INTERLEUKIN-2-INDUCIBLE T-CELL KINASE (ITK) INHIBITION PREVENTS HIV LATENCY REVERSAL

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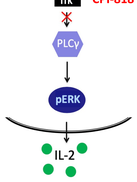
BACKGROUND

- ITK is a tyrosine kinase that is expressed in T cells, natural killer (NK) cells, and mast cells.
- ITK is involved in T cell receptor (TCR) signaling and regulates T cell development, activation, and differentiation.
- ITK is known to play a role in HIV replication, but its impact on HIV latency and persistence is as yet unknown.
- CPI-818 is a selective, covalent ITK inhibitor (Kd 2.5 nM) in clinical trials for therapy of T cell lymphomas which induces Th1 skewing and reduces T cell exhaustion markers.

OBJECTIVE

- **To determine the impact of ITK inhibition on HIV latency and proliferation of HIV-infected CD4+ T cells.**



Inhibit reversal of HIV latency
Block proliferation of HIV-infected cells
Protect target cells from HIV infection
Promote antiviral immunity through Th1 skewing
Reduce T cell exhaustion

CPI-818 mechanism of action and applications in HIV cure

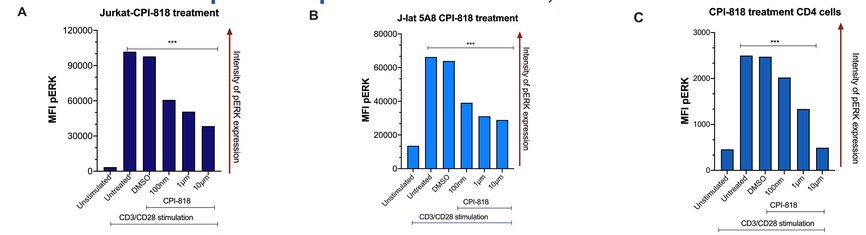
METHODS

- pERK (phosphorylated extracellular signal-regulated kinase) expression was measured by flow cytometry to assess the effects of CPI-818 on TCR signaling.
- HIV latency reversal was determined by pretreating cell lines, primary CD4+ T cells, and T cells from people living with HIV (PLWH) on antiretroviral therapy (ART) with CPI-818 (or DMSO control), followed by CD3/CD28 stimulation.
- Effects of CPI-818 on proliferation of HIV-infected CD4+ T cells were assessed in an *in vitro* infection model using eFluor670 dye to track cell division.
- The effect of ITK inhibition on establishment of HIV latency in primary CD4+ T cells was studied in an *ex vivo* infection model using a dual reporter virus enabling isolation and enumeration of latently- and productively-infected cells.

ITK inhibition blocks the TCR signaling pathway, preventing reversal of HIV latency in CD4+ T cells. ITK inhibitors can be explored as a block-and-lock strategy for HIV cure.

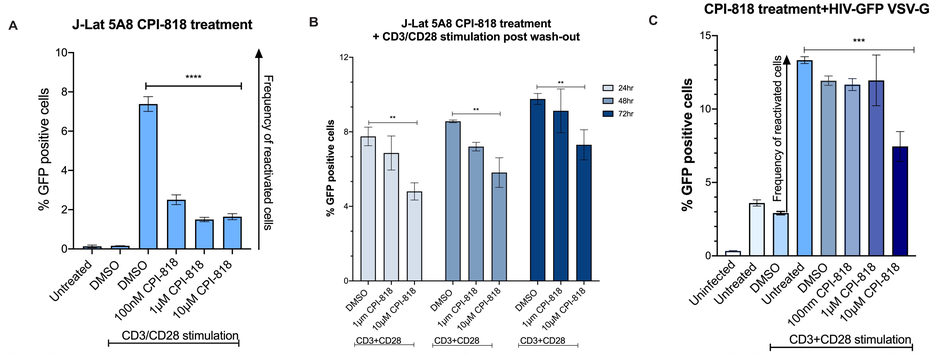
RESULTS

1) CPI-818 reduces pERK expression in Jurkat, J-Lat 5A8 and CD4+ T cells



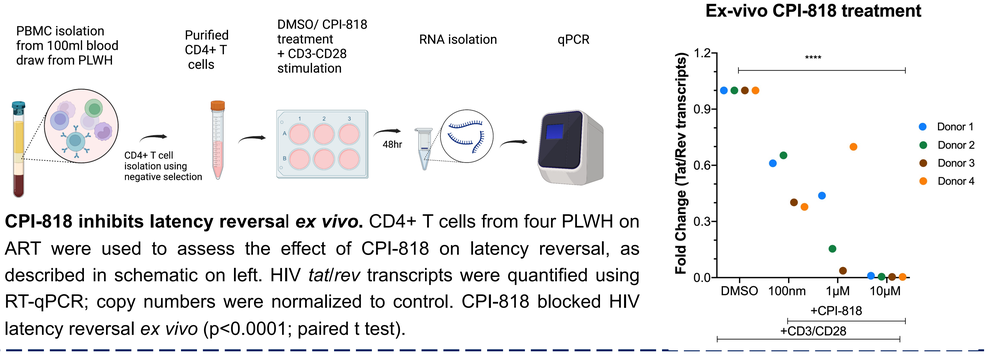
CPI-818 inhibits pERK expression. A) Jurkat, B) J-lat 5A8 (Jurkat cells latently infected with a GFP reporter HIV construct) and C) primary CD4+ T cells were treated with CPI-818 for 1 hour followed by CD3/CD28 stimulation. Flow cytometry was performed using pERK antibody. CPI-818 treatment resulted in a significant decrease in the mean fluorescence intensity of pERK in Jurkat (p=0.0002; t test), J-lat 5A8 (p=0.0003) and primary CD4+ T cells(p=0.006).

2) CPI-818 durably blocks CD3/CD28-mediated reversal of HIV latency



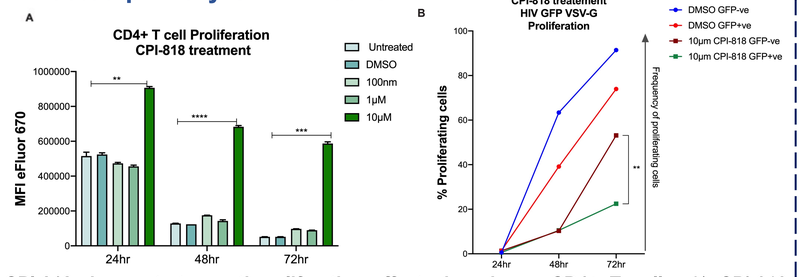
CPI-818 inhibits HIV latency reversal *in vitro*. A) J-Lat 5A8 cells were pretreated with CPI-818 followed by CD3/CD28 stimulation and GFP positive cells were assayed by flow cytometry. B) CPI-818 inhibited CD3/CD28-mediated latency reversal in J-Lat 5A8 cells in a dose-dependent manner for 72 hours post drug washout (p=0.0077). C) CPI-818 inhibited CD3/CD28-mediated latency reversal in primary CD4+ T cells infected with HIV-GFP VSV-G (p=0.0006).

3) CPI-818 blocks HIV latency reversal in CD4+ T cells from PLWH on ART



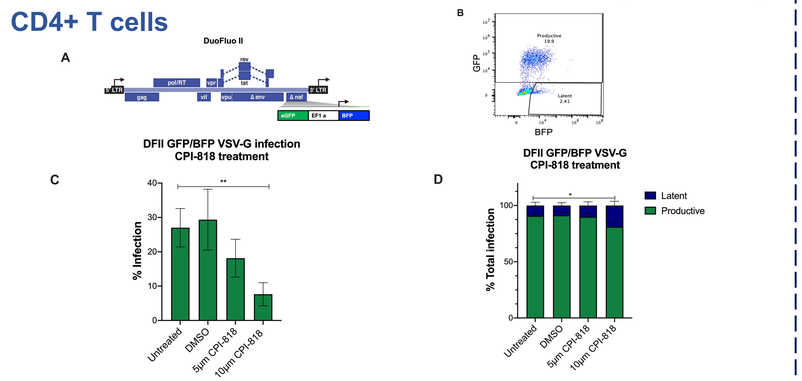
CPI-818 inhibits latency reversal *ex vivo*. CD4+ T cells from four PLWH on ART were used to assess the effect of CPI-818 on latency reversal, as described in schematic on left. HIV *tat*/*rev* transcripts were quantified using RT-qPCR; copy numbers were normalized to control. CPI-818 blocked HIV latency reversal *ex vivo* (p<0.0001; paired t test).

4) CPI-818 preferentially reduces proliferation of HIV-infected primary CD4+ T cells



CPI-818 demonstrates anti-proliferative effects in primary CD4+ T cells. A) CPI-818 inhibits proliferation of CD4+ T cells, as measured by eFluor670 dye to track cell division. B) HIV-infected primary CD4+ T cells proliferated at a lower rate than uninfected cells in the presence of CPI-818 (p=0.0052).

5) CPI-818 enhances establishment of HIV latency in primary CD4+ T cells



CPI-818 enhances the initial establishment of HIV latency. The effect of ITK inhibition on establishment of latency was studied in an *in vitro* infection model using the A) HIV DFII BFP/GFP dual reporter virus enabling B) measurement of latent and productive cells. C) Flow cytometry showed lower rates of infection in CPI-818 treated cells (p=0.0068). D) Flow cytometry data demonstrated that CPI-818 treatment before infection increased the ratio of HIV latently-infected to productively-infected cells(p=0.0248).

CONCLUSIONS

- ITK inhibition blocks HIV latency reversal in multiple latency models including treatment of CD4+ T cells from PLWH on ART *ex vivo*.
- ITK inhibition reduces the proliferation of HIV-infected CD4+ T cells.
- ITK inhibition promotes the establishment of latency in CD4+ T cells.
- ITK inhibition may offer a new approach to HIV cure by blocking several steps of the HIV life cycle and enhancing antiviral immunity.

ADDITIONAL KEY INFORMATION

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