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# Dose proportional inhibition of HIV-1 replication by mycophenolic acid and synergistic inhibition in combination with abacavir, didanosine, and tenofovir

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**Abstract**

Mycophenolate mofetil (MMF), a therapeutically used inhibitor of inosine monophosphate dehydrogenase is hydrolyzed to its active metabolite mycophenolic acid (MPA) in vivo. MPA exhibits anti-HIV activity in vitro. We tested MPA alone and in combination with abacavir (ABC), didanosine (DDI), lamivudine (3TC) and tenofovir (TFV) against wild-type human immunodeficiency virus type-1 (HIV-1) and nucleoside reverse transcriptase inhibitor (NRTI)-resistant HIV-1. MPA (62.5–500 nM), when combined with ABC or DDI, synergistically enhanced activity against wild-type HIV and the NRTI-resistant HIV clone DRSM34. MPA also enhanced the activity of TFV against both wild-type HXB2 and TFV-resistant strain $HIV_{K65R}$, in a more than additive manner. No significant antiproliferative effect of MPA ($\leq 0.25$ μM) alone or in the presence of ABC, DDI and TFV was observed. This indicates that the antiviral effects of MMF may be clinically achievable without fully blocking T-cell proliferation or inducing immunosuppression. These findings provide further rationale for the clinical testing of MMF in combination with ABC, DDI, and TFV. 

*Keywords:* Mycophenolate mofetil; Mycophenolic acid; Abacavir; Didanosine; Tenofovir; HIV-1; Antiretroviral resistance

## 1. Introduction

A key step of human immunodeficiency virus type-1 (HIV-1) infection is the conversion of single-stranded viral RNA genome into double-stranded proviral DNA by the action of viral reverse transcriptase (RT) (Mitsuya et al., 1987; Meyer et al., 1998). Inhibition of this viral enzyme

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has long been the cornerstone of antiretroviral therapy (ART). During drug treatment of HIV-infected patients incomplete suppression of virus replication can result in the emergence of drug-resistant viral mutants (Richman, 2001). Therapeutic options are currently limited for these patients who have failed therapies with nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). Further, the emergence of drug-resistant HIV in populations may impair the future utility of current antiretrovirals in untreated patients (Shirasaka et al., 1995; Hirsch et al., 1998; Yerly et al., 1999).

In this context, we have explored the use of mycophenolate mofetil (MMF) in combination with ART. MMF, used as an immunosuppressant in organ transplantation (Halloran et al., 1997; Allison and Eugui, 2000; Weigel et al., 2001), is hydrolyzed to its active metabolite mycophenolic acid (MPA) after oral uptake. It is a non-competitive, reversible inhibitor of inosine 5′-monophosphate dehydrogenase, a rate-limiting enzyme in the de novo synthesis of guanosine 5′-monophosphate (Allison et al., 1993; Mitchell et al., 1993; Allison and Eugui, 1996; Nelson et al., 1996; Sintchak et al., 1996). MPA has been shown to increase the activity of antivirals against herpesviruses (Neyts and De Clercq, 1998; Neyts et al., 1998a,b). Ichimura and Levy (1995) demonstrated that at 1–10 μM, which are similar to plasma concentrations obtained clinically, MPA inhibits replication of HIV-1 in vitro. We have previously demonstrated that MPA suppresses HIV replication in vitro and potentiates the antiviral activity of the guanosine analog inhibitor of RT, abacavir (ABC) (Margolis et al., 1999). We have also observed that MPA increased the antiretroviral effect of ABC against NRTI-resistant HIV strains but was antagonistic in combination with zidovudine and stavudine in primary mononuclear cell culture (Heredia et al., 1999). MPA also increased anti-HIV activity of didanosine (DDI) in vitro (Heredia et al., 1999).

Pilot clinical studies have suggested that MMF is safe when given as a component of ART in the setting of advanced acquired immunodeficiency syndrome (AIDS) and multidrug resistant HIV-1 infection (Coull et al., 2001). Chapuis et al. (2000) have reported that MMF decreases the ability to recover HIV from PBMCs of patients on ART, perhaps by a reduction in the number of latently infected T-cells.

Tenofovir (TFV), (*R*)-(9-[2-(phosphonylmethoxy)propyl] adenine (PMPA), is an acyclic nucleoside analog, related to adefovir, 9-[2-(phosphonylmethoxy)ethyl]adenine (PMEA), with HIV RT inhibitor activity (Pauwels et al., 1988; Smith et al., 1989; Balzarini et al., 1993, 1997). TFV has activity against a number of retroviruses including HIV and SIV (Tsai et al., 1997; Robbins et al., 1998; Silvera et al., 2000), and has been recently approved, as its oral prodrug, TFV disoproxil fumarate, for the treatment of HIV infections. Emergence of TFV-resistant HIV is infrequent (Deeks et al., 1998), although the K65R RT mutation has been shown to reduce the potency of TFV in vitro, and high-level NRTI resistance decreases viral susceptibility to TFV (Palmer et al., 1999; Wainberg et al., 1999; Van Rompay et al., 2000).

In this study, we investigated the effect of a range of MPA concentrations on the anti-HIV activities of DDI, TFV and the combination of ABC with DDI. We also assessed the interaction of MPA with these drugs against wild-type HIV and NRTI-resistant HIV strains. We found that MPA enhanced the anti-HIV activity of these drugs in a dose-dependent fashion, suggesting that MPA can potentiate the antiretroviral activity of selected NRTIs without blocking T-cell proliferation. Careful clinical trials are needed to test the relative risks and benefits of the use of MMF in ART.

## 2. Materials and methods

### *2.1. Antiviral drugs*

MPA was purchased from Calbiochem-Novabiochem Corporation (La Jolla, CA), DDI was obtained from Sigma (St. Louis, MO), TFV was provided by Gilead Sciences, Inc. (Foster City, CA), ABC and lamivudine (3TC) were provided by Glaxo-SmithKline Inc. (Research Triangle Park, NC).

### 2.2. HIV-1 strains

The antiviral activities of the drugs and combinations were assessed against six different HIV type (HIV-1) strains: a wild-type laboratory isolate (HXB2) and five recombinant isolates. The proviral clone HXB2 containing the K65R TFV resistance mutation (Gu et al., 1995) was kindly supplied by Wainberg. Multidrug resistant molecular clones: *DRSM34* (M41L, D67N, K70R, M184V, H208Y, T215Y, K219E); *MDRV-1* (K65R, L74V, Y115F, M184V), *RTMCY* (D67N, K70R, T215Y, K219Q) and *M184V* (Tisdale et al., 1997) were provided by Short and Lanier. $TCID_{50}$ were determined for each strain using MT-2 cells as described (Japour et al., 1993).

### 2.3. Antiviral assays

A total of $10^6$ activated peripheral blood mononuclear cells (PBMCs) were infected with 1000 $TCID_{50}$ of HIV-1 for 2 h, washed three times with PBS and resuspended in RPMI-1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine/penicillin/streptomycin (Gibco, Grand Island, NY) and 10 U/ml interleukin-2 (Boehringer Mannheim). Cells were seeded in 96-well flat-bottom tissue culture plates at a density of $2 \times 10^5$ PBMC/200 μl medium containing the appropriate drug dilution. Media were replaced at 3 days post-infection with fresh media containing IL-2 and drugs. On day 7, cell-free supernatants were harvested and HIV-1 p24 antigen was measured using an HIV-1 p24 antigen capture assay kit (AIDS Research Reagent Program, Frederick, MD). In all experiments, uninfected cell controls were maintained for determination of drug toxicity. Cell viability was assessed by trypan blue exclusion. Six days post-infection, cell proliferative capacity was assessed using the MTT assay as described (Denizot and Lang, 1986).

### 2.4. Drug interaction analysis

The interaction of MPA with ABC, DDI, and TFV was analyzed according to the definition of Loewe, using the interaction model of Greco et al. (1995). This model is fully parametric, and point estimates of the model parameters are obtained in a traditional weighted, non-linear least-square approach. The model follows the equation below:

$$1 = \frac{D_1}{IC_{50.1}[E/(E_{con} - E)]^{1/m1}} + \frac{D_2}{IC_{50.2}[E/(E_{con} - E)]^{1/m2}} + \frac{\alpha D_1 D_2}{IC_{50.1} IC_{50.2}[E/(E_{con} - E)]^{(1/2m1 + 1/2m2)}}$$

$IC_{50.1}$ and $IC_{50.2}$ are the drug concentrations resulting in 50% inhibition for drug 1 and 2, respectively; $D_1$ and $D_2$ are the concentration of drugs tested; $E_{con}$ is the control effect in the absence of either drug, $E$ is the observed effect in the presence of drugs; $m_1$ and $m_2$, the slope parameters for each drug, reflect the rate of rise of the effect curve for each single agent as in a sigmoid $E_{max}$ effect model. In this combination analysis of the point estimate of the slope parameter value is estimated for all the data. The $\alpha$ is the synergism–antagonism interaction parameter. If the estimate of $\alpha$ is zero, the drug combination is additive; if $\alpha$ is positive, the interaction is synergistic; if $\alpha$ is negative, the interaction is antagonistic. When the 95% confidence interval for $\alpha$ does not overlap with zero, this demonstrates statistical significance of the interaction, no matter how small the absolute $\alpha$ value. That is, if the 95% confidence interval encompasses zero, the interaction is additive. If it does not and $\alpha$ is positive, the interaction is significantly synergistic. If it does not and $\alpha$ is negative, the interaction is significantly antagonistic. While large $\alpha$ values, for example, illustrate a robustly synergistic interaction they are not directly correlated with the absolute potency of the drug combination studied. This model was implemented using the ADAPT II programs (D'Argenio and Schumitzky, 1990). Each drug combination was tested in triplicate so as to obtain an estimate of the variance of the effect at different drug concentration combinations.

## 3. Results

### 3.1. ABC and MPA synergize against multi drug-resistant HIV-1 in a concentration-dependent manner

The susceptibility of multidrug-resistant HIV clones DRSM34 and MDRV1 to ABC was previously shown to be enhanced by 0.25 μM MPA (Heredia et al., 1999). We tested the ability of MPA (0.0625, 0.125, 0.25 and 0.5 μM) in the presence and absence of ABC (4 μM) to suppress the replication of these viruses. Four micromolar is equivalent to the peak levels at ABC achieved by clinical dosage. We observed a dose-dependent antiviral effect of MPA against DRSM34 (Fig. 1) and MDRV1 (not shown) that was augmented by the addition of ABC. This level of augmentation was not as profound as observed with wild-type viral strains, but was significant. When the viral replication data obtained with DRSM34 were applied to a Greco model, an $\alpha$ value of 5.26 was obtained (Table 1). This value indicated synergy between MPA and ABC. Similar values for MDRV1 were also observed (not shown).

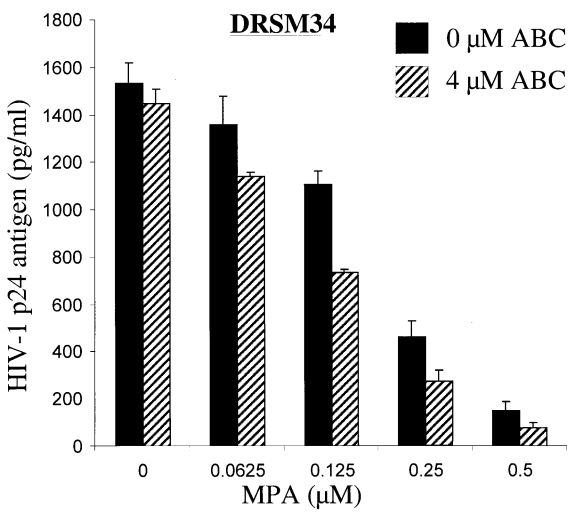


Fig. 1. MPA augments potency of ABC to inhibit NRTI-resistant HIV-1: PBMCs from a seronegative donor were infected with DRSM34 and cultured in the presence of MPA and ABC. HIV p24 antigen was assayed at 7 days post-infection. Values represent the mean ± standard deviation of triplicate determinations.

### 3.2. MPA and DDI exhibit synergy against both wild type and NRTI-resistant HIV in a concentration-dependent manner

Our previous studies did not address the question of the effect of MPA on the antiviral activity of DDI across a range of MPA concentrations against wild-type HIV or NRTI-resistant HIV. We tested the effect of low-dose MPA (62.5 nM, 0. 25 μM) in combination with DDI (0.5–15 μM) against HXB2 or DRSM34. High concentrations of DDI were tested to provide more complete data on the interaction of DDI with MPA.

Again we observed a dose-dependent antiviral effect of MPA. We also observed significant MPA augmentation of the potency of DDI against both viral strains, again illustrating a synergistic relationship (Fig. 2A and B). When these data were analyzed by the Greco model, we found that this combination of antiretrovirals exhibited synergy (Table 1). We observed $\alpha$ values of 0.69 for wild-type HXB2 and 7.74 for DRSM34. These values indicate that MPA amplifies the potency of DDI against DRSM34 and wild-type virus, although the effect appears less profound with wild type HIV.

### 3.3. ABC, DDI and MPA exhibit synergy against wild-type HIV-1 and multidrug-resistant HIV-1

We then tested the ability of low concentrations MPA (62.5 nM–0.5 μM) to inhibit the replication of wild-type HXB2 (not shown) and the multidrug resistant HIV-1 strain DRSM34 (Fig. 3) in the presence and absence of DDI (0–7.5 μM) and ABC (0–4.0 μM). In the clinic, DDI achieves peak plasma levels of 9 μM. As expected, we observed a dose-dependent augmentation of the combined effect of ABC and DDI across the whole MPA concentration range. However, this effect was much more pronounced compared to either MPA and ABC, or MPA and DDI. The triple combination also more potently inhibited replication of the DRSM34 multidrug-resistant virus as compared to the wild-type. We obtained an $\alpha$ value of 7.05 for HXB2, and an $\alpha$ value of 1847 for DRSM34 indicating synergy between these drugs. As MPA increases the potency of both ABC and DDI, this

Table 1
Parameter estimates and 95% confidence intervals for the $IC_{50}$ and interaction between ABC, DDI and MPA by a fully parametric analysis

| | $IC_{50}$ μM (95% C.I.) | | | α (95% C.I.) | | | |
|---|---|---|---|---|---|---|---|
| | MPA | ABC | DDI | MPA/ABC | MPA/DDI | ABC/DDI | MPA/ABC/DDI |
| HXB2 | 0.07 (0.06–0.07) | 1.41 (1.26–1.55) | 0.53 (0.33–0.73) | 0.76 (0.53–0.99) | 0.69 (0.47–0.91) | 2.21 (1.45–2.97) | 7.05 (4.87–9.24) |
| IIIB (Margolis et al., 1999) | 0.11 (0.60–0.72) | 0.63 (0.26–0.99) | n.d. | 8.2 (3.07–13.34) | n.d. | n.d. | n.d. |
| DRSM34 | 0.12 (0.11–0.12) | 45.34 (21.72–68.96) | 26.29 (15.97–36.6) | 5.26 (1.51–9.01) | 7.74 (3.37–12.13) | 60.43 (23.20–97.67) | 1847 (484.8–3210) |

α, synergism–antagonism interaction parameter; if the confidence interval does not overlap zero, the interaction is significantly synergistic (if positive) or antagonistic (if negative). For comparison, data using HIV $III_B$ (Margolis et al., 1999) are included. In that report, studies using DDI were not included [not done (n.d.)].

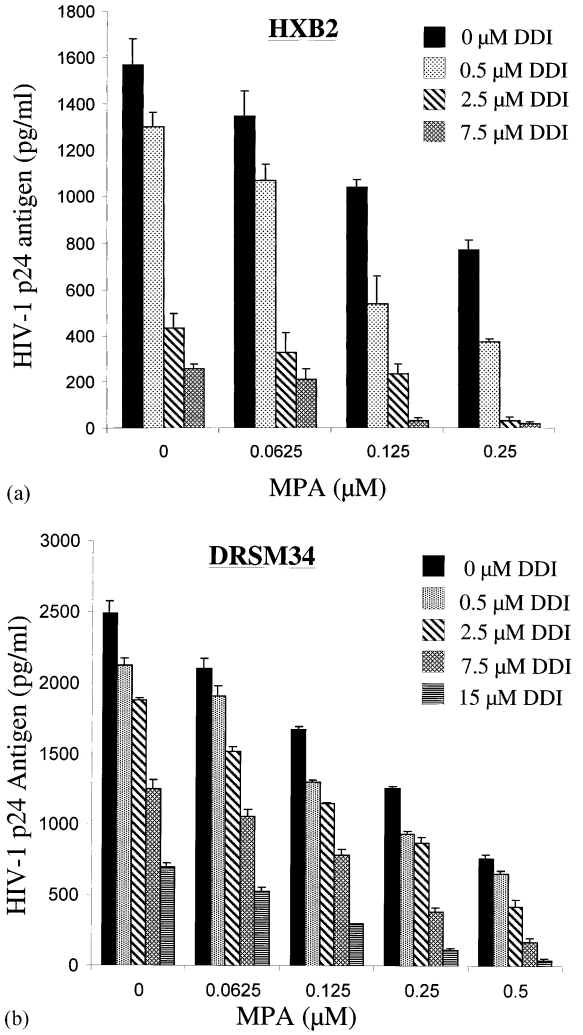


Fig. 2. MPA sensitizes NRTI-resistant HIV-1 to inhibition by DDI: PHA-activated PBMCs were infected with: (A) wild-type (HXB2); and (B) NRTI-resistant molecular clone (DRSM34) and cultured in the presence and absence of varying concentrations of DDI. HIV-1 replication was measured as p24 antigen in the culture supernatants on day 7 after infection, the time point at which peak of virus production was observed. Data represent mean ± standard deviation for triplicate determinations in each experiment.

combination may be of particular use in the setting of NRTI-resistant HIV.

We performed MTT assays to measure MPA-induced suppression of cell proliferation. (Fig. 4). As we have previously observed (Margolis et al., 1999; Heredia et al., 1999), 0.25 μM of MPA has

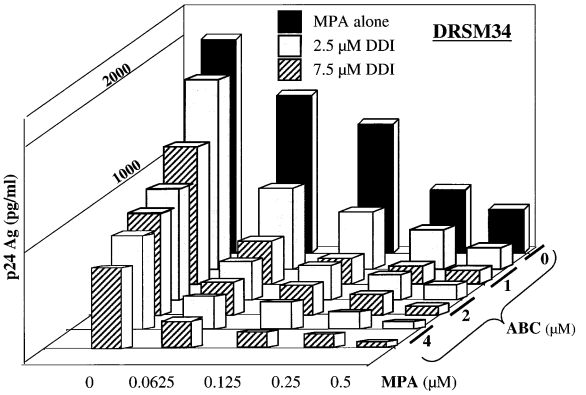


Fig. 3. MPA synergizes with ABC and DDI to inhibit replication of NRTI-resistant HIV (DRSM34): HIV p24 antigen was assayed at 7 days post-infection. Results are the means of triplicate determinations (standard deviations were on average 10% of the mean with a range of no more than 20% of the mean).

minimal effects on the viability and proliferation capacity of PBMCs. Only at concentrations of $\geq 0.5$ μM did we observe a substantial antiprolif-

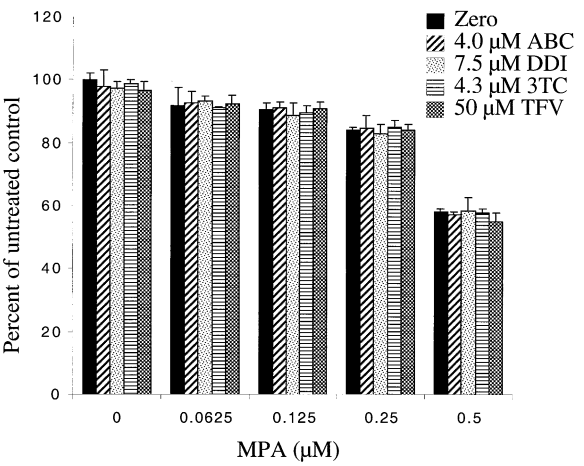


Fig. 4. Effect of MPA on seronegative donor PBMC in the presence and absence of nucleoside analogs (ABC, DDI, 3TC) and TFV: 3-day PHA-stimulated PBMCs were cultured in medium containing IL-2, 4 μM ABC, 7.5 μM DDI, 4.3 μM 3TC, 50 μM TFV and varying concentrations (as indicated) of MPA for 5 days without infection. MTT assay was performed on day 2 and 5. Percentage of cell proliferation was calculated from cells cultured in medium containing no drug. Data represent the mean ± standard deviation of three replicate cultures.

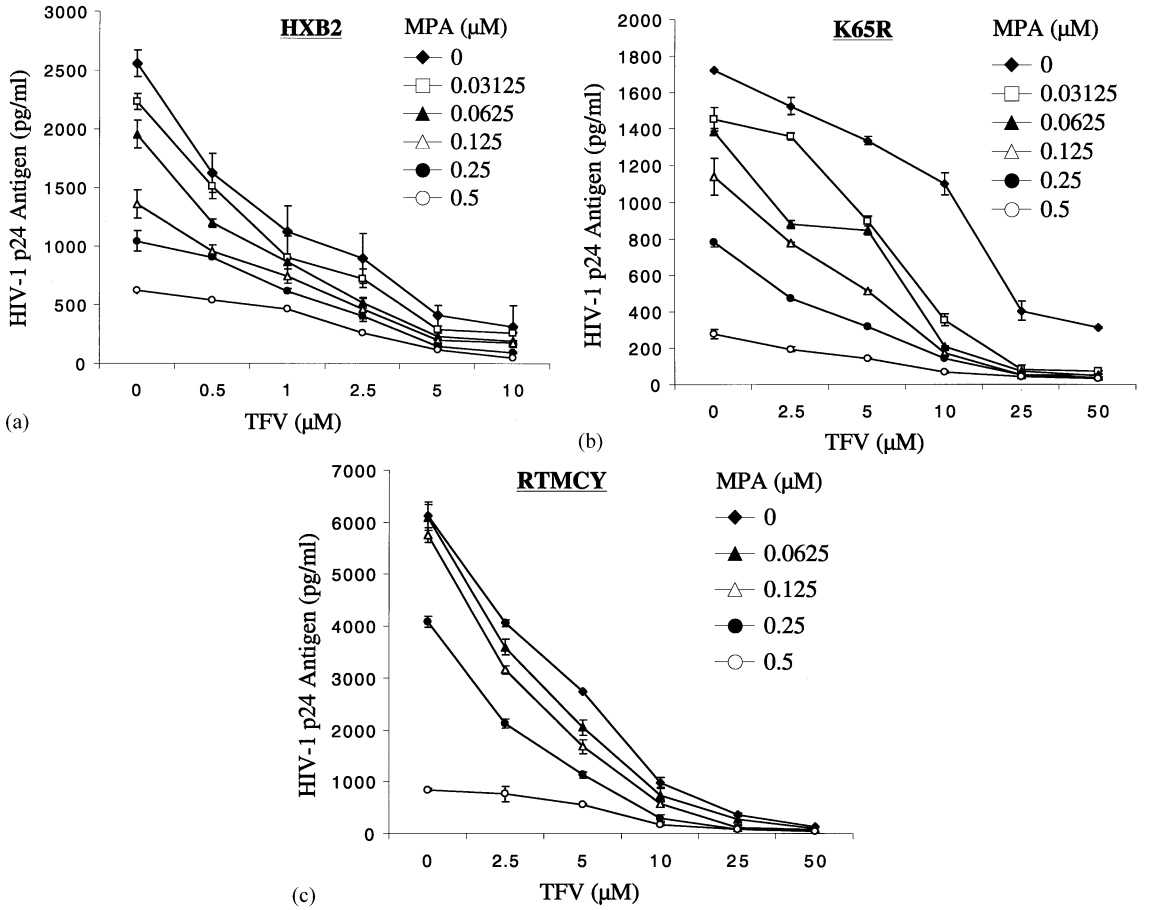


Fig. 5. Inhibition of wild-type HIV-1 (HXB2), TFV-resistant HIV molecular clone K65R and AZT-resistant HIV molecular clone RTMCY by MPA in the presence and absence of TFV: PHA-stimulated PBMCs from a seronegative donor were infected with HIV molecular clones cultured in the presence of MPA and TFV. HIV p24 antigen was assayed on day 7 after infection: (A) wild-type HXB2; (B) K65R; and (C) RTMCY. Values represent the mean ± standard deviation for triplicate determinations.

erative effect of MPA. No additional effects on cell viability/proliferation were observed in the presence of maximal concentrations of ABC, DDI or TFV when added to MPA.

### *3.4. TFV and MPA inhibit wild type, K65R, and AZT-resistant HIV clones*

PBMCs were infected with either HXB2, HIV-1 encoding the K65R TFV resistance mutation, or the NRTI-resistant RTMCY HIV-1 clones. While the K65R mutation confers resistance to TFV in vitro, it is rarely seen as an isolated mutation in clinical HIV isolates. Clinical isolates with substantial NRTI resistance can have decreased susceptibility to TFV. To examine the interaction of MPA and TFV, TFV was tested across the MPA concentration range of 0–10 μM for HXB2 and 0–50 μM for the K65R and RTMCY clones. The dose approved for clinical use, 300 mg daily (for TFV disoproxil fumarate), achieves a peak TFV concentration of 0.58 μM. MPA was again tested across the concentration range of 0–0.5 μM. MPA enhanced the antiviral effect of TFV across the range of MPA concentrations (Fig. 5A–C). While synergy was observed against HXB2, MPA contributed in a simply additive inhibition of K65R by TFV (Table 2). TFV and MPA had no

observable effect on cell viability or proliferation as measured by cell counts and MTT assay at concentrations less than 0.5 μM MPA (Fig. 4).

### 3.5. MPA adds little to the inhibition of M184V mutant HIV-1 by 3TC

HIV carrying the M184V mutation displays more than 100-fold decrease in susceptibility to 3TC (Wainberg et al., 1995). We tested the ability of MPA to augment the antiviral effect of 3TC against wild-type HXB2 and HIV-1 encoding the M184V mutation. The clinically utilized dose of 3TC results in steady state peak serum levels of 1.6 μM (Pluda et al., 1995). 3TC was tested across the concentration range of 0–4.3 μM for HXB2. MPA (0–0.5 μM) enhanced the antiviral effect of 3TC across the range of MPA concentrations with a calculated $\alpha$ value of 3.08 (95% C.I. 0.83–5.32) denoting synergy (Fig. 6A). We observed inhibition of M184V by MPA at concentrations $\geq 0.25$ μM. We saw only minimal additional suppression of viral replication of this strain with a concentration up to 4.3 μM for 3TC (Fig. 6B). As the $IC_{50}$ of M184V virus is too high, we could not reliably measure the interaction of 3TC and MPA. While there was no evidence of antagonism between 3TC and MPA, MPA did not appear to significantly augment the effect of the pyrimidine nucleoside analogue 3TC against the 3TC-resistant M148V HIV-1 molecular clone.

Table 2
Parameter estimates and 95% confidence intervals for the $IC_{50}$ and the interaction between TFV and MPA by a fully parametric analysis

| | $IC_{50}$ μM (95% C.I.) | | $\alpha$ (95% C.I) |
|---|---|---|---|
| | MPA | TFV | MPA/TFV |
| HXB2 | 0.47 (0.55–0.38) | 1.23 (1.01–1.46) | 3.23 (1.69–4.78) |
| K65R | 0.17. (0.16–0.18) | 6.37 (6.23–6.52) | 0.07 (0.02–0.12) |
| RTMCY | 0.31 (n.d.) | 3.40 (n.d.) | 0.04 (n.d.) |

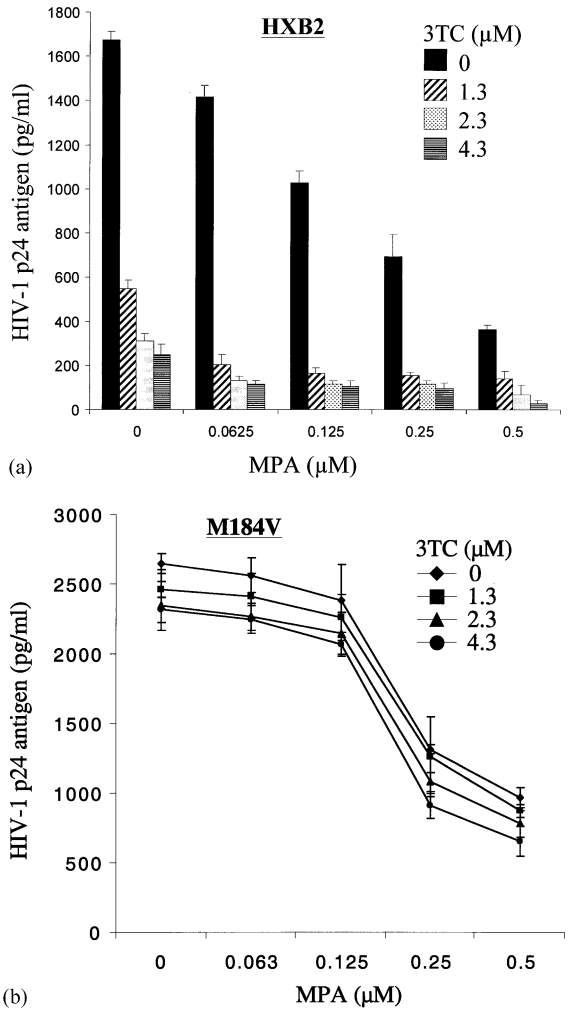


Fig. 6. Effect of MPA on the activity of a pyrimidine NRTIs: a total of $2 \times 10^5$ PHA-stimulated PBMCs from a seronegative donor were infected with: (A) wild-type (HXB2); and (B) 3TC-resistant HIV-1 molecular clone (M184V) and cultured in the presence of varying concentrations of MPA and 3TC. HIV-1 replication was measured as p24 antigen in the culture supernatants on day 7 after infection, the time point at which peak of virus production was observed. Values represent the mean ± standard deviation for triplicate determinations.

## 4. Discussion

We investigated the ability of MPA to augment the antiviral activity of a number of NRTIs against wild type and NRTI-resistant HIV-1. We have previously shown that MPA enhances the potency of ABC against wild type and NRTI-re-

sistant HIV-1 in vitro, at least in part by blocking de novo guanylate biosynthesis. This inhibition leads to depletion of the deoxyguanosine triphosphate (dGTP) pool within the cell and may result in an increase in the intracellular ratio of the active antiviral metabolite of ABC (i.e. carbovir triphosphate) to dGTP. As the isoform of IMP dehydrogenase expressed in activated T-cells has a higher affinity for MPA (Carr et al., 1993), this effect may be selective for such activated T-cells. However, MPA may blunt T-cell activation in vivo (Coull et al., 2001). Therefore, the direct effect of MPA in inhibiting HIV RT may be inextricably linked to its general effect, the blunting of T-cell activation and a non-specific inhibition of HIV replication.

In our previous studies, 0.25 μM MPA restored the ability of ABC to inhibit NRTI-resistant HIV (Heredia et al., 1999). Our observations now demonstrate that MPA alone and in combination with ABC results in inhibition of NRTI-resistant HIV clones at lower MPA concentrations, below that with measurable effect on proliferation. MPA may therefore augment the antiviral activity of selected NRTIs without completely blocking of T-cell proliferation.

A drug combination is deemed synergistic when the effect of dual therapy is greater than the product of the individual drug effects. Synergistic interactions yield $\alpha$ values $\geq 0$. We observed synergy of ABC and DDI in the absence of MPA when tested against wild-type HXB2. Of further interest, MPA/ABC, MPA/DDI and ABC/DDI were all synergistic when tested against multi drug-resistant HIV. The triple combination of MPA, ABC, and DDI displayed a high $\alpha$ value against this multidrug-resistant clone.

When tested against HIV $III_B$, the combination of ABC and MPA was synergistic ($\alpha$ value 8.2) (Margolis et al., 1999). We observed synergy with a smaller $\alpha$ value in our study of ABC and MPA tested against wild-type HXB2. Of interest, the measured $\alpha$ values for MPA in combination with DDI, ABC and DDI without MPA, and the triple combination of ABC, DDI and MPA all illustrated synergy when tested against wild-type HXB2.

A synergistic antiviral effect was observed when MPA and ABC, MPA and DDI, ABC and DDI without MPA, and the triple combination of ABC, DDI and MPA were tested against DRSM34, a NRTI multidrug-resistant HIV. The high $\alpha$ value of 1847 for the triple combination against DRSM34 is likely related to the two-way synergistic interactions of each component of the combination.

MPA augments antiretroviral activity at concentrations 32-fold below that shown to induce apoptosis in culture and fourfold below that found to be antiproliferative (Chapuis et al., 2000). Concentrations up to 10 μM of MPA did not inhibit neutrophil function (Allison et al., 1993), and high concentrations of MPA did not impair IL-2 receptor signaling (Dayton et al., 1992). Therefore, MPA may exert a selective antiviral effect in vivo without complete blockade of normal homeostatic immunological functions.

TFV may play an important role in ART following the development of resistance to first-line agents. As patients eligible for treatment with mycophenolate may also benefit from the use of TFV, it is important to test the interactions of these agents first in vitro. We observed that MPA added in a concentration-dependent fashion to the potential of TFV to inhibit both wild-type HIV and the K65R TFV-resistant mutant, and a NRTI-resistant strain RTMY, similar in genotype to clinical isolates with decreased sensitivity to TFV. We calculated an $\alpha$ value of 3.23 for TFV in combination with MPA for HXB2 HIV, indicating synergy. The confidence interval for the $\alpha$ value against TFV-resistant strains includes 0, suggesting that the effect of MPA on the potency of TFV is additive.

The ability of MPA to augment the activity of NRTIs against NRTI-resistant HIV strains, as expected, was restricted to the 'purine' type of NRTIs. We found that MPA added little to the antiviral activity of 3TC against HIV encoding the M184V mutation. This suggests that the primary antiviral effect of MPA at doses above 0.25 μM is the inhibition of cell proliferation. However, concentrations of MPA lower than 0.25 μM exerted up to 50% inhibition of viral production and decreased MTT activity by 10% or less. This observation implies that at concentrations of 0.25 μM or less, MPA may impair RT function to a greater degree than cell proliferation.

Our findings support the use of ABC, DDI, and TFV in investigational mycophenolate-containing regimens. Access to agents with antiviral activity is particularly important in salvage therapy. Given the recent clinical experience with hydroxyurea, in which antiviral activity came at the cost of toxicity, one must be cautious in interpreting these laboratory studies. The MPA concentrations used herein cannot be directly applied to clinical practice. Small, uncontrolled pilot studies performed in the setting of salvage therapy suggest that 500 mg bid of MMF can decrease viral load (Margolis et al., 2001). Controlled clinical trials are underway to validate the antiviral activity of MMF in salvage therapy, and test the long-term tolerability of MMF in HIV-infected patients.

## Acknowledgements

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