

PROTOCOL CLARIFICATION MEMORANDUM #3

HPTN 027: A Phase I Study to Evaluate the Safety and Immunogenicity of ALVAC-HIV vCP1521 in Infants Born to HIV-1 Infected Women in Uganda, Version 2.0, Dated 20 May 2005

Clarification Memorandum Date: 05 January 2007

Summary and Rationale

- It is clarified that, although the Day 1 post vaccination visit is intended to take place at the study clinic as indicated in Sections 4.2.2, a trained study clinician may perform the reactogenicity assessment at the infant's home if the mother cannot or does not bring him/her to the study clinic.
 - It is clarified that the criterion specified in Section 4.6.1 for withholding study vaccination in individual infants, "fever >38°C" pertains to axillary-measured temperature and that the corresponding non-axillary measured temperature to be applied for this criterion is >38.6°C. This clarification reflects the original intent which was to withhold vaccination if a grade 2 or higher fever was present as measured by the study staff.
 - It is clarified that references to 'potentially related' adverse events in Sections 4.6 and 4.6.1, which pertain to criteria for withholding study vaccination in individual infants, include events that are deemed possibly, probably or definitely related to the study vaccine. This clarification ensures the intended consistency with other sections of the protocol that refer more explicitly to the same set of AEs (e.g., Section 7.2).
 - It is clarified that the definition of "immunosuppressive therapy" as cited in Section 4.6.2 does not include low-dose steroids given for a short time between study vaccinations and more than a week prior to study vaccinations.
 - It is clarified that normal variations in typical neonatal conditions that are not regarded as unfavorable are not considered reportable adverse events as defined in Section 7.1; examples include clinical findings such as milia, miliaria and newborn peeling and laboratory findings such as slightly elevated or low monocyte, basophil or MCH counts, or elevated platelet, neutrophil or lymphocyte counts, as these are not toxicities.
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Implementation

This Clarification Memorandum has been approved by the DAIDS Medical Officer and may be implemented immediately upon issuance by the HPTN Coordination and Operations Center. IRB/EC approval of this Clarification Memorandum is not required by DAIDS; however, the site may submit the Clarification Memorandum to the responsible IRBs/ECs for their information, if desired, or for their approval, if required by the IRB/EC.

The modifications included in this Clarification Memo will be incorporated into the next full protocol amendment. Text appearing in bold below will be added where specified and text appearing with strike-through will be deleted.

Section 4.2.2 Evaluations During Immunization Phase (Weeks 0-14)

A clinic visit is to be scheduled the day after each immunization to allow for evaluation of any local or systemic reaction; **however this evaluation may be performed at the infant's home by a trained study clinician if necessary.**

Section 4.6 Toxicity Management/Severity Grading

The fourth paragraph will be modified as follows:

Close follow-up of infants after each study immunization will include observation for at least 1 hour immediately following administration, a clinic visit **or home visit by a trained clinician** on Day 1 and a clinic visit or home visit by a trained clinician on Day 2 following study immunization for identification of any local or systemic reaction, including but not limited to injection site erythema, induration, pain, and rash; symptoms of generalized allergic reaction, fever, sleepiness, lethargy, irritability, and seizures.

The following note will be added after the fifth paragraph:

Note that 'potentially related adverse events' as cited above are those deemed possibly, probably or definitely related to the study vaccine.

Section 4.6.1 Criteria for Withholding Vaccination

The second bullet will be modified as follows:

- **Grade 2 or higher** fever $\geq 38^{\circ}\text{C}$ documented by study staff within 3 days prior to scheduled immunization.

Section 4.6.2 Criteria for Permanent Vaccine Continuation

The fourth bullet will be modified as follows:

- Known or suspected disease of the immune system, active tuberculosis disease, measles, severe malnutrition (requiring hospital intervention), or

immunosuppressive therapy, **with the exception of low-dose steroidal therapy administered for fewer than five days between study vaccinations and more than one week (seven days) prior study vaccination.**

Section 7.1 Adverse Event Reporting and Safety Monitoring

The first paragraph will be modified as follows:

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily 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. Mothers will be instructed to report any AEs their infants may experience after randomization to the study staff. **Normal variations in typical neonatal conditions that are not regarded as unfavorable are not considered adverse events as defined above; examples include clinical conditions such milia, miliaria and newborn peeling and laboratory findings such as slightly elevated or low monocyte, basophil or MCH counts, or elevated platelet, neutrophil or lymphocyte counts.**

HIV Prevention Trials Network

PROTOCOL CLARIFICATION MEMORANDUM #2

HPTN 027: A Phase I Study to Evaluate the Safety and Immunogenicity of ALVAC-HIV vCP1521 in Infants Born to HIV-1 Infected Women in Uganda, Version 2.0, Dated 20 May 2005

Clarification Memorandum Date: 06 November 2005

Summary

To eliminate an inconsistency in the protocol, it is clarified that infant chemistries need not be performed with serum (i.e., plasma may be used); references to “serum” chemistries where they appear are eliminated. The schedule of evaluations and planned chemistry assessments remain unchanged.

Implementation

This Clarification Memorandum has been approved by the DAIDS Medical Officer and may be implemented immediately upon issuance by the HPTN Coordination and Operations Center. IRB/EC approval of this Clarification Memorandum is not required by DAIDS; however, the site may submit the Clarification Memorandum to the responsible IRBs/ECs for their information, if desired, or for their approval, if required by the IRB/EC.

The modification included in this Clarification Memo will be incorporated into the next full protocol amendment. Text bolded with strikethrough below will be amended where specified.

Section 2.3 Study Design

Page 39, first paragraph, fifth sentence will amend as:

- “A physical exam, interim history, and hematology and ~~serum~~ chemistry assessments...”

Section 4.2.2 Evaluations During Immunization Phase (Weeks 0-14)

The sixth bullet will amend as follows:

- ~~Serum~~ Chemistries (ALT (SGPT), bilirubin, creatinine) 2, 6, 10, 14

Section 4.2.3 Infant Post-Immunization Evaluations (Months 5-24)

The fifth bullet will amend as follows:

- ~~Serum~~ Chemistries (ALT (SGPT), bilirubin, creatinine) months 6,12

Section 9.1 Local Laboratory Specimens

First sentence will amend as:

- “Routine laboratory testing for hematology (CBC, differentials, CD4) ~~serum~~ chemistries...”

Section 9.3 Quality Control and Quality Assurance Procedures

Second paragraph, second sentence will amend as:

- “Plasma/~~serum~~ samples from all infants identified by the site...”

HIV Prevention Trials Network

PROTOCOL CLARIFICATION MEMORANDUM #1 for:

HPTN 027: A Phase I Study to Evaluate the Safety and Immunogenicity of ALVAC-HIV vCP1521 in Infants Born to HIV-1 Infected Women in Uganda Version 2.0, Dated 20 May 2005

Clarification Memorandum Date: 26 September 2005

Summary of Revision and Rationale

It is clarified that the interim history assessments to be performed at each follow-up visit will include the collection of limited information regarding potential social harms, as this is considered part of the overall assessment of participant safety.

Implementation

This Clarification Memorandum has been approved by the DAIDS Medical Officer and may be implemented immediately upon issuance by the HPTN Coordination and Operations Center. IRB/EC approval of this Clarification Memorandum is not required by DAIDS; however, the site may submit the Clarification Memorandum to the responsible IRBs/ECs for their information, if desired, or for their approval, if required by the IRB/EC.

The modification included in this Clarification Memo will be incorporated into the next full protocol amendment. Text appearing in bold below will be added where specified.

Section 4.2.2 Evaluations During Immunization Phase (Weeks 0-14)

The second bullet will be amended as follows:

- Targeted interim history, including assessments of infant feeding methods **and social harms**: weeks 2, 4, 6, 8 10, 12, 14

Section 4.2.3 Infant Post-Immunization Evaluations (Months 5-24)

The first bullet will be amended as follows:

- Targeted interim history, including assessments of infant feeding methods **and social harms**: months 5, 6, 9, 12, 15, 18, 21, 24

HPTN 027

A PHASE I STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF ALVAC-HIV VCP1521 IN INFANTS BORN TO HIV-1 INFECTED WOMEN IN UGANDA

A Study of the HIV Prevention Trials Network

Sponsored by

Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institute of Child Health and Human Development
US National Institute on Drug Abuse
US National Institute of Mental Health
US National Institutes of Health

Vaccine Provided by

Sanofi Pasteur

BB-IND 12023

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FINAL VERSION 2.0

20 May 2005

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SCHEMA

HPTN 027: A PHASE I STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF ALVAC-HIV vCP1521 IN INFANTS BORN TO HIV-1 INFECTED WOMEN IN UGANDA

Design: Phase I, randomized, double blind, placebo-controlled trial of ALVAC-HIV vCP1521 in infants born to HIV-1 infected women in Uganda.

Population: Infants born to HIV-1 infected Ugandan women with CD4 counts > 500 cells/ μ L attending the antenatal clinics at Mulago Hospital in Kampala, Uganda. As standard of care in Uganda, all HIV infected mothers and their infants attending these facilities are offered an antiretroviral regimen (e.g., Nevirapine) for prevention of mother to infant HIV transmission.

Study Size: 50 fully evaluable infants (40 vaccine, 10 control)

Study Treatment Regimen: Eligible infants will be enrolled and randomized after birth to one of two immunization arms as outlined below. The initial dose will be given on or before Day 3 after birth

Infant Group	Number	Birth (on or before Day 3 after delivery)	4 weeks	8 weeks	12 weeks
A	40	vCP1521	vCP1521	vCP1521	vCP1521
B	10	vCP1521 Placebo	vCP1521 Placebo	vCP1521 Placebo	vCP1521 Placebo
Total	50	<i>vCP1521 = ALVAC-HIV vCP1521 $\geq 10^{6.0}$ CCID₅₀/ml</i> <i>vCP1521 Placebo = Sodium Chloride Injection USP, 0.9%</i>			

Study Duration: Enrollment: approximately 3-6 months; Follow-up: 24 months post-partum.

Primary Objectives:

- To evaluate the safety and tolerance of ALVAC-HIV vCP1521 in infants born to HIV-1 infected Ugandan women with CD4 counts > 500 cells/ μ L.
- To evaluate the immunogenicity (cell-mediated and humoral responses) of ALVAC-HIV vCP1521 in infants born to HIV-1 infected Ugandan women with CD4 counts > 500 cells/ μ L.

Secondary Objectives:

- To monitor changes in CD4 cell counts in all vaccinated infants
- To evaluate the impact of receipt of ALVAC-HIV vCP1521 on the infant's immune response to standard UNEPI immunizations given in the first few months of life.

Vaccine Product Description: ALVAC-HIV (vCP1521) is a preparation of a live attenuated recombinant canarypox virus expressing gene products from the HIV-1 clade E *env* and clade B *gag* and clade B *protease* coding sequences and cultured in chick embryo fibroblast cells.

Vaccine Manufacturer: Sanofi Pasteur

Sponsoring Agency: Division of AIDS (DAIDS)
US National Institute of Allergy and Infectious Diseases (NIAID)
US National Institutes of Health (NIH)

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1.0 INTRODUCTION

The principal aim of HIV Prevention Trials Network (HPTN) protocol 027 is to determine the safety and immunogenicity of the ALVAC-HIV vCP1521 vaccine in infants born to women infected with human immunodeficiency virus (HIV) in Uganda. This is a first step in the long-term goal of identifying a safe, simple, cost efficient intervention in resource poor countries to prevent mother to infant HIV transmission during breastfeeding. Chemoprophylaxis of the mother and infant during the antepartum and/or peripartum period with antiretroviral therapy such as Zidovudine (ZDV) or Nevirapine (NVP) combined with immunoprophylaxis during the breastfeeding period with a recombinant ALVAC HIV-1 vaccine could be a safe, effective, feasible, and relatively inexpensive intervention for prevention of vertical HIV transmission while affording the well known benefits of breastfeeding.

The study of HIV vaccines in breastfeeding infants born to HIV infected women has the potential to provide significant benefit not only to the population being studied, but to the advancement of HIV vaccine development as well. Data from this trial in Ugandan neonates will complement data from Phase I/II and Phase III trials of this clade E/B vaccine in adults in Thailand, a trial of the clade B ALVAC-HIV vCP205 in Ugandan adults, and a similar trial of the clade B ALVAC-HIV vCP205 and ALVAC-HIV vCP1452 vaccines in infants conducted in the United States (US) by the National Institutes of Health's (NIH) Pediatric AIDS Clinical Trials Group (PACTG 326 - originally designated ACTG 326). ALVAC-HIV vCP1521 is currently undergoing efficacy testing for prevention of HIV infection in adults in Thailand.

1.1 Rationale

HIV-1 infection rates among women seeking antenatal services in some developing countries are well over 30%. HIV-1 transmission from an infected mother to her infant is estimated to be 21-45%, and in populations in which breastfeeding is the norm, postnatal transmission through breast milk has been shown to account for over one-third of all transmission.¹⁻⁵ Safe alternatives to breastfeeding are limited or infeasible for millions of HIV-infected women in the developing world and the benefits of breastfeeding are well established, therefore an intervention to decrease the risk of HIV-1 transmission during breastfeeding would be of utmost benefit to women and children in resource poor settings. Results of ACTG protocol 076 showed that an intensive regimen of ZDV starting at 14-34 weeks gestation, administered intravenously during labor and delivery, and orally to neonates for the first 6 weeks of life reduced the rate of HIV-1 vertical transmission by two-thirds in a non-breastfeeding study population in the U.S.⁶ A shorter ZDV regimen given to women a few weeks prior to and during labor was also found to reduce mother-to-infant HIV-1 transmission by 50% in a non-breastfeeding setting in Thailand.⁴ The same regimen achieved a 37% reduction in a breastfeeding population in Cote d'Ivoire after three months⁷ as did a similar regimen in a study in Cote d'Ivoire and Burkina Faso.⁸ An intrapartum/neonatal regimen of a single 200 mg dose of NVP given to the mother at the onset of labor and a single 2 mg/kg dose of NVP given to the infant within 72 hours of life reduced the risk of perinatal transmission among breastfeeding women in Uganda by 47% at 14-16 weeks and by 41% at 18 months compared to an intrapartum/neonatal regimen of ZDV (600 mg, then 300 mg every 3 hours during labor to mother, and 4mg/kg twice daily for one week to the infant).^{9,10} The South African Intrapartum/Neonatal Transmission (SAINT) study, compared a NVP regimen

modified from the one used in the HIV Network for Prevention Trials (HIVNET) study (HIVNET 012) including 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.¹¹ The overall estimated HIV infection rates by eight weeks in 1307 breast- and formula-fed 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.¹² The impact of these perinatal antiretroviral therapies is diminished in breastfeeding populations with continual exposure of the infant to HIV-1 through breast milk. While some studies continued to show efficacy at 18 to 24 month follow-up, additional infections through breast milk after the cessation of therapy lead to a decrease in overall efficacy.

The majority of HIV-1 infected women reside in the developing world where breastfeeding is practiced nearly universally, often for 1 to 2 years after delivery. Due in part to different populations studied and different ways of defining and calculating postpartum transmission, the reported rates of HIV-1 transmission through breastfeeding vary considerably, leaving the exact contribution to HIV-1 vertical transmission unclear. Both the European Collaborative Study¹³ and a Brazilian trial¹⁴ found a doubling in risk of transmission among breastfeeding women compared with non-breastfeeding women. A meta-analysis of data from five studies found a 14% additional risk of vertical transmission attributed to breast-feeding.¹⁵ In a randomized trial of formula vs. breastfeeding in Kenya, the risk of breastfed infants becoming infected was reported to be nearly twice as high as for those receiving formula (36.7% vs. 20.5%).⁵ Miotti and colleagues reported a relatively high risk of HIV transmission (0.7% incidence per month) from breastfeeding in the first 2-6 months and a lower but continuous risk from late breastfeeding transmission (0.3% incidence per month from 12-18 months or about 3% added risk from 12-24 months) in a prospective cohort study in Malawi.³ However, data from a retrospective analysis of infants participating a vitamin A study suggest that the risk of HIV transmission is significantly diminished in those who are exclusively breastfed compared to those in a mixed feeding group.¹⁶ Preliminary results have been presented from a randomized, non-controlled, open-label study entitled 'Stopping Infection from Mother-to-child from Breastfeeding in Africa' (SIMBA) conducted in Uganda and Rwanda in which women received ZDV/DDI for the last month of pregnancy and infants received either daily NVP or 3TC during and for 1 month after the breast feeding period.¹⁷ The overall rate of transmission reported of 8% by 6 months of age suggests that the combination therapy in the woman plus infant prophylaxis leads to decreased HIV transmission rates, but does not conclusively demonstrate the efficacy of infant prophylaxis alone in reducing breast milk HIV transmission. Several factors in this trial including the lack of a control arm, extended maternal therapy, low viral load in the mothers at delivery, short duration of breastfeeding, and limited safety information make it difficult to determine whether the use of antiviral regimens in infants during breastfeeding safely reduced the rate of breast milk transmission.

The high cost and difficult logistics of continuing antiretroviral therapy in the infant or mother for the duration of breast-feeding is likely to prohibit widespread and routine use in resource poor countries. Other options such as discouraging breastfeeding through the provision of infant

formula to HIV-1 infected women in developing countries are not only costly but also may pose a threat to the overall health and survival of infants if not properly administered. In most developing countries, where the majority of mother to child HIV transmission (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.¹⁸⁻²⁰ 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.^{21,22} 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 hallmark 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. 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 logistical difficulties, fear of disclosure of HIV status, cultural constraints and cost. Breastfeeding delays resumption of ovulation resulting in increased child spacing and confers psychosocial benefits through promotion of maternal-infant bonding. In addition to individual health benefits, there are economic and social benefits due to decreased health care costs associated with lowered rates of infant disease and to saving on the purchase of formula and other food for the child. There is, therefore, an urgent need for alternative interventions that provide protection from HIV infection to infants during breastfeeding.

Preventive and therapeutic HIV-1 vaccines are being evaluated in pediatric populations in the US. However, the immunogenicity of vaccines in industrial world populations does not predict the response in developing countries. Nutritional deficits, dietary differences, and microbial burden in individuals may alter immune constitution and the humoral and cellular responses to immunogens compared with those responses observed in U.S. populations receiving the same regimens. This has been documented with polio, rabies, and yellow fever vaccines. In addition, the distribution of major histocompatibility complex (MHC) Class I and Class II human leukocyte antigen (HLA) types is determined genetically and is highly divergent across populations of different racial origin. Both humoral and cellular responses to HIV vaccines are determined to a large extent by the particular MHC Class I and Class II types in vaccine recipients.

Many uncertainties remain about the most appropriate vaccines, the most effective timing of the immunizations, the extent of the neonatal immune response to active HIV-1 immunization, the duration of any present response, and the efficacy of HIV-1 vaccination in preventing vertical transmission. The goal of a vaccine intervention for the protection of infants from HIV-1 infection perinatally and through breast milk is to produce the broadest and most effective immune response as quickly as possible without jeopardizing vaccine immunogenicity with an administration schedule that is too accelerated. Critical immunologic and safety questions need to be answered in Phase I and II studies as soon as possible to prepare for rapid progression to efficacy trials with appropriate products. In addition, the high incidence of HIV in breastfeeding

infant populations enables rapid evaluation of vaccine efficacy.

1.2 Vaccine Choice

1.2.1 ALVAC Vaccines

HPTN 027 will continue evaluation of a candidate HIV-1 vaccine based on a live attenuated recombinant canarypox vector. The potential advantages of the live vector approach to HIV vaccine development include the ability of live vector recombinants to induce long-lasting humoral and cell-mediated immunity and thermostability. To date, canarypox vectors have been used in clinical trials of vaccines against rabies, measles, Japanese Encephalitis and cytomegalovirus (CMV) and for vaccines containing the HIV envelope or other genes. ALVAC HIV vaccines have been extensively studied in adults and have undergone Phase I evaluation in infants. Thus far, all ALVAC vaccines have a good safety profile in recipients (see Sections 1.3.2.2 and 1.3.3.1).

Like vaccinia virus, canarypox virus can accommodate large amounts of foreign deoxyribonucleic acid (DNA) in its genome, infect mammalian cells and cause them to produce foreign proteins. In contrast to vaccinia virus, canarypox virus is host-range restricted. In mammalian cells, it undergoes an abortive cycle of replication and does not produce infectious progeny virus. High doses of canarypox virus have not caused adverse effects in a wide variety of animals, even in profoundly immunosuppressed animals. This suggests that canarypox recombinants are not likely to disseminate and cause progressive disease in human recipients or be transmitted to unvaccinated contacts.

In addition, recombinant ALVAC vaccines were found to be safe in several small studies in immunocompromised hosts including those with HIV.²³⁻²⁵ A study of the safety of a recombinant ALVAC vaccine expressing the human carcinoembryonic antigen (ALVAC-CEA) given three times at a dose of $10^{7.4}$ plaque forming units (pfu) to 12 adults with advanced malignancies was well tolerated with no significant toxicities that could be attributed to ALVAC-CEA.²⁶ Another trial evaluated the safety of a recombinant ALVAC vaccine expressing both CEA and the B7.1 co-stimulatory molecule.²⁷ This vaccine (ALVAC-CEA-B7.1) was given to 18 subjects with advanced malignancies at increasing doses with 6 subjects in each group receiving $10^{6.7}$, $10^{7.7}$, and $10^{8.7}$ pfu, respectively. Clinical toxicity was minimal for these volunteers and only mild pain was noted at the injection site. Therefore, even high doses of recombinant ALVAC vaccines were well tolerated in a group of volunteers that are significantly immunocompromised.

1.2.2 Animal models of ALVAC vaccine efficacy against breast milk transmission

To mimic the repeated daily oral exposure to HIV that occurs during breastfeeding, Koen Van Rompay, Marta Marthas, and colleagues developed an infant rhesus macaque model in which animals are fed high doses of virulent simian immunodeficiency virus (SIVmac251) three times per day for five consecutive days.²⁸ This animal model was used to test the efficacy of active immunization administered at birth against subsequent oral challenge with SIVmac251 at 4 weeks of age. Six un-immunized infants were persistently viremic following SIVmac251 challenge; virus was detected in blood within 11 days after the last exposure to SIV. Eight animals were

immunized with recombinant canarypox (ALVAC) expressing *SIVgag-pol-env* at birth, 2 and 3 weeks of age. In contrast to 6 of the 6 controls, only 2 of the 8 vaccinated animals were virus positive within 4 weeks of SIVmac251 challenge ($p=0.02$; two-sided Fisher's Exact Test). There were no immune correlates of vaccine protection identified.

A second set of SIV studies in rhesus macaques was conducted by Marthas and colleagues using the same model with unvaccinated controls ($n=8$), the ALVAC *SIVgag-pol-env* ($n=8$), recombinant modified vaccinia virus Ankara (MVA)-*SIV gag-pol-env* ($n=8$), and the ALVAC vector alone ($n=4$) at birth, 2 and 3 weeks of age. The HIV infection rate for each group was 7/8 controls, 4/8 ALVAC SIV, 4/8 MVA SIV, and 1/4 ALVAC vector at 12-20 weeks post oral challenge at 4 weeks. The infected macaques in the ALVAC SIV group had significantly lower viremia and longer survival compared to control animals, but this was not seen with the MVA SIV vaccinated group. Although the numbers are small, these preliminary results suggest that an ALVAC-based anti-HIV vaccine may be promising to protect human infants against HIV infection acquired through breastfeeding. Most recently, Marthas and colleagues reported on extension of this macaque/SIV challenge model to evaluate the ability of neonatal vaccination to protect juvenile/adolescent animals against repeated SIV exposure.²⁹ After repeated oral challenge, 6 of 17 neonatally-vaccinated juveniles compared to 4 of 5 unimmunized juveniles were SIV-infected. These results are preliminary and the numbers are small, however they suggest that poxvirus-based anti-HIV vaccines may not only protect infants against breastmilk transmission, but also provide sustained protection through adolescence.

1.2.3 HIV Vaccine strategy using ALVAC alone

ALVAC-HIV vaccines alone do not induce a significant neutralizing antibody response, therefore in a number of studies, neutralizing antibody has been boosted by administration of a soluble protein antigen after ALVAC administration. However, humoral responses have been shown to be poorly cross-clade reactive and no protein antigen vaccines specific to clades A and D have been developed. Therefore no boost is proposed for HPTN 027. In addition, the animal data described above suggest potential protection with ALVAC vaccines alone. Similarly, ALVAC-based recombinant viruses expressing the feline leukemia virus (FeLV) subgroup A *env* and *gag* genes provided protection in the absence of detectable FeLV-neutralizing antibodies.³⁰ Clearly, an HIV vaccine strategy that provides protection with a single vaccine would be less costly, and more practical and easier to implement than a vaccine strategy that requires multiple clade-specific vaccine products to be administered in precise sequence.

1.2.4 ALVAC-HIV vCP1521

The only ALVAC product currently undergoing efficacy evaluation is ALVAC-HIV vCP1521. ALVAC-HIV vCP1521 is a preparation of canary pox virus expressing the products of HIV-1 *env* and *gag* genes. The genes are inserted into the C6 locus under the control of vaccine virus H6 and I3L promoters, respectively. The gp120 *env* sequence is derived from the subtype E strain TH023, but the anchoring part of the gp41 is derived from subtype B strain (LAI). ALVAC-infected cells present *env* and *gag* proteins in a near-native conformation.³¹ In addition, intracellular processing of foreign HIV-1 proteins via the MHC class I pathway facilitates stimulation of cytotoxic T-lymphocytes (CTL). Additional information on the physical, chemical

and pharmaceutical properties and the formulation and construction of the vaccine can be found in the Investigator's Brochure.³²

1.2.5 Cross-Clade Immunogenicity

The predominant HIV-1 strains isolated from Ugandans indicate that the great majority of incident cases are due to clade A virus and clade D virus with a small contribution from clade C and clade B virus.^{33, 34} The Ugandan clade D HIV-1 virus is genetically similar to the US clade B virus. The ideal HIV-1 vaccine would be capable of generating a protective immune response against all HIV-1 subtypes, affording widespread applicability. Genetic sequencing and immunotyping of HIV from different geographic areas suggest that there are important similarities and differences in these viruses relevant to vaccine design. The envelope glycoproteins have shown both genetic and immunological divergence across clades. The antibody induced by infection with a virus from one clade has not been shown to be consistently reactive across clades. One possibility to overcome the immunologic diversity of envelope proteins and to accelerate vaccine development for Africa is to include in vaccines additional proteins that may be more genetically conserved across clades. Studies to date suggest there is less genetic variation in the core proteins, *gag* and *pol*, than there is in the envelope proteins. Both envelope and core proteins have been shown to induce CD8+ CTLs. This strategy, therefore, is based on inducing cross-reactive CTLs rather than on antibodies, which can often be highly strain-specific. Demonstrated cross-clade CTL activity suggests that future vaccines can have common elements, thus potentially simplifying design. The role of heterogeneity in the immune response to HIV infection and the subsequent role in the immune response to HIV vaccination are not well understood. Extensive comparison of sequences of CTL and helper epitopes in viruses from the various clades and studies of infected individuals suggest that the immune responses induced by vaccines may be broad across different viral clades.³⁵⁻³⁷ Part of the rationale for use of *gag* from a subtype B in Uganda is that portions of the *gag* gene are conserved among virus subtypes. Therefore, *gag*-specific CTL elicited by vCP1521 may induce cross-reactivity in non-subtype B primary viruses. Data from an AIDS Vaccine Evaluation Group (AVEG) prime-boost trial (vCP205 alone or boosted with Chiron SF2 gp120/MF59) showed that CD8+ CTL from some vaccine recipients recognized target cells infected with non-subtype B viruses, including subtype E.³⁸

The HIVNET 007 trial of clade B-based ALVAC-HIV vCP205 in HIV-uninfected, low risk adults in Uganda provided relevant data on vaccine-induced cross-clade CTL response in vaccinees. Data from this study suggest that a clade B-based ALVAC-HIV vaccine candidate was able to elicit CTL response as well as detectable cross-reactivity to clade A and D antigens. The Elispot assay in this study was more sensitive in detecting HIV-specific responses compared to the chromium release assay (CTL) in this group of seronegative Ugandan volunteers.³⁹ Additional details are provided in Section 1.3.3.4.

Only by comparing vaccine efficacy in clade-matched populations to partially or completely unmatched ones can the impact of HIV diversity ultimately be understood. Significant scientific data that will be required prior to initiation of efficacy trials can be obtained from Phase I and II studies of the neonatal response to HIV-1 vaccines using a clade E vaccine. Essential preliminary information on the capability of the neonatal immune system to respond to an HIV-1 vaccine in the presence of maternally derived HIV antibodies, the optimal timing of immunization to maximize this response, and safety and tolerance of the ALVAC vaccine construct in neonates can be evaluated with a clade E/B vaccine. This will allow for

rapid progression from Phase I/II to Phase III testing of this ALVAC product or others when they are available for testing.

1.2.6 Clade Specific Vaccine Development

DNA, MVA, venezuelan equine encephalitis (VEE) and other HIV-1 vaccines based on strains predominant in Africa are in various stages of development and early evaluation. The status and design of human trials with these products are summarized at www.iavi.org/trialsdb. Sanofi Pasteur is developing a clade A ALVAC product that is expected to be available for human testing in 2005. The Walter Reed Army Institute for Research (WRAIR) in collaboration with Makerere University colleagues is planning a trial to evaluate this vaccine in Ugandan adults when it becomes available. Even with the development of a clade A product, approximately half of the HIV infections in Uganda will not be clade-matched to the vaccine. Determining the general applicability of an effective HIV vaccine will require testing in populations with both matched and unmatched circulating virus. If the ongoing Phase III trial of ALVAC-HIV vCP1521 in Thailand suggests efficacy in that population, future efficacy trials of this product alone or compared with other ALVAC products in non-clade E populations may be proposed. Our preliminary testing of the same product (ALVAC-HIV vCP1521) in Ugandan infants while the Thai trial is underway and while development of vaccines based on the virus subtypes most common in Uganda proceeds will provide an important component in the overall HIV vaccine development and evaluation strategy. Therefore, a stepwise process for evaluating ALVAC HIV vaccines in infants born to HIV infected Ugandan mothers is proposed, beginning with vCP1521.

1.3 Background

1.3.1 Neonatal Immune Response

Many issues related to the immune response of neonates must be considered in any HIV-1 vaccine trial. It is clear that neonates have different capacities for both cellular and humoral immune responses than adults.⁴⁰⁻⁴² The neonatal immune system is known to be immature, with decreased T and B cell functions. While neonatal T cells are capable of mature T cell responses, the responses differ both qualitatively and quantitatively from adult T cells.^{40,41} Neonatal lymphocytes have decreased function of T and NK cells and decreased production of type specific antibodies. Neonatal T cells have a much higher proportion of naive cells, are more difficult to activate, and produce altered cytokine profiles. The predominant T cell response to the majority of infections or immunizations in neonates is a Th2 allergic type humoral response rather than a Th1 cellular immune response. However, neonates are capable of producing antigen-specific cellular immune responses to infection. It is not clear whether the responses to immunization would be the same.

The neonatal response to early vaccination suffers from the same limitations as the response to neonatal infection. The success of neonatal immunization depends on many factors including the nature of the antigen presented, the timing of immunization, the mechanism of protection (humoral or cellular immunity), the presence or absence of maternally derived antibodies, vaccine dose, and type of adjuvant used. As the neonate ages, the development of the immune system progresses toward more adult-like responses. With infections such as HIV-1 in which early

protection is desired due to immediate exposure to the infecting agent, repeated dosing schedules are required to improve on what may be a minimal initial response. Early priming of the immune system may provide minimal initial protection, but lead to increased protection with subsequent vaccine exposures. Unlike most vaccine-preventable diseases, infants born to HIV-1 infected women who breastfeed are immediately and repeatedly exposed to the infectious agent in breast milk. Clearly, the likelihood of protection will be increased the earlier an immune response is generated.

All infants born to HIV-1 infected mothers will have HIV-1 antibodies acquired transplacentally. While some studies have found a correlation between the presence of specific maternal V3 loop antibodies and protection from infection, other studies have shown no such correlation. The exact mechanism of protection from HIV-1 infection is not clear. It appears that the CTL response may play an important role not only in controlling disease progression, but also in the protection from infection. Experience with other vaccines such as tetanus and measles in neonates have shown decreased humoral response to vaccination in the presence of maternally derived antibodies.⁴³ There are conflicting data from animal models suggesting and arguing against the possibility that maternally derived antibodies interfere with the development of pox virus vaccine expressed foreign antibody. However, in a mouse model of tetanus immunization in the presence of maternal antibodies to tetanus, tetanus-specific T cell responses to the vaccines were not inhibited, even when the antibody responses were completely inhibited.⁴³ This could explain the increased responses with each subsequent vaccine dose, and the ability of a three-dose vaccine schedule to overcome the inhibition due to the presence of maternal antibody. A vaccine that could prime the immune response at birth by eliciting strong T cell responses even in the absence of antibody responses could contribute to protection in the case of early exposure and increase the antibody response to subsequent doses. Neonates have varied abilities to respond to immunization with the greatest response to live vaccines, variable responses to protein vaccines, and negligible response to carbohydrate vaccines. In neonatal mice, live and DNA vaccines appear to stimulate Th1 and CTL responses better than inactivated or live recombinant canarypox vaccines. The initial poor response to vaccination may be overcome with repeated dosing which serves to prime the system for activation of memory cells rather than naive cells.⁴⁴

Experience with other childhood vaccines has documented successful immunization of neonates beginning immediately after birth. Active immunization with Hepatitis B vaccine combined with passive immunization with Hepatitis B immunoglobulin has been successful in preventing Hepatitis B infection in children born to Hepatitis B surface antigen positive mothers.⁴⁵ More recently, Hepatitis B vaccination has been added to the schedule of routine immunizations in childhood in the US and Uganda. The Hepatitis B vaccine used in the US is a yeast-recombinant subunit (HepBSAg) protein vaccine given at birth, 1, and 6 months of age. The majority of immunogenicity studies measure the presence of protective antibody levels rather than the presence of hepatitis B specific CTL activity. In the absence of maternally derived antibody, 82-95% of children attain protective antibody levels after 2 doses of vaccine and 95-100% of children attain protective levels after the 3 doses, depending on vaccine used.^{46,47} While the circumstances surrounding the use of the Hepatitis B vaccine are quite different from those of an HIV vaccine, the concept of successful early vaccination in neonates despite the immaturity of the immune response has been validated.

Once HIV proliferation begins in infected infants, it likely continues in a relatively unrestricted fashion, perhaps due to the paucity of HIV-specific cytotoxic T cells or other parameters of cell-mediated or humoral immune function in the first few months of life.⁴⁸ Compared to HIV-1 infected adults, HIV-1 infected neonates have a limited repertoire of antibody responses⁴⁹ and a paucity of HIV-1 specific circulating CTLs despite the presence of specific CTL precursors.^{50,51} Similar observations have been made after neonatal infections with CMV and RSV.⁵² Successful early sensitization to HIV epitopes might succeed in preventing HIV infection. Alternatively, the enhancement of HIV-specific immune function might also succeed in modifying HIV replication and affecting disease progression. The dosage of an HIV vaccine that is optimal in terms of reactogenicity is unknown. It is postulated that developmentally immature lymphocytes are activated by lower doses of antigen than more functionally mature lymphocytes.^{53,54}

1.3.2 Evaluation of HIV-1 Vaccines in Infants

1.3.2.1 Studies of gp120 subunit vaccines in pregnant women and infants

There are some data on the use of HIV vaccines in pregnant women or neonates in the US. Wright and colleagues studied the safety and immunogenicity of multiple doses of MN rgp120 vaccine given over the last half of pregnancy to 26 HIV-infected pregnant women in the US.⁵⁵ Vaccination was well tolerated with no significant local or systemic reactions in women and no adverse outcomes in the infants attributable to the vaccine. Earlier data suggested that therapeutic immunization with envelope-based vaccines might alter the clinical course of HIV infection and enhance the immune status of HIV-infected mothers or their infants, thus providing benefit to both. However, in this small study, vaccination of pregnant women did not alter plasma RNA reverse transcriptase-polymerase chain reaction copy number and was not associated with changes in CD4 counts or HIV binding and neutralizing antibody titers.

In ACTG 230, infants born to HIV infected women in the US were immunized at birth, 4, 12, 20 weeks with recombinant gp120 vaccines or adjuvant. The vaccines were found to be safe and well-tolerated in neonates, and there was no evidence of accelerated immunologic decline in HIV-infected infants.⁵⁶ Adjuvant alone recipients showed decreasing antibody titers to gp120 while vaccine recipients had stable or increasing titers to gp120 with decreasing titers of antibody to p31.⁵⁷ In addition, lymphoproliferative responses were present in approximately 70% of the infants immunized with the optimal dose of the Chiron gp120 vaccine and 50% of the infants immunized with the optimal dose of the Vaxgen gp120 vaccine.⁵⁸ An accelerated immunization schedule (birth, 2 weeks, 2 months, and 5 months) with the lowest dose of the SF-2 vaccine produced responses in all 11 vaccinees by 4 weeks. These data suggest that immune responses to vaccination are achievable at an early age when some perinatal or breastfeeding transmission may be prevented.

1.3.2.2 Studies of ALVAC HIV Vaccines in Infants

In PACTG 326, ALVAC-HIV vCP205 (the same ALVAC vaccine used in the adult trial in Uganda) was administered to infants of HIV-1 infected mothers in the US. The primary objectives were to determine the safety and immunogenicity of two dose levels ($10^{6.08}$ TCID₅₀/ml and $10^{6.33}$ TCID₅₀/ml) of ALVAC-HIV vCP205 given to infants at birth, 4, 8, and 12 weeks of age. Twenty-eight newborns

were randomized to receive the vaccine. Among 27 evaluable infants who received immunizations, 23 received 4 doses, 2 received 3 doses (1 placebo group and 1 study vaccine group), 1 received 2 doses (study vaccine group), and 1 received 1 dose (placebo group). No significant (>grade 3) vaccine-associated local or systemic reactions were observed. Both lymphoproliferative (LP) and CTL responses (to p24, ALVAC and gp160 antigens) were detected at rates similar to those found in ALVAC HIV vaccine trials in adults. LP responses after baseline (SI>3) to gp160, ALVAC and p24 were detected on at least one occasion in 1/5, 3/5, and 2/5 of the placebo subjects and in 10/18, 17/18, and 15/18 of the vaccinated subjects, respectively. Responses on two or more occasions were detected in 0/5, 0/5, and 0/5 of the placebo subjects and in 6/18, 15/18, and 8/18 of the vaccinated subjects, respectively. HIV-specific lytic activity was measured by Chromium release assay. CTL responses after baseline (to vp1291 (*env*, *gag*-pro MN), vp1174 (*env* MN) and vp1287 (*gag* MN) were detected on at least one occasion in 0/4, 1/5 and 0/5 of the placebo subjects and in 7/16, 4/17, and 6/17 of the vaccinated subjects respectively. Responses on two or more occasions were detected in none of the placebo subjects and in 2/14, 2/17, and 2/17 of the vaccinated subjects, respectively.

In late 2000, the PACTG 326 protocol was amended to include arms with the next generation ALVAC vaccine, ALVAC-HIV vCP1452 alone and combined with a subunit boost (VaxGen gp120 B/B). Thirty two mother-infant pairs were enrolled in the study, 16 in the vCP1452 vaccine/placebo group and 16 in the vCP1452 + VaxGen gp120 B/B vaccine/placebo group. Nine out of the 32 infants did not receive the full treatment; 1 received 3 doses, 1 received 2 doses, 5 received 1 dose, and 2 were determined to be ineligible to receive vaccine. There were only two vaccine-associated grade 3 adverse events (induration at week 6 and pain at week 2) and one grade 3 event thought to be possibly related to vaccine (1 neutropenia). LP and CTL data from Part 2 of the ACTG 326 study remain blinded, however CTL responses seen so far have been significantly less with ALVAC vCP1452 than those seen in Part 1 of the study using vCP205. Similar results have been seen in adults with the use of vCP1452 compared to vCP205. Preliminary LP data show more response in the vCP1452 + VaxGen gp120 B/B vaccine/placebo group than the ALVAC alone/placebo group.

HPTN 027 will expand the safety and immunogenicity data on the ALVAC-HIV products to a developing country infant population with ongoing exposure to HIV through breastfeeding in which future efficacy trials would be appropriate. The HPTN 027 design, including the vaccination schedule, mimics the design of the PACTG 326 study to the extent possible to enable comparison.

1.3.3 Summary of ALVAC-HIV Safety and Immunogenicity Data in Human Adult Trials

1.3.3.1 ALVAC HIV Vaccine Constructs

Table 1 below shows the different ALVAC-HIV constructs that have been developed and tested in human trials. Modifications to the ALVAC vaccines have been made over time in an attempt to elicit a broader immune response. These ALVAC HIV vaccines have been extensively studied in adult Phase I and II trials in several different countries. These trials are outlined briefly in Table 2 below.

Table 1: ALVAC HIV vaccine constructs

Vector	Env	Gag	Pro	Pol	Nef	E3L/K3L
vCP125	gp160 _{MN}					
vCP205	gp120 _{MN} gp41 _{TM}	gag _{LAI}	Pro _{LAI}			

vCP1521	gp120 ^{TH023} gp41 TM	gag ^{LAI}	Pro ^{LAI}			
vCP300	gp120 ^{MN} gp41 TM	gag ^{LAI}	Pro ^{LAI}	pol ^{CTL} 172-219, 325-383, 461-519	nef ^{CTL} 66-147 182-206	
vCP1433	gp120 ^{MN} gp41 TM	gag ^{LAI}	Pro ^{LAI}	pol ^{CTL} 172-219, 325-383, 461-519	nef ^{CTL} 66-147 182-206	
vCP1452	gp120 ^{MN} gp41 TM	gag ^{LAI}	Pro ^{LAI}	pol ^{CTL} 172-219, 325-383, 461-519	nef ^{CTL} 66-147 182-206	+

Table 2: Recombinant ALVAC Vaccines in Human Adult Trials

Candidate Vaccine	# Receiving ALVAC HIV	Protocol	Trial Site(s)	Principal Investigator(s)	Status
ALVAC-HIV (vCP125)	20	VAC01	ANRS ^a , France	Pialoux	Completed
ALVAC-HIV (vCP125)	10 (HIV ⁺)		Salpetriere, France	Katlama	Completed
ALVAC-HIV (vCP125) (Low and High Dose)	92	012A/012B	AVEG ^b 12A/12B, USA	Clements-Mann	Completed
ALVAC-HIV (vCP205)	25	VAC03	ANRS, France	Pialoux	Completed
ALVAC-HIV (vCP205) (Low and High Dose)	185	022/022A	AVEG 022, USA	Corey	Completed
ALVAC-HIV (vCP205)	22	029	AVEG 029, USA	Belshe	Completed
ALVAC-HIV (vCP205)	56	027	AVEG 027, USA	Wright	Completed
ALVAC-HIV (vCP205)	290	202	AVEG/HIVNET	Belshe, Stevens	Completed
ALVAC-HIV (vCP205)	56	032	AVEG	Mulligan	Completed
ALVAC-HIV (vCP205)	30	033	AVEG, USA	Evans	Completed
ALVAC-HIV (vCP205)	20	007	HIVNET, Uganda	Mugerwa, Ellner	Completed
ALVAC-HIV (vCP300)	20	VAC07	ANRS, France	Salmon	Completed
ALVAC-HIV (vCP300)	119	026	AVEG, USA	Evans	Completed
ALVAC-HIV vector (vCP205, 1433, 1452)	20/35/35	034	AVEG, USA	Schwartz	Completed
ALVAC-HIV (vCP205)/1452)	15/40	034A	AVEG, USA	Schwartz	Completed
ALVAC-HIV (vCP1452)	22 + 3	010	ANRS, France	Sicard	Completed
ALVAC-HIV (vCP1452)	26(HIV ⁺)	001	ADARC ^c , USA	Ho	Completed
ALVAC-HIV (vCP1452)	160	203	HVTN ^d , USA	Graham	Completed
ALVAC-HIV (vCP1452)	120	026	HIVNET, HVTN Haiti/Brazil/Trinidad/Peru	Wright	Completed
ALVAC-HIV (vCP1521)	203	RV132 and RV135	WRAIR ^e . Thailand	---	Completed
TOTAL	1624*				

*Total does not include subjects currently enrolled in the ongoing Phase III study of vCP1521 in Thailand.

^aANRS, Agence Nationale de Recherche sur le SIDA

^dHVTN, HIV Vaccine Trials Network

^bAVEG, AIDS Vaccine Evaluation Group

^eWRAIR, Walter Reed Army Institute of Research

^cADARC Aaron Diamond AIDS Research Center

1.3.3.2 ALVAC HIV Vaccine Safety

Studies of ALVAC-HIV vCP125, vCP205, vCP300, vCP1433, vCP1452, and vCP1521 with and without an rgp120 boost have shown excellent safety profiles in the above Phase I and II studies among low-risk and high-risk adult volunteers in France, the United States, Thailand, Uganda and the Caribbean.⁵⁹⁻⁶⁴ Over 1500 healthy volunteers have received ALVAC-HIV products to date without significant safety concerns. In a recent meta-analysis of data from previous AVEG and HIVNET studies of constructs vCP125, vCP205, vCP300, vCP1433 and vCP1452, Bruyn and

colleagues found that ALVAC-HIV vaccines, while producing frequent mild local and systemic reactions, were well tolerated and that the reactogenicity profile was similar for the different constructs.⁶⁵ Adverse reactions to immunization with the various ALVAC-HIV candidate vaccines have been similar to those observed in healthy adults who have received other licensed vaccines of similar types. These include transient pain, myalgia, tenderness, redness, and swelling at the injection site in some volunteers. Occasionally, volunteers have reported headaches, malaise, fever, dizziness, or nausea. Only a few volunteers felt sick enough to curtail normal activities, staying home from work or school. In addition, ALVAC HIV vaccines have been well tolerated when given to HIV infected volunteers.²³⁻²⁵

1.3.3.3 ALVAC HIV Vaccine Immunogenicity

After receiving 4 to 6 immunizations over a 12-month period in these studies, ALVAC-HIV candidate vaccines have induced HIV-specific CTL responses in 13-75% of recipients in some trials. Depending on the HIV-1 gene products expressed by the particular ALVAC-HIV candidate vaccine, anti-*env* ALVAC-HIV vCP125, ALVAC-HIV vCP205, and ALVAC-HIV vCP300, and anti-*gag* ALVAC-HIV vCP205, ALVAC-HIV vCP300, and ALVAC-HIV vCP1452, CTL activity has been detected. Although 3 to 4 immunizations with the different ALVAC-HIV experimental vaccines induced low level homologous HIV-1 neutralizing antibodies in volunteers, immunization with an ALVAC-HIV construct and an envelope subunit vaccine elicits CTL, ADCC, neutralizing and other antibody responses more often and in higher titers than either vaccine alone.

Evaluation of the most recent generation of ALVAC HIV vaccines, vCP1452, in the AVEG 034 studies, PACTG 326 and others showed disappointing results with respect to immunogenicity. HIV *pol* and *nef* genes and promoters E3L/K3L were added in ALVAC vCP1452 to improve immunogenicity and broaden the immune response. However, trials of vCP1452 in adults and infants revealed significantly less CTL responses than seen in earlier studies using vCP205 without these added genes. Therefore, vCP1452 vaccine will not proceed with further evaluation, and ALVAC products resembling the vCP205 product (such as vCP1521) will go forward.

1.3.3.4 Evaluation of ALVAC HIV Vaccine in Uganda

HIVNET 007 tested the clade B based ALVAC vCP205 in Uganda, a population where non-B clade HIV is endemic. Forty seronegative Ugandan volunteers were enrolled, 20 of whom received vCP205, 10 received ALVAC-rabies, and 10 received ALVAC placebo. Adverse reactions in this Ugandan cohort were similar to those in previous trials with these vaccines in HIV-uninfected volunteers in developed countries. No severe (grade 3 or 4) adverse reactions attributable to receipt of the vaccine were observed. Following immunizations on a 0, 1, 3, 6 month schedule, 4/20 volunteers receiving vCP205 demonstrated CD8+ CTL activity; 2 demonstrated *env*-specific CD8+ CTL activity and 2 had *gag*-specific CTLs. Cross-reactivity to clade A and D antigens was observed in 2 of these 4 responders using the standard chromium release assay. Confirmatory assays with the interferon-gamma (IFN- γ) Elispot method were performed using overlapping synthetic peptides corresponding to clade A, B and D on CD8+ cells that were expanded from cryopreserved PBMCs. The *gag*- and *env*-specific cumulative response was 40% and 35% respectively, using the Elispot assay, compared to 10% (both *gag* and *env*) in the standard chromium release assay. Of the 9 volunteers with vaccine specific responses on Elispot, 5 were found to

have cross clade reactivity to *gag* or *env* proteins from clade A and/or clade D viruses. HIVNET 007 suggested that a B-based ALVAC-HIV vaccine candidate was safe and able to elicit CTL response as well as detectable cross-reactivity to clade A and D antigens. The Elispot assay in this study was more sensitive in detecting HIV-specific response compared to the chromium release assay in this group of seronegative Ugandan volunteers.

1.3.4 Evaluation of ALVAC-HIV vCP1521 in Adults

1.3.4.1 Safety Evaluation

The safety data for ALVAC-HIV vCP1521 are derived from two WRAIR-sponsored studies in Thailand (RV132 and RV135).³² Overall, 268 subjects were enrolled in the two trials, of which 10 subjects received vCP1521, 193 subjects received vCP1521 + subunit boost, and 65 subjects received placebo (vCP1521 placebo or boost placebo). A total of 246 of the 268 subjects received all vaccinations and completed the follow-up.

Fourteen serious adverse events (SAEs) were reported in these two studies, all were considered unrelated to vaccination. Local and systemic reactogenicity events were reported separately from adverse events. The majority of these events were recorded as mild with few severe events. The most common potentially related events were injection site swelling and dizziness. Severe events included pain at site (3), erythema (11), induration (6), tiredness (3), headache (7), myalgia (5), nausea (1), and fever (26).

There were no grade 3 or 4 adverse events (AEs) judged to be possibly, probably or definitely related to the study vaccines. A total of 189 of 213 subjects with adverse events reported an AE occurring within 42 days vaccination. The percentages of subjects with AEs within 42 days were similar across vCP1521 + boost (69.4%) and placebo (69.2%) groups and just slightly below those with AEs over the entire study period (79.8% in vCP1521 + boost recipients and 75.4% in placebo recipients). Upper respiratory tract infection, nasopharyngitis, and myalgia were the most frequently reported AEs within 42 days of vaccination and during the entire study period.

To date, vCP1521 has been well tolerated and presents an acceptable safety profile, consistent with the other ALVAC-HIV constructs.

1.3.4.2 Evaluation of Immunogenicity

Immunogenicity data from the two WRAIR studies in Thailand are summarized below.³²

- In RV132, neutralizing antibodies to the TCLA subtype E strain NPO3 were found in 84% of vCP1521 + gp160 TH023 recipients and 89% of vCP1521 + gp120 CM235/SF2. Ninety-six percent of vCP1521 + gp160 TH023 and 100% of vCP1521 + gp120 CM235/SF2 prime-boost recipients had neutralizing antibodies against NPO3 or CM244 TCLA-adapted HIV-1.
- In RV135, neutralizing antibodies to the TCLA subtype E strains CM244 and NPO3 were found in 64% and 31%, respectively, of recipients who received vCP1521 + 300µg/300µg

AIDSVAX[®] B/E. Seventy-one percent of recipients had neutralizing antibodies against any clade E-adapted HIV-1 (either NPO3 or CM244). In contrast, neutralizing antibody to the TCLA subtype E strains CM244 and NPO3 was found in 44% and 23%, respectively, of recipients who received vCP1521 + 100µg/100µg AIDSVAX[®] B/E. Forty seven percent of prime-boost recipients had neutralizing antibodies against any clade E-adapted HIV-1 (either NPO3 or CM244). This indicates a clear dose-response relationship between induced neutralizing antibody and AIDSVAX B/E.

- No placebo recipients in either RV132 or RV135 developed neutralizing antibodies against the HIV-1 strains tested.
- In RV132, PBMC HIV-specific cumulative CTL responses were detected in 16% of vCP1521 + gp160 TH023 recipients and 25% of vCP1521 + gp120 CM235/SF2 recipients. HIV-specific cumulative CD8⁺ CTLs were detected in 11% and 25% of prime-boost recipients in the vCP1521 + gp160 TH023 and vCP1521 + gp120 CM235/SF2 arms of RV132, respectively. Among placebo recipients, PBMC and CD8⁺ HIV-specific CTLs were found in 10%.
- In RV135, PBMC HIV-specific cumulative CTL responses were detected in 23% of vCP1521 + 200 µg AIDSVAX[®] B/E recipients and 28% in the vCP1521 + higher dose (600 µg) of AIDSVAX[®] B/E prime-boost recipients. PBMC HIV-specific cumulative responses were found in 4% of placebo recipients. HIV-specific cumulative CD8⁺ CTLs were identified in 23% of subjects receiving either boost. No placebo recipient had CD8⁺ HIV-specific CTL responses. When all vCP1521 recipients in RV135 are considered as an aggregate (including five who received vCP1521 alone), 22 of 91 (24%) had CD8⁺ HIV-specific cumulative CTLs. Repeat positive CTL responses were observed in 9 (41%) of the 22 responders. HIV Gag activity to subtype A was assessed in eight responders; 3 (38%) showed cross-clade CTL activity. The CD8 CTL response to Gag was independent of p24 antibody response.
- In RV132, lymphoproliferative responses to the vaccine antigens gp120SF2, gp120CM235 and gp120TH023 were found in 75%, 68% and 55% of vCP1521 + CM235/SF2 recipients and 84%, 93% and 87% of vCP1521 + gp120 TH023 recipients, respectively. 93% percent of vCP1521 + gp160 TH023 and 75% of vCP1521 + gp120 CM235/SF2 prime-boost recipients had lymphoproliferative responses against any of the above antigens.
- In RV135, lymphoproliferative responses to gp120A244 and gp120MN were seen in 58% and 62% of the recipients, respectively. 71% of the recipients who received vCP1521 + 300µg/300µg AIDSVAX[®] B/E had lymphoproliferative responses against any of the above antigens.

ALVAC-HIV vCP1521 is currently being evaluated in a WRAIR-sponsored Phase III efficacy trial in Thai volunteers. ALVAC-HIV vCP1521 vaccine is now available for this Phase I trial in Ugandan infants born to HIV infected women. Data from these two complementary trials will contribute significantly to the advancement of HIV vaccine development.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

- To evaluate the safety and tolerance of ALVAC-HIV vCP1521 in infants born to HIV-1 infected Ugandan women with CD4 cell counts > 500 cells/μL.
- To evaluate the immunogenicity (cell-mediated and humoral responses) of ALVAC-HIV vCP1521 in infants born to HIV-1 infected Ugandan women with CD4 cell counts >500 cells/μL.

2.2 Secondary Objectives

- To monitor changes in CD4 cell counts in all vaccinated infants.
- To evaluate the impact of receipt of ALVAC-HIV vCP1521 on the infant's immune response to standard UNEPI immunizations given in the first few months of life.

2.3 Study Design

This is a phase I, randomized, double blind, placebo-controlled study to evaluate the safety and immunogenicity of a live recombinant Canarypox vectored HIV-1 vaccine, ALVAC-HIV vCP1521, in infants born to HIV-1 infected Ugandan women with CD4 cell counts > 500 cells/μL.

This study will take advantage of and benefit from the well-established research infrastructure at the Mulago Hospital HPTN study site in Kampala. This infrastructure has been developed and tested through conduct of Phase I-III HIV-related clinical trials including HIVNET 006, HIVNET 012 and the United Nations Joint Programme on AIDS (UNAIDS)-sponsored PETRA trial. Experience with these previous prospective HIV studies has shown that minimal loss to follow-up rates (6.5% over two years) can be maintained with the extensive home visiting and tracking systems that are currently in place at the study site. In addition, the CTL laboratory established at the Joint Clinical Research Center (JCRC) in Kampala for the HIVNET 007 adult vaccine trial will be used for HPTN 027.

HIV infected women attending the antenatal clinics at Mulago Hospital who provide written informed consent will first be screened as a proxy for their infants during the last trimester of pregnancy. Those women found eligible during initial screening will be asked to provide written informed consent for screening and subsequent enrollment of the infant. They will be asked to return to the hospital upon onset of active labor for delivery. After birth, infant eligibility for enrollment (randomization and study vaccination) will be assessed. A target of fifty eligible infants will be randomized to receive ALVAC-HIV vCP1521 or placebo (sodium chloride injection USP, 0.9%) on or before Day 3 after birth (Day 0 is the day of birth), and at 4, 8, and 12 weeks in a ratio of 4:1 (the same vaccination schedule used in PACTG 326 in the U.S.). Infants will be followed at the clinic every two weeks through week 14 and at months 5, 6, 9, 12, 15, 18, 21 and 24 after birth.

Assessment of product safety will include clinical observation and monitoring of hematological, chemical, and immunologic parameters. Primary safety evaluation will include close monitoring

of infants (history, physical exams and laboratory parameters) for local and systemic adverse reactions. Infants will be observed in the clinic for at least one hour after each immunization and will be scheduled to return to the clinic the next day (Day 1 post-immunization) and to be seen in the clinic or visited at home by a trained clinician on Day 2 post-immunization for assessment of local and systemic reactions to the vaccine including but not limited to injection site erythema, induration, pain, and rash; symptoms of generalized allergic reaction, fever, sleepiness, lethargy, irritability and seizures. If any reactions are present, the infant will continue to be followed closely until resolution. A physical exam, interim history, and hematology and serum chemistry assessments will be conducted two weeks after each immunization to assess toxicities/reactions and eligibility for continued immunization. Mothers will be advised to bring their babies to the clinic immediately or to otherwise notify study staff if any concerns arise between scheduled visits.

Secondary safety evaluation will include assessment of the impact of the ALVAC vCP1521 vaccination on immune status and response to childhood vaccination for all infants. CD4 cell counts will be performed to assess the effects of immunization on immune status. Antibody responses to standard UNEPI immunizations (polio, measles, tetanus, hepatitis B, and HIB) will be compared among infants in the two study arms. HIV proliferation and disease progression will also be evaluated by RNA PCR and CD4 cell count in infants subsequently found to be HIV infected.

Assessment of vaccine immunogenicity will begin with the assessment of T cell responses by interferon-gamma assays and CTL assays (cytometry-based degranulation assays) as indicated in Appendix I-B. The magnitude of vaccine-specific T cell responses as well as cross-clade activities will be measured. HLA typing will be performed using DNA extraction from cord blood. HIV antibody binding assays using vaccine specific antigens will be performed, however assessment of vaccine induced neutralizing antibodies will be limited, given the early presence of maternal antibodies and the absence of a protein boost. Other studies, such as more specific antibody assays may be performed as dictated by the infant's immune response to HIV antigens by standard rapid HIV-1 testing.

Infant HIV infection status will be determined using HIV-1 DNA and RNA PCR assays, as specified in Section 4.0 and in the Schedule of Evaluations in Appendix I-B. Therefore, there should be no difficulty distinguishing between natural infection with HIV and vaccine-induced antibody response. At 18 and 24 months, a rapid HIV test will also be performed; HIV uninfected infants who appear positive on this standard assay at 24 months will be offered post-study testing, approximately every six months, until this response disappears.

3.0 RECRUITMENT POPULATION AND SELECTION OF SUBJECTS

3.1 Recruitment Population

With 21,000 deliveries annually and an HIV-1 seroprevalence of approximately 12%, more than 2,500 HIV-1 infected pregnant women deliver at Mulago Hospital each year. The HIV-1 vertical transmission rate in this population was found to be 25% in over 800 HIV-1 infected women evaluated in natural history cohort studies. Use of the HIVNET 012 two-dose intrapartum/

neonatal regimen of nevirapine, now the standard of care at the study site, resulted in transmission rates of 13.5% by age 14-16 weeks and 15.7% by age 18 months in this population.

All women presenting for antenatal care are offered HIV counseling and testing by a trained nurse/counselor through the existing Mulago Hospital Prevention of Mother to Child Transmission (PMTCT) program. About 40 to 70 pregnant women each day accept testing. As standard of care, all HIV-1 infected women are offered an ARV regimen for PMTCT (currently the HIVNET 012 intrapartum/neonatal regimen of Nevirapine, which is approved for this indication in Uganda). All women are counseled about the risks and benefits of breastfeeding according to the World Health Organization (WHO) and Uganda Ministry of Health (MOH) recommendations. The majority of HIV infected women still choose to breastfeed their infants for many social and economic reasons. The MOH recommends that these women practice exclusive breastfeeding followed by early weaning at 3-6 months of age for those who can safely do so. With intensive ongoing counseling, the average duration of breastfeeding among women in the HIVNET 012 trial decreased from approximately 14 months to 9 months.

Immunization of infants for TB (BCG), polio (OPV), DPT, and measles is well accepted in Uganda. UNEPI has recently added Hepatitis B and Haemophilus Influenzae B (HIB) immunizations to the standard childhood immunization program. The current immunization schedule in the Makerere University-Johns Hopkins University (MU-JHU) research clinic (the study site) consists of BCG and polio at birth; polio and tetravalent DPT/HepB/HIB at 6, 10, and 14 weeks; and measles at 26 and 52 weeks.

3.2 Selection of Subjects

Screening for the study will proceed in a stepwise manner. If at any point during the process a mother or her infant is found ineligible, screening will be discontinued. HIV-infected women in the last trimester of pregnancy who have received their HIV test results and related counseling will be given general information about studies ongoing at the site and referred to appropriate study staff. They will first be provided introductory information about the vaccine study and, if interested, will be asked to consent for initial screening, which will include confirmation of HIV status with Western blot or DNA PCR, determination of CD4 cell count and a physical exam. For this Phase I study, women with a CD4 cell count of ≤ 500 cells/ μ L will not be eligible as they may be at high risk for vertical HIV transmission or illness which may limit their ability to bring their infants for follow-up as scheduled. Women who are ineligible for the study based on this criterion will be referred to local care and treatment programs as described in Sections 8.5.3 and 8.5.4. Women who meet the initial eligibility criteria and remain interested in their infant participating in the study will then receive intensive study informed consent counseling, including detailed information on the study procedures, visit schedule, risks and benefits. Thorough counseling of mothers will be emphasized. A staff member other than the counselor primarily responsible for the informed consent process will ask the volunteer a series of structured questions to assess her understanding. If the volunteer demonstrates full understanding of the study based on successful completion of the assessment of understanding (based on an algorithm to be specified in the study specific procedures manual) and she is willing to allow her infant to participate in the study, she will be asked to sign an informed consent form for screening and enrollment of her infant. Detailed locator/contact information will be collected and a home visitor will take each woman home to document her location for future follow-up.

After delivery, the mother will have a physical exam and her consent for the infant's participation

in the study will be verbally re-confirmed and this will be documented in the study records. The infant's eligibility for study enrollment will be assessed prior to randomization and vaccination. Infants who meet the eligibility requirements will be enrolled and assigned the next sequentially numbered vaccine kit on or before Day 3 post delivery. Assignment of the kit is the effective point of randomization. Study recruitment, screening, immunization and follow-up procedures are outlined in Section 4.0 and will be detailed in a study-specific procedures (SSP) manual.

Accrual will proceed slowly over a period of approximately 3-6 months to allow for careful assessment of vaccine toxicity and for scheduling and spacing of critical laboratory evaluations.

Note that mothers are screened as a proxy for their infants, but they are not enrolled in the study, therefore they undergo no study assessments after the infant's eligibility has been fully assessed and the screening process is complete.

3.3 Inclusion Criteria for HIV-1 Infected Women

- Third trimester of pregnancy, as judged by clinical exam and reported menstrual history
- Written informed consent
- ≥ 18 years of age
- Confirmation of HIV-1 infection documented by Western blot or DNA PCR
- CD4 cell count > 500 cells/ μL on the eligibility blood specimen
- Stated willingness to be taken home by a home visitor to document locator information and to be visited at home
- Stated intent to deliver at Mulago Hospital (the study site)

Note: All HIV infected women are offered NVP or other antiretrovirals for the prevention of mother to child HIV transmission as part of the standard of care at Mulago Hospital (outside of the study). A woman's choice of infant feeding method or her use or non-use of antiretrovirals for prevention of vertical transmission and/or for treatment of HIV will not affect her infant's eligibility for participation in HPTN 027.

3.4 Exclusion Criteria for HIV-1 Infected Women

- Prior participation in any HIV-1 vaccine trial
- Receipt of any investigational agent during this pregnancy
- Receipt of blood products, immunoglobulin, or immunotherapy during this pregnancy
- Evidence of clinically significant disease that would compromise the ability of the participant to complete the study or the study requirements as determined by the study clinician
- Known multiple gestation in current pregnancy

3.5 Inclusion Criteria for Infant Enrollment/Initiation of Vaccination

- ≤ 3 days of age (day of birth = Day 0)
- Born to an HIV-1 infected woman found eligible during screening
- Birth weight ≥ 2000 gm

3.6 Exclusion Criteria for Infant Enrollment/Initiation of Vaccination

- Mother discontinued prior to infant enrollment and randomization for any reason
- Maternal or infant receipt of any other active or passive HIV immunotherapy or investigational product other than the study vaccine. (Note that NVP is approved for use in pregnant women and neonates in Uganda, therefore it is not considered investigational or exclusionary in the context of this study.)
- Maternal or infant receipt of blood products, immunoglobulin, or immunosuppressive therapy during labor and delivery or prior to enrollment (infant)
- Documented or suspected serious medical illness or immediate life threatening condition in the mother that may interfere with the ability to complete study requirements, as judged by the examining clinician
- Documented or suspected serious medical illness, serious congenital anomaly, or immediate life threatening condition in the infant that may interfere with the ability to complete study requirements, as judged by the examining clinician. The clinical significance of any such abnormality is to be evaluated in the context of the safety of the volunteer and the objectives of this study.
- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction
- Multiple birth
- Baseline laboratory results (on or before Day 3 after birth/before enrollment):
 - Hemoglobin < 12.0 gm/dl
 - Platelet count < 100,000 cells/mm³
 - Absolute neutrophil count <1500 cells/mm³
 - Creatinine > 1.3 mg/dl
 - SGPT (ALT) > 3 times the upper limit of age adjusted institutional normal

3.7 Co-Enrollment Guidelines

Co-enrollment of infants in other trials of investigational agents will be discouraged. However, infants enrolled in HPTN 027 who are found to be HIV infected may participate in available treatment trials. Decisions about whether or not an infant enrolled in another trial will continue to receive the HPTN 027 study vaccine will be made on a case by case basis by the Protocol Chairs in consultation with the Protocol Safety Review Team (PSRT; described in Section 7.2), depending on the nature and timing of the other study in which s/he is enrolled. However, all infants receiving one or more study vaccinations will be asked to continue follow-up in HPTN 027, even if terminated early from the vaccination schedule.

Note: Mothers and HIV-infected infants may receive antiretroviral or other available therapy for treatment of HIV/AIDS and related illnesses.

3.8 Participant Withdrawal

A mother may withdraw her child from the study at any time for any reason. Extensive follow-up procedures used successfully in other studies at the site will be employed to maximize study retention and schedule adherence. Mothers who express concerns about their infants continuing in

the study will be counseled appropriately; however, continued participation in the study will be entirely voluntary. Participants may be withdrawn from the study if the study sponsor or government or regulatory authorities in the US or Uganda terminate the trial prior to its planned end date. Participants who are discontinued early from the vaccination schedule for any reason will not be withdrawn from the study and will remain in follow-up as scheduled.

4.0 STUDY PROCEDURES AND EVALUATIONS (See Appendix I A & B – Schedules of Evaluations)

4.1 Evaluations of the Mother

Written informed consent for screening of the mother must be obtained prior to undertaking study-specific assessments (Appendix II A).

4.1.1 Screening (third trimester of pregnancy)

- Targeted medical history (brief medical, obstetric, HIV)
- Targeted physical examination (for gestational age, multiple pregnancy, presence of serious illness)
- Hematology (CBC with differential, CD4 count)
- HIV-1 Western blot or DNA PCR (for HIV status confirmation)
- Quantitative HIV RNA PCR (stored plasma for later assay, if infant enrolled)
- Dried blood spot, plasma, and PBMC for storage
- Assessment of understanding (prior to signing consent for infant enrollment)

Women in the last trimester of pregnancy who are found to be eligible during screening will be asked to provide written informed consent for their infant's screening and participation in the study (Appendix II B) after successful completion of the assessment of understanding.

Note: Stored specimens from mothers of infants who are enrolled in the study may be used for later study-related immunologic/virologic evaluation depending on the results of the infant evaluations. These may include but are not limited to viral subtyping, HLA typing, specific HIV antibody testing, and other factors that may be associated with HIV transmission or protection. Specimens from mothers screened but whose infants are not enrolled will be stored only until it has been determined that her infant is not eligible for study enrollment, at which time the specimens will be destroyed. Any leftover specimens from enrolled infants and their mothers will be destroyed after all study-specified assays have been completed, including those for quality assurance.

4.1.2 After Delivery/Prior to Infant Enrollment

- Targeted history (for infant eligibility determination)
- Targeted physical exam (for infant eligibility determination)

4.2 Evaluations of the Infant

4.2.1 Pre-Immunization/Baseline Evaluations (After birth – prior to enrollment)

- History (for eligibility determination)
- Physical examination (for eligibility determination)
- Hematology (CBC with differential) (for eligibility determination)
- Chemistries (ALT (SGPT), creatinine) (for eligibility determination)
- Bilirubin
- HIV DNA PCR *Quantitative HIV-1 RNA PCR may be used if HIV-1 DNA PCR is not available. If positive, a second DNA PCR and/or RNA PCR on a subsequent specimen will be performed for confirmation.*
- Baseline CTL assay (from cord blood)
- IFN- γ assay (from cord blood)
- HLA typing (on DNA extracted from cord blood)
- Dried blood spot, specimen storage if blood quantity is sufficient

A total of 15-25 ml of cord blood will be obtained for the CTL and IFN- γ assays, and HLA typing. Peripheral blood (5ml) should only be obtained (at the Week 4 visit) for these assays if cord blood is not available.

If at any point during examination of the mother or infant prior to study enrollment/randomization, s/he is found ineligible, study-specific evaluations will be discontinued and specimens will not be taken for study-specific assays, with the exception of HIV status confirmation.

4.2.2 Evaluations During Immunization Phase (Weeks 0-14)

- Post-immunization monitoring: weeks 0, 4, 8, 12. Infants will be monitored for at least 1 hour immediately following each study immunization. Temperature and local and systemic reactions 30-60 minutes post administration will be recorded. A clinic visit is to be scheduled the day after each immunization to allow for evaluation of any local or systemic reactions. On the second day following each study immunization, a trained nurse will visit the infant at home (unless a clinic visit on Day 2 is indicated) to assess reactogenicity. Any reaction identified will be closely followed until resolution. Reactions to be assessed include, but are not limited to, those included in Appendix III. Mothers will be advised to bring their babies to the clinic immediately if any suspected reactions are observed between scheduled assessments.
- Targeted interim history, including assessment of infant feeding methods: weeks 2, 4, 6, 8, 10, 12, 14
- Targeted physical exam: weeks 2, 4, 6, 8, 10, 12, 14
- Hematology (CBC with differential): weeks 2, 6, 10, 14
- CD4 count: weeks 2, 6, 14
- Serum chemistries (ALT (SGPT), bilirubin, creatinine) 2, 6, 10, 14
- CTL: week 14
- IFN- γ : week 10
- Binding antibody ELISA: weeks 10, 14
- HIV DNA PCR: weeks 2, 6, 10, 14 - *Quantitative HIV-1 RNA PCR may be used if*

HIV-1 DNA PCR is not available. If positive, a second DNA PCR and/or RNA PCR on a subsequent specimen will be performed. If confirmed as infected, no further DNA PCR will be done but plasma will be obtained at each subsequent scheduled blood draw for quantitative RNA PCR. Dried blood spot, specimen storage if blood quantity is sufficient: weeks 2, 6, 10, 14

4.2.3 Infant Post-Immunization Evaluations (Months 5-24)

- Targeted interim history, including assessment of infant feeding methods: months 5, 6, 9, 12, 15, 18, 21, 24
- Targeted physical exam: months 5, 6, 9, 12, 15, 18, 21, 24
- Hematology (CBC with differential): months 6, 12, 18
- CD4 count: months 6, 12, 18
- Serum chemistries (ALT (SGPT), bilirubin, creatinine): months 6, 12
- Polio, tetanus, Hep B, HIB antibodies: month 6
- Measles antibodies: month 18
- CTL: months 6, 12, 18, 24 if indicated
- IFN- γ : months 6, 12, 18, 24 if indicated
- Neutralizing antibody: months 18, 24 (from stored plasma if HIV-1 antibody response present on rapid HIV-1 test)
- Binding antibody ELISA: months 6, 12, 18, 24
- HIV DNA PCR: months 6, 12, 18, 24 - *Quantitative HIV-1 RNA PCR may be used if HIV-1 DNA PCR is not available. If positive, a second DNA PCR and/or RNA PCR on a subsequent specimen will be performed. If confirmed as infected, no further DNA PCR will be done but plasma will be obtained at each subsequent scheduled blood draw for quantitative RNA PCR.*
- HIV-1 rapid test and, if positive WB: months 18, 24
- Dried blood spot and specimen storage if blood quantity is sufficient: months 6, 12, 18, 24

NOTE: Study vaccinations will be discontinued in infants found to be HIV-infected, however they will remain in the study and continue follow-up as scheduled. Uninfected infants with a positive antibody response on standard HIV-1 ELISA at 24 months will be offered post-study testing approximately every 6 months until this response disappears. Additional information on care of HIV-infected infants is included in Sections 8.5.3 and 8.5.4.

Urinalysis will be performed in infants with who present during follow-up with abnormal creatinine levels, edema, abnormal urine output or symptoms of urinary tract infection.

4.2.4 Infant Evaluations When Study Vaccination Permanently Discontinued

Infants who receive one or more doses of the study product and are subsequently discontinued from study vaccination due to an adverse event, HIV infection or any other reason will continue to be followed through 24 months as scheduled. Subjects will continue to have complete clinical and laboratory evaluations as shown in the schedule of evaluations (Appendix I), except that

immunological evaluations (CTL, IFN- γ , binding antibody ELISA, neutralizing antibody) are not required if the infant did not receive at least two doses of vaccine prior to discontinuation.

4.3 Interim Contacts and Visits

Interim (unscheduled) contacts and visits may take place at any time during the study. All interim contacts and visits will be documented in the participant's study source records and relevant data will be included on case report forms when appropriate. Windows for completion of scheduled visits will be defined in the study-specific procedures manual.

4.4 Concomitant Medications

All antiretrovirals received by the mother during pregnancy, during labor and delivery or postpartum for PMTCT or HIV treatment and all other medications, biologics, or blood products received by the mother during labor and delivery or by the infant at any time after birth will be reported in the participant's source records. This includes other immunizations, antiretrovirals (including nevirapine), and traditional herbs or medicinal products. Concomitant medications received by infants through six months of age (12 weeks after the last scheduled study immunization) will also be recorded on the DataFax CRF for inclusion in the study database.

4.5 Disallowed Medications

Immune modulators and IVIG are not allowed until 30 days after the last vaccination unless required for the health of the infant.

4.6 Toxicity Management/Severity Grading

It is anticipated that vaccine-associated side effects will occur, but that these side effects will rarely necessitate interruption of the immunization schedule.

The severity (the clinician's evaluation of intensity) of all adverse events will be classified based upon the standard Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, dated December 2004 (located at the following website: <http://rcc.tech-res-intl.com> and in the SSP Manual) and the Supplemental Toxicity Table for Reactogenicity Post-Vaccination Occurring within seven Days of Vaccination (Appendix III). Appendix III supersedes the standard DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, dated December 2004, when grading reactogenicity post-vaccination (within seven days). Also, malnutrition and axillary-measured fever will be graded as specified below.

Malnutrition (failure to thrive):

- Grade 1: Underweight: 60-80% of the 50th percentile expected weight for age and edema Absent
- Grade 2: Marasmas: <60% of 50th percentile expected weight for age and edema absent
- Grade 3: Kwashiorkor: 60-80% of the 50th percentile expected weight for age and edema present

Grade 4: Marasmic-kwashiorkor: <60% of 50th percentile expected weight for age and edema present

Fever (axillary-measured):

Grade 1: 37.1 - 38.0 °C

Grade 2: 38.1 - 38.7 °C

Grade 3: 38.8 - 39.9 °C

Grade 4: >39.9 °C

Any additional exceptions to the standard DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, dated December 2004, must be approved by the sponsor and will be provided to the US Food and Drug Administration (FDA) and to the Institutional Review Boards (IRBs)/Ethics Committees (ECs) in advance of implementation. When grading laboratory values, normal limits will be defined according to local institutional age specific values.

Close follow-up of infants after each study immunization will include observation for at least 1 hour immediately following administration, a clinic visit on Day 1 and a clinic visit or home visit by a trained clinician on Day 2 following study immunization for identification of any local or systemic reaction, including but not limited to injection site erythema, induration, pain, and rash; symptoms of generalized allergic reaction, fever, sleepiness, lethargy, irritability and seizures. Any reaction identified will be closely followed until resolution. Mothers will be also advised to bring their babies to the clinic immediately if they observe any suspected reactions.

All abnormal clinical events and laboratory values occurring in enrolled infants will be followed closely until resolution. The urgency and frequency of repeat evaluations will depend on the clinical significance of the specific abnormality. Study clinicians will provide appropriate clinical management of adverse events according to their best medical judgment and local practice. For grade 3 or 4 laboratory abnormalities, repeat evaluations will be performed within three days. If any persistent grade 3 or 4 clinical or laboratory abnormality is thought to be potentially due to the study vaccine, evaluations should be repeated approximately once every two weeks (or more frequently if necessary) until toxicity falls below grade 2 and will continue to be closely monitored thereafter as appropriate. Alternate explanations will be sought for all clinical and laboratory abnormalities, especially those considered potentially related to study vaccine that may lead to vaccine discontinuation. Safety of participating infants and close follow-up of all clinical and lab abnormalities – regardless of severity grade or potential relatedness - is of highest priority.

4.6.1 Criteria for Withholding Vaccination

(For exclusion criteria for the initiation of vaccination in infants, see Section 3.6)

Infants with any of the conditions listed below may not receive study vaccinations. Vaccination will be withheld until the condition resolves or improves to a less severe grade. Vaccinations withheld while awaiting resolution of a condition must be administered within one week (7 days) of the scheduled date, otherwise study vaccination will be permanently discontinued.

- Active serious intercurrent infection (such as meningitis, pneumonia, severe gastroenteritis, malaria) within 3 days prior to scheduled immunization, as determined by the examining

clinician. Normal childhood illnesses such as otitis media, URI, and gastroenteritis will not necessitate change in the vaccination schedule.

- Fever > 38°C documented by study staff within 3 days prior to scheduled immunization.
- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.
- One positive HIV DNA PCR result; if repeat testing confirms infection, vaccination will be permanently discontinued.
- Unresolved Grade 3 adverse events:
 - The next scheduled immunization will be held unless the AE resolves to Grade 2 or lower within one week of the scheduled immunization.
 - Subsequent vaccination of infants who have experienced a potentially related Grade 3 AE will be discussed with the Protocol Chairs and other members of the Protocol Safety Review Team prior to re-vaccination.
 - If that dose is given and there is no recurrence of the same toxicity afterward, subsequent immunizations will be given.
 - If the same Grade 3 AE recurs after subsequent immunization, immunization will be permanently discontinued.
- Other conditions that preclude further vaccinations due to safety or other concerns, as determined by the Protocol Chairs and the Protocol Safety Review Team.

For Grade 1 or 2 adverse events, no interruption in the vaccination schedule is necessary, even if possibly related, unless otherwise directed by the Protocol Chairs and the Protocol Safety Review Team.

4.6.2 Criteria for Permanent Vaccine Discontinuation in an Infant

(For overall stopping rules, see Section 7.2.)

Decisions regarding permanent discontinuation of study vaccine in an infant will be made by the PSRT after careful consideration of the specific case and all relevant data. Criteria for permanent vaccine discontinuation include the following:

- Selected Grade 3 adverse events (e.g., Grade 3 seizure or allergic reaction) as determined by the PSRT.
- Any Grade 4 adverse event
- Confirmed HIV infection
- Known or suspected disease of the immune system, active tuberculosis disease, measles, severe malnutrition (requiring hospital intervention), or immunosuppressive therapy
- Immunological decline defined as a confirmed CD4 value of <25%
- Noncompliance with study requirements, as determined by the site investigator in consultation with the PSRT
- Enrollment in another study that, in the judgment of the PSRT, interferes with participation in or interpretation of HPTN 027

Note: If an infant does not receive a scheduled study vaccination within the allowable window (+/- 7 days of the target date) for any reason, the vaccine will not be administered and all remaining study vaccinations will be discontinued.

5.0 STUDY PRODUCT

5.1 Regimen

Eligible infants will be enrolled and randomized after birth to one of two immunization arms (Group A or Group B) as outlined below:

Group A: ALVAC HIV-1 (vCP1521) 1 mL in right thigh muscle on or before Day 3 after birth and at weeks 4, 8, and 12.

Group B: vCP1521 placebo 1 mL in right thigh muscle on or before Day 3 after birth and at weeks 4, 8, and 12.

5.2 Study Product Formulation and Preparation

Additional detail about ALVAC HIV (vCP1521) can be found in the Investigator's Brochure.³²

ALVAC-HIV (vCP1521)

ALVAC-HIV (vCP1521) is a preparation of live attenuated recombinant canarypox virus expressing gene products from the HIV-1 *env* (clade E), *gag* (clade B), and protease (clade B) coding sequences and cultured in chick embryo fibroblast cells. The vaccine is manufactured by Sanofi Pasteur, Marcy L'Etoile, France. ALVAC-HIV (vCP1521) is formulated as a sterile, lyophilized product in single-dose vials. Each vial contains $\geq 10^{6.0}$ CCID₅₀/mL of ALVAC-HIV (vCP1521). The diluent supplied for reconstitution of ALVAC-HIV (vCP1521) consists of sterile sodium chloride solution (NaCl 0.4%). ALVAC-HIV (vCP1521) must be stored at 2-8°C and must not be frozen.

Prior to injection, each dose of vaccine (ALVAC-HIV (vCP1521)) is to be reconstituted with the diluent provided for this purpose (1 mL NaCl 0.4%). Using aseptic technique, the pharmacist will slowly inject the diluent into the vial containing the lyophilized vCP1521. The vial should then be swirled gently until complete dissolution of the lyophilisate. The vial should NOT be inverted. After dissolution, the pharmacist, using aseptic technique, will withdraw 1 mL of the reconstituted vaccine using a new needle and 3 mL syringe. The reconstituted vaccine must be kept at 2-8°C (NOT frozen) until just prior to injection. It must be used within two hours after reconstitution.

Each vaccine preparation is to be given intramuscularly with an appropriate sized pediatric needle into the right thigh muscle after preparation of the site with alcohol.

vCP1521 Placebo

Sodium Chloride Injection USP, 0.9% will be used as the placebo control. (Bacteriostatic 0.9% Sodium Chloride for Injection, USP will NOT be used.) To maintain blinding of study kits, the vCP1521 placebo will be stored at 2-8°C and must not be frozen.

Prior to injection, the pharmacist, using aseptic technique, will withdraw 1 mL of Sodium Chloride Injection USP, 0.9%, into a 3 mL syringe. To maintain blinding of study staff, the vCP1521 placebo must be used within two hours of preparation. Each vaccine placebo preparation is to be given intramuscularly with an appropriate sized pediatric needle into the right thigh muscle after preparation of the site with alcohol.

Since the vaccine and placebo products are not identical in appearance, the site Pharmacist of Record will prepare the doses for administration and provide them to the designated study staff for injection. The barrel of the syringe will be covered with an overlay, and the individual administering the injection to the participant will not be involved in the conduct of any study-related assessments. Additional procedures for maintaining blinding are described below and will be detailed in the study-specific procedures manual.

5.3 Study Product Supply and Distribution

The ALVAC-HIV (vCP1521) for this trial will be provided by Sanofi Pasteur and shipped to the NIAID Clinical Research Products Management Center (CRPMC). To preserve blinding, the products will be packaged by the NIAID CRPMC into sequentially numbered infant-specific kits. The sequential numbers (according to a computer generated randomization list) will be provided to the NIAID CRPMC by the HPTN Statistical and Data Management Center (SDMC). The number of kits supplied will be sufficient to accommodate the potential enrollment of additional infants to compensate for randomized infants who are not fully evaluable. Each kit will contain the complete set of vials of vaccine or placebo required for a single infant for all study immunizations. Study kits will be shipped to the Pharmacist of Record at the study site by the NIAID CRPMC. After final determination of infant immunization eligibility (Section 3.0), the next sequentially numbered kit will be assigned and associated with the infant's study ID number on site at the time of randomization. Each kit will be used to prepare immunizations only for the infant to whom it was assigned. A study pharmacist will be available at all times for preparation of study vaccine and placebo prior to administration.

5.4 Study Product Accountability

The site Pharmacist of Record is required to maintain complete records of all study kits and study products received from the CRPMC and subsequently dispensed. All unused study products must be returned to the CRPMC after the study is completed or terminated, unless otherwise instructed in writing. The procedures to be followed are in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* and in the study-specific procedures manual.

6.0 STATISTICAL CONSIDERATIONS

6.1 Overview

HPTN 027 is a Phase I randomized, double blind, placebo-controlled trial of ALVAC-HIV vCP1521 in infants born to HIV-1 infected Ugandan women with CD4 cell counts > 500 cells/ μ L.

Eligible infants will be randomized to receive ALVAC-HIV vCP1521 or placebo on or before Day 3 after birth, at 4, 8, and 12 weeks in a ratio of 4:1.

A total of 50 fully evaluable infants receiving all four scheduled study vaccinations is targeted. Infants who are excluded prior to assignment of a vaccine kit (the effective point of randomization) for any reason will not contribute to this number. A fully evaluable infant is defined as one who receives all four study vaccinations and is HIV uninfected at the 12-month timepoint. All infants who receive one or more study vaccinations will remain in the study for the full 24-month period of follow-up, even if terminated early from the vaccination schedule. However, an additional eligible infant may be enrolled for each infant that is not fully evaluable up to a maximum of 10 additional infants (for a total maximum of 60 participating infants), unless enrollment is stopped early due to the occurrence of unacceptable numbers or nature of toxicities. The decision to enroll an additional infant will be made by the Protocol Safety Review Team.

6.2 Study Endpoints

6.2.1 Primary endpoints

- **Safety:**
 - The primary safety endpoint is development of Grade 3 or higher adverse events attributable to the study product in each study group. Mild and moderate adverse events rates will also be tabulated.
- **Immunogenicity:**
 - Cell-mediated immunologic response as measured by HIV specific cytotoxic T lymphocyte activity against B *gag* and E *env* and/or by IFN-gamma assay responses to HIV antigens (*gag* and *env* overlapping peptides)
 - Humoral immunologic response as measured by detection of binding antibody to vaccine antigens by ELISA and by neutralizing antibody assay, if indicated by evidence of HIV antibody response to standard HIV-1 or binding antibody ELISA

6.2.2 Secondary Endpoints

- **Immunologic status:**
 - Immune status as measured by CD4 cell counts over the 24 month follow-up period in all immunized infants
 - Development of immunity to routine vaccination as measured by antibody levels to polio, tetanus, hepatitis B, HIB, and measles, and by monitoring BCG scar formation

6.3 Power Calculations

Statistical power calculations which assume a two-tailed Pearson Chi square test to detect a difference in toxicity rates between the treatment arm (P1) and the placebo arm (P2) (calculated for 1%, 5%, 10%, 15% and 20% placebo arm toxicity rates), with alpha levels (type 1 error) fixed at 0.05, reveal that to achieve a minimum of 80% power, true treatment group differences in rates

versus placebo of at least 57% would have to be observed (i.e., corresponding treatment arm rates would have to be greater than 42%, 51%, 60%, 67% and 73% respectively).⁶⁶ Similarly, comparisons of antibody and CTL responses will require at least a 57% group difference in positive response rates in the treatment arm versus the base comparison rates in order to achieve at least an 80% power to detect significantly different rates of immune response with a 2-sided 5% level test. Given the unknown reactogenicity in this age group and population, it is likely that the immunogenicity of the vaccine will not be statistically significant with this limited study size.

Safety will be assessed by comparing overall rates of grade 3 or 4 reactions attributable to receipt of the study product among those in the active agent and placebo groups as well as the rates of all adverse events, including those considered mild and moderate. With ALVAC vCP205 administration to Ugandan adults in HIVNET 007 and to infants in the US in PACTG 326, no severe (grade 3 or higher) adverse events attributed to receipt of the vaccine have been observed. There were only two vaccine-associated grade 3 adverse events (induration at week 6 and pain at week 2) in infants receiving ALVAC-HIV vCP1452. Among subjects receiving Canarypox vectors administered in AVEG Protocols 012A, 012B, and 022, 022A, 026, 029 and 202, there were infrequent (<10%) severe systemic and local reactions, meaning that to detect such differences, additional safety evaluation will be required in a phase II trial.

Immunogenicity (CTL, IFN- γ , binding and neutralizing antibody response) assessments will be completed on all participants to maintain blinding. HIV neutralization will be addressed by using log transformed titers as the analysis variable. CTL activity will be analyzed by looking for the presence or absence of HIV-specific CTL activity, as defined by CD8+ cell degranulation assays. The limited number of infants to be included in this phase I study may preclude adequately addressing this question if the vectors under study have an activity level lower than the study can detect.

The numbers of infants will be too small and the variability in infant response to standard immunizations will be too great to make statistically significant conclusions about the interaction between the HIV vaccines and the immune response to standard UNEPI vaccines. Only unexpected extremes of response (e.g., if all infants fail to develop protective antibody levels after vaccination) could be detected with this small number of infants studied.

Because of the difficulty of evaluating clinical disease progression in Uganda, evaluation of clinical/immunologic disease progression in HIV infected infants will primarily be based on mortality, HIV viral load by RNA PCR and CD4 cell counts at multiple time points. For CD4, the following criteria from the US Centers for Control and Prevention (CDC) definitions of immune suppression will be followed. If the CD4 absolute count and the CD4% result in different categories, the more severe category will be used.

For infants <12 months of age:

No evidence of suppression	CD4 \geq 1500/uL or \geq 25%
Moderate suppression	CD4 = 750-1499/uL or 15-24%
Severe suppression	CD4 < 750/uL or <15%

For infants 12 or more months of age:

No evidence of suppression	CD4 \geq 1000/uL or \geq 25%
Moderate suppression	CD4 = 500-999/uL or 15-24%
Severe suppression	CD4 < 500/uL or <15%

Analysis will employ the intent-to-treat principle in which all data from the enrolled participants will be analyzed according to the initial randomization assignment regardless of how many study vaccinations they receive. As enrollment and randomization are concurrent and do not take place until the infant's final eligibility has been determined and s/he is present at the clinic and ready to receive the first vaccination, it is anticipated that all randomized infants will receive at least one vaccination and will therefore contribute some safety data. All infants will also contribute to immunogenicity analyses; however, data from HIV-infected infants at or post infection will be excluded from the primary analysis.

6.4 Randomization and Blinding

In advance of the study, the HPTN SDMC will prepare randomization lists with computer-generated random numbers for use by the NIAID CRPMC in packaging of the study products. Blocked randomization will be used to ensure that the assignment sequence is not predictable and to ensure that the desired allocation between arms is maintained. Both the vaccine product and placebo product will be packaged in identical, sequentially numbered individual kits, each of which will contain a complete set of doses required for a single infant for all study immunizations. The same randomization scheme will be for all infants enrolled, including the 10 potential additional infants. Infants in both groups will be vaccinated according to the same immunization schedule (at 0, 4, 8, and 12 weeks) and have the same clinical and laboratory assessments.

After each infant's eligibility for vaccination is confirmed, the next sequentially numbered vaccine kit will be assigned to the infant and associated with his/her unique study ID number (assignment of the kit is the effective point of randomization). Additional procedures for maintenance of blinding are included in Section 5.0 and will be detailed in the study specific procedures manual.

Unblinding of participants and on-site study staff will occur after all infants have completed 24 months of follow-up, all protocol-specified lab assays and QA/QC activities have been completed and the data have been prepared for final analysis. In the event that the study is extended through a formal protocol amendment, mothers of infants who do not participate in the extension may be told the infant's treatment assignment when he/she has completed the original study follow-up period.

Interim safety and immunogenicity analyses may be performed to obtain an early assessment of the primary objectives and/or to inform planning for and design of future trials after a subset of infants prior to study completion, for example after 50% have been enrolled and received at least three study immunizations. Results will be provided at a group level only; no individual unblinding will occur.

7.0 ADVERSE EVENT REPORTING AND SAFETY MONITORING

7.1 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 does not necessarily 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. Mothers will be instructed to report any AEs their infants may experience after randomization to the study staff.

A serious adverse event (SAE) is any untoward medical occurrence that at any dose that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect (April 1996 International Conference on Harmonisation (ICH), Good Clinical Practice: Consolidated Guidance, (ICH E6). Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above may also be considered to be serious. (October 1994 ICH guidance (E2A), Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Note that for HPTN 027, congenital anomalies/birth defects are considered pre-existing conditions rather than AEs.

The Manual for Expedited Reporting of Adverse Events to DAIDS (referred to as the 'EAE Reporting Manual'), Version 1.0, dated 6 May 2004, will be followed for the duration of this study. Specifically, the 'intensive' level of reporting specified therein will be applied for the duration of each participant's follow period (from study enrollment until the participant completes the study or is terminated from the study for any reason). In addition, as a protocol-specific requirement, all grade 2 serious adverse drug reactions will also be reported in an expedited manner to DAIDS. For the duration of the study, all AEs that meet the criteria for expedited reporting to DAIDS must be documented on the DAIDS Expedited Adverse Event (EAE) Report Form and submitted to the DAIDS Safety Office within three business days of site awareness according to the procedures detailed in the EAE Reporting Manual. A copy of the EAE Report Form must also be sent at the same time to the Clinical Affairs Office at the SDMC.

Information on all AEs occurring in infants through 6 months of age (12 weeks after the last scheduled immunization), regardless of seriousness, severity or relatedness, will be recorded in the source documentation and on standard DataFax AE case report forms (CRFs) for entry into the study database. Thereafter, all illnesses will be recorded in the source documentation, however only serious AEs - regardless of severity or relatedness - and AEs that otherwise meet the criteria for expedited reporting to DAIDS will be reported on standard DataFax AE CRFs for entry into the study database. As noted above, adverse events that meet the criteria for expedited reporting to DAIDS will be reported according to the procedures specified in the EAE Reporting Manual throughout the duration of the study. Local and systemic reactogenicity events occurring within the first seven days after each study vaccination will be reported separately as adverse events (in addition to being reported on the Reactogenicity Assessment DataFax CRF) according to the procedures above if the criteria for expedited reporting to DAIDS are met; otherwise, these will be recorded only on the Reactogenicity Assessment DataFax CRF for entry into the study database.

These reporting procedures apply to all study infants, including those who discontinue the study vaccine early and remain in follow-up. There will be no active post-study reporting of adverse events; however, unexpected, serious adverse drug reactions must be reported if the study site staff become aware of the events on a passive basis, i.e., from publicly available information.

The study drug in HPTN 027 is the vaccine regimen begun in infants on or before Day 3 after birth (ALVAC-HIV vCP1452/Placebo); therefore it is the relationship of all AEs to this product that is to be considered in determining the reporting requirements for each AE (e.g., whether the AE must be reported in an expedited manner to DAIDS). Conditions or illnesses in infants occurring before randomization will be reported as pre-existing conditions (not as adverse events), including congenital anomalies. The severity of all adverse events will be graded in a standard manner as described in Section 4.6. The investigator or designee will assess the relationship of all AEs to the study product based on guidance provided in the EAE Reporting Manual, the Investigator's Brochure, and his/her clinical judgment. Both the EAE Manual and the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, dated December 2004, will be provided in the study-specific procedures (SSP) manual and are available at the following website: <http://rcc.tech-res-intl.com>. Any exceptions to the reporting criteria above or procedures outlined in the EAE Reporting Manual must be approved by the sponsor and provided to the US FDA and the IRBs/ECs in advance of implementation.

Information on all AEs included in the study database will be included in annual reports to the US FDA, and other applicable government and regulatory authorities. The investigators will report information on AEs and SAEs to the responsible Institutional Review Boards/Ethics Committees in the US and in Uganda in accordance with applicable regulations and individual IRB/EC requirements.

7.2 Safety Monitoring

Participant safety is of paramount importance to the HPTN. A multi-tiered safety review process consistent with that employed in DAIDS-sponsored adult vaccine trials will be followed for the duration of this study. The review process includes several levels of evaluation by various Network members and groups. This process, which is both timely and extensive in scope, includes review of medical history information, laboratory values, vaccination reactogenicity events, adverse events and concomitant medications. The study site investigators are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the Protocol Chairs and PSRT if unexpected concerns arise. The US Protocol Chair or designee will also review any Grade 3 or higher adverse event, regardless of relatedness. Participant safety is also monitored at the Network level through a series of routine reviews conducted by the SDMC Clinical Affairs staff, a Protocol Safety Review Team and the sponsor. Additional special reviews may also be conducted at each of these levels as dictated by the occurrence of certain events.

HPTN SDMC Clinical Affairs staff will review incoming safety and reactogenicity data on an ongoing (daily and weekly) basis. Values identified during review that are considered questionable, inconsistent, or unexplained will be queried for verification. AE reports submitted in an expedited manner to the DAIDS Safety Office (with a copy to the SDMC) will be forwarded upon receipt to the DAIDS Medical Officer and Safety Specialist for immediate review.

The Protocol Safety Review Team (PSRT) will convene routinely - at least every other week during the vaccination phase and approximately monthly thereafter - to review clinical and laboratory data reports (blinded by study treatment) generated by the HPTN SDMC. The PSRT will include the Protocol Chairs, the DAIDS Medical Officer for the study from the Prevention Sciences Branch, the Protocol Statistician, a Sanofi Pasteur representative, the National Institute of Child Health and Development (NICHD) Medical Officer and/or a DAIDS Vaccine Sciences Branch representative. 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. In addition to the routine safety data reviews, the PSRT will convene on an ad hoc basis to make decisions described above.

If one infant death judged to be probably not, possibly, probably or definitely related to the study product occurs or if two or more infants experience the same Grade 3 or higher adverse event judged to be possibly, probably or definitely related, further randomization/vaccination of infants will be suspended pending immediate review of all relevant safety data by the PSRT. Decisions regarding permanent discontinuation of study vaccines in individual infants and in the study overall will be made by the PSRT based on careful review of all relevant data and may involve sponsor consultation with the US FDA. The PSRT will also make decisions about whether to enroll additional infants for those who are not fully evaluable. If at any time, a decision is made to discontinue vaccination in an individual infant or in all participants due to toxicity, DAIDS will notify the US FDA and the Protocol Chairs will notify the responsible IRBs/ECs expeditiously.

8.0 HUMAN SUBJECTS CONSIDERATIONS

8.1 Institutional Review and Informed Consent

This protocol and the site informed consent documents and any subsequent modifications will be reviewed and approved by the sponsor and the Institutional Review Boards/Ethics Committees responsible for oversight of the study with respect to scientific content and compliance with applicable research and human subjects regulations prior to implementation.

Subsequent to initial review and approval, the responsible local Institutional Review Boards/Ethics Committees (IRBs/ECs) will review the study 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 the site. All changes to the informed consent forms must be approved by the responsible IRBs/ECs prior to implementation. All protocol amendments and changes to the consents forms associated with a protocol amendment must be approved by the sponsor (DAIDS) and the IRBs/ECs before implementation.

Thorough informed consent will be emphasized. Written informed consent will first be obtained from mothers for initial screening. The study will then be thoroughly explained to all women who are found eligible based on those initial screening tests. Before a mother is asked to sign a consent form for the screening and enrollment of the infant, she will be administered an assessment of understanding questionnaire which she must successfully complete. A copy of the consent forms

will be offered to the mother. Mothers will be encouraged to bring the father to the study clinic to participate in the consent process. If he is reasonably available at the study clinic, the study will also be thoroughly explained to the father and his written informed consent obtained; however, the father's written consent is not required for enrollment of the infant, unless otherwise directed by the IRB/EC. After the infant is born and his/her final eligibility is ascertained, the mother's consent will be verbally re-confirmed before enrollment and randomization of the infant. This will be documented in the infant's study records.

8.2 Subject Confidentiality

All study-specific records will be stored securely with access limited to authorized personnel only. Study-specific laboratory specimens, case report forms or documents that are transferred or transmitted off-site for processing will be identified by a coded number only, to maintain participant confidentiality. All local databases must be secured with password-protected access systems. The use of participant identifiers on study records will comply with the DAIDS SOPs for Source Documentation and Essential Documents.

Individual clinical information will not be released without the written permission of the participant, except as necessary for monitoring by the study sponsor (the U.S. National Institute of Allergy and Infectious Diseases), representatives of their authorized contractors (the DAIDS Clinical Site Monitoring Contractor (CSMC), the HPTN Coordinating and Operations Center (CORE), the HPTN SDMC, the HPTN CL, the US FDA, the vaccine manufacturer (Sanofi Pasteur), the IRBs/ECs and/or the Ugandan Ministry of Health.

8.3 Incentives

With prior approval of the IRBs, participants will be reimbursed only time and travel to scheduled study visits. This incentive has been used in previous studies at this site. Because the amount will vary according to transportation costs, the specific amount will not be stated in the informed consent to avoid misunderstanding. Participants also receive an elevated level of medical care through their study participation and receive free laboratory diagnostic testing, radiological examinations, and medications for illnesses.

8.4 Study Discontinuation

The study may be discontinued at any time by NIAID, the pharmaceutical sponsor, the US FDA, the IRBs/ECs, or the Ugandan government.

8.5 Access to HIV-Related Care

8.5.1 HIV Counseling and Testing

HIV pre-test and risk reduction counseling is routinely provided to all antenatal clinic attendees at Mulago hospital. Free HIV rapid testing and individual post-test counseling are provided routinely to all women who consent to undergo HIV screening (outside of the research project). As standard of care, all women identified as HIV infected receive counseling on the prevention of mother to child HIV transmission (PMTCT) including infant feeding counseling based on WHO

and Uganda Ministry of Health guidelines, and are offered an ARV regimen (e.g., the HIVNET 012 Nevirapine regimen) free of charge. Once identified as HIV infected by Mulago PMTCT program staff, pregnant women are referred to study staff to determine their eligibility for and potential interest in participating in this or other research studies. HIV counseling is provided in accordance with local Ministry of Health guidelines for counseling of HIV infected pregnant women with special reference to the prevention of mother to child transmission. Standard counseling about HIV transmission risks, condom use and safe sexual practices will be provided by antenatal clinic counseling staff and reinforced during study counseling sessions by study staff. In accordance with the policies of the US National Institutes of Health, participants must receive their HIV test results to take part in this study.

8.5.2 Care for Participants Identified as HIV-Infected at Screening

In addition to participation in the PMTCT program and as standard of care, antenatal clinic counseling staff routinely refer all women found to be HIV-infected on antenatal screening to available sources of medical and psychosocial care including but not limited to the Mulago HIV clinic (Infectious Disease Institute, Academic Alliance for AIDS Care in Africa), the Joint Clinical Research Center, the AIDS Information Center, Mildmay, and the AIDS Support Organization (TASO). A subset of HIV infected women are eligible to receive expanded ongoing HIV care and support including ARV through the pilot MTCT Plus Program, sponsored by Columbia University and run by staff of the Makerere University-Johns Hopkins University (MUJHU) Research Collaboration (where the study will be conducted). Standard access to free condoms, treatment for sexually transmitted infections, and treatment for illnesses will be provided by clinic staff for all women attending the antenatal clinics according to Mulago Hospital and MOH guidelines.

8.5.3 Provision of Antiretroviral Therapy (ARV) to HIV-infected Women and Children

Resources are not available through this trial to provide ARV to all HIV-infected women and children, nor would the funds be available for continuing therapy and monitoring after completion of the trial. The women participating in this trial will all have CD4 cell counts greater than 500 cells/ μ l, so they would not be eligible for ARV according to Uganda MOH guidelines during the trial period.

However, the MU-JHU Collaboration is committed to expanding access to antiretroviral treatment to all the HIV infected children and women seen at the study site. There has been an ongoing effort to develop programs at Mulago Hospital that will provide wider access to triple antiretroviral therapy. The MTCT plus program is expanding to provide family centered HIV care and support to more women identified as HIV infected in the Mulago PMTCT program. ARV care for this program is provided at the study site, and all women participating in HPTN 027 will also be referred to this program. In addition, there is an Elizabeth Glaser Pediatric AIDS Foundation funded program at MUJHU to provide ARV to HIV infected children from the research studies and free HIV care and ARV are available at the Mulago Pediatric HIV clinic. Therefore, any children found to be HIV infected during this trial would be referred to one of these programs for their ongoing HIV care.

In addition, the MU-JHU Collaboration investigators are working with the Academic Alliance for

AIDS Care in Africa, the US CDC, and other organizations to develop an HIV treatment facility where HIV infected adults and children in Kampala could receive subsidized antiretroviral therapy and high quality medical care. All study participants will be referred to these centers for treatment of their HIV infection outside of research protocols, as is typically done in the US. This is the ideal option, as limiting treatment for only infected children participating in certain clinical trials would pose a number of ethical problems. For example, it would not seem ethical that some of the infected children seen in the clinic receive free antiretroviral treatment whereas other infants, including siblings who were not in the trial, would not. Providing treatment for women in trials in the absence of provision of treatment for their male partners and other family members would be difficult.

8.5.4 Care Provided for HIV-infected Women and Their Children in the study

HIV infected women and their children participating in trials at the study site currently receive a number of therapeutic benefits including care for their HIV-associated and all other illnesses. For example, all women and children receive free immunizations and diagnosis and treatment for infections, including pneumonia, malaria, tuberculosis, and all other illnesses. HIV infected participants are provided Bactrim prophylaxis to prevent pneumocystis pneumonia and bacterial infections. In addition, children with growth faltering or failure to thrive receive nutrition education and counseling as well as small nutritional supplements such as soya, sugar, rice, beans etc to take home with them. All study women or children who require admission to the hospital receive close monitoring and follow-up, with provision of supplies (needles, syringes, catheters etc) and medications that may not be available in the hospital as well as a daily allowance to cover costs they may incur while hospitalized. Consequently, we believe that the women and children in our trials receive the best standard of care available.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Local Laboratory Specimens

Routine laboratory testing for hematology (CBC, differentials, CD4), serum chemistries (SGPT, bilirubin, creatinine) and DNA/ RNA PCR will be performed at the Makerere University-Johns Hopkins University Collaborative Core Laboratory (MU-JHU Corelab) at the Infectious Disease Institute of Makerere University. CTL and IFN- γ assays will be performed on fresh specimens (except baseline CTL assays which may be performed on cryopreserved cord PBMC) at the CTL lab at the Joint Clinical Research Center in Kampala. Binding and neutralizing antibody assays will be performed at the Uganda Virus Research Institute (UVRI) in Entebbe.

Cytotoxicity will be measured by degranulation flow cytometry-based assays. These assays measure CD107 surface expression⁶⁷ and provide more sensitive functional vaccine specific CD8+ T cytotoxic activity. DNA and RNA PCR testing will be performed by RT PCR using Roche AMPLICOR Monitor assays.

Each study laboratory will adhere to standards of good laboratory practice, the HPTN Manual of Laboratory Operations (distributed in hard copy to the study site by the CL); and local standard

operating procedures for proper collection, processing, labeling, transport, and storage of specimens to the local laboratories. Specimen collection, testing, and storage at the local laboratories will be documented using the HPTN Laboratory Data Management System (LDMS). All specimens will be shipped in accordance with International Air Transport Association (IATA) specimen shipping regulations.

9.2 Central Laboratory Specimens

Antibody testing to standard UNEPI vaccines will be performed through the HPTN Central Laboratory at JHU; either in laboratories at JHU or in JHU-contracted commercial laboratories. Tetanus antitoxoid, HIB, Hepatitis B, and measles antibody testing will be performed by enzyme immunoassay. Poliovirus (type 1,2,3) antibody will be determined by neutralization assay.

HLA typing and confirmatory immunology assays (if sufficient specimens are available) will be performed on cryopreserved cells at the Viral and Rickettsial Disease Laboratory of the California Department of Health in Richmond, California.

9.3 Quality Control and Quality Assurance Procedures

The HPTN Central Laboratory (CL) at JHU has established a proficiency-testing program at the study site and will be responsible for the oversight of quality control programs for all safety and HIV diagnostic laboratory evaluations for this study. The MU-JHU Corelab has College of American Pathology (CAP) certification and will be responsible for implementation of quality control programs for all safety and HIV diagnostic labs for this study. The CL at the Viral and Rickettsial Disease Laboratory of the California Department of Health in Richmond, California will be responsible for the training and oversight of quality control programs for all immunogenicity-related laboratory testing at both the JCRC and the UVRI. CL staff will also conduct periodic visits to the 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 staff 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, the HPTN SDMC will select a random sample of stored specimens to test for quality assurance (QA) purposes. Plasma/serum samples from all infants identified by the site as HIV infected and an equal number of randomly selected uninfected infants will be retested by the HPTN CL. In the event of any false positive or any false negative result that changes the endpoint infection status of the participant, a sample from the last visit from all participants will be retested. In addition, immunology assays may be repeated at the Viral and Rickettsial Disease Laboratory of the California Department of Health in Richmond, California, for quality assurance purposes.

The HPTN SDMC or CL will inform site staff of the samples selected for quality assurance testing, and site staff will ship the selected specimens to the CL.

9.4 Specimen Storage

Study site staff will store all specimens collected in this study at least through the end of the study and completion of all study relevant immunologic, safety and quality assurance testing. Any remaining specimens will be destroyed after the study team, the sponsor, the US FDA or other regulatory bodies have determined that all study related testing has been completed and that there will be no further specimen requirements.

9.5 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 US CDC. The recommendations may be found at the following website: <http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>.

10.0 ADMINISTRATIVE PROCEDURES

10.1 Study Activation

Following ethical review and approval, the study site will submit required documentation as listed in the DAIDS Protocol Registration Manual to HPTN CORE. CORE staff will work with study site staff and complete protocol registration in accordance with DAIDS procedures. Included in this step will be CORE and DAIDS review of the site-specific study informed consent forms.

Upon successful protocol registration and completion of all other study-specific site activation requirements as detailed in the HPTN Manual of Operations and the Study-Specific Procedures (SSP) Manual, the CORE will issue a study activation notice to the site. Implementation of the study may not proceed prior to receipt of this written notification.

10.2 Study Coordination and Oversight

This protocol will direct study implementation. In addition, a study-specific procedures (SSP) 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 posted on HPTN website (<http://www.HPTN.org>), which is publicly accessible, and submitted to the sponsor prior to implementation of the study and will be made available in hard copy to the IRBs/ECs, the US FDA and other regulatory authorities upon request.

Close cooperation between the Study Chairs, site investigators and staff, NIAID Medical Officer, HPTN CORE Protocol Specialist, SDMC Protocol Operations 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. Routine telephone conference calls will be held in which general site operations, participant accrual, and operational issues and toxicity data will be discussed.

Case report forms will be developed by the protocol team and the HPTN SDMC. Data will be transferred to the SDMC using DataFax for quality control checking, pooling and analysis. Quality control reports and queries will be routinely sent back to the site for verification and resolution of inconsistencies. All physical case report forms and other individual study related documents will remain securely stored on site. Site investigators will have access to data files maintained by the SDMC. Data processing and management procedures will be included in the study specific procedures manual.

Procedures for specimen collection, preparation, processing, testing and shipping will be included in the SSP manual. Oversight and direction for laboratory procedures, including QA/QC, will be provided by the HPTN Central Laboratory at Johns Hopkins University and the Viral and Rickettsial Disease Laboratory of the California Department of Health.

In addition to the close oversight of the Study Chair, NIAID Medical Officer and other members of the protocol team, the HPTN Study Monitoring Committee (SMC) will monitor the study regularly with a focus on issues relating to quality of trial conduct including rates of recruitment, adherence to study treatment and visit schedules, and retention. As described in Section 7.2, a Protocol Safety Review Team will closely monitor study safety data on a routine basis.

10.3 Study Site 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, Sanofi Pasteur, and US and in-country government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.4 Protocol Compliance

The study will be conducted in full compliance with the protocol and according to Good Clinical Practice. All protocol amendments must be submitted to and approved by DAIDS and the relevant IRB(s) prior to implementation.

10.5 Investigator Records

The Investigator will maintain, and store in a secure manner, complete, accurate and current study

records throughout the study. In accordance with US Federal regulations, the Investigator will retain all study records for at least two years following the date of marketing approval for the study product for the indication that was studied. If no marketing application is filed, or if the application is not approved, the records must be retained for two years after the US FDA is notified that the IND is discontinued. 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 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 protocol specified source documents).

10.6 Use of Information and Publications

Publication of the results of this trial will be governed by DAIDS and HPTN policies. Any presentation, abstract, or manuscript must be submitted to the HPTN Manuscript Review Committee and made available to DAIDS and Sanofi Pasteur for review prior to submission.

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Appendix I-A: Schedule of Maternal Evaluations

Evaluation/Procedure	Screening (third trimester of pregnancy)	After Delivery (Prior to infant enrollment)
Screening Informed Consent	X	
Assessment of Understanding (prior to enrollment consent)	X ¹	
Informed Consent for Infant Screening and Enrollment	X ^{1,2}	
Targeted History	X	X
Targeted Physical examination	X	X
Hematology (CBC with differential)	X	
CD4 cell count	X	
HIV-1 WB or DNA PCR	X	
Quantitative HIV RNA PCR (plasma storage for later testing only if infant enrolled)	X	
DBS, plasma, and PBMC for storage	X	

1 If found eligible during initial screening

2 If assessment of understanding questionnaire successfully completed

Appendix I-B: Schedule of Neonatal Evaluations

Month	0	1	2	3	5	6	9	12	15	18	21	24
Week	Birth (≤3 days after)	2	4	6	8	10	12	14				
Standard UNEPI immunization ¹	X (B,P)		X (P,D)		X (P,D)		X (P,D)		X (M)		X (M)	
Study vaccinations	X	X		X		X						
CLINICAL												
History	X											
Physical exam	X											
Targeted interim history		X	X	X	X	X	X	X	X	X	X	X
Targeted physical examination		X	X	X	X	X	X	X	X	X	X	X
Post immunization monitoring ²	X		X		X		X					
LABORATORY												
SAFETY												
Hematology (CBC, diff)	X	X		X		X		X		X		X
CD4 count		X		X				X		X		X
Chemistries (ALT [SGPT], bilirubin, creatinine)	X	X		X		X		X		X		
Dried blood spot, specimen storage, if possible	X	X		X		X		X		X		X
IMMUNOLOGY												
CTL	X ⁴							X		X ⁵		X ⁵
IFN-γ	X ⁴					X				X ⁵		X ⁵
HLA typing	X ⁴											
Binding antibody ELISA						X		X		X		X
Neutralizing Ab												X ⁶
Measles Ab												X
Polio, Tetanus, Hep B, HIB Ab ⁷									X			
VIROLOGY												
HIV DNA PCR (RNA PCR) ³	X	X		X		X		X		X		X
Rapid HIV test and WB												X

¹B = BCG; P = Polio; D = DTP+HIB+HepB; M = Measles

² Post-immunization monitoring includes observation for at least 1 hour after immunization, clinic assessment on Day 1 and a clinic or home assessment by a trained clinician on Day 2 following immunization.

³ Quantitative HIV-1 RNA PCR may be used if HIV-1 DNA PCR is not available. If positive, DNA or RNA PCR (for diagnosis confirmation) to be performed on a subsequent specimen; if confirmed, plasma to be obtained at each subsequent scheduled blood draw for quantitative RNA PCR in place of repeat DNA PCR testing. Study immunizations will be stopped in those found to be infected after enrollment/randomization.

⁴ From cord blood; obtain 5 ml of peripheral blood at the week 4 visit only if cord blood is not obtained. HLA typing will be performed using DNA extraction.

⁵ CTL/ IFN-γ may or may not be performed depending on results of earlier evaluations, to be determined by study team.

⁶ From stored plasma if evidence of HIV antibody response is present by rapid HIV-1 test.

⁷ If sufficient specimen is not available for all antibody tests, priority should follow the order listed above.

The amount of blood that can be obtained from infants is expected to be limited (approximately 1-5 ml per visit during the first 6 months and 5-15 ml per visit afterward). Therefore, priority for laboratory tests follows:

1. Hematology, including CD4
2. Chemistries
3. DNA PCR
4. CTL
5. IFN-γ assay
6. binding antibody ELISA
7. Neutralizing antibody

Appendix II-A Sample Informed Consent for Maternal Screening

SAMPLE INFORMED CONSENT FORM FOR SCREENING OF MOTHERS

HPTN 027: A PHASE I STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF ALVAC-HIV vCP1521 IN INFANTS BORN TO HIV-1 INFECTED WOMEN IN UGANDA Version [x.x], Dated [xx xx xx]

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Introduction

You are being asked to take part in screening tests to determine if your infant will be able to take part in the research study named above because you are HIV infected and in the last trimester of pregnancy. The research study will test an experimental vaccine against the human immunodeficiency virus (HIV) in infants. HIV is the virus that causes AIDS. The research study is sponsored by the US National Institutes of Health. The person in charge of the study at this site is Professor Francis Mmiro.

Before you decide if you want to participate in the screening tests, we would like to explain the purpose, the risks and benefits of participating, and what will be expected of you if you decide to participate. This informed consent form gives you information about the screening tests. This information will also be discussed with you. You are free to ask any questions. After the screening has been fully explained to you and if you agree to participate, you will be asked to sign this consent form or make your mark in front of a witness. You will be offered a copy of this form to keep.

What should you know about screening for the research study?

- Your participation in the screening is entirely voluntary.
- You may decide not to take participate in the screening tests or to withdraw from the screening at any time without losing the benefits of your standard medical care.
- If you decide not to participate in the screening, you and your baby cannot participate in this research study, but you can still join another research study later, if one is available and you qualify.
- Even if you agree to participate in the screening, it does not mean you have agreed to participate in the research study.
- Since the study relates to your HIV infection, we will explain what other programs or treatment are available outside the research.

Why is this research being done?

This research is being done to test an experimental vaccine against HIV in infants born to HIV infected Ugandan women. The vaccine in this study is called ALVAC-HIV vCP1521. The purpose of this study is to see if the HIV vaccine is safe in babies who are born to mothers with HIV. We also want to see how an infant's body reacts to the vaccine. There is a system in the body that makes special cells and materials that protect you from getting sick when germs get into your body. Vaccines are used to make this body system active in fighting certain germs to try to prevent a person from getting an infection with that germ. Many people are working very hard to try to find a vaccine that will work to fight against HIV infection. So far, no vaccine has been found that stops someone from getting HIV. There are studies that are being done to test several different HIV vaccines, including the one that will be used in this study, to see if they will work to prevent HIV infection.

Pregnant women who have HIV will participate in screening tests, but their babies will be the ones who join the study and get the study HIV vaccine after they are born. The purpose of the screening is to see if your baby will be able to participate in the study. The HIV vaccine to be tested in the study is experimental. This means it has not been approved by the Uganda National Drug Authority or the US Food and Drug Administration for general use and may first be used only in research with a small number of infants to make sure that the vaccine is safe and to see how it works.

If your baby is able to participate in the study, he or she will be put in one of two groups. Infants in one group will get the experimental HIV vaccine and infants in the other group will get the placebo injection that contains only salt water. About 40 infants will be in the experimental HIV vaccine group and 10 will be in the placebo group. Which group your infant is assigned to will be decided by chance (like a lottery). Your baby's chances of being in the experimental vaccine group instead of the placebo group are 4 to 1. Neither you nor the study staff will know which group your infant was assigned to until all of the infants have finished the study.

What will happen if you agree to the study screening?

The study staff will ask you some questions about your health and pregnancy, review your antenatal record, and do a physical examination. The study staff will draw about 2 teaspoons (10mls) of blood from you. Your blood will be tested to confirm your HIV status, to check to see if you have low blood counts, and to check to see how strong your body's system to fight infections is. You will be asked to return to the clinic to get the results of these blood tests. These screening tests are the first step in determining if your baby will be able to join the HIV vaccine study. Some infants will not be able to join the study because of information learned during the screening tests. We will not know for certain if your baby will be able to participate in the study until after he or she is born. If the screening tests show that your baby is not able to participate in the vaccine study, or if you choose not to have your baby participate, any leftover blood taken from you for the screening tests will be destroyed. If these first screening tests show that your baby may be able to join the study, more detailed information about the study will be given to you. You are encouraged to bring the baby's father to the study clinic so that we can explain the study to him also. You will be able to decide if you want your baby to join the experimental HIV vaccine study or not. You will be asked to come to Mulago Hospital for your delivery. After the

study has been fully explained to you, and you have asked all of your questions, the study worker will ask you some questions about the study to be sure that you understand. If you agree to have your baby participate in the experimental HIV vaccine study, you will be asked to sign another consent form after the study has been fully explained to you and you have asked all of your questions.

What are the risks of study screening?

Taking blood from you may cause slight pain, swelling, and bruising at the place where the blood is taken. Drawing blood can also cause fainting or infection, but this is rare. If you are screened for this study, some hospital and study staff will know that you have HIV. The study doctors and staff will protect information about you and your participation in these screening tests to the best of their ability. On your screening records, a code will be used instead of your name. Only the study staff will know this code. Study staff will make every possible effort to be sure that others do not learn your HIV status. However, sometimes if you receive special treatments or attend a special clinic, it may make others wonder if you have HIV.

What are the possible benefits of study screening?

These screening tests may or may not be of direct benefit to you. The results of the screening tests will be shared with the medical staff providing your antenatal care at this clinic and may help them know more about what care you need. They may refer you for additional care if they find that your body's system for fighting infections is weak.

What could make us take you out of the screening early?

You will be withdrawn from the screening if at any time the screening tests show that your baby is not able to participate in the study. You may also be withdrawn from the screening if the study is canceled by the study sponsor, the Ugandan Ministry of Health, the Ethics Committees overseeing the research, the U.S. Food and Drug Administration, or the company that makes the study vaccine.

What are the choices if you do not want to be screened for the study?

You do not have to agree to be screened for this research study. If you do not agree to the screening, your care at Mulago Hospital will not be affected. However, your baby will not be able to join the HIV vaccine study. If you agree to take part in the screening, you can change your mind at any time without losing the benefits of your standard medical care.

At this clinic, there is a special program for all pregnant women who are infected with HIV. You are advised to follow the Mulago Hospital program for HIV infected women whether or not you decide to be screened for these studies.

What about confidentiality?

The study doctors and staff will protect information about you and your participation in these screening tests to the best of their ability. On your screening records, a code will be used instead of your name. Only the study staff will know this code. The study staff will not give out any information that identifies you without your written consent. However, the Ugandan Ministry of Health, the U.S. Food and Drug Administration, the company that makes the study vaccine, the

study sponsor (the U.S. National Institutes of Health), and their authorized representatives will be allowed to inspect your screening records.

Will there be any costs or payments to you?

The screening procedures, physical examinations and blood tests will be done free - at no cost to you - but you will not receive any payment for having the screening tests done.

Antiretroviral treatment for HIV will not be provided through this study, however, study staff will refer you to available care and treatment programs (outside of the study) for which may you qualify.

What happens if you are injured during the screening?

Medical care will be provided for illness or injury directly related to the screening at no cost to you. Care or appropriate referral will be provided for any illness or injury that occurs during the screening that is not directly related to the screening, 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.

You will not give up your legal rights by signing this informed consent form.

What should you do if you have problems or questions about the screening?

If you ever have questions about this screening or if you have a study related medical problem or injury, you should contact the study investigator, Professor Francis Mmiro at the Makerere University-Johns Hopkins University Research Collaboration, Upper Mulago Hill Road. You may also ring Professor Mmiro at 041-541044.

If you have questions about your rights or your baby's rights as a research volunteer, you may contact [name and contact information of IRB member] Dr. _____ is a member of the Uganda AIDS Research Committee, one of the Ethics Committees that oversees the conduct of AIDS research in Uganda.

STATEMENT OF CONSENT

I have read (or someone has read and explained to me) this consent form. I understand the purpose of the screening, the procedures to be followed, and the risks and benefits as described in this consent form. I voluntarily agree to be screened for my baby's potential participation in the HIV vaccine study.

Participant's Name (print)

Participant's Signature or
Thumb Print

Date

For all volunteers: I have explained the purpose of the screening to the volunteer and have answered all of her questions. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study screening.

Name of person obtaining consent
(print)

Signature

Date

For illiterate volunteers: I attest that the information contained in this written consent form has been read and explained to the participant. She appears to understand the purpose, procedures, risks and benefits of the study screening and has voluntarily accepted to participate in this screening.

For those placing thumbprint **only:** I attest that the participant who states that her name is

_____ has placed her thumbprint on this consent form of her own free will
on this day: _____.

Name of witness to consent process
(print)

Witness' Signature

Date

Appendix II-B Sample Informed Consent for Screening and Enrollment of Infants

SAMPLE INFORMED CONSENT FORM FOR SCREENING AND ENROLLMENT OF INFANTS

HPTN 027: A PHASE I STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF ALVAC-HIV vCP1521 IN INFANTS BORN TO HIV-1 INFECTED WOMEN IN UGANDA Version [x.x], Dated [xx xx xx]

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Introduction

You are being asked to allow your baby to participate in the research study named above because you are HIV-infected and pregnant and because you had screening tests done that show that your baby may be able to participate in the research study. The study will test an experimental vaccine against the human immunodeficiency virus (HIV) in infants. The research study is sponsored by the US National Institutes of Health. The person in charge of the study at this site is Professor Francis Mmiro.

Before you decide if you want your baby to participate in the study, we would like to explain the purpose of the study, the risks and benefits of participating, and what is expected of you and your infant. This informed consent form gives you information about this study. This information will also be discussed with you. You are free to ask any questions. After the study has been fully explained to you, and if you agree that your baby can participate in the study, you will be asked some questions to determine whether you fully understand what is involved and the risks and benefits of participating. You will be asked to sign this consent form or make your mark in front of a witness. You will be offered a copy of this form to keep. You are encouraged to bring the baby's father to the study clinic so that we can also explain the study to him. If the father is available and comes to the study clinic to participate in the informed consent discussion, he will be asked to sign the consent form also.

If you qualify and agree to join the trial, your baby will have additional screening tests soon after birth to see if he or she can participate in the study. Some infants will not be able to participate in the study because of information learned during the screening.

What should you know about the research?

- Your participation in the study is entirely voluntary.

- You may decide not to take part in the study or to withdraw your baby from the study at any time without losing the benefits of your or your baby's standard medical care.
- If you decide not to have your baby participate in the study, you can still join another research study later, if one is available and you and/or your baby qualify.
- Since the study relates to your HIV infection, we will explain what other programs or treatment are available outside the research.

Why is this research being done?

This research is being done to test an experimental vaccine against HIV in infants born to HIV infected Ugandan women. HIV is the virus that causes AIDS. The vaccine in this study is called ALVAC-HIV vCP1521. Pregnant women who have HIV are screened for the study, but their babies will be the ones who are enrolled in the study and get the study HIV vaccine after they are born.

The purpose of this study is to see if the HIV vaccine is safe in babies who are born to mothers with HIV. We also want to see how an infant's body reacts to the vaccine. There is a system in the body that makes special cells and materials that protect you from getting sick when germs get into your body. Vaccines are used to make this body system active in fighting certain germs to try to prevent a person from getting an infection with that germ. Many people are working very hard to try to find a vaccine that will work to fight against HIV infection. So far, no vaccine has been found that stops someone from getting HIV. There are studies that are being done to test several different HIV vaccines, including the one that will be used in this study, to see if they will work to prevent HIV infection.

This study is just a first step in testing the HIV vaccine in infants. This HIV vaccine is experimental. This means that we do not know if this vaccine can protect your baby from getting HIV. It also means that first the vaccine may be used only in research with a small number of infants to make sure that the vaccine is safe and to see how it works. Then later we may be able to test the vaccine in many more infants to see if it can prevent HIV infection. The long-term goal is to find a vaccine to protect infants born to HIV infected mothers from getting HIV through breastfeeding. This experimental HIV vaccine has not been approved by the Uganda National Drug Authority or the US Food and Drug Administration for general use. However, they have allowed use of the vaccine in this study.

The vaccine we will use in this study has been tested in adults in Thailand, a country in Asia. It was found to be safe in those tests. Similar HIV vaccines have been tested in adults and infants in the U.S. and adults in France, Thailand, and Uganda and have been found so far to be safe.

This vaccine is NOT made from live HIV virus or from blood or other parts of people who have HIV. There is NO chance the vaccine contains the HIV virus. This means there is no chance your infant can get HIV or AIDS from this vaccine.

About fifty infants at this clinic will be included in the study. The total time each infant will be in the study is two years, but the vaccines are only given four times during the first three months of life. In this study, the study vaccine will be given to infants by injections in the thigh. Some infants (about 10) will receive a placebo injection instead of the experimental HIV vaccine. The placebo injection is an inactive substance (made of salt water) that has no vaccine or other

medicine in it. We will compare the response of infants who get the placebo injection to those who get the experimental HIV vaccine.

What will happen if you agree that your baby can participate in the study?

If you agree that your baby can be in this study, these are the things that will happen. After all of the study procedures and risks and benefits have been explained to you, you will be asked some questions about the study to be sure that you fully understand what is involved. If you agree that your baby can participate in the study and you sign this consent form, a home visitor will go with you to your home. It is important for the home visitor to know where you live so she can help remind you of clinic visits if you are not able to come to the clinic when you should and because study staff will visit your baby at home after each study vaccination.

We will ask you to come to Mulago Hospital for delivery. After delivery, you and your baby will have a physical exam and your baby will have some blood tests for a final check to see if he or she is able to participate in the study. A sample of blood from the placenta will also be taken at this time. Even if you qualify and agree to have your baby join the study, we will not know for certain whether your baby can participate in the study until after the final screening tests are done. If your baby is not able to participate in the study, you will continue to receive the standard postnatal care given at this clinic.

If your baby is able to participate in the study, he or she will be put in one of two groups. Infants in one group will get the experimental HIV vaccine and infants in the other group will get the placebo injection that contains only salt water. About 40 infants will be in the experimental HIV vaccine group and 10 will be in the placebo group. Which group your infant is assigned to will be decided by chance (like a lottery). Your baby's chances of being in the experimental vaccine group instead of the placebo group are 4 to 1. Neither you nor the study staff will know which group your infant was assigned to until all of the infants have finished the study.

Your infant will have four study vaccinations during the first three months of the study. The first study vaccination will be given within 3 days of your baby's birth. The other injections will be at about 1 month, 2 months and 3 months of age. Your infant will be watched in the clinic for at least one hour after each study vaccination. You will be asked to bring the baby to the clinic the day after each study vaccination, and a study clinician will visit your baby at home on the second day after each study vaccination to check on his/her health. You will be asked to report any side effects to the study staff right away. If your infant has symptoms, the study staff may ask you to bring your baby to the clinic for extra visits. It is very important that you follow the instructions the study medical staff gives you. If you give or want to give your infant any non-study drugs or treatments, you should report this to the study staff. If you wish to join or to have your baby join another research study, you should inform the study staff at this clinic.

Your infant will have about 20 clinic visits during the 2-year study, including a visit the day after each study vaccination. At each visit, your infant will be checked for any new signs and symptoms since your last visit. You will also be asked how your infant is doing, how you are feeding your baby, and about any drugs the baby has been given. Study staff will draw blood from your baby at about 8 of these visits for basic health testing and to check your baby's immune system and response to the study vaccines. No more than about one tablespoon of blood (15 mL) will be drawn at each of these visits. You might also be asked to bring your baby for extra tests between the regular visits. The study staff will give you the results of any tests related to your

baby's health. If your baby has serious side effects, the study doctor might decide that your baby should not get any further study injections. However, you will be asked to keep bringing your baby for the study follow-up visits as scheduled.

Your baby will be tested for HIV about 8 times during the study (about every 3-6 months). At any time, if test results show that your baby has HIV, the study staff will tell you in person as soon as possible. The study staff will talk to you about the meaning of the test results. An additional blood sample may be needed. If there are treatment programs or studies that you or your baby may be able to join, the study staff will tell you about these. If your baby is found to be HIV-infected, he or she will stop receiving the study vaccinations, but will be asked to continue in the study as scheduled.

If your baby is enrolled in the study, some of your blood from the first screening tests and some of your baby's blood that we take during the study will be stored for later study-related tests. To protect your privacy, these samples will be marked with a code only – not your name or your baby's name. After the study is finished and all related tests are completed, any of your blood or your baby's blood that is leftover will be destroyed.

If any new information is discovered about effects the study vaccine may have on your baby's health, the study staff will do their best to find you and give you the information, even when the study is over.

Study doctors and others will watch the study closely as it goes on. They will carefully review the information from the study. They will pay close attention to harmful reactions. If these people decide that too many problems have occurred, further study vaccinations may be delayed or canceled.

If you agree to take part in this study, it is important for you to keep all appointments for yourself and your baby. However, if you do not want your baby to stay in the study, you can leave at any time without losing the benefits of your standard medical care.

What are the risks of the study?

Study Vaccine Risks

The study vaccine and similar vaccines have been carefully tested in animals, human adults and infants. The testing included routine safety tests to see if anything bad happens to the immune system after getting this product. The studies have so far shown that this vaccine is safe.

However, we do not know if the study vaccine will protect your baby against HIV.

We also do not know what effect the study vaccine may have on the risk of your baby getting HIV or developing AIDS after birth. The risk could increase, decrease or stay the same. If your infant is infected with HIV, we do not know what effect the vaccine may have on the disease, but the study vaccinations will be stopped. If your baby has HIV, the time that it takes for your baby to become sick from AIDS may be the same, longer or shorter than expected. We also don't know if there will be any long-term effects on your baby's immune system if your baby gets the vaccine.

The side effects the baby might have are the same as those you might see with any vaccine. These include fever, chills, rash, weakness, change in sleep patterns and appetite. Injections into a

muscle can cause pain, soreness, redness, and swelling at the place where the baby gets the injection and, in rare cases, infection. These side effects can occur whether your baby gets the study HIV vaccine or the placebo (salt water) injection in this study. If your baby has a reaction, this does not mean that your baby got the experimental HIV vaccine. The side effects usually do not last long or require treatment. As with all vaccines or drugs, your baby could have an allergic reaction including skin rash, hives or even difficulty breathing. Allergic reactions are usually harmless but can sometimes be life-threatening. There may be other mild, or even serious, side effects that we do not know about yet. Therefore, it is important that you report any symptoms to the study staff as soon as they occur.

Blood Drawing Risks

Taking blood from your baby may cause slight pain, swelling and bruising at the place where the blood is taken. Drawing blood can also cause infection, but this is rare.

Social Risks

Babies born to mothers who have HIV will test positive for HIV for a while after birth because of the special anti-HIV materials that pass to them from their mother during the pregnancy, even if the baby is not HIV infected. In a similar way, the study vaccine may also cause babies to test positive for HIV by routine screening tests even if they are not infected with HIV. However, in this study, we will use special tests that will be able to tell if the baby is really infected with HIV. The special anti-HIV materials that a baby gets from his mother and those that can be produced after the study vaccine will go away over time. It is possible that the special tests we use will show that your baby does not have HIV, your baby's test results still appear positive on routine screening HIV tests at the end of the study. If this happens, the staff at this clinic will offer you additional HIV testing for your baby about every six months until the routine screening tests also show that your baby does not have HIV.

Others may treat your baby unfairly if the test vaccines make him seem to be HIV positive. To avoid problems (for example, if the baby has to go to the hospital and relationships with friends and family), the study staff can help you. We will offer you an ID card that proves that your baby was in this vaccine study. The card will have a phone number you can call and a place where you can go to get answers about the study while it is ongoing and when it is over. The study staff will prove that your child is in or has been in an experimental HIV vaccine study.

If you join this study, some hospital staff and all study staff will know that you have HIV. These workers are very serious about your privacy. Study staff will make every possible effort to be sure that others do not learn your HIV status. However, sometimes if you receive special treatment or attend a special clinic, it may make others wonder if you have HIV.

What are the possible benefits of being in the study?

This study may or may not have any direct benefit to you or your baby. Your baby may receive no benefit from the study vaccine because no one knows if vaccines against HIV work and because your baby may get the placebo. However, you and others may benefit in the future from the information that will be learned from the study.

What if the researchers learn something new?

The study doctors or staff will tell you any new information learned during the study that may

cause you to change your mind about having your infant continue participating in the study. Near the end of the study, you will be told when the study results will be available and how to learn about them.

What could make us take you out of the study early?

You and your baby may be withdrawn from the study before the study is done if the study is canceled by the study sponsor, the Ugandan Ministry of Health, the Ethics Committees overseeing the research, the U.S. Food and Drug Administration, or the company that makes the study vaccine.

Your baby may not be allowed to receive or continue the study vaccines if study doctors (or your doctor or the baby's doctor) decide that the vaccine would be harmful to your baby; if your baby needs a medicine not allowed on this study; if your baby has a serious reaction to the study vaccine, if your baby is found to be HIV infected or if you do not keep appointments or follow study procedures. Even if your baby must stop the study vaccines early, we ask that he or she remain in the study and complete the follow-up visits as scheduled.

What are the choices if you do not want your baby to be in the study?

You do not have to agree to let your baby join this study. If you choose not to have your baby participate, your care and your baby's care at Mulago Hospital will not be affected.

At Mulago hospital, there is a special program for all women who are infected with HIV. This program offers infant feeding counseling, a drug called Nevirapine or other drugs to reduce the risk of passing the virus to their babies during labor and soon after delivery, and other medical care. You are advised to follow the Mulago Hospital program for HIV infected women and receive the Nevirapine or other drugs to reduce the risk of passing HIV to your infant whether or not you decide to join this study. You may be in this study whether you decide to take the drugs offered to you or not.

The only way to be sure that your baby does not get HIV infected through breastfeeding is not to breastfeed your baby at all. The study staff will counsel you about the risks and benefits of breastfeeding and about safe options should you choose not to breastfeed. You do not have to breastfeed your baby to be in this study.

What about confidentiality?

The study doctors and staff will protect information about you and your baby's participation in the study to the best of their ability. On your baby's study records, a code will be used instead of a name. Only the study staff will know this code. The study staff will not give out any information that identifies you or your baby without your written consent. However, the Ugandan Ministry of Health, the U.S. Food and Drug Administration, the company that makes the study vaccine, the study sponsor (the U.S. National Institutes of Health), and their authorized representatives will be allowed to inspect your baby's study records.

Will there be any costs or payment to you?

The study vaccines, clinic visits, exams, and lab tests required in this study will not cost you any

money. Standard medical care for you and your baby will be provided by Mulago Hospital in accordance with Ugandan Ministry of Health policy.

We will not pay you or your baby to be in this study. However, we will reimburse you for your travel to the clinic for scheduled visits, or visits that we ask you to make.

Antiretroviral treatment for HIV will not be provided through this study. However, if your baby is found to be infected with HIV, we will refer you to available care and treatment programs for which he or she might qualify.

What happens if your baby is injured in this study?

Medical care will be provided for illness or injury directly related to this study at no cost to you. Medical 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.

You will not give up your legal rights by signing this informed consent form.

What should you do if you have study problems or questions?

If you ever have questions about this research or if your baby has a medical problem or injury in the study, you should contact the study investigator, Professor Francis Mmiro at the Makerere University-Johns Hopkins University Research Collaboration, Upper Mulago Hill Road. You may also ring Professor Mmiro at 041-541044.

If you have questions about your rights or your baby's rights as a research volunteer, you may contact [name and contact information for IRB member]. Dr. _____ is a member of the Uganda AIDS Research Committee, one of the Ethics Committees that oversees the conduct of AIDS research in Uganda.

The study counselors and other staff would be happy to help you contact the right person to answer any questions you have.

STATEMENT OF CONSENT

I have read (or someone has read and explained to me) this consent form. I understand the purpose of the research study, the clinical and research procedures to be followed and which ones are extra, and the risks and benefits as described in this written summary. I voluntarily agree to allow my baby to join this research study.

_____ Mother's Name (print)	_____ Mother's Signature or Thumb Print	_____ Date
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IF AVAILABLE:

_____ Father's Name (print)	_____ Father's Signature or Thumb Print	_____ Date
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For all volunteers: I have explained the purpose of this 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 of this study.

_____ Name of person obtaining consent (print)	_____ Signature	_____ Date
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For illiterate volunteers: I attest that the information contained in this written consent form has been read and explained to the participant. She appears to understand the purpose, procedures, risks and benefits of the study and has voluntarily accepted to have her baby participate in this study.

For those placing thumbprint **only:** I attest that the participant who states that her name is _____ has placed her thumbprint on this consent form of her own free will on this day: _____.

_____ Name of witness to consent process (print)	_____ Witness' Signature	_____ Date
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**Appendix III: Supplemental Toxicity Table for Grading Reactogenicity
Occurring within Seven Days of Study Vaccination**

Parameter		Grade 1	Grade 2	Grade 3	Grade 4
Localized injection site reaction		Erythema OR induration OR edema present but ≤ 2.5 cm diameter	Erythema OR induration OR edema >2.5 cm diameter but $<50\%$ surface area of the extremity segment (e.g. upper thigh)	Erythema OR induration OR edema involving $\geq 50\%$ surface area of the extremity segment (e.g. upper thigh) OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage	Necrosis (involving dermis and deeper tissue)
Pain/tenderness at the site of injection		Reaction to touch, grimace - no crying	Crying or protest to touch	Limitation/decrease in movement of affected extremity and/or crying on movement of site not touching	Inability to use affected extremity or requiring hospitalization
Cutaneous reaction - Rash at site of injection		Localized macular, papular, or vesicular lesions	Diffuse macular, maculopapular or morbilliform rash OR target lesions	Diffuse macular, maculopapular or morbilliform rash with vesicles or limited number of bullae OR superficial ulcerations of mucouse membrane limited to one site	Extensive or generalized bullous lesions OR ulceration of mucous membrane involving two or more distinct mucosal sites OR toxic epidermal necrolysis (TEN)
Acute systemic allergic reaction		Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR mild angioedema with no medical intervention indicated	Generalized urticaria OR angioedema with medical intervention indicated OR symptomatic mild bronchospasm	Acute anaphylaxis OR life-threatening bronchospasm OR laryngeal edema
Fever without alt. explanation	Axillary	37.1-38.0 °C	38.1-38.7 °C	38.8-39.9 °C	>39.9 °C
	Non-axillary	37.7-38.6 °C	38.7-39.3 °C	39.4-40.5 °C	>40.5 °C
Sleepiness, Lethargy, Irritability		Transiently lethargic, irritable or fussy (above usual norm), but otherwise normal routine	More sleeping or crying than usual, not on normal routine without alternative explanation	Prolonged crying, refuses to play/smile with parent/guardian or somnolent needs to be stimulated to take feedings	Inconsolable crying ≥ 3 hours, unusual high pitched crying/screaming
Seizure		Seizure, generalized onset with or without secondary generalization, lasting <5 minutes with <24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5-20 minutes with <24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting >20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation