

Identification of Nevirapine Resistant HIV-1 in the Latent Reservoir Following Single Dose Nevirapine

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Abstract

Background: A single dose of nevirapine (NVP) given to pregnant women during labor to prevent transmission of HIV-1 often leads to the emergence of NVP resistant virus in plasma. NVP resistant virus has been shown to fade from detection among plasma virus; however the presence of this virus in the latent reservoir in resting CD4+ T cells has not been determined.

Methods: Plasma and PBMCs were collected from women at least 6 months after they received single dose NVP. Highly purified resting CD4+ T cells were isolated and activated in the presence of reverse transcriptase and integrase inhibitors to prevent the completion of reverse transcription and integration in cells with unintegrated virus. Virus from the latent reservoir was isolated from 50 women (24 from Soweto, South Africa and 26 from Rakai, Uganda). Virus was also isolated from two women who did not receive NVP. In addition, analysis of replication competent virus released from the latent reservoir was performed on a subset of these women. A highly sensitive mutation specific assay (LigAmp) was used to identify virus containing any of three NVP resistance mutations (K103N, Y181C, G190A) among virus from the latent reservoir and virus present in a concurrent plasma sample.

Results: NVP resistant virus was identified in the latent reservoir of 4 of 50 women (8%) who received single dose NVP. Of these, 3 women (6%) did not have NVP resistant virus in the concurrent plasma sample. One woman had two NVP resistance mutations; one was present among both plasma virus and virus from the latent reservoir, while the other mutation was present only in virus from the latent reservoir. In addition one woman had low levels of K103N resistant virus present among virus isolated from plasma that was not found among virus of the latent reservoir. The K103N and G190A resistance mutations were identified among virus from women in this study, while the Y181C resistance mutation was not found. Control samples from women who did not receive NVP did not contain NVP resistant virus.

Conclusions: These results provide the first demonstration that NVP resistant virus that arises following a single dose of NVP can persist in the resting CD4+ T cell latent reservoir. The identification of NVP resistant virus suggests that this virus could re-emerge if an NNRTI is included in future HAART, providing a source of NVP resistant virus that could lead to future treatment failure and the development of further drug resistance mutations.

Introduction

The most widely used therapy to prevent mother-to-child transmission of HIV-1 in resource-limited settings is a single dose of the NNRTI NVP given to a pregnant mother at the time of delivery followed by a dose of NVP to the infant within 72 hours of birth. This therapy has been shown to be effective, however, NVP-resistant virus has been found in the plasma of up to 88% of women 6-8 weeks after treatment and has been shown to persist in the plasma of treated women for up to five years. Because first-line antiretroviral treatment regimens in developing countries consist of two nucleoside reverse transcriptase inhibitors (NRTIs) and one NNRTI, the presence of virus resistant to one of the components of this regimen could lead to future antiretroviral treatment failure.

Though the development and persistence of NVP-resistant virus in the plasma has been well studied, evidence of archived resistance in the latent CD4+ T cell reservoir is lacking. Following infection of activated CD4+ T cells by HIV-1 and integration of proviral DNA into the host genome, a fraction of infected cells return to a resting state forming a stable latent reservoir for HIV-1. The persistence of these cells in a quiescent state preserves the integrated HIV-1 proviral DNA for the life of the cell.

Previous studies have identified NVP resistance mutations among viral sequences isolated from total PBMCs. Because cells other than resting CD4+ T cells were included in these studies, these results do not directly prove the presence of virus containing the NVP resistance mutations in the stable latent reservoir. These studies may underestimate the number of women with virus containing NVP resistance mutations because the detection of all forms of HIV-1 DNA likely limits the identification of the minority of virus that represents the stable latent reservoir. To definitively determine the presence of NVP-resistant virus in the stable latent reservoir, we analyzed virus that could be released from highly purified resting CD4+ T cells from women who had received a single dose of NVP.

Methods

Women who had received single dose NVP during labor at least 6 months prior to sample collection with CD4 levels of >200 cells/mm³ were enrolled. Control samples were obtained from women who did not receive single dose NVP. All women included in this study had no other history of antiretroviral treatment. Purified resting CD4+ T cells were cultured in the presence of four antiretroviral drugs: two NRTIs (8.6 mM 3TC and 470 mM PMPA), one NNRTI (0.5 mM EFV) and one integrase inhibitor (4 mM E-87012, Merck). Resting CD4+ T cells were activated by the addition of PHA and irradiated PBMCs from an HIV-1 negative donor to stimulate production of integrated HIV-1 and cultured in RPMI containing IL-2 and cytokine-rich supernatant from activated T cells. Culture supernatants were collected daily and stored for analysis; fresh medium and drugs were added to the culture daily. LigAmp analysis for the presence of resistance mutations was performed with oligonucleotides designed for each mutation and HIV-1 subtype. A ligation reaction using mutation specific primers was followed by detection of ligated product by real-time PCR. Each sample was analyzed in duplicate and a standard curve included in each reaction was used to determine the amount of resistant virus present. The cutoff value was set at 1% resistant virus.

	Uganda	South Africa
Total participants who received sdNVP	30	30
LATENT RESERVOIR		
Total participants with virus isolated from cells of latent reservoir	26	24
Resistant virus present in cells of latent reservoir	2	2
PLASMA		
Total participants with plasma virus analyzed	29	29
Resistant virus present in plasma	1	1

Table 1. Identification of NVP resistant virus from the latent reservoir and plasma. Sixty total women who had received sdNVP were included in this study. Of these, virus was successfully isolated from the latent reservoir in 50 women. Four of these 50 women (8%) had NVP resistance mutations identified among virus from the latent reservoir. Plasma virus was analyzed for 88% of the 60 women (2 could not be analyzed due to low viral load levels). NVP resistant virus was identified among plasma virus from 2 of 58 women (3.4%).

Patient	Study site	NVP resistance in plasma virus	NVP resistance in virus from latent reservoir
LT1003	Uganda	None	G190A
LT1020	Uganda	K103N	K103N, G190A
LT2009	South Africa	None	K103N
LT2028	South Africa	None	K103N
LT2049	South Africa	K103N	None

Table 2. NVP resistance mutations detected among plasma virus and virus released from the latent reservoir. Specific NVP resistance mutations identified by LigAmp analysis are indicated for 5 patients who received a single dose of NVP.

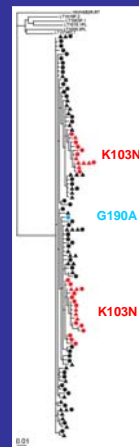


Figure 1. Phylogenetic analysis of patient LT1020 with sequences from virus released from the latent reservoir (circles) and plasma virus (triangles). Viral sequences containing the K103N resistance mutation are indicated in red. Viral sequences containing the G190A resistance mutation are indicated in blue. Sequence analysis also demonstrated that these two mutations are not present in the same viral variant as demonstrated by their separation on the phylogenetic tree. A single clone was isolated containing the G190A resistance mutation; in contrast, several clones containing the K103N resistance mutation were identified. Two phylogenetically distinct populations of virus containing the K103N resistance mutation were revealed by phylogenetic analysis. This may indicate that the K103N resistance mutation developed and persisted in two separate mutation events following sdNVP.

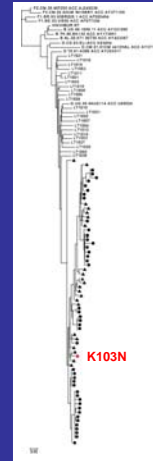


Figure 2. Phylogenetic analysis of patient LT2028 with sequences from virus released from the latent reservoir (circles) and plasma virus (triangles). LigAmp analysis of NVP resistant virus for patient LT2028 identified virus containing the K103N resistance mutation on only one day of the culture of eight days studied. As shown by the single red symbol on the phylogenetic tree from patient LT2028, a single viral variant containing the K103N resistance mutation was identified. The K103N resistance mutation was not found in any plasma virus sequences or by LigAmp analysis of plasma virus. A representative plasma viral sequence from each patient with the same subtype (D) that was analyzed in this study is included to show that all viral sequences isolated from patient LT2028 were phylogenetically distinct from those of other study participants.

Conclusions

NVP resistance mutations that arise following a single dose of NVP to prevent mother-to-child transmission of HIV-1 can be archived in the resting CD4+ T cell latent reservoir and persist for long periods of time and could provide a source of drug resistant virus that may contribute to future antiretroviral treatment failure and development of new resistance mutations. It is of vital importance to understand the makeup of the latent viral reservoir in women treated with single dose NVP to help understand the effects of future antiretroviral treatment for these women. A recent study showed that women who began NVP-based antiretroviral regimens that were initiated within 6 months of receiving single dose NVP (plus AZT from 34 weeks gestation through delivery) had higher rates of virologic failure than women without previous exposure to NVP (Lockman *et al.* NEJM 2007). Follow-up of these women lasted from 6 to 24 months after initiation of antiretroviral therapy. This study indicates that low levels of NVP-resistant virus among plasma virus may not affect virologic outcome of antiretroviral treatment regimens; however, further studies of the effectiveness of antiretroviral treatment in women treated with single dose NVP with longer follow-up periods are needed to determine the effect of NNRTI resistant virus archived in the latent reservoir.

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