Nonnucleoside Reverse Transcriptase Inhibitors Reduce HIV-1 Virus Production from Latently Infected Resting CD4+ T Cells Following Latency Reversal

Running Title: NNRTIs reduce latent HIV-1 virus production

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Therapeutic strategies that target the latent HIV-1 reservoir in resting CD4+ T cells of infected individuals are always administered in the presence of combination antiretroviral therapy. Using a primary cell of HIV-1 latency, we evaluated whether different antiviral drug classes affected latency reversal (as assessed by extracellular virus production) by anti-CD3/CD28 monoclonal antibodies or by bryostatin 1. We found that the nonnucleoside reverse transcriptase inhibitors efavirenz and rilpivirine significantly decreased HIV-1 production by ≥ 1 log.
Latently infected resting CD4+ T cells are a major reservoir of persistent HIV-1 infection (1-3).

During latent HIV-1 infection, the integrated HIV-1 provirus remains transcriptionally silent in the absence of stimulation or cellular activation. However, upon cellular activation HIV-1 RNA is transcribed and virus is produced. It has been widely speculated that reduction or elimination of the latent reservoir in resting CD4+ T cells may lead to an HIV-1 cure. One hypothesis in the field is that intervention strategies that reactivate latent HIV-1 infection will cause death of the infected cells by viral cytopathic effects or host cytolytic mechanisms, thereby decreasing the size of the latent reservoir. This approach, which has already been evaluated in several clinical studies (4), is typically referred to as “kick and kill” (5, 6). Importantly, this therapeutic strategy is always carried out in the presence of combination antiretroviral therapy to prevent de novo infection by the reactivated HIV-1. However, it is not known if antiretrovirals (ARVs) impact the efficacy of the “kick and kill” strategy, and consequently we addressed this important knowledge gap in this study.

To address whether ARVs impacted the “kick” phenotype, we used a primary cell model that utilizes direct HIV-1 infection of highly purified resting CD4+ T cells to generate latently infected cells (Fig. 1A), as described previously (7, 8). The resting CD4+ T cells were infected with either an X4-tropic strain of HIV-1, LAI (9), or the R5-tropic strain, BaL (10). Following the establishment of latency, the cells were treated with one of several different ARVs from five distinct drug classes, including attachment inhibitors (maraviroc (MVC) or AMD3100), nucleoside reverse transcriptase inhibitors (NRTIs; lamivudine (3TC) or tenofovir (TFV)), nonnucleoside reverse transcriptase inhibitors (NNRTIs; rilpivirine (RPV) or efavirenz (EFV)), an integrase strand transfer inhibitor (INSTI; raltegravir (RAL)), and protease inhibitors (PIs: darunavir (DRV) or atazanavir (ATV)). The concentration of each ARV used in this experiment was at least 20-fold greater than the reported 50 % inhibitory concentration (EC$_{50}$) determined in cell culture. Following the addition of each ARV, the
latently HIV-1-infected resting CD4+ T cells were stimulated with anti-CD3/CD28 monoclonal antibodies (mAbs, 3 beads per cell) to reactivate latent HIV-1. Virus production was quantified by measuring pelletable extracellular virion-associated HIV-1 RNA in the culture supernatant, as described previously (11). We found that equivalent amounts of X4-tropic (Fig. 1B) and R5-tropic (Fig. 1C) HIV-1 were generated from cells treated with attachment inhibitors, NRTIs, an INSTI, or PIs. In contrast, we observed a log or greater decrease in virus production from cells that had been treated with the NNRTIs, EFV or RPV (Fig. 1B, 1C). This decrease in HIV-1 production was not due to toxicity (Fig. S1A) or the NNRTI impacting global T cell activation (as assessed by CD25, CD69 or HLA-DR expression) in the absence (Fig. S1B) or presence (Fig. S1C) of anti-CD3/CD28 mAbs. Of note, more HIV-1 particle production was observed in the no ARV controls due to spread of the reactivated HIV-1 (Fig. 1B and 1C). The reduction in virus production following treatment of the latently HIV-1-infected resting CD4+ T cells with either EFV or RPV was dose-dependent for both the X4- (Fig. 1D) and R5-tropic (Fig. 1E) strains, with 50 % inhibitory concentrations (i.e., EC50) in the low nanomolar range, which is equivalent to their IC50 values for inhibition of reverse transcription (12). Consistent with the anti-CD3/CD28 mAb data, EFV and RPV also reduced virus production from latently-infected cells exposed to the protein kinase C agonist bryostatin 1 (100nM, Fig. 1F, 1G). Collectively, these data reveal that the NNRTIs EFV and RPV significantly attenuate the “kick” of latent HIV-1 from resting CD4+ T cells by inhibiting release of HIV-1 virus particles. This finding is consistent with our prior work which demonstrated that potent NNRTIs impact the late stages of HIV-1 replication (13), which leads to a decrease in virus production from HIV-1-transfected 293T or HeLa cells (14, 15). Specifically, NNRTIs enhance Gag-Pol polyprotein precursor dimerization, likely after plasma membrane targeting but before complete particle assembly, resulting in uniform distribution of p17 matrix to and dissociation of p24 capsid and reverse transcriptase from the plasma membrane (13-15). Interestingly, in the HeLa and 293T cells micromolar
concentrations of EFV were required to see a significant reduction in virus production (14, 15). In contrast, the concentrations of EFV or RPV required to decrease virus production from resting CD4+ T cells was in the nanomolar range (Fig. 1D, 1E), and is significantly lower than the peak plasma concentrations following a single oral dose in humans (1.6-9.1µM for EFV (16); 0.39-0.53µM for RPV (17)). This suggests that NNRTIs may decrease virus production in HIV-1-infected individuals using NNRTI-containing regimens enrolled in intervention studies aimed at eradicating the latent HIV-1 reservoir.

To assess whether ARVs impacted the “kill” phenotype due to the cytopathic effect of the reactivated virus, we quantified the number of HIV-1-infected cells for HIV-1LAI and HIV-1Bal with and without treatment with anti-CD3/CD28 mAbs or bryostatin 1 by quantitative PCR analysis of total HIV-1 DNA (Fig. 2). HIV-1 DNA was normalized to the total number of cells assayed (assessed by quantitative PCR amplification of the CCR5 gene (18)), as described previously (19). We observed significant decreases in the total number of X4- and R5-tropic HIV-1-infected cells exposed to anti-CD3/CD28 mAbs in the presence of AMD3100, MVC, 3TC, TFV, RPV, EFV, DRV, and ATV (Fig. 2A, 2B). A similar trend was observed for X4- and R5-tropic HIV-1-infected cells exposed to the latency reversing agent bryostatin 1 (Fig. 2C, 2D). Of note, RAL did not result in an apparent decrease in HIV-1 infected cells after treatment with either anti-CD3/CD28 mAbs or bryostatin 1. This is likely due to the total HIV-1 DNA assay quantifying reverse transcripts and 2LTR circles that are generated as the reactivated virus infects previously uninfected cells (all of the other ARVs result in the production of replication incompetent virus (DRV, ATV), block new infection cycles (AMD3100/MVC), or inhibit reverse transcription (3TC, TFV, EFV and RPV) and therefore do not interfere with the total HIV-1 DNA assay). The observation that EFV and RPV resulted in death of the HIV-1 infected cells post latency reversing agent administration is surprising, given that these cells did not produce many viral
particles (Fig. 1), which might suggest reduced viral cytopathic effects. Interestingly, further analyses revealed that the decrease in the number of HIV-1-infected cells treated with EFV or RPV was also found to be dose-dependent (Fig. 2E, 2F), and the slopes of these curves were similar to the curves reported in Fig 1D and 1E. In this regard, prior studies have illustrated that NNRTIs increase the intracellular processing of Gag and Gag-Pol (13-15), which leads to premature protease activation which is cytotoxic and can result in cell death (20, 21).

In summary, our data show that NNRTIs reduce HIV-1 production from latently infected resting CD4+ T cells. These findings have significant implications for HIV-1 eradication studies. For example, ex vivo studies that use NNRTIs to prevent virus spread, or resting CD4+ 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. Finally, our data suggest that there may be a therapeutic application for NNRTIs in helping to eliminate cells expressing HIV-1 Gag and Gag-Pol proteins, although additional studies are warranted to further explore this possibility.

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Conflict of Interest

None to declare
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Figure 1: NNRTIs reduce virus production following HIV-1 latency reversal in resting CD4+ T cells in vitro. (A) Schematic of experimental approach. (B) Copies of extracellular pelletable HIV-1 RNA in the culture supernatant are shown following treatment with anti-CD3/CD28 mAbs from cells infected with HIV-1LAI or (C) HIV-1BaL. Drug concentrations used: AMD3100 = 5μM; MVC = 1μM; 3TC = 50μM; TFV = 100μM; EFV = 1μM; RPV = 1μM; RAL = 500nM; DRV = 500nM; and ATV, 500nM. Error bars represent ± standard error of the mean. Data are from 5 independent experiments from different donors performed in duplicate for HIV-1LAI and 3 independent experiments from different donors performed in duplicate for HIV-1BaL. * indicates a significant reduction (p < 0.05) in virus production compared to all other treatment conditions. (D) Dose-dependent responses in virus production following treatment with RPV and EFV from cells infected with HIV-1LAI or HIV-1BaL. EC50 values were calculated using a three parameter nonlinear regression model in GraphPad Prism. (F) Copies of HIV-1 RNA in the culture supernatant are shown following treatment with bryostatin 1 from cells infected with HIV-1LAI or (G) HIV-1BaL. The experimental conditions are identical to those described in (B) and (C), and were obtained from 2 independent experiments from different donors performed in duplicate. Error bars represent ± standard deviation.

Figure 2: Changes in HIV-1 DNA following latency reversal when cells are treated with different ARVs. The fold change in (A) HIV-1LAI or (B) HIV-1BaL DNA per 10^6 cells in cells exposed to anti-CD3/CD28 mAbs are shown. The data compare cells exposed to anti-CD3/CD28 mAbs 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. Data are from 3-5 independent experiments performed in duplicate. The fold change in (C) HIV-1LAI or (D) HIV-1BaL.
DNA per 10^6 cells in cells exposed to bryostatin 1. The data compare cells exposed to bryostatin 1 versus unstimulated control cells under the same treatment conditions. Error bars represent ± standard deviation. Data are from 2 independent experiments performed in duplicate. Dose-dependent decrease in (E) HIV-1_LAI or (F) HIV-1_BaL–infected cells treated with RPV or EFV following stimulation with anti-CD3/CD28 mAbs are shown.


