

# Multi-class Drug Resistance Arises Frequently in HIV-infected Breastfeeding Infants Whose Mothers Initiate Highly Active Antiretroviral Therapy (HAART) Post-partum

J Lidström<sup>1</sup>, LA Guay<sup>1</sup>, P Musoke<sup>2</sup>, M Owor<sup>3</sup>, C Onyango-Makumbi<sup>3</sup>, JD Church<sup>1</sup>, SB Omer<sup>4</sup>, JB Jackson<sup>1</sup>, and SH Eshleman<sup>1</sup>

<sup>1</sup> Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, <sup>2</sup> Makerere University, Kampala, Uganda,

<sup>3</sup> Makerere University-Johns Hopkins University Research Collaboration, Kampala, Uganda, <sup>4</sup> Emory University, Atlanta, Georgia, USA

Abstract #: T-124

Poster #: 920

720 Rutland Ave  
Ross Building Rm 646  
Baltimore, MD 21205  
lidstrom@jhmi.edu  
Phone: 410-614-6498  
Fax: 410-502-9244

## SUMMARY

Some HIV-infected women begin highly active antiretroviral therapy (HAART) post-partum for their own health. If an HIV-infected infant is breastfeeding while its mother is receiving HAART, the infant may be at risk of developing resistance to drugs in the mother's HAART regimen. We analyzed antiretroviral (ARV) drug resistance in infants from the Six Week Extended NVP (SWEN)-Uganda study. At 1 year of age, the majority (6/7=85.7%) of infants who were exposed to maternal HAART had multi-class resistance.

## INTRODUCTION

The SWEN study, performed in Uganda, Ethiopia, and India compared the efficacy of maternal/infant single dose NVP (sdNVP) alone to sdNVP plus an infant regimen of up to 6 weeks of daily nevirapine (NVP) prophylaxis (from day 8 up to day 42) for prevention of mother-to-child HIV transmission (pMTCT) by breastfeeding [1].

Women in SWEN received sdNVP in labor, and some women received HAART post-partum for their own health.

ARV-resistant HIV can be transmitted to infants via breastfeeding. ARV-resistant HIV may also be selected in infants by transfer of non-suppressive levels of ARV drugs to infants via breast milk (Figure 1).

In this study, we analyzed ARV drug resistance in 7 HIV-infected, breastfeeding Ugandan infants in the SWEN study whose mothers started HAART post-partum.

## METHODS

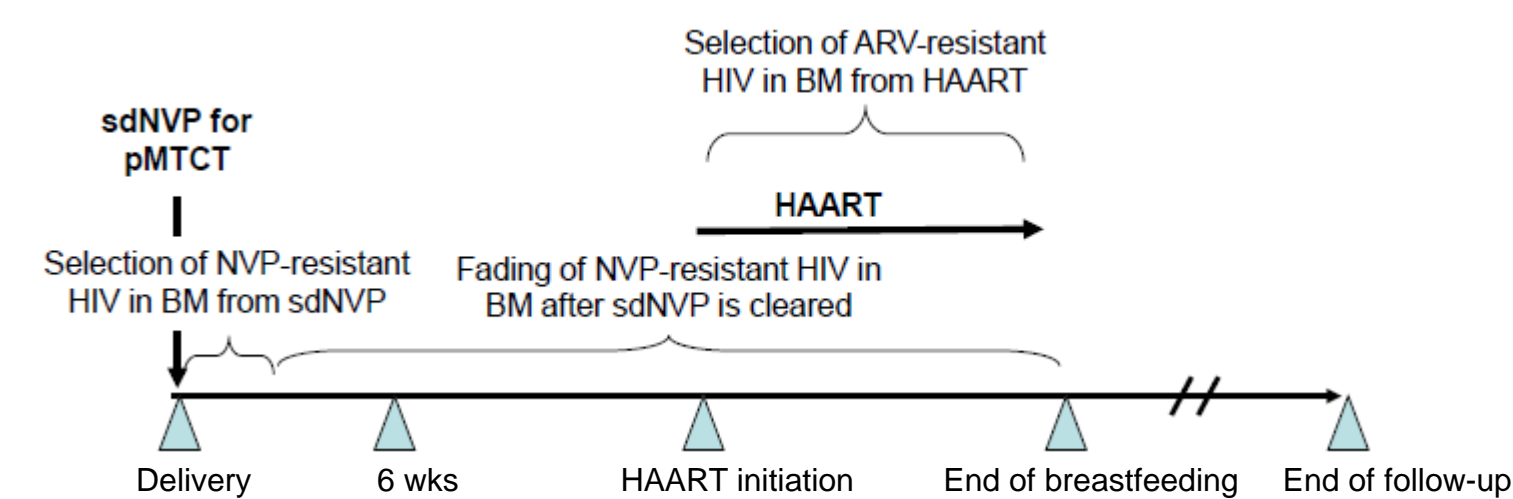
### Study Cohort

The infants in this study received either sdNVP (n=2) or sdNVP plus daily NVP from day 8 until HIV infection was confirmed (NVP was stopped between day 18 and day 36, n=5). The infants were diagnosed with HIV infection at birth (n=3), at 2 weeks (n=3), or at 6 weeks (n=1).

The mothers started HAART at 3 months (n=6) or 6 months (n=1) post-partum with the combination of either stavudine/lamivudine/nevirapine (d4T/3TC/NVP, n=3) or zidovudine/lamivudine/nevirapine (ZDV/3TC/NVP, n=4). None of the infants received HAART during the period studied.

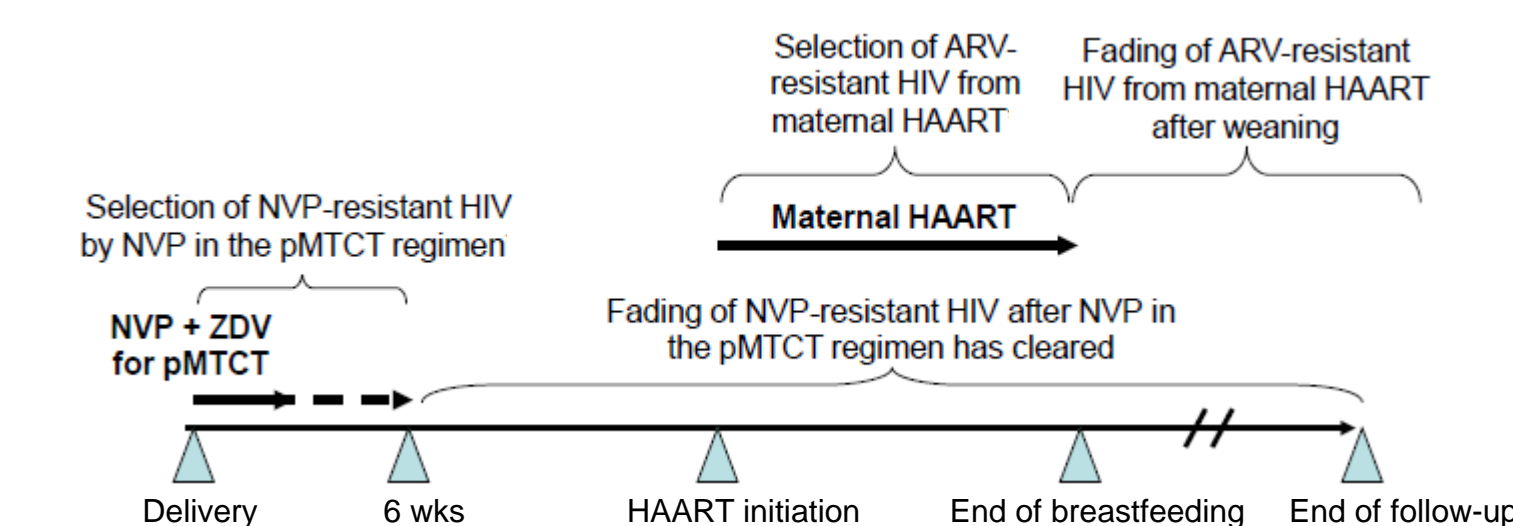
**Figure 1.** Potential mechanisms for development of ARV resistance in breast milk and infant plasma.

### 1A. Selection and fading of ARV resistance in breast milk.



Note: Resistant variants may also be selected in maternal plasma, and transferred to breast milk.

### 1B. Selection, transmission, and fading of ARV-resistance in infant plasma.



Note: The time of HIV infection varied among the infants studied; selection and fading of ARV resistance is relevant only for time periods after infant HIV infection.

### HIV Genotyping and Subtyping

Plasma samples (100 µl) from 1-year old infants were analyzed using the ViroSeq HIV-1 Genotyping System, v2.8 (Celera, Alameda, CA). Sequences were analyzed for the presence of mutations associated with resistance to NVP, d4T, ZDV, and 3TC (Stanford HIV Drug Resistance Database, 2009).

HIV *pol* sequences generated using ViroSeq were used for HIV subtyping. Sequence alignment was performed using the Clustal W algorithm (MegAlign Program, DNASTAR, Madison, WI) and phylogenetic analysis was performed using PHYLIP v.3.66.

**Table 1.** ARV resistance in HIV-infected breastfeeding infants whose mothers started HAART by 6 months.

Infant pMTCT regimen	Infant age at HIV diagnosis	Maternal HAART regimen	Time of HAART initiation	Mutations detected at 1 year of age
sdNVP + ext NVP day 8-28	Birth	d4T/3TC/NVP	≈ 0 months	K103N+ <b>M184V+M41L+T215F</b>
sdNVP + ext NVP day 8-21	2 weeks	d4T/3TC/NVP	≈ 6 months	Y181C+ <b>M184V</b>
sdNVP	6 weeks	d4T/3TC/NVP	≈ 3 months	Y181C
sdNVP	Birth	ZDV/3TC/NVP	≈ 3 months	K103N+ <b>M184V</b>
sdNVP + ext NVP day 8-18	Birth	ZDV/3TC/NVP	≈ 3 months	Y181C+ <b>M184V+D67N</b>
sdNVP + ext NVP day 8-36	2 weeks	ZDV/3TC/NVP	≈ 3 months	K103N+ <b>M184V</b>
sdNVP + ext NVP day 8-28	2 weeks	ZDV/3TC/NVP	≈ 3 months	K103N+Y181C+ <b>M184V+D67N+K70R</b>

These infants were HIV-infected by 6 weeks of age. Their mothers started treatment with d4T/3TC/NVP or ZDV/3TC/NVP by 6 months post-partum. Y181C and K103N are common NVP resistance mutations. NRTI resistance mutations are shown in bold.

## RESULTS

At 1 year of age, all 7 infants had NVP resistance and 6 (85.7%) of 7 also had resistance to nucleoside reverse transcriptase inhibitors (NRTIs). M184V, which is associated with resistance to 3TC and other NRTIs, was detected in 6/7 infants. Thymidine analog mutations (TAMS, M41L, D67N, K70R, T215F) were also detected in 3/7 infants (Table 1). All infants in this analysis were infected with subtype A HIV.

**Table 2.** Relevant data from other studies.

	PEPI (n=4)	KiBs (n=14)*
Source of data	[2]	[3]
Country	Malawi	Kenya
Maternal pMTCT regimen	sdNVP for early presenters	HAART (see below)
Infant pMTCT regimens	sdNVP + 1 week ZDV + daily ext NVP or daily ext NVP+ZDV up to 14 weeks	none
Time of infant diagnosis	<i>In utero</i>	By 24 weeks
Time of maternal HAART initiation	4-6 weeks postpartum	28 weeks gestation
Maternal HAART regimen	d4T/3TC/NVP	ZDV/3TC/NVP*
Major HIV subtype	C	C
Age at testing	14 weeks	14-24 weeks
Multi-class resistance	3/4=75%	4/14=29%

\*Other infants in KiBs received a nelfinavir-containing regimen.

## CONCLUSIONS

HIV-infected breastfeeding infants can acquire resistance to ARV drugs in their mothers' HAART regimen (Tables 1 and 2).

In this study, infants could have acquired non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance from the pMTCT regimen and/or from their mothers' HAART regimen. In contrast, NRTI resistance resulted from maternal HAART exposure, either from transmission of NRTI-resistant HIV through breast milk or from exposure to non-suppressive levels of NRTIs in breast milk.

Development of resistance to both NNRTIs and NRTIs (multi-class resistance) in HIV-infected infants is likely to significantly reduce their chance of responding to life-saving ARV therapy.

Further studies are needed to assess the risks and benefits of initiating ARV treatment in breastfeeding women whose infants are HIV-infected.

## REFERENCES

- [1] Six Week Extended-Dose Nevirapine (SWEN) Study Team. Lancet. 2008;372:300-13.
- [2] Lidstrom J, Kumwenda N, Kafulafula G, et al. XVIII Intl. HIV Drug Resist. Workshop, Fort Myers, FL. 2009. Abstract #135.
- [3] Zeh C, Weidle PJ, Nafisa L, et al. 15<sup>th</sup> CROI. 2008. Abstract #84LB.