A Comparison of 3 Regimens to Prevent Nevirapine Resistance Mutations in HIV-Infected Pregnant Women Receiving a Single Intrapartum Dose of Nevirapine

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Background. Intrapartum single-dose (SD) nevirapine (NVP) reduces perinatal transmission of human immunodeficiency virus (HIV) infection but selects for NVP-resistant virus, which compromises subsequent NVP-based therapy. A 1-week "tail" of lamivudine and zidovudine after SD-NVP decreases the risk of resistance. We hypothesized that increasing the duration or potency of the tail would further reduce this risk to <10%, using a sensitive assay to measure resistance.

Methods. HIV-infected pregnant Thai women with a CD4 cell count >250 cells/μL, most receiving zidovudine, were randomized at 28–38 weeks gestation to receive 1 of 3 intrapartum and postpartum regimens: (A) zidovudine plus enteric-coated didanosine plus lopinavir and ritonavir for 7 days, (B) zidovudine plus enteric-coated didanosine for 30 days, or (C) regimen 1 for 30 days. The incidence of NVP resistance mutations at day 10 or week 6 post partum in each arm was compared with that of a historical comparison group who received prenatal zidovudine and SD-NVP. NVP resistance was identified by consensus sequencing and a sensitive oligonucleotide ligation assay (OLA).

Results. At entry, the 169 participants had a median CD4 cell count of 456 cells/μL and an HIV load of 3.49 $\log_{10}$ copies/mL. The incidence of mutations in each of the 3 P1032 arms was 0% by sequencing and 1.8%, 7.1%, and 5.3% by OLA in arms A, B, and C, respectively, compared with 13.4% by sequencing and 29.4% by OLA in the comparison group ($P < .001$ for each study arm vs comparison group). Grade 4 anemia developed in 1 woman.

Conclusions. A 7-day tail of highly active combination therapy or 1 month of dual therapy after SD-NVP prevents most NVP resistance to minimal toxicity.

Clinical Trials Registration. The IMPAACT P1032 Clinical Trial is NCT00109590, and the PHPT-2 Clinical Trial is NCT00398684.

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The administration of single-dose (SD) nevirapine (NVP) at delivery to human immunodeficiency virus (HIV)–infected pregnant women and their newborns after antenatal zidovudine therapy decreases the risk of perinatal mother-to-child transmission (MTCT) of HIV infection to 1.9% among formula-fed infants [1]. However, SD-NVP commonly leads to the emergence of NVP-resistant virus in the mother, with ≥85% of mothers having resistant virus detectable using sensitive assays [2, 3]. The resistant virus becomes undetectable in most women 12–24 months after delivery but may persist at a low frequency in plasma or lymphocytes [2, 4–6]. Furthermore, receipt of SD-NVP increases the risk of failure of subsequent NVP-containing therapy, particularly if initiated within the first 6–24 months after receiving SD-NVP [7–13].

The Treatment Options Preservation Study (TOPS) study demonstrated that the addition of a short (4- or 7-day) postpartum course (''tail'') of zidovudine plus lamivudine after SD-NVP reduced the incidence of NVP resistance mutations from 59% to 12% and 7.3%, respectively, when measured by consensus sequencing, an insensitive assay [14]. Other effective tails include 3 days of zidovudine and lamivudine (1.4% NVP resistance by sequencing) [15], a single dose of tenofovir and emtricitabine (12% by sequencing and 19% by the sensitive oligonucleotide ligation assay [OLA]) [16, 17] and 7 days of tenofovir and emtricitabine (none by sequencing) [18, 19]. Extending the duration of the tail may increase its effectiveness, with 1 month of zidovudine and didanosine resulting in an incidence of resistance of 0% by sequencing and 1.8% by OLA [20]. Use of a zidovudine and lamivudine tail can also result in lamivudine resistance, particularly among women who receive prenatal lamivudine [15]. The World Health Organization recommends a 7-day tail of zidovudine plus lamivudine for women who receive SD-NVP [21].

The plasma half-life of NVP after SD-NVP is long, and plasma concentrations persist at subtherapeutic concentrations for 3–4 weeks, which in the presence of viral replication, favors the selection of resistant virus [22]. The rationale for the tail is to suppress viral replication until NVP plasma concentrations decrease to a level that will not promote the emergence of resistant virus. We hypothesized that increasing the duration or potency of the tail after SD-NVP therapy would decrease the incidence of NVP-resistant virus to <10% when measured with a sensitive assay.

METHODS

Study Design

International Maternal Pediatric and Adolescent AIDS Clinical Trials Group (IMPAACT) P1032 was a phase II, randomized, open-label, 3-treatment-arm study conducted in Thailand from June 2006 through June 2008. We included a historical comparison group without a postpartum tail.

Study Population

We recruited HIV-infected pregnant women (28–38 weeks gestation) at 7 IMPAACT-affiliated sites in Thailand. Participants were ≥18 years of age, had a CD4 cell count >250 cells/μL within 30 days before screening, and were antiretroviral naive except for zidovudine prophylaxis to prevent MTCT of HIV infection. Exclusion criteria included intent to breast-feed, anticipated need for antiretroviral therapy within 8 weeks after delivery, and use of specified contraindicated medications.

Historical Comparison Group

Participant data and specimens were selected from the PHPT-2 study conducted in Thailand from 2001 through 2003 at 5 P1032 sites [1]. PHPT-2 evaluated the efficacy of SD-NVP to prevent MTCT among HIV-infected pregnant women receiving zidovudine with no postpartum antiretroviral tail. Inclusion criteria included receipt of SD-NVP, CD4 cell count >250 cells/μL within 30 days before screening, and availability of plasma samples at 10 days and 6 weeks post partum.

P1032 Study Treatments

Use of zidovudine after 27 weeks gestation was encouraged. At enrollment, participants were randomized equally to 3 treatment arms. Assignment was blinded until the onset of labor, when a single oral dose of NVP (200 milligrams) was administered. For prolonged labor, a second NVP dose was administered 48 hours later. Participants in arm A received zidovudine, didanosine, and lopinavir plus ritonavir during labor and for 7 days post partum. Participants in arm B received zidovudine and didanosine during labor and for 30 days post partum. Participants in arm C received the drugs in arm A for 30 days post partum. Drug doses were zidovudine (300 milligrams every 3 hours during labor and every 12 hours post partum irrespective of food), didanosine delayed-release enteric-coated capsule (400 milligrams, 250 milligrams if body weight <60 kilograms) once daily during labor and post partum without food, and lopinavir (400 milligrams) and ritonavir (100 milligrams) fixed-dose soft-gel capsules twice daily during labor and post partum with food. We demonstrated adequate lopinavir levels through 30 days post partum with the 400/100 milligrams lopinavir/ritonavir dose [23]. Infants received SD-NVP within 3 days after birth and zidovudine for 1–6 weeks, according to the local standard of care.

Evaluation and Follow-Up

We obtained a maternal medical history, performed physical examination, and collected blood samples for quantitative plasma HIV load and resistance testing at entry; delivery; at postpartum days 10, 21, and 30; and at weeks 5, 6, 8, 12, and 24 and, if NVP

resistance mutations were identified, at weeks 36, 48, and 72. CD4 cell counts were obtained at entry, delivery, and week 24. Safety laboratory tests were performed at entry, delivery, days 10 and 30, and week 24. Adverse events were graded using the Division of AIDS criteria [24]. Adherence to study medications was obtained by self-report.

For infants, we obtained a infant medical history, performed physical examination, and collected blood samples within 48 hours after birth; at 3, 5, and 12 weeks of age; and, in HIV-infected infants, at 16 and 24 weeks of age. Safety laboratory tests were performed at birth and at 21 days of age. Infant HIV infection was defined as 2 separate blood samples positive for HIV-1 DNA or RNA (>10 000 copies/mL) by polymerase chain reaction testing.

Drug Resistance Testing

Resistance mutations were defined using the International Antiviral Society–USA 2008 guidelines [25]. Resistance testing was performed at the Institut de Recherche pour le Développement UMI 174/Programs for HIV Prevention and Treatment (PHPT) Laboratory in Chiang Mai, Thailand. Plasma samples with a viral load >500 copies/mL were first tested for NVP resistance by consensus sequencing, which detects mutations present in 20%–50% of the viral population [26]. If negative, the samples were tested by OLA (optimized for HIV subtype AE) for NVP resistance mutations in the reverse transcriptase gene (codons 103, 181, and 190) [27, 28]. The OLA detects mutations present in >5% of the viral population [29]. For participants to be considered to be free of NVP-resistance mutations, all samples had to test negative by both assays. Samples with a viral load <500 copies/mL were considered to be free of mutations.

Women with resistance mutations identified by either assay post-partum were tested for preexisting mutation at study entry and delivery. In addition, when mutations were identified by OLA at any time, plasma and peripheral blood mononuclear cell samples from weeks 12, 24, 36, 48, and 72 were tested by OLA. HIV-infected infants were tested for resistance by sequencing and OLA.

For women in P1032, we tested all week 5 samples by OLA for zidovudine resistance mutations (T215F/Y and K70R). In addition, the first sample with a viral load >500 copies/mL after discontinuation of study treatment was tested for didanosine (L74V) and lopinavir (V82 A/S/T) resistance by sequencing and OLA.

Statistical Analysis

The analysis included all randomized women who received intrapartum NVP. Group characteristics were compared using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. The primary efficacy analyses (1) estimated the incidence of new NVP resistance mutations in plasma HIV within 8 weeks post partum in each study arm with use of an exact binomial confidence interval (CI) and (2) compared the incidence of mutations at day 10 or week 6 post partum in each study arm with that in the comparison group, with use of Fisher's exact test and exact logistic regression to adjust for baseline differences in age, CD4 cell count, and viral load at entry. Safety analyses included maternal adverse events with grades of >2 or leading to a change in study medications and all infant adverse events. All P values were 2-sided, with differences considered significant at $P < .05$. Statistical analyses were performed using SAS software, version 9.1 (SAS Institute), and LogXact software, version 8.0 (Cytel).

The sample size of 42 evaluable women per arm provided ≥90% power to detect a difference in incidence of new mutations between any study arm and the comparison group of 40% versus 10%, with a 2-sided .05 type I error rate (with Bonferroni adjustment for 3 comparisons), allowing for 1 interim efficacy analysis. The target enrollment was initially 150 women (to allow for 15% nonevaluable) but, before data analysis, was increased to 175 to provide 42 evaluable women per arm with an entry viral load >500 copies/mL.

Ethics, Consent, and Monitoring

The protocol was approved by the institutional review committees of the Thai Ministry of Public Health, Chiang Mai University, Mahidol University, and Tulane University. All participants provided written consent using consent forms written in Thai. An independent Data and Safety Monitoring Board reviewed the study annually and recommended continuation.

RESULTS

Study Participants

Of 175 enrolled women, 2 were excluded before delivery (consent withdrawal and enrollment error). Four women did not receive study drugs (3 delivered before reaching the maternity unit, 1 had elevated liver enzymes). The remaining 169 women and 170 live-born infants (1 set of twins) are included in the analysis. The baseline characteristics in the 3 study arms were similar (Table 1). At entry, women in the PHPT-2 comparison group were slightly younger, less likely to be receiving zidovudine, and had a lower median CD4 cell count and higher HIV load, compared with the P1032 study participants (Table 1).

Completion of Study Treatment, Adherence, and Follow-Up

One woman in each P1032 arm did not complete study treatment because of use of disallowed medications. One woman in arm A was unavailable for follow-up after her 5-week study visit, and 1 in arm C after her 8-week visit. Only 4 women reported incomplete adherence to study medications.

Table 1. Characteristics of Mothers at Entry and Delivery[a]

Characteristic	P1032					
	Arm A (ZDV, ddI, and LPV/r × 7 days) (n = 56)	Arm B (ZDV and ddI × 30 days) (n = 56)	Arm C (ZDV, ddI, and LPV/r × 30 days) (n = 57)	Total (n = 169)	PHPT-2 Comparison Group (n = 119)	P^b
Age at entry, years	27 (19– 45)	28 (18– 39)	27 (18– 39)	28 (18– 45)	26 (15– 39)	.03
Gestational age at entry, week	32 (28– 37)	31 (27–38)	31 (28–37)	31 (27– 38)	31 (20– 41)	NS
Received ZDV, No. (%)						
During pregnancy	56 (100)	56 (100)	55 (96)	167 (99)	119 (100)	NS
Before entry viral load	45 (80)	42 (75)	44 (77)	131 (78)	23 (19)	<.001
Duration of ZDV during pregnancy, week						
At entry	3 (0–9)	2 (0–10)	2 (0–27)	2 (0–27)	2 (1–6)	NS
At delivery	10 (2–16)	11 (5–18)	10 (0–31)	10 (0–31)	10 (2–16)	NS
CD4 cell count, cells/μL						
At entry	431 (262–1233)	413 (251–1024)	481 (262–1183)	456 (251–1233)	414 (251–999)	.05
At delivery	484 (70–1001)	517 (198–1150)	566 (222–1182)	513 (70–1182)	NA	
Plasma HIV load, $\log_{10}$ copies/mL						
At entry	3.48 (2.60–4.98)	3.52 (1.00–4.99)	3.54 (2.60–5.05)	3.49 (1.00–5.05)	4.04 (1.65–5.91)	<.001
At delivery	3.63 (1.70–4.90)	3.36 (1.70–4.83)	3.48 (1.70–4.71)	3.48 (1.70–4.90)	NA	
Plasma HIV load ≤500 copies/mL, No. (%)						
At entry	15 (26)	14 (25)	14 (25)	43 (25)	9 (8)	<.001
At delivery	16 (29)	15 (27)	12 (21)	43 (25)	NA	

Abbreviations: ddI, didanosine; HIV, human immunodeficiency virus; LPV/r, lopinavir and ritonavir; NA, not available; NS, not significant (P > .05); PHPT, Programs for HIV Prevention and Treatment; ZDV, zidovudine.

[a] Data are expressed as median (range), unless otherwise specified.

[b] Comparison of P1032 total and PHPT-2. Fisher exact test was used for dichotomous variables, and Wilcoxon test for continuous variables.

Incidence of NVP Resistance Mutations in P1032 Study Arms

To determine the overall incidence of NVP resistance mutations among women enrolled in P1032, we included all samples obtained from day 10 through week 8 post partum. No mutations were detected by consensus sequencing. With both sequencing and OLA, 4 women had NVP resistance mutations present at baseline and 13 (7.7%) met the primary end point by acquiring a new NVP resistance mutation in the first 8 weeks after delivery (G190A developed in 10 women, K103N in 2, and Y181C in 1). (Table 2) We included 2 women with missing baseline samples who were considered to have new mutations. The incidence of NVP resistance mutations was 5.4%, 12.5%, and 5.3% in arms A, B, and C, respectively, with overlapping CIs. When the participant unavailable for follow-up after week 5 was conservatively imputed to have developed resistance, the incidence of resistance in arm A was 7.1%. One woman received 2 doses, and 1 woman 3 doses of NVP because of prolonged labor; neither woman developed NVP resistance mutations.

Incidence of NVP Resistance Mutations in P1032 and PHPT-2

To compare the incidence of NVP-resistance mutations in P1032 and PHPT-2, only P1032 samples from day 10 and week 6 post partum were considered, because these correspond to the available samples from PHPT-2. By consensus sequencing, the incidence of resistance mutations was 0% for P1032 and 13% for PHPT-2 (among 112 participants with available samples) (Table 3). By both resistance assays, the incidence of resistance in each P1032 arm was significantly lower than that in PHPT-2 (1.8%, 7.1%, and 5.3% for P1032 arms A, B, and C respectively, versus 29% for the PHPT-2 comparison group; all $P < .001$). When missing samples were imputed to have resistance, the results were similar (Table 3, Figure 1). We also limited the analysis to women with an entry plasma HIV RNA level >500 copies/mL to exclude women likely to have samples that could not be tested for resistance. Although the overall incidence of resistance was slightly higher, the differences between the 2 studies were similar (4.9%, 9.5%, and 7.0 % vs 33.6%; all $P < .001$). In logistic regression analyses adjusting for the baseline $\log_{10}$ plasma HIV RNA concentration, CD4 cell count, and age, the odds of resistance developing remained significantly lower in each study arm than in the comparison group (odds ratio, 0.11 [95% CI, .01–.48]; 0.30 [.07–.99]; 0.16 [.03–.58] and for arms A, B, and C, respectively, vs PHPT-2).

Table 2. P1032 Participants: Incidence of Nevirapine Resistance Mutations at Any Visit From Day 10 Through 8 Weeks

Variable	Arm A (ZDV, ddI, and LPV/r × 7 days) (n = 56)[a]	Arm B (ZDV and ddI × 30 days) (n = 56)	Arm C (ZDV, ddI, and LPV/r × 30 days) (n = 57)	Total (n = 169)
Mutations detected by sequencing				
New mutations, No.	0	0	0	0
95% CI, %	0–6.4	0–6.4	0–6.3	0–2.2
New mutations, (missing samples imputed as resistant), No. (%)[a]	1 (1.8)	0	0	1 (0.6)
95% CI, %	.07–9.5	0–6.4	0–6.3	.01–3.3
Mutations detected by both sequencing and OLA				
Present at baseline, No.[b]	3	1	0	4
New mutations, No. (%)[c]	3 (5.4)	6 (10.7)	2 (3.5)	11 (6.5)
Mutations with missing baseline results, No.	0	1	1	2
Total new mutations, No. (%)[d]	3 (5.4)	7 (12.5)	3 (5.3)	13 (7.7)
95% CI, %	1.1–14.9	5.2–24.1	1.1–14.6	4.2–12.8
Mutations detected by both sequencing and OLA, missing samples imputed as resistant				
New mutations, No. (%)[a]	4 (7.1)	7 (12.5)	3 (5.3)	14 (8.3)
95% CI, %	2.0–17.3	5.2–24.1	1.1–14.6	4.6–13.5
New mutations among subjects with RNA >500 copies/mL at entry, No. (%)	4/41 (9.8)	6/42 (14.3)	3/43 (7.0)	13/126 (10.3)
95% CI, %	2.7–23.1	5.4–28.5	1.5–19.1	5.6–17.0

Abbreviations: CI, confidence interval; ddI, didanosine; LPV/r, lopinavir and ritonavir; OLA, oligonucleotide ligation assay; ZDV, zidovudine.

[a] Week 6 and 8 samples were missing for 1 subject in arm A.

[b] Baseline samples were tested only if a mutation was found in a later sample.

[c] Mutations that were not present at baseline. In arms A, B, and C, respectively, 4, 8, and 6 subjects had RNA levels <500 copies/mL at all visits and were assumed to have no resistance mutations.

[d] Assuming that mutations with missing baseline results were new.

Maternal Follow-up and Persistence of Mutations in P1032

At 24 weeks post partum, the median CD4 cell count and viral load was similar in the 3 arms (460 cells/μL and 1260 copies/mL overall, respectively). At 72 weeks post partum, among women in whom resistance developed within 8 weeks post partum, no mutations were detected by OLA in plasma samples, and only 1 woman had a mutation (G190A) detected in peripheral blood mononuclear cells by OLA.

Incidence of Zidovudine, Didanosine, and Lopinavir Resistance Mutations

Only 1 woman (arm B) had a new zidovudine resistance mutation (K70R), first detected by sequencing and OLA at 5 weeks post partum. No major didanosine or lopinavir resistance mutations were identified.

Maternal Adverse Events

There were no significant differences between study arms in the frequency of clinical or laboratory abnormalities. Two women had grade 3 diarrhea; 1, grade 3 vomiting, and 2, anemia (grade 3 and grade 4 in 1 each).

Outcomes in Infants

The 170 infants (55% male) had a median gestational age of 39 weeks (8% preterm) and birth weight of 3.04 kg. Infant characteristics were similar in the 3 study arms. There were no statistically significant differences between study arms in the frequency of clinical or laboratory abnormalities. A grade 3 abnormal heart rhythm developed in 1 infant, and another infant died of complications of trisomy 21; 11 had grade 4 laboratory abnormalities (anemia in 3, hyperkalemia in 4, and hyperglycemia in 4).

Two infants (1.2%) became HIV-1 infected, 1 with a positive HIV-1 DNA polymerase chain reaction result at birth and 1 with a positive DNA test result at 24 weeks of age after completing study follow-up, with negative DNA test results at birth and weeks 3, 5, and 12. One hundred sixty-six infants were confirmed to be uninfected, and 2 had indeterminate infection status, although both had negative viral tests at 3 and 5 weeks of age (1 was unavailable for follow-up at 5 weeks, and 1 died at 8 weeks). No resistance mutations were detected in either of the infected infants.

Table 3. P1032 and PHPT-2 Participants: Incidence of Nevirapine Resistance Mutations at Day 10 or Week 6 Post Partum

Variable	P1032			PHPT-2 Comparison Group (n = 119)
	Arm A (ZDV, ddI, and LPV/r × 7 days) (n = 56[a])	Arm B (ZDV and ddI × 30 days) (n = 56)	Arm C (ZDV, ddI, and LPV/r × 30 days) (n = 57)	
New mutations detected by sequencing, No. (%)	0	0	0	15/112[b] (13.4)
95% CI, %	0.0–6.4	0.0–6.4	0.0–6.4	7.7–21.2
New mutations detected by sequencing, missing samples imputed as resistant, No. (%)[a]	1 (1.8)[a]	0	0	22/112[b] (19.6)
95% CI, %	.07–9.5	0–6.4	0–6.3	12.7–28.2%
Mutations detected by both sequencing and OLA				
Mutations present at baseline, No.[c]	2	1	0	2
New mutations, No. (%)[d]	1 (1.8)	3 (5.4)	2 (3.5)	35 (29.4)
95% CI, %	.07–9.5	1.1–14.9	.5–12.1	21.4–38.5
Mutations with missing baseline results, No.	0	1	1	0
Total with new mutations, No. (%)[e]	1 (1.8)[a]	4 (7.1)	3 (5.3)	35 (29.4)[f]
95% CI, %	.07–9.5	2.0–17.3	1.1–14.6	21.4–38.5
Mutations detected by both sequencing and OLA, missing samples imputed as resistant[a,f]				
Incidence of new mutations, No. (%)	2 (3.6)	4 (7.1)	3 (5.3)	36 (30.3)
95% CI, %	.5–12.3	2.0–17.3	1.1–14.6	22.2–39.3
95% CI for difference between each P1032 arm and PHPT-2, %	11.8–42.2 ($P < .001$)	7.8–38.9 ($P < .001$)	9.9–40.7 ($P < .001$)	…
New mutations in subjects with RNA >500 copies/mL at entry, No. (%)	2/41 (4.9)	4/42 (9.5)	3/43 (7.0)	36/107 (33.6)
95% CI, %	.6–16.6	2.7–22.6	1.5–19.1	24.8–43.4
95% CI for difference between each P1032 arm and PHPT-2 among subjects with RNA >500 copies/mL at entry, %	11.2–46.3 ($P < .001$)	6.4–41.9 ($P < .001$)	9.1–44.1 ($P < .001$)	…

Abbreviations: CI, confidence interval; ddI, didanosine; LPV/r, lopinavir and ritonavir; OLA, oligonucleotide ligation assay; ZDV, zidovudine.

[a] The week 6 sample was missing for 1 subject in arm A.

[b] There were 7 subjects with no results, 15 with new mutations (no missing baseline results), and 7 missing either day 10 or week 6 results.

[c] Baseline samples were tested only if a mutation was found in a later sample.

[d] Mutations that were not present at baseline. Five, 17, and 15 subjects in arms A, B, and C, respectively, and 21 in PHPT-2 had RNA levels <500 copies/mL at both visits and were assumed to have no resistance mutations.

[e] Assuming that mutations with missing baseline results are new.

[f] One PHPT-2 subject missing day 10 sequencing and OLA results.

DISCUSSION

By both consensus sequencing and the more sensitive OLA assay, the 3 arms of P1032 had similar low rates of NVP resistance after SD-NVP therapy, with resistance identified in 7.7% of women overall. Each arm had a rate significantly lower than that in the historical comparison group (1.8%, 7.1%, 5.3%, respectively, vs 30%). The finding that a short tail of highly active antiretroviral therapy was as effective as a longer tail suggests that early suppression of viral replication prevents the selection of resistant virus for the full duration of NVP exposure. No P1032 participant had resistance mutations detectable by consensus sequencing alone, in contrast to 13.4% of the comparison group, suggesting that the mutations in the P1032 participants were present in low frequency. It is likely that low-frequency mutations are archived at a low concentrations in long-lived cells and will decay relatively rapidly after NVP has cleared, making them less likely to compromise subsequent therapy with NVP.

We included didanosine rather than lamivudine in the tail, because didanosine is less likely to cause viral resistance when used with zidovudine to prevent MTCT of HIV infection [30, 31]. In addition, hepatitis B virus (HBV) infection is common in Thailand, with a prevalence of 10% [32]. Because lamivudine has activity against HBV, its use will promote HBV resistance to lamivudine, and a rebound in HBV disease has been reported when lamivudine therapy is discontinued [33–35].

Factors that increase the risk of developing NVP-resistant virus after SD-NVP include the virus subtype [36, 37], a higher plasma viral load [37], and a higher plasma NVP concentration, which prolongs the duration of NVP exposure [6, 38].

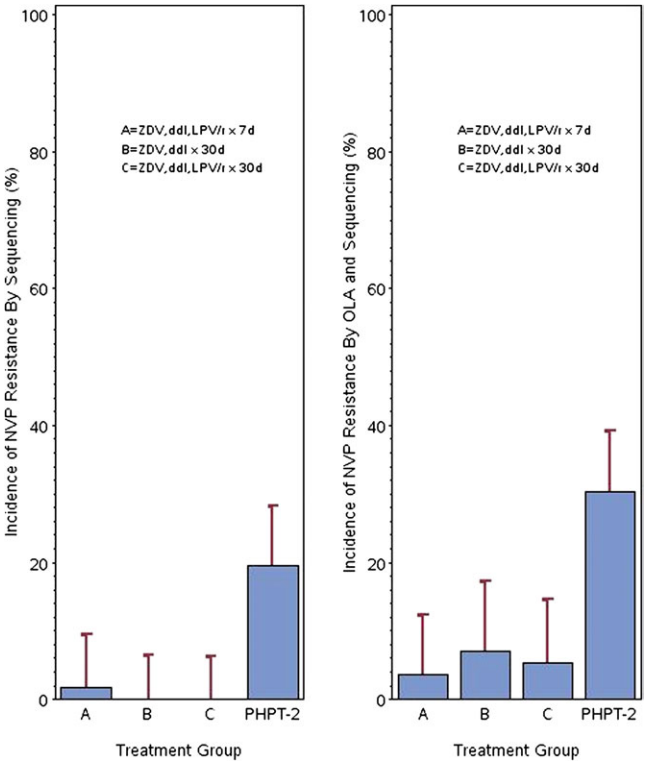

Figure 1. Incidence of nevirapine resistance mutations at day 10 or week 6 post partum for the 3 study arms in P1032 and for PHPT-2. Resistance identified by sequencing in left panel, oligonucleotide ligation assay and sequencing in right panel. The upper limit of the 95% confidence interval is shown. Missing samples are imputed as having resistance. Abbreviations: d, days; ddI, didanosine; LPV/r, lopinavir and ritonavir; NVP, nevirapine; OLA, oligonucleotide ligation assay; ZDV, zidovudine.

Participants in the comparison group, who were enrolled 5 years before P1032, were younger and had a slightly lower median CD4 cell count and higher median viral load. However, the results were unchanged when adjusted for these covariates in a logistic regression model.

Resistance mutations cannot be identified in specimens with a very low viral load, which we considered to be free of resistance mutations. Thus, in a planned secondary analysis, we estimated the incidence of resistance among only those women with a detectable viral load at entry. Among these women, the rates of resistance increased somewhat in all arms, but the differences between the P1032 arms and the comparison group remained significant (Table 3). Thus, the lower viral load in the P1032 participants is unlikely to account for their lower incidence of viral resistance.

Compared with 4–7 days of zidovudine plus lamivudine [14, 15] or a single dose of tenofovir and emtricitabine [17], the 3 regimens that we studied are more effective in reducing the risk of NVP resistance mutations as detected by consensus sequencing. Seven days of tenofovir and emtricitabine therapy also eliminated this risk [19]. In a recent study in Thailand, with use of the OLA assay, a 30-day tail of zidovudine and didanosine resulted in an incidence of NVP resistance of 1.8%, similar to the incidence in our study [20].

A limitation of the study is that it did not include a 7-day course of 2 nucleoside reverse-transcriptase inhibitors, as currently recommended by the World Health Organization. In the TOPS study, 3 and 7 days of zidovudine plus lamivudine resulted in a frequency of resistance of 13% and 9%, as shown by sequencing, whereas in P1032, no mutations were found by sequencing [14]. However, in the TOPS study, women did not receive zidovudine before labor. The risk of resistance in women receiving prepartum zidovudine and intrapartum NVP with a 7-day course of 2 reverse-transcriptase inhibitors remains to be evaluated.

In conclusion, all 3 P1032 regimens were well tolerated and, using a sensitive assay, markedly reduced the incidence of NVP resistance to a similar degree. In choosing among these options, 7 days of highly active antiretroviral therapy has the advantage of a short duration of therapy, but the cost, potential for intolerance, and limited availability of lopinavir and ritonavir in some locations may be a disadvantage. On the other hand, 30 days of dual reverse-transcriptase inhibitor therapy has the advantage of simplicity and low cost, but the longer duration of therapy has the potential for poorer adherence.

Notes

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Author contributions. The authors designed the study, wrote the protocol, had full access to the raw data, performed all analyses, wrote the manuscript, and had final responsibility for the decision to submit the manuscript for publication.

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References

1. Lallemant M, Jourdain G, Le Coeur S, et al. Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. N Engl J Med **2004**; 351: 217–28.
2. Loubser S, Balfe P, Sherman G, Hammer S, Kuhn L, Morris L. Decay of K103N mutants in cellular DNA and plasma RNA after single-dose nevirapine to reduce mother-to-child HIV transmission. AIDS **2006**; 20:995–1002.
3. Mofenson LM. Prevention in neglected subpopulations: prevention of mother-to-child transmission of HIV infection. Clin Infect Dis **2010**; 50(Suppl 3):S130–48.
4. Wagner TA, Kress CM, Beck I, et al. Detection of HIV-1 drug resistance in women following administration of a single dose of nevirapine: comparison of plasma RNA to cellular DNA by consensus sequencing and by oligonucleotide ligation assay. J Clin Microbiol **2010**; 48: 1555–61.
5. Palmer S, Boltz V, Martinson N, et al. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. Proc Natl Acad Sci U S A **2006**; 103:7094–9.
6. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS **2001**; 15: 1951–7.
7. Lockman S, Shapiro RL, Smeaton LM, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. N Engl J Med **2007**; 356:135–47.
8. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med **2004**; 351: 229–40.
9. Stringer JS, McConnell MS, Kiarie J, et al. Effectiveness of non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in women previously exposed to a single intrapartum dose of nevirapine: a multi-country, prospective cohort study. PLoS Med **2010**; 7:e1000233.
10. Coovadia A, Hunt G, Abrams EJ, et al. Persistent minority K103N mutations among women exposed to single-dose nevirapine and virologic response to nonnucleoside reverse-transcriptase inhibitor-based therapy. Clin Infect Dis **2009**; 48:462–72.
11. Chi BH, Sinkala M, Stringer EM, et al. Early clinical and immune response to NNRTI-based antiretroviral therapy among women with prior exposure to single-dose nevirapine. AIDS **2007**; 21:957–64.
12. Jourdain G, Wagner TA, Ngo-Giang-Huong N, et al. Association between detection of HIV-1 DNA resistance mutations by a sensitive assay at initiation of antiretroviral therapy and virologic failure. Clin Infect Dis **2010**; 50:1397–404.
13. Lockman S, Hughes MD, McIntyre J, et al. Antiretroviral therapies in women after single-dose nevirapine exposure. N Engl J Med **2010**; 363:1499–509.
14. McIntyre JA, Hopley M, Moodley D, et al. Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: a randomized clinical trial. PLoS Med **2009**; 6:e1000172.
15. Chaix ML, Ekouevi DK, Rouet F, et al. Low risk of nevirapine resistance mutations in the prevention of mother-to-child transmission of HIV-1: Agence Nationale de Recherches sur le SIDA Ditrame Plus, Abidjan, Cote d'Ivoire. J Infect Dis **2006**; 193:482–7.
16. Chi BH, Ellis GM, Chintu N, et al. Intrapartum tenofovir and emtricitabine reduces low-concentration drug resistance selected by single-dose nevirapine for perinatal HIV prevention. AIDS Res Hum Retroviruses **2009**; 25:1099–106.
17. Chi BH, Sinkala M, Mbewe F, et al. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. Lancet **2007**; 370:1698–705.
18. Arrive E, Chaix ML, Nerrienet E, et al. Maternal and neonatal tenofovir and emtricitabine to prevent vertical transmission of HIV-1: tolerance and resistance. AIDS **2010**; 24:2481–8.
19. Arrive E, Chaix ML, Nerrienet E, et al. Tolerance and viral resistance after single-dose nevirapine with tenofovir and emtricitabine to prevent vertical transmission of HIV-1. AIDS **2009**; 23:825–33.
20. Lallemant M, Ngo-Giang-Huong N, Jourdain G, et al. Efficacy and safety of 1-month postpartum zidovudine-didanosine to prevent HIV-resistance mutations after intrapartum single-dose nevirapine. Clin Infect Dis **2010**; 50:898–908.
21. World Health Organization. Rapid advice: use of antiretroviral drugs for treating pregnant women and preventing HIV infection infants—November 2009. Available at: http://www.who.int/hiv/pub/mtct/rapid_advice_mtct.pdf. Accessed 31 October 2011.
22. Cressey TR, Jourdain G, Lallemant MJ, et al. Persistence of nevirapine exposure during the postpartum period after intrapartum single-dose nevirapine in addition to zidovudine prophylaxis for the prevention of mother-to-child transmission of HIV-1. J Acquir Immune Defic Syndr **2005**; 38:283–8.
23. Cressey TR, Van Dyke R, Jourdain G, et al. Early postpartum pharmacokinetics of lopinavir initiated intrapartum in Thai women. Antimicrob Agents Chemother **2009**; 53:2189–91.
24. Center DRS. Table for grading the severity of adult and pediatric adverse events. Version 1.0-December 2004. Available at: http://rsc.tech-res.com/safetyandpharmacovigilance/. Accessed 31 October 2011.
25. Hirsch MS, Gunthard HF, Schapiro JM, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. Clin Infect Dis **2008**; 47:266–85.
26. Schuurman R, Demeter L, Reichelderfer P, Tijnagel J, de Groot T, Boucher C. Worldwide evaluation of DNA sequencing approaches for identification of drug resistance mutations in the human immunodeficiency virus type 1 reverse transcriptase. J Clin Microbiol **1999**; 37:2291–6.
27. Ellis GM, Mahalanabis M, Beck IA, et al. Comparison of oligonucleotide ligation assay and consensus sequencing for detection of drug-resistant mutants of human immunodeficiency virus type 1 in

peripheral blood mononuclear cells and plasma. J Clin Microbiol **2004**; 42:3670–4.
28. Beck IA, Mahalanabis M, Pepper G, et al. Rapid and sensitive oligonucleotide ligation assay for detection of mutations in human immunodeficiency virus type 1 associated with high-level resistance to protease inhibitors. J Clin Microbiol **2002**; 40:1413–9.
29. Beck IA, Crowell C, Kittoe R, et al. Optimization of the oligonucleotide ligation assay, a rapid and inexpensive test for detection of HIV-1 drug resistance mutations, for non-North American variants. J Acquir Immune Defic Syndr **2008**; 48:418–27.
30. Mandelbrot L, Landreau-Mascaro A, Rekacewicz C, et al. Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. JAMA **2001**; 285:2083–93.
31. Coffie PA, Ekouevi DK, Chaix ML, et al. Maternal 12-month response to antiretroviral therapy following prevention of mother-to-child transmission of HIV type 1, Ivory Coast, 2003–2006. Clin Infect Dis **2008**; 46:611–21.
32. Wiwanitkit V. An overview of hepatitis B serology screening check-up program among Thai workers. Viral Immunol **2002**; 15:647–9.
33. Wongprasit P, Manosuthi W, Kiertiburanakul S, Sungkanuparph S. Hepatitis B virus drug resistance in HIV-1-infected patients taking lamivudine-containing antiretroviral therapy. AIDS Patient Care STDS **2010**; 24:205–9.
34. Altfeld M, Rockstroh JK, Addo M, et al. Reactivation of hepatitis B in a long-term anti-HBs-positive patient with AIDS following lamivudine withdrawal. J Hepatol **1998**; 29:306–9.
35. Zhang JM, Wang XY, Huang YX, et al. Fatal liver failure with the emergence of hepatitis B surface antigen variants with multiple stop mutations after discontinuation of lamivudine therapy. J Med Virol **2006**; 78:324–8.
36. Eshleman SH, Guay LA, Mwatha A, et al. Characterization of nevirapine resistance mutations in women with subtype A vs. D HIV-1 6–8 weeks after single-dose nevirapine (HIVNET 012). J Acquir Immune Defic Syndr **2004**; 35:126–30.
37. Eshleman SH, Hoover DR, Chen S, et al. Nevirapine (NVP) resistance in women with HIV-1 subtype C, compared with subtypes A and D, after the administration of single-dose NVP. J Infect Dis **2005**; 192:30–6.
38. Chaix ML, Ekouevi DK, Peytavin G, et al. Impact of nevirapine (NVP) plasma concentration on selection of resistant virus in mothers who received single-dose NVP to prevent perinatal human immunodeficiency virus type 1 transmission and persistence of resistant virus in their infected children. Antimicrob Agents Chemother **2007**; 51: 896–901.