Background: The mechanistic target of rapamycin (mTOR) promotes HIV transcription. In line, we demonstrated that HIV preferentially targets guanine-rich CC68/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). Sigmoid colon biopsies (+32 biopsies/participant) were collected at Visits 1-2 from n=13 participants. HIV-DNA was quantified by real-time nested PCR. CD8+ T-cell replication-competent HIV was quantified by viral outgrowth assay (VOA). 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 ELSA.

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 HIV-DNA CD4+ T-cells in the colon (median: 7.3% vs 4.7%, p=0.015), significant reduction in correlated inflammation, and decreased mTOR phosphorylation in CD68+ T-cells (median: 13.0% vs 7.9%, p=0.0087), CD8+ T-cell frequency decreased by 3.1% (p=0.03). A lower frequency of CD8+ T-cell expression of the HIV coreceptors CD54 and integrin β7, and increased expression of the HIV restrictive factor SAMHD1 in colon CD68+CD4+ T-cells, and decreased sCD14 plasma levels (mean: 1,893 vs 1,517 ng/ml, p=0.03) at Visit 2 vs Visit 3.

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.

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 CD68+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 CD68+CD4+ T-cells.

Figure 4: Metformin treatment does not change the frequency of naive versus memory subsets within peripheral blood CD4+ and CD8+ T-cells.

Figure 5: 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 6: Level of integrated HIV-DNA and the frequency of cells carrying transcriptionally active HIV (TNA) in peripheral blood CD4+ T-cells remain stable upon 12 weeks of Metformin treatment and 12 weeks of subsequent followup.

Figure 7: Metformin treatment is associated with multiple changes in the expression of plasma markers of systemic inflammation and gut barrier dysfunction.

Table 1: Clinical information of study participants.

Table: Clinical information of study participants. TCR, T cells; CD4, CD8 T; % Naive, % of naive; % Total, % of total; % Viral load; HIV RNA copies per mL plasma; ND, not detected; ART, antiretroviral therapy; NA, information not available. *p, years.