

# Increased Resistance to Lamivudine Detected in HIV-Positive Pregnant Women Discontinuing Zidovudine (AZT), Lamivudine (3TC), and Nelfinavir (NFV): Results of PACTG 1022

Contact:

Lisa Frenkel, M.D.

Seattle Children's Research Institute

1900 Ninth Ave, MS C9S-8

Seattle, WA 98101

[lfrenkel@u.washington.edu](mailto:lfrenkel@u.washington.edu)

Phone: (206) 987-5140

Fax: (206) 884-7311

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Giovanina Ellis<sup>1</sup>, Jane Hitti<sup>2</sup>, Lisa Frenkel<sup>1,2</sup> for the P1022 Study Team

<sup>1</sup>Seattle Children's Hospital, Seattle, WA, USA; <sup>2</sup>University of Washington Medical Center, Seattle, WA, USA

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## ABSTRACT

**Background:** PACTG 1022 was a phase III, randomized open-label study of protease inhibitor-sparing or -including regimens in antiretroviral (ARV) naïve women initiating antiretroviral therapy (ART) during pregnancy. A total of 38 women with HIV RNA >1,000 cp/mL were randomized to AZT, 3TC, and either NVP or NVP to compare virologic suppression and tolerance in pregnancy. The trial closed early due to maternal toxicity. The rate of HIV genotypic resistance was compared by treatment group in women stopping study ART postpartum, to test the hypothesis that stopping ART would select resistance.

**Methods:** Specimens from women who stopped study ART and had viral loads >500cp/mL were tested for drug-resistance by oligonucleotide ligation assay (OLA) of HIV amplified from plasma and peripheral blood mononuclear cells and by consensus sequencing of virus from plasma using the ViroSeq Kit. When drug-resistance was detected, specimens obtained prior to therapy and during treatment were also evaluated. Resistance by treatment arm was compared using Fisher's exact test.

**Results:** Preliminary analysis of 20 women randomized to NVP (N=11) and NVP (N=9) detected M184V in 7/20 (35%) and K103N in 4/20 (20%) by OLA. OLA was initially indeterminate at M184 in 1/20 with subtype CR02.1G. M184V was selected primarily in women randomized to NVP (N=6/7), and K103N with NVP (N=3/4). Neither K103N nor M184V were detected at study entry. A trend was observed for greater M184V in NVP women (6/11 vs. 1/9, P=0.07). M184V was first detected after cessation of ART in 4/7, while 3 had viroemia during ART with M184V detected. K103N was detected only after discontinuation of ART. Consensus genotyping of plasma did not detect M184V and K103N in a 4 women position by OLA, suggesting low-level mutants. One or more major resistance mutations were detected after ART in 6/11 (55%) assigned to NVP and 4/9 (44%) to NVP.

**Conclusions:** The rate of HIV drug-resistance was high in women given NVP, and NVP during pregnancy for prophylaxis of mother-to-child transmission (pMTCT). Selection of 3TC resistance during and following the cessation of NVP-ART suggests that NVP pharmacokinetics in pregnancy may be suboptimal, and that staggered discontinuation of ARV may be warranted to reduce selection of 3TC resistance. Comparative studies of resistance during and following ART given for pMTCT and stopped postpartum are needed to identify regimens that minimize the risks of selecting HIV-1 drug-resistance in women.

## BACKGROUND

➤ PACTG 1022 was a phase III, randomized open-label study of protease inhibitor-sparing or -including regimens

➤ Antiretroviral (ARV) naïve women initiating antiretroviral therapy (ART) at 10-30 weeks gestation with HIV RNA >1,000 cp/mL were eligible for enrollment

➤ Goal was to randomize 220 women to each arm: AZT, 3TC, and either NVP or NVP to compare virologic suppression and tolerance in pregnancy

➤ The trial closed after enrolling 38 women due to maternal toxicity in the NVP group [1]

➤ We compared the rate of HIV genotypic resistance by treatment group in women stopping study ART postpartum, to test the hypothesis that stopping ART would select resistance.

## RESULTS

### DETECTION OF DRUG RESISTANCE BY STUDY ARM USING OLA AT DISCONTINUATION OF STUDY THERAPY

#### 11 WOMEN RANDOMIZED TO AZT, 3TC, NFV

Sample ID	Sample Type	Codon Tested					
		D30N	L90M	K103N	Y181C	M184V	T215F/Y
1	plasma						
2	pbmc						
2	plasma						
3	pbmc						
3	plasma						
4	pbmc						
4	plasma						
5	pbmc						
5	plasma						
6	pbmc						
6	plasma						
7	pbmc						
7	plasma						
8	pbmc						
8	plasma						
9	plasma						
10	plasma						
11	pbmc						
11	plasma						

#### 9 WOMEN RANDOMIZED TO AZT, 3TC, NVP

Sample ID	Sample Type	Codon Tested					
		D30N	L90M	K103N	Y181C	M184V	T215F/Y
12	pbmc						
12	plasma						
13	plasma						
14	pbmc						
14	plasma						
15	pbmc						
15	plasma						
16	pbmc						
16	plasma						
17	pbmc						
17	plasma						
18	pbmc						
18	plasma						
19	pbmc						
19	plasma						
20	plasma						

Table Legend: no resistance (yellow), Mutant & wildtype (pink), Mutant only (red), no reaction (grey)

➤ M184V was selected primarily in women randomized to NVP (N=6/7), and K103N with NVP (N=3/4)

➤ Neither K103N nor M184V were detected at study entry

➤ One or more major resistance mutations were detected after ART in 6/11 (55%) assigned to NVP and 4/9 (44%) to NVP

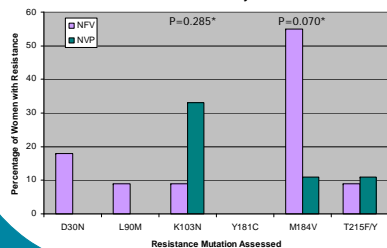
➤ Median interval between stopping study ARV's and resistance testing was 62 days (range 0-824)

➤ NVP group: Median: 60 days (range 0-268)

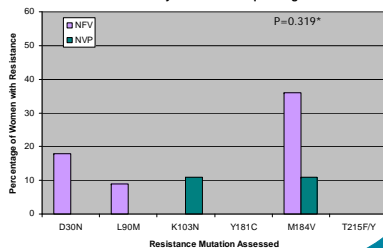
➤ NVP group: Median: 68 days (range 46-824)

➤ Drug-resistance was not always detected in both specimen types

Percentage of Women with Drug Resistance Detected in PBMC or Plasma by OLA



Percentage of Women with Drug Resistance Detected in Plasma by Consensus Sequencing



\*Fisher's Exact test used to calculate P values

## MATERIALS AND METHODS

➤ 20 women randomized to NVP (N=11) and NVP (N=9) had specimens available at or after end of study ART with viral loads >500cp/mL.

➤ 15 women had HIV RNA <500cp/mL at or following discontinuation of study medication  
3 women had HIV RNA >500cp/mL, but no available specimen

➤ HIV-1 drug-resistance was assessed in plasma and PBMC

➤ The ViroSeq HIV-1 Genotyping System v2.0 was used to extract, amplify, and consensus sequence HIV RNA from plasma

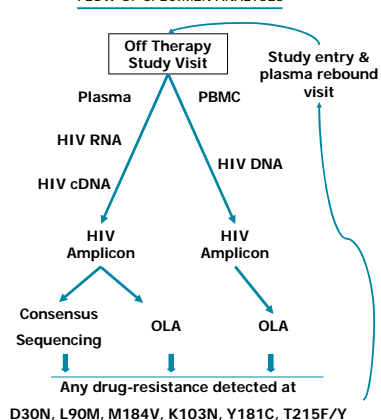
➤ Our in-house nested PCR protocol was followed for amplification of HIV *pol* from extracted DNA [2]

➤ OLA, a modified ELISA for sensitive detection of SNPs associated with HIV drug-resistance, was performed as previously described [2,3] but utilizing a different AP substrate with an increased dynamic range

➤ OLA detected mutations present in as little 2% of the population, whereas consensus sequencing detects mutations comprising 20-50% of the population

➤ Development of resistance from study participation was confirmed by analyzing study entry and/or intermediate specimens

### FLOW OF SPECIMEN ANALYSES



## CONCLUSIONS

➤ The rate of HIV drug-resistance was high in women given NVP- and NVP-ART during pregnancy for pMTCT.

➤ M184V was detected in 7/20 (35%) women and K103N in 4/20 (20%) women by OLA

➤ Selection of 3TC resistance during and following the cessation of NVP-ART suggests that NVP pharmacokinetics in pregnancy may be suboptimal

➤ A trend was observed for greater M184V in the NVP arm (6/11 vs. 1/9; P=0.07)

➤ These data reflect similar results described by Pilotto et al [4]

➤ Staggered discontinuation of ARV may be warranted to reduce selection of 3TC resistance

➤ Comparative studies of resistance during and following ART given for pMTCT and stopped postpartum are needed to identify regimens that minimize the risks of selecting HIV-1 drug resistance in women.

## REFERENCES

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