NNRTIs Decrease HIV-1 Production from rCD4+ T Cells Following Latency Reversal

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ABSTRACT

Background: Therapeutic strategies which target the latent HIV-1 reservoir in resting CD4+ T cells (rCD4+) in infected-individuals are always administered in the presence of combination antiretroviral (ARV) therapy. In this study, we evaluated whether the different therapeutic classes of ARV drugs impacted HIV-1 latency reversal.

Methods: Latency reversal was evaluated in a primary cell model of latency that utilizes direct infection of rCD4+ T cells. Two hours prior to addition of anti-CD3/CD28 monoclonal antibodies, cells were treated with an entry inhibitor (AMD3100 or maraviroc), a PI (atazanavir, darunavir), NRTI (lamivudine), NNRTI (rilpivirine, efavirenz) or INSTI (raltegravir). Controls included cells that were exposed only to antibody or the ARVs. Seven days post antibody administration, cell-associated HIV-1 DNA and extracellular virion production were quantified. T cell activation and cell viability were assessed by flow cytometry.

Results: NNRTIs were found to decrease both R5- and X4-tropic HIV-1 production (by ≥ 1-log fold-change) in rCD4+ T cells exposed to anti-CD3/CD28 monoclonal antibodies. This decrease was not due to toxicity, or changes in T cell activation in the presence or absence of NNRTI as assessed by CD25, CD69 or HLA-DR expression. In contrast, none of the other ARVs, including PIs which target the late stages of HIV-1 replication, had a significant impact on virus production. Further analysis in a cell line model of HIV-1 latency (J89GFP cells) revealed that the NNRTIs did not interfere with HIV-1 gene transcription or translation. Instead, consistent with published studies, we propose that the NNRTIs enhance premature activation of HIV-1 PR which results in intracellular processing of Gag and Gag-Pol leading to decreased viral particle production.

Conclusions: NNRTIs reduce HIV-1 production from latently infected cells. Ex vivo studies that use NNRTIs to prevent virus spread or cells from donors on NNRTI containing regimens should be cautiously interpreted.

METHODS

Primary cell model of HIV-1 latency and latency reversal

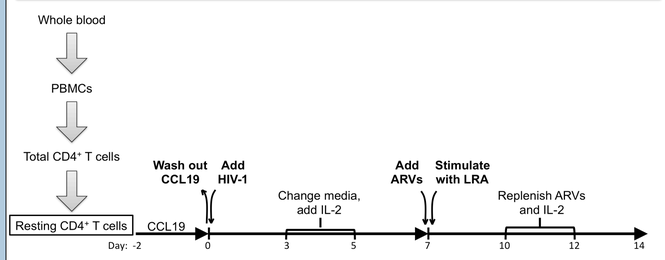


Figure 1: Resting CD4+ T cell isolation and primary cell model of HIV-1 latency. PBMCs were isolated from whole blood followed by two rounds of negative selection to purify resting CD4+ T cells. Resting CD4+ T cells were infected with HIV-1_{LAI} or HIV-1_{BaL} at an MOI = 1 following CCL19 (100nM) treatment. Cells were left in culture for 7 days to establish latent infection in the absence of any ARVs. 7 days post-infection, ARVs were added to the cells at designated concentrations. Two hours later, half of the cells were stimulated with either anti-CD3/CD28 antibodies or bryostatin 1, and half were left untreated. After 7 days of stimulation, cells and supernatant were harvested for HIV-1 DNA and pelletable virion-associated HIV-1 RNA, respectively. Cells were also used for flow analyses of cell viability and T cell activation.

Antiretroviral drugs (ARVs) used in this study

Drug name	Abbreviation	Class	Concentration
Darunavir	DRV	PI	0.5μM
Atazanavir	ATV	PI	0.5μM
Efavirenz	EFV	NNRTI	1μM
Rilpivirine	RPV	NNRTI	1μM
Lamivudine	3TC	NRTI	50μM
Tenofovir	TNV	NRTI	100μM
Raltegravir	RAL	InSTI	0.5μM
AMD 3100	AMD	CXCR4 entry inhibitor	5μM
Maraviroc	MVC	CCR5 entry inhibitor	1μM

Figure 2: Antiretroviral drugs used in this study. Multiple antiretroviral drugs from four different drug classes were used in this study. The concentrations used were at least 20-fold higher than the reported IC50 concentrations.

RESULTS

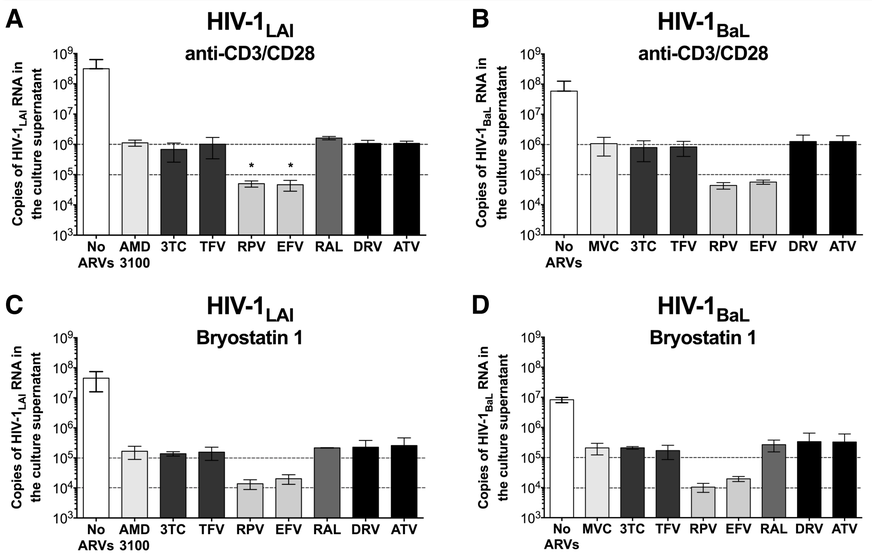


Figure 3: NNRTIs reduce virus production following HIV-1 latency reversal in rCD4+ T cells in vitro. Copies of pelletable virion-associated HIV-1 RNA in the culture supernatant are shown following treatment with anti-CD3/CD28 antibodies (3 beads per cell) from cells infected with (**A**) HIV-1_{LAI} or (**B**) HIV-1_{BaL}. * indicates a significant reduction ($p < 0.05$) in virus production compared to all other treatment conditions, evaluated by a Mann-Whitney test. Error bars represent ± SEM. N = 3-5 performed in duplicate. Copies of pelletable virion-associated HIV-1 RNA in the culture supernatant are shown following treatment with bryostatin 1 (100nM) from cells infected with (**C**) HIV-1_{LAI} or (**D**) HIV-1_{BaL}. Error bars represent ± SD. N = 2 performed in duplicate.

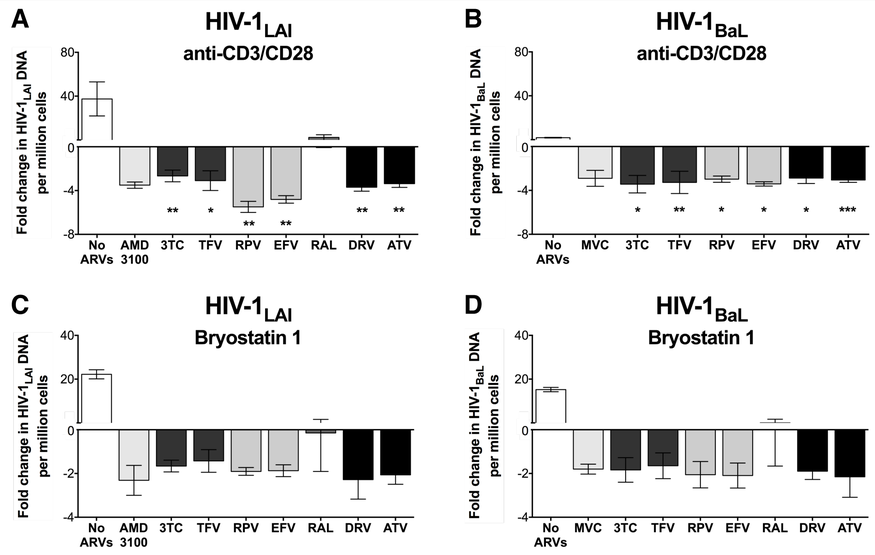


Figure 4: Changes in HIV-1 DNA following latency reversal when cells are treated with different ARVs. The fold change in (**A**) HIV-1_{LAI} or (**B**) HIV-1_{BaL} DNA per 10^6 cells is shown comparing cells stimulated with anti-CD3/CD28 versus unstimulated control cells under the same treatment conditions. Error bars represent ± SEM. * p value < 0.05, ** p value < 0.01, *** p value < 0.001, determined by a paired t test. N = 3-5 performed in duplicate. The fold change in (**E**) HIV-1_{LAI} or (**F**) HIV-1_{BaL} DNA per 10^6 cells is shown comparing cells stimulated with bryostatin 1 versus unstimulated control cells under the same treatment conditions. Error bars represent ± SD. N = 2 performed in duplicate.

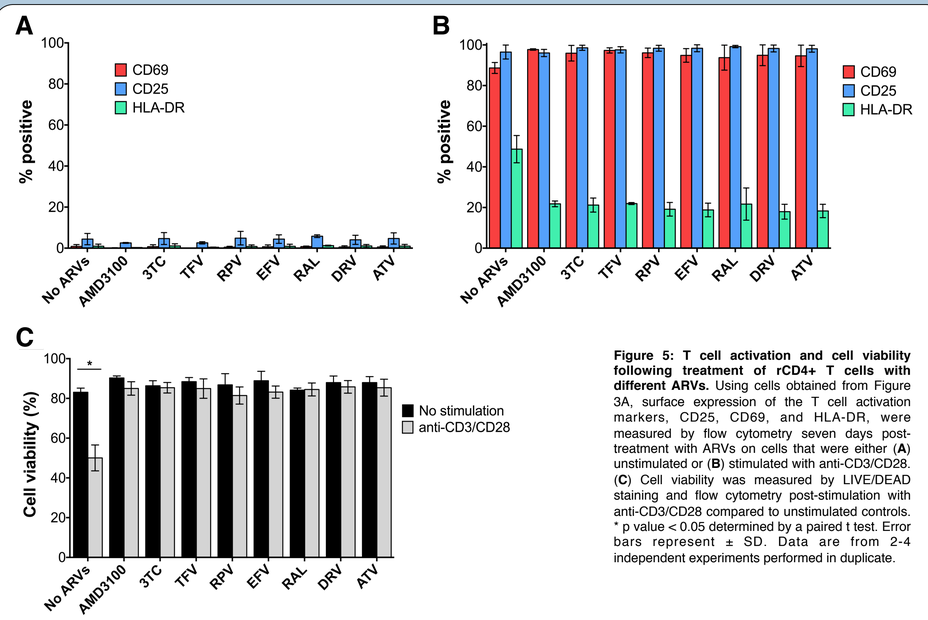


Figure 5: T cell activation and cell viability following treatment of rCD4+ T cells with different ARVs. Using cells obtained from Figure 3A, surface expression of the T cell activation markers, CD25, CD69, and HLA-DR, were measured by flow cytometry seven days post-treatment with ARVs on cells that were either (**A**) unstimulated or (**B**) stimulated with anti-CD3/CD28. (**C**) Cell viability was measured by LIVE/DEAD staining and flow cytometry post-stimulation with anti-CD3/CD28 compared to unstimulated controls. * p value < 0.05 determined by a paired t test. Error bars represent ± SD. Data are from 2-4 independent experiments performed in duplicate.

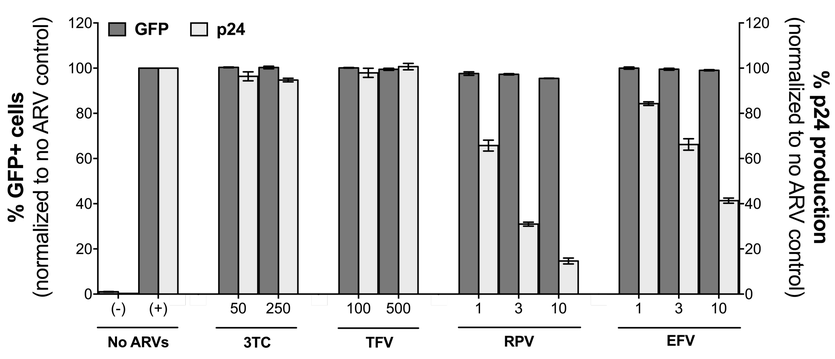


Figure 6: NNRTI-reduced virus production is not due to decreases in HIV-1 transcription or protein translation. In order to determine if the reduced virus production from cells treated with RPV or EFV was due to effects on transcription or translation, J89GFP cells were cultured in the absence or presence of different NNRTIs or NRTIs and were stimulated with PMA (30nM) for 48hrs. Following stimulation, the cells were analyzed by flow cytometry for cell viability, via LIVE/DEAD staining, and GFP expression, as a surrogate marker for protein translation. Virus production was quantified by p24 in the culture supernatant. (+) and (-) refer to with and without PMA stimulation, respectively, in the absence of any ARVs as controls. All drug concentrations shown are in micromolar. For both GFP expression and p24 production, samples were normalized to the no ARV (+) PMA control, which was set at 100%. Error bars represent ± SD. Data are from 2 independent experiments performed in duplicate.

CONCLUSIONS

NNRTIs reduce HIV-1 production from latently infected cells. As such, ex vivo and in vitro studies that use NNRTIs to prevent virus spread, or rCD4+ T cells from donors on NNRTI-containing regimens, should be cautiously interpreted, particularly if the only virological output is extracellular virus particle production.

Furthermore, it may be more difficult to observe an increase in plasma viral load following the administration of a latency reversing agent to an HIV-1-infected participant on a NNRTI based therapy, although additional studies assessing combinations of different ARV classes should also be assessed.

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