Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1 (Kesho Bora study): a randomised controlled trial

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Summary

Background Breastfeeding is essential for child health and development in low-resource settings but carries a significant risk of transmission of HIV-1, especially in late stages of maternal disease. We aimed to assess the efficacy and safety of triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis in pregnant women infected with HIV.

Methods Pregnant women with WHO stage 1, 2, or 3 HIV-1 infection who had CD4 cell counts of 200–500 cells per μL were enrolled at five study sites in Burkina Faso, Kenya, and South Africa to start study treatment at 28–36 weeks' gestation. Women were randomly assigned (1:1) by a computer generated random sequence to either triple antiretroviral prophylaxis (a combination of 300 mg zidovudine, 150 mg lamivudine, and 400 mg lopinavir plus 100 mg ritonavir twice daily until cessation of breastfeeding to a maximum of 6·5 months post partum) or zidovudine and single-dose nevirapine (300 mg zidovudine twice daily until delivery and a dose of 600 mg zidovudine plus 200 mg nevirapine at the onset of labour and, after a protocol amendment in December, 2006, 1 week post-partum zidovudine 300 mg twice daily and lamivudine 150 mg twice daily). All infants received a 0·6 mL dose of nevirapine at birth and, from December, 2006, 4 mg/kg twice daily of zidovudine for 1 week after birth. Patients and investigators were not masked to treatment. The primary endpoints were HIV-free infant survival at 6 weeks and 12 months; HIV-free survival at 12 months in infants who were ever breastfed; AIDS-free survival in mothers at 18 months; and serious adverse events in mothers and babies. Analysis was by intention to treat. This trial is registered with Current Controlled Trials, ISRCTN71468401.

Findings From June, 2005, to August, 2008, 882 women were enrolled, 824 of whom were randomised and gave birth to 805 singleton or first, liveborn infants. The cumulative rate of HIV transmission at 6 weeks was 3·3% (95% CI 1·9–5·6%) in the triple antiretroviral group compared with 5·0% (3·3–7·7%) in the zidovudine and single-dose nevirapine group, and at 12 months was 5·4% (3·6–8·1%) in the triple antiretroviral group compared with 9·5% (7·0–12·9%) in the zidovudine and single-dose nevirapine group (p=0·029). The cumulative rate of HIV transmission or death at 12 months was 10·2% (95% CI 7·6–13·6%) in the triple antiretroviral group compared with 16·0% (12·7–20·0%) in the zidovudine and single-dose nevirapine group (p=0·017). In infants whose mothers declared they intended to breastfeed, the cumulative rate of HIV transmission at 12 months was 5·6% (95% CI 3·4–8·9%) in the triple antiretroviral group compared with 10·7% (7·6–14·8%) in the zidovudine and single-dose nevirapine group (p=0·02). AIDS-free survival in mothers at 18 months will be reported in a different publication. The incidence of laboratory and clinical serious adverse events in both mothers and their babies was similar between groups.

Interpretation Triple antiretroviral prophylaxis during pregnancy and breastfeeding is safe and reduces the risk of HIV transmission to infants. Revised WHO guidelines now recommend antiretroviral prophylaxis (either to the mother or to the baby) during breastfeeding if the mother is not already receiving antiretroviral treatment for her own health.

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Introduction

In 2004, WHO issued guidelines[1] for the use of antiretroviral regimens for prevention of mother-to-child transmission (MTCT) of HIV-1 in resource-poor settings, which included recommendations for long-term antiretroviral therapy (ART) for women eligible for treatment (WHO clinical stage 4 or CD4 count <200 cells per μL); peripartum prophylaxis for women not eligible for treatment; and exclusive breastfeeding up to 6 months unless replacement feeding becomes acceptable, feasible, affordable, sustainable, and safe before that time. In practice, most HIV-infected

mothers breastfeed their infants, often not exclusively, which is associated with a significant risk of transmission.[2]

For immunocompromised mothers, ART is not only beneficial for maternal health but also reduces the risk of MTCT during pregnancy, delivery, and breastfeeding.[3–5]

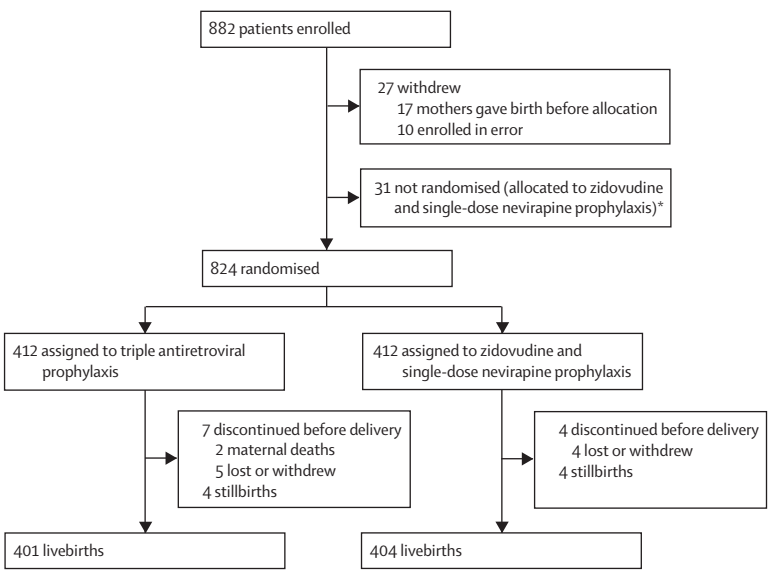

Figure 1: **Trial profile**
*Randomisation was suspended pending final approval of protocol amendments.

	Triple antiretroviral (n=412)	Zidovudine and single-dose nevirapine (n=412)
Study site		
Bobo Dioulasso, Burkina Faso	128 (31%)	123 (30%)
Durban, South Africa	93 (23%)	92 (22%)
Mombasa, Kenya	121 (29%)	124 (30%)
Nairobi, Kenya	20 (5%)	24 (6%)
Somkhele, South Africa	50 (12%)	49 (12%)
Age (years)	27 (24–31)	27 (23–31)
Education		
Never attended school	60 (15%)	63 (15%)
Completed primary school	136 (33%)	147 (36%)
At least some secondary school education	216 (52%)	202 (49%)
Occupation		
Unemployed	277 (67%)	298 (72%)
Self-employed	80 (19%)	69 (17%)
Salaried job	55 (13%)	45 (11%)
Marital status		
Married, monogamous	166 (40%)	184 (45%)
Married, polygamous	46 (11%)	51 (12%)
Not married, regular partner	180 (44%)	165 (40%)
Single	20 (5%)	12 (3%)
First pregnancy	74 (18%)	74 (18%)

Data are number (%) or median (IQR). Percentages do not add up to 100% in some cases because of rounding.

Table 1: **Demographics and baseline characteristics of the women**

For women infected with HIV who have a CD4 count greater than 500 cells per µL, the risk of transmission during breastfeeding is low, around 1%,[5,6] and any time-limited antiretroviral-based intervention was thought at the time of protocol development to potentially carry more risks (toxicity or emergence of resistance to antiretroviral drugs) than benefits in women who did not require ART for their own health.[7] For women with CD4 counts of 200–500 cells per µL, alternatives to replacement feeding to prevent postnatal HIV transmission are particularly important because breastfeeding is essential for child health and development in low-resource settings.[2] One alternative would be to provide antiretroviral prophylaxis to the mother or child during breastfeeding.[8–10]

We aimed to assess the efficacy and safety of triple antiretrovirals compared with those of zidovudine and single-dose nevirapine for prevention of MTCT during pregnancy and breastfeeding for pregnant women infected with HIV-1 who had CD4 cell counts of 200–500 copies per µL.

Methods

Patients

Full details of the rationale for the study and methods are provided elsewhere.[7] Key elements are summarised here. The Kesho Bora Study ("A better future" in Swahili) was a randomised controlled trial in antiretroviral-naive pregnant women infected with HIV-1 who visited antenatal clinics associated with five study sites (Centre Muraz, Bobo-Dioulasso, Burkina Faso; International Centre for Reproductive Health, Mombasa, Kenya; Kenyatta National Hospital, Nairobi, Kenya; University of KwaZulu-Natal, Durban, South Africa; and Africa Centre, University of KwaZulu-Natal, Somkhele, South Africa). Inclusion criteria were pregnancy less than 32 weeks' gestation (gestational age assessed with uterine height and date of last menstrual period; ultrasound was used when available); WHO clinical stage 1, 2, or 3 HIV infection; and CD4 cell count of 200–500 cells per µL. Women with CD4 counts of less than 200 cells per µL or greater than 500 cells per µL were offered enrolment into parallel prospective cohort studies.[5] Women with contraindications to rapid initiation of antiretrovirals were excluded—ie, those with a known allergy to antiretrovirals or benzodiazepines, those being treated with drugs that interact with antiretrovirals, or those with severe anaemia, neutropenia (grade 2 or above[11]), or liver or renal failure.

After receiving full oral and written information about the study at screening, eligible participants provided written informed consent when they returned for the enrolment visit. The study protocol was approved by the ethical and regulatory committees in Burkina Faso, Kenya, and South Africa (local and national, where required), and by WHO and the US Centers for Disease Control and Prevention.

Randomisation and masking

As soon as possible from 34 weeks, but not after 36 weeks, gestation, women were randomly assigned (1:1) to start either triple antiretroviral prophylaxis (triple antiretroviral group) or standard MTCT prophylaxis (zidovudine and single-dose nevirapine group).[1] Randomisation was stratified by centre and planned infant feeding mode, balanced in blocks of six or eight by a computer generated random sequence. Sealed envelopes containing the group assignment were prepared at the study coordinating centre and were marked externally only with the randomisation sequence number. The assigned envelope was opened only once all enrolment procedures had been completed. Patients and study investigators were not masked to treatment allocation.

Procedures

Women in the triple antiretroviral group received 300 mg zidovudine, 150 mg lamivudine, and 400 mg lopinavir plus 100 mg ritonavir twice daily until cessation of breastfeeding (to a maximum of 6·5 months post partum). Women in the zidovudine and single-dose nevirapine group received 300 mg zidovudine twice daily until delivery and a dose of 600 mg zidovudine plus 200 mg nevirapine (single-dose nevirapine) at onset of labour. From December, 2006, after WHO recommendations were updated,[12] the study protocol was amended and prophylaxis was initiated from 28 weeks' gestation for all patients enrolled after this date; and 1 week of zidovudine 300 mg twice daily plus lamivudine 150 mg twice daily post partum was added for women randomised to the zidovudine and single-dose nevirapine group.

All infants received a dose of 0·6 mL oral nevirapine suspension (about 2 mg/kg), preferably within 72 h of birth (no later than 7 days after birth). They also received co-trimoxazole prophylaxis from age 6 weeks to 12 months unless they were not infected with HIV after complete cessation of breastfeeding. From December, 2006, after the protocol amendment, they also received 1 week of zidovudine (4 mg/kg twice daily) from birth.[12]

All mothers were counselled on infant feeding as per 2003 WHO guidelines.[13] Women who opted for replacement feeding from birth received free formula up to 6 months and those who opted for breastfeeding were supported and counselled to exclusively breastfeed and rapidly wean over a 2-week period with complete cessation before the infant reached 6 months of age.

Women visited the antenatal clinics every 2 weeks from enrolment until delivery. Mothers and their babies attended antenatal clinics at 2, 4, 6, and 8 weeks after

	Triple antiretroviral (n=412)	Zidovudine and single-dose nevirapine (n=412)	p value*
CD4 count (cells per μL)†			
At enrolment	336 (282–408)	339 (267–408)	..
At delivery	463 (383–603)	416 (331–530)	<0·0001
At 6 months post partum	479 (367–597)	374 (292–473)	<0·0001
At 12 months post partum	401 (319–518)	378 (287–469)	0·002
Maternal viral load			
At enrolment (log_{10} copies per mL)‡	4·23 (3·66–4·75)	4·21 (3·58–4·74)	..
<300 copies per mL at delivery§	234/367 (64%)	111/373 (30%)	<0·0001
Mode of delivery¶			
Vaginal	360 (89%)	357 (88%)	0·14‖
Caesarean section before labour and rupture of membranes	19 (5%)	13 (3%)	..
Caesarean section after labour, rupture of membranes, or both	25 (6%)	38 (9%)	..
Duration of receipt of antiretrovirals from enrolment until delivery (weeks)	6·0 (4·1–8·4)	6·4 (4·3–8·8)	0·09
Adherence to antiretrovirals			
Reported no missed doses before delivery**	285/389 (73%)	290/396 (73%)	1·00
Received the nevirapine dose at the onset of labour††	NA	354/405 (87%)	..
Missed doses reported during post-partum prophylaxis‡‡			
0	207/383 (54%)	NA	..
1	38/383 (10%)	NA	..
>1	138/383 (36%)	NA	..

Data are median (IQR), n/N (%), or number (%). NA=not applicable. *χ^2 test for categorical variables, Student's *t* test for comparison of means, and sum rank test for comparison of medians. †Available for 412 women at randomisation, 375 of 405 at delivery, 371 of 390 at 6 months after delivery, and 357 of 374 at 12 months in the triple antiretroviral group and 412, 376 of 408, 362 of 390, and 357 of 368 in the zidovudine and single-dose nevirapine group. ‡Data missing for one woman in the zidovudine and single-dose nevirapine group. §Available for 367 of 405 women at delivery in the triple antiretroviral group and 373 of 408 in the zidovudine and single-dose nevirapine group. ¶Unknown timing of caesarean section for one woman in the triple antiretroviral group. ‖p for difference between all modes of delivery. **391 women in the triple antiretroviral group and 397 in the zidovudine and single-dose nevirapine group received at least 1 week of antiretrovirals before delivery; data unknown for two women in the triple antiretroviral group and one in the zidovudine and single-dose nevirapine group. ††Data unknown for three women. ‡‡29 women had less than 4 weeks of antiretroviral prophylaxis after delivery.

Table 2: **Laboratory findings, mode of delivery, and antiretroviral prophylaxis in mothers**

delivery and then monthly until 1 year and every 3 months thereafter. At each scheduled visit, clinical events, adherence to antiretrovirals, and nutritional status were recorded on standardised case-report forms. Infants' feeding patterns were assessed by an adaptation of the WHO infant feeding assessment.[14] Blood samples were taken from mothers for toxicity monitoring at enrolment, delivery, 2 and 6 weeks, and 3, 6, and 9 months after delivery; and from infants for toxicity monitoring and diagnosis of HIV infection at birth, 2 and 6 weeks, and 3, 6, 9, 12, 18, and 24 months.

The primary endpoints were HIV-free infant survival at 6 weeks and 12 months; HIV-free survival at 12 months in infants who were ever breastfed; AIDS-free survival in mothers at 18 months; and serious adverse events in mothers and babies. Data for AIDS-free survival in mothers at 18 months will be presented in another paper. The severity of clinical adverse experiences and laboratory abnormalities was graded with standard Division of AIDS toxicity tables.[11] All events that were grade 3 or higher and skin rashes and hepatic symptoms that were grade 2 or higher were deemed serious adverse events. Congenital abnormalities (except umbilical hernias) also were reported as serious adverse events.

Infants' infection status was assessed at age 6 weeks by a quantitative HIV-1 RNA real-time PCR assay with a lower detection limit of 300 copies per mL[15] (Generic HIV-1 Charge Virale, Biocentric, Bandol, France) in all sites except Nairobi, where a qualitative HIV-1 DNA PCR assay (Amplicor HIV-1 DNA v1.5 assay, Roche, Branchburg, NJ, USA) was initially used and the infection status of all infants thought to be HIV positive was subsequently confirmed with the Biocentric quantitative real-time PCR assay. Infants who were not infected with HIV at age 6 weeks were tested again at 12 months of age or a stored blood sample from their last visit was tested if the infant died or was lost to follow-up. If an infant was infected with HIV, earlier stored blood samples were tested to identify the time of infection (defined as the midpoint between the last negative and first positive PCR assay result). Infection was defined as a positive HIV test confirmed on a second sample, either by PCR or, if the infant was at least 18 months old, by serology. Infection was diagnosed with one sample only when a second sample was not available (eg, if the infant died before a second sample could be drawn). Disease transmission was judged to have occurred during the peripartum (in utero, intrapartum, or early postnatal) period if the PCR assay was positive at 6 weeks of age, and during the late postnatal period if the PCR was negative at 6 weeks and subsequently positive. Infants who died or were lost to follow-up before any blood sample could be taken were classified as unknown infection status.

	Triple antiretroviral	Zidovudine and single-dose nevirapine	p value*
Mothers†			
Clinical SAEs			
Women with at least one SAE (%)	57 (14%)	48 (12%)	0·35
SAEs	62 (15 per 100 women)	60 (15 per 100 women)	..
Infectious diseases	34	34	..
Obstetric pathology	23	15	..
Other SAEs	5	11	..
Deaths (%)	4 (1%)	4 (1%)	1·00
Infants‡			
Clinical SAEs			
Infants with at least one SAE (%)	107 (27%)	110 (27%)	0·86
Number of SAEs	141 (35 per 100 infants)	145 (36 per 100 infants)	..
Infectious diseases	91	87	..
Malnutrition/wasting	17	15	..
Congenital abnormalities	8	13	..
Neonatal problem	13	20	..
Other SAEs	12	10	..
Deaths (%)	24 (6%)	38 (9%)	0·09

SAE=serious adverse event. *χ^2 test. †n=412 in both groups. ‡n=401 in the triple antiretroviral group and n=404 in the zidovudine and single-dose nevirapine group.

Table 3: **Serious adverse events and deaths in mothers and infants up to 12 months**

Statistical analysis

A sample size of 869 was needed to achieve 90% statistical power to detect differences in HIV transmission rates at 12 months, and 80% power for infant HIV infection or death at 12 months. A sample size of 1574 was needed to achieve 90% power to detect differences in HIV transmission rates at 6 weeks. Group comparisons were made using Student's *t* test and χ^2 test (95% level, two-sided). The complement of the Kaplan-Meier product-limit estimates for remaining alive, free of HIV infection, or alive and free of HIV infection were used to assess mortality, HIV transmission rates, and rates of transmission or death combined; rates were compared with log-rank tests stratified by centre and intention to breastfeed.[16] Analyses were by intention to treat. Only the first liveborn infant from each multiple pregnancy was included in the analyses.[16] Infants lost to follow-up were censored at the date of their last negative HIV test for analyses of transmission, or date of last visit for mortality analysis and combined analysis of transmission or death. Factors associated with undetectable viral load at delivery, and peripartum and post-partum transmission were analysed with logistic regression stratified by centre.

This trial is registered with Current Controlled Trials, ISRCTN71468401.

Role of the funding source

Among the sponsors of the study, representatives of Agence nationale de recherches sur le sida et les hépatites virales, UNDP/UNFPA/World Bank/WHO Special Programme of Research, Development and Research Training in Human Reproduction, the Centers for Disease Control and Prevention, and Eunice Kennedy Shriver

National Institute of Child Health and Human Development were part of the study team and therefore these sponsors were involved in the study design, coordination, data collection, data analysis, data interpretation, and writing of the report. The study group had full access to all the data in the study and had final responsibility for the analysis and interpretation of the data and the decision to submit for publication.

Results

Between June, 2005, and August, 2008, 824 pregnant women were randomly assigned treatment and delivered 805 singleton or first, liveborn babies (figure 1). There were 11 multiple pregnancies (four in the triple antiretroviral group and seven in the zidovudine and single-dose nevirapine group). Maternal baseline characteristics were balanced across study groups (table 1). Overall, 73% of women reported no missed antenatal antiretroviral dose (table 2). 87% of women in the zidovudine and single-dose nevirapine group received a single dose of nevirapine at the onset of labour. After birth, 64% of women in the triple antiretroviral group reported having missed none or one dose.

The incidence of clinical and laboratory serious adverse events was similar in the two groups (table 3; webappendix pp 1–2). Eight women died (four in each group); causes of death included septic shock during prolonged labour (one woman), opportunistic infections (tuberculosis, severe gastroenteritis, anaemia with cardiac failure while on ART for HIV disease progression during follow-up, and acute pulmonary distress thought to be of infectious origin), two unexplained sudden deaths, and one probable intoxication by herbal medicines. The most common serious adverse events overall were infections (8·3 per 100 women), most notably tuberculosis (19 women), malaria (eight), and pneumonia (nine), with no significant differences between groups (data not shown). The next most common serious adverse events were obstetric (4·6 per 100 women). In the triple antiretroviral group, the antiretroviral regimen was stopped or changed in three women, all because of anaemia. Grade 3 or 4 anaemia (haemoglobin <7 g/L) was the most common laboratory abnormality, which occurred in ten (2·7%) of 377 women at delivery in the triple antiretroviral group compared with seven (1·8%) of 381 in the zidovudine and single-dose nevirapine group, and decreased to 0·8% and 0·5% at 3 months after delivery; the differences between treatment groups were not significant (webappendix p 1).

Maternal median CD4 cell counts were higher in the triple antiretroviral group than the zidovudine and single-dose nevirapine group at delivery, and at 6 months and 12 months (table 2). At delivery, the proportion of women with undetectable viral load was over two-times higher in

See Online for webappendix

	Triple antiretroviral			Zidovudine and single-dose nevirapine		
	n/N (%)	OR (95% CI)	Adjusted OR (95% CI)*	n/N (%)	OR (95% CI)	Adjusted OR (95% CI)*
Overall	234/367 (64%)	..	..	111/373 (30%)	..	..
Study site						
Bobo Dioulasso	80/113 (71%)	1·14 (0·51–2·52)	2·55 (1·06–6·15)	21/114 (18%)	0·40 (0·17–0·91)	0·73 (0·30–1·78)
Durban	62/90 (69%)	1·04 (0·45–2·37)	1·32 (0·55–3·16)	40/90 (44%)	1·41 (0·64–3·11)	1·63 (0·70–3·75)
Mombasa	52/97 (54%)	0·54 (0·24–1·19)	0·74 (0·31–1·77)	29/101 (29%)	0·71 (0·32–1·58)	0·79 (0·33–1·88)
Nairobi	8/20 (40%)	0·31 (0·09–1·05)	0·57 (0·16–1·99)	4/21 (19%)	0·42 (0·10–1·62)	0·36 (0·09–1·43)
Somkhele	32/47 (68%)	1·00	1·00	17/47 (36%)	1·00	1·00
Duration of use of antiretrovirals before delivery						
<5 weeks	63/135 (47%)	1·00	1·00	33/117 (28%)	1·00	1·00
5–6 weeks	66/92 (72%)	2·90 (1·59–5·32)	2·91 (1·52–5·57)	31/103 (30%)	1·10 (0·59–2·04)	1·10 (0·56–2·17)
7–8 weeks	47/66 (71%)	2·83 (1·44–5·59)	2·82 (1·36–5·86)	24/64 (38%)	1·53 (0·76–3·07)	1·53 (0·71–3·30)
>8 weeks	58/74 (78%)	4·14 (2·07–8·37)	3·60 (1·70–7·61)	23/89 (26%)	0·89 (0·45–1·73)	0·81 (0·39–1·72)
Prophylaxis as soon as possible from						
34 weeks' gestation	123/215 (57%)	1·00	..	54/213 (25%)	1·00	..
28 weeks' gestation	111/152 (73%)	2·02 (1·26–3·25)	..	57/160 (36%)	1·63 (1·02–2·61)	..
Plasma viral load at enrolment (log_{10} copies per mL)						
<3·5	59/71 (83%)	1·00	1·00	49/74 (66%)	1·00	1·00
3·5–3·9	51/65 (78%)	0·74 (0·29–1·89)	0·62 (0·25–1·52)	25/66 (38%)	0·31 (0·15–0·66)	0·29 (0·14–0·60)
4·0–4·4	55/87 (63%)	0·35 (0·15–0·79)	0·25 (0·11–0·57)	26/91 (29%)	0·20 (0·10–0·42)	0·20 (0·10–0·40)
4·5–4·9	47/79 (59%)	0·30 (0·13–0·68)	0·19 (0·08–0·45)	8/69 (12%)	0·07 (0·02–0·17)	0·07 (0·03–0·18)
≥5	18/56 (32%)	0·10 (0·04–0·24)	0·05 (0·02–0·12)	2/66 (3%)	0·02 (0·00–0·07)	0·02 (0·00–0·08)

OR for each term measured alone. No other factors from tables 1 or 2 were associated with undetectable viral load at delivery. OR=odds ratio. *Based on a model that included all terms in the column.

Table 4: **Factors associated with an undetectable viral load at delivery**

	Triple antiretroviral (n=401)	Zidovudine and single-dose nevirapine (n=404)	p value*
Girls	198 (49%)	213 (53%)	0·36
Antiretrovirals			
Nevirapine neonatal dose received	371/382 (97%)	374/384 (97%)	0·83
Postnatal week of zidovudine received†	162/165 (98%)	154/161 (95%)	0·33
Birthweight			
Low (<2500 g)	44/384 (11%)	28/379 (7%)	0·06
Very low (<1500 g)	1/384 (0%)	2/379 (1%)	0·62
Gestational age			
Preterm (<37 completed weeks of gestation)	53/401 (13%)	45/404 (11%)	0·39
Very preterm (<32 completed weeks of gestation)	2/401 (0%)	1/404 (0%)	0·62
Ever breastfed	307/401 (77%)	317/404 (78%)	0·55
Duration of breastfeeding in weeks	21·4 (8·6–25·4)	19·0 (9·0–25·7)	0·95
Exclusive breastfeeding up to last available visit before 3 months	135/298 (45%)	134/304 (44%)	0·80

Data are number (%), n/N (%), or median (IQR). *χ^2 test for categorical variables and sum rank test for comparison of medians. †Not applicable (early protocol version with no postnatal zidovudine) in 227 infants in the triple antiretroviral group and in 226 in the zidovudine and single-dose nevirapine group.

***Table 5*: Characteristics of the infants at birth and during follow-up**

the triple antiretroviral group than in the zidovudine and single-dose nevirapine group (p<0·0001; table 4). In the triple antiretroviral group, women with a shorter duration of treatment with antiretrovirals before delivery and those with higher viral load at enrolment were less likely to have undetectable viral load at delivery (table 4). In this group, the protocol amendment to initiate prophylaxis as soon as possible after 28 weeks instead of 34 weeks gestation increased the median duration of prophylaxis before delivery (7·7 weeks *vs* 5·0 weeks, p<0·0001) and the proportion of women with undetectable viral load at delivery (p=0·002; table 4). In an exploratory multivariate analysis, lower viral load at enrolment (p<0·0001) and longer duration of antiretroviral use before delivery (p=0·003) were significantly associated with undetectable viral load at delivery, after adjustment for centre in the triple antiretroviral group. In the zidovudine and single-dose nevirapine group, only lower viral load at enrolment was predictive of undetectable viral load at delivery after adjustment for centre (p<0·0001).

About 78% of infants were ever breastfed and the proportion was similar in both groups (table 5). Among infants who were ever breastfed, the median duration of breastfeeding and the proportion of infants exclusively breastfed up to age 3 months were similar between groups. In the triple antiretroviral group, 26 of 307 infants were still breastfed after cessation of the triple antiretrovirals. 27 infants from the triple antiretroviral group and 36 from the zidovudine and single-dose nevirapine group were lost to follow-up before 12 months, giving cumulative infant follow-up rates at 12 months of 93% in the triple antiretroviral group and 91% in the zidovudine and single-dose nevirapine group (p=0·25).

There were no statistically significant differences between the two groups in the proportions of infants with common laboratory serious adverse events (anaemia and neutropenia) or clinical serious adverse events (table 3; webappendix p 2). Grade 3 or 4 anaemia at birth occurred in 15% of infants in the triple antiretroviral group and 12% in the zidovudine and single-dose nevirapine group (p=0·18). Severe neutropenia occurred in 8% of infants in both groups at birth, but decreased to less than 1% by 3 months of age. Apart from preterm birth and low birthweight, 286 clinical serious adverse events were experienced by 217 (27%) of the 805 infants (table 3), an incidence rate of 35·6 per 100 infants. Incidence was similar between groups. 62% of all adverse events were infectious diseases (including 20 malaria episodes, 25 gastroenteritis cases, and 29 respiratory infections). There were fewer infant deaths before 12 months in the triple antiretroviral group than in the zidovudine and single-dose nevirapine group (table 3). Causes of death included four HIV-related, five neonatal, ten gastroenteritis, three pneumonia, and two malaria in the triple antiretroviral group; and there were eight HIV-related deaths, three neonatal deaths, seven gastroenteritis, ten pneumonia, two malaria, three other infections, one poisoning, one intestinal obstruction, one malnutrition, and two sudden, unexplained deaths in the zidovudine and single-dose nevirapine group. Cumulative 12-month mortality was 6·2% (95% CI 4·2–9·1%) in the triple antiretroviral group and 9·8% (7·2–13·2%) in the zidovudine and single-dose nevirapine group (p=0·06, log-rank test adjusted for centre and intention to breastfeed).

In the triple antiretroviral group, fewer infants were infected with HIV at 12 months (one had only one diagnostic assay) than in the zidovudine and single-dose nevirapine group (four only had one diagnostic assay; table 6; figure 2). The 12-month cumulative HIV infection rate was lower in the triple antiretroviral group than in the zidovudine and single-dose nevirapine group (p=0·029)—a relative risk reduction of 43%. The risk was not significantly different between groups at birth (p=0·52) or at 6 weeks of age (p=0·24). The number of infants infected after 6 weeks (when all transmissions are attributable to breastfeeding) but before 6 months after birth (end of breastfeeding with antiretroviral prophylaxis) was six in the triple antiretroviral group and 13 in the zidovudine and single-dose nevirapine group. The rates of infant HIV infection or death at 12 months were lower in the triple antiretroviral group than in the zidovudine and single-dose nevirapine group (p=0·017). Among the 75% of women who intended to breastfeed at the time of randomisation, of whom 571 of 597 (96%) actually initiated breastfeeding, cumulative rates of transmission at 12 months were lower in the triple antiretroviral group than in the zidovudine and single-dose nevirapine group (p=0·02; table 6); the relative risk reduction was 48%.

When stratified by CD4 count at enrolment (prespecified secondary objective), there was a significant difference at 12 months between the triple antiretroviral group and the zidovudine and single-dose nevirapine group when the CD4 count was less than 350 cells per µL (p=0·03; 48% risk reduction) but not when the CD4 count was 350 cells per µL or higher (p=0·33; 34% risk reduction; table 6). The number of infections averted was 12 in infants born to mothers with CD4 counts of less than 350 cells per µL versus four in those born to mothers with counts of 350–500 cells per µL. In infants born to mothers with CD4 counts less than 350 cells per µL there were four infections between 6 weeks and 6 months in the triple antiretroviral group versus nine in the zidovudine and single-dose nevirapine group, a 56% reduction. The risk of transmission was low in both groups when women had undetectable plasma viral loads at delivery, with a cumulative rate at 12 months of 2·7% in both groups (table 6). In an exploratory univariate and multivariate analysis, the only factor from the mother and infant characteristics (tables 1, 2, and 5) significantly associated with peripartum transmission

	Triple antiretroviral (n=401)		Zidovudine and single-dose nevirapine (n=404)		Relative risk reduction	p value
	Cumulative number of events/number at risk	Rate (95% CI)*	Cumulative number of events/number at risk	Rate (95% CI)*		
Infection						
Birth	7/394	1·8% (0·9–3·7%)	10/402	2·5% (1·3–4·6%)	28%	..
6 weeks	13/375	3·3% (1·9–5·6%)	20/374	5·0% (3·3–7·7%)	34%	..
6 months	19/349	4·9% (3·1–7·6%)	33/339	8·4% (6·0–11·6%)	42%	..
12 months	21/333	5·4% (3·6–8·1%)	37/305	9·5% (7·0–12·9%)	43%	0·029†
Infection or death						
Birth	11/399	2·8% (1·5–4·9%)	12/404	3·0% (1·7–5·2%)	7%	..
6 weeks	19/376	4·8% (3·1–7·4%)	25/376	6·2% (4·2–9·1%)	23%	..
6 months	33/352	8·4% (6·0–11·6%)	50/339	12·6% (9·7–16·3%)	33%	..
12 months	40/334	10·2% (7·6–13·6%)	63/304	16·0% (12·7–20·0%)	36%	0·017†
Infection from mothers who intended to breastfeed						
Birth	5/296	1·7% (0·7–4·0%)	8/301	2·7% (1·3–5·2%)	37%	..
6 weeks	8/284	2·7% (1·4–5·3%)	16/279	5·4% (3·3–8·6%)	50%	..
6 months	14/261	4·8% (2·9–8·0%)	28/251	9·5% (6·7–13·5%)	49%	..
12 months	16/249	5·6% (3·4–8·9%)	31/227	10·7% (7·6–14·8%)	48%	0·02‡
Infection, mothers' CD4 count <350 cells per µL						
Birth	4/219	1·8% (0·7–4·8%)	7/224	3·1% (1·5–6·3%)	42%	..
6 weeks	8/209	3·7% (1·8–7·2%)	14/205	6·3% (3·8–10·4%)	41%	..
6 months	12/195	5·5% (3·2–9·5%)	23/184	10·5% (7·1–15·4%)	48%	..
12 months	13/189	6·0% (3·5–10·1%)	25/164	11·5% (7·9–16·5%)	48%	0·03†
Infection, mothers' CD4 count ≥350 cells per µL						
Birth	3/175	1·7% (0·6–5·2%)	3/178	1·7% (0·5–5·1%)	0%	..
6 weeks	5/166	2·9% (1·2–6·7%)	6/169	3·4% (1·5–7·4%)	15%	..
6 months	7/154	4·1% (2·0–8·3%)	10/155	5·8% (3·1–10·5%)	29%	..
12 months	8/144	4·7% (2·4–9·3%)	12/141	7·1% (4·1–12·2%)	34%	0·33†
Infection, mothers' viral load at delivery <300 copies per mL						
Birth	0/229	0	2/110	1·8% (0·5–7·1%)	100%	..
6 weeks	3/224	1·3% (0·4–4·0%)	3/105	2·7% (0·9–8·2%)	52%	..
6 months	5/207	2·2% (0·9–5·3%)	3/97	2·7% (0·9–8·2%)	19%	..
12 months	6/200	2·7% (1·2–5·9%)	3/91	2·7% (0·9–8·2%)	0%	0·99†
Infection, mothers' viral load at delivery ≥300 copies per mL						
Birth	7/131	5·3% (2·6–10·9%)	8/260	3·1% (1·6–6·1%)	–71%	..
6 weeks	10/120	7·6% (4·2–13·7%)	16/239	6·2% (3·8–9·9%)	–23%	..
6 months	13/112	10·0% (5·9–16·5%)	28/217	11·0% (7·7–15·5%)	9%	..
12 months	14/108	10·8% (6·5–17·5%)	30/193	11·9% (8·5–16·6%)	9%	0·98†

*Complement of Kaplan-Meier product-limit estimates for remaining alive, free of HIV infection, or alive and free of HIV infection. †Log-rank test stratified by centre and intention to breastfeed. ‡Log-rank test stratified by centre.

Table 6: **Cumulative life table rates of HIV-1 infection or HIV-1 infection or death in infants**

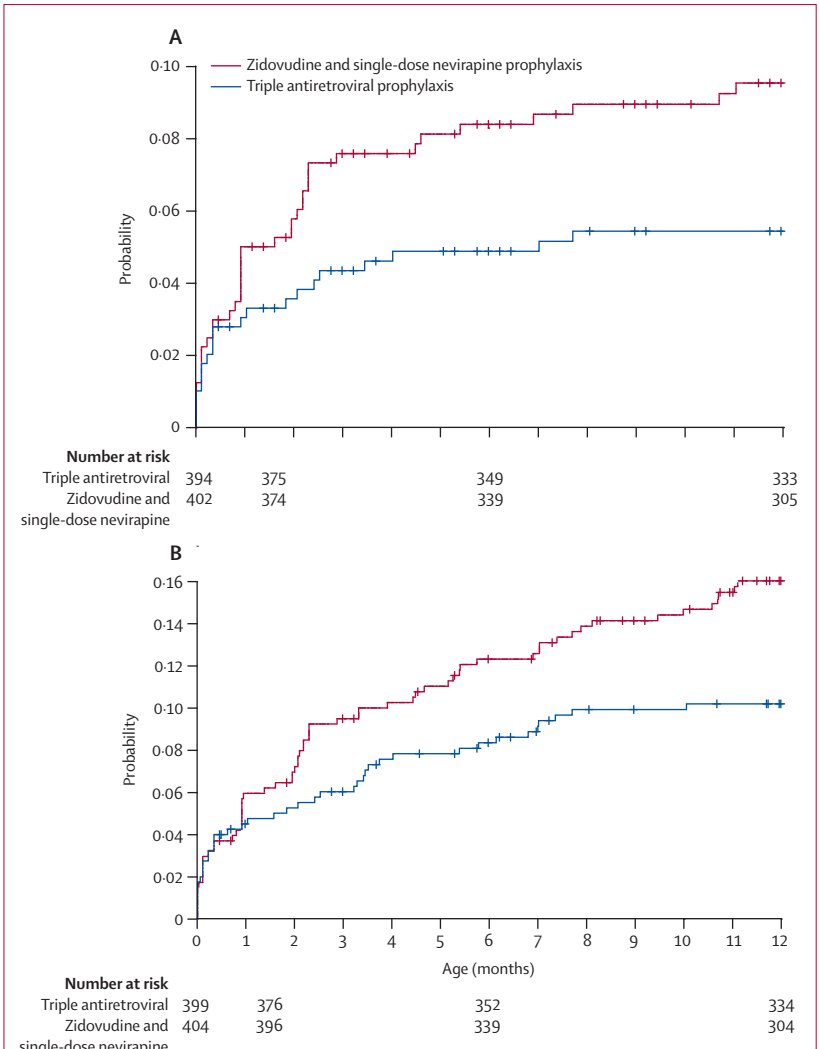

Figure 2: **Kaplan-Meier cumulative life table estimates of HIV-1 infection (A) and HIV-1 infection or death (B) in infants**

was detectability of virus at delivery (p=0·03; data not shown).

The rates of late postnatal HIV infections (after age 6 weeks) were 2·2% (95% CI 1·1–3·9%) in the triple antiretroviral group and 4·7% (2·9–7·3%; p=0·054) in the zidovudine and single-dose nevirapine group (relative risk reduction 53%), and only occurred in infants who were ever breastfed (data not shown). In both groups, the risk of late transmission after birth was associated with longer breastfeeding (median duration 22·4 [IQR 11·1–28·7] weeks in infants infected after birth compared with 13·7 [0·7–22·3] weeks in uninfected infants; p=0·001) and higher plasma viral load at delivery (median 3·3 [2·6–3·9] *vs* 2·5 [0·0–3·4] $\log_{10}$ copies per mL; p=0·006), but not with mode of breastfeeding (exclusive up to 3 months *vs* mixed; p=0·73; data not shown).

Discussion

Maternal triple antiretroviral prophylaxis reduced the risk of MTCT of HIV by 43% at 12 months compared with a standard zidovudine and single-dose nevirapine regimen. Women with CD4 cell counts of less than 350 cells per µL had a higher relative risk reduction and number of infections averted than did those with higher counts. Transmission rates at birth and age 6 weeks did not differ significantly between groups. However, because of slower than anticipated recruitment, the study did not have sufficient power to detect differences at age 6 weeks and antenatal duration of triple antiretroviral prophylaxis might have been too short (median 6 weeks) to have an effect on peripartum transmission over and above that provided by zidovudine and single-dose nevirapine prophylaxis.

Triple antiretroviral prophylaxis was not associated with significant increases in antiretroviral-related serious adverse events in mothers or their babies compared with zidovudine and single-dose nevirapine prophylaxis. Further analyses of the effect of triple antiretroviral prophylaxis on maternal health outcomes such as emergence of drug resistance and HIV disease progression are underway. Follow-up of mothers to 18–24 months (to be reported elsewhere) will provide important information.

Women and infants in Kesho Bora received intensive follow-up and active tracing. The follow-up rates at 12 months were 93% for infants exposed to triple antiretroviral and 91% for those exposed to zidovudine and single-dose nevirapine prophylaxis. Therefore, deaths or serious adverse events were probably not disproportionately missed in infants lost to follow-up. For five infants, HIV infection could not be confirmed with a second sample, but a misclassification of one or more of these five infants affecting the comparison between groups is unlikely.

The reported infant mortality rates in Kesho Bora were compatible with the national estimates of 10·5% in Burkina Faso, 7·4% in Kenya, and 4·5% in South Africa,[17] although comparison is difficult because, although mortality is expected to be higher in infants exposed to HIV, infants in clinical trial settings should receive a higher quality of care.

The only other randomised trial that has assessed a three-drug maternal prophylaxis regimen during breastfeeding was the breastfeeding, antiretrovirals, and nutrition (BAN) study[9] in Malawi (panel), although prophylaxis was only started after delivery. The relative risk reduction in HIV transmission at age 28 weeks was 25% (cumulative rate 8·2% with maternal prophylaxis *vs* 10·9% with no post-partum prophylaxis), compared with 42% in Kesho Bora (4·9% with triple antiretroviral prophylaxis *vs* 8·4% with zidovudine and single-dose nevirapine prophylaxis) at age 6 months. In infants who were not infected with HIV at age 2 weeks, the relative risk reduction was 49% in BAN (2·9% *vs* 5·7%) compared

with 47% in Kesho Bora (3·1% *vs* 5·9% at age 6 months among infants uninfected at birth). The Mma Bana randomised trial[10] in Botswana compared two different maternal prophylactic regimens that were started as soon as possible from the 26th week of pregnancy. 6-month cumulative rates of transmission were below 2%, with maternal viral load at delivery of less than 400 copies per mL achieved in over 90% of women (compared with 70% of women in Kesho Bora whose viral load was less than 400 copies per mL; data not shown). Viral load at enrolment was lower and the duration of antiretroviral prophylaxis before delivery longer in Mma Bana than in Kesho Bora. Ensuring an undetectable viral load by the time of delivery and during breastfeeding is the aim of maternal antiretroviral prophylaxis but cannot be achieved in all circumstances. Both duration of antenatal triple antiretroviral prophylaxis and baseline viral load were significantly associated with undetectable viral load at delivery in Kesho Bora in women receiving triple antiretroviral prophylaxis. However, with 8 weeks of prophylaxis viral load remained detectable in almost 30% of women.

In observational studies, cumulative rates of transmission from mothers receiving triple antiretroviral prophylaxis during breastfeeding irrespective of CD4 count (and with unknown viral load at enrolment) were 1·8% at 9 months in Rwanda,[18] 2·8% at 12 months in Mozambique,[19] 5·2% at 12 months in Kenya,[4] and 5·8% at 12 months in Tanzania.[20] These results are comparable to the 12-month transmission rate of 5·4% from mothers in the triple antiretroviral group in Kesho Bora.

Preliminary results from the Kesho Bora study[21] were considered in October, 2009, for the revision of the WHO guidelines,[22] which now recommend ART for all pregnant women infected with HIV who have CD4 counts of 350 cells per μL or less and antiretroviral prophylaxis during breastfeeding for women not on ART. Such prophylaxis can be given either to the mother as in Kesho Bora and BAN[9] or to the infant as in the Post-Exposure Prophylaxis of Infants[23] and BAN[9] trials, and seem to have similar effects on HIV transmission rates,[9] although no study has directly compared maternal with infant prophylaxis during breastfeeding (BAN was powered to compare each group to placebo but not against each other).

Although providing antiretrovirals until complete cessation of breastfeeding in Kesho Bora might have been preferable, the ethics committees restricted antiretrovirals to 6·5 months after birth to avoid encouraging women to prolong breastfeeding beyond the minimum period of recommended exclusive breastfeeding, the safety of which was unknown. However, in Kesho Bora and other studies that provided prophylaxis during breastfeeding,[4,9,10] prevention of all breastfeeding and thus transmissions after the WHO recommended weaning age of 6 months was impossible. WHO now recommends providing prophylaxis during the entire breastfeeding period.

Early initiation of ART is important to achieve undetectable viral load well before delivery; thus, women should be encouraged to plan pregnancies and attend antenatal care sufficiently early to diagnose HIV infection, assess the HIV stage, and initiate ART or antiretroviral prophylaxis as soon as possible. However, for programmes to ensure timely screening and staging, rapid access to antiretrovirals, and comprehensive but rapid counselling to ensure good adherence to treatment or prophylaxis will be a challenge.

***Panel:* Research in context**

Systematic review

We searched PubMed from inception to November, 2010, using the terms "breast feeding" AND "HIV-1" AND "prevention" AND "randomized clinical trial". The only other randomised controlled trial that assessed a three-drug maternal prophylaxis regimen during breastfeeding was the breastfeeding, antiretrovirals and nutrition (BAN) study,[9] in Malawi, although the prophylaxis in BAN was only started after delivery.

Interpretation

In children not infected with HIV-1 at age 2 weeks, the risk of postnatal transmission was reduced by almost half both in the BAN and Kesho Bora studies. However, the overall risk reduction at age 28 weeks was considerably higher in Kesho Bora than in BAN (42% *vs* 25%), which is consistent with the earlier start of maternal triple antiretroviral prophylaxis in Kesho Bora (28–36 weeks' gestation) than in BAN (after delivery).

Contributors

Isabelle de Vincenzi was the overall study coordinator and wrote the manuscript with support from Tim Farley and Philippe Gaillard, site principal investigators (Nicolas Meda, Nigel Rollins, Stanley Luchters, Ruth Nduati, Marie-Louise Newell), and Jennifer Read.

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Conflicts of interest

We declare that we have no conflicts of interest.

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