**ABSTRACT**

Background: The mechanistic target of rapamycin (mTOR) promotes HIV transcription. In line, we demonstrated that HIV preferentially targets gut-homing CCR6+Th17 cells for replication/persistence via mTOR-dependent mechanisms. Thus, mTOR inhibitors may limit residual HIV transcription during ART and subsequently reduce immune activation/inflammation. Here, we report results from a clinical trial conceived to evaluate the effect of 12 weeks of metformin (mTOR inhibitor) therapy on the size of HIV reservoirs (primary objective) and immune activation (secondary objective) in ART-treated HIV-infected adults (HIVART).

Methods: Metformin (850 mg bid) was administered orally for 12 weeks in n=22 HIV-ART. Participants were non-diabetic, on ART for >3 years, with <40 HIV-RNA copies/ml plasma for >3 months, and CD4/CD8 ratios ≤0.7. Blood was collected at baseline (Visit 1), after 12 weeks of metformin (Visit 2), and 12 weeks after metformin discontinuation (Visit 3). Sigmad colon biopsies (+32 biopsies/participant) were collected at Visits 1-2 from n=13 participants. HIV-DNA was quantified by real-time nested PCR. The chemotherapy-dependent HIV was quantified by viral outgrowth assay (VQA). Matched blood/colon memory CD4+ T-cells were analyzed for surface/intracellular molecule expression and simultaneously sorted by flow cytometry (BD AriaII). Plasma soluble factors were quantified using R&D Systems Multiplex Assay and ELISA.

Results: Metformin was well tolerated. Total HIV-DNA levels in blood/colon CD4+ T-cells and the frequency of blood/colon CD4+ T-cells carrying replication-competent HIV was stable between Visits 1-2. However, investigations on matched blood/colon samples revealed a positive effect of metformin as reflected by a decrease in the frequency of CD4+ T-cells in the colon (median: 7.3% vs 4.7%; Visit 1 vs 2: p=0.015), indicative of reduced HIV replication. A decreased mTOR phosphorylation (p-S6) and phosphorylated AMPK (Thr172) in the colon (median: 13.0% vs 7.9%; Visit 1 vs 2: p=0.0087) demonstrated a trend to decreased expression of the HIV co-receptors CCR5 and CXCR4, and increased expression of the HIV replicative factor SAMHD1 in colon CD4+CD4+ T-cells, and a decreased sCD14 plasma levels (mean: 1.893 vs 1.519 ng/ml; Visit 1 vs 2: p=0.03).

Conclusion: This pilot study reveals metformin-mediated benefits in controlling inflammation, in part via mTOR regulation, and prompts us further to investigate the immunological/cytokine benefits of long-term metformin supplementation in HIV-ART individuals.

**RESULTS**

Figure 1: Metformin treatment reduces colon infiltration of CD4+ T-cells with no impact on the expression of classical activation markers

Figure 2: Metformin reduces mTOR phosphorylation in colon-infiltrating T-cells, most robustly in CC56+CD4+ T-cells.

Figure 3: Metformin treatment promotes changes in the expression of molecules associated with cell survival, HIV permissiveness, and gut-homing in colon-infiltrating CC64+CD4+ T-cells.

Figure 4: Levels of Gag HIV-DNA remain stable in memory CD4+ T-cells from peripheral blood and colon biopsies upon 12 weeks of metformin treatment.

Figure 5: Level of integrated HIV-DNA and the frequency of carrying transcriptionally inducible HIV (TIA) in peripheral blood CD4+ T-cells remain stable upon 12 weeks of Metformin treatment and 12 weeks of subsequent follow-up.

Figure 6: Metformin treatment is associated with the expression of plasma markers of systemic inflammation and gut barrier dysfunction.

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Delphine Planas, PhD Student
CRCHUM Research Centre, Montreal, QC, Canada
Delphine.Planas@crchum.ulaval.ca

Rosalie Ponte, Rosalie Ponte
CRCHUM Research Centre, Montreal, QC, Canada
Rosalie.Ponte@crchum.ulaval.ca

Amélie Pagliuzzi, Amélie Pagliuzzi
CRCHUM Research Centre, Montreal, QC, Canada
Amélie.Pagliuzzi@crchum.ulaval.ca

Augustine Fert, Augustine Fert
CRCHUM Research Centre, Montreal, QC, Canada
Augustine.Fert@crchum.ulaval.ca

Laurence Marchand Raymond, Laurence Marchand Raymond
CRCHUM Research Centre, Montreal, QC, Canada
Laurence.Raymond@crchum.ulaval.ca

Annie Gosselin, Annie Gosselin
CRCHUM Research Centre, Montreal, QC, Canada
Annie.Gosselin@crchum.ulaval.ca

Franck Dupuy, Franck Dupuy
CRCHUM Research Centre, Montreal, QC, Canada
Franck.Dupuy@crchum.ulaval.ca

Vikram Mehraj, Vikram Mehraj
CRCHUM Research Centre, Montreal, QC, Canada
Vikram.Mehraj@crchum.ulaval.ca

Sylvie Lesage, Sylvie Lesage
CRCHUM Research Centre, Montreal, QC, Canada
Sylvie.Lesage@crchum.ulaval.ca

Maged Peter Ghali, Maged Peter Ghali
CRCHUM Research Centre, Montreal, QC, Canada
Maged.Peter.Ghali@crchum.ulaval.ca

Jonathan B. Angel, Jonathan B. Angel
CRCHUM Research Centre, Montreal, QC, Canada
Jonathan.B.Angel@crchum.ulaval.ca

Eric A. Cohen, Eric A. Cohen
CRCHUM Research Centre, Montreal, QC, Canada
Eric.A.Cohen@crchum.ulaval.ca

Nicolas Chomont, Nicolas Chomont
CRCHUM Research Centre, Montreal, QC, Canada
Nicolas.Chomont@crchum.ulaval.ca

Jean-Pierre Routy, Jean-Pierre Routy
CRCHUM Research Centre, Montreal, QC, Canada
Jean-Pierre.Routy@crchum.ulaval.ca

Petronela Anca, Petronela Anca
CRCHUM Research Centre, Montreal, QC, Canada
Petronela.Anca@crchum.ulaval.ca

**REFERENCES**

1. Department of microbiology, immunology and molecular genetics, University of Montreal, Montréal, QC, Canada.
2. HUM Research Centre, Montreal, QC, Canada.
3. Division of Gastroenterology and Hepatology, McGill University, Montréal, QC, Canada.
4. Ottawa Hospital Research Institute, Ottawa, ON, Canada.
5. Department of medicine, The Ottawa Hospital, Ottawa, ON, Canada.
6. Institut de Recherches Cliniques de Montréal, Montréal, QC, Canada. Equal contribution.