V. Cross-resistance of on-therapy HIV-1 isolates from patients with virologic failure in 908 treatment group to other PIs:

Table 5 shows that on-therapy HIV-1 isolates from two patients exhibited a 5.7- to 7.2-fold reduced susceptibility to APV. APV resistant HIV-1 isolates from one patient exhibited 3.6- and 4.4-fold reduced susceptibility to NFV and RTV, respectively. Similarly, APV resistant isolates from another patient exhibited 3.7- to 4.8-fold reduced susceptibility in vitro to IDV, LPV and NFV. However, these APV resistant isolates were susceptible to SQV.

Table 5: Cross-resistance of HIV-1 isolates from patients in 908 group with virologic failure to other PIs (Source : NDA 21-548, Vol 1, Table 21, Page 49)

Patient	Genotype		PI Phenotypic Susceptibility (FR[a])					
ID #	RT	PR	APV	IDV	LPV	NFV	RTV	SQV
2727	M184V	L10I V32I I47V	1.6	1.0	1.1	1.7	1.7	0.9
2610	M184V L74V	L10F I54L	2.1	0.6	1.0	1.6	0.9	0.8
2566	M184I	L10V I54M	2.9	1.1	1.5	2.0	1.7	1.2
2797	M184V	L10I K20R L33F I54L	5.7	1.2	2.1	3.6	4.4	1.5
2767	M184V	M46I I47V	7.2	3.7	4.8	3.7	3.0	1.1

[a] =Fold resistance

Study APV 30002:

Title: Analyses of HIV genotyping and phenotyping data collected during the course of a clinical study of APV 30002: A Randomized, Open-Label, Two Arm Trial to Compare the Efficacy, Safety and Tolerability of GW433908/Ritonavir QD to Nelfinavir BID When Used in Combination with Abacavir and Lamivudine BID for 48 Weeks in Antiretroviral Therapy Naïve HIV-1 Infected Subjects.

Objectives:

1. To assess the development of viral resistance arising during therapy with GW433908/RTV QD and NFV BID in antiretroviral therapy naïve HIV-1 infected adults.
2. To compare the incidence of NRTI and PI resistance between the GW433908/RTV QD and NFV BID arms at virological failure.

Study Design

APV 30002 was a randomized, open-label, two-arm study in HIV-1 infected ART-naïve subjects (defined as fewer than 4 weeks of therapy with an NRTI and no previous exposure to any PI or NNRTI). Randomization was stratified according to plasma HIV-1 RNA level at screening (≥1,000-10,000 copies/mL; 10,001-100,000 copies/mL; or >100,000 copies/mL). Six hundred and forty-nine subjects were randomized in a 1:1 scheme to one of the following two treatment groups:
Group 1: GW433908 1400 mg QD + RTV 200 mg QD + ABC 300 mg BID + 3TC 150 mg BID (n=322)
Group 2: NFV BID 1250 mg BID + ABC 300 mg BID + 3TC 150 mg BID (n=327)

Subjects underwent safety and efficacy assessments at the Screening Visit (up to Day -28), Day 1 (Entry) and Weeks 1, 2, 4, 8, 12, 16, 20, 24 and every 8 weeks thereafter. A follow-up visit was performed 4 weeks after permanent discontinuation from the study. Pharmacokinetic (trough level) sampling was performed at Weeks 4, 8 and 12 in a subset of subjects (n=50).

Virological Failure/Ongoing Replication: Criteria for virological failure were the same as that described for study APV 30001. The Virological Failure/Ongoing Replication population (n=101) constituted 39 subjects from the 908/RTV QD treatment group and 62 from the NFV BID treatment group. The median baseline HIV-1 plasma RNA was slightly higher for the NFV BID-than the 908/RTV QD-treated group in the Virologic Failure/Ongoing Replication Population (NFV BID 5.52: 908/RTV QD 5.04 $\log_{10}$copies/mL).

VI. Genotypes of baseline HIV-1 isolates from patients randomized to the 908/RTV QD and NFV BID arms:

Genotypes (RT, protease and Gag-Pol cleavage sites) were analyzed for baseline HIV-1 isolates from subset of patients including virological failure (random sample plus virological failure) randomized to 908/RTV QD (n=88) and NFV BID (n=112) arms.

VI (a). RT mutations:

None of the baseline HIV-1 isolates from patients randomized to the 908/RTV QD treatment arm (n=88) contained any mutation associated with resistance to ABC and 3TC (K65R, L74V, Y115F, M184V) [NDA 21-548; Study APV 30002; Vol 1, Table 10, Page 37). Additionally, mutations associated with multiple drug resistance (A62V, V75I,

F77L, F116Y and Q151) were not detected in baseline HIV-1 isolates. However, some of these baseline HIV-1 isolates contained the ZDV resistance-associated mutation M41L (n=1), and the NNRTI resistance-associated mutations L100I (n=1), K103N (n=1) V108I (n=1), and P236L (n=1).

None of the baseline HIV-1 isolates from patients randomized to the NFV arm (n=112) contained any mutation associated with resistance to ABC, 3TC or multiple drug resistance mutations. Similar to the 908/RTV QD arm, baseline HIV-1 isolates from these patients contained the ZDV resistance-associated mutations M41L (n=1), L210W (n=1), K219Q/E (n=1) and the NNRTI resistance-associated mutations V108I (n=1), Y181C (n=1), and P236L (n=3).

VI (b). Protease and Gag-Pol cleavage site mutations:

Genotypic analysis of baseline HIV-1 isolates from patients (n=88) randomized to 908/RTV treatment arm showed that isolates from one patient contained the APV resistance-associated mutation I50V. PI resistance-associated mutations were not detected in baseline HIV-1 isolates from other patients (NDA 21-548, study APV 30002, Vol 1, Table 11, Page 39). Protease accessory mutations L10F/I/R/V (n=12), K20M/R (n=4), M36I (n=37), L63P (n=46), A71V/T (n=5), and V77I (n=22) were also present in baseline HIV-1 isolates. Similarly, the p1/p6 P453 L cleavage site mutation was also detected in baseline HIV-1 isolates from three patients.

None of the baseline HIV-1 isolates from patients (n=109) randomized to the NFV treatment arm contained any primary mutation associated with PI resistance (NDA 21-548, study APV 30002, Table 11, Page 39). However, baseline isolates contained the accessory mutations: L10F/I/R/V (n=15), K20M/R (n=4), M36I (n=37), L63P (n=62), A71V/T (n=10), and V77I (n=30). In addition, baseline HIV-1 isolates contained the cleavage site mutations: p7/p1 A431V (n=1), p1/p6 L449F (n=3) and p1/p6 P453L (n=6).

VII. Genotypes and phenotypes of on-therapy HIV-1 isolates from virologic failure Patients in the 908/RTV QD and NFV BID treatment arms:

Genotypes of on therapy HIV-1 isolates were available only from patients with virologic failure. The virologic failure population constituted 39 of 322 patients who were initially randomized to receive 908/RTV QD and 62 of 327 randomized to the NFV treatment group. However, genotypes of baseline matched on-therapy HIV-1 isolates were available for 32 patients with virologic failure in the 908/RTV QD treatment group and 54 patients in the NFV BID treatment group.

VII (a). RT mutations:

Table 6 shows that on-therapy HIV-1 isolates from 4 patients with virologic failure on 908/RTV QD treatment regimen contained ABC/3TC resistance-associated mutations, M184V (n=2) and M184I (n=2), and ZDV resistance-associated mutations M41L (n=1), D67 (n=1), and T215Y (n=1). Some of these NRTI mutations were present in combination. However, on-therapy isolates from a majority of patients with virologic failure (n=31) receiving NFV BID regimen contained ABC/3TC resistance-associated mutations, K65R (n=2), L74V (n=1), M184V (n=22), and M184I (n=8), and the ZDV resistance-associated mutation K219E (n=1).

VII (b). Protease mutations:

Table 7 shows that none of the on-therapy HIV-1 isolates from patients (n=32) with virologic failure on 908 + RTV QD treatment contained APV resistance-associated mutations. However, isolates from one patient contained an I54V mutation. On-therapy HIV-1 isolates from 28 of 54 patients with virologic failure on NFV BID treatment contained the NFV resistance-associated mutation D30N. The Gag-Pol cleavage site mutation p1/p6 P449L was detected in isolates from one NFV BID treated patient.

Table 6: Summary of treatment emergent NRTI resistance-associated mutations in HIV-1 isolates from patients with virological failure (NDA 21-548, study APV 30002, Vol 1, Table 15, Page 47).

Mutation	908/RTV QD (n=32)	NFV BID (n=54)
M41L	1	0
K65R	0	2
D67N	1	0
L74V	0	1
M184I	2	8
M184V	2	22
T215Y	1	0
K219E	0	1

VII (c). Phenotypes of on-therapy isolates from patients with virologic failure

Phenotypic analysis showed that on-therapy isolates from 1 patient from 908/RTV group (1/32) were resistant to APV, IDV, LPV, NFV, RTV, and SQV (NDA 21-548, study APV 30002, Vol. 2, Tables 8.37.2, Pages 646-649). On-therapy HIV-1 isolates from this patient contained an I54V mutation. Baseline HIV-1 isolates from patients receiving 908/RTV with virologic failure were susceptible to APV, IDV, LPV, and SQV. However, baseline isolates from 1 patients with virologic failure were resistant to NFV and RTV. On-therapy HIV-1 isolates from 24/55 patients receiving NFV were resistant to NFV. On-therapy isolates from all these patients (n=55) were susceptible to APV, LPV and

SQV. However, on-therapy isolates from 2/55 patients with virologic failure from NFV BID group were resistant to IDV and from 1/55 patients to RTV. (NDA 21-548, study APV 30002, Vol. 2, Tables 8.37.2, pages 646-649). Baseline isolates from 3 of 61 evaluable patients with virologic failure were resistant to NFV. These baseline isolates were susceptible to APV, IDV, LPV, RTV and SQV (NDA 21-548, study APV 30002,Vol 2, Tables 8.36.2, pages 643-645).

Table 7: Summary of treatment-emergent PR resistance-associated mutations in on-therapy HIV-1 isolates from patients with virological failure (NDA 21-548, study APV 30002, Table 15, Page 47)

Mutation	908/RTV QD (n=32)	NFV BID (n=54)
L10F	0	1
L10I	0	1
L10V	0	1
K20M	0	2
D30N	0	18
V32I	0	0
L33F	0	0
M36I	0	8
M46I	0	7
M46L	1	0
I47V	0	0
I54L	0	0
I54M	0	0
I54V	1	0
L63P	12	28
A71T	0	2
A71V	0	1
V77I	1	3
V82A	1	0
N88D	0	2
N88S	0	4
L90M	0	4

VIII. Plasma APV exposure (pharmacokinetic parameters), IC_{50} value, and the development of resistance:

RTV has been used to boost the plasma concentration of APV following administration of GW433908 + RTV in studies APV 30002 and APV 30003 (described later on page 16). APV is metabolized by the CYP3A4 pathway, which is inhibited by RTV. RTV has been used in combination with GW433908 to increase the minimum plasma concentration for both wild type virus and APV-resistant viral strains. Increasing the

C_{min} prevents the development of PI-resistant HIV-1 variants in ART-naïve and PI-experienced patients.

Doses of GW433908 1400 mg /RV 200 mg QD and GW433908 700 mg/RTV 100 mg BID were selected for the pivotal clinical trials APV 30002 and APV 30003. The GW433908 700 mg/RTV 100 mg BID regimen delivers slightly higher plasma APV AUC $_{24,ss}$, slightly lower C $_{max,ss}$, and moderately higher $C_{\tau,ss}$ values, compared to the GW433908 1400 mg/RTV 200 mg QD regimens based on data generated in healthy adult subjects. Plasma APV PK parameter estimates for these two GW433908/RTV regimens are provided in Table 8.

Table 8: Steady-state plasma APV PK parameter estimates (geometric mean)for GW433908/RTV regimens (NDA 21-548, Volume 6.2, Page 14)

Plasma APV PK Parameter	**GW433908 1400 mg/RTV 200 mg QD N=22**	**GW433908 700 mg/RTV 100 mg BID N=24**
AUC $_{24,ss}$ (h*µg/mL)	69.4 (59.7-80.8)	79.2 (69.0-90.6)
C $_{max,ss}$ (µg/mL)	7.24 (6.32-8.28)	6.08 (5.38-6.86)
$C_{\tau,ss}$ (µg/mL)	1.45 (1.16-1.81)	2.12 (1.77-2.54)
$t_{max,ss}$ (h)•	2.1 (0.8-5.0)	1.50 (0.75-5.0)

•$t_{max,ss}$ data presented as median (range).

The plasma APV PK data collected in HIV-1 infected subjects receiving GW43390/RTV regimens in APV 30002 and APV 30003 were similar to the data collected in healthy adult subjects.

APV is approximately 90% bound to the α-1 acid glycoprotein (AAG). The antiviral potency of APV was reduced two to five fold at the maximum concentration tested (1.2 mg/mL). In APV 30002, plasma APV concentrations were maintained above the IC_{50} for APV against HIV-1 in all of the ART-naïve subjects who had both parameters (APV 30002 CSR-Table 17.7).

Comment:

The absence of key PI mutations which confer resistance to APV in on-therapy HIV-1 isolates from patients with virologic failure receiving 908/RTV BID suggested that APV plasma concentrations achieved in these patients were sufficiently high to prevent or delay the development of mutations. Additional support for the hypothesis that an increase in APV plasma concentration due to boosting effect of RTV either delayed or prevented PI-resistance development in patients treated with 908/RTV comes from the

study APV 30001. As described earlier, in APV 30001 patients were treated with 908 without RTV, and PI-resistance associated mutation were detected in post-therapy isolates from patients failing 908 therapy.

Study APV 30003

Title: Analyses of HIV genotyping and phenotyping data collected during the course of a clinical study APV 30003: A Phase III, randomized, multicenter, parallel group, open-label, three arm study to compare the efficacy and safety of two dosing regimens of GW433980/ritonavir (700 mg/100 mg twice daily or 1400 mg/200 mg once daily) versus lopinavir/ritonavir (400 mg/100 mg twice daily) for 48 weeks in protease inhibitor experienced HIV-infected adults experiencing virological failure.

Objectives:

1. To assess viral resistance patterns at baseline and those which emerge in subjects with virological failure.
2. To correlate these patterns with treatment outcome during therapy with GW433908/RTV QD or BID or LPV/RTV BID

Study Design

This was a randomized, parallel group, three-arm, open-label, multicenter, comparative study of two dosage regimens of 908/RTV versus LPV/RTV in combination with two active NRTIs, performed in the US, Europe, and other countries. Subjects were PI-experienced with at least 12 consecutive weeks of prior PI experience. PI experience was defined as exposure to either a single PI or pharmacokinetically enhanced regimens (i.e., regimens that include RTV at a dose of 400 mg or less). However, subjects could not have had previous exposure to more than two PIs (regimens containing a dose of 400 mg RTV or less count as 1 PI). Subjects must have had documented failure on a PI regimen, defined as having plasma HIV-1 RNA that never went below 1000 copies/mL after at least 12 consecutive weeks of PI therapy, or initial suppression of HIV-1 RNA which subsequently rebounded to ≥ 1000 copies/mL. Subjects could be either NNRTI experienced or naïve, however, subjects were not allowed to take an NNRTI during this trial. Subjects were required to be on therapy at the time of screening and had to remain on this therapy until Day 1. However, therapy at screen did not have to include a PI as long as evidence of prior PI failure was documented. In addition, to be eligible, subjects must have been experiencing virologic failure as defined by plasma HIV-1 RNA ≥ 1000 copies/mL at screening.

Subjects were stratified at randomization according to their plasma HIV-1 RNA level at screen (1000-10,000 copies/mL; 10,001-100,000 copies/mL; >100,000 copies/mL).
In this sub-study report, the three treatment groups are referred to as follows:

908/RTV QD: GW433908, 1400 mg once daily + RTV, 200 mg once daily + two active NRTIs.
908/RTV BID: GW433908, 700 mg twice daily + RTV, 100 mg twice daily + two active NRTIs.
LPV/RTV BID: lopinavir, 400 mg/ritonavir 100 mg twice daily + two active NRTIs.

Subjects underwent safety and efficacy assessments at the screening visit (up to 28 days prior to Day 1), a pre-baseline visit (within 7 days of Day 1), a baseline visit (Day 1), and at Weeks 1, 2, 4, 8, 12, 16, 20, 24, 32, 40 and 48. Subjects continued to be seen every 8 weeks thereafter until the last subject enrolled completed Week 48.

In the following descriptions the baseline resistance profile is defined as the resistance mutations detected in virus at the screening visit (plasma sample) and/or Day 1 plasma and/or Day 1 peripheral blood mononuclear cells (PBMC). There were three virology study populations. The three virology study populations were:

1. Intent-to-Treat Exposed (ITT[E]) population:

The ITT (E) population consisted of all subjects who were randomized into the study with documented evidence of having received at least 1 dose of randomized treatment. This population was used to describe the resistance profile present in the virus at baseline.

2. Resistance/virological response population:

The resistance/virological response population was defined as all subjects who received at least one dose of randomized treatment, excluding those who discontinued the randomized treatment at any time up to Week 48 for reasons other than virological failure (e.g. adverse event, consent withdrawn, lost-to-follow-up, protocol violation, clinical progression or other). This population was used to investigate correlations between treatment outcome at Study 48 and baseline resistance.

3. Virological failure/ongoing viral replication population:

The virological failure/ongoing viral replication population comprised of all subjects who took at least one dose of randomized treatment and who experienced virological failure or had evidence of ongoing replication. The subjects in this population comprised the following sub-populations:

(a). The virological rebound sub-population:
This sub-population included subjects with two or more consecutive samples with plasma HIV-1 RNA $\geq$1000 copies/mL and <1.0 $\log_{10}$ copies/ml decrease from baseline in vRNA and/or vRNA >1 $\log_{10}$ copies/mL above nadir at Week 16 or beyond, having previously responded to treatment by achieving either plasma HIV-1 RNA <400 copies/mL or having >1.0 $\log_{10}$ copies/mL decrease from baseline in plasma HIV-1 RNA.

(b). The inadequate virological response sub-population:
This sub-population included subjects who remained on randomized treatment to Week 24 or beyond, and failed to achieve either plasma HIV-1 RNA <400 copies/mL or a >1.0 $\log_{10}$ copies/mL decrease from baseline in plasma HIV-1 RNA, or subjects who remained on randomized therapy to Week 16 or beyond and failed to achieve either plasma HIV-1 RNA <400 copies/mL or a >0.7 $\log_{10}$ copies/mL decrease from baseline in plasma HIV-1 RNA.

(c). The ongoing viral replication despite adequate virological response sub-population:
This sub-population included subjects who achieved and maintained a >1.0 $\log_{10}$ copies/mL decrease in HIV-1 plasma RNA and did not demonstrate rebound as described above (i.e. to <1.0 $\log_{10}$ copies/mL below baseline), but had HIV-1 plasma RNA >1000 copies/mL.

Table 9 shows the subjects accountability grouped in different virology populations.

Table 9: Summary of subject accountability (virology population)

Population	908/RTV QD	908/RTV BID	LPV/RTV BID
ITT(E)	105	107	103
Resistance/ Virological Response	93	92	88
Virological Failure/On-going Replication	42	33	29

IX. Accountability of virology population for genotypic and phenotypic analysis:

Table 10 shows the genotype and phenotype data accountability for the three virology populations.

X. Prior antiretroviral treatment experience of virology population:

Table 11 lists the number and types of previously experienced PIs for ITT (E) virology population. A higher number of subjects in the 908/RTV QD and 908/RTV BID groups had received two prior PI treatments compared to the LPV/RTV BID group. NFV and IDV (with and without RTV) were the most widely used prior PIs, followed by SQV (with and without RTV). A limited number of subjects had used RTV alone. However, no subject had previously experienced APV or LPV.

Table 12 lists the number and type of prior NRTIs experience of ITT (E) virology population. The majority of subjects had prior experience of three or more NRTIs: 3TC, d4T, ddI, and ZDV. There was less prior experience of ABC, ddC, or TDF.
Table 13 lists the number and type of prior NNRTI experience of the ITT (E) virology population. A majority of the subjects had received either efavirenz (EFV), or nevirapine (NVP). A very limited number of patients had received prior treatment with delavirdine.

Table 14 lists the prior PI experience of virologic failure/on-going replication virology population. A majority of the patients on the 908/RTV QD or 908/RTV BID regimens with virologic failure had previously received two or more PIs: IDV, NFV and SQV. However, a majority of patients in LPV/RTV treatment arm with virologic failure had previously received one or two PIs.

Table 10: Summary of genotypic and phenotypic data accountability for subjects for the three virology populations

Population	**908/RTV QD**	**908/RTV BID**	**LPV/RTV BID**
ITT(E)	**N=105**	**N=107**	**N=103**
Plasma Day 1	104	101	96
PBMC Day 1	68	65	69
Baseline Genotype	105	107	103
Phenotype	104	102	97
Resistance/Virology Response	**N=93**	**N=92**	**N=88**
Plasma Day 1	93	88	84
PBMC Day 1	60	55	60
Baseline Genotype	93	92	88
Phenotype	93	89	85
Virological Failure/On-going Replication	**N=42**	**N=33**	**N=29**
Plasma Day 1	42	31	28
PBMC Day 1	25	21	22
Baseline Genotype	42	33	29
Genotype at Failure	39	33	28
Phenotype Day 1	42	31	28
Phenotype Failure	39	33	27
Phenotype Day 1 + Failure	39	31	26

Table 11: Summary of the prior PI-experience of the ITT E virology population

	908/RTV QD	**908/RTV BID**	**LPV/RTV**

No. of subjects	(N=105)	(N=107)	(N=103)
No. of previous PIs taken (regimen including RTVcounted as 1 PI)			
1	45	54	62
2	48	40	27
3	10	10	12
4	2	2	2
PIs Received Prior to Day 1			
IDV	49	48	46
IDV/RTV	12	10	18
NFV	68	63	58
RTV	6	11	5
RTV/NFV	0	2	0
SQV	29	23	19
SQV/RTV	15	15	14

Table 12: Summary of the prior NRTI experience of the ITT (E) virology population

	908/RTV QD	**908/RTV BID**	**LPV/RTV**
No of subjects	(N=105)	(N=107)	(N=103)
No of NRTI changes			
1	0	1	0
2	31	21	37
3	17	22	10
4	31	35	33
5	19	20	18
6	7	7	5
NRTIs			
ABC	31	25	26
Ddl	54	69	54
3TC	102	97	99
d4T	79	88	75
TDF	0	1	2
DdC	20	22	15
ZDV	87	88	85

Table13: Summary of prior NNRTIs experience of ITT (E) virology population

	908/RTV QD	**908/RTV BID**	**LPV/RTV**
No of subjects	(N=105)	(N=107)	(N=103)
Number of NNRTIs taken			
1	44	49	54
2	11	14	8

3	1	1	0
NNRTIs received prior to Day 1			
Delavirdine	4	5	2
Efavirenz	32	35	30
Nevirapine	31	35	32

XI. Genotypic analysis of baseline HIV-1 isolates from ITT (E) virology population:

XI (a). RT Mutations in baseline HIV-1 isolates:

Table 15 shows the frequency of NRTI-resistance associated mutations in the baseline HIV-1 isolates of the ITT(E) population. The NRTI resistance-associated mutations present in the baseline HIV-1 isolates from a majority of subjects randomized to 908/RTV QD, 908/RTV BID and LPV/RTV BID groups were the ABC/3TC/ddI resistance-associated mutations L74V, M184V, and Y115F; and the ZDV resistance-associated mutations M41L, D67N, K70R, L210W, T215YF and K219Q. The NNRTI resistance conferring mutations present in baseline HIV-isolates were L100I, K103N, V118I, Y181C, Y188C, G190A/S, P225H and M230L. In addition, baseline HIV-1 isolates contained the multi-nucleoside resistance conferring mutations A62V, V75I, V106A, F116Y and Q151M. Similarly, baseline HIV-1 isolates also contained T69D and T69INS. The mutation T69D confers resistance to ddC and T69INS in combination with T215Y/F confers resistance to tenofovir and other NRTIs.

Comment:

The prevalence of NRTIs and NNRTIs resistance-associated mutations in baseline HIV-1 isolates of patients (Table 15) is consistent with their prior NRTI and NNRTI treatment experience.

Table14: Summary of prior PIs experience of virologic failure/on-going replication population

	908/RTV QD (N=42)	**908/RTV BID (N=33)**	**LPV/RTV (N=29)**
Data Availability	**42**	**33**	**29**
PIs received prior to Day 1			
IDV	17	20	18
IDV/RTV	7	5	10
NFV	30	17	10
RTV	3	4	2
RTV/NFV	0	1	0
SQV	12	12	8
SQV/RTV	7	9	6

Table 15: RT mutations detected in baseline HIV-1 isolates from ITT(E) population

Mutations	908/RTVQD (N=105)	908/RTV BID (N=107)	LPV/RTV BID (N=103)
M41L	25	39	30
E44D	3	3	6
A62V	4	2	1
K65R	1	0	1
D67N	26	30	23
T69D	5	3	3
T69-Ins	0	1	1
K70R	21	26	24
L74V	11	15	16
V75I/T/M/	3	4	3
L100I	4	4	4
K103N	33	37	32
V106A	0	2	1
V108I	11	13	5
Y115F	0	0	3
F116Y	1	1	1
V118I	15	19	12
Q151M	1	1	2
Y181C/L/H	13	19	14
M184V	63	52	58
Y188C/L/H	2	4	1
G190A/S	10	16	20
L210W	12	23	11
T215Y/F	33	44	36
K219Q/E	18	20	19
P225H	6	3	4
M230L	0	0	2

XI (b) Protease mutations in baseline HIV-1 isolates:

The most common mutations detected were accessory mutations: L63P, A71V/T, V77I, L10I/F/V/R and M36I in decreasing order of prevalence. The most prevalent primary PI resistance-associated mutations detected in the baseline HIV-1 isolates of patients from each treatment group were L90M, M46I/L and D30N in decreasing order of occurrence. (Table 16). Other primary PI resistance-associated mutations detected in baseline HIV-1 isolates were V82A/F/T/S, N88D, I54V, I84V, N88S, G48V, V32I, I54L and I47V in decreasing order of occurrence. As mentioned earlier, mutations D30N, N88D and L90 M are associated with NFV resistance [redacted] Resistance Database). Mutations I54V and V882A/F/T/S confer resistance to RTV and IDV, respectively. Mutations G48V and L90M are associated with resistance to SQV. Mutations V32I, M46I/L, I47V, I54L, and I84V are associated with APV resistance. V32I also develops in concert with V82A mutation in HIV-1 isolates from patients treated with IDV.

Mutations I50V and I54M, also associated with APV resistance, were not detected in baseline HIV-1 isolates from any subjects in ITT(E) population. The I84V mutation was present in baseline HIV-1 isolates from 8/105, 8/107, and 7/103 subjects receiving 908/RTV QD, 908/RTV BID and LPV/RTV BID, respectively. The I84V mutation confers cross-resistance to most approved PIs and develops in HIV-1 isolates from patients treated with APV, IDV, and RTV.

The mutation I54L was present in baseline HIV-1 isolates of 4 subjects (#'s 3712, 4437 [908/RTV BID] and #'s 3060, 3993 [LPV/RTV BID]). Prior treatment history indicated both 908/RTV BID subjects entered the study (APV 30003) having failed a NFV containing regimen, whereas subjects 3060 and 3993 both entered the study having failed a SQV/RTV regimen. Subject 3993 had previously failed NFV and IDV/RTV.
Six subjects entered the study with a V32I mutation (908/RTV BID: Subject 3761; LPV/RTV BID: Subjects 3724,3446, 3711, 4436, 3591). All subjects had previously received IDV or IDV/RTV. Consistent with prior IDV exposure, the V82A mutation was present with the V32I mutation in HIV mutants from 4/6 patients.

Comment:
Presence of primary PI-mutations in baseline HIV-1 isolates from patients in ITT(E) population is consistent with their prior PI-treatment experience.

XII. Summary of virological responses

Virological response was defined as either reaching <400 copies/mL or having a 1.0 $\log_{10}$ copies/mL reduction in vRNA from baseline at Study Weeks 24 or 48. A summary of virological response (< 400 copies/mL) for the three study groups is shown in Table 17. Subjects discontinuing therapy for any reason other than virological failure were excluded from the analysis. The majority of subjects in all three groups had achieved a virological response at both study Week 24 [908/RTV QD 67%, 908/RTV BID 71%, LPV/RTV BID 80%] and Week 48 [908/RTV QD 56%, 908/RTV BID 67%, LPV/RTV BID 72%]. Subjects with a viral load of 1,000-10,000 copies/mL at entry were more likely than subjects with a viral load of >100,000 copies/mL to achieve <400 copies/mL at study Week 24 and 48.

Table16: Incidence of PI resistance-associated mutations in baseline HIV-1 isolates from ITT (E) population

Mutations	908/RTVQD (N=105)	908/RTVBID (N=107)	LPV/RTV (N=103)
L10F/I/V	39	39	37
K20M/R	9	15	11
L24I	7	6	3
D30N	16	24	21
V32I	0	1	5
L33F	2	3	2

M36I	37	37	37
M46I/L	30	24	24
I47V	0	0	2
G48V	4	0	3
I50V	0	0	0
F53L	3	3	1
I54L	0	2	2
I54V	12	11	12
L63P	78	80	82
A71V/T	44	37	46
G73S/A	13	5	9
V77I	39	41	42
V82A/F/T/S	17	10	18
I84V	8	8	7
N88D	12	21	11
N88S	3	3	4
L90M	29	32	31

- **Bold denotes primary PI-mutations**

Table 17: Summary of virological response at Week 24 and Week 48 for the resistance/virologic response population

	908/RTV QD	**908/RTV BID**	**LPV/RTV**
	N=93	**N=92**	**N=88**
Proportion of Subjects <400 copies/mL at Week 24			
1,000-10,000	30/38	32/37	29/35
10,000-100,000	27/42	26/40	32/40
>100,000	5/13	7/15	9/13
Proportion of Subjects <400 copies/mL at Week 48			
1,000-10,000	26/38	31/37	29/35
10,000-100,000	22/42	25/40	27/40
>100,000	4/13	6/15	7/13

XIII. Correlation of baseline phenotypic susceptibility to APV or LPV with virologic response at Week 48 (<400 copies/mL):

To evaluate the effect of baseline resistance on virological response, phenotyping susceptibility of baseline HIV-1 isolates to APV or LPV was determined at study entry (day 1). The phenotypic susceptibility of baseline HIV-1 isolates to study PI (APV or LPV) and virologic response (<400 copies/mL) at Week 48 is shown in Table 18. Baseline HIV-1 isolates from majority of the patients were susceptible (<0.4 to <2.5 fold susceptibility relative to reference strain) to APV (908/RTV QD, n = 81/93: 908/RTV BID, n = 71/88) or to LPV (LPV/RTV BID, n=63/85). Of these patients with baseline HIV-1 susceptibility of <0.4 to <2.5-fold, 46/81 patients from 908/RTV QD group, 56/71 from 908/RTV BID and 53/63 from LPV/RTV BID group had a virologic response of

<400 copies/mL at Week 48. Baseline HIV-1 isolates from 12/93 patients randomized to 908/RTV QD and 17/88 patients from 908/RTV BID exhibited a 2.5- to <6-fold decreased susceptibility to APV, 6/12 from 908/RTV QD group and 3/17 from 908/RTV BID group had virologic response of <400 copies/mL at Week 48.

Twenty-two patients (22/85) in the LPV/RTV BID group had baseline HIV-1 isolates with a 2.5- to ≥10-fold decreased susceptibility to LPV: 14/85 with LPV reduced susceptibility of 2.5- to <10-fold and 8/85 with ≥10-fold LPV reduced susceptibility. Ten of the 14 patients with a 2.5- to <10-fold reduced susceptibility to LPV at baseline had a virologic response of <400 copies/mL at Week 48. On the other hand, only 1 of the 8 patients with a ≥10-fold reduced susceptibility to LPV at baseline had virolgic response of <400 copies/mL at Week 48.

Table 18: Summary of phenotypic susceptibility of baseline HIV-1 isolates to APV or LPV and virological response (<400 copies/mL) at Week 48.

Fold-resistance Study PI	908/RTV QD N=93	908/RTV BID N=88	LPV/RTV BID N=85
<0.4	6/8	6/8	2/4
0.4 - <1.0	31/51	36/43	34/39
1.0 - <2.0	7/19	13/19	15/17
2.0 - <2.5	2/3	1/1	2/3
2.5 - <3.0	2/4	1/5	3/4
3.0 - <4.0	3/4	0/1	0
4.0 - <6.0	1/4	2/6	4/6
6.0 - <8.0	0	0	0/1
8.0 - <10.0	0	0/3	0/3
≥10.0	0	0/2	1/8

XIV. Correlation of primary protease mutations at baseline with virologic response:

An analysis was performed to correlate virologic response at Week 48 with primary protease mutations detected in baseline HIV-1 isolates (Table 19).

Table 19: Summary of baseline primary PI mutations to virological response (<400 copies/mL) at Week 48

Primary PI Mutation	908/RTV QD N=93	908/RTV BID N=88	LPV/RTV BID N=85
D30N	11/12	21/22	17/18
M46I/L	17/28	11/22	12/24
G48V	2/4	0	0/3
I50V	0	0	0

V82A/F/T/S	11/16	2/9	6/17
I84V	2/8	1/6	2/5
L90M	12/27	16/31	17/28

Comments:

1. Most patients from each treatment group (908 RTV QD, 908/RTV BID and LPV/RTV BID) achieved <400 copies/mL at Week 48 when D30N was present in baseline HIV-1 isolates.
2. In the presence of an L90M mutation in baseline HIV-1 isolates, patients receiving LPV/RTD BID (17/28) had a slightly better virologic response than those receiving 908/RTV QD (12/27) or 908/RTVBID (16/31).

Attempts were made to understand why there were differences in virologic response (<400 copies/mL at Week 48) in the presence of specific protease mutations, comparison of the viral genotype from subjects that received 908/RTV QD, 908/RTV BID or LPV/RTV BID and harbored virus containing the M46I/L, and /or V82A/S/T/F, and/or I84V and/or L90M at baseline was performed. Subjects failing to respond to therapy tended to have virus with phenotypic resistance to study PI, (>2.5- fold reduced susceptibility relative to the reference strain), sub-optimal NRTI and NNRTI backbone, poor adherence, high viral tier, or mutations associated with resistance to study PI. Analysis for some key baseline PI primary mutations is presented in Tables (20-23)

XIV (a): Effect of the M46I/L mutation in baseline HIV-1 isolates on virologic response of 908/RTV QD, 908/RTV BID and LPV/RTV BID at Week 48:

Table 19 shows the number of patients in each treatment arm with baseline HIV-1 isolates harboring M46I/L mutations which had virologic response at week 48. More patients harboring M46I/L mutations in baseline HIV-1 isolates and randomized to 908/RTV QD group showed virologic response (17/28) at Week 48 than those randomized to 908/RTV BID (11/22) and LPV/RTV BID (12/24).

Eleven patients (11/28) with the M46I/L mutation at baseline and receiving 908/RTV QD failed to achieve <400 copies/mL at week 48 (11/28). Of these, HIV-1 isolates from 3/11 had a 3.6- to 4-fold decreased susceptibility to APV relative to a reference strain at baseline (NDA 21-548, study APV 30003, Table 28, Page 78). However, HIV-1 isolates containing the M46I/L mutation at baseline from 6/17 patients responding to 908/RTV/QD therapy (HIV-1 RNA <400 copies/ml at Week 48) exhibited a 2.5- to 5.1-fold decreased susceptibility to APV (NDA 21-548, study APV 30003, Table 27, Page 77). Some of these baseline HIV-1 isolates with phenotypic resistance to APV contained (6/17) additional primary PI resistance-associated mutations: M46I + I84V + N88D (n=1), M46I + V82F + L90M (n=2), M46I + I54V + V82A (n=2), M46L + I54V + V82A (n=1).

Eleven patients receiving 908/RTV BID with M46I/L mutation in baseline HIV-1 isolates failed to achieve <400 copies/mL at Week 48. Of these, baseline HIV-1 isolates from 9/11 patients exhibited a 2.6- to 11-fold decreased susceptibility to APV (NDA 21-548, study APV 30003; Table 30, Page 80). Baseline HIV-1 isolates from these patients with reduced susceptibility to APV contained additional PI resistance-associated mutations: M46I + I84V + L90M (n=2), M46I + I54V + I84V + L90M (n=1), M46I/L + I54V + V82A/F (n=5) and M46I + V82A (n=1). Phenotypic analysis showed that baseline HIV-1 isolates from only 3/11 patients responding to 908/RTV BID therapy exhibited a >2.8- to 5.4-fold decreased susceptibility to APV. Baseline HIV-1 isolates from one of these 3 patient harbored an additional APV-resistance-associated mutation I54L (NDA 21-548, study APV 30003, Table 29, Page 79).

Twelve patients receiving LPV/RTV BID with the M46I mutation in baseline HIV-1 isolates failed to achieve <400 copies/mL at week 48 (NDA 21-548, study APV 30003, Table 32, Page 82). Baseline HIV-1 isolates from these patients exhibited a 2.6- to 93-fold decreased susceptibility to LPV. Baseline HIV-1 isolates from these patients contained additional primary PI resistance-associated mutations: M46I/L + I54V + V82A/T (n=4), M46I + I84V (n=2), M46I + V82A/T (n=2), V32I + M46I + V82A (n=2), V32I + M46I + I47V + I84V (n=1) and V32I + M46I + V82A (n=1). Phenotypic analysis showed that baseline HIV-1 isolates containing M46I/L mutations from 5/12 patients with virologic response on LPV/RTD BID exhibited a 2.7- to 18-fold decreased susceptibility to LPV (Table 31, NDA 21-548, study APV 30003,Table 31, Page 81). Baseline HIV-1 isolates from 3 of these 5 patients contained additional primary PI resistance-associated mutations: M46L + V82F (n=1), V32I + M46L + V82A (n=1), M46L + I54V + V82A (n=1).

XIV (b). <u>Effect of V82A/F/T/S mutations in baseline HIV-1 isolates on virologic response of LPV/RTV BID at Week 48</u>:

Table 20 shows the association of V82A/F/T/s mutations present in baseline HIV-1 isolates of responder and non-responder patients randomized to LPV/RTV BID. Baseline HIV isolates of 11 patients who failed to respond to LPV/RTD BID therapy had V82A/T mutations. In addition to V82A/T, baseline HIV-1 isolates from 10 of these 11 patients contained other primary PI resistance-associated mutations: I54V + V82A/T (n=4), V32I + V82A (n= 2), V32I + I47V + V82A (n= 1), G48V + V82A/T (n = 2), G48V + I54V + V82A (n=1). Baseline HIV-1 isolates from 10 of these 11 patients exhibited a 4.8- to 93-fold decreased susceptibility to LPV. Similarly, baseline HIV-1 isolates from 4/6 patients responding to LPV/RTV BID therapy had additional primary PI resistance-associated mutations: I54V + V82A (n=3), V32I + V82A (n=1). Phenotypic analysis showed that baseline HIV-1 isolates from 4 of these 6 patients exhibited a 2.8- to 18-fold reduced susceptibility to LPV (NDA 21-548, study APV 30003, Table 35, Page 85). Three subjects that responded to LPV/RTV BID had low viral load at entry (<10,000 to <25,000 copies/mL).

XIV (c). Effect of I84V mutation in baseline HIV-1 isolates on virologic response of 908/RTV QD, 908/RTV/BID and LPV/RTV BID at Week 48:

Two of 8 patients from 908/RTV QD group with baseline HIV-1 isolates containing the I84V mutation achieved virologic response of <400 copies/ml at Week 48 (Table 21). Six patients with HIV-1 isolates harboring I84V mutation were non-responders. Baseline HIV-1 isolates from only 2 of these 6 patients exhibited a 2.6- to 5.1-fold reduced susceptibility to APV. Similarly, baseline HIV-1 isolate from one of the responder patient had a low level of APV resistance (Table 21).

Table 20: Summary of viral genotype and response of subjects with HIV-1 isolates harboring V82A/T/F/S mutation at baseline receiving LPV/RTV BID and virological response at Week 48 (<400 copies/mL)

Subjects	Protease Mutations	Fold-resistance to LPV
Responders		
3931	I54V, L63P, A71V, V77I, V82A	2.8
3923	L10V, M46L, L63P, A71T, V82F	5.3
3720	V32I, M46L, L63P, A71V, V82A	4.4
4304	L10I, M36I, L63P, A71T, V77I, V82A	1.4
5512	L10I, M46L, F53L, I54V, L63P, A71V, V77I, V82A, L90M	18
3726	L10V, K20R, L24I, M36I, I54V, L63P, V77I, V82A	0.4
Non-responders		
4307	K20R, M36I, G48V, V82A	2.3
3071	L10I, L24I, M46I, I54V, V82A	14
4313	L10I, G48V, I54V, A71V, V82A	13
3444	L10I, M46L, L63P, V82A, L90M	2.6
3711	K20R, D30N, V32I, M36I, M46I, L63P,V82A, L90M	7.2
3446	L10I, K20R, V32I, M36I, M46I, L63P, A71V,V82A	9.4
4282	L10I, L24I, M36I, I54V, L63P, A71T, V82A/T	12
3599	L10I, M36I, M46L, I54V, L63P, A71V, G73S,V82A, L90M	13
4436	K20R, V32I, M36I, M46I, I47V, L63P, A71V, V77I, V82A, L90M	43
3473	L10I, K20R, M36I, M46I, G48V, L63P, A71V, G73S, V82T, L90M	4.8
3573	L10I/V, K20M, M36I, M46I, I54V, L63P, A71V, G73S, V82A, L90M	93

Table 21: Summary of viral genotype and response of subjects with HIV-1 isolates harboring the I84V mutation at baseline and receiving 908/RTV QD and virologic response at Week 48 (<400 copies/mL)

Subject	PI Mutations	Fold-resistance to APV
Responders		
3730	L10I, M46I, L63P, **I84V**, N88D	2.5
4942	M46I, L63P, G73S, V77I, **I84V**, L90M	1.1
Non-responders		
3732	L63P, A71V, G73S, **I84V**, L90M	0.6
3991	L10I, M36I, F53L, L63P, A71T, **I84V**	5.1
3424	L63P, A71T, G73S, V77I, **I84V**, L90M	2.4
3517	M36I, F53L, I54V, L63P, A71V, **I84V**, L90M	1
3701	L10V, K20M, M36I, M46I, L63P, A71V, **I84V**	0.5
3445	L10I, M36I, F53L,L63P, G73S, **I84V**, L90M	2.6

* Mutation I84V is bolded

Of the 6 patients with baseline HIV-1 isolates harboring I84V mutation and receiving 908/RTV /BID, only one patient achieved virologic response. The remaining 5 patients were non-responders. Phenotypic analysis showed that baseline HIV-1 isolates from these 5 patients exhibited a 2.7- to 14-fold decreased susceptibility to APV (Table 22).

Two of the 5 patients with baseline HIV-1 isolates containing the I84V mutation responded to LPV/RTV BID treatment (Table 23). Baseline HIV-1 isolates from one of these two patients had an additional APV resistance mutation I54L. The remaining three patients failed to respond to LPV/RTV BID at Week 48. Baseline HIV-1 isolates from all 3 patients with I84V mutation exhibited a high level of LPV-resistance (8.6 to 30- fold decreased susceptibility).

Table 22: Summary of viral genotype and response of subjects with HIV-1 isolates harboring I84V mutation at baseline and receiving 908/RTV BID and virologic response at Week 48 (<400 copies/mL)

Subject	PI Mutations	Fold – resistance to APV
Responders		
3472	L10I, M36I, I54V, L63P, V82A/T, **I84V**, L90M	2.1
Non-Responders		
3823	L10I, K20M, L63P, A71V, G73S, **I84V**, L90M	2.7

3063	L10I, M46I, L63P, **I84V**, L90M	8.8
3665	L10I, K20R, M36I, I54V, L63P, A71V, **I84V**, L90M	14.0
4994	L10I, M46I, L63P, G73S, V77I, **I84V**, L90M	4.9
3597	L10I, M36I, M46I, I54V, L63P, A71V, G73S, **I84V**, L90M	8.2

* The mutation I84V is bolded

Table 23: Summary of viral genotype and response of subjects with HIV-1 isolates harboring I84V mutation at baseline and receiving LPV/RTV BID and virologic response at Week 48 (<400 copies/mL)

Subject	**PI Mutations**	**Fold-resistance to LPV**
Responders		
4913	D30N, L63P, A71T, V77I, I84V, N88D, L90M	4.2
3060	I54L, L63P, I84V, L90M	0.8
Non-responders		
5535	L10I, M46I, L63P, A71V, I84V, L90M	8.6
4316	M36I, M46I, L63P, A71V, G73S, I84V, L90M	9
3591	L10F/I, V32I, M46I, I47V, L63P, V77I, I84V, L90M	30

XV. Treatment emergent PR-resistance associated mutations in HIV-1 from subjects experiencing virological failure and/on-going replication:

One hundred and four subjects (908/RTV QD (n=42), 908/RTV BID (n=33) and LPV/RTV BID (n=29) met the criteria of having virological failure and/or had on-going viral replication. The incidence of treatment emergent PI resistance-associated mutations in virologic failure patients is summarized in Table 24. The primary mutations that emerged in the protease gene of on-therapy HIV-1 isolates from virologic failure patients receiving 908/RTVQD treatment were: V32I (n=1), M46L (n=1), I47V (n=2), I54L (n=5), I84V (n=4) and L90 M (n=1). Mutations V32I, I47V and I54L were not present in baseline HIV-1 isolates of any of these patients.

The primary mutations that emerged in protease gene of on-therapy HIV-1 isolates from virologic failure patients receiving 908/RTV BID were: V32I (n=2), M46I/L (n=9), I47V (n=3), I50V (n=3), I54L (n=2), I54M (n=2) V82A (n=1), I84V (n=7), and L90M (n=2). The I50V mutation was observed only in 908/RTV BID group. A higher incidence of I84V was observed in the 908/RTV BID group (7/33) versus the 908/RTV QD group (4/39). Similarly, the incidence of M46I/L was higher in the 908/RTV BID group (9/33) compared to 908/RTV QD group (1/39).

The primary mutations that emerged in the protease gene of on-therapy HIV-1 isolates from virologic failure patients in LPV/RTV BID group were: I47A (n=2), I50V (n=1), I54V (n=1), and V82 T (n=1).

XVI. Treatment emergent NRTI resistance-associated mutations in HIV-1 from subjects experiencing virological failure and/on-going replication:

Table 25 shows the incidence of treatment emergent NRTIs resistance-associated mutations in virologic failure patients. The ABC/3TC resistance mutation M184V developed more frequently in virologic failure patients receiving 908/RTV BID (n=7) versus patients receiving 908/RTV QD (n=3) or LPV/RTV BID (n=3). The L74V was also present in on-therapy isolates from patients receiving 908/RTV QD (n=1), 908/RTV BID (n=1) or LPV/RTV BID (n=1). ZDV resistance-associated mutations M41L, L210W and K219Q/E emerged to varying degree. The T215Y/F mutation developed in on-therapy HIV-1 isolates from virologic failure patients in each treatment group: 908/RTV QD (n=2), 908/RTV BID (n=2) and LPB/RTV BID (n=1)

Table 24: Summary of treatment emergent protease mutations in virologic failure patients.

	908/RTV QD	**908/RTV BID**	**LPV/RTV BID**
	N=42	**N=33**	**N=29**
Genotype at Failure	**39**	**33**	**28**
L10I/F/R/V	9 (5F, 2I, 2V, 2X)	5 (4F, 1I)	2 (V)
K20M/R	0	0	1 (R)
L24I	0	1	0
V32I	1	2	0
L33F	3 (+2X)	5 (+1X)	0
M36I	0 (+1X)	1	1 (+1X)
M46I/L	1 (L)	9 (5I, 4L)	0
I47V	2	3	0 (2A)
I50V	0	3	1
I54V	0	0	1
I54L	5	2	0
I54M	1	2	0
I54S/X	0/2	1/3	0/1
L63P	0	0	1
A71V/T	1 (T)	2 (V/T)	1 (T)
G73S/A	1 (A)	1 (S)	0
V77I	1	0	0

V82A/F/T/S	0 (+3 V82I)	1 (A) (+1 V82I)	1 (T)
I84V	4	7	0
L90M	1	2	0

- Bold denotes primary PI mutations
- X= 3 or more amino acids detected, specific amino acid not determined.

3424	16	G73A/G (L10X, L33X)	I84V, L90M	11	2.4
3432	16	L10F/I, I84V	L10V, L90M	6.3	0.7
3470	16	L33F, I54M	D30N, N88D, L90M	27	1.9
3741	16	I54X, I84V	I54V, V82T, L90M	17	1.1
3803	16	M46L, I84V	G48V, I54V, V82A	6.4	2.9
3977	16	I54L, V82I	M46L, G73S, L90M	3	1
3991	16	(L33X)	I84V	48	5.1
4872	16	I54L	L90M	4.5	1.1
3303	20	I54L, I84V	M46L, L90M	3.7	0.7
3517	20	(L10X)	I54V, I84V, L90M	2.6	1
3445	24	(I54X)	I84V, L90M	17	2.6
3732	32	L10 F, I54L	I84V, L90M	14	0.6
3724	56	L10F, L33F	M46L, G73S, V77I	6.5	1.6
3832	56	V32I, I47V	L10I, M46L	19	0.9

X= 3 or more amino acids detected, specific amino acid not determined.

Phenotypic analysis data for baseline matched on-therapy HIV-1 isolates were available for 20 virologic failure/on going replication patients receiving 908/RTV QD (NDA 21-548, study APV 30003, Table 58, Pages 109-110). Of these, phenotypic analysis data for on-therapy and baseline isolates from 14/20 patients are shown in Table 26. Primary PI mutations detected in isolates at baseline and during therapy from these 14/20 patients are also shown in Table 26 for a possible correlation of genotypes with drug susceptibility. On-therapy HIV-1 isolates from 11/20 patients failing 908/RTV QD therapy exhibited a 2.6- to 19-fold decreased susceptibility to APV. On-therapy HIV-1 isolates from 8/11 contained APV resistance-associated mutations: V32I, M46L, I47V, I54L/M, and I84V, alone or in combination. On-therapy HIV-1 isolates from the other 3 patients failing therapy exhibited a 6.1- to 48-fold decreased susceptibility to APV (baseline isolates from these 3 patients had demonstrated 2.6 to 5.1 fold decreased susceptibility to APV). On-therapy HIV-1 isolates from these 3 patients contained either L33X (n=1), I54X (n=1) or M46L + I84V mutations (n=1). Both baseline and on-therapy HIV-1 isolates from an additional 6 patients were susceptible to APV (NDA 21-548, study APV 30003, Table 58, Pages 109-110). It should be noted that on-therapy isolates from 2 of these 6 patients with phenotypic susceptibility to APV contained APV resistance-associated mutations, I47V or I54L (patients # 3590 and #3105).

Table 27: Genotypes (PI mutations) and phenotypes of on-therapy and baseline HIV-1 isolates from virologic failure/on-going replication patients receiving 908/RTV BID (study APV 30003)

Subject	Time point (Wk)	Emerging PI-mutations	Baseline PI-mutations	APV resistance at failure	APV resistance at

Table 25: Summary of treatment emergent NRTI mutations in virologic failure patients

	908/RTV QD	908/RTV BID	LPV/RTV BID
	N=42	N=33	N=29
Genotype at Failure	**39**	**33**	**28**
M41L	1	2 (+1X)	0
E44D	0	2	0
K65R	0	1	0
D67N	0	1 (+1X)	1
T69D	0 (+1X)	0	0
L74V	1	2 (+2 V74I)	1
V75I/T/M/S/A	0	0	0 (+2 X)
F116Y	0	0	1
V118I	0	3	0
M184V	3	7 (+3 X)	3 (+1 X)
L210W	1	1	0 (+ 1 X)
T215Y/F	2 (F/Y) (+ 1X, 1V, 1I)	2 (Y) (+2X, 1S)	1 (Y)
K219Q/E	0	1 (E)	1 (Q)

X= 3 or more amino acids detected, specific amino acid not determined.

XVII. Phenotypic analysis of on-therapy HIV-1 isolates from virologic failure/on-going replication patients receiving 908/RTV/QD, 908/RTV BID and LPV/RTV BID:

On-therapy HIV-1 isolates from 16 of 39 virologic failure/on-going replication patients receiving 908/RTV QD, 25 of 33 on 908/RTV BID showed phenotypic resistance to APV and from 7 of 27 patients receiving LPV /RTV to LPV. As shown earlier, baseline HIV-1 isolates from some of these patients also exhibited resistance to APV or LPV.

The primary protease mutations and phenotype of on-therapy and matched baseline HIV-1 isolates from virolgoic failure/on-going replication patients from 908/RTV QD, 908/RTV BID and LPV/RTV BID groups are shown in Table 26-28.

Table 26: Genotypes (PI mutations) and phenotypes of on therapy and baseline HIV-1 isolates from virologic failure/on-going replication patients receiving 908/RTV QD (Study APV 30003)

Subject	Time point (Wk)	Emerging PI-mutations	Baseline PI mutations	APV resistance at failure	APV resistance at baseline

					baseline
3104	16	V32I, I47V	M36I, L90M	11	0.4
3302	16	I54M, I84V	D30N, N88D	16	0.7
3365	16	V32I, M46L, I47V	L10F, L63P	7.9	1.2
3761	16	L10F	V32I, M46L, L90M	6.4	1.1
3790	16	L33F, I84V	M46L, I54V, V82A	37	5.7
3840	16	L10F, M46I	L63P	6.8	ND
3872	16	I84V	I54V, V82A	12	0.9
3976	16	M46L, I84V	M46I, I54V,V82A	8.1	2.6
4252	16	M46L, N88G	I54V, N88S, L90M	24	1.1
4437	16	M46I, I47V	I54L, L90M	6.7	1.1
4994	16	(L33X, I54X)	M46I, I84V, L90M	29	4.9
5321	16	M46L,V82I	M36I, L90M	6	2.7
3114	20	L33F, I54S	M46I, I54A, V82A	13	4.2
3667	24	(I54X)	M46L, L90M	6.7	1.9
4401	24	(I54X)	V82I, L90M	14	2.7
3242	32	L33F, I50V, I54L	L63P, V77I	2.8	0.2
3393	32	M46I, I50V, I54L, V82A, I84V	L10I, V77I	4.9	0.8
3842	32	L33F, M46I, I50V, I84V	M46I, I54V, V82A	20	5.2
3341	40	L33F, M36I, I84V	M46I, I54V, V82F, L90M	52	11
3823	40	M46L, I54M	I84V, L90M	11	2.7

ND= Baseline data not available.
X= 3 or more amino acids detected, specific amino acid not determined.

Phenotypic analysis were available for baseline matched on-therapy HIV-1 isolates from 22 virologic failure/on going replication patients receiving 908/RTV BID. Isolates from 20/22 patients exhibited a 2.6- to 52-fold decreased susceptibility to APV. However, baseline isolates from 9/20 patients demonstrated decreased susceptibility (2.6- to 11-fold) to APV. Phenotypic analysis data for baseline HIV-1 isolates from one patient (#3840) were not available and isolates from 10/20 were susceptible to APV at baseline. Both on-therapy and baseline HIV-1 isolates from the remaining 2 patients (2/22) were susceptible to APV. On-therapy HIV-1 isolates from 15/20 patients contained APV resistance-associated mutations either alone or in combination: V32I, M46I/L, I47V, I50V, I54L, I54M and I84V. In addition, on-therapy isolates contained other mutations: L10F (n=1), L33X + I54 X (n=1; [X= 3 or more amino acids, specific amino acid not determined), I54X (n=2) and L33F, I54S (n=1). Patents with baseline HIV-1 isolates containing I54V, V82A mutations had on therapy HIV-1 isolates harboring I84V

mutations. Conversely, those with I84V mutation in baseline isolates developed I54L/M during therapy. Most HIV-1 isolates from patients with reduced susceptibility to APV at baseline remained APV-resistant during therapy.

Table 28: Genotypes (PI mutations) and phenotypes of on therapy and baseline HIV-1 isolates from virologic failure/on-going replication patients receiving LPV/RTV BID (study APV 30003)

Subject	Time point (Wk)	Emerging PI-mutations	Baseline PI-mutations	LPV resistance at failure	LPV resistance at baseline
3446	16	L10V/I, I47A	M46I, V82A	151	9.4
3473	16	I50V (I54X)	M46I, G48V V82T, L90M	99	4.8
3591	16	M36I (G73T)	V32I, M46I, I47V, I84V, L90M	68	30
3599	16	K20R	M46L, I54V, V82A, L90M	14	13
4436	16	I47A	M46I, I47V, V82A L90M	56	43
3071	20	(M36X)	M46I, I54V, V82A	16	14
3324	24	V82T	I54V, V82I	15	0.7
3065	32	L63P/H	L63H, L90M	0.6	ND
3444	32	I54V	M46L, V82A, L90M	11	2.6

X= 3 or more amino acids detected, specific amino acid not determined.

Table 28 shows that on-therapy HIV-1 isolates from 2 patients with virologic failure on LPV/RTV BID developed an I47A mutation that resulted in a 56- to 151-fold decrease in susceptibility to LPV. Baseline HIV-1 isolates from these 2 patients exhibited 9.4 to 43-fold decreased susceptibility to LPV and harbored IDV resistance-associated mutations M46I and V82A. On-therapy HIV-1 isolates from one patient (#3473) developed an I50V mutation that resulted in a 4.8- to 9-fold decrease in susceptibility to LPV. Baseline HIV-1 isolates from this patient contained M46I, G48V, L63P, V82T, and L90M mutations. On-therapy HIV-1 isolates from one patient (#3324) contained a V82T mutation and exhibited a 15-fold decrease in susceptibility to LPV. Baseline HIV-1 isolates from this patient were susceptible to LPV even though they harbored I54V mutations. In contrast, on-therapy HIV-1 isolates from another patient (#3444) harboring a I54V mutation exhibited a 11-fold decrease in susceptibility to LPV. Baseline HIV-1 isolates from this patient exhibited a 2.6-fold decrease in susceptibility to LPV and contained a V82T mutation.

CONCLUSIONS

With respect to microbiology, this NDA # 21-548 is supported. Virology data presented for studies APV 30001, 30002 and 30003 support the use of GW433908 in combination with NRTIs for the treatment of HIV-1 infection. In study APV 30001, One-hundred sixty-six antiretroviral naïve patients were treated with GW433908 in combination with ABC and 3TC. The PI-resistance-associated mutations observed in HIV-1 isolates from GW433908 treated virologic failure patients were similar to that reported for APV treated patients (Maguire et al., 2002; sNDA 21-007). Genotypic analysis of baseline-matched on therapy HIV-1 isolates from 29 patients with virologic failure (plasma HIV-1 RNA of $\geq$ 1000 copies/mL on two consecutive occasions on or after Week 12 treatment) on GW433908 therapy showed that isolates from 5/29 contained APV-resistance-associated mutations I54L/M (n=2), I54L + L33F (n=1), V32I + I47V (n=1) and M46I + I47V (n=1). In addition, these isolates contained ABC/3TC resistance-associated mutations: M184I (n=1), M184V (n=3), M184V + L74V (n=1). On-therapy HIV-1 isolates from another 11/29 patients contained ABC/3TC resistance-associated mutations M184V (n=9), M184V + L74V (n=1) or M184I (n=1).

Phenotypic analysis of on-therapy HIV-1 isolates harboring APV resistance-associated mutations (n=5) showed that isolates from two patients exhibited a 5.7- to 7.2-fold decreased susceptibility to APV in vitro. APV resistant HIV-1 isolates from one patient exhibited a 3.6- and 4.4-fold decreased susceptibility to NFV and RTV, respectively. Similarly, APV resistant isolates from another patient exhibited a 3.7- to 4.8-fold decreased susceptibility to IDV, LPV and NFV in vitro. However, these APV resistant isolates were susceptible to SQV.

Genotypic analysis showed that none of the baseline matched on-therapy HIV-1 isolates from patients (n=32) with virologic failure in study APV 30002 (n=322) contained any mutation associated with resistance to APV. However, on-therapy isolate from one patient contained an I54V mutation. Phenotypic analysis showed that isolates from this patient (1/32) were resistant to APV, IDV, LPV, NFV, RTV and SQV in vitro. The absence of key PI mutations which confer resistance to APV in HIV-1 isolates from patients with virologic failure receiving 908/RTV QD in study APV 30002 suggested that APV plasma concentrations achieved in these patients was sufficiently high to prevent or delay the development of mutations.

In study APV 30003, two doses of GW433908 + RTV in combination with 2 NRTIs were administered. Geometric mean plasma APV, $C_{\tau,ss}$ values for HIV-1 infected patients receiving GW 433908/RTV in APV 30003 were 1.69 µg/mL for the GW433908 700 mg BID + RTV 100 mg BID regimen and 1.35 µg/mL for the GW 433908 1400 mg QD + RTV 200 mg QD regimen (NDA 21-548; Vol 6.2, Page 14). Based on the plasma APV C_{min} values of 1.35 µg/mL and 1.69 µg/mL achieved with GW 433908 1400 mg QD + RTV 200 mg QD and GW433908 700 mg BID + RTV 100 mg BID, respectively in HIV-1 infected patients, and 50% human serum binding adjusted IC_{95} of 0.322 µg/mL, it is likely that APV plasma concentration would exceed by 4- to 5-fold above the 95%

inhibitory concentration (Condra et. al., 2002) in patients treated with RTV boosted GW433908. Thus, boosting with RTV would delay or prevent the development of APV resistant HIV-1 mutants in 908/RTV QD and 908/RTV BID treated patients.

In study APV 30003 virologic failure patients with prior experience with one or two PI- were randomized to 908/RTV QD (n=105), 908/RTV BID (n=107) or LPV/RTV BID (n=103) treatment groups. The majority of patients had received prior treatment with IDV, NFV or SQV (with or without RTV). No patient had previously received APV or LPV. Genotypic analysis showed that accessory mutations L63P, A71V/T, V77I , L10I/F/V/R and M36I, in decreasing order of prevalance, were present in baseline HIV-1 isolates from most patients.. The primary PI resistance-associated mutations prevalent in baseline HIV-1 isolates of patients from each treatment group were L90M, M46I/L and D30N, in decreasing order of occurrence. Other primary PI mutations detected in baseline HIV-1 isolates were V82A/F/T/S, N88D, I54V, I84V, N88S, G48V, V32I, I54L, and I47V in decreasing order of prevalance. As mentioned earlier, mutations D30N, N88D/S and L90 M are associated with NFV resistance (Shafer 2002; Stanford University Resistance Database 2002). The I54V, V82A/F/T/S mutations confer resistance to IDV and RTV (Molla et al.,1996; Zhang et al., 1997). Mutations G48V and L90M are associated with resistance to SQV. Mutations V32I, M46I/L, I 47V, I54L, I84V are associated with APV resistance. V32I also develops in concert with V82A mutation in HIV-1 isolates from patients treated with IDV. APV resistance-associated mutations I50V and I54M were not detected in baseline HIV-1 isolates from any of the patients enrolled in study APV 30003. The I54L mutation, associated with APV resistance, was detected in baseline isolates from 2/105 patients randomized to 908/RTV BID and 2/107 patients in the LPV/RTV group. The I54V mutation was present in baseline HIV-1 isolates from 12/105, 11/107 and 12/103 patients receiving 908/RTV QD, 908/RTV BID and LPV/RTV BID, respectively. The mutation I54V confers resistance to APV, IDV, LPV, RTV, and SQV. The mutation I84V was present in baseline HIV-1 isolates from 8/105, 8/107, and 7/103 patients receiving 908/RTV QD, 908/RTV BID and LPV/RTV BID, respectively. Similar to the mutation I54V, the I84V mutation confers cross-resistance to most approved PIs and develops in HIV-1 isolates from patients treated with APV, IDV, RTV and SQV. The PI mutations observed in baseline HIV-1 isolates from patients in study APV 30003 were consistent with their prior PI use.

Phenotypic analysis showed that baseline HIV-1 isolates of 81/93 and 71/88 patients randomized to the 908/RTV QD and 908/RTV BID groups, respectively, were susceptible to APV in vitro. Baseline HIV-1 isolates from 12/93 and 17/88 patients receiving 908/RTV QD and 908/RTV BID, respectively, exhibited a 2.5- to <6-fold decreased susceptibility to APV in vitro. Baseline HIV-1 isolates from 63/85 patients receiving LPV/RTV BID were susceptible to LPV in vitro. However, isolates from 22/85 patients exhibited a 2.5 to ≥10-fold decreased susceptibility to LPV in vitro.

On-therapy HIV-1 isolates from 14/39 and 20/33 virologic failure/on-going replication patients receiving 908/RTV QD or 22/33 receiving 908/RTV BID showed reduced susceptibility to APV. Similarly, on-therapy isolates from 8/27 virologic failure/on-going

replication patients receiving LPV/RTV BID showed reduced susceptibility to LPV. As shown earlier, baseline HIV-1 isolates from some of these patients also exhibited resistance to APV or LPV.

Phenotypic analysis showed that baseline matched on-therapy HIV-1 isolates from 11/20 patients failing 908/RTV QD therapy developed a 2.6- to 19-fold decreased susceptibility to APV. On-therapy HIV-1 isolates from 8 of these 11 patients contained APV-resistance-associated mutations: V32I, M46L, I47V, I54L/M, and I84V, either alone or in combination. On-therapy HIV-1 isolates from 3 other patients in 908/RTV QD group exhibited a 6.1- to 48-fold decreased susceptibility to APV. On-therapy isolates from these 3 patients contained either L33X (n=1), I54X (n=1) or M46L + I84V mutations (X= 3 or more amino acids). Isolates from these patients at baseline demonstrated reduced susceptibility to APV.

Phenotypic analysis of baseline matched on-therapy HIV-1 isolates from 22 virologic failure/on going replication patients receiving 908/RTV BID showed that isolates from 20 patients exhibited a 2.6- to 52-fold decreased susceptibility to APV. However, baseline HIV-1 isolates from 9/20 patients exhibited a 2.6- to 11-fold decreased susceptibility to APV. On-therapy HIV-1 isolates from 15/20 virologic failure/on going replication patients from 908/RTV BID group contained APV resistance-associated mutations either alone or in combination: V32I, M46I/L, I47V, I50V, I54L, I54M and I84V. In addition, on-therapy isolates contained other PI mutations: L10F (n=1), L33X and I54 X (n=1), I54X (n=2), and L33F and I54S (n=1). Patients with baseline HIV-1 isolates containing I54V and V82A mutations had on therapy HIV-1 isolates harboring I84V mutations. Conversely, those with I84V mutation in baseline isolates acquired an I54L/M mutation during therapy. Most HIV-1 isolates from patients with reduced susceptibility to APV at baseline remained resistant to APV during therapy.

Genotypic and phenotypic analyses of matched baseline and on-therapy HIV-1 isolates from patients with virologic failure on LPV/RTV BID showed that isolates from 9/11 patients exhibited a 2.6- to 151-fold increased resistance to LPV. Most of these isolates at baseline contained V82A/T mutations in combination with M46I, I54V, I47V, I84V, or L90M. On-therapy HIV-1 isolates from 2 patients developed an I47A mutation that resulted in a 56- to 151-fold decrease in susceptibility to LPV. On-therapy HIV-1 isolates from another patient developed an I50V mutation that resulted in a 4.8- to 99-fold decrease in susceptibility to LPV. Baseline HIV-1 isolates from this patient contained M46I, G48V, L63P, V82T and L90M mutations. On-therapy HIV-1 isolates from two patients with V82T and I54V mutations exhibited an 11- to 15-fold decrease in susceptibility to LPV.

A possible correlation of PI mutations in baseline and on-therapy HIV-1 isolates with virologic response (<400 copies/mL) can be made for patients receiving 908/RTV/BID in study APV 30003. On-therapy isolates from 13 of the 33 virologic failure patients contained mutations I54L/M/S, I84V and V82A/T. Baseline HIV-1 isolates from 7/33 patients contained I54V and V82A/T mutations and from 5/33 patients I84V mutations.

Other APV-resistance-associated mutations: I47V (n=3) and I50V (n=3) were also present in on-therapy HIV-1 isolates from virologic failure patients receiving 908/RTV BID.

An analysis of the effect of baseline primary PI-resistance mutations on virologic response (<400 copies/mL) showed that only 17-22% patients in 908/RTV BID group with isolates harboring mutations I54V, V82A/T/F/S and I84V had virologic response. The majority of patients with mutations D30N and N88D/S were responders. However, this analysis is limited since it does not account for the contribution of the other PI-resistance-associated mutations selected during therapy. Primary PI-resistance mutations in concert with accessory mutations selected during therapy would contribute towards treatment failure. On the other hand, PI-resistance associated mutations present in baseline HIV-1 isolates from patients with prior PI-treatment failure could persist during therapy when patients are switched to an alternate PI-regimen (Kantor et al., 2002). Several studies have tried to correlate the presence of primary PI-resistance- associated mutations in baseline HIV-1 isolates with virologic response or phenotypic susceptibility in PI-experienced patients. The presence of I84V mutation in isolates from PI-experienced virologic failure patients was most significantly ($p<0.0001$) associated with phenotypic resistance to APV (Schmidt et al., 2000; Paulsen et al. 2002). The mutations I54V and V82T were significantly associated with decreased phenotypic susceptibility to LPV.

METHODOLOGY:

Sample Collection, Shipping and Handling:

Plasma and PBMC samples were collected, stored and shipped to the [redacted] Subject samples identified for analyses were requested from [redacted], and shipped either to GSK International Clinical Virology (ICV) Department, Stevenage, UK or to [redacted] For genotypic analyses carried out at GSK [redacted] was performed as described in the manufacturer's instructions using either the [redacted] for HIV-1 RNA determination from plasma or the [redacted] for DNA extraction from PBMCs. Aliquots of the extracted nucleic acid were used as starting material for sequence determination.

Methodology for Genotypic Analysis:

Genotypic analysis of the viral protease (PR), reverse transcriptase (RT) and p7/p1 and p1/p6 gag cleavage sites (CS) of virus present in plasma samples obtained from all subjects at Day 1, and specific post-baseline plasma samples was performed by [redacted], using the [redacted] technology according to their standard procedures. Genotypic analyses of viral PR, RT and CS were performed at GSK ICV Department on virus present in Day 1 PBMC samples from all subjects for whom a sample was available, and on selected plasma samples using [redacted] DNA sequencing.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA 21,548; SN-000 Review Completed 09/25/03

Genotypic Analysis (GSK ICV Assay):

[redacted]

Primer Nucleotide Sequence

[redacted]

Sequencing reactions were resolved on the [redacted] according to the manufacturer's instructions ([redacted] Data were analyzed using the [redacted] software. Mixtures of mutant and wild-type sequences with electropherogram peak size ratios of approximately 40-80 % were recorded as mixed populations. Mutant: wild-type ratios greater than 80 % were designated as the mutant. Mutant present less than 40% of wild type were not reported.

Genotypic summaries were based on differences in the plasma virus sequence from the molecular wild-type strain HXB2. An assessment was made of the total number of changes across all amino acids within the RT, PR and p7/p1 - p1/p6 Gag cleavage site regions, as well as an assessment of specific amino acid changes associated with the development of resistance to antiretrovirals.

Genotypic Analysis [redacted] Assay):

[redacted] utilizes DNA sequence analysis to detect mutations in HIV-1 protease (PR) and reverse transcriptase (RT) associated with resistance to PR and RT inhibitors,

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[redacted]

Methodology for Phenotypic Analysis:

Phenotypic analysis to determine drug susceptibility of virus in baseline samples for all subjects from whom they were available, and post-baseline samples obtained from subjects experiencing virological failure/on-going viral replication was performed by [redacted] using the [redacted] according to their standard procedures.

[redacted]

The results from this assay are expressed as IC_{50} values (concentration of drug which inhibits the virus by 50%). If the patient virus requires a significantly higher drug concentration to inhibit its replication, as compared to the drug sensitive reference virus, then the patient virus has reduced susceptibility to that drug (Petropolous et al., 2000). Based on the fold-increase in IC_{50} concentration relative to wild-type HIV-1, samples were described as susceptible (<2.5-fold increase), intermediate resistance (>2.5-fold and <10-fold increase) or high level resistance (>10-fold) to each study drug.

Methodology for HIV RNA copy number determination:

The approved Amplicor HIV-1 Monitor® test (version 1.5) 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. The linear range of ultrasensitive assay is 50 to75,000 copies/mL.

5 Draft Labeling Page(s) Withheld

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GlaxoSmithKline Document Number SH2002/00014/00. Study ID: APV 30002 Virology Report. Analyses of HIV genotyping and phenotyping data collected during the course of a clinical study of APV 30002: a randomized, open-label, two arm trial to compare the efficacy, safety and tolerability of GW433908/ritonavir QD to nelfinavir BID when used in combination with abacavir and lamivudine BID for 48 Weeks in antiretroviral therapy naïve HIV-1 infected subjects.

GlaxoSmithKline Document Number SH2003/00023/00. Study ID APV 30003 VirologyReport. Analyses of HIV genotyping and phenotyping data collected during the course of a clinical study APV 30003: a Phase III, randomized, multicenter, parallel group, open-label, three arm study to compare the efficacy and safety of two dosing regimens of GW433908/ritonavir (700mg/100mg twice daily or 1400mg/200mg once daily) versus lopinavir/ritonavir (400mg/100mg twice daily) for 48 Weeks in protease inhibitor experienced HIV-Infected adults experiencing virological failure.

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PHASE 4 COMMITMENTS

1.

2. Please provide data on the in vitro anti-HIV-1 activity of amprenavir against HIV-1 clades and multiple isolates of HIV-2.
3.

RECOMMENDATIONS

With respect to microbiology, this NDA # 21-548 is approvable.

Lalji Mishra, Ph.D.
Microbiologist

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