Presentation: 0340
Cure Strategies: Humans and Animal Studies – P-E06
2:30pm-4:00pm. Tuesday March 10, 2020

Mycophenolate Mofetil for Depletion of the HIV Reservoir

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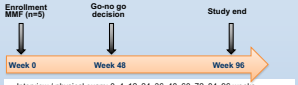
Introduction

- The HIV reservoir consists of ~1-10 million latently infected cells containing intact, replication-competent virus, which persists despite decades of ART.[1,2]
- A 100-1000 fold larger number of cells contain non-intact, replication incompetent HIV DNA.[3,4]
- HIV integrates into a unique chromosomal location within each newly infected cell.[5]
- The detection of multiple infected cells with the same viral sequence and / or chromosomal integration site demonstrates that many cells in the reservoir were generated via cellular proliferation rather than new viral replication events.[6,9]
- Mathematical modeling suggests that >99% of latently infected cells are members of proliferative clones.[10]
- Central memory CD4+ T cells proliferate every ~1-2 months in persons with and without HIV, while naïve CD4+ T cells proliferate every 500 days.[11-12]
- Cellular proliferation may therefore be a viable therapeutic for reservoir reduction.
- Mathematical models project a 5-10-fold reduction in CD4+ lymphocyte proliferation sustained over a year may lower the reservoir by > 2 logs.[13]
- Mathematical models project that a 5-10-fold reduction in CD4+ lymphocyte proliferation for a year may shift the reservoir from central (T_{CM}) and effector memory (T_{EM}) to naïve (T_N) T cell predominant.[13]
- A prior clinical trial demonstrated a > 1 log reduction in reservoir volume, as well as a ~50% reduction in proliferation markers (Ki67+) in circulating CD4+ T cells, after 24 weeks of mycophenolate mofetil (MMF) 1 g twice daily, in 3 of 6 participants.[14]
- Another trial utilizing MMF 250 mg twice daily was associated with a delay in time to viral rebound following ART analytical treatment interruption (ATI) among documented responders to the drug.[15]

Methods

Trial Design

- Phase 2 open-label study of MMF for reduction of the HIV reservoir (NCT03262441)
- Planned enrollment n=5
- University of Washington, Seattle ACTU / Harborview Medical Center
- One week lead in of MMF 500 mg daily followed by MMF 500 mg twice daily for 48 or 96 weeks
- Inclusion criteria of documented 80% anti-proliferative effect *ex vivo* at peak drug levels (see below)
- Frequent safety labs and assessment of reservoir size **(Fig 1)**
- Go-no go assessment at 48 weeks based on at least a >0.25 log 10 reduction in HIV DNA



- Interview / physical exam: 0, 4, 12, 24, 36, 48, 60, 72, 84, 96 weeks
- Safety labs: 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 , 28, 32, 36, 40, 44, 48, 60, 72, 84, 96 weeks
- Anti-proliferative assays: 0 (inclusion test), 4, 24, 48, 96 weeks
- Plasma RNA viral load: 0, 4, 8, 12, 16, 24, 36, 48, 72, 96 weeks
- MMF PK: 0, 1, 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96 weeks
- TREC levels: 0, 4, 24, 48, 96 weeks
- HIV DNA ddPCR: 0, 4, 8, 12, 16, 24, 36, 48, 72, 96 weeks
- Rectal biopsies for reservoir assays: 0, 48 & 96 weeks

Fig 1. Study schema.

Participation

- Serologically and virologically confirmed HIV-1 infection
- Continuous fully suppressive ART for > 2 years
- CD4+ T cell count > 350/mm^3
- Documentation of anti-proliferative effect at peak MMF dose (see below)
- No malignancy, autoimmune disease, prior diagnosis of AIDS, active infection, substance abuse, medical non-compliance, abnormal lab values
- No proton pump inhibitors, estrogen or progestin contraceptives or other interacting medicines.
- No pregnancy / intention to become pregnant / breast feeding

Trial oversight

- Local DSMB.
- Trial sponsor: amfAR, The Foundation for AIDS Research
- Performed in accordance with the principles of the Declaration of Helsinki
- Approved by the University of Washington IRB

Methods *continued*

End points

- HIV reservoir size measured by total and intact HIV DNA using ddPCR
- HIV reservoir subset composition including effector memory CD4+ T cells (T_{EM}) and central memory CD4+ T cells (T_{CM}) and naïve CD4+ T cells (T_N)
- HIV RNA
- Peripheral CD4+ T cell counts
- Excess opportunistic infections
- Drug-related adverse events

Documentation of anti-proliferative effect of MMF

- Reduction in CD4+ T cell proliferation 1 hour following MMF dose
- Assessed by total antiproliferative test (TAPT) assay (see below)
- Decision tree:
  - Day 7 MMF 500 mg orally twice daily: test 1-hour post MMF serum against participant PBMCs
    - If >80% reduction in proliferation on 500 mg twice daily, then proceed to one year of 500 mg twice daily
    - If <80% reduction in proliferation on 500 mg twice daily, then increase to 750 mg twice daily and re-test in one week
  - Day 7 MMF 750 mg orally twice daily: test 1-hour post MMF serum against participant PBMCs
    - If >80% reduction in proliferation on 750 mg twice daily, then proceed to one year of 750 mg twice daily
    - If <80% reduction in proliferation on 750 mg twice daily, then end participation in study

Assays

- *TAPT:*
  - Serum and PBMCs collected at peak and trough dosing
  - Proliferating cells gated based on CellTrace Violet kit (Invitrogen)
  - Stimulation with anti-CD3/CD28 beads at 1:1 bead:cell ratio
  - Gating into proliferation based on CellTrace Violet fluorescence
  - Reduction in proliferation = 1 – (proliferation in presence of patient serum / proliferation in absence of patient serum)
- *ddPCR:*
  - T cells per uL estimated with 5'RPP30 early target and deltaD target
  - Primer targets = *5'pol, gag, env*
  - 6 replicates per time point
  - Data processing with QuantaSoft AP
  - See poster 00311 for details
- *Flow cytometry / sorting:*
  - Whole PBMCs thawed -> CD4+ T cells selected by negative selection and sorted
  - Surface stains: CD45RA, CCR7, CD3, CD4, CD8, Live/dead, CCR7
  - Permeabilized and stained for Ki67
  - Fluorescence quantification on BD Biosciences LSRII
  - Cells pelleted for ddPCR
- *Mesoscale discovery:*
  - Analyses for cytokine detection and concentration on plasma
  - Low CVs (<30%) identified with all replicates

Results

Enrollment

- 5 enrolled participants
- One participant self discharged early during the trial for personal reasons.

ID#	Age, Years	Sex	Race/ Ethnicity	Entry CD4/ mm^3	48 week CD4/ mm^3	HIV RNA copies/ml	Time on ART/ years	Current ART Regimen	MMF dose
9252	54	M	Caucasian	492	382	Undetectable	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	Undetectable	5	DTG/RPV	750 mg bid
9232	62	M	Caucasian	799	739	Undetectable	11	TAF/FTC/E VT/COBI	500 mg bid

Table 1. Study participant data.

TAPT assay results

- 3 of 4 participants met "go" criteria" for continuation at 500 mg twice daily
- One participant (9282) had inadequate inhibition of proliferation at drug peak (78%) and was boosted to 750 mg twice daily; he met continuation criteria at that dose with higher trough inhibition of cellular proliferation **(Fig 2)**
- Participant 9232 did not have a TAPT performed during drug trough

Results *continued*

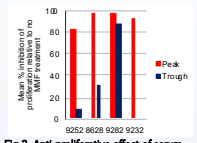


Fig 2. Anti-proliferative effect of serum against contemporaneously sampled CD4+ T cells.

MMF tolerability and toxicity

- Well-tolerated with no nausea, vomiting or dyspepsia
- One SAE (9252): post-operative finger cellulitis requiring one-week oral antibiotics
- HIV RNA suppression throughout the study
- Peripheral CD4+ T cell counts stable during the study

HIV reservoir kinetics

- Study stopped in all 4 participants due to no reduction in reservoir at 48 weeks
- Total HIV DNA and intact HIV DNA levels stable in all participants **(Fig 3)**
- Intact HIV DNA undetectable at all time points in one participant (9282) **(Fig 3)**

Uninfected and infected CD4+ T cell subset kinetics

- No impact on overall proportion of T_{EM}, T_{CM} or T_N among total circulating CD4+ T cells **(Fig 4)** or CD8+ T cells (not shown)
- No persistent effect on proliferation marker expression (Ki67+) in circulating T_{EM}, T_{CM} or T_N CD4+ T cells **(Fig 5)**
- Per capita total HIV DNA and intact HIV DNA levels stable in T_{EM}, T_{CM} or T_N **(Fig 6)**.

Exploratory analyses

- MPA levels predict results of TAPT assay: 2 of 3 troughs sub-therapeutic **(Fig 7)**
- No effect of MMF on cytokines related to T cell proliferation **(Fig 8)**

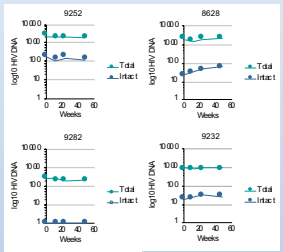


Fig 3. Stable total and intact HIV DNA kinetics during 48 weeks of MMF therapy. Hollow circles are undetectable.

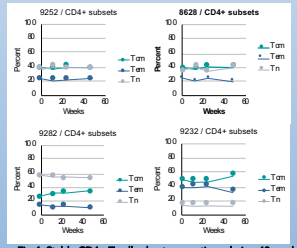


Fig 4. Stable CD4+ T cell subset proportions during 48 weeks of MMF therapy.

Results *continued*

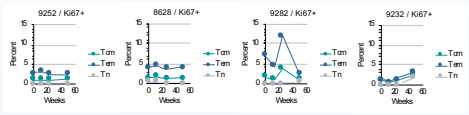


Fig 5. Lack of persistent change in Ki67+ expression in CD4+ T cell subsets (bottom row) during 48 weeks of MMF therapy.

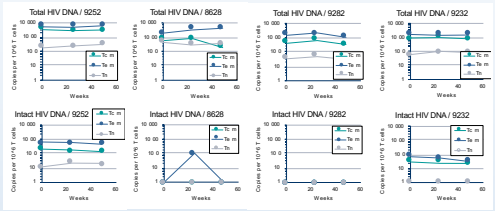


Fig 6. Stable per capita total and intact HIV DNA kinetics within CD4+ T cell subsets during 48 weeks of MMF therapy. Hollow circles are undetectable.

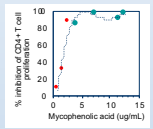


Fig 7. Correlation between mycophenolic acid levels and TAPT results. Blue dots are peak levels; red dots are trough levels. Trend line indicates a sharp drop off between inhibition and no inhibition between 2 and 3 ug/mL *ex vivo*.

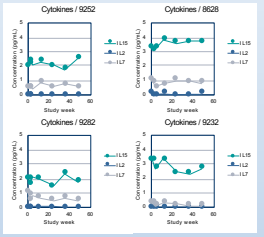


Fig 8. Stable cytokine levels during 48 weeks of MMF therapy.

Discussion

- 48 weeks of MMF well tolerated and not associated with virologic or immunologic failure on ART
- No reduction in total or intact HIV DNA
- No shift in the HIV reservoir towards a predominance of slowly proliferating naïve CD4 T cells
- An explanation for lack of reservoir reduction and decrease in proliferation *in vivo* not identified
- Sub-therapeutic drug levels may explain lack of efficacy
  - MMF anti-proliferative effect at trough was minimal in 2/3 tested participants
  - MMF has a steep dose response curve with a sharp cut-off between potent and absent effect[14]
  - Past studies showed variable effect of MMF on reservoir volume and Ki67 expression[15]
  - Modeling suggests >50% reduction in proliferation is required to achieve reservoir reduction[14]
- Other possible explanations: include compensatory survival mechanisms in non-proliferating CD4+ T-cells, compensation with thymic emigrants or drug resistance
- Future studies with higher doses are warranted

References

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