

## Original article

# Antiviral activity and safety of aplaviroc with lamivudine/zidovudine in HIV-infected, therapy-naive patients: the ASCENT (CCR102881) study

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**Background:** This Phase IIb study explored the antiviral activity and safety of the investigational CCR5 antagonist aplaviroc (APL) in antiretroviral-naive patients harbouring R5-tropic virus.

**Methods:** One hundred and forty-seven patients were randomized 2:2:1 to one of two APL dosing regimens or efavirenz (EFV). All dosage arms were administered twice daily and in combination with lamivudine/zidovudine (3TC/ZDV; Combivir, COM). Efficacy, safety, and pharmacokinetic parameters were assessed.

**Results:** This study was prematurely terminated due to APL-associated idiosyncratic hepatotoxicity. The primary endpoint of the study was the proportion of patients with plasma HIV-1 RNA <400 copies/ml who remained on randomized treatment through week 12. Of the 147

patients enrolled, 145 patients received one dose of treatment and were included in the intention-to-treat population. The proportion of patients with HIV-1 RNA <400 copies/ml at week 12 was 53%, 50% and 66% in the APL 600 mg twice daily, APL 800 mg twice daily, and EFV arms, respectively. Common clinical adverse events (AEs) were diarrhoea, nausea, fatigue and headache. APL demonstrated non-linear pharmacokinetics with high interpatient variability. In addition to the hepatic findings, there was an apparent dose-response relationship in the incidence of diarrhoea.

**Conclusions:** Whereas target plasma concentrations of APL were achieved, the antiviral activity of APL as the third agent in a triple drug regimen did not appear to be comparable to EFV in this treatment-naive patient population.

## Introduction

Although highly active antiretroviral therapy (HAART) results in a profound and sustained reduction in plasma HIV-1 RNA in many individuals, the side effects of currently available antiretrovirals (including toxicity, dyslipidaemia and body dysmorphic changes) and the emergence of multidrug-resistant viral strains continue to represent major challenges for the management of HIV infection. As such, new targets for therapy (such as inhibitors of the chemokine receptors CCR5 and CXCR4) could provide improvements in the care of HIV-infected individuals.

CCR5 antagonists are active against viral isolates that use the CCR5 coreceptor for viral entry. CCR5-tropic (R5-tropic) virus has been found to be present in at least 50% of viruses in both antiretroviral therapy (ART)-naive and -experienced, HIV-1-infected patients in several studies [1–6]. There is considerable interest in the potential utility of CCR5 antagonists when used in potent combinations with other antiretroviral drugs.

Aplaviroc (APL) is a CCR5 antagonist being developed for the treatment of HIV-1 infection in combination with other ART regimens. *In vitro* data

showed that APL was highly specific for the CCR5 receptor and inhibited R5-tropic HIV-1 replication at low to subnanomolar concentrations [7]. Multiple passage experiments (>48 weeks) using R5-tropic HIV-1 from both laboratory-derived and clinical isolates demonstrated that reduced susceptibility to APL was slow to develop *in vitro* [8,9]. Data from a single and repeat dose-escalation study in HIV-negative healthy adult patients suggested that APL was well-tolerated at individual doses up to 800 mg [10]. Of note, 24 h after a single dose, when plasma drug concentrations were undetectable, significant CCR5 receptor occupancy by APL (median percentage of 68–88% across the doses studied) was still observed. Furthermore, at 12 h after final dose administration during the multiple-dose phase, receptor occupancy by APL exceeded 97% [10]. A 10-day APL monotherapy study completed in HIV-1-infected adults demonstrated acceptable short-term safety. A mean 1.66 log<sub>10</sub> copies/ml at nadir decrease in viral load from baseline was observed in the highest dosage arm (600 mg twice daily), demonstrating potent antiviral activity [11].

The antiviral efficacy observed with APL in this monotherapy study compared favourably with the short-term efficacy of other potent antiretrovirals in ART-naïve HIV-1-infected patients. Short-term monotherapy studies evaluating nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) have also demonstrated a 1.5–2.0 log<sub>10</sub> copies/ml decrease in viral load from baseline [12–14]. Therefore, the short-term antiviral potency demonstrated with the CCR5 antagonist APL supported the investigation of its addition as a third agent to a standard-of-care dual NRTI backbone in ART-naïve patients.

ASCENT (A Study of Combivir and Entry Inhibitor in Naïve Treatment – CCR102881) was a randomized clinical trial designed to define an APL dosage regimen that could be potentially evaluated in subsequent Phase III trials.

## Methods

### Study participants

HIV-1-infected, antiretroviral-naïve male and female patients aged ≥13 years (or ≥18 where required by local regulatory agencies) with a screening plasma HIV-1 RNA ≥10,000 copies/ml, CD4<sup>+</sup> T-cell count ≥100 cells/mm<sup>3</sup> and R5-tropic virus, as determined by the PhenoSense HIV Entry™ assay, (Monogram Biosciences, South San Francisco, CA, USA) were eligible for study enrolment. Furthermore, patients could not have major NRTI or NNRTI mutations based on the mutation list defined by the International AIDS Society [15], which were determined by the GeneSeq™ assay (Monogram

Biosciences). The study was conducted from 14 January 2005 until 15 September 2005.

### Study design and treatment regimens

This Phase IIb, randomized, partially double-blinded, multicentre, parallel-group, dose-ranging study was conducted at 33 centres in the United States, four centres in Canada and 24 sites in the European Union. Patients were randomized 2:2:1 to one of the two double-blinded APL dosage regimens (APL 600 mg twice daily or APL 800 mg twice daily) or an efavirenz (EFV) control regimen; all regimens were administered in combination with lamivudine (3TC) and zidovudine (ZDV), which were dispensed as one fixed-dose combination tablet of Combivir®. APL was administered as 200 mg tablets. Randomization was stratified according to each patient's plasma HIV-1 RNA level at screening (<100,000 copies/ml versus ≥100,000 copies/ml). Originally designed to be a 96-week study, ASCENT was stopped prematurely due to the occurrence of treatment-emergent idiosyncratic hepatotoxicity in some patients participating in this and a parallel Phase IIb study [16]. Thus, most patients were evaluated over a 12-week treatment period only.

### Study assessments and monitoring

Patients enrolled in the study were evaluated at screening, days 1 (baseline, BL), 3, 5, and 10, and weeks 2, 4 and every 4 weeks thereafter. An additional follow-up visit was to be made within 4 weeks following the withdrawal visit for resolution of any ongoing adverse events (AEs) and new serious AEs (SAEs). Screening evaluations included informed consent, demographics (which included Centers of Disease Control and Prevention [CDC] classification), medical history, physical examination, triplicate electrocardiograms with heart rate, and determination of HIV-1 coreceptor tropism and reverse transcriptase (RT) genotype. Clinical evaluations of AEs and SAEs were also performed at screening, BL, weeks 2, 4 and every 4 weeks thereafter through to week 24, and every 8 weeks thereafter through to withdrawal. Laboratory testing performed at a central laboratory included complete blood count with lymphocyte subset determination, serum chemistry panel and plasma HIV-1 RNA by PCR. In addition, hepatitis B surface antigen and hepatitis C serology were performed for all patients at the baseline visit. Clinical and laboratory AEs were graded according to the 2004 DAIDS toxicity grading scale [17]. Efficacy was evaluated by measuring plasma HIV-1 RNA in blood samples (by both the Roche [Van Nuys, CA] COBAS Monitor Amplicor Standard PCR Assay [lower limit of detection 400 copies/ml] and the Roche [Heston, UK] PCR UltraSensitive Assay [lower limit of detection 50 copies/ml]). Lymphocyte subsets were assessed by flow cytometry. CDC-associated conditions and clinical

disease progression were also monitored. New or recurrent category C clinical disease progression events were confirmed by an independent reviewer. Safety was assessed by monitoring of clinical AEs and SAEs, clinical laboratory tests, HIV-associated conditions, concomitant medications, ECGs and vital signs during the treatment phase. The treatment phase included all data collected while the patients were on randomized treatment, plus data collected through 30 days following treatment discontinuation when additional antiretrovirals could have been initiated.

Analysis of viral tropism was carried out for all patients at all visits (with the exception of days 3, 5 and 10) when the plasma HIV-1 RNA was above the validated cutoff of the PhenoSense HIV Entry assay ( $\geq 1,000$  copies/ml). For virological failures, the PhenoSense GT<sup>TM</sup> Assay (Monogram Biosciences) was performed on day 1 and virological failure samples to assess RT and protease (PR) genotype and phenotype. In addition, APL susceptibility was determined for the day 1 and virological failure samples using the PhenoSense HIV Entry Assay.

#### Statistical analysis

The primary endpoint of the study was the proportion of patients with plasma HIV-1 RNA  $< 400$  copies/ml remaining on their randomized treatment regimen through week 12 of the study; a time-to-loss-of-virological-response algorithm was used in the analysis. The intention-to-treat (ITT) population was defined as all patients who met study criteria and were randomized into the study with documented evidence of having received at least one dose of randomized treatment. The primary analysis was to compare the proportion of responders (intent-to-treat) in each APL dosage regimen to the maximum observed response rate among the APL regimens. The control (EFV) arm was included as an internal control. For the clinical management of study participants, virological failure was defined as either an incomplete virological response (did not achieve a  $1 \log_{10}$  copies/ml decrease in plasma HIV-1 RNA by week 4 relative to baseline value or two consecutive measurements of plasma HIV-1 RNA  $\geq 400$  copies/ml on or after week 24) or virological rebound (plasma HIV-1 RNA rebounds to  $\geq 400$  copies/ml on two consecutive measurements after being previously  $< 400$  copies/ml on or after week 4, or two consecutive viral load determinations that are  $> 0.5 \log_{10}$  copies/ml plasma HIV-1 RNA from the nadir value on study, where nadir is the lowest HIV-1 value on or after week 4).

This study was not designed to evaluate formal statistical hypotheses. Rather, the design was based on an approach of estimation of early response in order to screen out ineffective regimens of APL. The planned sample size was 125 patients (50 patients in each of the APL groups and 25 patients in the EFV group).

#### Pharmacokinetic/pharmacodynamic analysis

Serial plasma samples for APL, 3TC and ZDV pharmacokinetic (PK) evaluations were collected from a subset of patients at week 12 and the concentrations of APL, 3TC and ZDV were measured by previously described liquid chromatography tandem mass spectrometry methods at GlaxoSmithKline (Research Triangle Park, NC, USA) [18,19]. The following APL, 3TC and ZDV PK parameters were estimated by standard non-compartmental methods using WinNonlin Professional v4.1 (Pharsight, Mountain View, CA, USA): maximum plasma concentrations ( $C_{max}$ ), area under the plasma concentration curve over the dosing period ( $AUC_{0-\tau}$ ) and trough concentrations ( $C_{\tau}$ ) during each dosing period. The PK parameters were summarized as geometric means with 95% confidence intervals (CI). Relationships between various PK parameters and pharmacodynamic measures (for example, HIV-1 RNA, receptor occupancy or safety measure) were assessed using simple correlation analyses.

## Results

#### Patient characteristics and disposition

Of the 145 patients randomized and treated, 83% were male, 75% were white, 80% were CDC class A and 12% were coinfecting with HBV or HCV (demographic characteristics for the total study population are shown in Table 1). The mean duration of exposure was similar across the treatment arms (~100 days or 14 weeks duration). However, a slightly longer duration of exposure was noted in the EFV arm because of the implementation of the termination of the study (that is, patients who were on the control arm were allowed more flexibility in determining the cessation of EFV than patients who were receiving APL as their randomized treatment). Similar numbers of patients had baseline plasma HIV-1 RNA  $< 100,000$  and  $\geq 100,000$  copies/ml (48% versus 52%). Median BL HIV-1 RNA and CD4<sup>+</sup> T-cell counts were well matched among the treatment arms. Of the 145 patients who received at least one dose of study medication, 115 (81%) completed  $\geq 12$  weeks prior to premature study termination. Based on the study termination date, three patients were randomized but started treatment too late to have been able to complete 12 weeks on treatment. The reasons for premature discontinuation prior to week 12 were generally similar across the treatment arms (AEs, lost to follow-up, an individual's decision and protocol-defined virological failure).

#### Virological response

In the ITT analysis at week 12, the response rates were similar with both APL dosage regimens; however, these rates were generally lower than the response rate observed with EFV (Figure 1), especially in the higher

viral load stratum (Figure 2). For the change from baseline in plasma HIV-1 RNA, a mean decline of approximately 3 log<sub>10</sub> copies/ml was seen across all treatment groups at the week 12 time point (range -2.80--3.12 log<sub>10</sub> copies/ml). However, there was greater variability in response in the two APL treatment arms relative to the control as evidenced by the range of responses observed (Table 2).

This is also illustrated in Figures 3A, B and C, which shows the change from baseline over time. From these data it appears that some individuals had little or no response to APL, whereas other patients had a rapid decline in plasma HIV-1 RNA from baseline. PK/PD analyses of the week 12 data did not correlate. In addition, tropism and resistance to study medications at BL did not appear to be an associated response.

### Immunological response

CD4<sup>+</sup> T-cell counts increased from baseline to week 12 in all treatment groups: 100 cells/mm<sup>3</sup> for APL 600 mg twice daily, 117 cells/mm<sup>3</sup> for APL 800 mg twice daily and 87 cells/mm<sup>3</sup> for EFV (Table 2).

### Safety

More patients treated with APL experienced a treatment-emergent gastrointestinal AE (45/58 [81%] and 45/58

[83%] for APL 600 mg and 800 mg twice daily, respectively) than patients in the EFV control arm (8/29, 28%). Specifically, diarrhoea, nausea, and vomiting were each more than twice as likely to be observed in the APL treatment arms. However, these events were rarely treatment limiting, although a trend toward a slightly higher discontinuation rate for gastrointestinal events was noted among APL 800 mg twice daily patients. The most frequent treatment emergent grade 2–4 AEs reported by at least five patients were diarrhoea, nausea, fatigue, vomiting and anaemia (Table 3). There were more patients with grade 2 or higher diarrhoea, nausea and vomiting in both APL arms than in the control arm.

There was a slightly higher rate of infections reported in the APL-containing arms (37/116, 31%) relative to the EFV arm (6/29, 21%), with upper respiratory tract infections (5% APL recipients versus 0% control), bronchitis and sinusitis (each 4% in APL recipients versus 0% in controls) most commonly reported (data not shown). However, the occurrence of grade 3 and 4 infections was rare in both APL (2/116 patients) and control (0/29 patients) arms.

Overall, the median changes from baseline in clinical chemistry and haematology parameters to week 12 were generally small and similar between treatment

**Table 1.** Baseline demographic and disease characteristics (intention-to-treat population)

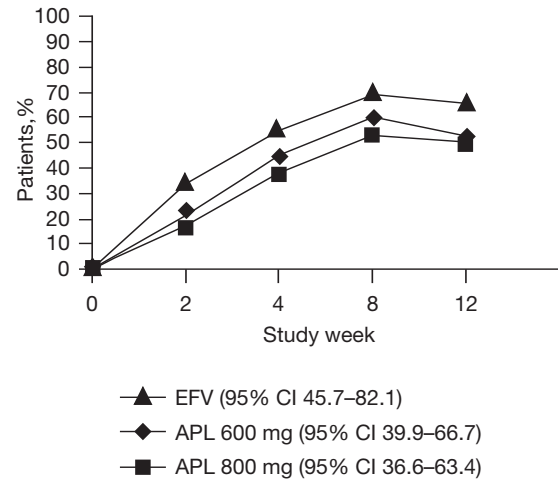
Characteristic	APL 600 mg* n=58	APL 800 mg* n=58	EFV* n=29	Total n=145
Median age, years (range)	40 (22–65)	37 (20–63)	36 (21–62)	38 (20–65)
Gender, n (%)				
Male	46 (79%)	48 (83%)	26 (90%)	120 (83%)
Female	12 (21%)	10 (17%)	3 (10%)	25 (17%)
Race, n (%)				
African American/African heritage	11 (19%)	12 (21%)	7 (24%)	30 (21%)
White	45 (78%)	43 (74%)	21 (72%)	109 (75%)
Other	3	3 (5%)	1 (3%)	7 (<1%)
Ethnicity, n (%)				
Hispanic or Latino	9 (16%)	12 (21%)	10 (34%)	31 (21%)
Not Hispanic or Latino	49 (84%)	46 (79%)	19 (66%)	114 (79%)
CDC classification, n (%)				
Class A: asymptomatic	50 (86%)	43 (74%)	23 (79%)	116 (80%)
Class B: symptomatic, no AIDS	7 (12%)	10 (17%)	2 (7%)	19 (13%)
Class C: AIDS	1 (2%)	5 (9%)	4 (14%)	10 (7%)
Baseline plasma HIV-1 RNA				
Median, log <sub>10</sub> copies/ml (range)	4.98 (3.95–6.63)	5.07 (4.00–6.29)	5.08 (3.80–6.20)	5.03 (3.80–6.63)
<100,000 copies/ml, n (%)	28 (48%)	27 (47%)	14 (48%)	69 (48%)
≥100,000 copies/ml, n (%)	30 (52%)	31 (53%)	15 (52%)	76 (52%)
Median baseline CD4 <sup>+</sup> T-cell count, cells/mm <sup>3</sup> (range)	265.0 (87–477)	227.0 (89–663)	264.0 (133–633)	256.0 (87–663)
Hepatitis B, n (%)	3 (5%)	0	2 (7%)	5 (3%)
Hepatitis C, n (%)	2 (3%)	3 (5%)	2 (7%)	7 (5%)

\*Twice daily dosage. APL, aplaviric; CDC, Centers of Disease Control and Prevention; EFV, efavirenz.

groups. Median total bilirubin increased from baseline to week 12 in both APL arms, while falling slightly in the EFV arm (data not shown). Grade 3–4 laboratory abnormalities are shown in Table 4. Although the majority of patients had no treatment-emergent clinical chemistry abnormalities, the APL clinical programme was prematurely terminated because some patients developed treatment-emergent hepatotoxicity, specifically alanine aminotransferase and/or total bilirubin increases consistent with Hy's Law [20], in ASCENT and a parallel study of APL + lopinavir/ritonavir (LPV/r) in ART-naive, HIV-infected patients. In ASCENT, one patient withdrew due to severe hepatic cytolysis after 59 days of therapy with APL 800 mg twice daily. Liver biopsy and investigations for alternative causes were negative, and thus suggestive of acute drug toxicity; the investigator attributed the hepatic cytolysis to APL. Four weeks later, the patient was asymptomatic; enzymes returned to normal 8 weeks after treatment discontinuation. Overall, there was a higher frequency of grade 2 or higher alanine aminotransferase increases (>2.5× upper limit of normal) in patients receiving APL (8/116, 7%) when compared with the EFV patients (0/29 patients); the frequency of grade 2 or higher treatment-emergent toxicity in total bilirubin also occurred more frequently in APL recipients (13/116, 11%) than in those receiving EFV (1/29, 3%). A complete analysis of these findings has been presented elsewhere [16].

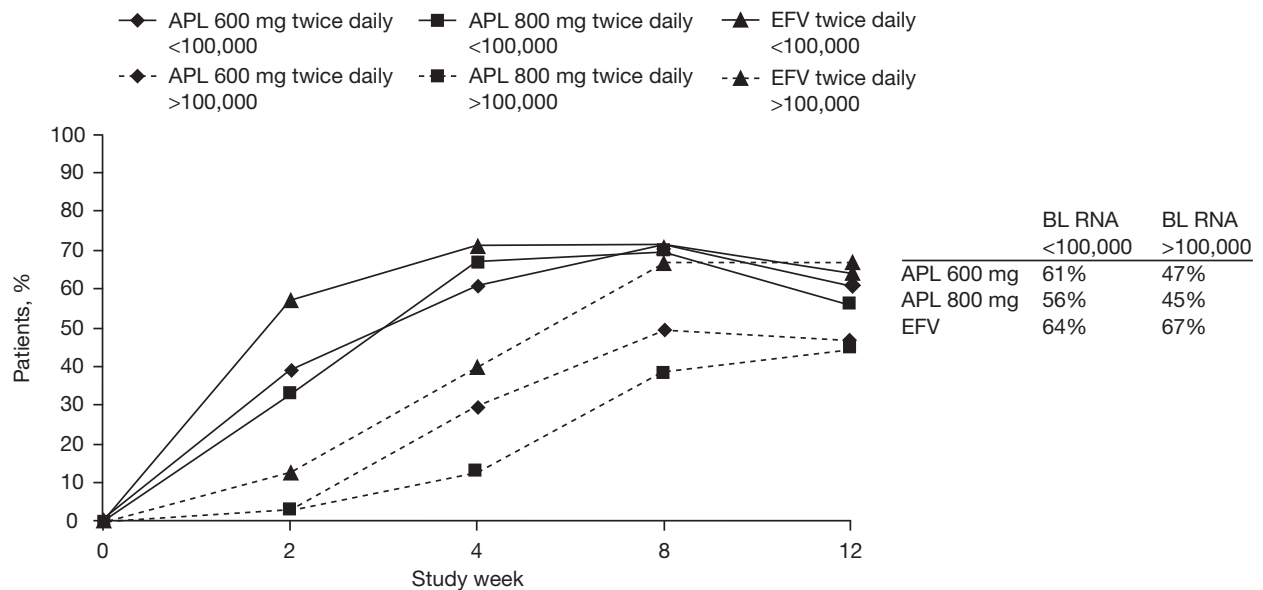
SAEs unrelated to hepatotoxicity were rarely reported in this study and none were attributed to APL. One patient on APL was diagnosed with cervical carcinoma that was judged to be unrelated to APL. The patient had vaginal bleeding before entering the study and a biopsy obtained on day 10

Figure 1. Proportion with HIV-1 RNA <400 copies/ml



At week 12, 66%, 53% and 50% of efavirenz (EFV), aplaviroc (APL) 600 mg and APL 800 mg patients, respectively, had <400 copies/ml HIV RNA. CI, confidence interval.

Figure 2. Proportion with HIV-1 RNA <400 copies/ml by strata (intention-to-treat population)



	BL RNA <100,000	BL RNA >100,000
APL 600 mg	61%	47%
APL 800 mg	56%	45%
EFV	64%	67%

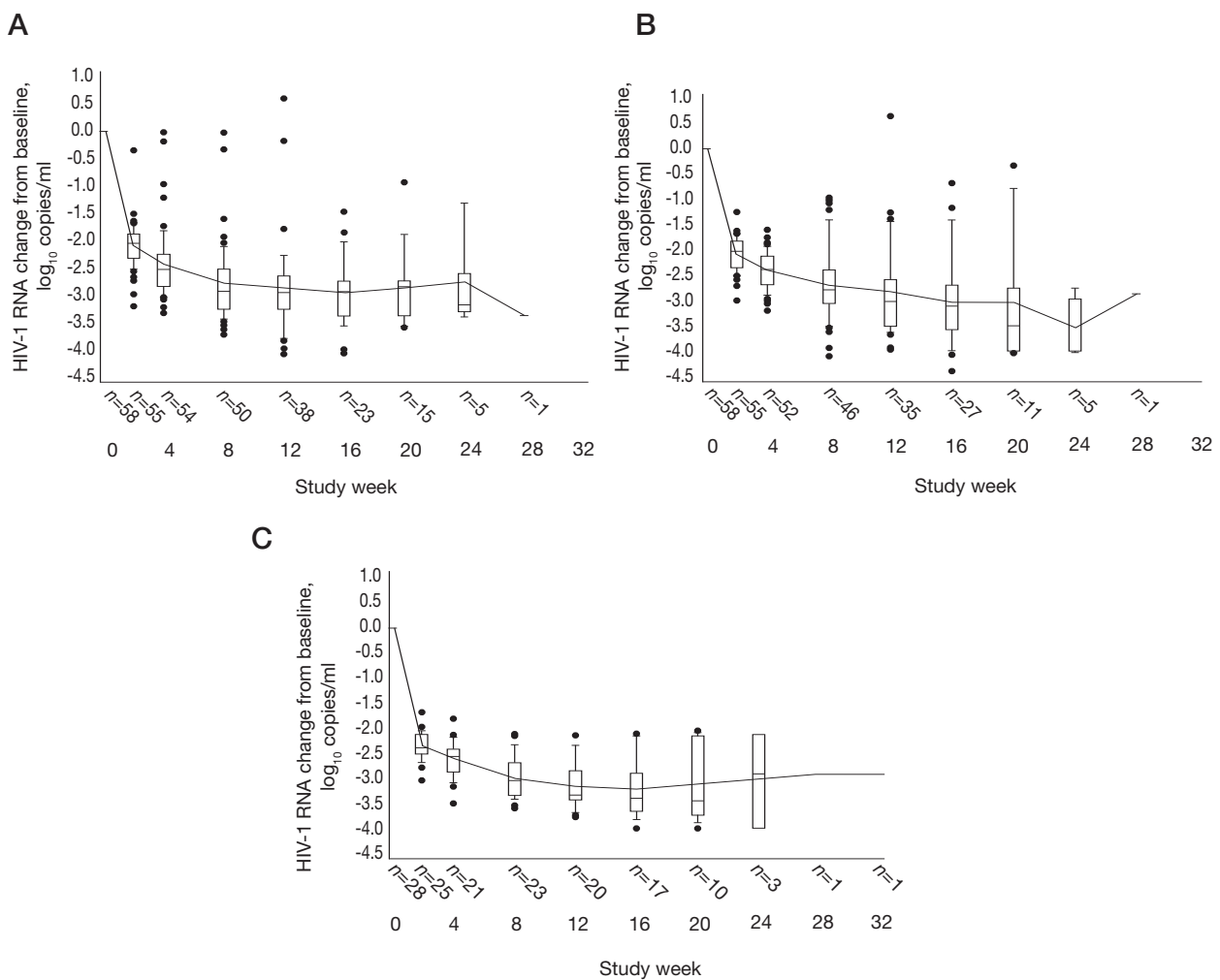
The table highlights the proportion of patients that had <400 copies/ml HIV RNA at week 12. APL, aplaviroc; BL, baseline; EFV, efavirenz.

**Table 2.** Week 12 HIV-1 RNA and CD4<sup>+</sup> T-cell change from baseline

	APL 600 mg twice daily (n=38)	APL 800 mg twice daily (n=35)	EFV twice daily (n=20)
RNA change, log <sub>10</sub> copies/ml			
Mean (sd)	-2.85 (0.881)*	-2.80 (0.937) <sup>†</sup>	-3.12 (0.483) <sup>‡</sup>
Median (range)	-2.94 (-4.06-0.60)*	-3.00 (-3.94-0.63) <sup>†</sup>	-3.31 (-3.73--2.11) <sup>‡</sup>
CD4 <sup>+</sup> T-cell change, cells/cm <sup>3</sup>			
Median (range)	100 (-122-617) <sup>§</sup>	117 (-39-465) <sup>§</sup>	87 (-12-257) <sup>‡</sup>

\*n=38, <sup>†</sup>n=35, <sup>‡</sup>n=20 and <sup>§</sup>n=37. APL, aplaviroc; EFV, efavirenz.

**Figure 3.** Plasma HIV-1 RNA change from baseline (intention-to-treat population)



(A) Aplaviroc (APL) 600 mg twice daily. (B) APL 800 mg twice daily. (C) Efavirenz twice daily.

confirmed the diagnosis. One patient died during this study (APL 800 mg twice daily) from Burkitt's lymphoma, which was deemed by the investigator to be non-study drug related.

**Pharmacokinetics/pharmacodynamics**

APL demonstrated non-linear PKs with greater than dose-proportional increases in the week 12 APL AUC<sub>0-τ</sub> and C<sub>max</sub> values (Table 5). APL PK parameters

**Table 3.** Most common grade 2–4 adverse events reported in >10% of patients (treatment phase, safety population)

	APL 600 mg twice daily n=58	APL 800 mg twice daily n=58	EFV twice daily n=29
Diarrhoea	38 (66%)	39 (67%)	2 (7%)
Nausea	31 (53%)	31 (53%)	7 (24%)
Fatigue	15 (26%)	13 (22%)	8 (28%)
Headache	18 (31%)	9 (16%)	4 (14%)
Dizziness	6 (10%)	7 (12%)	8 (28%)
Vomiting	9 (16%)	7 (12%)	1 (3%)
Rash	3 (5%)	4 (7%)	8 (28%)
Abdominal pain	3 (5%)	9 (16%)	2 (7%)
Anaemia	7 (12%)	2 (3%)	1 (3%)
Vertigo	3 (5%)	0	3 (10%)

All results are presented as n (%). APL, aplaviroc; EFV, efavirenz.

**Table 4.** Treatment-emergent grade 3–4 laboratory abnormalities reported by >2% patients (treatment phase, safety population)

	APL 600 mg twice daily		APL 800 mg twice daily		EFV twice daily	
	3*	4*	3*	4*	3*	4*
<b>Clinical chemistry</b>						
Increased ALT	0	0	1 (2%)	1 (2%)	0	0
Increased AST	0	0	1 (2%)	1 (2%)	0	0
Hyperglycaemia	1 (2%)	0	2 (3%)	0	0	0
Increased lipase	0	1 (2%)	0	1 (2%)	0	0
Hyperbilirubinaemia	4 (7%)	1 (2%)	0	2 (3%)	0	0
Hypercholesterolaemia	0	0	0	0	1 (3%)	0
Increased creatine kinase	1 (2%)	0	0	2 (3%)	1 (3%)	0
<b>Haematology</b>						
Neutropenia	5 (9%)	1 (2%)	2 (3%)	1 (2%)	1 (3%)	1 (3%)
Decreased haemoglobin	0	2 (3%)	0	1 (2%)	1 (3%)	0
Decreased total WBC	0	0	0	0	1 (3%)	0
Decreased platelets	0	0	0	0	0	0

All results are displayed as n (%). \*Maximum grade on treatment. ALT, alanine aminotransferase; AST, aspartate aminotransferase; APL, aplaviroc; EFV, efavirenz; WBC, white blood cells.

for the 600 mg twice daily regimen were similar to those previously observed in the 10-day monotherapy study of HIV-infected patients receiving the same APL dosage regimen [11]. The geometric mean plasma APL  $AUC_{0-\tau}$  values of 1,184 and 2,733 ng.h/ml for the 600 mg and 800 mg twice daily dose groups, respectively, both exceeded the antiviral target AUC. High interpatient variability was noted, especially for the APL 800 mg twice daily regimen (coefficient of variation range 124–174% for  $C_{max}$ ,  $C_{\tau}$  and  $AUC_{0-\tau}$ ). Overall, within a given treatment group, APL PK parameters appeared similar between the two baseline HIV-1 RNA strata and the geometric mean plasma 3TC and ZDV  $AUC_{0-\tau}$  values were consistent with previously reported values (data not shown). Although APL PK was characterized by high interpatient variability, no consistent relationships between APL  $AUC_{0-\tau}$ ,  $C_{max}$  or  $C_{\tau}$  and measures of

antiviral response were detected in the subset of patients who provided week 12 serial PK samples.

### Resistance

Although the study was prematurely terminated, protocol-defined virological failure was relatively infrequent (8/145 patients or 6%). An in-depth, detailed population and clonal analysis of the virological failures from ASCENT will be presented in a forthcoming manuscript (J Demarest *et al.*, personal communication); however, population level results will be briefly summarized here. Although the overall efficacy between APL-containing groups was comparable, the relative rate of protocol-defined virological failure was higher in the 600 mg APL group (6/58 [10%] patients in the 600 mg APL twice daily group versus 2/58 [3%] patients in the 800 mg APL twice daily

**Table 5.** Pharmacokinetics of aplaviroc at week 12 (intensive sampling subset)

Parameter	APL 600 mg twice daily ( <i>n</i> =15)	APL 800 mg twice daily ( <i>n</i> =18)
<b>AUC<sub>0-τ</sub></b>		
Geometric mean, ng.h/ml	1,184	2,733
95% Confidence interval, ng.h/ml	923–1,517	1,468–5,088
Coefficient of variance of baseline, %	47.2	137
<b>C<sub>max</sub>, ng/ml</b>		
Geometric mean, ng.h/ml	314	853
95% Confidence interval, ng.h/ml	240–411	418–1,740
Coefficient of variance of baseline, %	51.6	174
<b>C<sub>τ</sub></b>		
Geometric mean, ng.h/ml	11.8	16.9*
95% Confidence interval, ng.h/ml	8.01–17.5	9.16–31.3
Coefficient of variance of baseline, %	80.5	124

\**n*=12. AUC<sub>0-τ</sub>, area under the plasma concentration curve over the dosing period; C<sub>max</sub>, maximum plasma concentration; C<sub>τ</sub>, trough concentration.

group). No protocol-defined virological failures were observed in the control group.

The primary characteristic of virological failure in this study was the detection of the M184V mutation in 6/8 patients. Reduced susceptibility (>threefold change in median inhibitory concentration) to APL at the population level was not observed in any of the eight patients with protocol-defined virological failure. Two of these eight patients had dual/mixed-tropic virus detected at the time of virological failure; both also harboured the M184V mutation. Tropism readout changes were also detected in a minority of patients in the absence of therapy as well as a minority of responders on randomized treatment; more detail about the observed tropism readout changes will be presented in a forthcoming manuscript (HA Madsen *et al.*, unpublished observations).

## Discussion

Although the combination of two NRTIs and a third agent (NNRTI or PI) represents the regimen of choice for many HIV-infected patients who are naive to anti-retroviral therapy, the response rates observed in this study, while similar between the APL dosage regimens, demonstrated a moderately diminished response relative to EFV overall, especially in the higher viral load stratum. These results were not statistically significant. The study was not designed to evaluate formal hypothesis tests overall or in the high viral load stratum. The original intent of the study was to estimate dose response for the two APL doses. The control group was included as a contemporary cohort for the purposes of estimating relative response and potentially designing future trials to evaluate APL-containing regimens. The observed results suggest a trend toward diminished response for APL regimens,

although the results do not reach a definitive level of statistical significance.

While target plasma concentrations of APL were achieved, the antiviral activity of APL as the third agent in a triple drug regimen did not appear to be comparable to EFV. The reason for the diminished response over 12 weeks in this study are unclear, and are not consistent with the excellent antiviral activity demonstrated with short-term APL monotherapy. Though APL demonstrated non-linear PKs with high interpatient variability, no consistent relationships between APL AUC<sub>0-τ</sub>, C<sub>max</sub> or C<sub>τ</sub> and measures of antiviral response were detected in the subset of patients who participated in week 12 intensive PKs. This could be because plasma concentrations consistent with maximal response were achieved in the vast majority of patients; indeed, AUC<sub>0-24 h</sub> values met or exceeded the target AUC<sub>0-24 h</sub> of 1,900 ng.h/ml determined in the 10-day monotherapy study [11]. PK parameters for COM were also consistent with previously reported values. The high interpatient variability may be attributed to genetic variation in several drug-metabolizing enzymes and transporters as suggested by a previous pharmacogenetic analysis of APL PKs in healthy patients [21], as well as interindividual differences. Finally, baseline virological characteristics, such as tropism and pre-existing resistance to study medications, also did not appear to correlate with the lack of virological response in some patients. The major characteristic of viruses at the time of confirmed virological failure was the detection of genotypic and phenotypic resistance to 3TC, which was detected in 6/8 virological failures. These results are consistent with what has been observed from treatment-naive patients failing a regimen of COM and vicriviroc, another CCR5 entry inhibitor in development. In the vicriviroc study, M184V/I was detected in all 22 patients successfully



genotyped [22]. Overall, these data are consistent with the hypothesis that ART first selects pre-existing mutants that confer the greatest benefit to the virus with the fewest mutations (that is, the lowest genetic barrier).

The safety profile of CCR5 antagonists has been the subject of intense scrutiny. As discussed elsewhere [16], the idiosyncratic hepatotoxicity associated with APL appears to be a compound-related (rather than mechanism-related) event. If hepatotoxicity were a class effect, one would also expect hepatotoxicity to have occurred in other trials of CCR5 antagonists. Although confounded by several other factors, only a single case of serious hepatotoxicity has been observed in a patient in the treatment-naïve study of maraviroc [23]. Likewise, differences in liver enzyme levels were not reported in Phase II studies of vicriviroc. In the case of APL, the relatively low proportion of affected individuals among those treated is consistent with idiosyncratic drug hepatotoxicity. This observation suggests one of two possibilities – APL toxicity may only occur in some patients with an as-yet undefined cofactor, consistent with idiosyncratic hepatotoxicity, or the treatment duration was not sufficient to allow hepatotoxicity to emerge in the majority of APL-treated patients. It appears clear that hepatitis B or C coinfection and/or concomitant medications cannot explain our findings in isolation, particularly since the index case (with the most severe hepatic cytolysis) had no such confounding factors. Genetic predictors of toxicity are currently undergoing investigation, and will be the subject of a separate report.

On the other hand, the data from this study are reassuring with regards to mechanism-based toxicity with CCR5 antagonists, which as a class could theoretically impair immune response or surveillance in this patient population. Although infections appeared to occur at a slightly higher rate in APL-treated than control patients, these findings appeared to be driven by low-grade, community-acquired infections rather than more severe and/or HIV-associated conditions. In addition, treatment-emergent malignancies did not emerge on this study, in contrast to the equivocal signal with the CCR5 antagonist vicriviroc [24]. However, because exposure to APL was relatively short-term in this study, definitive conclusions cannot be drawn from these data.

In conclusion, although not powered to show significant differences between treatments, this study demonstrated a reduced virological response in ART-naïve R5-tropic patients treated with APL when compared with EFV. The occurrence of idiosyncratic hepatotoxicity in several APL-treated patients resulted in premature closure of this study, and ultimately led to the termination of APL clinical development.

## Acknowledgements

Data presented in part at the 10th European AIDS Conference, Dublin, Ireland, 17–20 November 2005.

This study was financially supported by GlaxoSmithKline. The authors would like to thank the study participants who gave their informed consent, the study coordinators, the GlaxoSmithKline staff and the clinical investigators (name of individual investigators can be found in an additional file) who participated in this study.

## Disclosure statement

The authors declare that they have no competing interests.

## Additional file

The additional file, which contains the name of the clinical investigators who participated in this study, can be accessed via the Volume 13 Issue 2 contents page for *Antiviral Therapy*, which can be found at [www.intmedpress.com](http://www.intmedpress.com) (by clicking on ‘Antiviral Therapy’ and then ‘Journal PDFs’)

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Accepted for publication 19 November 2007