

The use of β -D-2,6-diaminopurine dioxolane with or without mycophenolate mofetil in drug-resistant HIV infection

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Objective: We evaluated the safety, tolerability and antiretroviral activity of β -D-2,6-diaminopurine dioxolane (DAPD; amdoxovir) with or without mycophenolate mofetil (MMF) in HIV-1 infection following extensive antiretroviral therapy (ART).

Methods: Oral DAPD 500 mg twice daily with placebo or MMF 500 mg twice daily was added to failing ART. HIV-1 RNA viral load (VL) decline to week 2 was analyzed by intent-to-treat, using rank-based tests. Patients with VL decline $> 0.5 \log_{10}$ copies/ml at week 2 (virologic response, VR) optimized ART and continued therapy for up to 96 weeks.

Results: Forty adults with median VL $4.5 \log_{10}$ copies/ml, median 184 CD4⁺ cells/ μ l, and a median of 6 nucleoside reverse transcriptase inhibitor (NRTI) mutations (range, 1–8) were randomized. Median VL reduction at week 2 was $-0.26 \log_{10}$ copies/ml ($P < 0.0001$). Response to DAPD/placebo (median $-0.37 \log_{10}$ copies/ml) was unexpectedly greater than to DAPD/MMF (median $-0.23 \log_{10}$ copies/ml), although this difference was not statistically significant ($P = 0.59$). MMF appeared to lower concentrations of DAPD and its metabolite dioxolane guanosine. Of 10 patients with VR (DAPD 7, DAPD/MMF 3), four persisted beyond week 24. VR was more frequent with ≤ 5 baseline NRTI mutations ($P = 0.12$) or < 4 thymidine-associated mutations (TAMs) without E44D or V118I ($P = 0.08$). Twenty-three patients received extended DAPD +/- MMF; five beyond week 24. Few adverse events were related to study medications.

Conclusions: The addition of DAPD +/- MMF to failing therapy appears safe and well tolerated. DAPD had significant activity at week 2 (mean $-0.35 \log_{10}$) in heavily pretreated patients that was not augmented by MMF.

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Introduction

The evolution of HIV-1 in the face of antiretroviral therapy requires continuous efforts to develop new agents with activity against drug-resistant HIV-1. β -D-2,6-diaminopurine dioxolane (DAPD; amdoxovir) is a purine

nucleoside analogue with in-vitro antiviral activity against HIV-1, HIV-2, and hepatitis B virus. DAPD is deaminated by adenosine deaminase to its 2'-deoxyguanosine analog, dioxolane guanosine (DXG). Thence DXG is phosphorylated by cellular kinases to DXG-triphosphate (DXG-TP), the active metabolite, which

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acts as a chain terminator. DXG has potent in-vitro activity against wild-type and zidovudine (ZDV)/lamivudine (3TC) and/or stavudine (D4T)/3TC-resistant HIV-1 variants and those with the multi-nucleoside reverse transcriptase inhibitor (NRTI) resistance insert at codon 69 [1].

The emergence of HIV-1 resistance to DXG has been examined *in vitro* in lymphocytic cell lines. Resistance develops slowly, with prolonged exposure to increasing concentrations of DXG in MT-2 cells. One of two mutations (K65R or L74V) within the reverse transcriptase (RT) gene is selected, conferring low-level (three to sixfold) phenotypic resistance to DXG [2]. Clinical HIV-1 isolates obtained from patients who had failed ZDV, 3TC, and/or non-nucleoside reverse transcriptase inhibitor (NNRTI) therapy remained DXG-sensitive. DXG has synergistic in-vitro anti-HIV activity when used in combination with a variety of other antiretroviral agents, but has little activity against recombinant variants with the 65R/151M double mutation [3,4].

An open-label, phase I study of DAPD conducted in HIV-infected, treatment-naïve and treatment-experienced patients explored the antiviral activity of DAPD monotherapy and add-on DAPD therapy over 15 days. Treatment-experienced patients with a median time on antiretroviral therapy (ART) of 4 years and exposure to a median of seven ART drugs had a median reduction in viral load (VL) of 0.66 log₁₀ copies/ml with DAPD dosed at 500 mg twice daily. Therapy-naïve patients receiving DAPD at 500 mg twice daily experienced a median 1.3 log₁₀ VL reduction [5].

The major toxicity of DAPD observed in animal toxicology studies was obstructive nephropathy at high doses, secondary to the precipitation of DAPD/DXG due to the poor aqueous solubility of DXG. In a 52 week study, cynomolgus monkeys, receiving DAPD 800 or 1200 mg/kg per day, developed obstructive nephropathy and resultant uremia; in five monkeys, after 26 weeks of treatment, islet cell atrophy and hyperglycemia occurred, and lens opacities were observed which were thought to be secondary to hyperglycemia, and not a direct effect of DAPD [6]. All doses of DAPD tested have been well tolerated in five human phase I/II studies [5–7]. In one study of 18 patients, four patients from the same study site were withdrawn from study when nongradable opacities in the lens were discovered between weeks 6.8 and 15 of DAPD 300 mg twice daily or 500 mg twice daily administration, and one patient with a mild posterior subcapsular cataract was found at week 3.6. It is not known if these findings were present at study entry, since ophthalmologic examinations were added mid-study. None of the five patients had renal or glucose abnormalities and, for all, the lens abnormalities were visually insignificant [6]. The other thirteen participants in that study did not have lens findings with DAPD

administration for up to 96 weeks and no individual receiving DAPD 300 mg twice daily (*n* = 9) for up to 48 weeks in AIDS Clinical Trials Group study A5118 had lens abnormalities [7].

Mycophenolic acid (MPA) is a potent, selective, non-competitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and thus inhibits the *de novo* synthesis of guanosine nucleotides [8]. This effect is specific for lymphocytes, because the predominant pathway for 5'-guanosine triphosphate (GTP) synthesis in lymphocytes is the *de novo* pathway, whereas other cell types are able to efficiently use the salvage pathway of GTP synthesis. The addition of MPA has been shown to lower the *in vitro* anti-HIV 50% effective concentration (EC₅₀) values of guanosine (G)-analogue NRTI including abacavir, DAPD, and DXG [9–12]. MPA reduces the intracellular concentration of GTP, which increases the ratio of the G-analogue-TP to dGTP. This effect increases the likelihood that the chain terminating GTP analogue will be incorporated by HIV-1 reverse transcriptase. *In vitro*, MPA has been observed to antagonize the antiviral effect of ZDV and D4T against NRTI-sensitive HIV-1 [11]. Preliminary, uncontrolled studies have suggested that mycophenolate mofetil (MMF), converted to MPA after oral absorption, could augment antiretroviral therapy in HIV-infected patients [9,10,13,14].

We therefore performed a phase I/II, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the safety, tolerability, and antiretroviral activity of DAPD as add-on therapy, alone or in combination with MMF, in HIV-infected adults with extensive prior ART treatment experience and limited treatment options.

Design and methods

Study design

Antiretroviral-experienced HIV-infected men and women at least 18 years of age who were experiencing virologic failure (plasma HIV-1 RNA \geq 2000 copies/ml) on their current antiretroviral regimen enrolled in ACTG A5165. Prior treatment experience with three antiretroviral drug classes was required, as defined by exposure for at least 3 months each to two or more NRTIs, two or more non-boosted protease inhibitors (PI) or a dual PI regimen, and one NNRTI.

Patients had been on their failing antiretroviral regimen, which did not include abacavir, for at least 30 days prior to entry, and had a screening plasma HIV-1 RNA level \geq 2000 copies/ml, and a screening CD4+ cell count \geq 50 cells/ μ l. Enrollees were required to have absolute neutrophil count \geq 750/mm³, hemoglobin \geq 7.0 g/dl,

platelet count $\geq 50\,000/\text{mm}^3$, creatinine $\leq 1.5 \times$ upper limit of normal (ULN), estimated creatinine clearance of ≥ 80 ml/min, serum albumin > 2.5 mg/dl, AST (SGOT), ALT (SGPT), and alkaline phosphatase $\leq 2.5 \times$ ULN, total bilirubin $\leq 2.5 \times$ ULN, serum lipase $\leq 1.5 \times$ ULN, random or fasting glucose < 121 mg/dl, hemoglobin A1c \leq ULN, urinalysis showing $< 2+$ proteinuria and < 2 cellular casts per high powered field, a negative pregnancy test if of reproductive potential, agreement not to participate in a conception process and to use two contraceptive methods, and Karnofsky performance score ≥ 70 within 45 days prior to entry.

Informed consent, reviewed and approved by the NIAID Division of AIDS and each local site institutional review board, was obtained from study volunteers. Upon study entry, individuals received DAPD (500 mg orally, twice daily) with either MMF (500 mg orally, twice daily) or matched MMF placebo (twice daily). Assignment to study drug was stratified by screening plasma HIV-1

RNA level $< 40\,000$ or $\geq 40\,000$ copies/ml. At week 2, patients and providers optimized antiretroviral therapy using a Virtual Phenotype (Virco Lab, Raritan, New Jersey, USA) obtained 2 weeks before DAPD initiation. Patients having a virologic response, defined as a reduction in plasma HIV-1 RNA by week 2 to ≥ 0.5 \log_{10} copies/ml below baseline (the geometric mean of the pre-entry and entry values) or to < 400 copies/ml, continued on blinded study treatment until week 24 (Fig. 1).

Patients without a virologic response at week 2 or with a confirmed rebound of plasma HIV-1 RNA after week 2 to $< 0.5 \log_{10}$ below baseline were unblinded to study therapy. Patients who were assigned to DAPD and placebo were offered DAPD and open label MMF after first confirmed virological failure. Patients assigned to DAPD and MMF discontinued MMF after first confirmed virologic failure, but could continue DAPD, if a phenotypic resistance assay (Virco Lab, Inc., Durham,

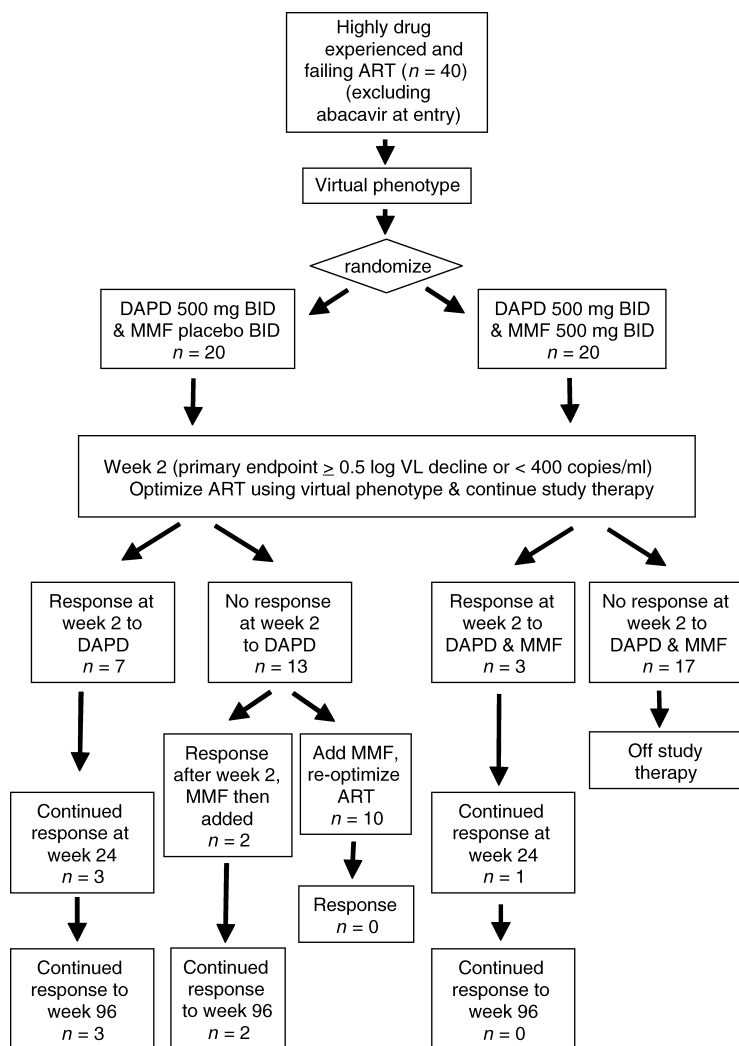


Fig. 1. Study assignment and outcome for 40 patients randomized to β -D-2,6-diaminopurine dioxolane (DAPD)/placebo and DAPD/mycophenolate mofetil (MMF). ART, antiretroviral therapy; BID, twice daily; VL, viral load.

North Carolina, USA; ref. [15]) showed less than fivefold resistance to DXG. Given the available clinical data on DXG resistance and clinical response, this cutoff was chosen based on the known resistance conferred by the RT mutations K65R and/or L74V. At week 24, all patients were unblinded to study therapy. Patients with continued virologic response to optimized ART including DAPD +/- MMF remained on study medication for up to 96 weeks.

Pharmacokinetic substudy

Intensive pharmacokinetic sampling was performed on the first 26 patients enrolled, sampling plasma eight times from 0 to 8 h after an observed dose at steady state. Plasma DAPD and DXG concentrations were determined by mass spectroscopy [16]. The primary pharmacokinetic parameters analyzed were area under the concentration-time curve (AUC) within the dosing interval expressed as from time zero to 12 h (AUC_{0-12}), and the maximum observed plasma concentration (C_{max}). AUC_{0-12} was calculated according to the trapezoidal rule where AUC_{0-12} is the sum of the measured AUC from time zero to the last measured concentration at 8 h post dose, plus the extrapolated AUC from 8 to 12 h post dose (WinNonLin, version 5.0.1; Pharsight Corporation, Mountain View, California, USA). The AUC between 8 and 12 h post dose was determined by estimation of the patient's DAPD or DXG concentration at 12 h post dose using their own calculated elimination rate constant (k_{el}) and decay of the last measured concentration to 12 h post dose according to the formula:

$$C_{12} = C_{last} \times e^{[-k_{el}(12-T_{last})]}$$

where C_{12} is the predicted concentration 12 h post dose, and C_{last} is the last measured concentration obtained at a nominal time of 8 h post dose (T_{last}). The extrapolated AUC between these two points was then calculated employing the trapezoidal rule. C_{max} was taken as the maximum observed plasma concentration following the observed dose [17]. Samples for quantification of trough total mycophenolic acid, mycophenolic acid glucuronide, and free (unbound) mycophenolic acid concentrations by high performance liquid chromatography (HPLC) mass spectroscopy were obtained during weeks 1 and 2.

Data analysis

Analyses were conducted using an intent-to-treat approach. Baseline HIV-1 RNA was calculated as the geometric mean of the pre-entry and the entry values. The primary endpoint was compared using Wilcoxon rank-sum test. Toxicities were graded using the standardized ACTG toxicity grading criteria (0 - none, 1 - mild, 2 - moderate, 3 - severe, 4 - potentially life threatening, 5 - lethal). If an adverse experience was reported at both baseline and during follow-up, then the follow-up adverse experience was included in the analysis only if it was at least one grade worse than that at baseline.

Comparison of the intensive pharmacokinetic data between the two arms was performed using a *t*-test. The 50% inhibitory concentration (IC_{50}) fold-changes for DXG at baseline were compared between the two arms using Wilcoxon rank sum test. The relationship between baseline DXG phenotype and virologic response was examined using Spearman correlation test.

Results

Study population characteristics

Between February 2003 and June 2005, 40 study volunteers enrolled; 20 in each study arm (DAPD with MMF placebo, and DAPD with MMF). Due to slow enrollment, accrual to the study was closed prior to achieving the enrollment target of 56 participants. Study patients were predominantly male (90%) and white (55%), and 70% were between 40 and 49 years of age (Table 1). The median plasma HIV-1 RNA at enrollment was 4.5 \log_{10} copies/ml, and median CD4+ cell count was 184 cells/ μ l. Fifty-three percent of patients enrolled with ≤ 200 cells/ μ l. Study patients had a median of 6 NRTI mutations (IAS-USA guidelines) at entry

Table 1. Baseline characteristics by treatment.

	Total (n = 40)	Study treatment arm	
		DAPD (n = 20)	DAPD and MMF (n = 20)
Sex			
Male	36 (90%)	18 (90%)	18 (90%)
Female	4 (10%)	2 (10%)	2 (10%)
Race/ethnicity			
White non-Hispanic	22 (55%)	11 (55%)	11 (55%)
Black non-Hispanic	7 (18%)	3 (15%)	4 (20%)
Hispanic	8 (20%)	5 (25%)	3 (15%)
Asian, Pacific Islands	2 (5%)	0 (0%)	2 (10%)
Other	1 (3%)	1 (5%)	0 (0%)
Age (years)			
Median	45	45	45
30-39	4 (10%)	3 (15%)	1 (5%)
40-49	28 (70%)	12 (60%)	16 (80%)
50-59	7 (18%)	2 (25%)	2 (10%)
60-69	1 (3%)	0 (0%)	1 (5%)
Intravenous drug use			
Never	36 (90%)	17 (85%)	19 (95%)
Currently	1 (3%)	1 (5%)	0 (0%)
Formerly	3 (8%)	2 (10%)	1 (5%)
HIV-1 RNA level (copies/ml)			
Median	31563	33691	25499
$< 40\,000$	23 (58%)	12 (60%)	11 (55%)
$\geq 40\,000$	17 (43%)	8 (40%)	9 (45%)
CD4+ cell count (cells/ μ l)			
Median	184	184	203
51-200	21 (53%)	11 (55%)	10 (50%)
201-300	9 (23%)	3 (15%)	6 (30%)
301-400	2 (5%)	0 (0%)	2 (10%)
401-500	4 (10%)	3 (15%)	1 (5%)
> 500	4 (10%)	3 (15%)	1 (5%)

DAPD, β -D-2,6-diaminopurine dioxolane; MMF, mycophenolate mofetil.

Table 2. Nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations at baseline.

Number of IAS-USA NRTI mutations at entry	Frequency		
	Number of patients	Percentage of patients	
1	2	5	
3	4	10	
4	5	12.5	
5	6	15	
6	10	25	
7	7	17.5	
8	6	15	
Stanford algorithm score ^a			
< 10	3TC	TDF	D4T
10–29	2	1	1
30–59	7	6	1
≥ 60	1	29	16
	30	4	22
Stanford algorithm score	ZDV	DDI	ABC
< 30	2	1	2
30–59	7	13	10
≥ 60	31	26	28

ABC, abacavir; DDI, didanosine; D4T, stavudine; TDF, tenofovir; ZDV, zidovudine; 3TC, lamivudine.

^aIncreases with greater resistance: <http://hivdb.stanford.edu>.

(Table 2). The Stanford algorithm predicted high levels of resistance to all available NRTIs (Table 2; ref. [18]).

Virologic response to β -D-2,6-diaminopurine dioxolane

Virological response to the addition of DAPD to failing therapy, with or without MMF, was evaluated 2 weeks after study entry to provide a rapid assessment of DAPD activity in this population with advanced HIV-1 disease and extensive NRTI resistance. When the responses of both study arms were pooled, the median change in HIV-1 RNA was $-0.26 \log_{10}$ copies/ml, and the mean change was $-0.29 \log_{10}$ copies/ml ($P < 0.0001$).

When analyzed separately, the HIV-1 RNA change from baseline in each arm was statistically significant, but there was no significant difference in the response between arms. The average HIV-1 RNA response in the DAPD with placebo arm (median -0.37 and mean $-0.35 \log_{10}$ copies/ml) was greater than in the DAPD with MMF arm (median -0.23 and mean $-0.24 \log_{10}$ copies/ml), but this difference was not statistically significant ($P = 0.59$). Overall, 10 of 40 patients had a decline of VL $> 0.5 \log_{10}$ or more at week 2, seven of these in the DAPD arm and three in the DAPD/MMF arm. After two weeks of study therapy, CD4+ cell counts increased in 19 patients (nine in DAPD arm and 10 in DAPD/MMF arm) and decreased in 21 (11 in DAPD arm and 10 in DAPD/MMF arm). The median change in CD4+ cells was a decline of 4 cells/ μ l.

All patients optimized background therapy after 2 weeks of study therapy (Fig. 1). Following optimization, six of

10 patients who were responding at week 2 to the addition of DAPD or DAPD/MMF had rebound of viremia to within $0.5 \log_{10}$ copies/ml of baseline before week 24. The mean and median change in CD4+ cell counts was 122 and 56 cells/ μ l, respectively, for seven of these patients who remained on study treatment to week 24 (Fig. 1). Ten patients who did not respond to DAPD at week 2 re-optimized their background ART and added MMF. There were no prolonged virologic responses to re-optimized background ART with DAPD and MMF in these 10 patients.

In the six patients who had rebound of viremia between weeks 2 and 24, five patients had less than fivefold resistance to DAPD by phenotype assay, and so were allowed to re-optimize background therapy and continue DAPD. Out of these, three patients (one receiving DAPD, two receiving DAPD and MMF) remained on study and had continued virologic response to week 96. The mean and median increases in CD4+ cell count in these three patients were 276 and 255 cells/ μ l, respectively. The mean and median increases in CD4+ cell count in five patients who continued study treatment up to week 96 was 153 and 216 cells/ μ l, respectively.

Plasma drug concentrations

Trough total mycophenolic acid concentrations, measured during weeks 1 and 2, were within the expected range: median 795 ng/ml, mean 859 ng/ml. The average free fraction or percentage unbound of mycophenolic acid was 1.3%, with a range from 0.3 to 3.1%. Analysis of DAPD and DXG AUC_{0–12} and C_{max} revealed no statistically significant differences ($P < 0.05$) between these two parameters in patients receiving DAPD when compared with those who received DAPD and MMF (Table 3). There was, however, a trend towards lower DXG AUC_{0–12} ($P = 0.06$) in patients who received DAPD plus MMF compared with those who only received DAPD (Fig. 2 and Table 3).

Impact of reverse transcriptase mutations on response

Consistent with preclinical studies, four patients with K65R and/or the Q151M complex did not respond to DAPD or DAPD with MMF. Among the remaining patients, there was a statistical trend toward better response in patients with less than six NRTI mutations ($P = 0.12$) and with less than four TAMs in the absence of E44D or V118I ($P = 0.08$).

Phenotypic resistance to DXG was assayed in 33 available baseline samples (Virco). The DXG fold-change ranged from 0.5 to 113.6; median fold-change was 1.4 and the mean was 7.2. There was no significant difference in baseline DXG susceptibility between the two treatment arms ($P = 0.56$) and no significant correlation between baseline DXG susceptibility and virologic response ($P = 0.36$). DXG susceptibility was determined again at

Table 3. Pharmacokinetic study results.

	Mean	Median	Quartiles [Q ₁ , Q ₃]	Standard deviation	Coefficient of variation
DAPD arm					
DAPD					
AUC _{0-12h} ^a	5.67 ^b	4.47	0.92, 9.94	5.11	90.24
C _{max} ^a	2.33 ^c	1.73	0.39, 4.15	2.08	89.04
DXG					
AUC _{0-12h}	23.67 ^c	22.01	15.7, 28.6	11.65	49.22
C _{max}	5.10 ^d	4.89	3.40, 6.92	2.40	47.06
DAPD and MMF arm					
DAPD					
AUC _{0-12h}	3.11 ^b	1.95	1.42, 5.31	2.45	78.80
C _{max}	1.34 ^c	0.86	0.51, 2.34	1.10	81.82
DXG					
AUC _{0-12h}	16.32 ^d	15.14	10.8, 20.0	6.18	37.88
C _{max}	3.66 ^e	3.37	2.55, 4.56	1.47	40.16

AUC_{0-12h}, area under the curve for time zero to 12 h; DAPD, β-D-2,6-diaminopurine dioxolane; DXG, dioxolane guanosine; MMF, mycophenolate mofetil.

^aAUC (μmol/l*h), C_{max} (μmol/l).

^bP=0.23.

^cP=0.18.

^dP=0.06.

^eP=0.12.

first virologic failure. In 16 available samples at failure, the DXG fold-change ranged from 0.2 to 24.8, the median fold-change was 1.5 and mean was 3.4, which was not significantly different from baseline.

Toxicities

Two grade 3 events (an asymptomatic lipase elevation and flatus) were reported during blinded study treatment prior to the primary study endpoint for analysis of virologic response (week 2). Thirty-one patients with adequate virologic response, or who switched to open label MMF after failure of DAPD with placebo, continued on study treatment after week 2. Of these, three discontinued study therapy for grade 3 events (at week 4 and two at week 16). These events were flatus (onset week 1), hyper-bilirubinemia (week 12), and hepatic transaminase elevation (week 13). No other study

participant terminated therapy prematurely due to MMF or DAPD toxicity or intolerance.

Nine patients (eight on DAPD and one on DAPD + MMF) had grade 3 or 4 elevations of lipase after week 2, but only two (in DAPD arm) were judged possibly related to study drugs. Eight patients (four in each arm) were reported to have grade 3 or 4 creatine phosphokinase elevations; one (in DAPD arm) was judged as possibly related to study drug. Three patients (two on DAPD and one on DAPD + MMF) were reported to have grade 3 or 4 elevation of total bilirubin, but only one of these (in DAPD arm) was deemed to be study drug related. Four patients (two in each arm) were reported to have grade 3 or 4 aspartate aminotransferase elevations. Three patients receiving DAPD had elevation of triglycerides and one of lactic acid; these events were judged as unrelated to DAPD.

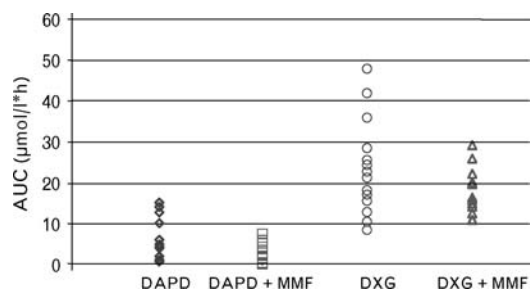


Fig. 2. β-D-2,6-diaminopurine dioxolane (DAPD) and dioxolane guanosine (DXG) exposures [area under the curve for time zero to 12 h (AUC₀₋₁₂)] in patients randomized to DAPD/placebo and DAPD/mycophenolate mofetil (MMF). DAPD (squares) and DXG (triangles) exposure in patients receiving MMF tended to be lower than DAPD (diamonds) and DXG (circles) without MMF (P=0.06, NS).

Potential drug-related toxicities that might be associated with the administration of DAPD or MMF such as CD4+ decline < 50% of baseline, or decline of renal function (≥ grade 2) were not observed. One grade 2 elevation of glucose reported as possibly/probably related to study treatment was observed at week 1 and 3 of study therapy. This individual discontinued DAPD and MMF at week 12 due to virologic failure.

Due to lens opacities observed after prolonged, high-dose administration of DAPD sufficient to induce renal damage in a 52-week cynomolgus monkey study, intensive ophthalmologic monitoring was also performed. Centrally trained ophthalmologists performed validated ophthalmologic examinations using the Lens Opacities Classification System III (LOCS III) every 3 months [19]. One patient on DAPD with MMF was

noted to have an increase of LOCS III score of > 1 that was not felt to be study-drug related, and that did not persist. No other significant ophthalmologic toxicities or persistent and significant changes in lens opacity were observed.

Discussion

Optimal treatment of drug-resistant HIV-1 infection is thought to require more than two active drugs. Although antiretrovirals with novel mechanisms of action are in development, many patients currently failing therapy have HIV-1 resistant to three of the classes of drugs in wide use: NRTI, NNRTI, and protease inhibitors. Drugs with residual activity despite NRTI resistance, or strategies to augment the activity of NRTI would be useful for the treatment of such individuals.

The present study has demonstrated that DAPD, with or without MMF, was safe and well tolerated as add-on therapy, and had significant antiretroviral activity ($-0.35 \log_{10}$) in heavily pretreated patients with extensive NRTI resistance (median = 6 mutations in HIV RT). A small subset of heavily pretreated patients in this trial achieved persistent clinical benefit for many weeks using combination regimens including DAPD. Very few adverse events were observed in temporal association with DAPD use.

Nevertheless, in contrast to synergy observed *in vitro*, DAPD activity over 2 weeks was not increased by MMF in this study. In fact, a trend towards poorer response was seen in the first 2 weeks in patients receiving DAPD with MMF. Although two pilot trials suggested that MMF could augment the activity of abacavir [11,13], these trials were uncontrolled. Another uncontrolled study by Chapuis *et al.* in aviremic patients on HAART that contained abacavir showed that the use of MMF led to a depletion of peripheral blood mononuclear cells carrying HIV [9].

Vrisekoop *et al.*, however, found that there was neither a detrimental nor beneficial effect of MMF when added to HAART in acutely or chronically infected patients [20]. In the same cohort of patients, Sankatsing *et al.* did not find a consistent effect of MMF on intracellular nucleoside pools [21], using the same assays performed in a prior study that reached the opposite conclusion [13].

In this study, plasma concentrations of total mycophenolate were found within the expected range. Further, as MPA is a highly protein-bound drug and plasma protein levels may be altered in HIV-infected patients, it is notable that free MPA levels were within the expected range. Measurement of DAPD and the active

DAPD metabolite DXG also showed levels of exposure broadly within the expected range. Although both DAPD and MPA exposure appeared to be within the expected ranges, a trend towards lower exposure to DAPD and DXG in patients who also received MMF was observed, but this trend did not reach statistical significance ($P=0.06$). The pilot nature and small sample size of this study may have contributed to our inability to definitively document a negative drug-drug interaction. Nevertheless the trend toward lower DXG concentrations among participants who received DAPD plus MMF suggests an adverse drug-drug interaction between DAPD and MMF as a possible explanation for failure of MMF to enhance DAPD activity. The mechanism of this negative interaction is not known.

In summary, DAPD was safe and well tolerated. In a prior study, DAPD 500 mg twice daily was active in treatment-experienced patients, of which 96% had at least one NRTI mutation at baseline, lowering HIV-1 RNA by a median of 0.7 log copies/ml after 15 days of therapy [5]. In the current study, DAPD also had significant antiretroviral activity in patients with extensive NRTI resistance (median six NRTI mutations). The smaller antiretroviral effect of DAPD in the current study could be due to the greater number of NRTI mutations and hence possible DXG cross-resistance at baseline compared with the prior study. Further studies with DAPD are warranted to define the role of DAPD in treatment-experienced patients. This study did not provide support for further investigation of DAPD in combination with MMF.

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