

HPTN 046

A Phase III trial to determine the efficacy and safety of an extended regimen of nevirapine in infants born to HIV-infected women to prevent vertical HIV transmission during breastfeeding

A Study of the HIV Prevention Trials Network

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LIST OF ABBREVIATIONS AND ACRONYMS

3TC	lamivudine
AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
ART	antiretroviral therapy
AZT	azidothymidine
CL	(HPTN) Central Laboratory
CORE	(HPTN) Coordinating and Operations Center
CRF	case report form
CRPMC	Clinical Research Products Management Center
DAIDS	Division of AIDS
DSMB	Data Safety and Monitoring Board
ddI	didanosine
EC	ethics committee
FDA	(United States) Food and Drug Administration
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
IRB	institutional review board
LDMS	Laboratory Data Management System
LL	local laboratory
MTCT	mother-to-child HIV transmission
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NICHHD	(United States) National Institute of Child Health and Human Development
NIH	(United States) National Institutes of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	nevirapine
NVPR	nevirapine resistance
PACTG	Pediatric AIDS Clinical Trials Group
PEP	post-exposure prophylaxis
PSRT	Protocol Safety Review Team
SAE	serious adverse event
SDMC	(HPTN) Statistical and Data Management Center
SJS	Stevens-Johnson Syndrome
SSP	Study Specific Procedures
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
US	United States
WHO	World Health Organization
ZDV	zidovudine

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SCHEMA

HPTN 046: A Phase III Trial to Determine the Efficacy and Safety of an Extended Regimen of Nevirapine in Infants Born to HIV-Infected Women to Prevent Vertical HIV Transmission During Breastfeeding

Purpose: To evaluate the efficacy and safety of an extended regimen of nevirapine (NVP) for 6 months or through cessation of breastfeeding, whichever is earliest, compared to placebo for prevention of mother-to-child human immunodeficiency virus (HIV) transmission (MTCT) in breastfeeding infants who are born to HIV-infected women.

Design: Phase III, multi-site, randomized, double blind, placebo-controlled trial.

Study Population: HIV-1 infected women and their breastfeeding infants. *As standard of care (external to the study) all HIV-infected women at the study sites are offered the HIVNET 012 two-dose intrapartum/neonatal regimen of NVP for prevention of maternal to child HIV-1 transmission.*

Study Size: 1576 mother/infant pairs

Stratification: Randomization will be stratified by maternal antiretroviral exposure during this pregnancy, with three levels: mothers receiving antiretroviral therapy (ART) for treatment of HIV; mothers receiving antiretroviral regimen (e.g. intrapartum NVP, short course azidothymidine (AZT) for prevention of MTCT; or neither. (If a mother received ART and an antiretroviral regimen for prevention of MTCT, the default will be to the ART group).

Treatment Regimen: Infants will be randomized on or before day 3 after birth to one of two arms and receive either NVP or placebo on a daily schedule as described below.

The infant study drug regimen will begin 5 days after birth (± 2 days). The duration of study treatment for infants will be for the first 6 months of life or until cessation of breastfeeding, whichever is earliest. Infants determined to be HIV-infected will be taken off of study treatment but will remain in follow-up.

Treatment Arms	Infant Dosing Regimen
NVP Suspension (10 mg/ml) (n = 788)	0.6 ml (6 mg) once daily from 5 days after birth (± 2 days) to 2 weeks of age 1.5 ml (15 mg) once daily from 2 to 4 weeks of age 1.8 ml (18 mg) once daily from 4 to 6 weeks of age 2.0 ml (20 mg) once daily from 6 to 8 weeks of age 2.2 ml (22 mg) once daily from 8 weeks to 3 months of age 2.4 ml (24 mg) once daily from 3 to 4 months of age 2.6 ml (26 mg) once daily from 4 to 5 months of age 2.8 ml (28 mg) once daily from 5 to 6 months of age
NVP Placebo (n = 788)	0.6 ml once daily from 5 days after birth (± 2 days) to 2 weeks of age 1.5 ml once daily from 2 to 4 weeks of age 1.8 ml once daily from 4 to 6 weeks of age 2.0 ml once daily from 6 to 8 weeks of age 2.2 ml once daily from 8 weeks to 3 months of age 2.4 ml once daily from 3 to 4 months of age 2.6 ml once daily from 4 to 5 months of age 2.8 ml once daily from 5 to 6 months of age

Note: The study drug regimen does not include the two-dose intrapartum/neonatal HIVNET 012 nevirapine regimen that is standard of care in the study sites.

Study Duration: Total duration will be approximately 3.5 years. Participants will be enrolled over a period of approximately 18 to 24 months; mothers and infants will be maintained in follow-up through 18 months postpartum.

Study Objectives:

The primary objectives of this study are to:

1. Compare the rate of HIV infection at 6 months in infants determined to be HIV uninfected at birth in each arm.
2. Evaluate and compare the safety and tolerance in infants in each arm.

The secondary objectives of this study are to:

1. Compare the proportion of infants who are alive and free of HIV at 6 months and 18 months of age in the two arms.
2. Compare the relative rates of HIV infection in infants over 18 months in the two arms.
3. Compare the infant survival rates (mortality regardless of HIV infection) over 18 months in the two arms.
4. Determine the frequency and duration of NVP-resistant HIV strains in maternal plasma and breast milk and the relationship with MTCT.
5. Determine the relationship of maternal plasma and breast milk RNA levels to risk of MTCT.
6. Determine the frequency, type, and duration of NVP-resistant HIV strains in the plasma of infants who become HIV-infected.
7. Compare the rates of HIV disease progression, as defined by CD4+ cell count, HIV-1 RNA copy number, and mortality between the two arms in infants who become HIV-infected.
8. Determine NVP concentrations in infants who become infected with HIV and a sample of HIV uninfected infants as an index of adherence to the NVP regimen.

Study Sites:

- Prince Mshiyeni Hospital; Durban, South Africa
- Muhimbili Hospital; Dar es Salaam, Tanzania
- Mulago Hospital; Kampala, Uganda
- Chitungwiza Clinics; Harare, Zimbabwe

1.0 INTRODUCTION AND BACKGROUND

In 2002, an estimated 800,000 children became infected with HIV, mainly in developing countries. Since the beginning of the HIV epidemic, an estimated 5.1 million children worldwide have been infected with HIV. Mother to child HIV transmission (MTCT) is responsible for more than 90% of these infections, in which HIV is transmitted during pregnancy, labor, delivery, or during breastfeeding. Among infected infants who are not breastfed, about two-thirds of the cases of MTCT occur around the time of delivery and the rest during pregnancy (mostly during the last 2 months) (1). In populations where breastfeeding is the norm, postnatal transmission through breast milk accounts for more than one-third of all transmission (2-6). Therefore, even though antepartum and intrapartum antiretroviral regimens have been shown to significantly reduce MTCT, these substantial gains may be lost in breastfeeding populations in which infants have continued exposure to HIV through breast milk.

In most developing countries, where the majority of MTCT continues to take place, promotion of breastfeeding has been central to maternal and child health and to reducing infant mortality by providing optimal nutrition, by protecting against common childhood diseases, such as diarrheal and respiratory infections, and by promoting child spacing (7,8). However, HIV infection is also transmitted through breast milk, leading to the dilemma that replacement feeding, while protecting against HIV infection, may also place the infant at greater risk of dying from other infections (9). This is particularly true in rural areas and regions where limited access to clean water increases the risk of diarrheal disease if replacement feeding is used. Additionally, the societal norm in most developing countries is breastfeeding, and there are extraordinary social and family pressures to breastfeed. The use of formula feeding may be viewed as a surrogate for HIV infection and lead to social stigmatization, discrimination, and even violence and abandonment of the woman and her infant. Concerns for confidentiality may significantly influence a woman's decision to breastfeed (10). Thus, the universal promotion of formula feeding as an alternative to breastfeeding for HIV-infected women in developing countries is often not a feasible or even desirable option. Even in those areas where an HIV-infected mother might have the choice to use formula, the decision to breastfeed appears to be influenced by considerations such as the cost of formula, fear of disclosure of HIV status, and cultural constraints. It is, therefore, the goal of this study to evaluate an intervention that may enable HIV-infected mothers who wish to breastfeed, or who have no other option, to do so while still protecting their infants from HIV infection.

This Phase III trial will evaluate the efficacy and safety of a daily regimen of NVP provided to infants for 6 months or through the cessation of breastfeeding (whichever is earliest) compared to placebo for prevention of MTCT in breastfeeding infants who are born to HIV-infected women. As standard of care (external to the study) all HIV-infected women at the study sites are offered the HIVNET 012 two-dose intrapartum/neonatal regimen of NVP. The daily regimen of NVP was chosen for this study based on the safety and pharmacokinetic data from HIVNET 023 in which daily, weekly, and twice weekly dosing regimens were compared (see Section 1.5). A placebo controlled study design will be used because there are currently no conclusive data on potential efficacy of postpartum interventions to diminish transmission when the infant is breastfed. There may also be side effects of administering drug to healthy infants, therefore it is essential to ascertain that the benefit is greater than any risks incurred. A primary objective of the study is to evaluate and compare the safety and tolerance in the two arms. The placebo-controlled design allows for the most accurate and appropriate assessment of both efficacy and safety.

1.1 Breastfeeding and Mother to Child Transmission

1.1.1 Benefits of Breastfeeding

The many advantages of breastfeeding have been well documented. The practice is known to confer health, nutritional, immunological, developmental, psychological, social, and economic benefits (7,8,11).

Studies have shown that breastfeeding provides general health, growth, and development benefits to the infant, while significantly decreasing the risk of a number of acute and chronic diseases. Breastmilk provides secretory antibodies against specific pathogens as well as non-specific broad-spectrum protection from pathogens by milk constituents such as leukocytes, fatty acids, lactoferrin, glycoconjugates (such as lactadherin and oligosaccharides), nucleotides, immunomodulators, and anti-inflammatory agents (12). Breastfeeding has an important role in regulation of the immune response during infancy and has been shown to lower infant morbidity and mortality and decrease the incidence and/or severity of gastrointestinal and lower respiratory tract infections, otitis media, and necrotizing enterocolitis (11,13-15). These benefits are greatest among infants who exclusively breastfeed; exclusively breastfed infants are less likely than mixed breastfed infants to have diarrheal or respiratory illness or to develop atopic disease (7,16).

In a meta-analysis, breastfeeding was associated with a six-fold (95% confidence interval [CI] 6-10) decrease in mortality due to infectious diseases for infants less than 2 months of age. Protection persisted but declined with age during infancy with a four--fold (95% CI 3-6) decrease for ages 2 to 3 months, a three-fold (95% CI 2-4) decrease for ages 4 to 5 months, a two-fold (95% CI 1-3) for ages 6 to 8 months and 1.4-fold (95% CI 1-3) for ages 9 to 11 months (15). Others have similarly shown that the protective effects of breastfeeding are greatest in the first 6 months of life (17,18). Breastmilk contains carbohydrates, fats, amino acids, minerals and vitamins as well as various growth-promoting factors, enzymes, hormones and substances such as epidermal growth factor, insulin and somatomedin and provides optimal nutrition to infants, particularly during the first 12 months of life (18,19).

Breastfeeding also provides benefits to the mother. Breastfeeding delays resumption of ovulation resulting in increased child spacing. There are also psychosocial benefits of breastfeeding through promotion of maternal-infant bonding. In addition to individual health benefits, there are economic and social benefits due to savings for formula purchases for the child and decreased health care costs due to lowered rates of infant disease.

Nduati and colleagues have reported a post-hoc observation that maternal deaths were increased over 24 months in infected mothers who breastfed compared to those who fed with formula, with cumulative mortality 10.5% vs. 3.8%, respectively (20). It was hypothesized that the increased metabolic demands of breastfeeding in a population with already marginal nutritional status secondary to HIV infection could result in substantial nutritional impairment and wasting. However, a vitamin A intervention study from Durban, South Africa, did not detect any significant effect of breastfeeding on maternal morbidity, maternal deaths, CD4+ cell counts, or hemoglobin (21).

1.1.2 HIV Transmission through Breastfeeding.

A large number of studies have provided definitive evidence that HIV is transmitted through breast milk; these data have been reviewed in depth in several recent papers and will not be discussed here (9,16,22).

A meta-analysis of data from five studies found the overall additional risk of MTCT attributed to breastfeeding from chronically infected women to be 14% (95% CI: 7-22%) (23). A prospective study in South Africa reinforces these results with the estimated rate of breastfeeding transmission being 12% by 15 months of age (21). In a randomized trial of formula vs. breastfeeding in Kenya, the cumulative probability of HIV-1 infection at 24 months was 36.7% (95% CI: 29-44%) in the breastfeeding arm and 20.5% (95% CI: 14-27%) in the formula arm; the estimated rate of breastfeeding transmission at 24 months was 16.2% (95% CI: 6.5%-25.9%) (6). Transmission of HIV through breast milk accounted for 44% of overall HIV transmission in this study.

While data show a correlation of duration of breastfeeding with transmission, the risk of HIV transmission over time while breastfeeding has been difficult to determine, in part due to the difficulty in distinguishing between intrapartum and early breastmilk HIV transmission and to methodological differences in studies. In a meta-analysis based on four cohorts in Africa, Leroy and colleagues found a risk of breast milk transmission (defined as transmission occurring after 2.5 months of age) of 3.2 per 100 child years of breastfeeding (3). In a prospective cohort study in Malawi that evaluated breastmilk transmission starting at an earlier timepoint (transmission occurring after 1 month of age), Miotti and colleagues reported a relatively high risk of HIV transmission, 0.7% per month, from early breastfeeding between ages 1-5 months and 0.6% per month between 6-12 months, with a lower but continuous risk from late breastfeeding transmission (0.3% incidence per month from 12-17 months) (4). The overall rate of breastfeeding transmission in the meta-analysis was less than half that observed in the Malawi study (3.2 vs. 6.9 per 100 person years, respectively) with the greatest difference in reported transmission being in the first 1-5 months (3.5% in Malawi compared with 0.7% in the meta-analysis). These differences may be due to different definitions of postnatal transmission, with the Malawi study including earlier time points that were associated with the highest rates of transmission. Consistent with the Miotti study, data from two randomized clinical trials (the Kenya and SAINT trials) also suggest that breastfeeding transmission is highest in the first 2 months of feeding. In the Kenya study, the breastfeeding group had 6.3% more infections between delivery and 6-8 weeks than the formula fed group; similarly, in the SAINT trial in South Africa (described in Section 1.3.2), there were 5.6% more new HIV infections among breastfed infants in the same time period (6,24).

In addition to duration of breastfeeding, other risk factors for breastfeeding transmission include maternal viral load, cell-free virus in breast milk, breast pathology (mastitis, breast abscess, cracked nipples), and infant thrush before age 6 months (25-27). Sub-clinical mastitis may also increase the risk of breastfeeding transmission (26,28). A few observational studies have found that exclusive breastfeeding may be safer than mixed breastfeeding (29,30). However, this finding requires further investigation.

1.1.3 Preliminary SIMBA Study Results

In July 2003, results of the SIMBA (Stopping Infection from Mother-to-Child from Breastfeeding in Africa) study were presented at the International AIDS Society (IAS) Meeting, Paris, France. SIMBA was a randomized, open-label study sponsored by the International Antiviral Therapy Evaluation Center (IATEC) conducted in Uganda and Rwanda in which 405 women received dual antiretroviral therapy with zidovudine (ZDV) and didanosine (ddI) from 36 weeks of pregnancy, intrapartum, and for 1 week postpartum. Infants were randomized to NVP (N=198) vs. 3TC (N=199) throughout

breastfeeding and for 1 month after cessation. Overall, 43 of 397 (11%) infants had HIV infection or died by age 6 months. In Kaplan-Meier analyses, the overall rate of HIV infection was 8% (95% CI, 5-10%) and the overall rate of either HIV-infection or death was 10% (95% CI, 7-12%). Excluding infants positive at birth, 3 of 367 (0.8%) infants became infected between birth and age 4 weeks, and another 3 of 358 (0.8%) became infected between 4 weeks and 6 months (2/179 [1.1%] on 3TC, and 1/179 [0.6%] on NVP), for a total of 1.6% infected following birth (comparison between 3TC and NVP was not statistically significant).

The preliminary report from this Phase II trial and its relevance to the HPTN 046 design have been carefully considered by the HPTN 046 team as well as by the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine and Prevention Data and Safety Monitoring Board (DSMB). The 12-15% late postnatal transmission reported in most prior studies was in the context of ongoing breastfeeding into the second year of life whereas the median length of breast feeding in SIMBA was only about 3 months. In addition, critical covariates for HIV transmission such as maternal viral load at delivery (median RNA = 2.74 log copies/mL) were substantially different from those in other African studies. SIMBA used a background of maternal AZT/ddI treatment during pregnancy, intrapartum, and one week postpartum in the mothers, whereas most perinatal studies and most governmental programs implementing MTCT prevention programs in the international arena are using the HIVNET 012 two-dose intrapartum/neonatal NVP regimen. The SIMBA regimen is more complex and is therefore unlikely to be implemented as national policy in Tanzania, Zimbabwe, South Africa or Uganda at this time. The duration of breastfeeding reported in SIMBA was 3.3-3.5 months. Thus, breastfeeding and risk of transmission ceased on average approximately 2.5 months before the 6-month study endpoint. In contrast, the median duration of breastfeeding in HIVNET 012 conducted in Uganda was 9 months. Additionally, since there was such early weaning in SIMBA, it will be critical to also evaluate infant growth and morbidity data in SIMBA participants to adequately address the risk of the intervention. The percentage of exclusive breastfeeding is much higher in SIMBA (>90%) than seen in other breastfeeding studies. Whether this intervention without any antiretroviral prophylaxis would also lower the risk of breast milk HIV transmission is controversial, but some researchers believe exclusive breastfeeding lowers risk. An important covariate in the risk of postnatal transmission is maternal RNA at delivery. The median reported RNA in the NVP arm in SIMBA was 2.74 (IQR 2.6-3.5) log, compared with 4.4 log in HIVNET 012. Plasma RNA levels have been shown in several studies to be an important determinant of breast milk transmission and to correlate with breast milk viral load. Consider the subgroup of HIVNET 012 women who had HIV RNA less than the median level of 4.4 log copies/mL. While these women still had median HIV RNA (3.8 log) that was more than 1 log higher than the median level in SIMBA, these two groups likely had similar median viral loads before receiving antivirals, (assuming AZT/ddI leads to a 1.1 decrease in log HIV RNA). In this group from HIVNET 012, the postnatal rate of infant infection was only 1.4%.

Thus, while the preliminary results from this Phase II trial are encouraging, the HPTN 046 team as well as the NIAID DSMB concluded that available SIMBA data are too limited to make any formal conclusions. The SIMBA study did not include a control arm in which no infant prophylaxis was provided, therefore it is not possible to differentiate whether the observed rate of breast milk transmission is due to the AZT/ddI treatment of the mother, which included postnatal therapy; the low viral load in the mothers at delivery; the short duration of breastfeeding; or other factors. This Phase II study does

not conclusively demonstrate the efficacy of infant prophylaxis in reducing breast milk transmission, nor are the regimens currently applicable to a broad population of breastfeeding women, such as late presenting women who could not be offered prenatal antiretroviral interventions.

1.1.4 Breastfeeding in Developing Countries in Africa

Breastfeeding is the norm in the countries in which this study will be conducted. A representative national study in South Africa on childhood nutrition found that 88% of mothers initiated breastfeeding and that 95% of those mothers continued breastfeeding for at least 3-5 months. In Zimbabwe, 98.7% of mothers initiated breastfeeding; at 6-7 months, only 0.8% had weaned their infants. In a study conducted in Dar es Salaam, Tanzania, 90% of women were still breastfeeding at 3 months and 87% at 6 months. In recent studies of antiretrovirals to reduce MTCT in West and East Africa and KwaZulu Natal, South Africa, the overwhelming majority of HIV-infected women chose to breastfeed their babies despite receiving counseling on the risks and benefits of breast and formula-feeding. In the HIVNET 012 trial in Uganda (described in Section 1.3.2), 99% of women breastfed their infants; 80% were still breastfeeding at 6 months and 12% were still breastfeeding at 18 months (31). In the SAINT trial in South Africa (described in Section 1.3.2), 43% of infants were breastfed, with 75% still breastfeeding until 3 months, 11% until 6 months and 14% until 1 year (24).

Adherence to formula feeding in populations where breastfeeding is the norm may be problematic. For example, in the trial of breastfeeding versus formula feeding of infants born to HIV-infected women in Kenya, in which women were provided with considerable support to initiate and maintain formula feeding, more than 25% of those randomized to the formula feeding group were reported to have also breastfed their infants (6). The Kenya trial revealed the difficulties of balancing risks between breast and formula-feeding for HIV-infected women in developing countries. Infant mortality at 24 months was similar for the formula-fed and breastfeeding groups, despite a 40% lower risk of HIV transmission in the formula fed group. Furthermore, the morbidity and mortality during the first few months of life were higher in the formula group; this applied to both HIV-infected and HIV-uninfected infants. The cost to HIV-exposed but uninfected infants is therefore a serious consideration in policies on breast versus replacement feeding in poor populations.

Current World Health Organization (WHO) recommendations emphasize that breastfeeding should continue to be supported and promoted in all populations, irrespective of HIV infection rates; that there should be improved access to HIV counseling and testing; and that HIV-infected women should be fully counseled about the benefits of breastfeeding, the risk of HIV transmission through breastfeeding, and the risks and possible advantages associated with other methods of infant feeding; and should be supported in their choice of infant feeding (32).

1.2 Rationale for Extended Regimen of Infant NVP to Prevent MTCT

Given the many advantages of breastfeeding, the significant obstacles to substituting formula for breast milk in developing countries, and the documented risk of HIV transmission through breastfeeding, there is an urgent need to make breastfeeding by HIV-infected women safe from transmission of the virus to their infants. To that end, this study proposes to test the efficacy of providing an extended prophylactic antiretroviral regimen to breastfeeding infants born to HIV-

infected women over a maximum period of 6 months for prevention of MTCT through breast milk. Because the maximal benefit from breastfeeding is during the first 6 months of life, there is continued risk of HIV transmission for the duration of breastfeeding, so weaning by age 6 months will be encouraged (32). Others have similarly shown that the protective effects of breastfeeding are greatest in the first 6 months of life (17,18).

NVP was selected for evaluation as the antiretroviral drug candidate for prevention of vertical HIV transmission based on its relatively long half-life, excellent bioavailability, potent antiretroviral activity, ability to be administered in a once-daily dosing regimen, established safety with single dose neonatal administration in over 1,600 mother-infant pairs, and established safety when used in multiple doses for treatment of infected children (33,34).

NVP is a non-nucleoside reverse transcriptase inhibitor (NNRTI). It is a benzodiazepine derivative that is a potent inhibitor of HIV-1 replication with an inhibitory concentration (IC)₅₀ of 10 micrograms/ml. It has excellent oral absorption and bioavailability, and a high therapeutic index. NVP is highly lipophilic and widely distributed throughout the body, and has been shown to penetrate cell-free HIV-1 and inactivate virion-associated reverse transcriptase *in situ*. NVP prophylaxis has been shown to prevent infection in chimpanzees challenged with HIV-1 (35). It has been well tolerated when used therapeutically in HIV-infected adults, children, infants, and as a single dose in the first week of life for prophylaxis of MTCT in HIV-exposed neonates (31,33). The main route of NVP elimination is hepatic, with metabolism by the liver cytochrome p450 metabolic enzymes (primarily CYP3A4 and CYP2B6, and to a lesser extent, CYP2D6 and CYP2C9). It is an inducer of hepatic cytochrome p450, resulting in an “autoinduction” phenomenon characterized by an approximately 1.5 to 2-fold increase in apparent oral clearance and decrease in terminal half-life after 2-4 weeks of daily dosing in children and adults (36).

1.2.1 Rationale for NVP Resistance Testing

Resistance with monotherapy is a concern. The rate of NVP resistance in women and infants receiving NVP prophylaxis in this trial could be higher or lower than that observed in HIVNET 012 (described in Section 1.3.2). HIV-infected children in this trial are likely to receive NVP before their infection is discovered. This provides an opportunity for *de novo* selection of NVP resistance in infants infected at birth or post-partum. NVP-resistant HIV could also be transmitted to infants via breastfeeding. Prolonged use of NVP in the post-partum period could further increase the rate of NVP resistance in infants who are infected at birth or post-partum. The types of mutations that arise in infants receiving 6 months of NVP and the rate at which NVP-resistant HIV fades in women and infants in this trial may also differ from findings in HIVNET 012.

It is important to assess if NVP resistance has an effect on the efficacy of NVP prophylactic regimens. First, if NVP-resistant virus is selected in a mother, it may increase the probability that a breastfeeding infant would be infected despite NVP prophylaxis. Second, selection of resistant virus may affect the utility of NVP prophylaxis in a subsequent pregnancy. Third, selection of resistant virus in women could lead to transmission of NVP resistant HIV to the community. Fourth, as discussed above, it is probable that some children will become HIV infected while continuing to receive NVP but before their HIV status is determined. Finally, emergence of NVP resistance in women or infants receiving NVP prophylaxis could also limit their future options for antiretroviral treatment. These issues emphasize the importance of resistance testing in this cohort.

There is currently no evidence that NVP resistance influences the health of women or infants. However, in HIVNET 012 (described in Section 1.3.2) it was not possible to address those issues because the number of HIV-infected infants was small, and women were followed systematically for only 6 weeks after delivery. The larger size of this cohort, extended duration of the infant regimen and longer follow-up of women and infants may offer an opportunity to address these important issues.

1.3 Intrapartum/Neonatal NVP for Reducing Intrapartum MTCT

1.3.1 Pharmacokinetics of Intrapartum/Neonatal NVP

Prophylactic use of NVP has targeted achievement of drug levels of 100 mcg/ml, 10 times the *in vitro* IC₅₀ of the drug for HIV. It is likely that lower NVP concentrations are required to prevent infection than to treat established infection. However, no data are available that indicate the NVP drug concentration required to prevent transmission.

The pharmacokinetics of NVP following initial dosing during the third trimester are similar to those in non-pregnant adults (37). In Pediatric AIDS Clinical Trials Group (PACTG) 250, done in the U.S., NVP elimination was found to be prolonged when administered to pregnant women during labor, with the half-life increasing from 43 hours during the third trimester to 73 hours during labor. NVP crossed the placenta readily, and the ratio of cord blood to maternal NVP blood concentration averaged approximately 80%. Because of immature liver metabolism, the half-life of NVP in neonates was prolonged compared to older children; the half-life of a single dose of NVP administered at age 48-72 hours in neonates was 36.8 hours (range 27.3-49.5 hours) (37). A pharmacokinetic study in African pregnant HIV-infected women and their infants studied in Uganda (HIVNET 006) yielded similar results (38). As a result of the rapid absorption and distribution of NVP and its relatively slow elimination in women in labor and newborns, administration of a single 200 mg dose of NVP during labor and a single dose of 2 mg/kg to the infant at 48-72 hours after birth maintains NVP concentrations in the infant above 100 mcg/ml (10 times the *in vitro* IC₅₀) throughout the first week of life; this regimen was shown in HIVNET 012 (described below) to significantly decrease MTCT (31).

1.3.2 Efficacy of Intrapartum/Neonatal NVP for Preventing MCTC

The efficacy of NVP in reducing MTCT of HIV during labor and delivery was evaluated in three Phase III efficacy trials: HIVNET 012 (in Uganda); SAINT (in South Africa); and PACTG 316 (in the U.S., Europe, Bahamas and Brazil). These studies are described below.

HIVNET 012 was a perinatal trial in breastfeeding women in Uganda that compared two intrapartum/neonatal regimens: a NVP regimen consisting of a single 200 mg dose of oral NVP to the mother at the onset of labor and a single 2 mg/kg dose of NVP to the infant within 72 hours of life, and an ultra-short ZDV regimen consisting of 600 mg orally to the mother at the onset of labor and 300 mg every 3 hours until delivery and 4 mg/kg orally twice daily to the infant for 7 days after birth (31). The study enrolled 626 HIV-infected pregnant women and their infants, 313 in the NVP and 313 in the ZDV group. The NVP regimen was found to reduce the risk of HIV-1 transmission by 47% (95% CI: 20-64%) at 14-16 weeks compared to the ZDV regimen. The estimated risks of MTCT in the NVP and ZDV groups were 8.2% and 10.4% at birth; 11.9% and 21.3% by age 6-8

weeks; and 13.1% and 25.1% by age 14-16 weeks, respectively. Follow-up has been reported through age 12 and 18 months (39,40). At 18 months, transmission was 15.7% in the NVP group compared to 25.8% in the ZDV group. Thus, NVP efficacy was maintained through 18 months of age, with an estimated efficacy of 41% (95% CI: 16-59%). However, postpartum MTCT through breastfeeding continued to occur in both treatment groups. Almost one-quarter of all infant infections in the NVP group (an absolute rate of 4%) occurred postnatally between 6-8 weeks and 18 months of age.

The SAINT study, conducted in South Africa compared a modified HIVNET 012 NVP regimen (a single 200 mg dose of NVP given to the mother at onset of labor, another 200 mg dose to the mother 24-48 hours after delivery, and a single 6 mg dose given to the infant 24-48 hours after delivery) to a combination intrapartum/postpartum ZDV and lamivudine (3TC) regimen (given orally to the mother at onset and during labor and for 7 days to the mother and infant) that had been shown to be effective in reducing mother-to-child transmission in the PETRA study (24,41). Breast- and formula-fed infants at 11 sites in South Africa were tested for HIV-1 at birth, 4 weeks and 6 to 8 weeks by DNA PCR. A total of 1318 women and 1330 infants were randomized. The overall estimated HIV infection rates by eight weeks in 1307 infants (654 NVP, 653 ZDV/3TC) were 12.3% (95% CI: 9.6-15.0) in the NVP group and 9.3% (95%CI: 7.0-11.7) in the ZDV/3TC group. Excluding infections detected within 72 hours of birth, which reflect *in utero* transmission (42), 5.7% (95%CI: 3.6-7.7) of infants in the NVP group and 3.6% (95%CI: 2.0-5.2) in the ZDV/3TC group were infected within 8 weeks after birth. Maternal viral load, CD4+ cell count, timing of maternal dose, prolonged rupture of membranes were risk factors for infant infection. Infection-free survival rates at 8 weeks were 85.9% (95%CI: 82.8-89.1) in the NVP and 87.7% (95%CI: 84.9-90.5) in the ZDV/3TC group. Thus, a comparison of two short-course intrapartum/neonatal antiretroviral regimens showed both were similarly effective.

PACTG 316 was a randomized, double-blind placebo controlled trial conducted in the U.S., Europe, Bahamas and Brazil, designed to evaluate the safety and efficacy of intrapartum/postpartum NVP compared to NVP placebo added to standard antiretroviral therapy for the prevention of MTCT (43). HIV-infected pregnant women receiving standard antiretroviral therapy were randomized to receive 200 mg NVP or NVP placebo during active labor; infants received 2 mg/kg NVP or placebo at 48-72 hours. Seventy-seven percent of women received combination therapy (28% ZDV/3TC, 8% other combinations without and 41% combinations with protease inhibitors) and 23% received ZDV monotherapy as per the PACTG 076 regimen; infants received the 6-week ZDV PACTG 076 prophylaxis regimen. Thirty-four percent of women had elective cesarean delivery. Twelve hundred and seventy mother-infant pairs received study drug (642 in the NVP group) and had infection status known. No infant was breastfed. Overall transmission was 1.4%, with 1.4% in the NVP arm and 1.5% in the placebo group; 56% of the infected infants were infected *in utero*. The study was discontinued prior to full accrual because the perinatal transmission rate was significantly lower than expected and the planned sample size was not sufficient to meet the primary objective of the study. Thus, among HIV-infected women who have adequate prenatal care, are treated with standard antiretroviral antenatal therapy (generally combination ART), do not breastfeed, and for whom elective cesarean section is a safe option, the risk of perinatal transmission was very low and administration of the 2-dose intrapartum/neonatal NVP regimen did not produce a clinically relevant further lowering of transmission.

1.3.3 Safety of Intrapartum/Neonatal NVP

Safety data on the NVP maternal intrapartum/neonatal regimen are available from two Phase I studies, PACTG 250 and HIVNET 006, and the three Phase III studies discussed above.

PACTG 250 was a Phase I safety and pharmacokinetic study of the intrapartum/neonatal NVP regimen in women and infants in the U.S (37). PACTG 250 enrolled 17 HIV-infected pregnant women and their babies. No clinical or laboratory toxicities related to NVP were observed in either mothers or babies, and no severe rashes were noted.

This Phase I study was repeated in 21 Ugandan HIV-infected women and their infants in the HIVNET 006 study (38). No serious adverse experiences or grade 3 or 4 clinical or laboratory toxicities related to NVP were seen in the mothers. There was one serious adverse experience in an infant thought by the investigators to be possibly but not likely related to study drug. This was an infant who developed respiratory distress at birth and seizures after a difficult and prolonged labor requiring forceps. No other serious adverse experiences that were attributable due to study drug were observed in the infants and no women or infants had rash toxicity.

In the HIVNET 012 trial in Uganda (described in Section 1.3.2), 313 HIV-infected pregnant women and their infants received NVP (31). CBC and chemistries were monitored in the infants at age 24 hours, 7 days and 6 weeks. Mothers had a CBC at 24-48 hours postpartum and chemistries done at 7 days postpartum. The rates of maternal serious adverse experiences were similar between the NVP and ZDV groups (4.7% and 4.4%, respectively) and the occurrence of clinical and laboratory abnormalities were again similar in both groups. Nine women (4 in the ZDV and 5 in the NVP group) had maculopapular rash but none were serious. Reported serious adverse experiences among infants within 56 days of birth were balanced between the two groups (12.3% in the AZT group and 10.9 % in the NVP group). Of the 64 babies with at least one serious adverse experience reported within 56 days of birth, seven (2.3%) in the AZT group and two (0.6%) in the NVP group were thought to be possibly but unlikely due to study drug. In the two NVP group infants, these adverse experiences were transient respiratory distress at birth with meconium staining requiring oxygen, and a non-macerated stillbirth to a mother who had received NVP 3.5 hours before delivery. Eighteen babies, balanced between the two groups had maculopapular rash, no case of which was serious. The frequency and severity of laboratory adverse effects were similar between the two ZDV and NVP arms as well (including hematologic and chemistry abnormalities). Reports of serious adverse experiences through 18 months of follow-up were also balanced between the two groups (40). There were 76 deaths among 629 babies in follow-up through 18 months, 13.6 % in the AZT group and 10.6% in the NVP group. The most frequent cause of death was pneumonia, followed by gastroenteritis/diarrhea/dehydration, anemia, and malaria. Nevirapine was associated with significantly longer HIV-1 free survival at 18 months. When stratified by babies' infection status at age 6-8 weeks, survival rates at 18 months were similar in both groups.

In the SAINT study in South Africa (described in Section 1.3.2), there was no difference in the frequency of maternal serious or non-serious adverse experiences or deaths between women receiving ZDV/3TC or a modified HIVNET 012 NVP regimen described above NVP (44). The most common maternal adverse experiences through 28

days post-delivery were related to obstetrical procedures (23.7% in NVP group; 26.4% in ZDV/3TC group; $p = 0.25$). The rate of rash was similar in both arms. Nine women died in the study: 5 in the NVP arm and 4 in the ZDV/3TC arm. None of these deaths were considered to be treatment related. Twelve (9%) infants weighing less than 2 kilograms at birth received study medication. The non-serious adverse experiences reported for infants through 28 days of life were also similar in both treatment groups and most were respiratory system disorders. There was no difference in the occurrence of rash in the two groups of infants. The rates of serious adverse experiences (clinical or laboratory abnormalities) were similar in the two treatment groups, 8.5% in the NVP and 9.8% in the ZDV/3TC group. The most frequent serious adverse experiences were respiratory system disorders (NVP 3.8%, ZDV/3TC 4.2%) and infections (NVP 2.3%, ZDV/3TC 3.0%). There were 13 infant deaths in the NVP arm; 10 of which were related to HIV infection, 5 to gastroenteritis, 3 to pneumonia and 1 to birth asphyxia. None of the deaths was related to drug toxicity.

In PACTG 316 (described in Section 1.3.2), the toxicity monitoring included a CBC and SGPT in the mothers at delivery and 4-6 weeks postpartum and in the infant at birth, 6-9 days and 4-6 weeks of life. Overall, significant toxicity was rare in both mothers and infants and did not differ between NVP and placebo groups (43). Only two women had a severe (grade 3 or higher) rash after study drug, one in each treatment group. Non-rash serious toxicity (grade 3 or higher) after study drug was rare and similar in both arms (6% in each treatment group). Grade 3 or higher hepatic toxicity (elevated transaminases or bilirubin) was reported in 10 women, 5 in each treatment group (4 women, 2 per group, with known underlying liver disease). Three women died during postpartum follow-up (2 NVP, 1 placebo); none of the causes was attributed to study drug (ischemic cardiomyopathy; sickle cell disease, and disseminated histoplasmosis). Four infants (1 NVP, 3 placebo) had a reported grade 3 or higher rash, 2 between birth and 3 days and 2 at ≥ 4 days of age; no rash was attributable to NVP. Serious non-rash toxicity (grade 3 or higher) was observed in 30% of infants, and was similar between the two treatment arms; most were judged unrelated to study drug. The most frequent experiences included anemia (13%) and neutropenia (13%). Three infants (2 NVP and 1 placebo) had grade 3 grade or higher hepatic toxicity. All 3 were attributed to cytomegalovirus infection with hepatitis. Eight infants died (3 NVP, 5 placebo); deaths were due to SIDS (3), bacterial infection (2), post-operative gastric hemorrhage (1), trauma (1) and unknown (1) and none were attributable to study drug.

1.3.4 NVP Resistance with the Intrapartum/Neonatal NVP regimen

High-level NVP resistance can be induced by a single point mutation in the HIV reverse transcriptase coding region (K103N or Y181C). HIV variants with these mutations are present at low background levels in HIV-infected individuals prior to NVP exposure (45). Exposure to NVP in the presence of replicating virus rapidly selects for these pre-existing NVP-resistant variants, which may become the major quasispecies as replication of NVP-sensitive virus is inhibited. NVP resistance is seen within 4 weeks in almost all patients receiving NVP monotherapy (46).

Emergence of NVP resistance after single dose NVP prophylaxis was first reported in 3/15 (20%) of women in the Phase I/II Ugandan HIVNET 006 trial (47). In that study, detection of NVP resistance 6-8 weeks after single dose NVP was associated with a longer median NVP elimination half-life, decreased median oral clearance, and increased median area under the concentration time curve.

The emergence and fading of NVP resistance was further evaluated in women and infants in HIVNET 012, described in Section 1.3.2, (41/48 women with infected infants, 70 randomly-selected women with uninfected infants, and 33/49 infected infants) (48,49). NVP resistance mutations were detected in 19% (21/111) of women tested 6-8 weeks after delivery. The rate of NVP resistance was similar among women whose infants were and were not infected by 6-8 weeks. Development of resistance was associated with longer NVP clearance and half-life, higher maternal baseline HIV RNA and lower maternal CD4+ cell counts. Furthermore, the rate of NVP resistance was higher in women with subtype D than subtype A, suggesting that resistance rates may vary from one geographical region to another, depending on which subtypes are prevalent (50). Follow-up samples collected 12-24 months postpartum were available for 11/18 women with resistance at 6-8 weeks postpartum, and none had resistant virus detectable.

Of the 24 infected infants in the NVP group on whom specimens were available for resistance testing, 11/24 (46%) were found to have NVP resistance mutations at 6-8 weeks of age. Of the 11 infants with NVP resistance detected at 6-8 weeks, 91% were infected at birth compared to 69% without resistance. Mortality within the first year of life was similar for infants with and without NVP resistance. In infants with resistance who had follow-up samples, 4 out of 9 had no detectable resistance by 14-16 weeks of age. Among the infants who still had resistance at 14 -16 weeks, 3 out of 3 tested at 12 months no longer had resistance detected. Late HIV transmission in HIVNET 012 (described in Section 1.3.2) was also evaluated. Twelve infants developed infection after 6-8 weeks of age (likely breast milk-transmitted infection; median age at diagnosis 10 months). Samples were available from 9 of these 12 infants collected 2-9 months after diagnosis; 8 out of 9 had no NVP resistance, including 2 infants whose mothers had resistant virus. One infant with NVP resistance was born to a mother who had NVP resistance detected at 6-8 weeks.

With the exception of the one infant with late infection, in those instances in which infected infants had resistant virus, the mothers either had wild type virus or virus with a different resistance mutation pattern. This suggests that the infants developed resistance *de novo* (when NVP prophylaxis was administered to an already infected infant), as opposed to resistant virus being transmitted from the mother. The one infant who developed late infection whose mother had NVP resistance detected at 6-8 weeks postpartum had the same pattern of mutations as the mother. Interestingly, the type of mutations that developed differed in women vs. infants, with K103N mutation more common in women and the Y181C mutation more common in infants. This may be relevant to future treatment, since subtype B HIV viruses with the K103N vs. Y181C mutation differ in their cross-resistance to efavirenz (51,52). Viruses with the Y181C mutation alone have little resistance to efavirenz (Y181C can enhance the level of resistance of viruses containing additional NVP mutations), whereas viruses with the K103N mutation are cross-resistant to other NNRTIs (53).

These studies demonstrate that NVP resistance can develop with single dose NVP in women and in infants who become infected despite NVP; following the single-dose of NVP without continued NVP exposure, resistance fades from detection in women and infants by 12-24 months among single dose recipients using standard genotyping methods. However, HIV with NVP resistance mutations may continue to circulate in these women and infants as minor variants, and may be maintained as provirus in infected cells. This may influence the efficacy of NVP in a subsequent pregnancy, or the

efficacy of subsequent treatment of women or infants with an NNRTI-containing regimen.

Emergence of NVP resistance mutations was also analyzed in Zimbabwean women enrolled in HIVNET 023 8 weeks following administration of single dose NVP prophylaxis (54). For that study, HIV-1 genotyping was done with the TRUGENE™ HIV-1 Genotyping Kit (Visible Genetics Inc.) with modified v1.5 primers. NVP resistance mutations were detected in 8/30 (27%) women at 8 weeks following treatment with sd-NVP (2/4=50% vs. 6/26=23% of women whose infants were or were not HIV-1 infected, $p=0.26$). NVP resistance mutations included K103N, V106A, Y181C, and G190A. The predominant mutation was K103N. Women in HIVNET 023 all had subtype C HIV-1. The rate of resistance among women with subtype C appeared to be similar to that observed in HIVNET 012 (described in Section 1.3.2) for women with subtype D, and higher than that observed in HIVNET 012 among women with subtype A. However, because different HIV-1 genotyping assays were used in the two trials (ViroSeq for HIVNET 012 and TRUGENE™ for HIVNET 023), the resistance rates among women in the two trials cannot be directly compared.

In PACTG 316 (described in Section 1.3.2), where all women were receiving antenatal antiretroviral therapy in addition to the single dose intrapartum study drug, resistance was analyzed in 51 NVP arm women in the U.S. with delivery RNA >400 copies/ml, 46 of whom got study drug, and 43 NPV arm women in France with delivery RNA >200 copies/ml (55,56). NVP resistance was detected at 6 weeks postpartum in 12 of the 89 women (13%) who received single dose NVP and had some level of viral replication, despite the receipt of other antiretroviral drugs antenatally.

In the SAINT study (described in Section 1.3.2), a modified single-dose NVP regimen was given, where the women received a single dose of NVP at onset of labor and a second dose at 48 hours postpartum; their infants received a single 48 hour NVP dose. This regimen was compared to the PETRA ZDV/3TC regimen, where ZDV/3TC was given to women during labor and to both women and infants for 1 week postpartum. NVP resistant mutations were detected in 74 (67%) of the 111 women who received two doses of NVP. The predominant NVP mutations found were K103N (62%) and Y181C (45%). Studies of 40 infected infants [4-6 wks/age] demonstrated NVP mutations in 21 of 40 (53%). The predominant mutation was Y181C present in 53%. Paired data from 26 mother-infant pairs suggested that in a few instances NVP resistant virus may have been transmitted through breastfeeding. Long-term follow-up [9-12 months] samples were available from 57 women who were studied at 4-6 weeks postpartum. Of 36 women with NVP resistant mutations at 4-6 weeks, 28 (78%) reverted to wildtype, and eight (22%) retained NVP resistance. The K103N mutation was the most durable. No ZDV or 3TC mutations were detected in the 37 women who received the multiple dose ZDV/3TC regimen. Therefore, the use of a 2 dose maternal NVP regimen for prevention of MTCT is associated with a selection frequency (67%) of resistance mutations which is 3X greater than that observed with a single dose maternal regimen (19% HIVNET012). The majority of women who have NVP resistance mutations revert to wild type after 9-12 months (57).

1.4 Chronic Use of NVP for Treatment of HIV-Infected Children

1.4.1 Pharmacokinetics of NVP Treatment in HIV-Infected Infants and Children

During long-term therapy in children >2 months of age, NVP clearance is rapid, averaging ~120 ml/hr/kg in children during the first 2 years of life; subsequently there is a gradual decrease in NVP clearance, with average clearance decreasing to ~60 ml/hr/kg by age 8-10 years (33,34,58). NVP was tested in PACTG 356 in HIV-infected infants as young as age 2 weeks with a starting dose for infants age 15 days to ≤ 3 months of 5 mg/kg as a single daily oral dose for 14 days, increasing to 120 mg/m² twice daily for 14 days, and then to 200 mg/m² twice daily. For children aged 3 months to 2 years, the dose used is 120 mg/m² as a single oral daily dose for 14 days, and then 200 mg/m² twice daily. This dose has been well tolerated in infants. The pediatric NVP administration schedule approved for use in HIV-infected children aged 2 months up to eight years by the U.S. Food and Drug Administration (FDA) is 4 mg/kg once daily for the first 14 days, followed by 7 mg/kg twice daily thereafter. For children aged 8 years or more, the recommended dose is 4 mg/kg once daily for 2 weeks followed by 4 mg/kg twice daily thereafter. The dose of 2 mg/kg used in this protocol during the first 2 weeks of life is 40% of the initial PACTG 356 dose. The dose of 4 mg/kg used in this protocol after the first 2 weeks of life is 29% of the US FDA-approved NVP dose for HIV infected infants over age 2 months. Because the objective of NVP use in this study is prevention of infection rather than treatment of established infection, reduced doses will be administered.

1.4.2 NVP for Treatment of HIV Infection in Adults and Children

NVP has been used for treatment of HIV-infected adults and children. The drug is well tolerated and produces a rapid rise in CD4+ cell count and reduction in HIV replication. However, antiviral responses are transient when NVP is used as monotherapy, with viral levels rebounding in some individuals by 4 weeks after starting therapy due to the development of NVP resistant virus (46). In contrast, when it is administered in combination with other antiretroviral drugs, antiviral response has been profound and sustained (59). Administration of higher doses of NVP (more than 400 mg per day) in adults appears to be safe and to result in plasma levels above the IC₅₀ of resistant virus with resultant sustained antiviral responses (60,61). NVP is commonly used in combination antiretroviral regimens for treatment of HIV-infected children, including infants as young as age 2 weeks in a clinical trial (PACTG 356) (58,62,63).

1.4.3 Safety of NVP Used for Treatment of HIV Infection

The most frequently reported adverse experiences related to NVP are rash, fever, nausea, headache and abnormal liver function tests (33,34). Severe, life-threatening skin reactions, including fatal cases, have been reported with NVP therapy, occurring most frequently during the first 6 weeks of therapy. These have included cases of Stevens-Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity reactions characterized by rash, constitutional findings, and organ dysfunction. Rashes are usually mild to moderate maculopapular erythematous cutaneous eruptions with or without pruritus and located on the trunk, face and extremities. Stevens-Johnson Syndrome (SJS) has occurred in 0.3% of 2861 adult patients exposed to NVP (34). Dose escalation, using a lower "lead-in" dose of NVP for the first 2 weeks of therapy, has been shown to reduce the frequency of NVP-associated rash.

Severe, life threatening, and, in some cases, fatal hepatotoxicity, including fulminant and cholestatic hepatitis, hepatic necrosis and hepatic failure, have been reported in patients treated with NVP. In clinical trials the overall NVP-attributable risk of hepatitis is approximately 1%. Increased transaminase (ALT) values before starting therapy or history of hepatitis B or C infections have been associated with a greater risk of hepatic adverse experiences in patients on chronic NVP therapy (64). Acute hepatotoxicity has progressed to hepatic failure with transaminase elevation, with or without hyperbilirubinemia, prolonged prothrombin time or eosinophilia. Hepatic dysfunction can be isolated or associated with signs of hepatotoxicity. Serious hepatic events occur most frequently during the first 12-16 weeks of NVP therapy, and have been reported to occur as early as within the first few weeks of therapy. Approximately one-third of cases have occurred after the critical 12-week period.

NVP has occasionally been given to HIV-uninfected individuals as post-exposure prophylaxis (PEP) for occupational and non-occupational exposures to HIV. When given as PEP, NVP is administered for 4 weeks in standard doses used for treatment of HIV-infected individuals, and often given without the 2-week induction period recommended for treatment. There have been twelve cases of reported hepatotoxic reactions among these individuals; one developed liver failure (requiring liver transplantation), seven had clinical hepatitis and four had elevations in serum liver enzymes without reports of clinical hepatitis. Data on the number of persons taking NVP for PEP is insufficient to calculate accurate rates of adverse event (65).

In initial clinical trials of NVP treatment in HIV-infected children, rash was observed in 24% (34). When a 2-week lower dose "lead in" period was used, the incidence of rash was decreased (58). While grade 2 or higher skin rash has been reported in up to 33% of children receiving nevirapine (66), serious rash is less common. In a study of 4-drug therapy including NVP (given with 2-week lead in), serious rash was observed in 6% of children (67). In another study of nevirapine based therapy in 74 HIV-infected children, rash occurred in 20% of patients but serious grade 3 or 4 rash occurred in only 5% (68). Granulocytopenia has also been reported in children receiving nevirapine (68); in the above study in 74 children, 7% of children had grade 3 or 4 neutropenia, and in other studies neutropenia has been reported in 9% to 38% of children receiving nevirapine (66,69). However, it should be noted the children in these studies were also receiving nucleoside analogue reverse transcriptase, such as ZDV, a known cause of granulocytopenia.

1.4.4 Development of NVP Resistance with NVP Used for Treatment

Viral isolates with high-level NVP resistance were obtained as early as day 14 from peripheral blood mononuclear cells of children receiving NVP monotherapy (58). Development of resistance was generally associated with rebound in viremia; however, some children had durable reduction in viral load despite the emergence of resistance.

The most common mutations seen in clinical isolates of patients treated with NVP are Y181C and K103N. Recent studies have indicated that mutations associated with NVP resistance result in measurable biochemical abnormalities in affected HIV variants, with the most common effect being a change in the ratio of viral RNase H to polymerase activities (70). In particular, reduced RNase H cleavage is observed. This can result in reduced replicative fitness of the resistant viral strain compared to wild type virus; this was particularly true for NVP resistant virus with the Y181C mutation. This is consistent

with the theory that drug-resistant mutants rarely predominate in the absence of drug pressure because they have reduced replication fitness relative to wild-type virus. Additionally, mutations in the non-nucleoside binding pocket appear to affect the conformation of residues at the dNTP binding site and can result in a partial phenotypic reversal of multi-nucleoside resistance (71). The Y181C mutation has been shown to lead to resensitization of ZDV-resistant virus to ZDV treatment (71).

More detailed information regarding NVP can be found in the Investigator's Brochure and the Package Insert.

1.5 HIVNET 023: Safety and Pharmacokinetics of Extended NVP Regimen

HIVNET 023 was a Phase I/II randomized open label clinical trial to evaluate the safety and pharmacokinetics of three different NVP dosing regimens from birth to 6 months in breastfeeding infants born to HIV-infected women. The primary pharmacokinetic objective was to determine a NVP dosing regimen that is safe and maintains NVP plasma concentration above 100 micrograms/ml continuously from birth to 6 months of life for use in a Phase III efficacy trial.

The study was conducted in general antenatal clinics in Chitungwiza, Zimbabwe and Durban, South Africa. HIV-infected women were enrolled from April 2000 to January 2001 and given an oral dose of 200 mg of NVP to administer to themselves at the onset of labor. Seventy-five of their infants (including 1 set of twins) were randomized within 48 hours of birth to 1 of 3 study arms and received either weekly, twice weekly or daily regimens of NVP from birth through 6 months of age. Infants in the weekly and twice weekly arms received 4 mg/kg/dose for the first 2 weeks of life, then 8 mg/kg/dose through 24 weeks. In the daily arm, infants received 2 mg/kg/dose for the first 2 weeks of life, followed by 4 mg/kg/dose through 24 weeks.

1.5.1 NVP Concentration Results

The primary NVP concentration outcome measures were trough (pre-dosing) NVP concentrations assessed in all infants. The therapeutic target was a trough concentration greater than 100 ng/ml, which is 10 times the in vitro IC₅₀ against HIV. Pre-dose (trough) concentrations were determined at 2, 8, 16, 20 and 24 weeks of age. Deficiencies in sample processing occurred at the Durban site from study initiation in April 2000 through November 2000. Inspection of the NVP concentration values and their assigned sampling times from this period reveals many sets of pre and post dose samples that are not consistent with our current understanding of NVP pharmacology. Therefore the study team decided to exclude all NVP concentration data from the Durban site from the period prior to correction of the sample processing deficiencies.

Concentrations are available for between 10 and 17 infants in each treatment arm (daily, twice-weekly and weekly) at each time point. Overall, none of the pre-dose samples from the daily dosing arm infants were below the therapeutic target of 100 ng/ml, while 3 of 65 (4.6%) of the twice-weekly samples and 48 of 75 (64%) of the weekly samples fell below the target. When all concentrations from all time points are combined for each treatment arm, median pre-dose NVP concentration was 1348 ng/ml (range: 108 - 4843 ng/ml) with daily dosing compared to 459 ng/ml (range: <25 - 1386 ng/ml) with twice-weekly dosing and 64 ng/ml (range: <25 - 1519 ng/ml) with weekly dosing. Although the twice-weekly arm maintained trough concentrations above the target concentration nearly as well as with daily dosing, the protocol team has decided to use daily dosing in this protocol, as explained below in section 1.6.

1.5.2 Safety Results

Clinical Safety and Tolerance

All infants enrolled at both sites are included in the clinical safety and toxicity analyses. No severe (Grade 3 or higher) skin rash, hepatic or renal toxicity related to NVP were observed. Three infants died (2 during follow-up, 1 after the 32-week visit). The causes of death included early-onset neonatal sepsis (1 infant, HIV-uninfected) and pneumonia (2, both HIV-infected with clinical evidence of AIDS). None of the deaths were attributable to study drug. The most frequent serious adverse clinical events (Grade 3 or 4 events) unrelated to study treatment were sepsis, pneumonia, meningitis, gastroenteritis, bronchiolitis, AIDS-defining conditions and congenital defects (1 polydactyly, 1 atrial septal defect). Two infants (both HIV-infected) developed hepatomegaly and 2 infants (both HIV-infected) developed splenomegaly; these events were deemed unrelated to study drug.

Laboratory abnormalities

Analysis of laboratory abnormalities was performed only for the 36 infants enrolled at the Harare site due to deficiencies in sample collection and processing present at the Durban site. The most frequent serious laboratory abnormality was neutropenia (Grade 3 or higher) noted in 8 infants. Grade 3 or higher neutropenia was reported in 4 infants in the once-weekly arm, 4 in the twice-weekly arm, and none in the daily arm. Seven of the 8 infants were asymptomatic. Faulty machine calibration at this laboratory site may have accounted for some of the reported neutropenia abnormalities. One infant had a clinical event (presumed viral infection) temporally associated with the neutropenic episodes; the neutropenia was detected when symptoms of viral infection was noted. The neutropenia resolved in all 8 infants. In seven infants, the neutropenia was transient, and infants continued on NVP dosing through 24 weeks. In 1 infant with persistent neutropenia, the abnormality resolved after NVP was permanently discontinued. Grade 3 or higher anemia was observed in 2 infants. In both infants, anemia was judged to be possibly, but unlikely to be related to study drug. Two infants experienced Grade 3 or higher thrombocytopenia, unrelated to study drug; both infants were HIV-1-infected and the etiology was felt to be secondary to HIV infection. None of the enrolled infants had Grade 3 or higher elevations in serum transaminase values. Thus, NVP was deemed safe and well tolerated by the infants in this study.

1.6 Rationale for Infant Daily Dosing Regimen

Based on the results of HIVNET 023 (described in the section above), the daily dosing regimen of NVP was chosen for this protocol for the following reasons. First, infants in the daily arm had fewer reported toxicities than those in either the once- or twice weekly arms. Second, based on experience with therapeutic and prophylactic drug regimens, adherence with daily dosing is likely to be better than with twice-weekly dosing. Third, consequences, in terms of maintaining consistent NVP plasma concentrations, of an occasional missed dose with the daily regimen would be negligible compared to an occasional missed dose with the twice-weekly regimen. If one dose with the twice-weekly regimen is missed, then a baby's trough level is more likely to fall below the IC₅₀ compared with missing one daily dose. Finally, the NVP trough concentrations in babies in the daily dosing arm were on average more than twice as high as those in the twice a week dosing arm. While we do not know the precise protective level, this may be

very important for efficacy, as inhibitory NVP plasma concentrations must be consistently maintained during exposure.

To simplify infant NVP dosing in HIVNET 046, the level of NVP doses will be adjusted based on age rather than weight. This schedule was designed to administer an infant of average birth weight a daily dose of approximately 2 mg/kg during the first 2 weeks and 4 mg/kg thereafter. The chart below presents the NVP mg/kg doses that would be administered at the time of each HIVNET 046 visit to an infant of average weight as well as the lowest weight and heaviest infant if this schedule was used in infants with the same weight distribution as those in HIVNET 012 and 023 described in the table below.

Age	Dose (mg)	NVP: mg/kg		
		Average weight infants NVP mg/kg	Lowest weight infant NVP mg/kg	Heaviest infant NVP mg/kg
Birth	6	2.01	3.00	1.33
1 week	6	1.83	3.75	1.20
2 weeks	6	1.70	2.40	1.30
	15	4.26	6.00	3.26
6 weeks	15	3.25	6.00	2.27
	18	3.90	7.20	2.73
8 weeks	18	3.45	5.14	2.81
	20	3.84	5.71	3.13
10 weeks	22	4.03	6.88	2.75
12 weeks	No weight data available			
14 weeks	24	3.93	7.50	2.58
16 weeks	24	3.59	4.80	2.82
	26	3.89	5.20	3.06
20 weeks	26	3.57	5.31	2.95
	28	3.85	5.71	3.18
24 weeks	28	3.66	5.19	2.95

An infant weighing the same as an average weight infant in HIVNET 012/023 would receive a mean mg/kg NVP dose of 1.85 mg/kg (range: 1.70-2.01 mg/kg) during the first 2 weeks of life and 3.77 mg/kg (range: 3.25 to 4.26 mg/kg) from 2 weeks through 6 months of age. An infant with a weight equal to the lightest weight infant enrolled in HIVNET 012/023 would receive a mean mg/kg NVP dose of 3.05 mg/kg (range: 2.40 to 3.75 mg/kg) during the first 2 weeks of life and 5.89 mg/kg (range: 4.80 to 7.50 mg/kg) from 2 weeks through 6 months. An infant with a weight equal to the heaviest weight infant enrolled in HIVNET 012/023 would receive a mean mg/kg NVP dose of 1.28 mg/kg (range: 1.20 to 1.33 mg/kg) during the first 2 weeks of life and 2.87 mg/kg (range: 2.27 to 3.26 mg/kg) from 2 weeks through 6 months. The mg/kg NVP dose approved for treatment of HIV infected infants is 4 mg/kg once a day for the first 2 weeks of treatment followed by 7 mg/kg twice a day. The NVP doses in the above schedule will be administered once per day in HIVNET 046. An infant weighing the same amount as the smallest HIVNET 012/023 infant would receive the largest mg/kg doses using the HIVNET 046 schedule. The total daily dose administered to these infants would be considerably less than the total daily dose approved for use in infected infants. An infant weighing as much as the heaviest HIVNET 012/023 infant would receive the smallest mg/kg NVP dose using the HIVNET 046 schedule. The daily dose arm of HIVNET 023 used mg/kg doses of 2 mg/kg for the first 2 weeks of life followed by 4 mg/kg from 2 weeks through 6 months. These doses produced NVP plasma concentrations well in excess of the therapeutic target of 100 ng/ml, so that the plasma

concentrations achieved with the age-adjusted dosing schedule should well exceed the therapeutic target in the heaviest infants in HIVNET 046.

2.0 STUDY DESIGN

This is a Phase III, multi-site, randomized, double blind, placebo-controlled trial to determine the efficacy and safety of an extended NVP regimen provided for 6 months or through cessation of breastfeeding, whichever is earliest, compared to placebo, for prevention of MTCT in breastfeeding infants who are born to HIV-infected women.

At all study sites, the HIVNET 012 intrapartum/neonatal two-dose regimen of NVP is standard of care for prevention of MTCT. All pregnant HIV-infected women presenting at antenatal clinics at these sites are offered this regimen. Eligible infants will be randomized on or before day 3 post birth to receive either the extended active agent regimen of NVP suspension or placebo for the first 6 months of life or through cessation of breastfeeding (whichever is earliest). Cessation of breastfeeding is defined as completely stopping all exposure to breast milk. Randomized infants that are determined to be HIV-infected while on study treatment will be taken off study treatment but will remain in follow-up.

It is anticipated that most women will receive intrapartum single dose NVP, but some women may miss the intrapartum dose (e.g. due to precipitous delivery). It is also anticipated that antiretroviral therapy may become available at some sites for treatment of HIV-infected pregnant women during the conduct of this study. Therefore, since some women may receive intrapartum NVP and/or another antiretroviral regimen for prevention of MTCT (MTCT prophylaxis) and/or some may receive antenatal antiretroviral therapy for treatment of HIV, and some may receive neither, randomization will be stratified by site with 3 levels based on maternal therapy during this pregnancy: MTCT prophylaxis; antenatal antiretroviral therapy; or neither. In the event that a mother received both antenatal antiretroviral therapy for treatment and MTCT prophylaxis, the infant will be assigned to the antiretroviral group.

A total of 1576 breastfeeding infants born to HIV-infected eligible mothers will be randomized to one of the two study arms in a 1:1 ratio. Randomized infants and their mothers will be followed for 18 months.

2.1 Maternal Screening, Enrollment and Follow Up

At all study sites, as part of standard of care, pregnant women will be offered HIV counseling and testing and women determined to be HIV-infected will be provided the HIVNET 012 intrapartum/neonatal 2-dose NVP prophylaxis regimen for prevention of MTCT.

Also, HIV-infected pregnant women will receive general counseling on breastfeeding and safe alternatives from designated infant feeding counselors. These counselors will undergo training consistent with WHO and local Ministry of Health (MOH) guidelines. The training will focus on providing accurate information and balanced options on infant feeding choices that are acceptable, feasible, affordable, safe, and sustainable for each individual. Only HIV-infected women who, after thorough counseling, clearly wish to breastfeed will be referred to the study counselors to receive information regarding the study. Someone other than the designated infant feeding counselor will administer the informed consent process. Women who choose to breastfeed will be advised to breastfeed exclusively because this has been shown to be the optimal type of breastfeeding for child growth and development, and it is recommended by WHO, UNICEF, and UNAIDS.

Described below are the required clinical and laboratory procedures for mothers; see also Appendix I A for a schedule of maternal evaluations. HIV-infected women who provide informed consent may be screened for the study at any time during the third trimester of pregnancy or on or before day 3 after birth. Women screened prior to delivery will have medical history and physical exam documented for study purposes and will undergo one blood draw for CBC with differential, CD4+ cell count, stored plasma for later NVP resistance, HIV-1 RNA PCR testing. At labor and delivery (on or before day 3 post delivery) blood will be drawn for CBC with differential, CD4+ cell count, and plasma (storage for NVP resistance and HIV-1 RNA PCR testing). Post-delivery, enrolled mothers will be seen at 2 and 6 weeks and 3, 6, 12, and 18 months. Medical history and physical examinations, as outlined in the Study Specific Procedures (SSP) manual, will be conducted at every follow-up visit. Maternal blood for CBC with differential, CD4+ cell count, and plasma storage for NVP resistance and HIV-1 RNA PCR testing will be drawn at 2 and 6 weeks and 3, 6 and 12 months. At 18 months, a plasma sample will be stored for NVP resistance testing.

Weaning will be encouraged at 6 months; however, the timing of breastfeeding cessation will be determined by the mother and is likely to depend on a number of economic and social factors. Independent counselors that are not involved directly with the study procedures will be available to women throughout the study to offer continuous support on the feeding choice to the women. Throughout the study, breastfeeding status and practices will be assessed by interview. Breast milk will be collected and stored at 2 and 6 weeks and 3, 6 and 12 months. The breast milk sample will only be collected from women who are still breastfeeding at each timepoint, despite counseling regarding early cessation of breastfeeding at 6 months. These samples may be tested at a later date for NVP resistance and HIV-1 RNA PCR copy number.

Mothers will be instructed to begin administration of the study drug to the infant 5 days after birth (± 2 days). Adherence to the infant dosing regimen will be assessed at each maternal/infant visit by interview. (In addition, adherence will be assessed by measurement of NVP concentration levels in stored plasma samples from infants who become infected and from a sample of uninfected infants.)

See section 9.3 for a description of the clinical care that will be provided to mothers.

2.2 Infant Randomization and Follow Up

Randomization of eligible infants will take place on or before day 3 post birth. See section 4.2 for randomization criteria. Study drug will be dispensed to the mothers prior to discharge with the instruction to have the infant swallow the first dose of study drug 5 days after birth (± 2 days). See section 6.2 for treatment dose and administration procedures.

Described below are the required clinical and laboratory procedures for infants; see also Appendix I B for a schedule of infant evaluations. Physical examinations and medical histories, as outlined in the SSP manual, will be conducted on infants at 2, 4, 6, and 8 weeks and 3, 4, 5, 6, 9, 12, and 18 months. Blood samples for the following assays will be drawn at birth, 2, and 6 weeks and 3, 6 and 12 months: CBC with differential, alanine aminotransferase (ALT) (up to 6 months only), and HIV-1 DNA PCR. CD4+ cell counts will be done on infants with confirmed HIV infection at 2 and 6 weeks and 3, 6, 12 and 18 months. At 9 months, blood will be drawn for HIV-1 DNA PCR. At 18 months, an HIV EIA assay will be done. At birth, 2 and 6 weeks and 3, 6, 12 and 18 months plasma will be stored for retrospective evaluations of NVP resistance, HIV-1 RNA PCR copy number and NVP concentrations in infants determined to be HIV-infected. A sample of HIV uninfected infants will also be retrospectively tested for NVP concentrations.

The HIV-1 DNA PCR assay will be run at the sites in real time. Any infant with a positive virologic assay (either positive HIV-1 DNA PCR or EIA at 18 months) will have a repeat assay done on another specimen to confirm infection status. Infants identified as HIV-infected will be taken off of study treatment and remain in follow-up and undergo all scheduled assessments with the exception of adherence and HIV DNA PCR or EIA. See section 9.3 for a description of the clinical care that will be provided to HIV-infected infants. Evaluation of clinical, immunologic and virologic disease progression in infected infants will be based on physical examination, medical history, CD4+ cell counts and HIV-1 RNA PCR. CBC with differential will be drawn on HIV-infected infants at 18 months of age.

Note: Infant blood amounts are expected to be limited; therefore priorities for laboratory assays will be specified in the SSP Manual.

2.3 Use of Roche Amplicor 1.5 HIV-1 DNA PCR Assay to Determine HIV Infection

The DNA test to be used for diagnosis of infant HIV infection is currently an unlicensed assay in development by Roche Diagnostic Systems. It uses the same primers that are now incorporated into the Amplicor HIV-1 Monitor assay version 1.5, which is the most current version of the RNA PCR assay that is in use to quantitate plasma RNA copy number. The currently available version 1.0 Amplicor DNA and FDA-licensed RNA PCR assays, which are highly sensitive for detection of subtype B HIV, are less sensitive for detecting some HIV non-B subtypes, including the subtypes commonly found in Africa (72-74). The Amplicor 1.5 RNA PCR assay has been shown in several studies to have increased sensitivity to non-subtype B strains, including those subtypes A, C and D that commonly occur in the HPTN 046 study sites (73,75,76). In a study of the Roche Amplicor version 1.0 and 1.5 DNA PCR assays in 106 HIV-infected and 55 seronegative Tanzanian pregnant women with HIV subtype A, C and D infection, the Amplicor version 1.5 had higher sensitivity for detection of HIV-1 DNA than the standard version 1.0 assay (99.1% versus 97%, respectively) (74).

Some studies have suggested that HIV-1 RNA assays may be more sensitive for diagnosis of infant HIV infection than DNA PCR (77,78). However, the studies have not evaluated the sensitivity of such tests in the presence of maternal antiretroviral therapy (in some sites, antiretroviral therapy may become available for treatment during the course of the study) or in the case of continued antiretroviral prophylaxis as will be studied in HPTN 046. It is known in antiretroviral treated individuals that although free virus as detected by HIV-1 RNA can become undetectable, cell-associated HIV as detected by HIV-1 DNA remains positive. Therefore, the HIV-1 Amplicor DNA 1.5 PCR assay will be used for diagnosis of HIV infection in infants in this study.

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

The primary objectives of this study are to:

1. Compare the rate of HIV infection at 6 months in infants determined to be HIV uninfected at birth in each arm.
2. Evaluate and compare the safety and tolerance in infants in each arm.

3.2 Secondary Objectives

The secondary objectives of this study are to:

1. Compare the proportion of infants who are alive and free of HIV at 6 months and 18 months of age in the two arms.
2. Compare the relative rates of HIV infection in infants over 18 months in the two arms.
3. Compare the infant survival rates (mortality regardless of HIV infection) over 18 months in the two arms.
4. Determine the frequency and duration of NVP-resistant HIV strains in maternal plasma and breast milk and the relationship with MTCT.
5. Determine the relationship of maternal plasma and breast milk RNA levels to risk of MTCT.
6. Determine the frequency, type, and duration of NVP-resistant HIV strains in the plasma of infants who become HIV-infected.
7. Compare the rates of HIV disease progression, as defined by CD4+ cell count, HIV-1 RNA copy number, and mortality between the two arms in infants who become HIV-infected.
8. Determine NVP concentrations in infants who become infected with HIV and a sample of HIV uninfected infants as an index of adherence to the NVP regimen.

4.0 STUDY POPULATION

This study targets enrollment of HIV-infected women and 1576 of their breastfeeding infants. HIV-infected pregnant women will be recruited from antenatal clinics at HPTN sites in Zimbabwe, South Africa, Uganda, and Tanzania.

4.1 Maternal Eligibility Criteria

Women must meet all of the following criteria to be eligible for the study:

- ≥ 18 years of age
- Willing and able to provide study informed consent
- Third trimester of pregnancy or on or before day 3 after delivery
- HIV-infected, as evidenced by 2 positive EIA's; or 1 positive EIA and 1 positive WB; or 2 separate rapid tests (WHO acceptable diagnostic HIV-1 infection criteria for adults)
- No serious current or previous complications of this pregnancy, as judged by the on-site clinician
- Free of active serious infection other than HIV or other serious illnesses, as judged by the on-site clinician
- Intend to breastfeed
- If not already delivered: Intend to deliver at a facility where the study is based

Note: Women who are receiving or have received antiretrovirals (including NVP) for treatment or for prevention of MTCT *are* eligible. Mothers will be considered enrolled in the study at the point of infant randomization. Mothers of infants who are not randomized will not be considered enrolled in the study.

4.2 Infant Randomization Criteria

Infants must be randomized on or before day 3 post delivery. Infants must meet the following criteria for randomization:

- Born to an HIV-infected mother who is eligible and has consented to take part in this study
- Blood sample obtained for HIV-1 DNA PCR, CBC with differential and ALT (Note: Results of these laboratory tests are not required prior to randomization or study drug dosing.)
- Birthweight of at least 2000 gm

Infants who meet any of the following criteria will be excluded from randomization:

- Not able to breastfeed (e.g. mother died or otherwise unable or unwilling to breastfeed despite original intentions)
- Skin rash grade 2B (urticaria), grade 3 or above
- Confirmed or suspected clinical hepatitis, defined as clinical signs and symptoms of clinical hepatic dysfunction including enlarged liver (>4 cm below right costal margin), hepatic tenderness, ascites, portal hypertension (e.g., varices, splenomegaly, caput medusae), or hepatic encephalopathy (e.g., asterixis, changes in level of consciousness)
- Serious illness or condition that would prohibit compliance with study procedures as judged by site clinician

Note: Other conditions listed in Section 6.2.1 are also exclusionary if known prior to randomization. Infants who are excluded prior to randomization for any reason and their mothers will not be considered enrolled in the study. Infants who are randomized and their mothers will remain in the study even if they do not initiate study drug dosing or discontinue dosing prematurely for any reason.

In the case of a multiple birth, infants will be included in the study only if both/all are eligible and will be randomized to the same study arm.

5.0 STUDY PROCEDURES

An overview of the study visit and procedures schedule is presented in Sections 2.0-2.2 and Appendix I A and B. Presented below is additional information on visit-specific study procedures.

5.1 Screening and Follow-up Maternal Evaluations

5.1.1 Maternal Eligibility Evaluations: third trimester of pregnancy/prior to labor and delivery

Clinical Evaluations and Instructions

- Documentation of HIV-infected status*
- Infant feeding options counseling
- Study informed consent
- Specimen storage consent (optional)
- Demographics
- Confirmation of intent to breastfeed
- Medical history
- Physical exam

*Note: If documented confirmation of the mother's HIV status (as evidenced by 2 positive EIA's; or 1 positive EIA, and 1 WB; or 2 separate rapid tests) is not available as part of standard of care at the study clinic, then confirmatory testing will be performed after study informed consent has been obtained and prior to enrollment (infant randomization).

Laboratory Evaluations

- Confirmatory HIV test (if needed)
- CBC with differential
- CD4+ cell count
- Plasma storage for HIV-1 RNA PCR and NVP resistance

Note: The specimen storage consent (see Appendix II B) is for storage and testing of samples that are not required by the study protocol. Women who choose to participate in the study do not have to provide consent for specimen storage to be enrolled. Storage of specimens not required by the study protocol is optional for study sites.

5.1.2 Maternal Labor and Delivery Evaluations: as close to delivery as possible but on or before day 3 after delivery

Note: For women who are screened after labor and delivery, the evaluations for the Screening and Labor/Delivery visits can be *combined into one visit*; duplicate blood work does need not to be done.

Clinical Evaluations and Instructions

- Infant feeding options counseling
- Medical history
- Physical exam
- Instructions for administration of infant study drug

Laboratory Evaluations

- CBC with differential

- CD4+ cell count
- Plasma storage for HIV-1 RNA PCR and NVP resistance

5.1.3 Maternal Follow-up Evaluations

Note: If the infant is not randomized for any reason, mothers will not be considered enrolled and maternal follow-up evaluations will not be carried out.

Clinical Evaluations and Instructions (2 and 6 weeks and 3, 6, 12, and 18 months)

- Interim medical history
- Symptom directed physical exam
- Infant feeding options counseling
- Instructions for administration of infant study drug (2 and 6 weeks and 3 months only or as long as the infant is receiving study drug)

Laboratory Evaluations (2 and 6 weeks and 3, 6, and 12 months)

- CBC with differential
- CD4+ cell count
- Plasma storage for HIV-1 RNA PCR and NVP resistance testing
- Breast milk storage (as long as the infant is breastfeeding or through 12 months, whichever is sooner.)

Laboratory Evaluations (18 months)

- Plasma storage for NVP resistance testing

5.2 **Infant Randomization and Follow-up Evaluations**

5.2.1 Infant Evaluations Prior to Enrollment/Randomization (on or before day 3 of life)

Clinical Evaluations

- Birth and neonatal history, including antenatal and or NVP exposure.
- History: general and potential drug reactions
- Physical examination
- Confirmation of mother's intent/ability to breastfeed

Laboratory Evaluations

- CBC with differential
- ALT
- Real-time Roche Amplicor HIV-1 DNA PCR testing
- Plasma storage for NVP resistance, HIV-1 RNA PCR, and NVP concentrations

5.2.2 Follow-up Infant Evaluations

Note: The following evaluations will be performed only on enrolled/randomized infants.

Clinical Evaluations (2, 4, 6, and 8 weeks, and 3, 4, 5, 6, 9, 12, and 18 months)

- History (general and potential drug reaction)
- Infant feeding practices assessment
- Physical examination
- Adherence assessment (maternal interview) (at 2,4,6 and 8 weeks, 3, 4, 5, and 6 months only)

Laboratory Evaluations (at 2 and 6 weeks and 3, 6, 12, and 18 months)

- CBC with differential (at 2 and 6 weeks and 3, 6, and 12 months in all infants and at 18 months in infants determined to be HIV-infected)
- ALT (at 2 and 6 weeks and 3, and 6 months only)
- Real-time Roche Amplicor HIV-1 DNA PCR testing (at 2 and 6 weeks and 3, 6, 9 and 12 months only.) If positive, repeat test on a second sample on or before the participant's next scheduled visit for confirmation, see section 8.6.1.
- Plasma storage for NVP resistance, HIV-1 RNA PCR and NVP concentrations (at 2 and 6 weeks and 3, 6, 12, and 18 month), See section 8.6.1
- HIV EIA (at 18 months only). If positive, confirm with a different EIA then first test or Western Blot on a second sample on or before the participant's next scheduled visit.
- CD4+ cell count (for infants with confirmed HIV infection only at 2 and 6 weeks and 3, 6 12, and 18 months)

Note: Should an infant become HIV infected at any time during the study, that infant will be taken off of study drug but will remain in follow-up. If study drug is discontinued early for any reason (e.g. HIV infection, cessation of breastfeeding), the randomized infant will remain in follow-up and undergo all scheduled evaluations as described in Section 5.4.

5.3 Maternal Evaluations in the Case of Early Withdrawal

All mothers completing the 18-month evaluation will have fulfilled the maternal clinical and laboratory evaluation requirements for the study. Enrolled mothers of randomized infants who discontinue the study prior to the 18-month evaluation will have the following clinical and laboratory evaluations performed, if possible, but will also be discontinued from the study:

- Interim medical history
- Symptom directed physical exam
- Plasma storage for NVP concentration and resistance

5.4 Infant Evaluations in the Case of Treatment Discontinuation or Study Withdrawal

All infants completing the 18-month evaluation schedule have fulfilled the infant clinical and laboratory evaluation requirements for the study.

Randomized infants off study drug/on study

All randomized infants who are either prematurely discontinued from study drug or who never initiate study drug will be considered *off study drug/on study* and will follow the same schedule of events as those infants who continue study treatment except adherence assessment. All of these infants will be followed through 18 months as scheduled.

Randomized infants prematurely discontinued from the study before the 6-month evaluation will have the following clinical and laboratory evaluations performed, if possible:

- History
- Physical exam
- Infant feeding practices assessment
- CBC with differential and platelet count
- ALT
- Roche Amplicor HIV-1 DNA PCR
- Plasma for storage (for NVP resistance, HIV-1 RNA PCR and NVP concentration)

Infants prematurely discontinued from the study at any time after the 6-month evaluation will have the following clinical and laboratory evaluations performed, if possible:

- History
- Physical exam
- Roche Amplicor HIV-1 DNA PCR
- Plasma for storage (for NVP resistance, HIV-1 RNA PCR and NVP concentration)

5.5 Participant Retention

Once an infant is randomized, the study site will make every reasonable effort to follow the infant and mother for the entire 18-month study period. (Eligible mothers and infants are considered enrolled in the study at the point of infant randomization.) It is projected that the rate of loss-to-follow-up on an annual basis will be at most 5% (see Section 8.3.1). Study site staff are responsible for developing and implementing local standard operating procedures to achieve this level of follow-up.

5.6 Participant Withdrawal

Participants may withdraw from the study for any reason at any time. The investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures after consultation with the Protocol Chair, National Institutes of Health (NIH) Medical Officers, Statistical and Data Management Center (SDMC) Protocol Statistician, and Coordinating and Operations Center (CORE) Protocol Specialist.

Participants also may be withdrawn if the study sponsor or government or regulatory authorities terminate the study prior to its planned end date.

Note: Early discontinuation of study product for any reason is not a reason for withdrawal from the study.

5.7 Toxicity Management in the Infant

Management of any adverse event will be according to the best clinical practice available and the judgment of the site investigator or designated clinician. If adverse events occur, study drug may be temporarily held or permanently discontinued. Clinical decision making for temporarily holding or permanently discontinuing study drug will be based on clinical judgment and on the severity grade of the AE observed as outlined in the Toxicity Management Table in Appendix IV.

The first 12-16 weeks of therapy with NVP is a critical period during which intensive monitoring of patients is necessary to detect potentially life-threatening hepatic events and skin reactions.

All randomized infants will complete the 18 months of follow-up as scheduled even if study drug is not started or is discontinued for any reason.

5.8 Infant Concomitant Medications

Infant systemic medications including only antibiotics, antiretrovirals, antifungals, and antimicrobicides used in infants will be recorded on applicable study case report forms through 8 weeks post dosing. Mothers' reported receipt of other antiretrovirals will also be assessed at each infant follow-up visit while breastfeeding to record infant exposure via breast milk.

6.0 STUDY TREATMENT/PRODUCT/INTERVENTION

6.1 Treatment Formulation and Content

NVP (supplied as 10 mg/ml oral suspension) and placebo will be provided by Boehringer Ingelheim Pharmaceuticals Inc. and is to be stored at the study site at 15°C - 30°C. Daily temperature monitoring is required to document storage conditions.

6.2 Treatment Dose and Administration

Infants who meet randomization criteria (see Section 4.2) will be randomized to one of two treatment arms described in the table below. The duration of study treatment will be for the first 6 months of life or until cessation of breastfeeding whichever is earliest. Cessation of breastfeeding is defined as completely ceasing all exposure to breast milk. Mothers will receive syringes and instructions for dosing the oral suspension to their infants. The study drug regimen will be based on infant age and administered using an oral syringe with calibrations to 0.2 ml. Infants determined to be HIV-infected will be taken off of study drug but will remain in study follow-up.

The infant study drug regimen is:

Treatment Arms	Infant Dosing Regimen
NVP Suspension (10 mg/ml) (n = 788)	0.6 ml (6 mg) once daily from 5 days after birth (\pm 2 days) to 2 weeks of age 1.5 ml (15 mg) once daily from 2 to 4 weeks of age 1.8 ml (18 mg) once daily from 4 to 6 weeks of age 2.0 ml (20 mg) once daily from 6 to 8 weeks of age 2.2 ml (22 mg) once daily from 8 weeks to 3 months of age 2.4 ml (24 mg) once daily from 3 to 4 months of age 2.6 ml (26 mg) once daily from 4 to 5 months of age 2.8 ml (28 mg) once daily from 5 to 6 months of age
NVP Placebo (n = 788)	0.6 ml once daily from 5 days after birth (\pm 2 days) to 2 weeks of age 1.5 ml once daily from 2 to 4 weeks of age 1.8 ml once daily from 4 to 6 weeks of age 2.0 ml once daily from 6 to 8 weeks of age 2.2 ml once daily from 8 weeks to 3 months of age 2.4 ml once daily from 3 to 4 months of age 2.6 ml once daily from 4 to 5 months of age 2.8 ml once daily from 5 to 6 months of age

Note: The study drug regimen does not include the two-dose intrapartum/neonatal HIVNET 012 Nevirapine regimen that is standard of care in the study sites.

6.2.1 Conditions for Exclusion from Initial Study Drug Dosing

See Section 7.0 and the DAIDS Standard Toxicity Tables for grading criteria. The Toxicity Tables may be accessed at the following website:

http://www.hptn.org/network_information/regulatory_resources.htm.

If any of the conditions bulleted below are known prior to randomization, the infant should not be randomized.

If any of the conditions bulleted below are known prior to dosing in an infant who has been randomized, the first dose of study drug should be withheld and the following procedures should be followed. If the mother has left the clinic with the study drug, study staff will attempt to contact her, and she will be instructed to withhold dosing and to return to the clinic for a repeat blood test and/or further clinical assessments.

- ◆ Abnormal lab results as specified below. Test should be repeated within 72 hours or as soon as possible. If the repeat lab test result does not show resolution to Grade 1 for ALT or Grade 2 for blood count parameters, as judged by the on-site clinician, the tests can be repeated. If repeated results do not show resolution of the abnormalities within 7 days of when the study drug was to be first initiated, then dosing should not be started. The infant is considered off drug, on study.
 - ALT from birth specimen is Grade 2 or higher.
 - Hemoglobin, absolute neutrophil count or platelet count from birth specimen is Grade 3 or higher
- ◆ Suspected clinical hepatitis, defined as clinical signs and symptoms of clinical hepatic dysfunction, regardless of ALT values, including enlarged liver (>4 cm below right costal margin), hepatic tenderness, ascites, portal hypertension (e.g., varices,

splenomegaly, caput medusae), or hepatic encephalopathy (e.g., asterixis, changes in level of consciousness).

If clinical hepatitis is confirmed, dosing should not be started. The infant is considered off drug, on study.

- ◆ Grade 2B rash (urticaria): Study drug should not be started. If rash resolves within 7 days of when study drug was to be first initiated, study drug can be started. If rash remains at Grade 2B or higher for more than 7 days, study drug should not be started and the infant is considered off drug, on study.
- ◆ Grade 3 or 4 skin rash: study drug should not be started and the infant is considered off drug on study.

Note: The occurrence of these baseline abnormalities is expected to be very rare based on data from HIVNET 012, the PETRA Study, and HIVNET 023.

6.2.2 Conditions for Exclusion from Subsequent Doses of Study Drug

Once an infant has initiated study drug, subsequent doses may be temporarily held or permanently discontinued if:

- Continued dosing is contraindicated according to the Toxicity Management Table in the Appendix IV
- Continued dosing is contraindicated for any reason, as judged by the on-site clinician
- Required concomitant use of rifampin or oral ketoconazole
- Infants are determined to be HIV infected
- Infant ceases to breastfeed (all exposure to breast milk is stopped)

Note: Infants determined to be HIV infected will have study drug permanently discontinued but will remain in follow-up as described in Section 5.4. They will also be referred for appropriate care; see Section 9.3.

6.3 **Treatment Supply and Accountability**

Nevirapine 10 mg/mL suspension will be provided by Boehringer Ingelheim Pharmaceuticals, Inc.

Study drugs and oral syringes will be supplied by the NIAID Clinical Research Products Management Center (CRPMC). The site pharmacist is required to maintain records of all study drugs received from the CRPMC and subsequently dispensed to study participants. All unused study drugs are to be held until the study is completed or terminated or until otherwise instructed by the sponsor. Specific instructions will be provided by the sponsor for return or destruction of the study products.

6.4 **Adherence Assessment**

Adherence to the infant dosing regimen will be assessed at every mother/infant visit by interview. Education about dosing will be reinforced at each visit. Whenever possible, remedies will be sought to facilitate dosing in those cases that non-adherence is suspected, for example with home visits and directly observed therapy by the study staff. Adherence will also be evaluated by

measurement of NVP concentrations in stored plasma samples obtained during study visits from those infants who become infected with HIV and in a sample of HIV uninfected infants.

7.0 SAFETY MONITORING AND ADVERSE EVENT REPORTING

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which may or may not have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. Study participants will be instructed to contact the study clinician to report any AEs their infants may experience.

A serious adverse event (SAE) is defined as any adverse experience occurring at any dose that results in any of the following outcomes: death, a life-threatening condition, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, or an important medical event that, based upon appropriate medical judgment, may jeopardize the patient or subject and may require intervention to prevent one of the outcomes listed above (21 CFR 312.32(a), April, 1998).

Information on all non-serious and serious AEs in infants through 8 months of life (8 weeks after maximum study dosing duration) - regardless of relatedness - will be recorded in the participant source records and on standard Datafax AE case report forms (CRFs) for entry into the study database. After 8 months of life, information on all concurrent illnesses will be recorded in the participant source records, but only SAEs will be reported on standard Datafax AE CRFs for entry into the study database. Throughout the entire 18 month follow-up period, SAEs that are judged by the on-site study clinician to be possibly, probably or definitely related to the study drug, or for which a relationship cannot be determined will also be reported on the standard DAIDS SAE Report Form and sent within three days of site awareness to the DAIDS Adverse Experience Reporting (AER) Office.

The following typical childhood illnesses will be recorded in participant source records and captured in the study database as interim medical history or physical examination findings, but will not be reported separately as adverse experiences: diaper rash, otitis media, and afebrile upper and lower respiratory tract infections including bronchiolitis. However, if one of these conditions results in death, it will be reported as an SAE according to the procedures outlined above.

The study drug in HPTN 046 is the daily NVP or NVP placebo regimen begun in infants at 5 (+2) days postpartum. Conditions or illnesses in infants occurring before randomization will be reported as pre-existing conditions, including congenital anomalies.

The study site Investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the study team if unexpected concerns arise. The HPTN SDMC will prepare routine clinical data reports (blinded to treatment assignment) for review by a Protocol Safety Review Team (PSRT) including the NIAID and National Institute of Child Health and Human Development (NICHD) Medical Officers, the Protocol Chair and the Protocol Statistician. The content, format and frequency of the clinical data reports will be agreed upon by the PSRT and the SDMC in advance of study implementation. The PSRT will convene via conference call routinely (approximately every month) or as needed throughout the study to review the data and discuss any potential safety concerns. In addition, the study will be monitored by the NIAID DSMB, as described in Section 8.7.

Information on all AEs included in the study database will be included in reports to applicable government and regulatory authorities. Site staff will report information on all AEs and SAEs to their

Institutional Review Board (IRB)/Ethics Committee (EC) in accordance with applicable regulations and local IRB/EC requirements.

Any exceptions to the reporting criteria or procedures outlined above must be approved by the sponsor and provided to the IRBs in advance of implementation.

7.1 Severity Grading

Severity of AEs will be graded according to the Standard DAIDS Toxicity Tables for infants <3 months and children >3 months of age (located at: http://www.hptn.org/network_information/regulatory_resources.htm) with the following exception:

Cutaneous/Skin Rash/Dermatitis AEs will be graded according to the DAIDS Supplemental Toxicity Table for Grading Severity of Cutaneous/Skin Rash/Dermatitis Adverse Experiences (Appendix III).

Any additional exceptions to the Standard DAIDS Toxicity Tables must be approved by the sponsor and provided to the IRBs in advance of implementation. When grading laboratory values, normal limits will be defined according to local institutional values for infants and children. Protocol-specified local laboratory results will be reported on standard Datafax local laboratory results CRFs for entry into the study database, and abnormalities will be graded. Lab abnormalities that are asymptomatic or not attributable to a clinical diagnosis will also be reported separately on a standard Datafax AE CRF if the severity grade is ≥ 3 .

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design

This is a Phase III, multi-site, randomized, double blind, placebo-controlled trial. The main purpose of the trial is to evaluate the efficacy and safety of an extended regimen of NVP provided for 6 months or through cessation of breastfeeding, whichever is earliest, compared to no study drug (standard of care only), for prevention of MTCT in HIV uninfected infants at birth who are born to HIV-infected women, and are breastfeeding.

8.2 Endpoints

8.2.1 Primary Endpoints

1. HIV infection at 6 months in infants determined to be HIV-uninfected at birth enrolled in each arm of the study, as determined by HIV-1 DNA PCR confirmed by a positive assay on a different specimen.
2. Frequency and severity of adverse reactions among participating infants.

8.2.2 Secondary Endpoints

1. Proportion of infants who are alive and free of HIV at 6 months and 18 months of age in the two arms.
2. Relative rates of HIV infection 18 months in the two arms.

3. Infant survival rates (mortality regardless of HIV infection) over 18 months in the two arms.
4. Frequency and duration of maternal plasma and breast milk NVP-resistant HIV strains and the relationship with HIV transmission.
5. Relationship between maternal plasma and breast milk RNA levels and the risk of MTCT.
6. Frequency and duration of NVP-resistant HIV strains in the plasma of HIV-infected infants
7. Rates of disease progression as defined by CD4+ cell counts, HIV-1 RNA PCR and mortality in the infected infants in the two arms.
8. NVP concentrations in infants determined to be HIV-infected and sample of HIV-uninfected infants.

8.3 Accrual, Follow-up, and Sample Size

8.3.1 Power Calculations for Monitoring the Primary Efficacy Outcome

The primary efficacy endpoint in this trial is the rate of HIV-1 infection at 6 months, in infants determined to be HIV-uninfected at birth. Samples for HIV DNA PCR testing will be drawn from infants on or before day 3 postpartum and results will be available in real-time. Infants determined to HIV-infected at birth will be excluded from the analysis of the primary and secondary endpoints. Importantly, these samples to address possible exclusion of mother/infant pairs will be obtained before randomization and before any study doses are delivered, thereby removing the risk that these exclusions would introduce bias between the intervention groups. All statistical analyses to be discussed hereafter will be applied to the cohort of mother/infant pairs in which the infants have been determined to be HIV-uninfected at birth.

To ensure high quality study results, intensive follow-up procedures will be implemented. It is projected that the rate of loss-to-follow-up on an annual basis will be, at most, 5% (based on experience in HIVNET 012 and HPTN 023 trials). Although bias induced by loss-to-follow-up cannot be eliminated by increases in sample size, the sample size calculations in this section have been adjusted upward to remove the increase in variability induced by the projected level of loss-to-follow-up.

Based on results from HIVNET 012, it is anticipated that the overall rate of HIV-infection in the control arm of this trial will be approximately 8% at three days post birth, and approximately 15% at six months of age. Therefore, in the control arm of this trial, the rate of HIV transmission through 6 months will be approximately 7%. A trial with 1450 mother/infant pairs (adjusting for a 5% loss-to-follow-up) will provide 90% power to detect a reduction in the rate of HIV infection in infants who are uninfected at birth from 7% to 3%, using a Pearson chi-square test statistic having a (one-sided) false positive error rate of 0.025.

Since this trial will need to enroll a cohort of 1450 pairs of mothers and infants where the infant is determined to be HIV uninfected at birth, the actual number of mother/infant pairs that will need to be randomized will be higher. Specifically, for example, if 92% of randomized infants are determined to be HIV uninfected in samples drawn on or before day 3 post birth, then 1576 mother/infant pairs will need to be randomized in the trial.

Total duration of the study will be approximately 3 to 3.5 years. It is estimated that the period to enroll the 1450 eligible mother/infant pairs (i.e., to randomize approximately 1576 mother/infant pairs) at four sites will be approximately 18 to 24 months; follow-up of mothers and infants will be through 18 months postpartum.

8.3.2 Power Calculations for Safety Monitoring

Based on results from HIVNET 012, prevention of MTCT should result in an improvement in 18-month survival from nearly 60% for infected infants to approximately 92.5% for uninfected infants. Hence a regimen that reduces MTCT by 4%, through that effect, should reduce the death rate by 18 months of age from approximately 10% to approximately 8.7%, corresponding to a reduction in the first 18 months of approximately 13 deaths per 1000 infants.

As a result, the surveillance of safety in these trials should be able to detect a regimen-induced increase in serious non-reversible safety risks that would be of that order of magnitude. A trial of 1450 mother/infant pairs would provide approximately 80% power (using the standard 2.5% false positive error rate) to detect the following increases in rates of such serious AEs in mother/infant pairs:

from 10/1000 to 30/1000, or
from 1/1000 to 11/1000

Therefore, with safety surveillance that will be conducted in the cohort of mother/infant pairs participating in the HPTN 046 trial, the calculations above indicate that this study will be adequately powered to identify (or rule out) any serious safety risks that would be of the order of magnitude of the clinical benefit that would be attained by the 4% reduction in MTCT.

8.4 Random Assignment and Stratification

Infants will be randomized on or before day 3 post birth. Randomization will be by site and employ permuted block algorithms with randomized block size. These procedures will be coordinated by the HPTN Statistical and Data Management Center.

It is anticipated that most mothers will have received at least intrapartum NVP, and yet some may not have due to precipitous delivery or other reasons. Likewise, it is anticipated that most infants will have received a single dose of nevirapine within 72 hours of birth as standard of care. To maximize the generalizability of the trial conclusions, maternal use of any other antiretrovirals as ongoing therapy is not an exclusion criterion for enrollment. Because it is possible that the use of such therapy could substantially decrease the rate of MTCT, there will be a balanced randomization across all study sites that is stratified by 3 levels of maternal antiretroviral exposure at the time of randomization: mothers receiving ART for treatment of HIV; mothers receiving antiretroviral regimen (e.g. intrapartum NVP, short course AZT) for prevention of MTCT; or neither. (If a mother received ART and an antiretroviral regimen for prevention of MTCT, the default will be to the ART group).

8.5 Blinding

Individual study drug kits will be prepared and labeled by the DAIDS CRPMC according to the randomization assignments determined by the HPTN Statistical Center and then sent to the study sites. Randomization and blinding procedures will be coordinated with the Statistical Center and detailed in the study specific procedures manual. Randomized infants will not be unblinded to their randomization assignment unless there is clear justification that such information is required to make decisions about care for that infant.

8.6 Data Analysis

8.6.1 Primary Analyses

To account for incomplete follow-up information as well as the stratification at randomization, the primary analysis of the primary endpoint, HIV infection at 6 months, will be based on the difference between intervention groups in Kaplan-Meier estimates of percent free of HIV infection at 6 months, stratified by maternal use of antiretrovirals, and with variances estimated by Greenwood's formula.

The definitions below will be used to determine HIV infection in infants.

Definition of in utero HIV infection.

Infants will have blood drawn for HIV testing on or before day 3 post birth for HIV DNA assay. If negative, the infant will be considered to be HIV uninfected at birth (41). If the initial HIV DNA PCR is positive, then the infant will be considered to have been infected *in utero* and any data collected will be excluded from the primary and secondary analyses of efficacy and safety. However, a second specimen will be obtained and tested, by HIV DNA PCR for confirmation.

Definition of HIV infection other than through in utero transmission.

An infant less than 15 months of age will be considered to be HIV infected if two separate peripheral blood specimens drawn on different days are each positive by HIV DNA PCR.

Infants older than 15 months of age who are reactive for HIV-1 antibody by two different EIAs or HIV-1 Western Blot will be considered to be HIV-1 infected.

8.6.2 Secondary Analyses

1. *Proportion of infants who are alive and free of HIV at 6 months and at 18 months of age in the two arms.*

Based on data from HIVNET 012, approximately 9% of infants on the control regimen will have HIV infection or will die by 6 months of age. If the extended NVP regimen reduces this risk from 9% to 5%, this benefit will be detected with 80%

power using a chi-square test statistic having the standard (one-sided) false positive error rate of 0.025.

In turn, approximately 12% of infants on the control regimen will have HIV infection or will die by 18 months of age. If the extended NVP regimen reduces this risk from 12% to 8%, this benefit will be detected with 70% power using a chi-square test having the standard (one-sided) false positive error rate of 0.025.

2. *Relative rates of HIV infection at 18 months in the two arms.*

Based on data from HIVNET 012, approximately 8% of infants on the control regimen are expected to become infected with HIV by 18 months of age. If the extended NVP regimen reduces this risk from 8% to 4%, this benefit will be detected with 85% power using a chi-square test statistic having the standard (one-sided) false positive error rate of 0.025.

Note: Infants will be censored at infection.

3. *Infant survival rates (mortality regardless of HIV infection) over 18 months in the two arms.*

Based on data from HIVNET 012, approximately 9% of infants on the control regimen are expected to die by 18 months of age. Furthermore, the 18-month death rates in that trial were 7.5% for uninfected infants and were greater than 40% for infected infants. If the extended NVP regimen reduces the 18-month HIV infection rate by 4%, then the 18-month death rate should be reduced from approximately 10% to 8.7%, mediated through this effect on reduced risk of HIV infection.

HPTN 046 will estimate the actual level of reduction in 18-month death rates between the extended NVP and control regimens. Since the standard error for this estimated difference will be approximately 1.5%, this study is not powered to conclusively address the effect of the extended NVP regimen on 18-month mortality.

4. *Frequency and duration of maternal plasma and breast milk NVP-resistant HIV strains and the relationship with HIV transmission*

4A. Frequency of NVP resistance (NVPR) HIV in maternal plasma

Based on results from 111 women in the NVP arm of HIVNET 012, approximately 19% of women who receive single dose NVP only (and no other antiretroviral therapy) will have detectable NVPR at 6-8 weeks post-partum.

The rate of resistance in HPTN 046 may be different than in HIVNET 012 for the following reasons:

- Some women may receive antiretroviral therapy other than single dose NVP before or after delivery.
- The rate of resistance may be influenced by subtype. Rates of NVPR in HIVNET 012 were higher for women with subtype D than A (10/35 vs. 6/50,

OR=2.93; (71)). There is no information for other subtypes (e.g, C), which will be prevalent in this study.

- In HIVNET 012, NVPR was associated with higher baseline viral load and lower CD4+ cell count. These variables may be different in HPTN 046 (different stage of disease).

The focus of this analysis will be women who received only the 012 regimen. It is likely that the number of women exposed to different antiretroviral regimens will not be sufficient for an informative statistical analysis of resistance rates with these different antiretroviral regimens. To allow a comparison with data for HIVNET 012, analyses will first be completed on samples collected 6-8 weeks post-partum. It may also be of interest to assess the rate of resistance at 2 weeks, which might be higher, if some samples fade between 2-8 weeks.

Subtypes can be determined from analysis of the sequence data. Comparison of the rates of NVPR among women with different subtypes (e.g. A, D, C) is not a stated objective of the study. However, data from the resistance analysis may be sufficient to address this, depending on the proportions of subtypes prevalent at each site. In such comparisons by subtype, adjustments would be made for covariates such as baseline viral load, baseline CD4+ cell count, and study site.

4B. Duration of NVPR HIV in maternal plasma

Based on results from HIVNET 012, it is expected that most NVPR detected at 6-8 weeks will fade within 12-24 months. However, those data were available on only 11 evaluable women, many of whom did not have samples collected between 6-14 weeks and 24 months. In HPTN 046, it is planned to collect samples from all women through 18 months post-partum. For women with NVPR at 6-8 weeks (among those tested), samples from later time points will be analyzed to determine when NVPR fades from detection. The number of women available for this analysis, of course, will depend on the NVPR rate. Covariates such as viral load, CD4+ cell count, and receipt of other antiretroviral drugs will be studied to determine whether they are associated with fading.

4C. Frequency, type and duration of NVPR HIV in breast milk

Breast milk was not analyzed for NVPR in HIVNET 012, so it is unclear what rate of detection and duration of NVPR in breast milk should be expected in HPTN 046. The breast milk from 2 and 6 weeks post-partum will be examined first. If NVPR is detected in breast milk, subsequent samples from those women will be analyzed. The association of NVPR in breast milk with maternal baseline viral load and CD4+ cell count, and with breast milk RNA levels will be explored.

4D. Relationship of maternal plasma and breast milk NVPR with transmission

In this trial, resistance testing will be performed on all women with infected infants. Based on calculations in secondary endpoint analysis in section 6A (below), it is estimated that there will be approximately 103 such mother/infant pairs in the control arm and 76 pairs in the extended NVP arm of this trial. In addition, resistance testing

will be performed on at least 100 randomly selected women in each arm who did not transmit.

As noted in 4A, approximately 19% of women who receive single dose NVP only (and no other antiretroviral therapy) will have detectable NVPR in plasma at 6-8 weeks post-partum. The rates of NVPR will be compared between the transmitters and non-transmitters, with adjustments made for covariates such as viral load and CD4+ cell count, study arm, and study site. Again, the main analysis will focus on women who received only the HIVNET 012 regimen. The analysis will consider separately those transmissions at birth versus later transmissions by breastfeeding.

Similar analyses to those discussed in the previous paragraph for maternal plasma will be conducted to address the relationship of maternal breast milk NVPR and transmission. There are no data from HIVNET 012 to project the rate of NVPR in breastmilk.

5. *Relationship between maternal plasma and breast milk RNA levels and the risk of MTCT.*

Association of maternal plasma RNA and MTCT should be similar to that observed in HIVNET 012 and other studies. However, the HPTN 046 trial should provide important additional insights into the association of breastmilk RNA levels and transmission.

6. *Type, frequency and duration of NVP-resistant HIV strains in the plasma of HIV-infected infants*

6A. Determine the type and frequency of NVPR HIV strains in the plasma of infants who become HIV-infected.

With 725 mother/infant pairs in each arm, and with a loss-to-follow-up rate of at most 5%, the HPTN 046 trial should have approximately 689 mother-infant pairs in each arm.

In the control arm: With an estimated HIV transmission rate of 8% at birth, and an additional 7% by 6 months, it is expected in the control arm that there will be approximately 55 infants infected at birth and 48 infected after birth. Based on HIVNET 012, approximately 50% of the 55 infants infected at birth (n=27) will have NVPR at 6-8 weeks. Among infants infected after birth, some may acquire NVPR directly from NVP dosing, and some will have a NVPR strain transmitted from the mother by breastfeeding. In HIVNET 012, there were only 9 evaluable infants in the NVP arm with late HIV infection; 1 had NVPR. It is estimated that approximately 1/9 of these 48 infants (n=5) will have NVPR.

In the extended NVP arm: With an estimated HIV transmission rate of 8% at birth and an additional 3% by 6 months, it is expected in the extended NVP arm that there will be approximately 55 infants infected at birth and 21 infected after birth. In infants infected at birth whose mothers elect to drop out of the study, it is expected the NVPR rate will be approximately 50%. In infants infected at birth whose mothers elect to stay on study, it is expected that most or all will develop NVPR by

6-8 weeks, with continued NVP dosing. Finally, in infants infected after birth, it is expected that most or all will develop NVPR by 6-8 weeks, with continued NVP dosing.

6B. Determine the duration of NVP-resistant HIV strains in the plasma of infants who become HIV-infected

The duration of NVPR in the plasma of infected infants will be estimated. Based on HIVNET 012, it is expected that most infants in the control arm will have NVPR fade by 12 months (approximately half of those by 14-16 weeks). However, data in HIVNET 012 were available for only 7 evaluable infants. The time of fading may be different in the extended NVP arm, since more fit NVPR variants may be selected by continued NVP dosing.

7. *Rates of disease progression as defined by CD4+ cell counts, HIV RNA PCR and mortality in the infected infants in the two arms.*

For each intervention group, the cohort of infected infants will be followed for each of several measures of disease progression. Descriptive summaries of these disease progression measures will be provided. However, these data will not be used to make formal comparisons between these two groups of infected infants, since any differences found could well be due to important intrinsic differences between these two non-randomized cohorts rather than to the effect of the extended NVP regimen.

8. *NVP concentrations in infants determined to be HIV-infected and a sample of HIV uninfected infants.*

NVP concentrations will be measured in stored plasma samples obtained during study visits from those infants who become infected with HIV and a sample of uninfected infants. These concentrations will be compared to simulated concentration-time plots derived from a population pharmacokinetic analysis of the HIVNET 023 data. Infants whose concentrations fall more than 2 standard deviations from the mean of the HIVNET 023 simulated concentration-time plots will be presumed to have not adhered to the dosing regimen. Rates of adherence in infected and uninfected infants will be compared.

8.7 Data and Safety Monitoring Procedures

The duration of the study will be approximately 3.5 years. Accrual will require approximately 18 to 24 months. The primary efficacy assessments will be performed when infants are 6 months of age, and extended efficacy and safety of mothers and infants will be assessed through 18 months postpartum.

The HPTN Study Monitoring Committee (SMC) will monitor study regularly with a focus on issues relating to quality of trial conduct, such as overall and site-specific rates of recruitment, adherence to study interventions and visit schedules, and retention. A Protocol Safety Review Team including the NIH Medical Officers, the Study Chair and other members of the protocol team will closely monitor clinical study data on a routine basis.

The study will also be monitored by the NIAID DSMB. Administrative and safety data will be reviewed approximately every four to eight months during the first eighteen months of the trial.

Primary and secondary endpoint data as well as safety data will be monitored at least annually thereafter.

For interim analyses of efficacy data, the symmetric O'Brien-Fleming group sequential design will be used. It is expected that two formal interim analyses will be conducted at intervals of eight months after the first twelve months of the trial. This symmetric boundary not only provides a guideline for early termination recommendations when interim data are strongly positive, but also guides recommendations for early termination when interim data are sufficiently unfavorable to rule out targeted levels of beneficial effects on the rate of HIV infection.

The NIAID DSMB also could provide a recommendation to terminate or alter the design or conduct of the trial if unacceptable safety results emerge. While the reviews of safety will consider both expected or unexpected adverse events, particular focus will be given to monitoring occurrence of grade 4 hepatitis and serious (e.g., Stevens Johnsons) rash, and the relative rates of death. The DSMB will also give careful consideration to emerging evidence about safety and toxicity in relation to infant weight within age strata. The multiplicity of safety measures precludes the development of formal statistical monitoring procedures to guide recommendations about termination. If significant safety concerns emerge, the DSMB will have full access to relevant efficacy and safety data to assess the relative benefit-to-risk profiles of the study regimens when developing their recommendations.

9.0 HUMAN SUBJECTS CONSIDERATIONS

9.1 Ethical Review

This protocol and the template informed consent forms contained in Appendix II A and B will be reviewed and approved the sponsor and the applicable IRBs/ECs with respect to scientific content and compliance with applicable research and human subjects regulations. All informed consent forms used by the site (local language and English versions) must be approved by the responsible IRBs/ECs prior to consenting any study subjects.

The protocol, site-specific informed consent forms (local language and English versions), participant education and recruitment materials, and other requested documents — and any subsequent modifications — also will be reviewed and approved by the ethical review bodies (IRBs/ECs) responsible for oversight of research conducted at the study site prior to implementation.

Subsequent to initial review and approval, the responsible local Institutional Review Boards/Ethical Committees (IRBs/ECs) will review the protocol at least annually. The Investigator will make safety and progress reports to the IRBs/ECs at least annually, and within three months of study termination or completion at his/her site. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, all unanticipated problems involving risks to human subjects or others, and summaries of each DSMB review of safety and/or efficacy.

9.2 Informed Consent

Informed consent will be obtained from each study participant (or the parent or legal guardian of participants who cannot consent for themselves) before any study specific procedures are performed. Each study site is responsible for developing a study enrollment informed consent

form for local use, based on the template in Appendix II A, which describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. The study site also is responsible for translating the template form into local languages, and verifying the accuracy of the translation by performing an independent back-translation. The specimen storage consent (see Appendix II B) is for storage and testing of samples that are not required by the study protocol. Women who choose to participate in the study do not have to provide consent for specimen storage to be enrolled. Storage of specimens not required by the study protocol is optional for study sites. All informed consent forms used by the site (local language and English versions) must be approved by the responsible IRBs/ECs prior to consenting any study subjects.

Participants (or their parent or legal guardian) will be offered a copy of the informed consent form(s). Study staff will document the informed consent process as described in the study-specific procedures manual. The informed consent form and discussion will outline the risks and benefits of participating in this protocol.

9.3 Access to HIV-Related Care

This study will enroll women who are infected with HIV. At all study sites HIV counseling and testing are provided as part of standard of care (external to the study). All HIV-infected participants – mothers and infants identified during the course of the study - will be referred to available sources of medical and psychosocial care.

Clinical care provided to HIV-infected mothers and infants may vary by site. At a minimum, mothers and infants will be offered a number of therapeutic benefits including free diagnosis and treatment for their infections, malaria, tuberculosis, and other illnesses. All infants determined to be HIV-infected, are offered bactrim prophylaxis to prevent pneumocystis pneumonia and bacterial infections. All study women or children who require admission to the hospital will receive close monitoring and follow-up. Mothers will be offered nutritional counseling, multivitamins, iron and folate. Each site will develop a plan for the provision of medical care and support to mothers that is consistent with host country standards and policies. Currently, at the sites there are no resources to provide ART for all the HIV infected children and women in the study. However, as programs for provision of ART and treatment trials are implemented in these settings, formal links will be established for rapid referral of both infected mothers and infants, as appropriate.

9.4 Incentives

Pending IRB/EC approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits. Site-specific reimbursement amounts will be specified in the study informed consent forms.

9.5 Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored securely in areas with access limited to study staff. To maintain participant confidentiality a coded number will identify all study specific laboratory specimens, reports, study data collection, process, and administrative forms. All study records that contain names or other personal identifiers will be stored separately from other study records. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate area with limited access.

A participant's study information will not be released without the written permission of the participant, except as necessary for monitoring by the NIAID and/or its contractors; Boehringer Ingelheim; representatives of the HPTN CORE, SDMC, and/or Central Laboratory (CL), the relevant IRBs/ECs, and/or other government and regulatory authorities.

9.6 Study Discontinuation

The study may be discontinued at any time by the sponsor (US NIAID), Boehringer Ingelheim Pharmaceuticals, the relevant IRBs/ECs or by in-country government or regulatory authorities.

10.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

10.1 Local Laboratory Specimens

The following tests will be done at the local laboratory (LL):

- ALT
- CBC with differential
- CD4+ cell count
- Roche Amplicor HIV-1 DNA PCR
- HIV-1 EIA
- HIV-1 Western blot

Each study site will adhere to standards of good laboratory practice, the HPTN Manual of Laboratory Operations; and local standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens to the LL. Specimen collection, testing, and storage at the LL will be documented using the HPTN Laboratory Data Management System (LDMS).

The Study Specific Procedures will outline in greater detail the requirements for obtaining and processing these samples.

10.2 Central Laboratory Specimens: Storage and Future Testing

As described in Section 5.0, the following types of specimens will be collected and stored locally. Samples that are identified by the HPTN Central Laboratory (CL) as needed for testing will be requested from the sites and shipped to the CL as follows:

- Plasma (maternal and infant) for determination of NVPR and HIV-1 RNA copy number.
- Plasma (infant) for determination of NVP concentration.
- Breast milk for determination of NVPR and HIV-1 RNA copy number

Each study site will adhere to standards of good laboratory practice and the HPTN Manual of Laboratory Operations for proper collection, processing, labeling, and transport of specimens for the CL. All specimens will be shipped in accordance with the International Air Transport Association (IATA) specimen shipping regulations. All shipments will be documented using the HPTN LDMS.

The Study Specific Procedures will outline in greater detail the requirements for obtaining these samples.

10.3 Quality Control and Quality Assurance Procedures

The HPTN CL has established a proficiency-testing program at each study site. CL staff also will conduct periodic visits to each site to assess the implementation of on-site laboratory quality control procedures, including proper maintenance of laboratory testing equipment, use of appropriate reagents, etc. CL staffs will follow-up directly with site staff to resolve any quality control or quality assurance problems identified through proficiency testing and/or on-site assessments.

Throughout the course of the study, plasma/serum samples from all HIV infected infants and an equal number of randomly selected uninfected infants will be retested by the Central Lab. In the event of false positive or false negative HIV result, which changes the endpoint infection status of the subject, a sample from the last visit from all subjects will be retested. In addition, 5% of women enrolled will be retested by the CL for HIV antibody in order to confirm HIV-1 infection. Site laboratory inspections will be done to check for adequate and appropriate collection, handling, storage and shipping of specimens, and general site lab QA.

The CL will inform site staff of the samples selected for quality assurance testing, and site staff will ship the selected specimens to the CL. All specimens will be shipped in accordance with the HPTN CL Manual of Operations (which will be kept at each site) and IATA specimen shipping regulations. All shipments will be documented using the HPTN LDMS.

The CL will test the specimens for HIV antibody and compare the results of their tests with the results obtained by the local labs. CL staff will follow-up directly with site staff to resolve any quality assurance problems identified through this process.

10.4 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the United States (U.S.) Centers for Disease Control and Prevention (the CDC recommendations are located at <http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>).

11.0 ADMINISTRATIVE PROCEDURES

11.1 Study Activation

Following ethical review and approval, study sites will submit required administrative documentation — as listed in the SSP manual — to the HPTN CORE. CORE staff will work with study site staff to complete “protocol registration” in accordance with DAIDS procedures. Included in this step will be CORE and DAIDS review of each site-specific study informed consent form, including translated and backtranslated versions.

Pending successful protocol registration and submission of all other required documents (e.g. local drug authority approval for import of study products), the CORE staff will issue a site-specific “study activation notice” to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site.

11.2 Study Coordination

This protocol will direct study implementation. In addition, a Study-Specific Procedures Manual will outline procedures for conducting study visits; data and forms processing; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations. The SSP will be submitted to the sponsor prior to implementation of the study and will be made available to the IRBs/ECs, the US FDA and other regulatory authorities upon request.

The study team and HPTN SDMC will develop study case report forms. Data will be transferred to the HPTN SDMC, entered, and cleaned using the DataFax data management system. Quality control reports and queries will be routinely sent back to the site for verification and resolution.

Close cooperation between the Study Investigators, NIAID and NICHD Medical Officers, Protocol Coordinator, Biostatistician, Data Managers, and other study team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. The study team will monitor rates of accrual, adherence, follow-up, and AE incidence closely. Representatives of the HPTN CORE and SDMC on a regular basis also will evaluate these rates. If necessary, a Protocol Clarification Team — comprised of the protocol co-chairs, NIH Medical Officers, HPTN CORE, SDMC and CL representatives — will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management, documentation, and information sharing.

11.3 Study Monitoring

On-site study monitoring will be performed in accordance with DAIDS policies. Study monitors will visit the site to:

- verify compliance with human subjects and other research regulations and guidelines;
- assess adherence to the study protocol, study-specific procedures manual, and local counseling practices; and
- confirm the quality and accuracy of information collected at the study site and entered into the study database.

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the HPTN CORE, SDMC, CL, NIAID, Boehringer Ingelheim, and US and in-country government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

11.4 Protocol Compliance

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chair and the study sponsor (DAIDS). All protocol amendments must be submitted to and approved by the relevant IRB(s)/EC(s) and the DAIDS Protocol Registration Office prior to implementing the amendment.

11.5 Investigator's Records

The Investigator will maintain and store in a secure manner complete, accurate, and current study records throughout the study. The Investigator will retain all study records for at least three years

after submission of the site's final Financial Status Report to DAIDS, which is due within 90 days after the end of the site's cooperative agreement with DAIDS, unless otherwise specified by DAIDS or the HPTN CORE. Study records include administrative documentation — including protocol registration documents and all reports and correspondence relating to the study — as well as documentation related to each participant screened for and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

11.6 Use of Information and Publications

Publication of the results of this study will be governed by HPTN policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to the HPTN Manuscript Review Committee, DAIDS, and Boehringer Ingelheim for review prior to submission.

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APPENDIX I A SCHEDULE OF MATERNAL EVALUATIONS

<i>Evaluations</i>	Screening	Labor / Delivery (on or before day 3 pp) ¹	2 wks pp	6 wks pp	3 mos pp	6 mos pp	12 mos pp	18 mos pp
	3rd trimester or on or before day 3 pp							
Documentation of HIV Status	X							
Confirmatory HIV test (if required)	X							
Study Informed Consent	X							
Demographics	X							
Confirmation of intent to breastfeed	X							
Medical history	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
Infant feeding options counseling	X	X	X	X	X	X	X	X
CD4+ cell count	X	X	X	X	X	X	X	
HIV-1 RNA PCR (stored plasma)	X	X	X	X	X	X	X	
NVP resistance (stored plasma)	X	X	X	X	X	X	X	X
CBC with differential	X	X	X	X	X	X	X	
NVP resistance and HIV-1 RNA (stored breast milk) ²			X	X	X	X	X	

wks=weeks, mos=months, pp=postpartum

¹ For women screened prior to labor and delivery only (on or before day 3 postpartum)

² Breast milk to be collected and stored as long as infant is breastfeeding from all mothers for future evaluation of NVP resistance and HIV-1 RNA copy number.

APPENDIX I B SCHEDULE OF INFANT EVALUATIONS

Evaluations	Enrollment/ Randomization (on or before day 3 post birth)	2 wks	4 wks	6 wks	8 wks	3 mos	4 mos	5 mos	6 mos	9 mos	12 mos	18 mos
Birth and neonatal medical history	X											
Confirmation of mother's intent/ability to breastfeed	X											
Infant feeding practices assessment		X	X	X	X	X	X	X	X	X	X	X
History (general, potential drug reaction)	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of adherence to infant study regimen		X	X	X	X	X	X	X	X			
CBC with differential	X	X		X		X			X		X	X ¹
CD4+ cell count ¹		X		X		X			X		X	X
ALT	X	X		X		X			X			
Roche Amplicor HIV-1 DNA PCR ²	X	X		X		X			X	X	X	
HIV EIA ³												X
Plasma Storage ⁴ NVP resistance HIV-1 RNA PCR NVP concentration	X	X		X		X			X		X	X

wks=weeks mos=months

NOTE: Infant blood amounts are expected to be limited; therefore priorities for laboratory assays will be specified in the SSP Manual.

¹ To be done on infants with confirmed HIV infection only

² If HIV-1 DNA PCR positive, confirm with a repeat HIV-1 DNA PCR on a second sample obtained on or before the participant's next scheduled visit.

³ If reactive, confirm with a different EIA or Western Blot on a second sample obtained on or before the participant's next scheduled visit.

⁴ Plasma is being obtained and stored on all infants, and will be used to evaluate NVP resistance, HIV-1 RNA copy number and NVP concentrations among those infants found to be HIV-infected. NVP concentration will also be evaluated on a sample of HIV-uninfected infants.

APPENDIX II A SAMPLE STUDY ENROLLMENT CONSENT FOR HPTN 046

HPTN 046: A Phase III trial to determine the efficacy and safety of an extended regimen of nevirapine in infants born to HIV-infected women to prevent vertical HIV transmission during breastfeeding, Version [X.X]

PRINCIPAL INVESTIGATOR: [Name and Contact Information for the site PI]

INTRODUCTION

You are being asked to take part in the research study named above. This is a study for women with HIV who plan to breastfeed their babies. HIV is the virus that causes AIDS. The person in charge of the study at this site is [insert name of PI]. This study is sponsored by the U.S. National Institutes of Health.

Before you decide whether or not to take part in this research study, you need to know the purpose, the possible risks and benefits to you and your baby and what will be expected of you and your baby. The study staff will discuss this with you. They will answer any questions that you have. After the study has been fully explained to you, you can decide whether or not you want to participate. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form or make your mark in front of someone. You will be offered a copy to keep.

Please note that:

- Your participation in this research is entirely voluntary;
- You may decide not to participate or to withdraw from the study at any time without losing the benefits of your and your baby's standard medical care;
- If you decide not to participate in this study, you can still join another research study later, if one is available and you qualify.

PURPOSE OF THE STUDY

There are two purposes for this research study. The first is to determine if giving a drug named nevirapine to babies may help prevent them from getting HIV during breastfeeding. The second is to make sure that nevirapine is safe for babies when given once a day for 6 months.

Mothers who have HIV can pass HIV to their babies before delivery, during delivery, and after delivery through breastfeeding. Other research studies have shown that giving one dose of nevirapine as a pill to mothers at the time of delivery and one dose to babies as a syrup soon after birth can cut the chances of passing HIV to babies during delivery by about half.

Every women with HIV who comes to the clinic is offered nevirapine to help prevent passing HIV to her baby during delivery. You will be offered this drug regardless of your decision to participate in this research study.

We do not know if nevirapine or any other drug given to the baby every day for 6 months is safe or can prevent a baby from getting infected with HIV while breastfeeding. This study will help find that out. One recent study has suggested that nevirapine and other similar anti-HIV drugs given to babies during breastfeeding may help to reduce the chance of a mother passing the HIV virus to her baby, but this has not be proven. Currently, the only certain way to prevent passing HIV through breastfeeding is not to breastfeed. As the counselors have discussed with you, there are health risks and benefits to both breastfeeding and not breastfeeding.

This study will include about 1600 mothers and infants in different countries in Africa. In this country, about 400 mothers and infants are expected to participate in the study.

PROCEDURES

If you agree to participate in this study and are eligible to participate, you will be part of the study from now until your baby is 18 months old. You and your baby will be asked to come back to the clinic about 11 times. At birth, your baby will be put into one of two study groups by chance (like tossing a coin). One group of babies will be given nevirapine syrup and the other group will be given a syrup called a placebo. The placebo syrup looks like nevirapine syrup but does not contain nevirapine or any other medicine. Neither you nor the staff at your clinic will know to which group your baby was assigned until the end of the study. Your baby has an equal chance of being in either group.

No matter whether you decide to participate in the study or which group your baby is in, staff at this clinic will offer you a single dose of nevirapine to take at the onset of labor and a single dose of nevirapine to be given to your baby within 72 hours of birth to decrease passing HIV during delivery. This dose of nevirapine does not prevent the chance of passing HIV to your baby through breastfeeding.

Mother's Procedures

Options for feeding your infant have been discussed with you and will be discussed with you throughout the course of the study. The study staff will discuss with you the risks and benefits of both breastfeeding and not breastfeeding. If you decide that you want to breastfeed your baby, you can take part in the study if you otherwise qualify.

Screening Procedures: If you agree to take part in this study and sign the informed consent form, we will first need to determine if you and your baby are eligible. This will include asking you some questions about your health and doing a physical examination. Some people may not be eligible for the study due to information learned during the screening. You will also be asked to give a blood sample (about 10 ml which is equal to about one teaspoon) to check your health and to be stored for later HIV-related tests. If your previous HIV test has not yet been confirmed by a second test, we will counsel you about this and use some of the blood sample you gave to do another HIV test. If so, you will need to come back to the clinic to find out the results of your HIV test and receive counseling. If the second HIV test is negative, you and your baby cannot participate in the study. If your second HIV test result is not negative but is also not positive, you will be offered additional HIV counseling and testing.

If you have not already delivered your baby, you will be instructed to come the clinic as soon as your labor contractions begin. You will come to this hospital or clinic to give birth to your baby. When you arrive, you will be asked questions about your health and be asked to give a blood sample (about 10 ml which equals about one tablespoon) to check your health and to be stored for later HIV-related tests. The study staff will inform you if you and your baby are eligible for the study.

Study Procedures: If you and your baby are eligible to participate in the study, you will be given study syrup in a bottle and instructions for how and when to give the syrup to your baby. You will be asked to give the study syrup to your baby to swallow once a day beginning five days after birth. Neither you nor the staff at your clinic will know whether your baby is swallowing the nevirapine syrup or the placebo syrup. The study syrup is given to the baby using a syringe that will be provided to you by the study staff. You may choose to ask someone in your household to help you remember to give the study syrup to your baby. The clinic and study staff and the home visitors will be aware that you are HIV infected, but they will tell no one. If you choose someone in your household to help you to remember to give the study syrup to your baby, you may or may not decide to tell this person that you are infected with HIV. That is your decision.

You will be asked to bring your baby back to the study clinic about eleven times during the 18 months of the study. These study visits will take place every other week until your baby is 2 months old. After that, the visits will be once a

month through 6 months and then every 3 months until 12 months. A final visit will be when your baby is 18 months old.

At some of the study visits, you will give a blood sample (about 15 ml, which is equal to about 1 tablespoon) to check your health. You will be asked to give a sample of breast milk (20 ml, which is less than 2 tablespoons), to be stored for later HIV-related tests. At all of the visits, you will have a physical exam and will be asked about your health. You will be given the results of all tests done during the study related to your health.

Baby's Procedures

At birth, your baby's health and weight will be checked to be sure that he or she is eligible for the study and a small amount of blood will be drawn (about 5 ml or one teaspoon). If you and your baby are eligible for the study, you will be asked to give your baby the study syrup every day for the first 6 months of life or as long as you are breastfeeding. If you choose to completely stop breastfeeding your baby before 6 months, you will also stop giving your baby the study syrup.

At some of the study visits your baby will have a small blood sample (about 5 ml, which equals one teaspoon) taken to check his or her health. You will be given the results of all tests performed during the study that are related to your baby's health. Some of the blood taken will be tested for HIV. Your baby will be tested for HIV about 7 times over the 18-months of the study. If one of the tests shows that your baby may be infected with HIV, a second test will be done to confirm this result. If your baby is found to be HIV infected, you will be informed as soon as possible. You will stop giving your baby the study syrup. However, you and your baby will remain in the study and be asked to return for all study visits as scheduled.

If your baby stops taking the study syrup for any reason (even after only one dose), you will be asked to remain in the study and return with your baby for all of the study visits as scheduled.

Weaning

You will be encouraged by the study staff to stop breastfeeding at the end of 6 months or earlier if you choose. Your baby's last dose of nevirapine will be given at 6-months or soon after you stop breastfeeding, whichever comes first. If you choose to continue breastfeeding after 6 months you will be given additional counseling on the risk of giving HIV to your baby by continued breastfeeding.

RISKS and/or DISCOMFORTS

A number of serious side effects have been associated with nevirapine used in adults and children for treatment of HIV. However, these side effects have only been reported with use of much higher doses of nevirapine than will be used in this study. These side effects include inflammation of the liver that in rare cases may lead to severe or life-threatening liver damage and death. An infant with liver disease may seem tired or sleepy, feed poorly, have pale stool, darkened urine, yellowing of the eyes or skin, tenderness of the liver, or abnormal tests of the liver. An infant with active hepatitis B or C infection or abnormal liver tests is at higher risk for worsening liver disease.

Rash is the most common side effect of nevirapine. The rash may be severe and has resulted in hospitalization but rarely in death. One of the risk factors for developing serious skin reactions includes failure to take the nevirapine properly.

Hypersensitivity reactions may occur and may be associated with rash, fever, muscle or joint tenderness, blisters, mouth lesions, facial swelling, red eyes and irritation of the eyes, general irritability, hepatitis, kidney problems and/or changes in white blood cell levels. These conditions have rarely been fatal.

If your infant develops symptoms of any of the serious side effects listed above, no matter how long he or she has been receiving study syrup, you must bring your infant to the study clinic immediately or contact the medical staff at the site. The study staff will examine your baby and advise you whether to stop giving the study syrup. If you or the study doctor decide to stop your infant's study syrup because of symptomatic hepatitis, hypersensitivity or severe skin reactions, you should not give your child the study syrup again. Other side effects include fever, headache and upset stomach.

In a study of babies who received one dose of nevirapine within 2-3 days after birth, no serious rashes or liver problems related to nevirapine were reported. The doses of nevirapine to be given in this study are much lower than those given for treatment of HIV. At these doses, we do not expect babies to experience bad effects from this drug. In a study of babies who received nevirapine every day, once a week or twice a week for up to 6 months while breast feeding, no serious rash, liver or kidney disease were reported. In that study of infants, nevirapine was found to be safe and well-tolerated. However, the number of babies that have received nevirapine for several months after birth is very small, so we do not know for sure. We will carefully monitor the effects of study syrup on the babies. At the time you and your baby are discharged from the hospital or clinic, you will receive instructions on what to do if you see any rash on you or your baby. It is very important that you come to the study clinic or tell the study doctor or nurse right away about any rash or other problems.

You may feel discomfort when blood is drawn and may feel dizzy or even faint. A bruise may form or swelling may occur where the needle goes in your arm. Your baby will feel discomfort when blood samples are taken. Redness, pain in the area or a bruise may form and swelling may occur where the needle goes into your baby's skin.

If your baby has the HIV virus in his or her body at birth, we do not know if giving your baby nevirapine during breastfeeding will make the infection better or worse. Studies in other countries show that adults and children who have the HIV virus and receive only nevirapine do not get any long-term problems or benefits. Nevirapine may prevent HIV infection, but is not good enough to treat HIV infection by itself. If your baby becomes infected with HIV during this study and is swallowing nevirapine, it may mean that nevirapine and other drugs like nevirapine will not work as well to treat HIV. This is because the HIV virus can adjust to the drug and become resistant to it. This means that drug may not be as useful as part of possible future HIV treatment for your baby. If your baby is found to be infected with HIV during the study, you will be advised to stop giving him or her the study syrup. However, it is likely that your baby will receive the study syrup before it is known that your infant is infected with HIV.

POTENTIAL BENEFITS

You and your baby may receive no benefit from this study. However, knowledge gained from this study may in the future help others remain HIV uninfected. You and your baby will receive information about your health from the study examinations and laboratory tests.

Being in this study may reduce the chances of your baby getting HIV, but no guarantee can be made. Your baby may be receiving the study syrup called placebo with no medicine in it. It is also unknown whether the study syrup with nevirapine will prevent your baby from getting HIV while breastfeeding.

REIMBURSEMENT

At each scheduled visit you will receive [insert site-specific amount of money] to pay for your transport costs.

NEW FINDINGS

You will be told of any new information learned during the course of the study that might cause you to change your mind about staying in the study.

REASONS WHY YOU OR YOU BABY MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You or your baby may be removed from the study without your consent for the following reasons:

- the study doctor or study staff decides that continuing in the study would be harmful to you or your baby;
- you are unable to keep appointments or take the study product as instructed;
- the study is cancelled by the sponsor (the US National Institutes of Health), the Ministry of Health in this country, [insert other relevant in-country authorities], or the pharmaceutical company supplying the nevirapine.
- The Institutional Review Board or Ethics Committee or Data Safety and Monitoring Board (DSMB) recommends that the study be stopped early. (A DSMB is an outside group of experts who monitor the study.)

If your baby discontinues the study drug early for any reason (even after only one dose), you and your baby will be asked to remain in the study for all of the study visits and assessments as scheduled. If you or your baby withdraw from the study early for any reason, you will be asked to undergo a final assessment including a physical examination and blood draw, if possible.

ALTERNATIVES TO PARTICIPATION

You do not have to be in this study if you do not want to. The only known way to prevent passing HIV from a mother to her baby during breastfeeding is not to breastfeed. The clinic and study staff will explain the risk and benefits of breastfeeding to you and about safe alternatives. You will be provided information about where formula may be obtained. If you decide not to participate in the study, you will not lose the benefits of your standard medical care.

The study staff will also tell you refer you to HIV treatment programs that may become available at this medical facility or in your area. You have a right to consider all options available to you and your baby.

COSTS TO YOU

There is no cost to you for participating in this study. All of study visits and tests and the study drugs for your baby will be provided free of charge.

CONFIDENTIALITY

Your and your baby's research records will be confidential to the extent permitted by law. You will be identified by a code. Personal information from your records will not be released without your written permission. You will not be personally identified in any publication about this study. Blood and breastmilk collected from mothers, and blood collected from babies as part of this research study will be stored for study tests done later. These specimens will be stored in containers that do not have names on them but rather a code to protect your and your baby's privacy. These samples will be used for tests that are not checking your baby's health. These tests are for learning more about HIV and nevirapine treatment.

Your records may be reviewed by the United States National Institutes of Health (the agency that sponsors this research), the study monitors, Boehringer Ingelheim (the manufacturer of nevirapine), and [insert name of site IRB].

RESEARCH-RELATED INJURY

Medical care will be provided for illness or injury directly related to this study at no cost to you. Care or appropriate referral will be provided for any illness or injury that occurs during the study that is not directly related to the study, but you may have to pay for this care. There are no plans to give you money if there is a research-related complication or injury.

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you ever have questions about this study or in case you are injured as a result of participation in this research study, you should contact (Name of local investigator or study clinician) at (telephone number and physical address). If you ever have questions about your rights as a research subject you may call (name and title of IRB member) at (telephone number and physical address).

SIGNATURE PAGE

HPTN 046: A Phase III trial to determine the efficacy and safety of an extended regimen of nevirapine in infants born to HIV-infected women to prevent vertical HIV transmission during breastfeeding, VERSION [X.X]

If you have read the informed consent or had it read and explained to you and understand the information, and if you voluntarily agree to join this study, please sign your name or make your mark below.

Mother's name [print] Mother's signature Date

Father's Name (print) Father's Signature or mark Date
(If he is reasonably available)

I observed the informed consent discussion and, to the best of my knowledge, the information provided was complete and accurate, the potential study participant understands the information provided, and she willingly agrees to take part.

Witness' name [print] Witness' signature Date

I have fully explained the purpose of this study, the procedures, the risk and benefits and other information provided in this consent form to the potential study participant. I have answered all of her questions. To the best of my knowledge, she understands the information provided and willingly agrees to participate in the study.

Investigator's or Designee's Name Investigator's or Designee's Signature Date
(print)

APPENDIX II B SAMPLE CONSENT FOR STORAGE AND FUTURE USE OF BLOOD SAMPLES HPTN 046

HPTN 046: A Phase III trial to determine the efficacy and safety of an extended regimen of nevirapine in infants born to HIV-infected women to prevent vertical HIV transmission during breastfeeding, Version [X.X]

Principal Investigators : [Insert name and contact information for the site PI]

INTRODUCTION

You have decided to take part in the study named above, which is sponsored by the US National Institutes of Health. While you are in this research study, there may be some blood and breastmilk samples taken from you and your child that might be useful for future research. You are being asked to agree to the storage of these samples. This consent form gives you information about the collection, storage and use of your samples. The study staff will talk with you about this information. Please ask if you have any questions. If you agree to the storage of your own and your child's samples, you will be asked to sign this consent form. You will get a copy to keep. You may participate in the follow-up study even if you do not agree to storage of your own and your child's specimens.

HOW WILL YOU GET THE SAMPLES FROM ME?

There will be NO ADDITIONAL samples taken from you for storage. After all the tests are done for this research study, there may be some left over samples of blood and breastmilk. If you agree, left over samples will be kept and used for future HIV-related research.

HOW WILL YOU USE MY SAMPLES?

Your own and your child's samples will only be used to look for additional evidence of infection with HIV or other agents, damage caused by infection, or your body's response to infection (such as examining cells, proteins, and other chemicals in your body). Tests may also include examining your genes (DNA), since they might affect your response to disease in important ways. Your genes might make you more or less susceptible to becoming infected, your responses to infection or to treatment stronger or weaker, or make HIV progress more rapidly or slowly. No other kinds of genetic test will be done by anyone on your stored specimens without first explaining the test to you and obtaining your permission.

The researchers do not plan to contact you with any results from tests done on the stored samples. This is because research tests are often done with experimental procedures, so the results from one research study are generally not useful for making decisions on managing your health. Should a rare situation come up in which the researchers decide that a specific test result would provide important information for your own or your child's health, the researchers will try to contact you. If you wish to be contacted with this type of test result, you must give the study doctor or nurse any change to your address and/or phone number.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by the sponsor of this study (the US National Institutes of Health) and a special committee at the researcher's institution (an Institutional Review Board).

HOW LONG WILL YOU KEEP MY SAMPLES?

There is no time limit on how long your samples will be stored.

HOW WILL MY SAMPLES BE STORED?

Your samples will be stored at special facilities that are designed to store samples safely and securely. The storage facilities are designed so that only approved researchers will have access to the samples. Some employees of the

storage facilities will need to have some access to your samples in order to store them and to keep track of where they are, but these people will not have information that directly identifies you.

DOES STORAGE OF MY SAMPLES BENEFIT ME?

There are no direct benefits to you. The benefit of doing research on stored samples includes learning more about HIV infection.

WHAT ARE THE RISKS?

There are few risks related to storing your samples. When tests are done on the stored samples there is a small but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems getting a job.

WHAT ABOUT CONFIDENTIALITY?

To keep your information private, your own and your child's samples will be labeled with a code that can only be traced back to this research clinic. Your personal information (name, address, phone number) will be protected by the research clinic. When researchers are given your stored samples to study they will not be given your personal information. The results of future tests will not be included in your health records or the health records of your child. Every effort will be made to keep your personal information confidential, but we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

WHAT ARE MY RIGHTS?

Allowing your own and your child's samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study.

If you decide now that your samples can be stored for future research, you may change your mind at any time. You must contact your study doctor or nurse and let them know that you do not want your samples used for future research. **Your samples will then not be used.**

WHAT DO I DO IF I HAVE QUESTIONS?

For questions about the storage of your samples, contact [insert name of site investigator] at [insert telephone number].

If you have questions about your own and your child's rights as research volunteers, contact [insert the name or title of person on the Institutional Review Board] at [insert telephone number].

SIGNATURE PAGE

HPTN 046: A Phase III trial to determine the efficacy and safety of an extended regimen of nevirapine in infants born to HIV-infected women to prevent vertical HIV transmission during breastfeeding, VERSION [X.X]

Please carefully read the statements below or have them read to you and think about your choice. No matter what you decide it will not affect your care.

I agree to have my own and my child's left over blood and breastmilk samples stored and tested for future research related to HIV infection.

_____ Yes

_____ No

Volunteer's Name (print)

Volunteer's Signature or mark

Date

Father's Name (print)
(If he is reasonably available)

Father's Signature or mark

Date

Witness' Name (print)
(as appropriate)

Witness' Signature

Date

I have explained the purpose of storing specimens from the study to the volunteer and have answered all of her questions. To the best of my knowledge, she understands the purpose, procedures, risks and benefits.

Printed name of Study Staff
Conducting Consent Discussion

Study Staff Signature

Date

APPENDIX III TOXICITY TABLE FOR CUTANEOUS/SKIN RASH/DERMATITIS

GRADE 1	GRADE 2	GRADE 3	GRADE 4
CUTANEOUS/SKIN RASH/DERMATITIS			
<p>Erythema, with or without pruritis</p>	<p>A. Diffuse erythematous macular or maculopapular cutaneous eruption or dry desquamation with or without pruritis; OR typical target lesions without blistering, vesicles, or ulcerations in the lesions.</p> <p>B. Urticaria</p>	<p>A. Diffuse erythematous macular or maculopapular cutaneous eruption or moist desquamation with or without pruritis together with any of the following constitutional findings considered related to study drug:</p> <ol style="list-style-type: none"> 1. 5 x ULN AST, ALT or 2 x baseline if baseline > ULN. 2. fever, >39°C 3. blistering and/or vesiculation of cutaneous eruptions 4. any site of mucosal lesions; OR <p>B. angioedema; OR</p> <p>C. exfoliative dermatitis defined as severe widespread erythema and dry scaling of the skin, with generalized superficial lymphadenopathy, and with other constitutional findings such as fever, weight loss, hypoproteinemia possibly related to study drug; OR</p> <p>D. diffuse rash and serum sickness-like reactions defined as a clinical symptom complex manifested as fever, lymphadenopathy, edema, myalgia, and/or arthralgia; OR</p> <p>E. diffuse cutaneous eruptions, usually starting on the face, trunk or back, often with prodromal symptoms plus one of the following:</p> <ol style="list-style-type: none"> 1. cutaneous bullae, sometimes confluent with widespread sheet-like detachment of skin (<10% body surface area), (Nikolski's sign) (SJS) 2. two or more anatomically distinct sites of mucosal erosion or ulceration not due to another cause. 	<p>Diffuse cutaneous eruptions, usually starting on the face, trunk or back, often with prodromal symptoms plus cutaneous bullae with widespread sheet-like detachment of skin (>10% of body surface area), (Nikolski's sign), (SJS/Toxic Epidermal Necrolysis (TEN) overlap syndrome; TEN)</p>

APPENDIX IV TOXICITY MANAGEMENT PROCEDURES

Randomized infants, active on study drug, may have study treatment temporarily held or be discontinued from study treatment according to the guidelines in section 6.2.2 or according to criteria listed in the following table. Management of any adverse event will be according to the best clinical care available and the judgment of the site investigator or designated clinician.

CONDITION	SEVERITY ¹	STUDY DRUG USE	FOLLOW-UP AND MANAGEMENT
HEPATIC TOXICITY MANAGEMENT			
Suspected clinical hepatitis ²	Any Grade	Hold (<i>regardless of ALT grade</i>)	Observe and evaluate. If clinical hepatitis is confirmed: Study drug should be permanently discontinued. If clinical hepatitis is ruled out and ALT has returned to baseline: Study drug can be restarted.
Asymptomatic ALT	Grade 2	May be continued pending repeat assessment based on the clinician's judgment.	Repeat laboratory assessment as soon as possible, ideally within 72 hours.
Asymptomatic ALT	Grade 3/4	Hold	Repeat laboratory assessment as soon as possible, ideally within 72 hours. If repeat assessment is Grade 2 or less. Study drug may be restarted. If repeat assessment confirms Grade 3 or higher toxicity: Continue to hold study drug and re-evaluate. If Grade 3 or higher toxicity recurs in a participant who returned to Grade 2 or less, study drug should be permanently discontinued.
SKIN RASH MANAGEMENT			
Erythema with or without pruritus during first 2 weeks of study treatment	Grade 1	May be continued	Pruritis and minor accompanying symptoms may be managed with antihistamines, antipyretics and/or non-steroidal anti-inflammatory medications. If rash does not resolve within 14 days of onset, contact protocol team.
Diffuse erythematous macular or maculopapular rash or dry desquamation with or without pruritus but without constitutional findings or target lesions without blister/vesicle or ulceration in lesion	Grade 2A	May be continued	As above; however if rash occurs within the first two weeks of life, the dose should remain at 0.6ml per day and not be escalated until the rash resolves.
Urticaria	Grade 2B	May be continued	As above; however, if study drug is interrupted, DO NOT reintroduce.
Grade 3 or 4 Skin rashes		Immediate and permanent discontinuation	
EVENTS OTHER THAN HEPATIC TOXICITY OR RASH MANAGEMENT			
Any Grade 3 event other than hepatic toxicity or rash		May be continued or held pending repeat assessment based on the clinician's judgment.	Repeat assessment as soon as possible, ideally within 72 hours. If repeat assessment is Grade 2 or less: Study drug may be restarted. If repeat assessment confirms Grade 3 toxicity and alternative explanations for the abnormality have not been determined: Hold study drug for up to 7 days and reassess. If Grade 3 or higher toxicity persists or recurs and alternative explanations for the abnormality have not been determined: Study drug should be permanently discontinued. Note: If alternative explanations for the abnormality have been determined then study drug may be continued.
Any Grade 4 event other than hepatic toxicity or rash		Hold	Repeat assessment as soon as possible, ideally within 72 hours. If repeat assessment confirms Grade 4 toxicity: Permanently discontinue study drug

¹ See Section 7.0 and Appendix III for grading criteria.

² Clinical Hepatitis is defined as clinical signs and symptoms of clinical hepatic dysfunction regardless of ALT values, including enlarged liver (> 4cm below right costal margin), hepatic tenderness, ascites, portal hypertension (e.g., varices, splenomegaly, caput medusae), or hepatic encephalopathy (e.g., asterixis, changes in level of consciousness).