

# Stable HIV Reservoir Despite Prolonged Low-Dose Mycophenolate to Limit CD4+ T-cell Proliferation

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**Background.** The HIV reservoir of latently infected CD4+ T cells represents the barrier to cure. CD4+ T-cell proliferation is a mechanism that sustains the reservoir even during prolonged antiretroviral therapy (ART). Blocking proliferation may therefore deplete the reservoir.

**Methods.** We conducted an unblinded, uncontrolled clinical trial of mycophenolate, a T-cell antiproliferative compound, in people with HIV on chronic suppressive ART. Study drug dose selection was based on calibration to an observed ex vivo antiproliferative effect. The primary outcome was clinically significant reduction ( $>0.25$  log<sub>10</sub>) in the HIV reservoir, measured by total and intact HIV DNA per million T cells in blood over 48 weeks.

**Results.** Five participants enrolled in the trial. Four participants took mycophenolate mofetil (MMF). One had a per-protocol switch to enteric-coated mycophenolate sodium (Myfortic) due to nausea but left the study for personal reasons. One participant developed finger cellulitis, but there were no opportunistic infections. In the 4 participants who completed the protocol, there was no clinically significant reduction in total or intact HIV DNA. There was no change in blood CD4+ T-cell subset composition within the HIV reservoir or the entire CD4+ T-cell population, although total CD4+ T cells decreased slightly in all 4 participants. An ex vivo antiproliferative effect was observed using participant serum obtained 1 hour after dosing, but this effect was severely diminished at drug trough.

**Conclusions.** Mycophenolate given over 48 weeks did not reduce the volume or composition of the HIV reservoir.

**Clinical Trials registration.** NCT03262441.

**Keywords.** HIV reservoir; HIV cure; MMF; clinical trial.

The HIV reservoir consists of a widely anatomically distributed population of latently infected CD4+ T cells usually containing a single molecule of genetically intact, replication-competent HIV DNA integrated into human chromosomal DNA [1–3]. Even after decades of suppressive antiretroviral therapy (ART), the abundance of these cells is only slightly reduced [4–7]. When ART is stopped, viral replication resumes within a small number of latently infected reservoir cells and ultimately spreads throughout the entire body. The HIV reservoir is therefore the barrier to HIV cure.

Substantial evidence suggests that cellular proliferation is fundamental to reservoir stability [8–16]. HIV DNA from the reservoir frequently contains identical sequences within the exact same chromosomal integration site. These clonal sequences are unlikely to have arisen via error-prone HIV viral replication followed by integration into the same chromosomal location within different CD4+ T cells, but instead are likely copied by human cellular DNA polymerase during mitosis. While most HIV proviruses sampled during ART are replication incompetent due to mutation, methylation, or fragmenting, clonal populations of CD4+ T cells with replication-competent, intact HIV proviruses have been observed over protracted periods of time [17–22]. Mathematical modeling demonstrates that most intact and defective HIV DNA sequences detected after 1 year of fully suppressive ART were likely generated by cellular proliferation [23].

Even though CD4+ T cells in the reservoir proliferate every 2–3 months [24, 25], reservoir volume does not expand but instead remains stable over years on ART [4–7]. The frequent CD4+ T-cell proliferation in the reservoir must therefore be balanced by a nearly equivalent cellular death rate. If proliferation could be pharmacologically inhibited without altering this

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natural death rate, then reservoir volume would be expected to decline more rapidly over time [26].

Agents that target lymphocyte proliferation therefore represent a promising approach for HIV cure. Modeling studies based on CD4+ T-cell turnover rates measured in people with HIV [24, 25] suggest that sustained inhibition of proliferation in the absence of compensatory mechanisms that lower the CD4+ T-cell death rate could shrink the reservoir by 100- to 1000-fold in a year [26]. Several small molecular therapies that specifically inhibit lymphocyte proliferation are licensed for the treatment of autoimmune diseases and prevention of graft rejection following transplantation. The most common of these is mycophenolate mofetil (MMF), whose active agent (mycophenolic acid [MPA]) is a reversible, noncompetitive inhibitor of inosine-5'-monophosphate dehydrogenase, an enzyme essential for the synthesis of guanosine-5'-monophosphate in lymphocytes. An enteric-coated formulation of mycophenolic acid, Myfortic, is often prescribed to people who develop nausea or vomiting on MMF [27].

MMF has been safely given to people with HIV on ART [28] and demonstrated possible reservoir reduction. In 1 study, the reservoir, measured by quantitative viral outgrowth assay, decreased by 1–2 logs in 3 of the 6 people who received MMF for 6 months [29]. In a second study, the time to HIV rebound following ART interruption (ATI) was prolonged in participants who had a demonstrated reduction in T-cell proliferation capacity following 4 months of MMF [30]. While the reservoir was not explicitly measured in this study, this result suggests that MMF might have depleted the reservoir. To investigate these preliminary results, we conducted a clinical trial of MMF/Myfortic in individuals on suppressive ART.

## METHODS

### Clinical Trial Design

This open-label, nonrandomized, noncontrolled, pilot clinical trial (NCT03262441) was conducted at the University of Washington (UW), Seattle, AIDS Clinical Trial Unit at Harborview Medical Center. Trial oversight was provided by a local data safety monitoring board. The trial was performed in accordance with the principles of the Declaration of Helsinki and approved by the UW Institutional Review Board (IRB; Study00002182). It fulfilled all 22 items on the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) list (<https://www.cdc.gov/trendstatement/>).

Recruitment occurred through local and online advertisement and with assistance from the Seattle HIV Cure Community Advisory Board. We sought to enroll people with serologically confirmed HIV infection who were on ART for at least 2 years with undetectable HIV including no single event with >1000 HIV RNA copies/mL blood and <40 copies/mL on at least 4 occasions. Inclusion criteria also

included age >17 and <66 years, CD4+ T-cell count >350/mm<sup>3</sup> within the last 365 days, and Karnofsky score >80. Exclusion criteria included active malignancy or myeloproliferative disorder, autoimmune disease, use of other immunosuppressive agent, active opportunistic infection, pregnancy, vomiting or diarrhea, substance abuse, excessive intake of ethanol, medical noncompliance, anemia, leucopenia, thrombocytopenia, liver enzyme elevation, or renal creatine clearance <60 mL/min.

All participants signed informed consent at enrollment. Participant flow is outlined in Figure 1. Adverse events were followed according to the study schedule and recorded. All participants received study drug in the clinic, consisting of a 1-week lead-in of MMF 500 mg once daily followed by MMF 500 mg twice daily for 48 or 96 weeks. Participants who did not tolerate daily MMF during the lead-in due to nausea or vomiting were switched to Myfortic 360 mg daily, which was then increased to twice daily after a week. Study drug was taken at home.

After the first week of twice-daily dosing, all participants underwent an ex vivo total antiproliferation test (TAPT; see

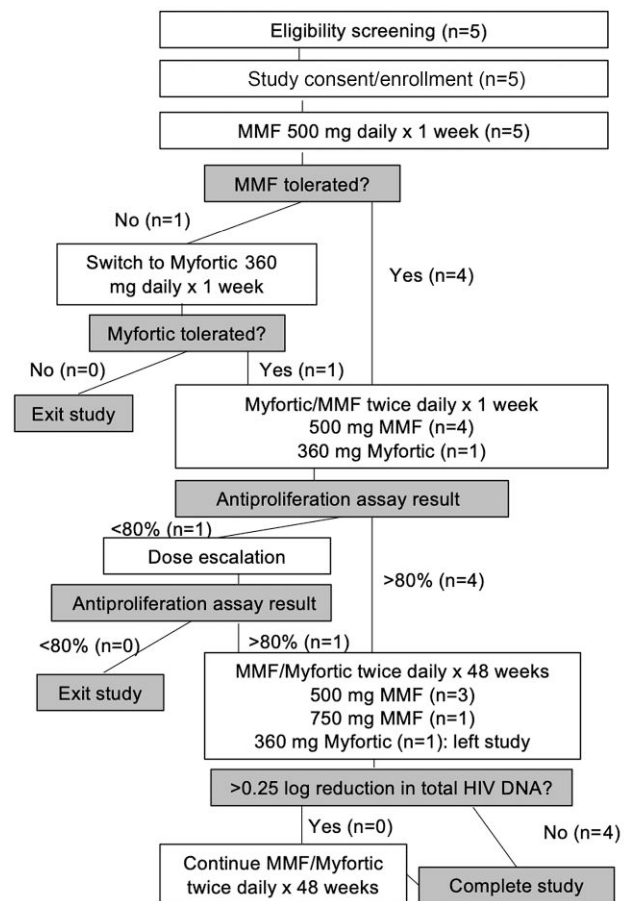


Figure 1. Study flowchart. Abbreviation: MMF, mycophenolate mofetil.

below) at drug peak (60 minutes after receiving study drug) and trough (30 minutes before dosing) to determine the effect of MMF treatment on participants' T-cell proliferation [30]. Based on mathematical modeling estimating the amount of suppression sufficient to reduce reservoir volume [26], continuation in the study required  $\geq 80\%$  suppression of T-cell proliferation at peak levels by TAPT (Figure 1). If  $\geq 80\%$  reduction in proliferation was observed, the participant proceeded to 48 weeks of 500 mg twice daily. If  $< 80\%$  reduction was observed, the dose increased to 750 mg twice daily for 1 week. If  $\geq 80\%$  reduction was observed at 1 week, the participant proceeded to 48 weeks of 750 mg twice daily. If  $< 80\%$  reduction was observed, the participant ended study participation. A similar outline was planned for Myfortic transition from 360 mg twice daily to 540 mg twice daily.

The primary end point was HIV reservoir volume measured by total HIV DNA using droplet digital polymerase chain reaction (ddPCR) per million T cells in blood. The study protocol included a go/no/go assessment at 48 weeks requiring a  $\geq 0.25$  log<sub>10</sub> reduction in HIV DNA. The 0.25 log threshold was surmised to be a biologically relevant reduction relative to historical controls, based on data from studies of reservoir reduction on suppressive ART by Siciliano et al. and Crooks in which few participants achieved this threshold on ART alone [4, 6].

The secondary end points included intact HIV DNA, HIV reservoir subset composition, HIV RNA, peripheral CD4<sup>+</sup> T-cell counts, excess opportunistic infections, and drug-related adverse events. Several exploratory outcomes included TAPT assay outcomes at later time points, proliferation assessed by Ki67 expression, and cytokine measurements. Details on these assays are available in the [Supplementary Methods](#).

#### Patient Consent

The patient's written consent was obtained. The design of the work was approved by the local ethical committees (University of Washington IRB) and conforms to standards currently applied in the United States.

#### Total Antiproliferation Test Assay

We stained cells with a cell division tracking dye, then stimulated them for 96 hours with  $\alpha$ CD3/ $\alpha$ CD28 coated beads in the presence of interleukin (IL)-2 and autologous serum from the corresponding time points. Because this setup tests the combined effects of in vivo mycophenolic acid (MPA)-exposed blood T cells and MPA-containing autologous serum, we termed it the total antiproliferation assay. After culture, we stained the cells with T-cell phenotyping antibodies and analyzed by flow cytometry (details in the [Supplementary Methods](#)).

#### Digital Droplet PCR

We measured the HIV proviral reservoir in genomic DNA from CD4<sup>+</sup> T cells and from central memory, effector memory, and naïve T-cell subsets. We also measured HIV in rectal biopsies. Methods used for isolating and sorting T-cell subsets are described in the [Supplementary Methods](#). We used the Bio-Rad ddPCR platform to run an assay targeting 3 regions in the HIV genome (within *gag*, *pol*, and *env*). This assay provides a good approximation of intact and defective provirus copy numbers, has been validated in a clinical laboratory, and is described in detail in 2 prior publications [31, 32]. A determination of proviral "intactness" by this method requires the detection of all 3 HIV targets in a single droplet. To avoid false negatives from true intact provirus being mechanically sheared and distributed into different droplets, we used a method modified from Wiegand et al. to extract high-molecular weight genomic DNA [33]. For rectal biopsies, we used the adapted Wiegand protocol and a Qiagen QIAamp DNA micro kit. Additional details are available in the [Supplementary Methods](#).

#### MPA Levels

MPA levels were measured by the UW clinical lab using mass spectrophotometry.

#### Statistical Analysis

A small study sample size was selected based on safety considerations and available funding. The unit of statistical analysis was the individual participant. Adequate power was not a prerequisite for this pilot study given that any intervention that substantially reduces the reservoir in even a small number of patients would be a major step forward for the HIV cure field and that funding was not available to support a control group. To account for all data points, we calculated regression slopes of total and intact HIV DNA in blood for all participants and calculated the mean of this outcome.

## RESULTS

#### Study Overview

Among 5 ART-suppressed men with HIV (Table 1) enrolled into the trial between January 2018 and September 2019, 4 tolerated MMF 500 mg daily for 1 week and escalated to 500 mg twice daily in the subsequent week. The single participant who developed nausea switched to 360 mg Myfortic daily, which was well tolerated, and then escalated to twice daily for a week.

After a week of twice-daily MMF or Myfortic therapy, 1 participant on MMF required dose escalation (Figure 1), but all 5 ultimately achieved per-protocol antiproliferative thresholds and progressed to the next step in the study, consisting of 48 weeks of either MMF or Myfortic. The single participant receiving Myfortic de-enrolled from the study for personal reasons soon after initiating study drug and before assessment of the

**Table 1. Study Participant Data**

ID#	Age, y	Sex	Race	Entry CD4/mm <sup>3</sup>	48-wk CD4/mm <sup>3</sup>	HIV RNA, copies/mL	Time on ART/y	Current ART Regimen	MMF Dose
9252	54	M	Caucasian	492	382	ND	16	DTG/FTC/TAF	500 mg bid
8628	60	M	Caucasian	573	460	<7	19	EVT/COBI/FTC/TDF	500 mg bid
9282	26	M	Latino	606	468	ND	5	DTG/RPV	750 mg bid
9232	62	M	Caucasian	799	739	ND	11	TAF/FTC/EVT/COBI	500 mg bid

Abbreviations: ART, antiretroviral therapy; DTG, dolutegravir; EVT/COBI, elvitegravir/cobicistat; FTC, emtricitabine; MMF, mycophenolate mofetil; RPV, rilpivirine; TAF, tenofovir alafenamide.

primary outcome. The remaining 4 participants had undetectable HIV viral loads and high CD4+ T-cell counts at the time of study initiation (Table 1). All 4 completed the trial.

### Total Antiproliferation Test Results

We used the TAPT to calibrate dosing to individual participants. Among the 4 participants who initiated MMF at 500 mg twice daily for 1 week, 3 demonstrated a sufficient reduction of proliferation at peak dosing (Figure 2A). One participant (9282) had inadequate reduction of proliferation (78%, not shown in Figure 2A) at peak dose and required a per-protocol dose escalation to 750 mg twice daily. After 1 week of this higher dose, this participant also met the inclusion criteria, with 98% suppression of T-cell proliferation at peak. While on the higher dose, participant 9282 also had the highest antiproliferative effect (88%) at the drug's trough time point. There was concordance between measured levels of drug (MPA) and the antiproliferative effect observed using the TAPT assay (Figure 2B).

The single participant receiving Myfortic 360 mg twice daily had 99.4% and 91.8% reduction in proliferation at peak and trough before exiting the study.

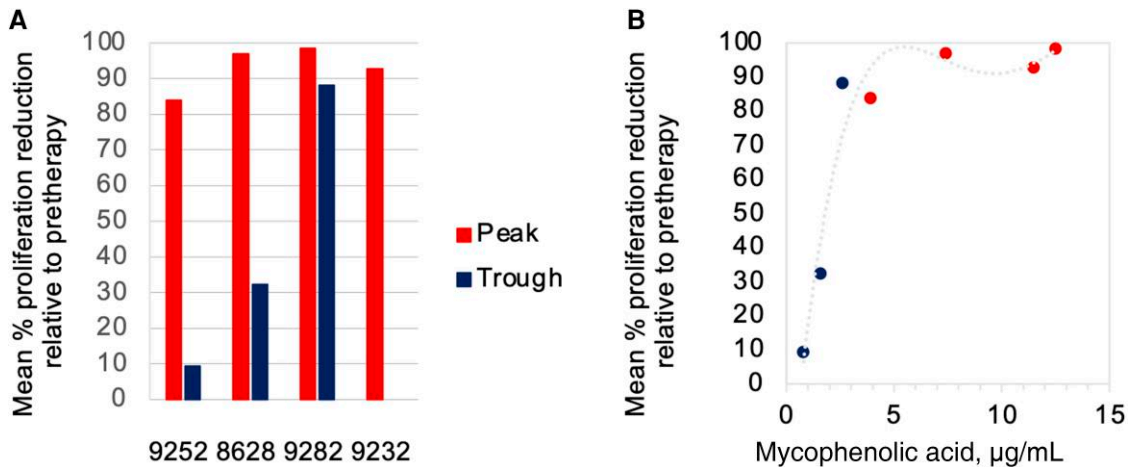
### Limited Impact of MMF on HIV Reservoir Volume

In all 4 participants on MMF, total HIV DNA and intact HIV DNA remained stable during MMF treatment at 12, 24, and 48 weeks after initiation (Figure 3). No participant had the 0.25 log reduction in total HIV DNA required to continue the study after 48 weeks. Participants 9252, 8628, 9282, and 9232 had  $-0.05$ ,  $+0.05$ ,  $-0.10$ , and  $-0.02$  log<sub>10</sub> changes in total HIV DNA volume per million T cells, respectively, over 48 weeks.

In participant 9282, intact HIV DNA was undetectable throughout the study. The remaining 3 participants had no significant decrease in intact HIV DNA levels while on MMF.

To assess the rate of HIV clearance, we fit exponential models to total HIV DNA trajectories separately in each participant. We calculated clearance half-lives of 2.7 years for participant 9282 ( $R^2 = 0.82$ ) and 6.7 years for participant 9252 ( $R^2 = 0.46$ ) and a reservoir doubling time of 3.3 years ( $R^2 = 0.82$ ) for participant 8628. Participant 9232 had stable total HIV DNA levels, precluding calculation of a doubling or halving time. These estimates are like those previously reported for people with HIV treated with ART, suggesting little effect of MMF treatment.

Similarly, we fit exponential models to the intact HIV DNA trajectories. We calculated a clearance half-life of 2.2 years for



**Figure 2.** Antiproliferative effect of MMF in vivo. A, TAPT results from each trial participant demonstrating the degree of antiproliferative effect of patient serum against their own time point–matched CD4+ T cells. Participant 9282 required a dose escalation to achieve >80% reduction in T-cell proliferation at peak (1 h postdose). The result after dose escalation is shown. Participant 9232 did not provide a trough (30 min pre–morning dose) sample. B, Correlation between mycophenolic acid levels and TAPT results. Red dots are peak levels; blue dots are trough levels. The dotted trend line indicates a sharp drop off between inhibition and no inhibition, between 2 and 3 µg/mL. Abbreviations: MMF, mycophenolate mofetil; TAPT, total antiproliferative test.

9252 ( $R^2 = 0.33$ ) and doubling times of 0.6 years ( $R^2 = 0.96$ ) for participant 8628 and 2.2 years ( $R^2 = 0.19$ ) for participant 9232. Participant 9282 had undetectable intact HIV DNA levels, precluding calculation of a doubling or halving time.

Mean regression slopes for total HIV DNA and intact HIV DNA among the 4 participants were  $-0.00033$  (95% CI,  $-0.0020$  to  $0.0014$ ) and  $+0.0024$  (95% CI,  $-0.0030$  to  $0.0078$ )  $\log_{10}$  cell-associated HIV DNA per 1 million T cells per week.

### Impact of Therapy on the Tissue HIV Reservoir

We quantitated the HIV tissue reservoir in rectal mucosal tissue at weeks 0 and 48 in 3 participants (Supplementary Figure 1). Participant 8628 demonstrated a slight increase in total HIV DNA, participant 9252 exhibited stable total HIV DNA levels, and participant 9282 had a nearly 10-fold decrease in total HIV DNA. Regarding intact HIV DNA levels, participant 9252 demonstrated a roughly 2-fold decrease over the course of the study, while participants 8628 and 9282 did not have detectable intact HIV DNA in rectal tissue.

### Impact of Therapy on HIV Reservoir Subset Composition

We hypothesized that because different CD4+ T-cell subsets proliferate at different frequencies, levels of HIV DNA would decrease faster in frequently proliferating subsets such as effector memory ( $T_{EM}$ ) and central memory ( $T_{CM}$ ) CD4+ T cells relative to slower proliferating subsets such as naïve ( $T_N$ ) CD4+ T

cells. However, total and intact HIV DNA levels within each of these subsets were stable over the course of the year (Figure 4).

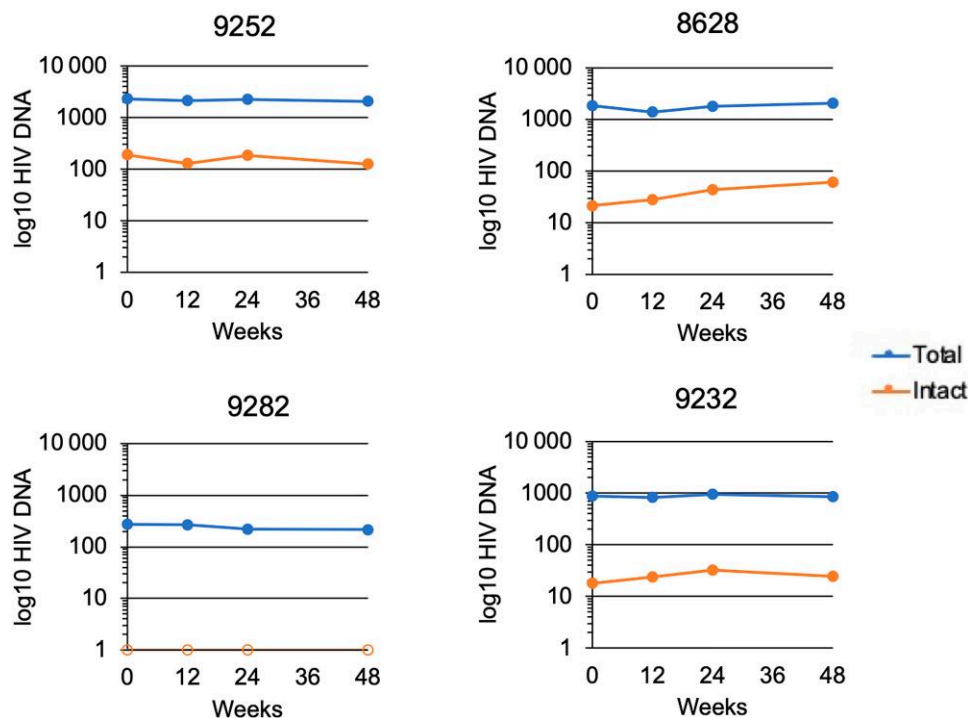
Total and intact HIV DNA levels were generally lower in naïve than memory T cells, as expected. Participant 8628 had a 4-fold reduction in  $T_{CM}$ -associated total HIV DNA between weeks 24 and 48 and transient detectable intact HIV DNA in  $T_{EM}$  at week 24. Participant 9282 had no detectable intact HIV DNA in any subset at any time point.

### Impact of Therapy on CD4+ T-cell Compartment

Total CD4+ T-cell counts decreased slightly in treated individuals ( $P = .007$  by paired  $t$  test) (Table 1), but no participant had a documented CD4+ T-cell count  $<350/\text{mm}^3$ . MMF did not affect the proportion of CD4+ T cells within each memory compartment (Supplementary Figure 2). Overall, we conclude that therapy had no disproportionate effect on any CD4+ T-cell subset composition within or outside of the HIV reservoir.

### Exploratory Analyses to Explain Lack of MMF Efficacy

We explored several possible reasons for the failure of MMF to meaningfully reduce the amount of intact or total HIV DNA or impact subset composition of the HIV reservoir. First, based on lack of a strong antiproliferative effect on CD4+ T cells during times of trough drug levels at trial initiation (Figure 2A and B), we tested serum for antiproliferative effects later in the trial. In participants 9252, 8628, and 9232, trough antiproliferative effects



**Figure 3.** Stable total and intact HIV DNA kinetics in T cells during 48 weeks of MMF therapy. Measurements are per 1 million T cells in blood. Hollow circles signify undetectable. Abbreviation: MMF, mycophenolate mofetil.

were consistently low and substantially less than at peak (Supplementary Figure 3). Participant 9282 had a less consistent pattern, with trough antiproliferative effects often exceeding peak effects, which were consistently lower than in other participants. This result suggests that our dosing protocol failed to achieve adequate, sustained inhibition of CD4+ T-cell proliferation.

We next looked indirectly for evidence of proliferation in vivo by assessing the proportion of CD4+ T cells expressing Ki67 (Supplementary Figure 4). Ki67 levels were generally stable within participants during the trial. One participant (9282) had a significant increase in Ki67 expression at the 24-week time point, which did not coincide with any known illness or symptomatology. This result suggests that MMF did not decrease in vivo proliferation.

Another possibility is that compensatory mechanisms may have countered MMF's antiproliferative effect by decreasing CD4+ T-cell death rate. To test this hypothesis indirectly, we measured interleukin-7 (IL-7) in plasma over time and found that levels were extremely stable (Supplementary Figure 5). IL-15 levels were also stable, while IL-2 levels remained low.

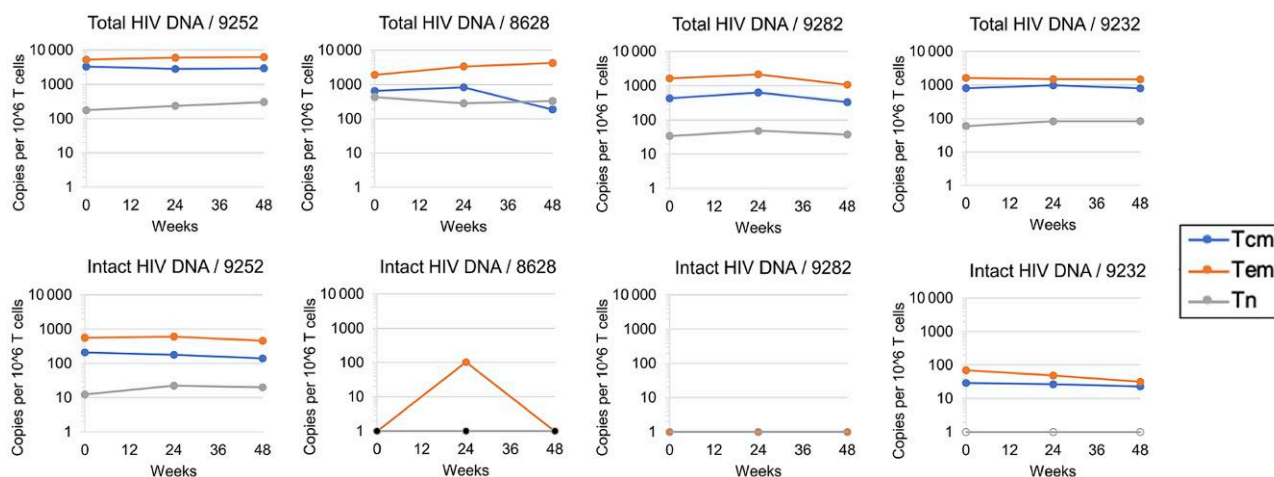
## DISCUSSION

MMF given over 48 weeks failed to reduce either total or intact HIV DNA in the blood of 4 study participants. This outcome suggests that MMF given at the relatively low doses used in our study is unlikely to be a reliable backbone for HIV cure approaches. However, this result does not disprove the widely supported hypothesis that cellular proliferation is important for maintaining the HIV reservoir [11, 16, 17, 19, 22, 23]. This distinction is vital because trials with higher doses of MMF or other antiproliferative strategies might still demonstrate depletion of the HIV reservoir.

We also did not observe a shift in CD4+ T-cell subset composition during ART and MMF treatment, in general or within the HIV reservoir. Our study provides some of the first evidence that these subsets are remarkably stable during chronic ART and likely reflect the immunologic status and age of the infected host [24]. Because antiproliferative therapies would preferentially impact more rapidly proliferating subsets, we recommend that CD4+ T-cell subset composition be considered as an end point for all future studies in which reservoir reduction is an intended outcome.

Our prior work demonstrated an ex vivo effect of MMF against all CD4+ T-cell subsets [26]. We therefore surmise that the drug likely had minimal effect on any subset in our 4 study participants. The drug's mechanism of action suggests that there should also be equal activity against homeostatic and antigen-driven proliferation. Our results are unlikely to be explained by differential effects on these 2 processes.

There are several possible reasons for the lack of observed efficacy of MMF in our study. One might relate to the pharmacokinetics of MMF. A small study in which participants on ART received MMF for 6 months demonstrated a reduction in cells harboring replication-competent HIV [29]. MMF-treated participants in another small study had delayed time to viral rebound following ART interruption [30]. These results conflict with ours and suggest that there may be a threshold level below which MMF rapidly loses its effect. We demonstrated low trough antiproliferative activity in all study participants and variable peak activity throughout the study. In addition, Ki67, an indicator of in vivo proliferation, was unaffected by MMF treatment despite ex vivo evidence that mycophenolic acid lowers Ki67 expression [34]. Our mathematical modeling suggests that an antiproliferative effect must be potent and sustained to achieve true reservoir reduction [26]. Thus,



**Figure 4.** Stable per capita total and intact HIV DNA kinetics within CD4+ cell subsets during 48 weeks of MMF therapy. Hollow circles are undetectable. Abbreviations: MMF, mycophenolate mofetil; Tcm, central memory CD4+ T cells; Tem, effector memory CD4+ T cells; Tn, naïve CD4+ T cells.

inadequate inhibition of CD4+ T-cell proliferation may explain the lack of efficacy we observed.

In addition, we did not measure drug levels or activity in target tissues, and discrepancies between drug levels or activity in whole blood and lymphatic tissues may be critical. That there was 1 participant who had a substantial reduction in total HIV DNA in the rectal compartment may suggest that this mechanism is important, though substantially more evidence is needed to assess this possibility. A single participant who did not complete the study on Myfortic had very high trough inhibition of proliferation, which may suggest that Myfortic, likely due to its extended-release formulation, has greater potential to achieve HIV reservoir reduction. In summary, it is possible that higher dosages, or more even release kinetics of active drug, might have resulted in the predicted levels of reservoir reduction.

There are also possible physiological reasons that MMF failed to reduce the volume of the HIV reservoir in our study. MMF is an effective agent for limiting graft rejection following transplantation as well as recurrences of rheumatologic diseases [35, 36]. Given that the drug works via a single mechanism, it is likely that its antiproliferative effect underlies its efficacy. However, lymphopenia is not a predictable or common side effect of MMF, which suggests that B and T lymphocytes may have compensatory mechanisms to maintain stable numbers despite the prevention of both homeostatic and antigen-driven proliferation. We looked for this indirectly by measuring IL-7 but observed no therapy-induced differences [37]. This does not rule out the possibility of a compensatory effect.

It is possible that MMF had more specific effects on individual CD4+ T-cell clones in the reservoir that expand and contract at much different rates and have been demonstrated to wax and wane over time [17]. Future antiproliferation studies should consider secondary end points based on analysis of the full genome, integration site, and/or T-cell receptor sequencing to assess whether the reservoir clonal structure is impacted by prolonged lymphocyte antiproliferation therapy. For an antiproliferative strategy to be acceptable in the clinic, there would need to be a substantial reduction in reservoir volume without significantly decreasing total CD4+ T-cell counts. A prerequisite for this outcome is that HIV is disproportionately integrated into cells that proliferate more frequently than the uninfected CD4+ T-cell population, as has been suggested by several studies [12, 18, 24, 38]. Truly assessing this would require a longitudinal analysis of the CD4+ T-cell subset and clonal composition of the reservoir.

Another consideration for the antiproliferative approach is that a reduction in reservoir volume may not be permanent. Following myeloablative conditioning during stem cell transplant, there is a massive observed reduction in HIV reservoir volume that is sometimes but not always followed by a re-expansion in levels of intact and total HIV DNA [39]. Similar

phenomena might occur following lymphocyte antiproliferative therapy. In future trials, it will be important to follow reservoir dynamics in participants for many months after treatment is stopped.

Our study has notable limitations. First, it was a small, uncontrolled pilot trial with only 4 participants. Our results may not be generalizable, and therapeutic efficacy may have been demonstrated with a larger prospective cohort. Had we observed a meaningful reduction in reservoir volume, a larger controlled study would have been proposed. Second, we did not obtain lymph node samples during the study and therefore may be missing key pharmacokinetic and immunologic observations. Finally, study participants were all men, and we cannot rule out the possibility of different results in a gender-balanced study.

## CONCLUSIONS

In conclusion, low-dose MMF did not reduce or alter the composition of the HIV reservoir when given for almost a year despite strong evidence that cellular proliferation maintains the reservoir. Further studies using higher doses, extended-release formulations, or other combinations of T-cell antiproliferative agents are warranted.

## Supplementary Data

**Supplementary materials** are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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**Potential conflict of interest.** J.T.S., C.L., S.M.H., U.P., M.P., K.R.J., H.Z., K.P., E.H., R.D.H., and F.H. report no conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**Author contributions.** J.T.S. conceived the study, designed the protocol, enrolled participants, followed participants, and wrote the manuscript. C.L. and S.M.H. quantified intact and total HIV DNA within CD4+ T-cell subsets and performed other T-cell assays. U.P. performed cytokine quantitation assays. M.P., K.P., E.H., and R.D.H. assisted in participant enrollment, participant follow-up, protocol design, and drug dispensation. F.H. conceived the study, designed the protocol, supervised all laboratory analyses, and wrote the manuscript.

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