

## Short communication

# Amdoxovir versus placebo with enfuvirtide plus optimized background therapy for HIV-1-infected subjects failing current therapy (AACTG A5118)

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**Background:** Amdoxovir (2,6-diaminopurine dioxolane; DAPD) is a nucleoside reverse transcriptase inhibitor (NRTI) of human immunodeficiency virus-1 (HIV-1) with activity against wild-type and NRTI-resistant viruses.

**Methods:** ACTG A5118 assessed the antiretroviral activity and safety of DAPD (300 mg orally, twice daily) versus placebo in combination with enfuvirtide (ENF) plus an optimized background (OB) regimen in subjects with failure of two or more antiretroviral (ARV) regimens. The primary endpoints for comparison were time-averaged area under the curve minus baseline (AAUCMB) of plasma HIV-1 RNA concentration at 24 weeks and time to first serious (DAIDS toxicity table Grade  $\geq 3$ ) adverse event (AE). An unplanned interim review recommended closing enrollment because the study was unlikely to demonstrate a difference between arms. The 18 subjects

on study, nine in each arm, were unblinded and allowed to continue study treatment through 48 weeks.

**Results:** Intention-to-treat analysis showed the median AAUCMB was  $-0.9 \log_{10}$  copies/mL (95% CI =  $-2.2, -0.1$ ) in the DAPD arm and  $-0.9 \log_{10}$  copies/mL (95% CI =  $-1.1, -0.1$ ) in the placebo arm ( $P=0.69$ ). Median CD4<sup>+</sup> T-cell increase was 79 cells/mm<sup>3</sup> (95% CI = 1, 115) in the DAPD arm and 60 (95% CI = 1, 101) in the placebo arm ( $P=0.45$ ). Time to first serious AE did not differ between arms ( $P=0.91$ ). Mild decreases of creatinine clearance were observed with similar frequency between arms; no subject developed lens opacities.

**Conclusions:** Addition of DAPD to ENF plus OB in advanced subjects with highly resistant virus appeared safe, but did not add statistically significant antiretroviral activity at 24 weeks in this small study.

## Introduction

Amdoxovir (2,6-diaminopurine dioxolane; DAPD) is a guanosine analogue that retains *in vitro* activity against HIV-1 isolates resistant to other nucleoside reverse transcriptase inhibitors (NRTIs) [1]. The presence of Q151M, K65R or L74V reduces susceptibility to DAPD [1–3]. AIDS Clinical Trials Group (ACTG) protocol A5118 evaluated the antiretroviral activity and safety of DAPD with an optimized background (OB) regimen containing enfuvirtide (ENF) in triple class-experienced individuals who had not received ENF before.

## Methods

### Design

A5118 was a multicentre, randomized, double-blind, pilot study of DAPD or placebo in combination with ENF plus an OB regimen. Primary objectives were to compare the antiviral activity of the DAPD- and placebo-containing arms, and to explore the safety and tolerability of DAPD. The OB regimen was selected based on treatment history and genotypic (TruGene version 8.0; Visible Genetics, Bayer HealthCare, Berkeley, CA, USA) and phenotypic (Phenosense; Virologic, Inc., San Francisco, CA, USA) resistance

testing. Individuals were randomized to DAPD (Gilead Sciences, Foster City, CA, USA) 300 mg or matching placebo (both given orally as two 150 mg capsules twice daily) plus ENF (Trimeris, Morrisville, NC, USA) 90 mg subcutaneously twice daily plus OB therapy of three to five approved antiretroviral agents (ARVs). Ritonavir (RTV) at a dose of 200 mg twice daily or less was not counted as an ARV.

### Individuals

Eligible individuals were at least 18 years old and had been on stable, failing ARV regimens for at least 8 weeks, with plasma HIV-1 RNA levels above 5,000 copies/ml. Individuals must have failed at least two regimens containing three or more drugs, including at least two NRTIs, two protease inhibitors (PIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). Individuals must have received ARV regimens for at least 24 months, had no prior DAPD or ENF exposure, a calculated creatinine clearance (Cockcroft-Gault [4]) greater than 80 ml/min, no significant lens opacities, and absence of the K65R or Q151M mutations in HIV-1 by genotype.

### Endpoints

Primary study endpoints were time-averaged area under the curve minus baseline (AAUCMB) in plasma HIV-1 RNA copies/ml from baseline to week 24 and time to first Grade 3 or worse sign, symptom or laboratory abnormality [5] at least one grade higher than baseline. Secondary endpoints included the proportion of individuals with plasma HIV-1 RNA below 200 copies/ml at week 24, and change in plasma HIV-1 RNA level and CD4<sup>+</sup> T-cell count from baseline to week 24.

### Enrollment and monitoring

Individuals were assessed at screening, pre-entry, entry, weeks 1, 2, 4, 8 and every 4 weeks thereafter through to week 48. Ophthalmological examinations were performed at screening and at weeks 8, 16, 24, 32, 40 and 48 to assess presence or absence of lenticular opacities using the Lens Opacities Classification System III [6]. The protocol was approved by local institutional review boards; written informed consent was obtained from all individuals.

When only 18 individuals enrolled over 32 weeks and Gilead Sciences communicated that it was releasing rights to DAPD, an unplanned interim analysis was requested by the study team in January 2004. That review found no safety concerns, but noted that even with complete enrollment the study was unlikely to demonstrate a statistically significant difference between the two arms. The review committee recommended closing the study to accrual, unblinding

individuals and allowing individuals the option to continue study treatment through to week 48.

### Statistical analysis

The primary efficacy endpoint (AAUCMB to week 24) was compared across study arms using a Wilcoxon rank sum test. Assuming the standard deviation of the week 24 AAUCMB was between 0.5 and 0.8, a sample size of 25 individuals per treatment arm was expected to give 80% power to detect a difference of 1 standard deviation. The AAUCMB was calculated by the trapezoidal rule on the log<sub>10</sub> scale using data up to week 24. HIV-1 RNA values below the limit of detection were imputed at 50 copies/ml. The secondary endpoints of change in HIV-1 RNA and CD4<sup>+</sup> T-cell count from baseline to the average of the week 20 and 24 evaluations were compared across arms using a Wilcoxon rank sum test. The proportions of individuals with HIV-1 RNA below 200 copies/ml were compared using a Fisher exact test.

Time to first Grade 3 or worse symptom or toxicity was compared by an exact log rank test. Only toxicities occurring while an individual was taking study treatment or within 8 weeks of study treatment discontinuation were included. All *P* values were two-sided.

## Results

### Baseline characteristics of individuals

Between 23 May and 18 November 2003, 18 individuals were enrolled in the study. Table 1 shows the individuals' demographics and baseline characteristics. Most individuals had advanced HIV-1 disease and extensive ARV resistance at baseline. Baseline samples from four individuals in the DAPD arm and two in the placebo arm had L74V. Genotypic sensitivity scores (GSS) were calculated by adding the number of active drugs in the OB regimen (excluding ENF and DAPD) as determined by the baseline genotypic resistance assay (no resistance =1, possible resistance =0.5, resistant =0, with RTV-boosting not a separate drug). Median GSS was 0.5 (range 0–3); only five individuals had a GSS above 1. There were no statistically significant differences in any of the baseline characteristics between the two arms.

### Study follow-up

Thirteen out of the 18 individuals (six on the DAPD arm and seven on the placebo arm) completed 48 weeks of follow-up. Two individuals (one from each arm) died after 39 weeks on study but off study drugs. Neither cause of death (pneumonia or cytomegalovirus encephalitis) was considered related

Table 1. Baseline characteristics

	Treatment arm		
	All subjects	DAPD (n=9)	Placebo (n=9)
Sex, n (%)			
Male	15 (83%)	7 (78%)	8 (89%)
Female	3 (17%)	2 (22%)	1 (11%)
Age, years, mean (sd)	43.8 (8.9)	41.7 (10.5)	45.9 (6.8)
Race/ethnicity			
White non-Hispanic, n (%)	11 (61%)	4 (44%)	7 (78%)
Black non-Hispanic, n (%)	2 (11%)	1 (11%)	1 (11%)
Hispanic (regardless of race), n (%)	5 (28%)	4 (44%)	1 (11%)
HIV-1 RNA, log <sub>10</sub> copies/ml, median (Q1, Q3)	4.8 (4.5, 5.5)	4.7 (4.3, 5.4)	5.3 (4.7, 5.7)
CD4 <sup>+</sup> T-cell count, cells/mm <sup>3</sup> , median (Q1, Q3)	36 (18, 123)	72 (15, 163)	25 (18, 79)
Number of NRTI mutations, median (Q1, Q3)	6 (4, 7)	6 (1, 8)	7 (5, 7)
Number of NNRTI mutations, median (Q1, Q3)	1 (0, 2)	1 (0, 2)	1 (1, 2)
Number of PI mutations, median (Q1, Q3)	8 (7, 10)	7 (5, 10)	9 (8, 10)
Antiretroviral drugs in OB			
Mean number of drugs	3.3	3.2	3.3
Frequency of use, n (%)			
PI (boosted)	14 (78%)	5 (55%)	9 (100%)
NNRTI	4 (22%)	2 (22%)	2 (22%)
NRTI only	3 (17%)	3 (33%)	0 (0%)
Tenofovir	18 (100%)	9 (100%)	9 (100%)
Didanosine	7 (39%)	3 (33%)	4 (44%)
OB GSS, n (%)			
0.0	7 (39%)	3 (33%)	4 (44%)
0.5	4 (22%)	2 (22%)	2 (22%)
1.0	2 (11%)	1 (11%)	1 (11%)
1.5	2 (11%)	0 (0%)	2 (22%)
2.0	1 (6%)	1 (11%)	0 (0%)
3.0	2 (11%)	2 (22%)	0 (0%)
OB GSS (</>1), n (%)			
<1	11 (61%)	5 (56%)	6 (67%)
1+	7 (39%)	4 (44%)	3 (33%)

DAPD, 2,6-diaminopurine dioxolane (amdoxovir); GSS, genotypic sensitivity score; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OB, optimized background; PI, protease inhibitor; Q, quartile; sd, standard deviation.

to study treatment. Four individuals (two from each arm) discontinued study drugs prior to week 24. All individuals remained blinded to treatment assignment through week 24.

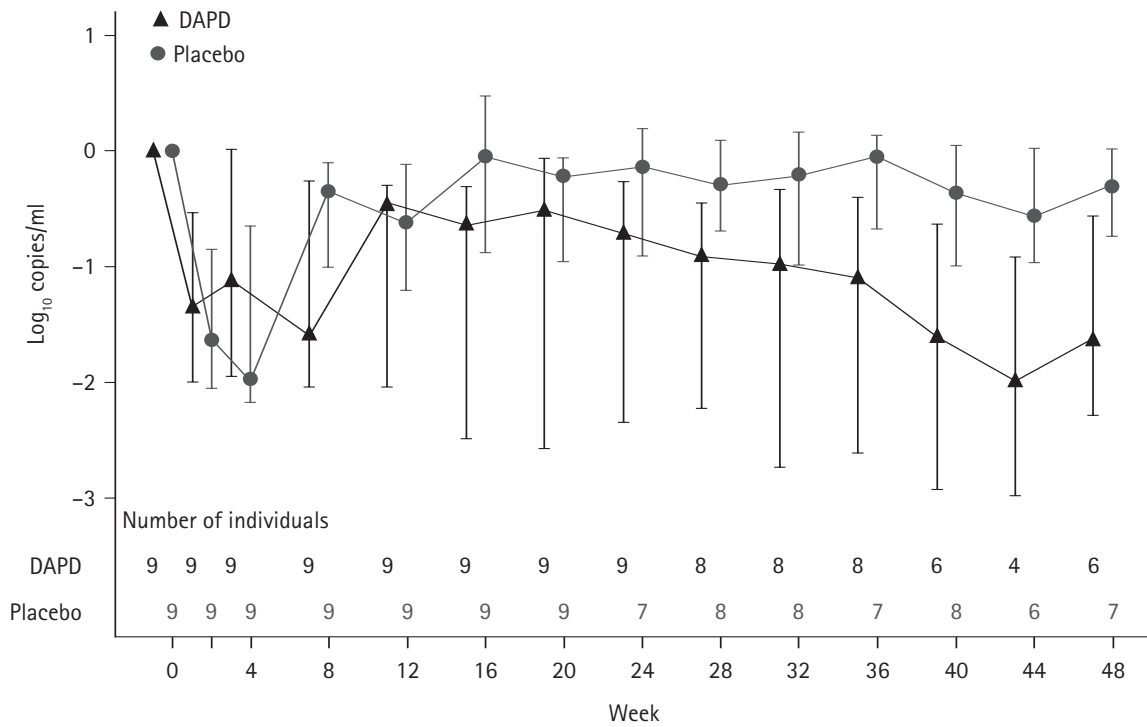
### Efficacy

Figure 1 shows the median change from baseline in plasma HIV-1 RNA over time by treatment arm (intent-to-treat). There was no difference between treatment arms in the AAUCMB (median  $-0.9$  log<sub>10</sub> copies/ml in each group,  $P=0.69$ ) or in change in median HIV-1 RNA from baseline at week 24 ( $-0.4$  log<sub>10</sub> copies/ml versus  $-0.1$  log<sub>10</sub> copies/ml,  $P=0.35$ ) or number of individuals with HIV-1 RNA  $\leq 200$  copies/ml (three in DAPD versus one in placebo,  $P=0.58$ ). A *post-hoc* analysis suggested that individuals

with the best responses tended to have a higher GSS: the 11 individuals with a GSS less than 1 had a median week 24 AAUCMB of  $-0.5$  log<sub>10</sub> copies/ml (95% confidence interval (CI) =  $-0.9, -0.1$ ) whereas individuals with a GSS of 1 or more had  $-2.1$  log<sub>10</sub> copies/ml (95% CI =  $-3.4, 1.2$ ;  $P=0.11$ ). The four individuals in the DAPD arm with L74V at baseline tended to have a poorer response than those without (median AAUCMB  $-0.62$  versus  $-2.1$ ); these individuals also had the four lowest genotypic sensitivity scores (median 0.25).

Comparable median increases in CD4<sup>+</sup> T-cell count occurred in both arms. At week 24 (intent-to-treat) the median increase from baseline was 79 (95% CI = 21, 372) cells/mm<sup>3</sup> in the DAPD arm versus 60 (95% CI = 1, 101) cells/mm<sup>3</sup> in the placebo arm ( $P=0.45$ ).

Figure 1. Median (1st and 3rd quartile) HIV-1 RNA change (log<sub>10</sub> copies/ml) over time by treatment arm (intent-to-treat)



DAPD, 2,6-diaminopurine dioxolane (amdoxovir).

### Safety

Table 2 shows the adverse events that occurred during the median 48-week study follow-up period. Few Grade 3 or worse signs or symptoms were reported, with no difference in time to first Grade 3 or worse adverse event between treatment arms ( $P=0.9$ ).

Because of toxicities observed in prior studies of DAPD conducted in animals (unpublished data, Gilead), fasting glucose, creatinine clearance below 80 ml/min and quantitative changes in lens opacity were targeted for review. No individuals developed fasting hyperglycaemia or an increase in lens opacity score. Six individuals (two in the DAPD arm and four in the placebo arm) experienced a decrease in creatinine clearance to 50–80 ml/min while on study treatment. Study treatment was temporarily suspended in two individuals (one in each arm) due to decreased creatinine clearance, with subsequent normalization of the creatinine clearance in both cases.

HIV-related morbidities were frequent in this highly drug-resistant population, and were evenly distributed across both arms: 44% (8/18) of individuals developed a serious infection or malignancy during the 48-week follow-up (Table 2). All but one occurred in individuals without a good virological response.

### Discussion

This pilot study closed to accrual early and therefore was no longer powered to detect a difference in efficacy between arms. Although the virological response at 24 weeks was not significantly different in the DAPD and placebo arms, addition of DAPD to ENF plus OB did appear to be safe. Obstructive nephropathy, hyperglycaemia and lenticular opacities observed at high DAPD doses in monkey studies (unpublished data, Gilead) were not observed in our study. The small number of individuals who experienced a decreased creatinine clearance was not different between DAPD and placebo.

Study individuals harboured highly resistant strains of HIV-1, as demonstrated by the baseline median GSS of 0.5. Nevertheless, individuals in both arms had an approximately 1-log<sub>10</sub> drop in HIV-1 RNA at 24 weeks, and a doubling of their CD4<sup>+</sup> T-cells per mm<sup>3</sup>, a response similar to that seen among ENF recipients in the TORO studies [7,8]. Whereas L74V has been associated with moderately decreased *in vitro* susceptibility of HIV-1 to DAPD in some [2,3] but not all [1] studies, the small number of individuals in this study does not allow us to assess the impact of L74V on response to DAPD.

In conclusion, this small pilot study did not demonstrate benefit from the addition of DAPD to ENF plus

Table 2. Safety

Adverse events	DAPD (n=9)	Placebo (n=9)
DAIDS Grade 3 or 4 events		
ENF injection site reaction	3	2
Headache and fatigue	1	0
Fever	0	3
Bilirubin	1	3
Fasting triglycerides	1	1
Lipase	1	0
Other adverse events		
Creatinine clearance 50–80 ml/min	3	4
Increase in lens opacity score (LOCS III)	0	0
Subjects with a serious clinical event		
<i>Pneumocystis pneumonia</i>	2	1
CMV retinitis	1*	1 <sup>‡</sup>
<i>Mycobacterium avium</i> complex	1*	
Squamous cell carcinoma	1 (anus)*	1 (skin)
Bacterial pneumonia	1 (died) <sup>†</sup>	1
HSV encephalitis	1	

\*These three events occurred in one subject. <sup>†</sup>This subject also had *Pneumocystis jirovecii* pneumonia. <sup>‡</sup>Later died with cytomegalovirus (CMV) encephalitis. DAPD, 2,6-diaminopurine dioxolane (amdoxovir); ENF, enfuvirtide; HSV, herpes simplex virus.

an OB regimen in individuals with advanced HIV disease and highly resistant virus, but DAPD at 300 mg twice-daily appeared to be safe.

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