JAK INHIBITORS TOFACITINIB AND RUXOLITINIB EMPLOY A NOVEL MECHANISM TO BLOCK T-CELL ACTIVATION MEDIATED HIV REPLICATION

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Background

Despite the successful suppression of plasma viremia, persistent immune activation and inflammation in HIV infection is associated to HIV persistence and increased morbidity. Several gamma-c (γc) cytokines (IL-2, IL-7, IL-15) upstream of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathways can influence HIV infection in various target cells **(Figure 1)**. IL-7 is associated with reservoir size in successfully treated individuals through the mechanism of homeostatic proliferation of central and transitional memory CD4+ T-cells, maintaining a stable pool of latently infected cells. In addition, cytokines IL-2, IL-7 and IL-15 may contribute to HIV persistence by promoting survival and/or proliferation of latently infected cells during antiretroviral treatment. These cytokines have also been shown to induce expression of the negative regulator PD-1, a maker of CD8+ T-cell dysfunction and is associated with CD4+ T-cells harboring integrated proviral HIV DNA. Tofacitinib and ruxolitinib are FDA-approved JAK1/2 inhibitors for treatment of rheumatoid arthritis and myelofibrosis, respectively.

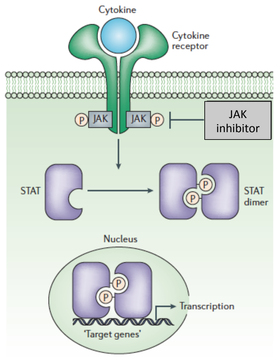


Figure 1. Janus kinase/signal transducer of activated transcription pathway (Adapted from *Nat Rev Drug Discov.* 2006 Jul;5(7):549-63)

We evaluated tofacitinib and ruxolitinib for their ability to block cytokine-mediated activation of JAK/STAT signaling pathway(s), T-cell activation and viral replication in HIV-infected primary human lymphocytes.

Ruxolitinib and Tofacitinib Inhibit γc Receptor Cytokine Induced Stat5 Phosphorylation and Bcl-2 Expression

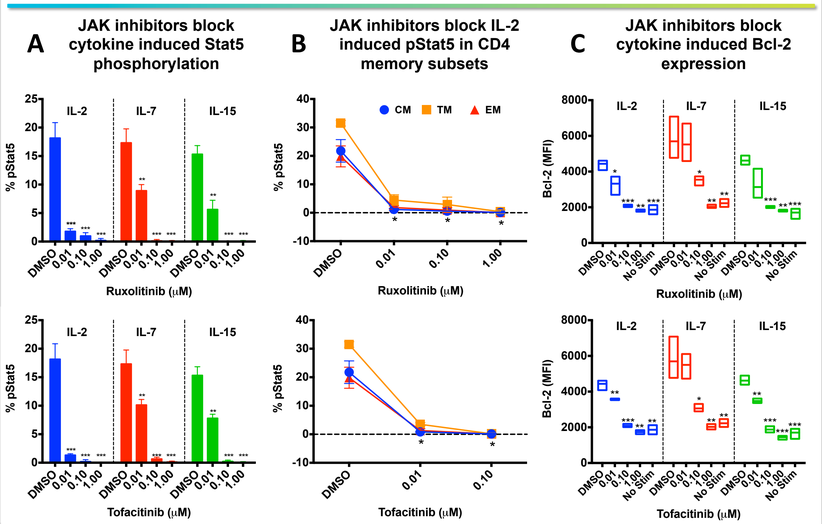


FIGURE 2. STAT5 phosphorylation (pY694) in CD4 cells **(A)** and CD4 memory subsets **(B)** measured by FCM in PBM cells from 3 healthy subjects after 15 minute stimulation with 50 ng/mL IL-2 (A and B), 2 ng/mL IL-7 or 5 ng/mL IL-15 and 0.01, 0.1 and 1 μM ruxolitinib, tofacitinib or DMSO. **(C)** Bcl-2 expression in CD4 cells measured by FCM in PBM cells from 3 healthy subjects after 6 day culture in the presence of 100 ng/mL IL-2, 5 ng/mL IL-7 or 10 ng/mL IL-15 and 0.01, 0.1 and 1 μM ruxolitinib, tofacitinib or DMSO. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Ruxolitinib and Tofacitinib Reduce HIV Production and Replication

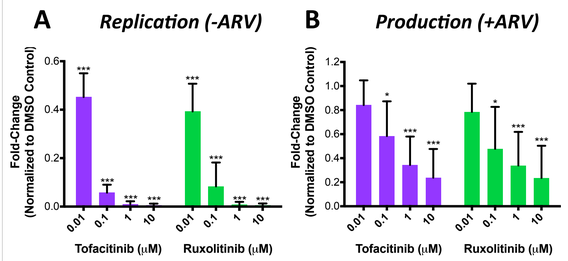


FIGURE 3. (A) Viral production/replication measured by p24 ELISA in primary CD4 cells isolated from 3 viremic donors stimulated 6 days with anti-CD3/28 in the presence of 0.01-20 μM tofactiinib, ruxolitinib or DMSO; **(A)** without antiretrovirals (ARV) or **(B)** with ARVs (180 nM zidovudine, 100 nM efavirenz, 200 nM raltegravir).

Ruxolitinib and Tofacitinib Inhibition of anti-CD3/CD28 Induced Activation, Proliferation and Survival of CD4 T Lymphocytes

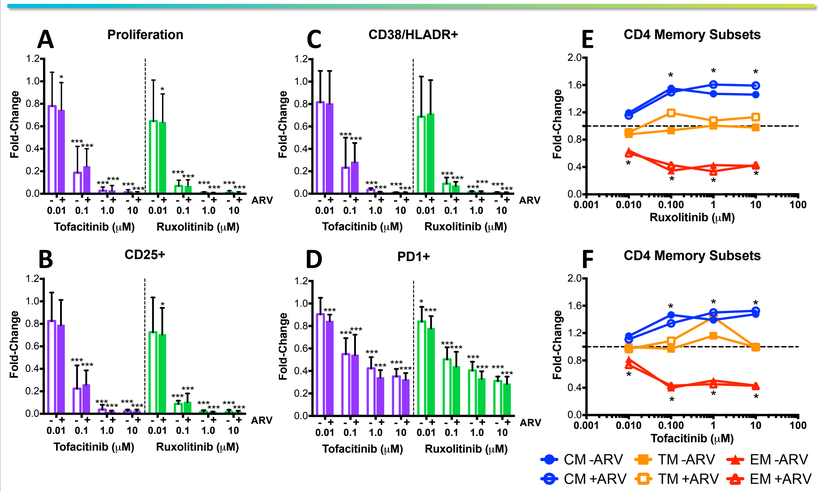


FIGURE 4. Activation (CCR5, CD25, CD38, HLA-DR, PD-1) and proliferation (CellTrace™ Violet) markers as measured by FCM in primary CD4 cells **(A-D)** and CD4 memory subsets **(E-F)** isolated from 5 viremic donors, stimulated for 6 days with anti-CD3/28 +/- ARV as in Figure 3 and 0.01-10 μM ruxolitinib, tofacitinib or DMSO. Percent cells were normalize to DMSO control and graphed as a fold-change. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Exogenous Addition of IL-2 Overcomes JAK Mediated Inhibition

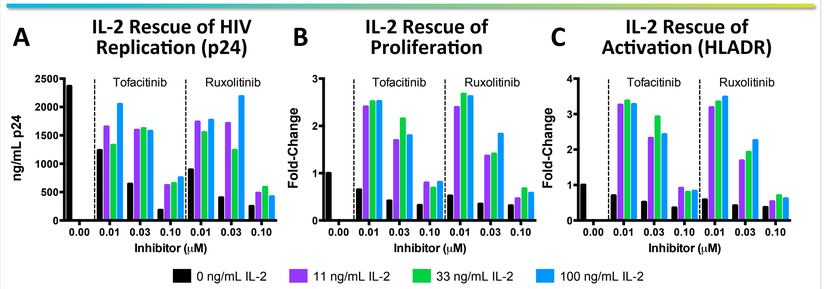


FIGURE 5. (A) Viral replication measured by p24 and **(B)** proliferation (CellTrace™ Violet) and **(C)** activation (HLA-DR) as measured by FCM in primary CD4 cells isolated from a viremic donor, stimulated for 6 days with anti-CD3/28, 0-100 ng/mL IL-2 and 0.01-10 μM ruxolitinib, tofacitinib or DMSO. Percent cells were normalize to DMSO control and graphed as a fold-change for **B** and **C**.

Ruxolitinib Inhibits Bystander Infection of Target Cells

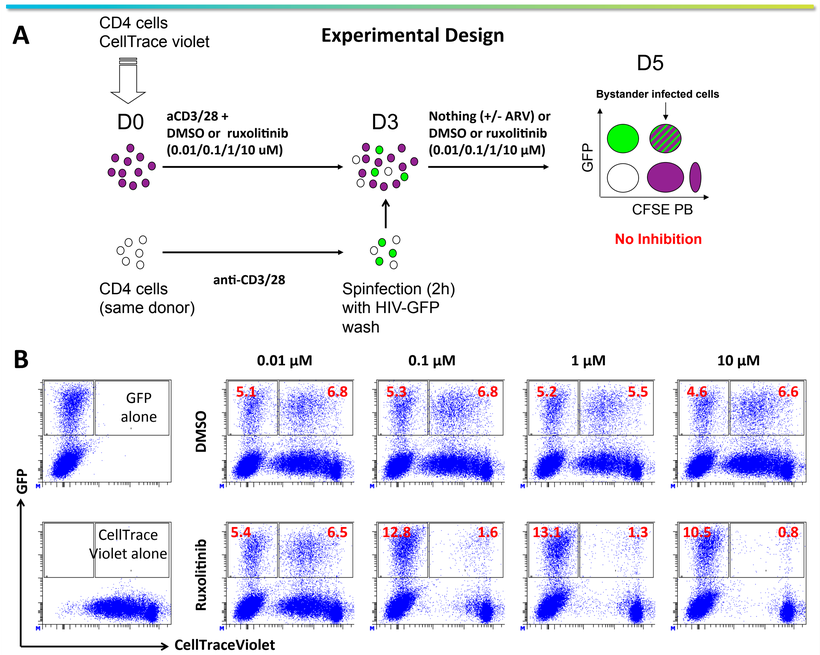


FIGURE 6. (A) Schematic of experimental design. **(B)** Day 5 flow cytometry plots generated with BD FACSDiva™ Software.

Conclusions

Physiologically relevant concentrations of tofacitinib and ruxolitinib inhibit multiple signaling pathways associated with viral persistence.

- Inhibit seeding of the HIV reservoir by γc receptor cytokines (IL-2, IL-7, IL-15) triggered by chronic T-cell receptor activation and bystander immune activation
- Prevent expression of negative regulators for T-cell activation (PD-1) which may result in restoration of innate and adaptive immune function
- Future work will investigate if ruxolitinib or tofacitinib also decrease expansion/maintenance of the HIV reservoir by inhibition of homeostatic proliferation driven by cytokines such as IL-7 and IL-15

Inhibition was prevented by excess IL-2 suggesting that JAK inhibitors act by either:

1. Decreasing IL-2 mediated expansion/survival of productively infected cells
2. Prevention of priming of bystander cells for infection

Selective blocking of JAK/STAT signaling pathway(s) could prevent persistence of cells producing virus, thereby reducing viral reseeding and reservoir size mediated by persistent immune activation and inflammation, leading to a "functional cure."

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