

Breastfeeding Plus Infant Zidovudine Prophylaxis for 6 Months vs Formula Feeding Plus Infant Zidovudine for 1 Month to Reduce Mother-to-Child HIV Transmission in Botswana

A Randomized Trial: The Mashi Study

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Context Postnatal transmission of human immunodeficiency virus-1 (HIV) via breastfeeding reverses gains achieved by perinatal antiretroviral interventions.

Objective To compare the efficacy and safety of 2 infant feeding strategies for the prevention of postnatal mother-to-child HIV transmission.

Design, Setting, and Patients A 2 × 2 factorial randomized clinical trial with peripartum (single-dose nevirapine vs placebo) and postpartum infant feeding (formula vs breastfeeding with infant zidovudine prophylaxis) interventions. In Botswana between March 27, 2001, and October 29, 2003, 1200 HIV-positive pregnant women were randomized from 4 district hospitals. Infants were evaluated at birth, monthly until age 7 months, at age 9 months, then every third month through age 18 months.

Intervention All of the mothers received zidovudine 300 mg orally twice daily from 34 weeks' gestation and during labor. Mothers and infants were randomized to receive single-dose nevirapine or placebo. Infants were randomized to 6 months of breastfeeding plus prophylactic infant zidovudine (breastfed plus zidovudine), or formula feeding plus 1 month of infant zidovudine (formula fed).

Main Outcome Measures Primary efficacy (HIV infection by age 7 months and HIV-free survival by age 18 months) and safety (occurrence of infant adverse events by 7 months of age) end points were evaluated in 1179 infants.

Results The 7-month HIV infection rates were 5.6% (32 infants in the formula-fed group) vs 9.0% (51 infants in the breastfed plus zidovudine group) ($P = .04$; 95% confidence interval for difference, -6.4% to -0.4%). Cumulative mortality or HIV infection rates at 18 months were 80 infants (13.9%, formula fed) vs 86 infants (15.1% breastfed plus zidovudine) ($P = .60$; 95% confidence interval for difference, -5.3% to 2.9%). Cumulative infant mortality at 7 months was significantly higher for the formula-fed group than for the breastfed plus zidovudine group (9.3% vs 4.9%; $P = .003$), but this difference diminished beyond month 7 such that the time-to-mortality distributions through age 18 months were not significantly different ($P = .21$).

Conclusions Breastfeeding with zidovudine prophylaxis was not as effective as formula feeding in preventing postnatal HIV transmission, but was associated with a lower mortality rate at 7 months. Both strategies had comparable HIV-free survival at 18 months. These results demonstrate the risk of formula feeding to infants in sub-Saharan Africa, and the need for studies of alternative strategies.

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ACCORDING TO SOME ESTIMATES, approximately 25% to 48% of infants born to human immunodeficiency virus (HIV)-infected breastfeeding women in certain populations may become infected with HIV-1.^{1,2} A relatively high proportion of mother-to-child transmission in breast-

feeding populations may occur through breastfeeding, perhaps more than 40%.^{3,4} Perinatal antiretroviral prophylaxis can greatly reduce the risk of mother-

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to-child transmission of HIV,⁵⁻¹⁰ but this efficacy is eroded by transmission via breastfeeding.^{3-6,10-12} Exclusive breastfeeding with abrupt early weaning after 3 to 6 months,^{4,13} pasteurization,^{14,15} hot water bath,¹⁶ and microbicidal treatment of breast milk with alkyl sulfates¹⁷ have been proposed as methods to make breastfeeding safe. However, so far they have been either unsuccessful or cumbersome and expensive to implement.

Although avoidance of breastfeeding can eliminate HIV transmission through breast milk,^{8,9,12,18} excess infant morbidity and mortality have been associated with the use of infant formula,¹⁹⁻²³ particularly where access to clean water is limited. In much of the world, cost and stigma also limit the use of formula feeding. However, a trial conducted among HIV-infected women and their infants in Nairobi, Kenya, found that formula feeding prevented an estimated 44% of infant HIV infections without leading to excess infant mortality, and that infants randomized to formula feed were therefore more likely to survive and to be HIV-uninfected at age 2 years than infants randomized to breastfeed (70% vs 58%, respectively, $P = .02$).¹² It should be noted that this study population was urban, had to have access to clean municipal water in order to participate in the Kenyan study, and did not receive antiretroviral treatment or mother-to-child transmission prophylaxis.

Only 2 unpublished studies^{24,25} have assessed the use of extended antiretroviral prophylaxis (lamivudine and nevirapine) in breastfed infants for the prevention of postnatal transmission of HIV. Both studies reported low HIV transmission rates, but they did not include a control group. Zidovudine has been shown to effectively prevent mother-to-child transmission of HIV when taken during pregnancy,^{9,26,27} when given as post-exposure prophylaxis to newborns,²⁸⁻³⁰ or in the setting of occupational exposure to HIV-1.³¹ There have been no previous trials of infant zidovudine prophylaxis throughout the breastfeeding period, however. To date, there have also been no comparisons of a formula-

feeding strategy with a strategy in which breastfed infants were given an intervention aimed at preventing infection from exposure to HIV in breast milk.

In 2001, we initiated the Mashi (milk) study to evaluate both perinatal and postnatal intervention strategies for reducing mother-to-child transmission of HIV in Botswana, where a national program for the prevention of mother-to-child transmission had been implemented in 1999. This program offered antenatal zidovudine to mothers, followed by 1 month of prophylactic infant zidovudine and the provision of infant formula, and was the first perinatal HIV prevention program in Africa to provide infant replacement feeding nationwide.

METHODS

Trial Design

The Mashi study was a randomized 2 × 2 factorial clinical trial for HIV-infected pregnant women and their infants, designed to compare interventions for both preventing perinatal HIV transmission (part 1) and reducing postnatal HIV infection and mortality (part 2).

The results of part 1, which was designed to assess the efficacy of adding single-dose nevirapine to maternal and infant zidovudine to reduce perinatal mother-to-child transmission, are presented elsewhere.³² In the original design of part 1 of the study, 1 dose of nevirapine (200 mg for women at labor onset; 6 mg for infants within 72 hours of birth) or placebo was taken. In August 2002, as a result of efficacy data from Thailand,⁸ the peripartum intervention was revised to eliminate infant placebo and provide all infants with open-label nevirapine immediately after being born. The maternal intervention remained unchanged.

Part 2, which is the focus of this paper, was randomized, nonblinded, and the first study to compare the efficacy and safety of breastfeeding plus infant zidovudine prophylaxis for 6 months (breastfed plus zidovudine) to formula feeding from birth plus 1 month of infant zidovudine (formula fed) for reducing postnatal transmission of HIV. Prior studies had informed the exist-

ing strategies for reducing postnatal HIV transmission, specifically the recommendation of exclusive formula feeding or exclusive breastfeeding with early weaning of infants.^{12,13,33}

The Health Research Development Committee from Botswana and the Harvard School of Public Health Human Subjects Committee approved the study protocol and amendments.

Study Population

HIV-infected pregnant women attending antenatal clinics were referred to study locations at the district hospitals in the southern region of Botswana in 1 city, 1 town, and 2 large villages. Of the 11 881 pregnant women screened for HIV infection, 3935 (33%) had positive results and 1200 (30%) of these women were enrolled in the study between March 27, 2001 and October 29, 2003.

Eligibility criteria included being between 33 and 35 weeks' gestation; having a positive HIV-1 enzyme-linked immunosorbent assay (ELISA) on 2 separate samples; being aged 18 years or older; having levels of hemoglobin at 80g/L or above, absolute neutrophil count of 1000 or more cells/mm, alanine aminotransferase and aspartate aminotransferase at 10 or less times upper limit of normal, and creatinine 1.5 mg/dL (132.6 μmol/L) or less; and not having known intolerance to zidovudine or nevirapine.

All HIV-positive women who agreed to join the study signed a written consent form explaining the purposes of the study, the schedule of clinical and laboratory evaluations, the risks and benefits of both feeding strategies, the option to withdraw from the study at any given time without prejudice, the option to join the national prevention of mother-to-child transmission program at any time, that no identifying information would be included in any publications or presentation of the results, and the names of key people to contact about their rights or for any questions about the study. These women were encouraged to discuss the study or elements of the consent form with their partners or family members before joining the study. Study participants signed updated informed consent

Table 1. Comparison of Interventions for Preventing Perinatal HIV Transmission (Part 1) and Reducing Postnatal HIV Infection and Mortality (Part 2): The Mashi Study*

Study Factor	N = 1200†	
	Group	Group
Original design March 27, 2001 (study opening) to August 11, 2002 perinatal factor (part 1)‡	Single-dose placebo to mother plus single-dose placebo to infant (placebo/placebo)	Single-dose nevirapine to mother plus single-dose nevirapine to infant (nevirapine/nevirapine)
Postnatal factor (part 2) No. Breastfed plus zidovudine	122	120
Formula-fed	123	126
Revised design August 12, 2002, to October 29, 2003 (completion of study accrual) perinatal factor (part 1)§	Single-dose placebo to mother plus single-dose nevirapine to infant (placebo/nevirapine)	Single-dose nevirapine to mother plus single-dose nevirapine to infant (nevirapine/nevirapine)
Postnatal factor (part 2) No.§ Breastfed plus zidovudine	179	177
Formula-fed	176	177

*All mothers received antenatal zidovudine and all babies received zidovudine during the first month of life.
 †All mother/infant pairs randomized.
 ‡In the original design of part 1 of the study, mothers and infants were randomized to single-dose nevirapine or placebo (200 mg for mothers at labor onset and 6 mg for infants within 72 h of birth).
 §In August 2002, in response to efficacy data from Thailand,⁸ the perinatal intervention (part 1) was revised to eliminate infant placebo and provide all infants with open-label nevirapine immediately following birth. The maternal intervention remained unchanged. The postnatal intervention (part 2) also remained unchanged.
 ||Infants were randomized to 6 mo of breastfeeding plus prophylactic infant zidovudine (breastfed plus zidovudine), or formula feeding plus 1 mo of infant zidovudine (formula-fed).

forms for amendments that affected the information in the consent form they originally signed for their participation in the study.

Randomization and Study Interventions

Centralized randomization to both part 1 and part 2 groups occurred at study enrollment (34 weeks' gestation), using permuted blocks of size 8 within each site. All women received zidovudine 300 mg orally twice daily from 34 weeks' gestation until labor onset, and every 3 hours during labor until delivery. Mothers and infants were assigned to single-dose nevirapine or placebo, depending on the randomized perinatal intervention group (part 1). See TABLE 1 for a schematic representation of the factorial design, including the revision of the part 1 design.

Infant zidovudine syrup was administered 4 mg/kg/12 hours from birth until 1 month of age in all infants, and discontinued at 1 month in the formula-fed group. For the breastfed plus zidovudine group, infant zidovudine prophylaxis continued from 1 to 2 months of age at 4 mg/kg/8 hours, and from 2 to

6 months of age (while still breastfeeding) at 6 mg/kg/8 hours. Exclusive breastfeeding was recommended (as per Botswana guidelines), although the introduction of foods/liquids other than formula was not considered nonadherence to breastfeeding. Mothers were instructed to begin and complete weaning between 5 and 6 months of age. Free infant formula was provided from 5 through 12 months of age to facilitate safe weaning. For infants in whom zidovudine was discontinued due to toxicity, mothers were instructed to simultaneously discontinue breastfeeding and begin formula. Mothers randomized to the formula-fed group were supplied with formula for 12 months. All mothers were educated about safe formula preparation and administration, and provided with high-protein food for infants from 6 through 12 months of age.

Antiretroviral Treatment

In October 2002, combination antiretroviral treatment consisting of zidovudine, lamivudine, and nevirapine (highly active antiretroviral treatment [HAART]) became accessible through a national program. Women with a CD4 cell count

of less than 200 cells/mm³ or an AIDS-defining illness at enrollment or during follow-up were offered HAART, as were all HIV-infected infants.

Follow-up and Evaluation

Infant evaluations were scheduled at birth, monthly until age 7 months, at age 9 months, and then every third month through age 18 months. Peripheral blood was obtained at birth, at age 4 weeks, and at ages 4, 7, 9, and 12 months for HIV testing by polymerase chain reaction (PCR) DNA assay, and at age 18 months by 2 independent ELISAs. Full blood counts with differential were performed for all infants at birth and at ages 1, 4, 7, 9, and 18 months, and additionally at ages 2, 3, 5, and 6 months for those in the breastfeeding plus zidovudine group. Quantifications of alanine and aspartate aminotransferases (by enzymatic method according to the International Federation of Clinical Chemists without pyrodoxal-5'-phosphate) and serum creatinine (by buffered kinetic Jaffe reaction without deproteinization) were performed at age 1 month for all babies, and at age 6 months for those in the breastfeeding plus zidovudine group. Signs, symptoms, and diagnoses were collected based upon targeted clinical assessment.

Adherence to infant feeding strategy and zidovudine was assessed by maternal report at each scheduled visit using standardized questionnaires. For zidovudine, adherence was assessed by collecting information on study drug intake and the primary reason for any missed doses. For infant feeding strategy, adherence was assessed by recording the date of weaning; frequency of breast milk and formula intake; purpose and time of the introduction of fluids, solid foods, and other nonhuman milk; and water source since the previous visit.

The following definitions were used when describing infant feeding: (1) exclusive formula feeding (formula feeding only, never breastfed); (2) exclusive breastfeeding (breastfeeding only, no other fluids, milks, or foods); (3) mixed breastfeeding (breastfeeding with the ad-

dition of fluids, solid foods, and nonhuman milks); and (4) predominant breastfeeding (breastfeeding with the addition of only fluids other than milks).

Definition of HIV Infection and Primary Study End Points

Infants with a positive PCR were retested on a separate sample (or by ELISA at age 18 months); time of HIV infection was based on the date of the earliest positive test result. Infants who died or were lost to follow-up after a single positive PCR test were considered HIV positive. The primary efficacy end points were infant HIV infection through the 7-month visit window (up to 243 days of age), and the composite end point of HIV infection or death (or HIV-free survival) through the 18-month visit window (up to 593 days of age). The primary safety end point was infant toxicity by the 7-month visit.

Statistical Methods and Interim Monitoring

The trial was designed to enroll 1200 mother/infant pairs to provide adequate power for the main objectives of both the peripartum and the feeding strategy components. The latter was designed as a superiority study to detect differences between the formula-fed and breastfed plus zidovudine groups. Based on a 2-sided type I error of .05, and an annual loss to follow-up rate of 10%, 1200 mother/infant pairs provided 80% power to detect a 7% difference in rates of HIV infection by age 7 months, and more than 90% power for a 10% difference in HIV-free survival at 18 months of age, based on reference rates of 17% and 79%. While the formula-fed group was regarded as the best strategy to prevent breastfeeding-associated mother-to-child transmission at the time the trial was initiated, neither regimen had been evaluated in Botswana at the time; thus, 2-sided tests were used to assess differences between the formula-fed and breastfed plus zidovudine groups in either direction. Additionally, the power of the study for differences of 7% and 10% is

relatively stable across a range of reference rates. The power for the peripartum comparisons is presented elsewhere.³²

The study was monitored by an independent data and safety monitoring board. Two preplanned interim efficacy reviews occurred in June 2002 and April 2003 using the O'Brien-Fleming spending function to control type I error rates (false positives) due to multiple statistical tests carried out in the interim and final analyses.³⁴

This report is based on all study visits occurring and specimens collected through October 24, 2005, which corresponded to the latest specimen date collected for an HIV test (ELISA). All infants were born at least 18 months prior to this date and therefore, all study end points were potentially observable. All analyses were based on live, first-born infants, and included all observed HIV transmissions (and other follow-up data), regardless of timing. All part 1 perinatal randomized participants were grouped together in order to test between feeding strategies (part 2). Because of the factorial design, tests of interactions between part 1 and part 2 groups were performed. Since the part 1 groups were modified during the trial, assessments of interactions were conducted separately for the original and revised part 1 groups. All statistical comparisons used a nominal, 2-sided .05 significance level, and have not been corrected for multiple testing.

Comparisons of treatment groups for baseline characteristics used the Fisher exact test (Wilcoxon rank-sum test) for discrete (continuous) measurements. Time-to-event data were analyzed using the Kaplan-Meier estimator, the log rank test, and Cox proportional hazards modeling. Estimated rates of HIV infection at age 7 months and HIV-free survival at age 18 months were obtained from Kaplan-Meier estimators. For HIV infection, death was a censoring event, and for HIV-free survival, the time of the event was the earlier of HIV infection or death. Infants who were lost to follow-up before reaching study end points were censored at their last nega-

tive HIV test (for the transmission end point and the HIV-free survival end point), and were censored at their last study visit for the infant mortality end point. Treatment group comparisons for these end points are based on standardized differences between the Kaplan-Meier estimates, stratified by part 1 group. The standard error of the difference, obtained as the square root of the estimated variance of the difference, was also used to compute 95% confidence intervals (CIs) for the differences.

Secondary efficacy end points included time to HIV infection and time to the earlier of HIV infection or death, stratified by part 1 group. Treatment group comparisons of these end points used log rank tests and the Cox proportional hazards model (with the proportional hazards assumption assessed for each model). *P* values were obtained from the corresponding Wald tests, and estimated relative risk (RR) and corresponding CIs were obtained from the estimated coefficients of the model and their estimated standard errors. Time to infant death is compared by an unstratified log rank test, and tests of interactions are based on the Cox model. Covariate-adjusted Cox models included maternal baseline HIV RNA and CD4 cell count, mode of delivery, and infant gestational age and weight. Covariate-adjusted treatment group comparisons gave similar results to unadjusted comparisons and are omitted.

Statistical software used to carry out the final analysis included SAS version 9.1 (SAS Institute Inc, Cary, NC) and StatXact version 5 (Cytel, Inc, Cambridge, Mass). The primary analysis was originally carried out (as specified by the study protocol) between December 2004 and January 2005, following evaluation of all infant participants for a minimum of 7 months. The analyses presented in this report have been updated to March 2006, allowing 18 months of follow-up for all randomized infants.

RESULTS

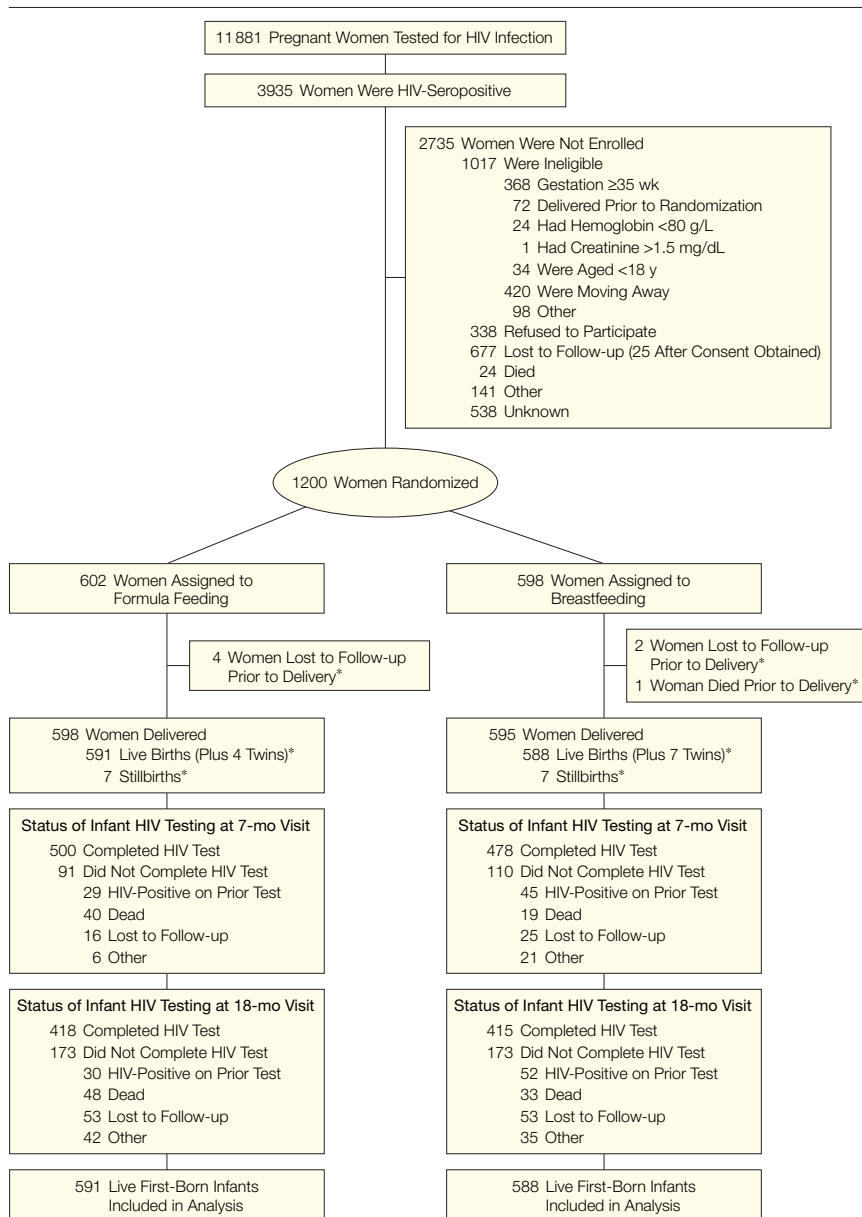
There were 1200 HIV-positive pregnant women randomized between

March 27, 2001 and October 29, 2003, of whom 1193 reached delivery, resulting in 591 and 588 live first-born infants in the formula-fed and breastfed plus zidovudine groups, respectively (FIGURE 1). Maternal characteristics at enrollment and delivery and infant characteristics at birth were

well balanced between the formula-fed and breastfed plus zidovudine groups (TABLE 2; $P > .05$ for all comparisons other than sanitation facilities, for which $P = .04$). These infants were born between April 22, 2001 and December 23, 2003. By the 7- and 18-month evaluations, 16 (2.7%)

and 53 (9.0%) of the 591 formula-fed and 25 (4.3%) and 53 (9.0%) of the 588 breastfed plus zidovudine infants were lost to follow-up, respectively (Figure 1). There were also very few (<1%) missed DNA PCR tests for reasons other than loss to follow-up (Figure 1).

Figure 1. Enrollment and Randomization of HIV-1–Infected Pregnant Women and Delivery of Their Infants According to Study Group and Completeness of HIV Testing at Scheduled Infant Evaluation Time Points



HIV denotes human immunodeficiency virus. *Infants from mothers lost to follow-up or dying before delivery, second-born twins, and stillbirths were excluded from all analyses.

HIV Infection

A total of 32 and 51 infants in the formula-fed and breastfed plus zidovudine groups, respectively, became HIV positive by 7 months of age (TABLE 3), corresponding to cumulative positivity rates of 5.6% (formula fed) and 9.0% (breastfed plus zidovudine) ($P = .04$). The estimated treatment hazard ratio for time until HIV infection (FIGURE 2A) is 1.65 ($P = .02$; 95% CI, 1.07-2.55).

There was a statistically significant interaction between feeding strategy and the original part 1 group ($P = .04$; FIGURE 3A), with a greater difference in 7-month HIV infection rates among infants in the nevirapine/nevirapine part 1 group (3.4% vs 14.0%) than in the placebo/placebo part 1 group (8.6% vs 11.0%). There was no suggestion of an interaction ($P = .75$) between feeding strategy and the revised part 1 groups when all infants received nevirapine and maternal HAART was available for qualifying women, and the HIV infection rates for the formula-fed and breastfed plus zidovudine groups were lower and more similar (Figure 2D).

When only infants who were alive and HIV-free at age 1 month were analyzed, the estimated cumulative proportions of infants with a diagnosis of HIV infection occurring between ages 1 and 7 months in the breastfed plus zidovudine and formula-fed groups were 4.5% and 0.6%, respectively. These results should be interpreted with caution because the infants included in the analysis were selected on the basis of an event (being HIV-1-free at age 1 month) that may have been affected by feeding assignment, making the comparison susceptible to postrandomization selection bias.³⁵

Mortality

One hundred fourteen infants died after birth, 63/591 (10.7%) from the formula-fed group and 51/588 (8.7%) from the breastfed plus zidovudine group. Of the 77 infants who died with an HIV-negative status, 45 (58%) had a negative HIV test result within the 2 weeks preceding death, and 73 (95%) had a negative HIV test result within the 3 months preceding death. A total of 5 infants died before an initial PCR result was obtained. The remaining 32 infant deaths (15 from the formula-fed group and 17 from the breastfed plus zidovudine group) were among babies who tested HIV positive before death. The most common causes of infant death were diarrhea and pneumonia. The deaths in the breastfed plus zidovudine group were more likely to be in HIV-infected infants and at older ages as compared with the deaths in the formula-fed group. There were 3 deaths due to anemia, of which 2 (1 in each group) were judged as possibly related to zidovudine.

The cumulative incidence of infant death by month 7 was significantly higher in the formula-fed group than in the breastfed plus zidovudine group (9.3% vs 4.9%; $P=.003$), but this difference diminished beyond month 7, such that the time-to-mortality distributions through 18 months of age were not significantly different ($P=.21$) (Figure 2B). There was a significant interaction ($P=.03$) between feeding strategy groups and the original part 1 group with respect to time to infant death, with a larger formula-fed vs breastfed plus zidovudine difference among infants in the placebo/placebo group (RR=2.75; 95% CI, 1.15-6.57) than among infants in the nevirapine/nevirapine group (RR=0.75; 95% CI, 0.34-1.66) (Figure 3B). This interaction was no longer statistically significant ($P=.12$) after adjustment for covariates. There was no interaction between feeding strategy and the revised part 1 groups ($P=.14$), and the rates of death in the formula-fed and breastfed plus zidovudine groups were somewhat lower than in the original study period and similar to each other

Table 2. Baseline and Delivery Characteristics of All Randomized Mothers (N = 1200) and All Live, First-Born Infants (N = 1179)*

Variables	Total*	Randomized Feeding Strategy	
		Formula-Fed	Breastfed Plus Zidovudine
Enrollment site, No./total (%)	1200 (100)	602 (50)	598 (50)
Molepolole	349/1200 (29)	176/602 (29)	173/598 (29)
Mochudi	315/1200 (26)	157/602 (26)	158/598 (26)
Lobatse	268/1200 (22)	136/602 (23)	132/598 (22)
Gaborone	268/1200 (22)	133/602 (22)	135/598 (23)
Part 1 randomized treatment group, No./total (%) †	1200 (100)	602 (50)	598 (50)
Placebo/placebo	245/1200 (20)	123/602 (20)	122/598 (20)
Nevirapine/nevirapine	246/1200 (20)	126/602 (21)	120/598 (20)
Placebo/nevirapine revised	355/1200 (30)	176/602 (29)	179/598 (30)
Nevirapine/nevirapine revised	354/1200 (30)	177/602 (29)	177/598 (30)
Maternal age median, y	26.78	26.76	26.87
No./total (%)	1200 (100)	602 (50)	598 (50)
Highest education level completed, No./total (%)	1181 (100)	591 (50)	590 (50)
None	40/1181 (3)	20/591 (3)	20/590 (3)
Primary	308/1181 (26)	163/591 (27)	145/590 (24)
Secondary	804/1181 (68)	397/591 (67)	407/590 (69)
University	29/1181 (2)	11/591 (2)	18/590 (3)
Monthly person income (USD), No./total (%)	1175 (100)	587 (50)	588 (50)
None	717/1175 (61)	360/587 (61)	357/588 (61)
≤100	243/1175 (21)	121/587 (21)	122/588 (21)
101-1000	215/1175 (18)	106/587 (18)	109/588 (18)
Source of drinking water, No./total (%)	1063 (100)	532 (50)	531 (50)
Piped into home	75/1063 (7)	42/532 (8)	33/531 (6)
Tap in the yard	587/1063 (55)	278/532 (52)	309/531 (58)
Communal standpipe	388/1063 (37)	205/532 (39)	183/531 (34)
Others	13/1063 (1)	7/532 (1)	6/531 (1)
Sanitation facilities most used, No./total (%)‡	1181 (100)	591 (50)	590 (50)
Indoor toilet	157/1181 (13)	89/591 (15)	68/590 (11)
Private latrine/house	872/1181 (74)	428/591 (72)	444/590 (74)
Shared latrine/compounds	118/1181 (10)	55/591 (9)	63/590 (11)
No latrine facilities	28/1181 (2)	13/591 (2)	15/590 (3)
Other	6/1181 (1)	6/591 (1)	0/590 (0)
Electricity in home, No./total (%)	280/1181 (24)	150/591 (25)	130/590 (22)
Refrigerator in home, No./total (%)§	350/1181 (30)	173/591 (29)	177/590 (30)
Maternal baseline CD4 ⁺ count, cells/mm ³ No./total (%)	1151 (100)	576 (50)	575 (50)
Median	366.0	358.5	372.0
Maternal baseline plasma HIV-1 RNA, log ₁₀ copies/ml No./total (%)	1182 (100)	591 (50)	575 (50)
Median	4.35	4.33	4.39
Male infants	625/1179 (53)	312/590 (53)	313/589 (53)
Premature (<37 wks gestational age [Dubowitz]), No./total (%)	61/1160 (5)	24/581 (4)	37/579 (6)
Birth weight median, kg	3.1	3.1	3.1
<2.5 kg, No./total (%)	92/1173 (8)	45/590 (8)	47/583 (8)
Congenital abnormalities	20/1175 (2)	9/589 (2)	11/586 (2)

*Baseline characteristics of mothers and infants were well balanced across feeding groups ($P>.05$) except for sanitation facility (groups compared using χ^2 [Wilcoxon Rank-Sum] tests for categorical [continuous] measurements). †In part 1, mother/infant pairs were randomized to receive blinded maternal and infant single-dose nevirapine (nevirapine/nevirapine) or maternal and infant placebo (placebo/placebo). The study was revised 17 mo after initiation to compare maternal and infant single-dose nevirapine (nevirapine/nevirapine revised) with maternal placebo and infant single-dose nevirapine (placebo/nevirapine revised). ‡ $P = .04$ for sanitation facilities most used. §Refrigerator powered by electricity or gas.

(Figure 2E). Birth weight (RR=2.77 for <2.5 kg vs normal; 95% CI, 1.69-4.54) and maternal delivery viral load (RR=1.46/1 log₁₀ higher viral load; 95% CI, 1.19-1.78) were significantly associated with time to infant death.

HIV-Free Survival

A total of 166 infants died or became HIV positive through the 18-month visit. Of these, 80 and 86 were in the formula-fed and breastfed plus zidovudine groups, respectively, corresponding to a cumulative 18-month rate of HIV infection or mortality of 13.9% and 15.1% (Table 3; P=.60; 95% CI for difference, -5.3% to 2.9%) (see also Figure 2C). There was a statistically significant interaction (P=.02) between feeding strategy and original part 1 group with evidence of a greater difference in HIV-free survival between the formula-fed and breastfed plus zidovudine groups among patients receiving nevirapine/nevirapine than among patients receiving placebo/placebo (Figure 3C).

There was no suggestion of an interaction between feeding strategy and the revised part 1 group (P=.42) when HAART was available. Rates of HIV-free survival for the formula-fed and

breastfed plus zidovudine groups were similar and higher during the revised study period when HAART was available (Figure 2F). When only infants who were alive and HIV-free at 1 month were analyzed, the breastfed plus zidovudine/formula-fed hazard ratio of infant death or HIV infection between 1 and 7 months of age was 1.30 (P=.28; 95% CI, 0.81-2.10). These results should be interpreted with caution for the same reason noted previously.

Safety

TABLE 4 summarizes the occurrence of any grade 3 (severe) or worse laboratory toxicities and clinical adverse events within infants' first 7 months of life. The rates of grade 3 or higher signs or symptoms (17.6% vs 13.1%; P=.03) and of hospitalization (20.3% vs 15.6%; P=.04) by 7 months were significantly higher among infants in the formula-fed group than in the breastfed plus zidovudine group. The rate of grade 3 or higher laboratory abnormality associated with zidovudine toxicity was significantly higher in the breastfed plus zidovudine group than in the formula-fed group (24.7% vs 14.8%; P<.001).

Adherence to Infant Zidovudine and Feeding Strategy

Of the 1179 live-born babies, 1172 (99.4%) initiated study zidovudine following birth. The median duration of infant zidovudine was 5.9 months in the breastfed plus zidovudine group, and 84% (479 of 567) of responding mothers in the breastfed plus zidovudine group reported never missing 1 or more full days of infant zidovudine; 95% (562 of 591) of live-born infants in the formula-fed group received at least 2 weeks dosage of zidovudine. Full adherence to exclusive formula feeding was self-reported by 93% of mothers in the formula-fed group. Three infants in the formula-fed group were infected between months 1 and 7, presumably because they were exposed to breast milk.

Among mothers in the breastfed plus zidovudine group, self-reported adherence to exclusive breastfeeding was 57.1% at month 1, 31.3% at month 3, and 17.5% at month 5. Predominant breastfeeding was practiced by 21.2%, 20.1%, and 7.5% of mothers in the breastfed plus zidovudine group by 1 month, 3 months, and 5 months, respectively. Mixed breastfeeding was practiced by 21.7%, 48.6%, and 75.0%

Table 3. Primary Efficacy End Points for Infants According to Intervention Group

Infant Age	HIV Infection				Death				HIV Infection or Death			
	Cumulative HIV Infection Rate, No. (%) [*]		Rate Difference, % (95% CI) [†]	P Value [‡]	Cumulative Death Rate, No. (%) [*]		Rate Difference, % (95% CI) [†]	P Value [‡]	Cumulative HIV Infection or Death Rate, No. (%) [*]		Rate Difference, % (95% CI) [†]	P Value [‡]
	Formula-Fed	Breastfed + Zidovudine			Formula-Fed	Breastfed + Zidovudine			Formula-Fed	Breastfed + Zidovudine		
	No. at risk	No. at risk	No. at risk	No. at risk	No. at risk	No. at risk						
Birth§	22 (3.8)	19 (3.3)	0.5 (-1.6 to 2.6)	.62	15 (2.5)	8 (1.4)	1.2 (-0.4 to 2.8)	.14	36 (6.1)	27 (4.6)	1.5 (-1.1 to 4.1)	.25
1 mo§	29 (5.0)	27 (4.6)	0.4 (-2.1 to 2.9)	.76	25 (4.3)	9 (1.5)	2.7 (0.8 to 4.6)	.005	52 (8.9)	36 (6.1)	2.7 (-0.3 to 5.7)	.08
7 mo§	32 (5.6)	51 (9.0)	-3.4 (-6.4 to -0.4)	.04¶	54 (9.3)	28 (4.9)	4.4 (1.5 to 7.4)	.003	73 (12.5)	74 (12.9)	-0.4 (-4.2 to 3.5)	.86
18 mo§	33 (6.0)	53 (9.5)	-3.6 (-6.7 to -0.5)	.02	62 (10.7)	48 (8.5)	2.2 (-1.2 to 5.6)	.21	80 (13.9)	86 (15.1)	-1.2 (-5.3 to 2.9)	.60¶
	No. at risk 493	No. at risk 481			No. at risk 512	No. at risk 529			No. at risk 493	No. at risk 483		

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.
^{*}Cumulative rates of each end point were estimated by Kaplan-Meier method for the time points listed.
[†]The rate difference is calculated as the difference in the Kaplan-Meier estimates, and SEs of the differences (to form 95% confidence intervals) were calculated using the Greenwood formula.
[‡]P values are from unstratified Z tests.
[§]Birth, months 1, 7, and 18 are defined as up to days 15, 45, 243, and 593, respectively.
^{||}Number at risk (by Kaplan-Meier method) at the end of the previous listed visit.
[¶]P values at primary end point times (age 7 mo for HIV infection, age 18 mo for HIV infection or death) are based on Z tests stratified by all (4) unique part 1 groups.

of mothers in the breastfed plus zidovudine group by 1, 3, and 5 months, respectively. Sixty-one events (54 due to infant zidovudine toxicity) requiring cessation of breastfeeding occurred through 5 months postpartum.

Use of HAART

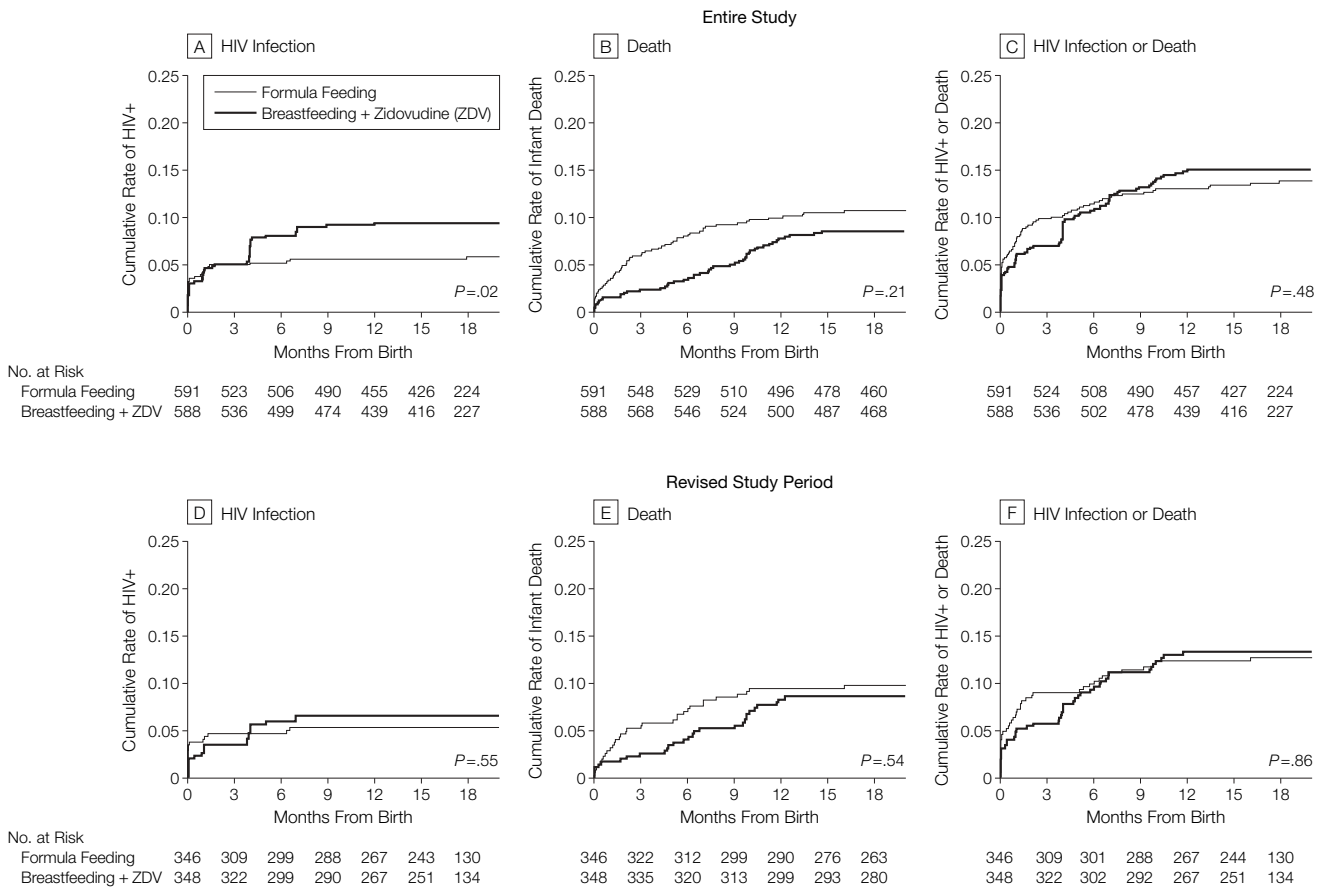
Seventy-one women (37 in the formula-fed group and 34 in the breastfed plus zidovudine group) initiated HAART before delivery, 82 between delivery and 7 months postpartum, and 216 at more than 7 months postpartum (similar numbers started HAART during each of these periods in the formula-fed and breastfed plus zidovudine groups). Four

infants (2 each assigned to the formula-fed and breastfed plus zidovudine groups) had an initial positive HIV test result following their mother's initiation of HAART. For the 2 infants assigned to the formula-fed group, mothers started HAART close to time of delivery and HIV positivity was identified on days 1 and 33 of life, respectively. For the 2 infants assigned to the breastfed plus zidovudine group, positivity was established in the fourth month of life, and both of their mothers had recently begun HAART (21 and 50 days prior to the initial positive DNA PCR). A total of 56 infants (38 breastfed plus zidovudine and 18 formula-

fed) started HAART following confirmed HIV infection.

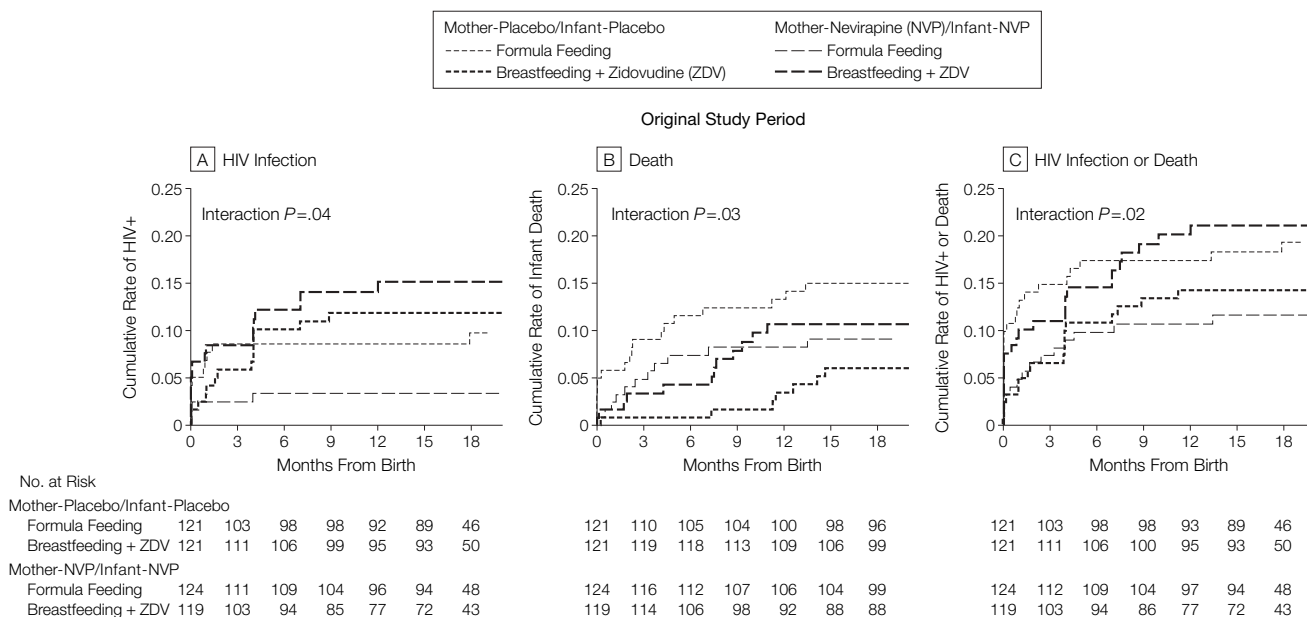
Exclusion of live-born infants whose mothers initiated HAART before delivery did not appreciably change feeding strategy comparisons for any study end point: at age 7 months, the cumulative rate of HIV positivity was 5.6% and 9.6% ($P=.02$) in the formula-fed and breastfed plus zidovudine groups, respectively; at 18 months, the rate of HIV-positivity or mortality was 13.9% and 15.5% ($P=.48$) in the formula-fed and breastfed plus zidovudine groups, respectively; and the cumulative incidence of infant death by month 7 was 9.3% and 4.6% ($P=.002$) in the formula-

Figure 2. Cumulative Rate (Proportion) of Infant End Points (Entire Study and Revised Study Period)



HIV denotes human immunodeficiency virus. Top row (panels A,B,C) shows cumulative event rate by randomized feeding strategy (and collapsing over peripartum groups) over the entire study period (including 591 infants assigned to the formula-fed group and 588 infants assigned to the breastfed plus zidovudine group). Bottom row (panels D, E, F) shows cumulative event rate for the revised study period (births after August 12, 2002) reflecting change in perinatal intervention study group (active, open-label nevirapine to all infants and availability of HAART to qualifying mothers and infants). The analyses in the bottom row consist of 348 infants assigned to the breastfed plus zidovudine group including 177 whose mothers were assigned to single-dose placebo, and 346 infants assigned to the formula-fed group including 172 whose mothers were assigned to single-dose placebo. Rates over time are calculated from the Kaplan-Meier method. P values are based on stratified log rank test (efficacy end points) and log rank test (mortality).

Figure 3. Cumulative Rate (Proportion) of Infant End Points (Original Study Period)



Cumulative event rate during the original study period (births prior to August 12, 2002), where for each end point a significant interaction between the 2 randomized factors (perinatal intervention of active nevirapine to mothers/infants [mother-nevirapine/infant-nevirapine] vs placebo [mother-placebo/infant-placebo] and feeding strategy) was detected; analyses include 121 infants assigned to formula feeding and placebo/placebo, 124 infants assigned to formula feeding and nevirapine/nevirapine, and 119 infants assigned to breastfeeding plus zidovudine and nevirapine/nevirapine. Rates over time are calculated from the Kaplan-Meier method. *P* values are based on stratified log rank test (efficacy end points), log rank test (mortality), and the Cox model (interaction).

Table 4. Grade 3 or 4 Infant Adverse Events in Formula-Fed and Breastfed plus Zidovudine Groups by Age 7 Months*

Adverse Event	Formula-Fed, No. (Rate)†	Breastfed plus Zidovudine, No. (Rate)†	<i>P</i> Value
Grade 3 or 4 signs and symptoms	100 (17.6)	74 (13.1)	.03
Hospitalization	116 (20.3)	89 (15.6)	.04
Grade 3 or 4 laboratory abnormalities	84 (14.8)	142 (24.7)	<.001
Toxicity leading to cessation of zidovudine‡	10 (1.7)	55 (9.2)	.001
Anemia	5	6	
Neutropenia	2	46	
Thrombocytopenia	1	0	
Others	2	3	

*Information on toxicity criteria is provided in the manual of operations for the study.
 †Cumulative rates by age 7 months estimated by Kaplan-Meier method and groups compared by log-rank test.
 ‡Zidovudine was administered for 1 month to infants in the formula-fed group as compared with 6 months to infants in the breastfed plus zidovudine group.

fed and breastfed plus zidovudine groups, respectively. Exclusion of the 56 infants who started HAART also does not appreciably change infant mortality comparisons: at ages 7 and 18 months, the cumulative infant mortality rates were 9.3% and 10.7%, respectively in the formula-fed group vs 4.9% and 8.5%, respectively, in the breastfed plus zidovudine group. The rate is still significantly higher ($P=.003$) in the

formula-fed group at 7 months of age, but is not significantly different ($P=.21$) by 18 months of age.

COMMENT

This trial compared 2 approaches for reducing postnatal HIV infection and infant mortality and found similar HIV-free survival rates. Overall, infants in the formula-fed group experienced lower rates of HIV infection and in-

creased rates of early mortality and adverse events from infectious etiologies than those in the breastfed plus zidovudine group, such that HIV-free survival at 18 months was similar in the 2 groups. Our expectation was that formula-fed infants would have better HIV-free survival at age 18 months since we anticipated that deaths after 6 months of age would be predominantly due to HIV-1 infection.

External results led to the modification of the perinatal intervention component (part 1) of our trial almost midway through enrollment, and the modification coincided with HAART availability for qualifying women and infected infants. As a result, the trial can be viewed as two 2×2 factorial trials, one using the original study period perinatal groups (nevirapine/nevirapine and placebo/placebo) in a non-HAART setting, and the other using the revised study period perinatal groups (nevirapine/nevirapine and placebo/nevirapine) in a setting where HAART was available. Because HAART reduces vi-

ral load and mother-to-child transmission is directly associated with viral load, use of HAART likely reduced overall transmission rates in the revised study period.

In any factorial trial, it is important to consider the possibility of interaction between the factors, specifically whether the relative efficacy of the formula-fed and breastfed plus zidovudine groups depended on the perinatal intervention. We found a statistically significant interaction between the feeding strategies and the original perinatal interventions (nevirapine/nevirapine and placebo/placebo) with respect to HIV infection, with a greater difference favoring formula feeding over breastfeeding plus zidovudine among mother/infant pairs receiving nevirapine/nevirapine. Conversely, the interaction between factors for the infant mortality end point was for a greater difference favoring breastfeeding plus zidovudine over formula feeding among mother/infant pairs receiving placebo/placebo. Moreover, because the effects of formula feeding and breastfeeding plus zidovudine on HIV infection and mortality were in opposite directions for these 2 end points, HIV-free survival was comparable between the 2 groups. One possibility for the interaction with the part 1 intervention with respect to HIV infection is that maternal and infant nevirapine prevented perinatal HIV transmission in a subset of high-risk infants, and that those high-risk infants in the breastfed plus zidovudine group subsequently became infected from exposure to HIV in breast milk. If this were true, this interaction would likely have been reduced after the introduction of HAART, because HAART could decrease the risk of transmission among the high-risk subset of mother/infant pairs. In fact, none of the 34 women in the breastfeeding plus zidovudine group who received HAART from delivery have yet transmitted HIV to their infants.

For all end points, there was no suggestion of an interaction between feeding strategy and the revised perinatal intervention groups, which were implemented at the time HAART became

available. Rates of HIV infection and infant mortality in the formula-fed and breastfed plus zidovudine groups were lower and more similar in the revised study than in the original study period (Figure 2D, E, and F and Figure 3). The availability of infant HAART may have lowered infant mortality in the revised study period. However, because infant HAART was initiated only after HIV infection, it did not affect our HIV-free survival end point, and thus our results for this end point are generalizable to regions where infant HAART may not be available.

Self-reported adherence to formula feeding was high (93%). While we cannot confirm this adherence, it is consistent with only 3 HIV infections diagnosed in the formula-fed group from 15 days to age 7 months. Although virtually all women in the breastfed plus zidovudine group breastfed, many did not do so exclusively, despite educational efforts. The early introduction of water was common, with 31% of infants receiving some water by age 1 month. Low adherence to exclusive breastfeeding in Botswana has been noted not only during the pilot period of our study,³⁶ but also during the 2001 evaluation of the national prevention of mother-to-child transmission program.³⁷ If mixed or non-exclusive breastfeeding were associated with increased risk of HIV transmission as reported by Coutoudis et al,^{33,38} the efficacy of 6 months of infant zidovudine in reducing breastfeeding infection in an exclusively breastfeeding population would be underestimated in our study. Although the degree of exclusivity for breastfeeding could have influenced the results, our goal in this study was to compare formula feeding to breastfeeding plus zidovudine under local conditions that would best reflect the potential value for future implementation if warranted by the results.

Ethical considerations and Botswana national policy excluded the option of an untreated control group as a comparator to the 2 feeding strategy groups. Thus, this study was unable to compare the additional benefit of either strategy to one in which infants were breastfed with no

postnatal intervention prophylaxis. Comparison of our 12- and 18-month rates of HIV infection and HIV-free survival to those achieved in other studies^{4,5,39,40} suggest that our combined perinatal/feeding strategy approach was relatively effective in preventing HIV infection and reducing mortality. However, HIV transmission rates due to breast milk in the breastfed plus zidovudine group of our study were similar to rates reported by some prevention of mother-to-child transmission studies with breastfeeding alone.^{5,6,11} For example, mother-to-child transmission rates among breastfeeding infants in Cote d'Ivoire whose mothers received short-course zidovudine were 14.8% at age 6 weeks and 18% at age 6 months,¹¹ while an individual patient data meta-analysis of mother-to-child transmission occurring after 4 weeks of age in breastfeeding infants revealed an estimated rate of breastfeeding transmission of 0.6% to 0.9% per month.⁴

Comparison across studies that test divergent interventions among differing populations should be undertaken with caution, as the relative effects of antepartum/intrapartum and postnatal interventions may complicate comparisons of incremental transmission rates between trials with different interventions in 2 ways. First, antepartum/intrapartum interventions, by preventing early infections, increase the pool of HIV-negative infants at risk for infection through breastfeeding, which must be taken into account when interpreting incremental infection rates. Second, antepartum/intrapartum interventions may delay (to the breastfeeding period) transmissions to those infants at highest risk of becoming infected. Earlier studies have shown that mothers with the highest levels of viremia have an increased risk for infecting their infants during both the peripartum and breastfeeding periods.⁴¹⁻⁴⁴ Other regional differences, potentially including differences in viral subtype and feeding practices, may also complicate cross-study comparisons.^{38,45-47} Thus, we cannot recommend the use of extended infant zidovudine prophylaxis.

laxis for the prevention of breastfeeding-related mother-to-child transmission based upon the results of this study, given the significant number of infant HIV infections that occurred after 1 month of age in the breastfed plus zidovudine group compared with the formula-fed group.

Higher morbidity and mortality rates among formula-fed infants compared with breastfed infants in the developing world have previously been described.^{21,22,48} One notable exception is the only other randomized study (in addition to Mashi) comparing these strategies among HIV-infected women, which was conducted in Nairobi, Kenya.¹² This latter study showed a similar 2-year mortality rate but a significantly lower HIV-free survival rate in the breastfeeding group.¹² In contrast, we found significantly higher rates of infant morbidity and mortality (mostly related to diarrheal diseases and pneumonia) in formula-fed infants than in breastfed infants. The differences in our findings compared with those of the Kenyan study may be explained by the fact that the Kenyan participants were urban and had to have access to clean municipal water in order to participate (and may therefore be less representative of women in much of the developing world).

CONCLUSIONS

In summary, our study showed that both formula feeding and breastfeeding with prophylactic infant zidovudine gave similar rates of HIV-free survival at 18 months. Formula feeding had a higher risk of early mortality, but breastfeeding with zidovudine prophylaxis had a higher risk of HIV transmission. Our study, which was the first to compare formula feeding to breastfeeding with extended antiretroviral prophylaxis, revealed relatively high morbidity and mortality rates associated with formula feeding among infants of HIV-infected mothers, but did not lend definitive support to the use of infant zidovudine prophylaxis to prevent breastfeeding-related mother-to-child transmission. Our study results highlight the need for

a careful assessment of the local management of childhood illnesses (mostly diarrheal and respiratory diseases) before the implementation of a formula feeding strategy for the prevention of mother-to-child transmission of HIV in developing countries.

Breastfeeding with zidovudine prophylaxis was a feasible prevention of mother-to-child transmission strategy in Botswana, but further study is warranted to determine the efficacy and safety of other interventions to prevent mother-to-child transmission related to breastfeeding, such as the use of maternal HAART during the breastfeeding period.

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