MORBIDITY AND MORTALITY OF HIV EXPOSED UNINFECTED CHILDREN IN SUB-SAHARAN AFRICA

by

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ABSTRACT

Background: Several sub-Saharan Africa countries are on a fast-track to elimination of mother-to-child transmission of HIV through rapid scale-up of antiretroviral therapy (ART) programs. Peripartum-ART exposures have been associated with increased childhood morbidity.

Objectives: To compare anthropometric, and clinical outcomes among exposed (maternal-HIV and ART), versus unexposed children.

Methods: Prospective cohort of exposed children enrolled from the PROMISE randomized-clinical-trial (combination-ART (cART) versus non-cART), and age-and-gender-matched controls separately enrolled from child-well clinics, in Malawi, and Uganda. WHO growth-standards (2006) were used to derive weight-for-age (WAZ); length-for-age (LAZ); weight-for-length (WLZ); and head-circumference-for-age (HCAZ) z-scores; and the DAIDS toxicity tables (version 1.0, 2004/2009), to classify hematological parameters. Wilcoxon Rank-Sum/Fischer’s exact tests were used to compare variables, and Generalized-Estimating-Equations, and Cox proportional hazards models to measure associations.

Results: Overall, 471(50.5%) exposed and 462(49.5%) control-children were enrolled. Ugandan exposed verses controls had lower mean-Z-scores: LAZ (p<0.001), and WAZ (p<0.001) at 12 and 24 months-of-age, respectively; and HCAZ (p=0.016) at 24 months. Similar trends were observed in Malawi (p>0.05). Adjusted relative-risk (RR), 95% confidence interval (CI) of stunting was higher among exposed versus control-children: 2.11 (1.14, 3.90), p=0.017, at 12-months, and 1.83 (1.03, 3.24), p=0.039, at 24-months-of-age, in Uganda; and 1.57 (1.18, 2.10), p=0.002, at 24-months-of-age, in Malawi. Relative-risk of HCAZ below WHO median was higher among exposed versus controls at 24-months-of-age, RR (95 CI) = 1.78 (1.10, 2.90), p=0.019, in Malawi; and 1.28 (0.82, 2.01), p=0.279, in Uganda. Hematological parameters, and hospitalization risks, were similar (p>0.05) across exposure groups, or more favorable among exposed versus controls. Grade 2 or higher anemia risk was lower among exposed versus control-
children: adjusted RR (95% CI) = 0.33 (0.17, 0.64), p=0.001 in Uganda, and RR (95% CI) = 0.56 (0.26, 1.16), p=0.119, in Malawi. Similar trends were observed with grade 3 or higher risk. Risk-estimates were homogeneous across cART and non-cART exposure-groups (p>0.05); and in-utero versus cumulative (in-utero and postpartum)) models.

**Conclusions:** In-utero, but not postpartum, exposures to maternal-HIV and ART, are associated with lower LAZ (including stunting), WAZ and HCAZ at 24 months-of-age. Hematological patterns and hospitalization risk were homogeneous across exposure groups.
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<tr>
<td>3TC</td>
<td>lamivudine</td>
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<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
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<td>ART</td>
<td>Anti-Retroviral Therapy</td>
</tr>
<tr>
<td>ABC</td>
<td>abacavir</td>
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<tr>
<td>ANC</td>
<td>antenatal care</td>
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<tr>
<td>ART</td>
<td>antiretroviral therapy</td>
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<tr>
<td>ARV</td>
<td>antiretroviral drug</td>
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<tr>
<td>AZT</td>
<td>zidovudine</td>
</tr>
<tr>
<td>CD4</td>
<td>A marker found on the surface of T Helper Cells</td>
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<tr>
<td>CTX</td>
<td>co-trimoxazole</td>
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<tr>
<td>d4T</td>
<td>stavudine</td>
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<tr>
<td>DHS</td>
<td>Demographic and Health Survey</td>
</tr>
<tr>
<td>DPT3</td>
<td>third dose of diphtheria, pertussis, and tetanus vaccine</td>
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<tr>
<td>EBF</td>
<td>exclusive breastfeeding</td>
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<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
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<tr>
<td>EFV</td>
<td>efavirenz</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<tr>
<td>FTC</td>
<td>emtricitabine</td>
</tr>
<tr>
<td>GARPR</td>
<td>Global AIDS Response Progress Reporting HIV human immunodeficiency virus</td>
</tr>
<tr>
<td>HIVDR</td>
<td>HIV drug resistance</td>
</tr>
<tr>
<td>HTC</td>
<td>HIV testing and counselling</td>
</tr>
<tr>
<td>IF</td>
<td>infant feeding</td>
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<tr>
<td>IMPAACT</td>
<td>International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network funded by the US NIH</td>
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<tr>
<td>L&amp;D</td>
<td>labor and delivery</td>
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<tr>
<td>LPV/r</td>
<td>lopinavir boosted by ritonavir</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MDG</td>
<td>Millennium Development Goal</td>
</tr>
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<td>MF</td>
<td>mixed feeding</td>
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<tr>
<td>MTCT</td>
<td>mother-to-child transmission</td>
</tr>
<tr>
<td>NAP</td>
<td>National AIDS Programme</td>
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<tr>
<td>NIH</td>
<td>The United States National Institutes of Health</td>
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<tr>
<td>NNRTI</td>
<td>non-nucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>NRTI</td>
<td>nucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>PEPFAR</td>
<td>United States President's Emergency Plan for AIDS Relief</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PITC</td>
<td>provider-initiated testing and counselling</td>
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<tr>
<td>PLHIV</td>
<td>people living with HIV</td>
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<tr>
<td>PMTCT</td>
<td>prevention of mother-to-child transmission</td>
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<tr>
<td>PROMISE</td>
<td>Promoting Maternal and Infant Survival Everywhere (PROMISE)</td>
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<tr>
<td>RF</td>
<td>replacement feeding</td>
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<td>TDF</td>
<td>tenofovir</td>
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<tr>
<td>UNAIDS</td>
<td>United Nations Joint Programme on HIV/AIDS</td>
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<td>UNGASS</td>
<td>United Nations General Assembly Special Session on HIV/AIDS</td>
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<td>UNICEF</td>
<td>United Nations Children’s Fund</td>
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<td>UNPD</td>
<td>United Nations Population Division</td>
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<tr>
<td>VCT</td>
<td>voluntary counselling and testing</td>
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<tr>
<td>VL</td>
<td>viral load</td>
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<td>WHO</td>
<td>World Health Organization</td>
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INTRODUCTION
1.1 INTRODUCTION

In Sub-Saharan Africa, home to about 10% of the world’s population, the burden of the HIV pandemic on the health of children remains a major global health concern, with high attributable morbidity and mortality rates. Despite the substantial progress and widespread implementation of proven effective interventions, the incidence of mother-to-child transmission of HIV (MTCT) remains high. In 2015, the region contributed more than 80% of the 150,000 new pediatric infections globally.\textsuperscript{1,2}

A major driver for the relatively slow decline of the MTCT risk in sub-Saharan Africa is the slow uptake of efficacious antiretroviral (ARV) drug based strategies for prevention of mother-to-child transmission of HIV (PMTCT). Similar ARV-based strategies were successfully implemented in resource-replete settings in North America and Europe where HIV vertical transmission is now virtually eliminated.\textsuperscript{3,4} In addition, high rates of HIV infection among women of reproductive-age persist in some sub-Saharan African countries. In 2015, the region contributed majority of the prevalent (69.5%) and incident (65.2%) HIV cases globally, the female-to-male ratio (three-to-two) was higher than any other region in the world, and had more than 90% of all pregnant women living with HIV worldwide. There were considerable variations across countries. The eastern and southern Africa countries accounted for approximately 45.7% of the total, and 37.3% of the pediatric incident HIV infections globally.\textsuperscript{1} Other key factors for the slow MTCT risk decline include high unintended pregnancy rates, and losses to follow up of mothers and their infants throughout the PMTCT cascade.\textsuperscript{1,5} Furthermore, prolonged breastfeeding which is crucial for infant survival in the region is associated with 30-40% MTCT risk in the absence of any intervention, as well as increased vertical transmission risk associated with maternal seroconversion during breastfeeding.\textsuperscript{6,7}
While efforts for rapid scale-up of ARV-based PMTCT strategies are ongoing in sub-Saharan Africa, there are growing concerns that exposures to ARV drugs during critical *in-utero* and prolonged postpartum periods could potentially be associated with both short and longer term complications related to development, physical growth, and overall health. This would be counterproductive to the overall PMTCT goal of HIV-free survival among HIV-exposed uninfected (HEU) children. In addition, given the established increased morbidity and mortality risk associated with exposure to maternal HIV infection among the HEU children compared to their HIV-unexposed uninfected (HUU) counterparts, the added risk attributed to ARV exposures could pose a double jeopardy for ARV-exposed HEU children.

According to the World Health Organization (WHO) antiretroviral therapy (ART) based PMTCT guidelines for resource limited settings issued in 2009/2010 and later updated in 2013 and 2016, HEU children in sub-Saharan Africa will have prolonged exposures to complex ARV drug regimens, potentially up to 31 months (6-9 months *in-utero* plus 18-24 months postpartum during breastfeeding). Although the ARV-based PMTCT coverage gap remains wide in many countries in sub-Saharan Africa, remarkable progress has been reported in several others in response to the 2009 UNAIDS call for elimination of mother-to-child transmission of HIV (eMTCT). Indeed, the estimated 1.2 million new HIV infections averted between 2009 and 2015 among children in the Global Plan Priority (GPP) countries is attributed to the rapid scale-up of ART which more than doubled during the same period in 21 of the 22 GPP countries: 36% in 2009 compared to 80% in 2015. Therefore, substantial reductions in vertical HIV transmission as a result of successful ART-based PMTCT programs in sub-Saharan Africa, will result in a generation of HEU children with long-term exposure to complex ARV regimens.

However, the current evidence in the literature linking peripartum ARV exposures and morbidity during early childhood is inconclusive and limited to: 1) animal models; 2) few human studies restricted to European and North American cohorts; 3) shorter duration (antenatal only
ARV exposures); 4) limited range of ARV-type exposure studied; and 5) study design limitations that precluded appropriate group comparisons between exposed and unexposed children. Proposed biologically mediated pathways include mitochondrial damage, toxicity to nuclear DNA of hematopoietic stem cells, and dysregulation of bone metabolism in the developing fetus and the young infant.

This research will attempt to help fill this knowledge gap through a longitudinal cohort study of ARV-exposed HEU children and age-and gender-matched HUU controls, followed through 12 and 24 months of age, at two international sites in Malawi and Uganda. Both countries located in southern and eastern Africa, respectively, have a high burden of MTCT risk and a demonstrated commitment to scaling-up ART-based PMTCT programs. In addition, the assessment of morbidity and mortality outcomes by: a) ARV regimen type and b) duration of exposures to maternal HIV and prophylactic ARV drugs will be critical to the identification of opportunities for intervention if sequelae to prolonged ARV exposure are found.

1.1.1 Specific Aims

The specific aims of this analytic research using data from an ongoing cohort study are:

1. To compare children with dual exposures to maternal HIV-infection and prophylactic ARV drugs (exposed-group) to controls on anthropometric outcomes (age and gender appropriate Z scores for weight, length, and head circumference) at 12 and 24 months of age respectively.
   a. Compare the anthropometric outcomes of exposed versus control-group children by type of ARV regimen exposure (sub-aim 1A) within a 2-Factor (antepartum and postpartum randomization) model.
   b. Compare the anthropometric outcomes of exposed versus control-group children by duration of ARV regimen exposure (sub-aim 1B) within the 2-Factor model.
2. To evaluate differences in the risk of negative hematological outcomes between exposed and control-group children at 12 and 24 months of age. For this analyses, anemia, neutropenia, and thrombocytopenia will be assessed as separate outcomes.
   a. Evaluate if there are differences in risk of hematological complications between exposed versus control children, by ARV exposure type (sub-aim 2A) within the 2-Factor model.
   b. Evaluate if there are differences in risk of hematological complications between the exposed versus control-group children, depending on type of ARV exposure duration (sub-aim 2B) within the 2-Factor model.

3. To determine the differences in morbidity (hospitalizations) and mortality between exposed versus control-group children through 24 months of age.
1.2 REVIEW OF THE LITERATURE

1.2.1 Pediatric Exposures to Maternal HIV and Prophylactic Antiretroviral Drugs

1.2.1.1 Scope of the Epidemic

Globally, an estimated 1.4 million HIV-infected women become pregnant every year. In 2015, approximately 150,000 cases of mother-to-child transmission of HIV (MTCT) and about 1.25 million new HIV exposed uninfected (HEU) children were reported worldwide. Among 21 of the 22 UNAIDS Global Plan Priority (GPP) countries targeted for elimination of mother-to-child transmission of HIV (eMTCT), an estimated 80% of HIV-infected women received antiretroviral (ARV) drugs for prevention-of-mother-to-child transmission of HIV (PMTCT). Therefore, approximately 1.0 million HEU children had in-utero and postpartum exposure to ARV drugs. The incidence of HEU children with peripartum exposure to ARV drugs is expected to increase to about 1.5 million new cases per year by 2020. In 2016, the UNAIDS and key global partners established the “super fast-track targets” to ensure at least 95% of pregnant women living with HIV globally are receiving ART by 2018, and the number of new MTCT cases per year are reduced to less than 20,000 by 2020.

Sub-Saharan Africa, home to about 10% of the world’s population, is disproportionately affected with more than 90% of all pregnant women living with HIV globally. Of the 22 UNAIDS GPP countries targeted for rapid-scale up of ART based programs 21 are in sub-Saharan Africa.

1.2.1.2 Maternal HIV Biology

The Human immunodeficiency virus (HIV) is a retrovirus of the lentivirus family with a single strand RNA genome that causes HIV infection in humans. HIV infects vital cells of the immune system with cell surface expression of target proteins including CD4 and either of two chemokine co-receptors – CXCR4 or CCR5. The immune cells targeted include helper T cells
particularly CD4+ T cells, macrophages and dendritic cells. HIV infection leads to the destruction of CD4+ cells through a number of ways: programmed self-cell destruction (both pyroptosis and apoptosis), direct killing of infected cells by HIV, or CD8 cytotoxic killing of HIV-infected cells. Cumulative CD4+ cell destruction manifests with progressive HIV disease and ultimately Acquired Immuno-Deficiency Syndrome (AIDS), a syndrome characterized by increased susceptibility to life-threatening opportunistic infections and diseases.

1.2.1.3 Antiretroviral Therapy (ART)

Antiretroviral (ARV) drugs are potent in decreasing the HIV viral load (VL) and are used in the management of HIV/AIDS and control of HIV infection. There are five classes of currently licensed ARV drugs that act on four critical stages of the HIV life-cycle. These include Nucleoside Reverse Transcriptase Inhibitors (NRTI); Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI); Protease Inhibitors (PIs); Integrase Nuclear Strand Transfer Inhibitors (INSTIs) and Entry inhibitors. To optimize drug efficiency, multiple ARV drugs that act on different stages of the HIV life cycle are used in combination, an approach known as highly active antiretroviral therapy (HAART) or antiretroviral therapy (ART). In most cases the combinations include a ‘backbone’ of 2 NRTIs plus 1 NNRTI or PI or INSTI as a ‘base’. Antiretroviral therapy decreases the VL and in doing so reinstates and maintains the immune function and ultimately decreases the risk of opportunistic infections which often result in death among HIV infected individuals. Use of ART at all stages of HIV disease progression (early or late) is associated with a reduction of HIV-related morbidity and mortality. Maternal VL (plasma or breastmilk) is the strongest predictor of MTCT risk, and maternal ART and/or infant ARV drug use during the MTCT risk period is associated with MTCT risk reduction.
Nucleoside reverse transcriptase inhibitors (NRTI) are nucleoside analogues which inhibit reverse transcription by competitively binding and therefore blocking the HIV reverse transcriptase enzyme. Reverse transcription is a critical step for HIV survival by which the virus reverse transcribes from RNA to DNA, a pre-requisite for its integration into the DNA in the nucleus of the human cell. Examples of currently used NRTIs include zidovudine (ZDV), abacavir (ABC), lamivudine (3TC), emtricitabine (FTC), tenofovir (TDF) and tenofovir alafenamide (TAF) a prodrug of TDF. Zidovudine was the first ARV to be developed and licensed to treat HIV in 1987. Previously licensed NRTIs that have been phased out of use include didanosine (ddI), stavudine (d4T) and zalcitabine (ddC).

Non-Nucleoside reverse transcriptase inhibitors (NNRTI) which were first approved for use to treat HIV in 1996 inhibit reverse transcription through non-competitive inhibition of reverse-transcriptase enzyme by binding to an allosteric site of the enzyme. First generation NNRTIs include nevirapine (NVP) and efavirenz (EFV), and second generation NNRTIs are etraviirine and rilpivirine. Other NNRTIs licensed include rilpivirine (RPV), etravirine (ETV) and delavirdine (DLV).

Protease inhibitors (PIs) impede the maturation of HIV virions by blocking the HIV protease enzyme. The HIV protease serves as a catalyst to the cleavage of gag and gag/pol precursor proteins of the non-infectious nascent virion particles in a transformational process to the infectious mature virions. The PIs were first approved for use in 1995 and examples include lopinavir in combination with ritonavir (LPV/r), indinavir (IDV), nelfinavir (NFV), amprenavir (APV) fosamprenavir (FAV), saquinavir (SQV), tiprinavir (TPV) and ritonavir (RTV). Darunavir (DRV) and atazanavir (ATV) are recommended first line drugs.

Entry inhibitors (or fusion inhibitors) target the binding, fusion and entry steps of the HIV life cycle. The viral component gp120 attaches to the host cell CD4 receptor. The ensuing
conformational changes in the virion enable gp120 to interface with either of two host cell surface co-receptors, CCR5 or CXCR4. This in turn induces further changes in the virion which activates the gp41 component of the virus which attaches to the host cell to serve as a conduit for transfer of the viral material into the host cell. Fusion inhibitors block the transfer of viral material by binding the gp41. This class includes enfuvirtide (T-20) which was first licensed in 2003.

Maraviroc (MVC) which was first licensed in 2007 is a CCR5 co-receptor antagonist which prevents the interaction between the gp120 and the CCR5 co-receptor.

Integrase inhibitors which are also known as integrase nuclear strand transfer inhibitors or (INSTIs) are the most recent class of ARV drugs, approved in October 2007. Integrase inhibitors inhibit the HIV integrase enzyme, which is responsible for integration of viral DNA into the DNA of the host cell. Examples include raltegravir (RAL) which was the first of this class to be approved by the FDA, as well as elvitegravir (EVG) and dolutegravir (DTG) approved in 2014.

**Fixed Dose Combinations of ARV drugs**

A Fixed-dose combination of ARV drugs refers to a combination of multiple ARV drugs (different classes or a single class) into a single pill which helps reduce the pill burden. The approved fixed-dose combinations that constitute ART – complete regimen for the treatment of HIV – include Atripla (FTC+TDF+EFV), Complera/Eviplera (FTC+RPV+TDF), Stribild (EVG+ cobicistat(COBI)+FTC+TDF), Triumeq (ABC+DVG+3TC), Genvoya (EVG + COBI + FTC + TAF) and odefsey (FTC+RPV+TAF). The other fixed-dose combinations that must be combined with one or more additional pills to complete a regimen include Combivir (3TC+ZDV), Truvada (TDF+FTC), Kaletra (LPV+RTV), Epzicom (ABC+3TC), Descovy (FTC+TAF), Dutrebis (3TC+RAL), Precobix (DRV+COBI), Evotaz (ATV+COBI).
**Pediatric Antiretroviral drugs**

Specific formulations for ARV drug dosing are needed for children which depend on the body surface area or body weight or age bands. For ease of administration in children, manufacturers have developed syrups or oral suspensions. The FDA approved pediatric formulations include NRTIs (ZDV, 3TC, FTC, d4T, ABC, ddI, TDF) as well as fixed dose formulations of NRTIs including Combivir (3TC+ZDV), Epzicom (ABC+3TC), Retrovir (ZDV+azidothymidine(AZT)), Trizivir (ABC+ZDV+3TC) and Truvada (TDF+FTC); NNRTIs including NVP, EFV, RPV, ETV and DLV; PIs including APV, TPV, IDV, SQV, LPV/r, FPV, RTV, DRV, ATV and NFV; 1 fusion inhibitor (T-20 / ENF); 1 entry inhibitor (MVC); and integrase inhibitors RAL and DTG. Approved pediatric Fixed Dose Combinations that provide the complete combination regimen include Atripla (EFV+FTC+TDF), Complera (FTC+RPV+TDF) and Stribild (elvitegravir + cobicistat + FTC+TDF).28

1.2.1.4 In-utero and Postpartum Exposures to Maternal HIV and Prophylactic ARV drugs

Maternal HIV infection accounts for essentially all reported HIV exposures among HEU infants worldwide. Suffice to say that the timing of exposures to maternal HIV infection and prophylactic ARV drugs among HEU children directly mirrors the MTCT risk window. The MTCT risk continuum spurns three physiologically distinguished periods: 1) the early in-utero period (< 28 weeks gestation), a period of transplacental MTCT risk; 2) peripartum period (third trimester of pregnancy after 28 weeks gestation through 7 days of infant life), a period when transmission is mediated through contact with maternal fluids such as maternal-fetal blood transfusions or direct contact to maternal blood, amniotic fluid or birth canal secretions; 3) postpartum MTCT risk primarily through infant ingestion of infected maternal breast milk and colostrum.33,34 Colostrum-mediated transmission can also be classified under peripartum considering the timing of breastfeeding initiation. The postpartum breastfeeding period is further
classified into an early (first 6-8 weeks of life) and late (from 6-8 weeks of life through the duration of breastfeeding). Over the years, as the scientific understanding of the MTCT risk period has improved coupled with the need for practical considerations for setting PMTCT policy guidelines, their implementation as well as monitoring and evaluation of their impact, a simpler classification of the MTCT risk period – antenatal, delivery and postnatal periods – has emerged.

In the absence of any PMTCT intervention among breastfeeding populations, about 25-30% of the total MTCT risk is attributed to the early in-utero period, 50-60% to the peripartum period and 30-40% to the postnatal period. Known predictors of MTCT risk include maternal HIV viral load (plasma and breastmilk), vaginal deliveries and breastfeeding practices such as prolonged breastfeeding, mixed feeding, and abrupt weaning. It is biologically plausible that the same risk factors are directly correlated with increased risk to maternal HIV exposures (in-utero and postpartum/breastmilk) among HEU children. Several studies previously reported the presence of HIV-specific cytolytic T cell activity in apparently uninfected children born to HIV-infected mothers, including a recent Kenyan study that demonstrated a dose-response relationship between maternal viral loads (plasma and breastmilk) and HIV-specific immune activation among HEU children.

### 1.2.2 Prevention of Mother-to-Child Transmission of HIV (PMTCT) Strategies

#### 1.2.2.1 Antiretroviral Therapy (ART) based strategies for PMTCT

A comprehensive approach to effective PMTCT strategies entails access and utilization of HIV testing services by women during the MTCT risk continuum (antenatal, intrapartum, and postpartum) followed by initiation of life-long ART for HIV-infected women; safe delivery practices; safe infant feeding practices; and appropriate postpartum healthcare for child survival including infant HIV testing for early identification and initiation of ART for HIV-infected infants.
In resource-replete settings in North America and Europe where large-scale implementation of ART-based PMTCT strategies has emerged as a major public health success story, vertical transmission of HIV is virtually eliminated. Combination maternal ARV drug coverage during pregnancy and labor/delivery along with infant zidovudine (ZDV) prophylaxis, modified obstetric care and avoidance of breastfeeding have been implemented since the late 1990s. However, the progress in the scale-up of ARV drugs for PMTCT in resource-limited settings has been slower.

1.2.2.2 Antiretroviral (ART) based PMTCT strategies in sub-Saharan Africa (2001 – 2016)

The World Health Organization (WHO) recommends a four-pronged PMTCT programmatic approach: 1) prevention of new infections among reproductive age women; 2) prevention of unintended pregnancies among women living with HIV; 3) identification of HIV infected pregnant women followed by efforts to prevent vertical transmission of HIV; and 4) family-centered management and care for mothers living with HIV. Many settings in sub-Saharan Africa with urgent need for effective PMTCT programs, have limited-resources. In addition, prolonged breastfeeding is pervasive and crucial for infant survival. The WHO ARV-based PMTCT guidelines appropriate for such settings have evolved rapidly over the last decade, from the simpler single ARV drug regimens to more complex ARV drug cocktails and more recently, life-long ART-based strategies. The WHO PMTCT guidelines and the corresponding ARV-drug combinations are summarized in Table 1.1.

The earliest ARV-based PMTCT guidelines issued in 2004 comprised of a maternal single-dose nevirapine (sdNVP) taken at labor onset, and infant sdNVP administered within hours of birth, followed by daily infant ZDV for one week to minimize the risk of emergence of NVP-resistant HIV strains. The 2006 WHO guidelines introduced an antepartum component (third-trimester maternal ZDV taken as a daily dose through delivery) followed by a more complex maternal intrapartum ARV drug cocktail (ZDV, 3TC and sdNVP at labor onset) followed by a
one-week tail of ZDV plus 3TC to minimize emergence of NVP resistant HIV strains. Infants received sdNVP followed by 1 week ZDV.48

However, the most dramatic changes were made in 2009/2010, following randomized-controlled trial data demonstrating efficacious (MTCT rates as low as 1-2% throughout the risk-period); safe and relatively affordable ARV drug combinations suitable for PMTCT in resource-limited breastfeeding settings.22,49–51 This revitalized the call for elimination of mother-to-child transmission of HIV (eMTCT) globally.46 In furtherance of this mandate, the WHO issued recommendations with two-options ('Option A' or 'Option-B') for ARV-based PMTCT strategies for HIV-infected pregnant or breastfeeding women deemed not in need of ART for their own health based on CD4 cell count test.46 ‘Option-A’ entailed use of maternal ZDV initiated as early as 14 weeks of gestation through labor/delivery; plus sdNVP at labor onset followed by one week of ZDV or 3TC. The infant regimen contained a daily NVP dose taken for the duration of breastfeeding. On the other hand, ‘option-B’ included a triple ARV regimen initiated as early as 14 weeks of gestation, through labor/delivery and the duration of breastfeeding, regardless of maternal CD4 cell counts.

Subsequent revisions to the WHO PMTCT guidelines in 2013, and more recently in 2015, have evolved to a test-and-treat approach by which pregnant or breastfeeding women diagnosed with HIV infection, regardless of CD4 cells count, are immediately initiated on ART for life beyond the MTCT risk period (Option B-plus).5 These revisions were influenced in part by research findings suggestive of better survival among HIV-infected individuals with early verses delayed ART initiation;15,16,52 HIV-prevention benefits of ART among HIV-infection discordant couples;52 higher PMTCT efficacy associated with ‘option B’ versus ‘option A’;53 and more programmatic challenges including loss of HIV-infected women to care associated with cessation of PMTCT care ('options A or B').54,55
1.2.2.3 Rapid scale-up of ART-based PMTCT programs in sub-Saharan Africa – past, present and future

Based on the new-found optimism for potential eMTCT, in 2011 the UNAIDS set a global target to achieve less than 40,000 new pediatric HIV cases annually by 2015 down from the 260,000 incident cases estimated in 2009. To achieve this goal, 22 Global Plan Priority (GPP) countries, home to more than 90% of pregnant women living with HIV globally, were targeted for rapid scale-up of the 2009/2010 WHO PMTCT guidelines, primarily to achieve more than 90% national coverage of ARV-based PMTCT strategies by 2015. Except for India, all the GPP countries were from sub-Saharan Africa, including Angola, Botswana, Burundi, Cameroon, Chad, Côte d’Ivoire, the Democratic Republic of the Congo, Ethiopia, Ghana, Kenya, Lesotho, Malawi, Mozambique, Namibia, Nigeria, South Africa, Uganda, the United Republic of Tanzania, Swaziland, Zambia, and Zimbabwe. Although wide coverage gaps persist in some countries like Nigeria, several other countries in the region have made remarkable progress (figure 1.1). Botswana, Mozambique, Namibia, South Africa, Swaziland, and Uganda achieved the UNAIDS target of more than 90% coverage of pregnant women living with HIV with ARV drugs for PMTCT.

The PMTCT benefits of ART-based programs are evident and many countries in the region are on a fast-track to eMTCT. An estimated 1.2 million childhood infections were averted between 2009 and 2015 in the 21 GPP countries in the region, although the estimated 110,000 new childhood HIV infections in 2015 alone remains unacceptably high. In 2016, a renewed global eMTCT commitment was launched to reduce the number of new MTCT infections to less than 20,000 per year by 2020 by ensuring at least 95% of all pregnant women living with HIV globally are on lifelong-ART by 2018. Therefore, a receding MTCT epidemic following successful ART-based PMTCT programs will result in a generation of HEU children with long-term exposure to complex ARV drug regimens.
1.2.3 Morbidity and Mortality among HIV-Exposed Uninfected (HEU) Children

Good nutrition and healthy growth during the first 1000 days of existence – from conception throughout the in-utero and the first 24 months after birth – are associated with lasting health benefits throughout life. In-utero and postpartum environmental exposures to maternal HIV infection and/or prophylactic antiretroviral drugs have the potential to tip the scale and potentially drive HEU children into a negative nutritional balance consequently resulting in sequelae related to growth, neurocognitive development, susceptibility to infectious diseases and ultimately overall survival during the fetal period, early childhood and beyond.

1.2.3.1 Morbidity and Mortality among HEU children in resource-replete settings

Some of the earliest studies looking at the morbidity and/or mortality among HEU children were based on European and North American pediatric cohorts, although findings are heterogeneous. In 1999 Blanche and Tardieu reported that several NRTI perinatally exposed HEU infants in the French perinatal cohort had unexplained severe neurologic disease; and related this to possible mitochondrial dysfunction from in-utero exposure to NRTIs. In a larger study of over 4000 uninfected HIV and ARV exposed children, Barret and Tardieu reported that the 18-month incidence of symptoms compatible with mitochondrial dysfunction was 0.26%, and 0.07% for mortality. Transient neonatal hyperlactatemia as well as deficits in mitochondrial respiratory chain complex enzyme function in muscle tissue were reported among HEU children with NRTI exposures. However, analyses of longitudinal data from several U.S. perinatal cohort studies conducted in the 1990’s, and the European Collaborative Study did not find evidence of mitochondrial degenerative disease among ARV exposed uninfected infants. A seven-year follow-up of infants exposed to ZDV alone during pregnancy and for 6 weeks in the Pediatric AIDS Clinical Trial Group (PACTG) 076 study found no evidence of negative late effects on physical growth or development and no increased cancer risk. The use of PI based combination triple
ARVs during pregnancy has been associated with increased risk of premature delivery in some but not all studies.\textsuperscript{64} In addition, clinically asymptomatic but statistically lower median hemoglobin, lymphocyte, neutrophil and platelet counts among ARV exposed infants than control unexposed infants have been reported. More hematologic effects were noted among children with \textit{in utero} exposure to combination ARVs, than to zidovudine alone.\textsuperscript{61,65,66}

Data from the Pediatric HIV/AIDS Cohort Study (PHACS) conducted between 2007-2010 among 2029 US infants, suggests that a TDF-based ART regimen versus non-TDF-based ART regimen was associated with a significantly lower adjusted mean length-for-age Z scores and head circumference for age Z scores at 1 year, respectively.\textsuperscript{67} More recent European studies that assessed infectious morbidity reported a high hospital admission rate due to serious infections (\textit{Streptococcus pneumoniae} and \textit{Haemophilus Influenza}) among HEU children during the first year of life;\textsuperscript{68} higher rates of invasive group B streptococcal disease among HEU versus HUU;\textsuperscript{69} higher morbidity among HEU children born during the ART era versus those born prior;\textsuperscript{70} and higher blood-disorder associated hospitalizations among HEU versus HUU children.\textsuperscript{71}

Although majority of studies that previously assessed morbidity of HEU children, particularly in the context of perinatal ARV drug exposures, were based in resource-replete settings in Europe and North America, most studies lacked a demographically comparable HUU reference group or had no comparison at all, and some studies had limited sample sizes. In these settings, infectious diseases are rare which made it difficult to ascertain potential impact of maternal HIV exposure on infant susceptibility to infectious diseases. Infant exposure to ART was routine and therefore these studies could not isolate the independent effects of maternal HIV exposure; and breastfeeding was mostly avoided as a PMTCT strategy another key consideration that limits the generalizability of these findings to HEU children in sub-Saharan Africa.
1.2.3.2 Morbidity and Mortality among HEU children in sub-Saharan Africa – (pre-ART era)

In resource-limited settings in sub-Saharan Africa with the highest global burden of HEU children, other determinants of early-childhood morbidity and mortality include small-for-gestation-age; infectious diseases (IDs) such as measles, diarrhea, pneumonia, meningitis, and malaria; environmental/tropical enteropathy, an acquired syndrome characterized by altered gut immunity and diminished capacity to absorb nutrients, associated with poor sanitary conditions; and environmental and socio-economic cofactors such as household level food-insecurity; water hygiene and sanitation (WASH) factors and household level socio-economic stability.72–74

In 2013, more than half of the estimated 6.3 million under-5-year-old child deaths globally were attributed to IDs with pneumonia, diarrhea, and malaria as the leading causes of ID-related deaths. The majority (about 67%) of deaths occurred before the first birthday, and about 44% during the neonatal period (within the first 28 days after birth), the leading causes being preterm birth complications; pneumonia; and intrapartum-related complications. Similarly, severe IDs are associated with early-childhood growth impairment (wasting, stunting and underweight), diarrhea notably as the strongest ID predictor of stunting in children.75 Other etiological factors of growth impairment include fetal growth restriction, and child undernutrition (tropical enteropathy and food-insecurity).72–75

While 25% of the births globally occurred in sub-Saharan Africa, the region contributed half of the under-5-year old deaths in 2013, and these startling statistics are projected to get worse with an estimated 33% of births and 60% of deaths by 2030.74 Pre-ART era studies that assessed morbidity and mortality outcomes among HEU children in sub-Saharan Africa are summarized below.
Physical growth:

Evidence based on studies conducted in low-resource settings in sub-Saharan Africa prior to the ART era were ambivalent (table 1.2). A Kenyan study reported inferior height-for-age Z scores at 6 weeks of life; superior weight-for-height Z scores at 6 and 18 months of life, respectively, among HEU compared to their HUU counterparts, while a Zambian study reported HEU compared to HUU children were more likely to be stunted, which persisted despite nutritional supplements. These studies had limited sample sizes. More recent reports based on retrospective analyses of a cohort of Zimbabwean HEU children, the largest pre-ART HEU study in sub-Saharan Africa, suggests that HEU versus HUU children had lower measures of weight-for-age, weight-for-height, and height-for-age through 24 months of age; and inferior head growth through 12 months of life. However, several other smaller studies conducted in Democratic Republic of Congo, Malawi, Rwanda, and Zambia consistently reported homogeneous physical growth outcomes among HEU and HUU children: height-for-age; weight-for-age; weight-for-height; and head circumference-for-age. The smaller HEU children at birth reported in the Malawian study eventually caught up with their HUU counterparts. Height-for-age, weight-for-age and weight-for-height are standard anthropometric measures for stunting (impaired linear growth), underweight and wasting (impaired ponderal growth), respectively.

Mortality:

Research findings on maternal HIV exposure and early childhood mortality during the pre-ART era are heterogenous based on separate studies conducted in Rwanda (1988-1991), Uganda (1994-1998), Gambia (1993-1997), Zimbabwe (1997-2002), and Zambia (2000-2002). There was a tendency towards higher mortality differences, as high as 2-3 times among HEU versus HUU children observed among younger age groups which leveled off among older age
group children: at 6 months but not at 12 months of age in the Ugandan study,\textsuperscript{88} and at 12 months of age with smaller mortality differences at 24 months of age in the Zimbabwean study.\textsuperscript{90} The Zambian study which followed older children from 9 months of age, although limited by sample size, reported non-statistically significant three times higher HEU versus HUU child mortality at 3 years of age. The Rwandan and Gambian studies with longer follow-up reported no mortality differences at 60 and 72 months of age, respectively.\textsuperscript{87,89}

1.2.3.3 Morbidity and Mortality among HEU children in sub-Saharan Africa – (ART era)

To appreciate the implications of morbidity and mortality studies among HEU children conducted during the ART era, it is prudent to consider the secular trends over the last 10 years as the ARV-based PMTCT landscape has transitioned from the earliest and simplest PMTCT strategies based on intrapartum single dose NVP (sdNVP); followed by sdNVP with a tail of ZDV with or without 3TC; and later the era of longer duration and more complex ARV regimens (WHO ‘options A or B’), and more recently WHO ‘Option B-plus’ (lifelong ART for all HIV-infected women).

Physical growth:

Recent reports based on analysis of data from two large completed randomized clinical trials in Botswana suggest that in-utero exposures to ART versus ZDV monotherapy, were associated with inferior weight-for-age and inferior length-for-age through 24 months of life among HEU children.\textsuperscript{92} This study had no HUU comparison group. Two recent cross-sectional studies, a hospital-based survey in Uganda\textsuperscript{93} conducted between 2010-2011 during an era of ‘WHO option-A or B’, and a population-based survey in Botswana,\textsuperscript{94} conducted between 2013-2014 during an era of ‘WHO option B-plus’, both reported inferior length-for-age among HEU compared to HUU children through 6-12 months, and through 12-24 months, respectively. In addition, the Ugandan study reported inferior weight-for-age and inferior weight-for-length
among HEU versus HUU children. However, there were no differences in weight-for-age through 24 months of age in an earlier South African study in which sdNVP-based PMTCT strategies were used.95 A summary is presented in table 1.2.

Mortality

The current literature on ARV exposures and mortality among HEU children in the region is based on studies among HEU children with limited ARV drug exposures, mostly peripartum sdNVP with or without a tail of a NNRTI monotherapy. Therefore, previous studies have limited generalizability to HEU children in the current ART era who experience exposures to complex ART regimens for prolonged periods, potentially up to 20 months (9 months in-utero and 18-24 months postpartum). Because of the limited ART prophylaxis during the postpartum period, HEU children compared to their HUU counterparts in the respective studies tended to have shorter breastfeeding durations.

In populations with sdNVP exposure alone, the mortality rate at 20 months-of-age was 18.7% among HEU compared to 4.3% among uninfected unexposed controls in a Malawian cohort;57 and similar findings of high cumulative mortality of 13.6% between 1 and 24 months-of-age among HEU children in a Zambian study.58 In a large multinational trial conducted between 2001 and 2003 in Malawi, Tanzania and Zambia, HEU children with perinatal sdNVP exposures and who received cotrimoxazole prophylaxis had higher mortality at 12 months compared to HUU children.96 However, two South African studies with similarly simple and short duration PMTCT ARV regimens, sdNVP or ZDV monotherapy, reported similar mortality rates across HEU and HUU groups, through 9 months of age97 and 12 months of age.98 One of the studies, conducted between 2002 and 2004, reported markedly lower rates of breastfeeding in the HEU versus HUU infant groups, although the deleterious effects might have been minimal since about 30% of the participants were from wealthier areas where formula feeding is relatively
safe.\textsuperscript{97} The larger study on the other hand reported high rates of exclusive breastfeeding across the HEU and HUU groups which might explain the homogenous mortality rates across the two groups.\textsuperscript{98} Two other studies with longer postpartum ARV exposures: sdNVP followed by 6 months of infant ZDV in a Botswana study (2002-2004);\textsuperscript{99} and sdNVP followed by 4 weeks of ZDV in a Mozambican surveillance program (1996-2009);\textsuperscript{100} independently reported increased mortality among HEU versus HUU children.

1.2.3.4 Mechanisms of Action

Although evidence of increased morbidity and mortality among HEU children relative to their HUU counterparts remains inconclusive based on the current literature, environmental, socio-economic, and biological differences have been postulated as probable etiological factors. It is likely that the potential relative vulnerability of HEU children is a manifestation of a multilevel interplay between several factors. Black et al,\textsuperscript{72} describe a three tier relationship of proximal, intermediate and distal factors, a modification of the UNICEF conceptual framework,\textsuperscript{101} for ideal in-\textit{utero} and early childhood growth and development (figure 1.2). The proximal factors include behavioral (parenting, caregiving, feeding, weaning and child stimulation); dietary (breastfeeding, weaning diets, food nutrition) and health determinants (infectious disease burden). The intermediate factors include food security (availability, economic access, and consumption); caregiving resources (maternal health, household, and community levels) and environmental (access and utilization of health services, as well as hygienic households and communities) factors. The intermediate factors are in turn influenced by more distal and global factors such as socio-economic and financial resources, politics, and governance. Previous studies based on populations in resource limited settings in sub-Saharan Africa demonstrated differences between HEU and HUU children with regard to caregiving (maternal health, maternal death),\textsuperscript{102-104} dietary (duration of breastfeeding, quality of breastmilk),\textsuperscript{99,103} or health determinants (colonization or vertical transmission of pathogens).\textsuperscript{103,105}
Beyond behavioral, socio-economic and micro-environmental factors, several studies previously reported correlations between maternal HIV disease severity (low CD4 cell counts, high plasma viral load or maternal death) and respiratory infections, as well as mortality among HEU children. These findings suggest that under homogeneous behavioral, socio-economic, and environmental exposures, non-exchangeability of morbidity and/or mortality outcomes across HEU and HUU children may be attributable to maternal HIV and/or prophylactic ARV drug exposures.

**Biological mechanisms of childhood morbidity attributed to maternal HIV exposure**

Immunological perturbations including immune activation, decreased number and function of T-cells, and deranged humoral immune responses have been posited as potential mechanisms for *in-utero* and postpartum childhood morbidity attributed to maternal-HIV exposure.

The detection among HEU children of a wide array of HIV-specific cytotoxic T lymphocyte (CTL) response types are considered a signature marker for infant exposure to maternal HIV. HIV-infected mothers are in a chronic state of immune activation as a result of HIV infection, recurrent or chronic opportunistic infections and/or infestations, which is associated with a pro-inflammatory *in-utero* and postpartum (breastfeeding) milieu. Normal pregnancy is associated with a state of immunological tolerance. The mounted immune responses among HEU relative to HUU children, including both CTL (breakdown of HIV infected cells and increased secretion of interferon (IFN)-γ) and CD4 helper T-cell response (proliferation, interleukin (IL)-2 and IFN-γ secretion) potentially accounts for the apparent protection against HIV acquisition among ARV-exposure naïve HEU children. However, according to *in-vitro* studies immune-activated cells are associated with increased susceptibility to HIV and theoretically other intracellular infections. This suggests that the increased pro-inflammatory
state among HEU relative to HUU children potentially results in a relatively greater vulnerability to common childhood infections.

The support for an impaired immune response as the biological basis for the reported higher morbidity and mortality, mostly of infectious etiology, among HEU compared to HUU children is derived from several observations of immunological differences (phenotypic and functional). Phenotypical perturbations among HEU children have been reported by several studies the most frequent of which reported antigen-experienced cellular phenotypes, reduced thymic function with fewer naïve T cells, expanded memory T cell subsets, and increased immune activation with increased cell death which could lead to immune senescence in the infant immune system.

A consistently reported functional difference between HEU and HUU infants is the reduction in the transplacental transfer of maternal antibodies as well as diminished concentrations of specific antibodies to common childhood vaccine-preventable infections. This may be due to a severely impaired maternal B cell function and/or poor IgG transplacental transfer among HIV-infected women. This idea is further supported by findings of similar responses to vaccine-specific antibodies among HEU and HUU children suggestive of comparable infant B cell function. Therefore, differences in vaccine response are likely mediated by differences in concentration of the pre-vaccination vertically acquired maternal antibodies. Vertically acquired immunity based on maternal immunoglobulin (Ig) G protects the infants for several months after birth, and therefore diminished passive immunity has implications of a potentially protracted relative vulnerability of HEU children. For example, while in general the IgA, IgM and IgG levels are higher among HEU compared to HUU children, the specific antibodies against tetanus among neonates and measles antibodies among older children were reportedly significantly lower.
**Biological mechanisms of childhood morbidity attributed to ARV drug exposures**

Biologically mediated pathways for pediatric morbidity attributed to ARV drug exposures include mitochondrial degeneration particularly following exposure to nucleoside reverse transcriptase inhibitors, toxicity to nuclear DNA of hematopoietic stem cells, and dysregulation of bone metabolism and/or mineralization, in the developing fetus and the young infant.

The NRTI-induced inhibition of DNA polymerase-g interrupts the process of mitochondrial DNA replication which leads to mitochondrial damage and dysfunction. There is evidence to suggest that the degree of mitochondrial DNA depletion varies by the type of NRTI, with ddC, ddl and d4T as the strongest inhibitors of DNA polymerase-g. In a milieu of mitochondrial damage and dysfunction, pyruvate metabolism is skewed towards lactate production and clinical syndromes manifested with hyperlactatemia and lactic acidosis have been reported.

In 1999, Olivero et al utilized a neonatal monkey model to study mitochondrial changes in cord and infant tissues among infant monkeys exposed to NRTIs. The team reported evidence of mitochondrial dysfunction in tissue culture studies. In a lab based study, mitochondrial dysfunction was demonstrated in cord blood drawn from monkey infants without retroviral infection, as well as blood drawn from HEU infants, after the respective samples had transplacental exposures to NRTI drugs. In terms of human studies, findings have been conflicting. In 1999, Blanche and Tardieu reported that several infants in the French perinatal cohort who were HIV negative but exposed to perinatal NRTIs had unexplained severe neurologic disease; and related this to possible mitochondrial dysfunction from in utero exposure to NRTI drugs. In a larger study of over 4000 uninfected HIV and ARV exposed children Barret and Tardieu reported that the 18-month incidence of symptoms compatible with mitochondrial
dysfunction was 0.26% and 0.07% for mortality.\textsuperscript{59} Other clinical syndromes suggestive of NRTI-induced mitochondrial damage that have been reported include transient neonatal hyperlactatemia as well as deficits in mitochondrial respiratory chain complex enzyme function in muscle tissue.\textsuperscript{59,60} However, analyses of longitudinal data from a number of U.S. perinatal cohort studies conducted in the 1990s, and the European Collaborative Study have not found evidence of mitochondrial degenerative disease among ARV exposed HEU infants.\textsuperscript{61,62}

In-vitro studies suggest ZDV-induced deleterious effects on the pluripotent hematopoietic progenitor cells.\textsuperscript{149,150} Hematopoiesis is a dynamic and complex process of constant and balanced replacement of various blood cell lines by proliferation and differentiation of the appropriate daughter germ cell lines. Independent studies that assessed hematological outcomes among newborns and infants with peripartum exposures to antiretroviral drugs reported a mild and significant inhibition of erythroblastic progenitors which was persistent.\textsuperscript{61,140,151}

Based on pre-clinical animal studies, TDF is associated with dysregulation of bone metabolism and mineralization, mediated through renal toxicity pathways: a) hypophosphatemia secondary to proximal tubular epithelial cell damage and resultant tubular dysfunction; and b) decreased function of alpha-1-hydroxylase an enzyme of vitamin D metabolism as a result of diminished glomerular filtration.\textsuperscript{152} The TDF-induced renal toxicity is dose dependent.\textsuperscript{141} Nephrotoxicity with hypophosphatemia has been reported among HIV-infected patients on TDF, with rare reports of Fanconi syndrome particularly among patients with TDF and lopinavir/ritonavir cocktails.\textsuperscript{153,154}

1.3 RESEARCH METHODOLOGY
1.3.1 Conceptual Framework

We hypothesize that dual exposures to maternal HIV infection and prophylactic ARV drugs during critical \textit{in-utero} and prolonged postpartum periods, a phase of rapid cell growth and
differentiation is associated with negative physical growth, hematological complications and ultimately decreased survival among exposed-group children compared to the unexposed uninfected controls. The potentially mediating biological mechanisms are as summarized in section 1.2.3.4 above. The postulated pathways and potential co-factors are summarized in figure 1.3.

### 1.3.2 Study Design

This was a prospective-cohort study nested within the ongoing NEURODEV (ND) study, a US National Institutes of Health (NIH) funded study number HD 073296, Dr. Mary Glenn Fowler, and Dr. Michael Boivin as Co-Principal investigators. The ND study is a five-year longitudinal follow-up study evaluating developmental, neuropsychological, neurologic, physical growth and hematological group differences between HEU children with prior exposures to ARV drugs for PMTCT, and age-and-gender matched HUU controls. Children were considered HEU if they were determined to be HIV-uninfected, and born to HIV-infected mothers. The control group HUU children were HIV-uninfected, born to HIV-uninfected mothers, and therefore no prior history of ARV drug exposures. To minimize heterogeneity across the two groups regarding socio-economic, cultural practices and other environmental factors critical to child health and survival, HUU children and their mothers were identified from well-child/immunization clinics within the same hospital settings that served as the source population for the PROMISE clinical trial participants.

The ND study is ongoing at two international sites in Blantyre, Malawi and Kampala, Uganda. The two countries are part of the 21 global plan priority countries earmarked for rapid scale-up of ARV drugs for PMTCT by the UNAIDS. The ND study is funded by the US National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health, and Human Development (NICHD). The institutional review boards (IRBs) at the Johns Hopkins
Medical Institute (JHMI) and at the respective implementing clinical research sites, as well as the relevant national regulatory councils in Malawi and Uganda, approved the ND study.

1.3.3 Study, source, and target populations

1.3.3.1 Study population

Exposed-group children

From August 2013 through October 2014, 480 children (240 at each site) co-enrolled in an ongoing PMTCT ARV drug trial called PROMISE – ‘Promoting Maternal and Infant Survival Everywhere’, were targeted for enrollment into the ND study at both sites in Blantyre, Malawi and Kampala, Uganda. The eligibility criteria were: 6-12 months of age at enrollment; birth weight ≥ 2000 gm; negative infant HIV-1 DNA PCR; no known serious chronic condition; confirmed maternal HIV-infection status; and written informed consent from the mother.

Unexposed control-group children

A total of 480 (240 at each site) control group children were targeted for recruitment via well-child/immunization clinics between August 2013 and October 2014. Age (+/- 4 months), and gender-matched controls were enrolled if they met the eligibility criteria: birth weight ≥ 2000 gm; no known serious chronic clinical condition; written consent from the mother and confirmed HIV-1 negative mothers (HIV-1 rapid testing).

1.3.3.2 Source population

1.3.3.2.1 Exposed-group children – PROMISE clinical trial

The ND study HEU children and their mothers were identified from a source population of mother-baby pairs co-enrolled and followed in the PROMISE trial between 2011 and 2016, at the respective clinical research sites in Blantyre, Malawi and Kampala, Uganda.
The PROMISE clinical trial number NCT01061151 (clinicaltrials.gov registry), a multicenter international research conducted in India, Malawi, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe, was an NIH supported randomized controlled trial designed to compare the efficacy, safety, and compliance of different ARV regimens appropriate for PMTCT in resource limited settings. A detailed description of the trial has been previously reported. In summary, HIV-infected pregnant women who were otherwise healthy were recruited early during pregnancy and followed under controlled trial settings through antepartum and delivery periods; and together with their babies through the postpartum period.

Trial enrollment began in 2011. In both Malawi and Uganda, the WHO ‘option A’ was the standard-of-care for healthy HIV-infected women with a CD4 count of more than 350 cells per cubic millimeter and therefore considered not in need of ART for their own health. Women were enrolled in the trial if they met the following eligibility criteria: CD4 count of at least 350 cells per cubic millimeter (or a country specific threshold for initiating triple-drug ART, if that threshold was higher), gestation of 14 weeks and beyond and not in labor, no previous use of triple-drug ART (one or two ARV drugs based PMTCT in previous pregnancies and for 30 days or fewer during the current pregnancy before enrollment was permitted), no indications for ART for their own health, a hemoglobin level of at least 7.5 g per deciliter, an absolute neutrophil count (ANC) of at least 750 cells per cubic millimeter, an alanine aminotransferase level of less than 2.5 times the upper limit of the normal range, an estimated creatinine clearance of more than 60 ml per minute, and no serious pregnancy complications. Women were excluded if they had active tuberculosis or on treatment for tuberculosis within 30 days prior to enrollment, were in need for hepatitis B virus treatment, had heart defect, fetal congenital malformation.

The trial which employed a non-masked (open-label) strategy had two sequential randomizations. The antepartum randomization (first 14 weeks of gestation or later) had 3 arms: ‘arm-A’ (antepartum and intrapartum ZDV followed by intrapartum maternal sdNVP and
TDF+FTC); ‘arm-B’ (ZDV+3TC+ LPV/r) and ‘arm-C’ (TDF+FTC+LPV/r). All the maternal ARV regimens per the antepartum randomization, and infant nevirapine initiated at birth, were continued through the time of postpartum randomization. The postpartum randomization was performed within one week (6-14 days) of delivery/birth and included two arms: ‘arm-A’ (infant prophylaxis with daily NVP for the duration of breastfeeding) and ‘arm-B’ (maternal triple ARV regimens for the duration of breastfeeding, plus infant NVP through 6 weeks of life). The postpartum ‘arm-B’ maternal triple ARV regimens were prescribed in accordance with the country-specific standard-of-care. The antepartum and postpartum PROMISE trial ARV-based randomizations and treatment regimens are depicted in figure 1.4.

1.3.3.2.2 Control-group children – child-well clinics

HIV-unexposed uninfected (HUU) children and their mothers in the ND study were identified via well-child/immunization clinics within the same hospital settings that served as the source population for the PROMISE clinical trial participants at the respective clinical research sites, in Blantyre, Malawi and Kampala, Uganda.

1.3.3.2.3 Clinical Research Sites (CRSs)

The research was conducted at two international clinical research sites (CRSs), the College of Medicine-Johns Hopkins University (CoM-JHU) CRS in Blantyre, Malawi; and the Makerere University-Johns Hopkins University (MU-JHU) CRS in Kampala, Uganda. Through collaborative efforts with their respective Clinical Trials Units (CTUs) at the Johns Hopkins University, both CRSs have developed research capacity over the last two decades including structures for research development; administrative and financial management; monitoring and evaluation as well as personnel and core-resources (clinics; pharmacy; laboratory; ethics and regulatory; data management). In addition, strong relationships with the communities at the respective sites have been built through ongoing community engagements with key stakeholders.
such as the Community Advisory Boards (CAB). Such effective collaborations with stakeholders who represent the community needs, ensure in part, that the conducted research meets the research priorities of the communities, and provides the basis for establishing appropriate study populations. Currently, the CoM-JHU CRS is engaged in more than 25 studies, and MU-JHU CRS more than 20 studies, at various stages from initiation to completion, mostly international collaborative research efforts funded through the US National Institutes of Health.

1.3.3.3 Target population

The target population comprises of Malawian and Ugandan HEU children with peripartum exposures to ARV drugs. In addition, the study findings are potentially generalizable to other HEU children in sub-Saharan Africa in settings with similarly high PMTCT need, where scale-up of ART programs is ongoing, and with concurrent high burden of early childhood co-morbidity and mortality. Both Malawi and Uganda are part of the 22 UNAIDS Global Plan Priority (GPP) countries for scale-up of ART-based programs, a United Nations led global eMTCT effort. Malawi is situated in Southeast-Africa and Uganda in East-Africa, shown in Figure 1.5.

1.3.3.3.1 Malawi profile

Malawi has an area of about 118,484 square kilometers, and an estimated population of 17.7 million people (2015). In the recent 2015/2016 Malawian Demographic Health Survey (DHS), about 58.5% women and 59.6% of men were younger than 30 years of age; 12% women and 5% men reported having no formal education; and about 19.3% women and 15.9% men were in the lowest wealth quintile. The World Bank classifies Malawi as a low-income level country and the estimated gross domestic product per-capita in 2015 was 340 US dollars.

The HIV epidemic in Malawi is generalized with an estimated adult (15-64 years) HIV prevalence of 9.1% (2015), one of the highest globally. Women are disproportionately affected,
with a prevalence of 12.8% compared to 8.2% among men.\textsuperscript{155,158} Malawi was the pioneer in sub-Saharan Africa to launch a nationwide HIV test-and-treat ART-based program for PMTCT (2011), and has since made remarkable progress. More than 76% pregnant women living with HIV in 2015 were on life-long ART, compared to 21% in 2009. Consequently, the 4,800 new pediatric HIV infections in 2015 was a remarkable 71% reduction compared to the 2009 estimates.\textsuperscript{57} Prolonged breastfeeding is common and about 91% of children breastfeed through 12-17 months-of-age and 77% through 18-23 months of age.\textsuperscript{157} Therefore, the annual incidence of HEU children with peripartum exposures to ARV drugs is expected to continue increasing in the coming years.

Childhood morbidity and mortality rates in Malawi are very high. Among under-5-year-old children, 37% were short for their age/stunted (below 2 standard deviations (SD)), and 11% were severely stunted (below -3 SD), while 12% were underweight, and 3% severely underweight. Approximately, 63% had some degree of anemia (less than 11g/dl), mild (27%); moderate (34%) and severe (2%) anemia. The estimated neonatal mortality rate was 27 deaths per 1,000 live births; infant mortality rate was 42 deaths per 1,000 live births; child mortality rate was 23 deaths per 1,000 children surviving to age 12 months. Overall, under-5 mortality rate was 64 deaths per 1,000 live births, a decline compared to 112 deaths per 1,000 live births in 2001-2005. The Malawian universal child immunization program targets common vaccine-preventable diseases including tuberculosis (BCG), diphtheria, whooping cough (pertussis), tetanus; polio; measles, hepatitis B, Haemophilus influenza type b (Hib), Streptococcus pneumoniae and rotavirus. However, among 12-23 months-old children, only 71% were fully vaccinated, and 2% had not received any vaccinations.\textsuperscript{157}

Additionally, ‘Water, Sanitation, and Hygiene’ (WASH) factors are crucial indicators of childhood growth, morbidity, and mortality in resource-limited settings. In Malawi, about 87% of the households nationally (98% urban, and 85% rural) obtain drinking water from an improved
source including piped source, public tap or standpipe, tube well or borehole, and protected well or spring. About 78 percent urban and 67% rural households did not have access to treated water. Toilet/latrine facilities reported by 51.1% urban and 27.6% rural households were improved but shared (flush/pour flush to septic tank, flush/pour flush to pit latrine, ventilated improved pit (VIP) latrine, pit latrine with slab, composting toilet); while 4.2% rural and 19.4% rural households reported using facilities considered to be non-improved (pit latrine without slab/open pit, bucket, no facility/bush/field).\textsuperscript{157}

### 1.3.3.3.2 Uganda profile

Uganda has an area of 241,039 square kilometers with a population of about 39.0 million people (2015). Per the 2011 Ugandan Demographic Health Survey (DHS), about 75.0% women and 76.6% of men were younger than 30 years of age; 64.0% women and 83.0% men were literate; and about 1.9% urban and 23.1% rural individuals were in the lowest wealth-index quintile. Uganda is classified as a low-income country, and the estimated GDP per capita in 2015 was 571 US dollars.\textsuperscript{156,159}

About 1.5 million (7.1%) of the Ugandan population in 2015 were living with HIV. Women are disproportionately affected.\textsuperscript{155} Uganda made the greatest progress of all the 22 UNAIDS GPP countries targeted for global eMTCT efforts, with an estimated MTCT risk through breastfeeding cessation of 3.0% in 2015, a reduction of more than 86% compared to the 2009 estimates. This is attributed to the successful scale-up of ART-based programs for PMTCT. More than 95% of pregnant women living with HIV received ARV medicine for PMTCT, in 2015.\textsuperscript{57}

As noted with the Malawian MTCT epidemic, successful ART-based PMTCT programs in Uganda will result in a generation of HEU children with peripartum ARV drug exposures since prolonged breastfeeding is the norm. The median duration of breastfeeding is 19 months, and
more than 94% of children breastfeed beyond 12 months-of-age. However, background rates of child morbidity and mortality rates remain unacceptably high. Per the 2011 DHS report, about 33% and 14% of under-5-year old children were stunted and severely stunted, respectively. About 5% of under-5 children were wasted, and 14% underweight. One in two children between 6-59 months-of-age had anemia, 22% mildly, 26% moderately and 2% severely anemic. Acute respiratory infection (ARI) and diarrheal diseases are the leading causes of childhood morbidity and mortality. Of the surveyed under-5-year-old children, nearly 15% and 25% had ARI and diarrheal symptoms reported during the preceding two weeks. The neonatal mortality rate was 27 death per 1,000 live births; infant mortality rate was 54 per 1,000 live births and child mortality rate was 38 per 1,000 live births. The under-five mortality rate was 90 per 1,000 live births.159

There are several precipitating factors for the sustained high child morbidity and mortality. Despite efforts to combat common vaccine-preventable childhood killer infectious diseases, only one in two children between 12-23 months-of-age are fully vaccinated against the vaccine-preventable childhood killer infectious diseases, and about 4% not vaccinated at all. In addition, in many settings in the country, environmental WASH factors are key mediators of childhood infectious diseases. About 90.6% of urban but only 65.6% of rural households (where more than 80% of the population resides) have access to improved water source (piped source, public tap or standpipe, tube well or borehole, and protected well or spring). About 26.7% and 58.9% urban and rural households, respectively, do not have access to treated water. About 51.6% urban and 11.3% rural households reported using improved but shared toilet facilities (flush/pour flush to septic tank, flush/pour flush to pit latrine, ventilated improved pit (VIP) latrine, pit latrine with slab, composting toilet). An estimated 55.4% of urban and 5.3% of rural households were using electricity for lighting, while only 4.6% of urban and 0.2% rural households reported using electricity or gas for cooking.159
1.3.4 Study Procedures

The ND study staff were frequently trained on standardized data collection and documentation (structured questionnaires) procedures per routinely updated Standard Operating Procedures (SOP) manuals. Clinical (history and physical examination), anthropometric; neurodevelopmental and laboratory procedures were performed at the enrollment (baseline visit) and routinely during study scheduled follow-up visits at 24, 30, 36, 42, 48, 54 and 60 months of infant age. This research is based on clinical, anthropometric and laboratory assessments obtained at the 12 and 24 months-of-age study visits. The ND study recruitment, enrollment and follow-up procedures are summarized in the appendix. The recruitment flow diagrams for the exposed and control group children respectively are summarized in appendix 1.4 and 1.5, respectively.

1.3.4.1 Informed consent procedures

Written informed consent for study participation was obtained from the mothers prior to study enrollment. Counselling and informed consent procedures were conducted and documented by experienced counselors with training on the local Ministry of Health and WHO HIV counselling guidelines, Good Clinical Practice (GCP) and study standardized operating procedures (SOP) across the two sites in Blantyre, Malawi and Kampala, Uganda.

In addition, written informed consent was obtained prior to study screening of potential controls. Mothers with documented screening informed consent were offered HIV pre-test counseling and testing using a rapid-test in accordance with the local Ministry of Health guidelines. Documentary evidence of prior HIV testing (1 negative Enzyme Linked Immunosorbent Assay (EIA) or 1 negative rapid test within 3 months prior to time of consent) was considered acceptable.
1.3.4.2 Enrollment evaluations (Exposed-Group)

Mother-baby pairs identified via the PROMISE trial underwent a clinical review including medical history, physical examination, and collection of socio-demographic characteristics. Documentary evidence of the following information was abstracted from the PROMISE study records: confirmed maternal HIV-infection status; maternal plasma viral load; fetal and postpartum infant ARV drug exposures; infant birth history, birth weight, infant and maternal medical history, infant growth, and development progress.

Confirmation of maternal HIV infection

Confirmed HIV infection was defined as documented positive results from two samples collected at different time points prior to study entry. Any of the following tests were acceptable for the respective samples: sample # 1 (two rapid antibody tests from two different manufacturers or based on different principles and epitopes; One EIA OR Western Blot OR immunofluorescence OR chemiluminescence; One HIV DNA PCR; One quantitative HIV RNA Polymerase Chain-Reaction (PCR) (> 5,000 copies/mL); One qualitative HIV RNA PCR; One total HIV nucleic acid test); and sample #2 (one EIA confirmed by Western Blot OR immunofluorescence OR chemiluminescence; One HIV DNA PCR; One quantitative HIV RNA PCR (> 5,000 copies/mL); One qualitative HIV RNA PCR; One total HIV nucleic acid test). Sample # 2 had to be tested in the laboratory approved by the PROMISE trial central lab, participates in appropriate external quality assurance programs, and follows Good Clinical Laboratory Practice (GCLP) guidelines.

In-utero and postpartum exposures to maternal HIV and ARV drugs

Evidence and quantification of antepartum-fetal and postpartum-child exposures to maternal HIV infection are based on the documented maternal plasma HIV RNA concentration (quantified by real-time PCR in the ART groups and by batch testing of stored samples in the non-ART
groups), measured at antepartum trial entry, week 4 post-entry, and delivery; and at postpartum trial entry, weeks 6, 26 50, and thereafter quarterly.

Documentary evidence of in-utero and postpartum child exposure to maternal and/or infant prophylactic ARV drugs is based on the antepartum and postpartum ARV drug based randomizations, as well as maternal reported mother and child ARV drug adherence. Standardized questionnaires were routinely administered to document drug adherence, drug switch (for toxicity, ART initiation among women randomized non-ART regimens, etc.), drug discontinuation (temporary or permanent), and breastfeeding (medium of postpartum child exposures to maternal HIV and prophylactic ARV drugs) status.

1.3.4.3 Enrollment evaluations (Control-Group)

Mother-baby pairs identified via the well-child/immunization clinics underwent preliminary screening to confirm the maternal HIV-uninfected status; and willingness to participate in a long-term follow-up study including home-visits. Eligible mother-child pairs underwent a clinical review including medical history, physical examination, and collection of socio-demographic characteristics. The control-group mothers provided their children’s birth records (standard WHO Child Health passport/Ministry of Health card/), which served as the source documentation for the infant date of birth and birth-weight.

1.3.4.4 Infant follow-up evaluations

Clinical assessments and anthropometric measurements at 12, and 24 months-of-age, were assessed by experienced study nurses trained on standardized study procedures. The standard tape was used to measure the head circumference and mid-upper arm circumference, the WHO weight measure with a calibrated scale to determine the weight, a stadiometer was used to measure length of the younger children and the ordinary height scale used for older children. The study doctor performed a full medical exam to elicit past illnesses; hospitalizations; medications;
and a physical exam based on a standardized questionnaire. For purposes of standardized assessment of infant nutrition (including severity of malnutrition) the WHO criteria for evaluation of malnutrition and Failure to Thrive adapted to the PROMISE trial was used (Appendix 1.3). The clinical history at each follow-up visit was documented on a standardized form (Appendix 1.6).

*Complete Blood Count (12 and 24 months of age)*

Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell count (WBC), differential count, absolute neutrophil count (ANC), and platelet count at 12 and 24 months of age was documented for both exposed group and control group children. Control group children had blood drawn at 12 and 24 months of age study visits for CBC measurement. Complete blood count results for the exposed group children were abstracted from the PROMISE trial records.

*HIV screening at 24 months-of-age*

HIV screen results at 24 months of age were documented for both exposed and control group children. Control group children were screened with the HIV-rapid test. If the HIV-rapid test was positive, a second rapid test on a separate sample collected at a different time point was performed. A third rapid test was used as a tie breaker if the first and second rapid tests yielded discordant test results.

HIV screen results for the exposed group children were abstracted from the PROMISE Trial records. PROMISE trial children who had not achieved complete cessation of breastfeeding received a Nucleic-Acid-Test (NAT) using HIV DNA PCR or a second-choice HIV RNA PCR. If the initial HIV NAT was positive, a confirmatory test was performed as soon as possible with a repeat HIV NAT on a second sample drawn on a different day. Among children who had achieved complete cessation of breastfeeding, HIV antibody testing was done using an Enzyme
Linked Immunosorbent Assay (EIA) or HIV rapid test. If HIV antibody test was negative, no further HIV testing was done. Children with a positive HIV antibody test had a follow-up HIV NAT test performed as soon as possible on a separate sample on a different day.

1.3.4.5 Quality Control and Quality Assurance

To ensure that procedures are standardized across the two sites, the same versions of the protocol, informed consent forms, study source documentation and Standard Operating Procedures were maintained across the two sites. This was the responsibility of the protocol coordinator (PC) through back and forth e-mail communication and conference calls (at least once a month) involving the respective site study teams and the protocol team. This also serves the purpose for real-time protocol query resolution. In addition, a protocol retreat for refresher protocol training is held annually at either of the two sites, in alternate pattern, and is attended by the protocol team members and representatives from both sites.

1.3.5 Data Management and Statistical Analysis

1.3.5.1 Sample size calculation

The sample size calculations for the parent-ND study were based on neurodevelopment outcomes, the primary study objective. The accrual target was 960 mother-child pairs, 480 per site (240 in each exposure-group). These analyses are therefore based on a fixed sample size. We compared study outcomes by exposure-status (exposed versus controls), as well as disaggregated exposure categories versus unexposed control reference, respectively. It is important to assure that even simple comparisons had sufficient power. We estimated a-prior that the study was well powered (≥ 80%) to detect practically meaningful effect sizes, defined as exceeding 1/3 of the standard deviation, based on Cohen’s difference between means expressed in standard deviation units. Minimum detectable effect sizes based on 5% (two-sided) type I error and the fixed sample sizes are depicted in table 1.3. With the available sample sizes, even smaller differences of 0.25 to
0.20 of the standard deviation are detectable with power 0.80 or greater. The only exception is N=120, where differences need to be 0.52 of the standard deviation or greater to be detected as statistically significant.

1.3.5.2 Data management

Data collected concurrently at enrollment and study follow-up visits was entered and validated in real-time using ‘FileMaker 12 and Server 14’ at the respective sites, and routinely migrated by the site data managers to the study data management team based in Kampala, Uganda. In addition, we obtained data on in-utero and postpartum exposures to maternal-HIV and prophylactic antiretroviral drugs from the PROMISE randomized trial database at the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) Statistical and Data Analysis Center at the Harvard School of Public Health. The exposure data included PROMISE trial randomizations (antepartum and postpartum); ARV drug-adherence; maternal HIV-viral load at delivery, and infant birthweight. We abstracted infant birth weight for the control-group children from the immunization card or WHO Health passports obtained from study participants. We assessed the association between maternal-HIV and prophylactic ARV drug exposure and anthropometric, hematological and morbidity outcomes, respectively.

1.3.5.2.1 Exposure to maternal-HIV and prophylactic ARV drugs

Exposed (maternal-HIV and prophylactic ARV drugs) status was based on confirmatory documentation from the PROMISE randomized clinical trial records of the mother’s HIV-infection status, prophylactic ARV drug exposures, and child’s HIV-free status through 24-months-of-age. Duration of exposure was computed as the accumulated time between antepartum ARV drug initiation through breastfeeding cessation, when exposure was effectively ceased. Unexposed status of the control-group children was based on confirmed maternal HIV-negative report at ND study pre-entry, and confirmed infant HIV-free status through 24 months-of-age.
We further classified disaggregated exposure by the PROMISE trial randomization schemes: antepartum-randomization Arm-A (ZDV alone) versus Arm-B (ZDV+3TC+LPV/r) versus Arm-C (TDF+FTC+LPV/r); and postpartum-randomization arm-A (daily infant NVP) versus arm-B (maternal TDF+FTC+LPV/r), respectively. We also classified disaggregated exposures by combination ART (cART), regimen with a cocktail of three or more ARV drugs, versus non-cART regimens. Lastly, we conducted sensitivity analyses, exploring in-utero exposures regardless of postpartum exposures, and separately, cumulative (in-utero + postpartum) exposures.

1.3.5.2.2 Growth outcomes

Anthropometric (weight, height, and head circumference) measurements at birth, 12 and 24 months-of-age were used to calculate z-scores for length-for-age (LAZ); weight-for-length (WLZ); and weight-for-age (WAZ) using the WHO age-and gender-based Growth standards (2006). Based on the derived Z-scores, anthropometric outcomes defined by standard deviations (SDs) below the median of the WHO population standards were compared across the exposure groups versus the control-group reference, respectively. Anthropometric z-scores < 2 SD below the WHO reference-population median are indicative of moderate growth impairment, while z-scores < 3 SD are suggestive of severe growth impairment. We defined stunting, wasting, and underweight as LAZ, WLZ and WAZ below minus two SDs from the medians of the respective references; and severe stunting, wasting and underweight defined as below minus three SDs from the reference population medians, respectively.

1.3.5.2.3 Morbidity and mortality outcomes

We examined the following childhood morbidity outcomes: childhood hospitalization history (yes versus no) as reported by the mother/primary care-giver at a subsequent study visit; hematological outcomes (≥ grade 3 decreased hemoglobin (anemia); ≥ grade 3 decreased absolute
neutrophil counts (neutropenia) or (≥ grade 3 decreased platelet counts (thrombocytopenia); and a composite morbidity/mortality index including child hospitalization, grade 3 or higher decreased hematological outcomes, or mortality.

1.3.5.2.4 Key covariates

We considered key covariates that have previously been associated with childhood growth, morbidity or mortality including: maternal factors (age, maternal viral load, primary child-care giver); household-level social-economic and/or environmental factors (income, water source, electricity/gas use, refrigerator use); and infant factors (age, gender, and breastfeeding duration). In addition, maternal well-being was assessed per the standardized Hopkins Symptoms Checklist (HSCL) score for anxiety and depression domains. The household-income-stability index score was based on a composite of five binary (yes/no) questions of the standardized AFASS questionnaire: “Is mother currently working?”, “Is mother the primary bread-winner?”, “Is pooled income sufficient?”, “Is pooled income sufficient for 12-months?”, “Is there enough to spend on infant nutrition and transport to health care?”. Equal weights (yes=1; no=0) were applied to each question and based on the total score, household-income was considered relatively-stable (5, 4 or 3); fairly-stable (2) or unstable (1 or 0).

1.3.5.3 Statistical Analyses

All the statistical analyses were conducted using Stata version 14.2 (Stata, College Station TX, USA). Wilcoxon Rank Sum and Fischer’s exact tests were used to compare continuous and categorical variables, respectively. The Mantel-Haenszel chi-square ($\chi^2$) was used to assess for trends within categorical variables. All the p-values were based on a two-sided hypothesis test with a type-1 error ($\alpha = 0.05$).

Generalized Estimating Equation (GEE) regression models with a log-link were used to compute the relative risk of binary outcomes (stunting, wasting, underweight, and small heads
(HCAZ below the median of the WHO reference population); anemia, and neutropenia) at 12 and 24 months-of-age visits, with 95% Confidence Intervals (CI) computed with robust variance estimators, while accounting for within individual correlations.

Poisson regression model with robust error variance was used to estimate the relative risk of morbidity (at least one hospitalization since birth) at 12 months-of-age, by exposure groups. We used the Kaplan-Meier survival curves to assess the time-to-event (hospitalization, hospitalization/death composite), after 12 months-of-age through 24 months-of-age, and tested differences by exposure using the log-rank test. The Greenwood’s formula was used to estimate the 95% confidence intervals for the proportion of children ‘surviving’ (free of the event-of-interest) through 24 months-of-age. Cox proportional hazards model was used to estimate relative hazards while adjusting for key confounding variables.

For the respective GEE and survival analyses, univariate and multivariate analyses were used to explore covariates defined a-prior, both correlated with exposure and predictors of infant health. In addition to maternal viral load, exposure duration, breastfeeding status (yes/no), breastfeeding duration, infant age and gender, covariates with a p-value ≥ 0.10 in univariate analysis with the respective outcomes, were considered for the multivariate models to control for potential confounding bias. Product terms of site (Malawi versus Uganda) and exposure (maternal HIV plus ARVs) variables were added to the multivariate regression models to assess whether geographical location modified the exposure and outcome associations. Likelihood ratio tests were used to compare null and extended models. We also performed a mediation analysis exploring the potential for low birthweight (<2500 grams) as a mediator for the growth outcomes at 12 and 24 months-of-age.
1.4 SUMMARY OF INTRODUCTION

Several of the 22 Global Plan Priority countries (all but India in sub-Saharan Africa) are on a fast-track to eMTCT, and by 2015, more than 95% of HIV-infected women in Uganda, and 80% in Malawi, had combination-ART (cART) coverage during the vertical transmission risk-period. It is projected that more than 90% of 1.5 million new cases of ART-exposed HEU children worldwide per year, by 2020, will come from sub-Saharan Africa. Therefore, substantial reductions in vertical HIV transmission because of successful ART-based PMTCT programs in sub-Saharan Africa, will result in a generation of HEU children with long-term exposures to complex ART regimens. However, there are growing concerns that peripartum ART exposures are associated with increased morbidity and mortality among HEU children. This presents a potential for dual and perhaps complimentary epidemics in sub-Saharan Africa, since the region contributed more than 50% of overall under-five child deaths globally in 2013. Proposed biologically mediated pathways of ART effects include mitochondrial damage, toxicity to nuclear DNA of hematopoietic stem cells, and dysregulation of bone metabolism in the developing fetus and the young infant. However, evidence based on African populations is inconclusive, and had ultra-short ART regimens, short or no breastfeeding, and study-design limitations. Our research attempts to fill this knowledge gap through a longitudinal cohort study of ARV-exposed HEU children and age-and gender-matched HUU controls, followed through 12 and 24 months of age, at two international sites in Malawi and Uganda. To the best of our knowledge, no prior study assessed morbidity and mortality outcomes through 24 months-of-age among HEU children with a history of in-utero and prolonged postpartum ART exposures beyond 12-18 months-of-age.
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<table>
<thead>
<tr>
<th>Antepartum</th>
<th>Intrapartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 2013, 2015&lt;sup&gt;5&lt;/sup&gt; : combined antiretroviral therapy (cART) regardless of maternal CD4 cell count, taken for life (Option B+)</td>
<td>Maternal cART (14 weeks to labor)</td>
<td>Maternal cART during labor</td>
</tr>
<tr>
<td>ZDV+3TC+LPV/r</td>
<td>ZDV+3TC+LPV/r</td>
<td>ZDV+3TC+LPV/r; ZDV+3TC+ABC</td>
</tr>
<tr>
<td>ZDV+3TC+ABC</td>
<td>ZDV+3TC+ABC</td>
<td>ZDV+3TC+EFV; TDF+FTC+EFV</td>
</tr>
<tr>
<td>ZDV+3TC+EFV</td>
<td>ZDV+3TC+EFV</td>
<td>TDF+FTC+EFV</td>
</tr>
<tr>
<td>TDF+FTC+EFV</td>
<td>TDF+FTC+EFV</td>
<td></td>
</tr>
</tbody>
</table>

| WHO 2009/2010<sup>6</sup>: antiretroviral (ARV) prophylaxis by maternal CD4 cell count (Option A or B) |
|---|---|---|
| **Option A** | Maternal ZDV (14 weeks gestation through labor onset) | Maternal sdNVP (labor onset) | Maternal: ZDV/3TC 1 week postpartum |
| | Maternal ZDV/3TC (labor) | | BF infant: NVP daily from birth until 1 after all exposure to breast milk |
| | | | Non-BF infant: ZDV first 6 weeks of life |
| **Option B** | Maternal cART (14 weeks gestation through labor onset) | Maternal cART (Throughout labor) | Maternal cART through BF cessation |
| | ZDV+3TC+LPV/r | ZDV+3TC+LPV/r | ZDV+3TC+LPV/r; ZDV+3TC+ABC |
| | ZDV+3TC+ABC | ZDV+3TC+ABC | ZDV+3TC+EFV; TDF+FTC+EFV |
| | ZDV+3TC+EFV | ZDV+3TC+EFV | TDF+FTC+EFV |
| | | | Infant: daily NVP from birth through BF cessation |
| | ZDV+3TC/FTC+NVP/EFV | | |
| | * irrespective of gestational age | | |
| | * no EFV during first trimester | | |
| Maternal CD4 ≤350cells/mm³/clinical reason indication | maternal: ZDV+3TC/FTC+NVP/EFV | BF infant: NVP birth to 6 weeks |
| | | Non-BF: ZDV/NVP for 6 weeks | |
| Maternal CD4 <350cells/mm³ | Maternal cART | ZDV+3TC/FTC+NVP/EFV | Maternal: ZDV+3TC/FTC+NVP/EFV |
| | ZDV+3TC/FTC+NVP/EFV | | Non-BF: ZDV/NVP for 6 weeks |
| | * irrespective of gestational age | | |
| | * no EFV during first trimester | | |

3TC-lamivudine; ABC-Abacavir; ART (Antiretroviral Therapy); ARV, antiretroviral; BF (Breast Feeding); BF cessation, one week after all exposure to breast milk; cART, combined ART; EFV, efavirenz; FTC, emtricitabine; LPV/r, lopinavir-ritonavir; NVP, nevirapine; sdNVP, single dose nevirapine; ZDV, zidovudine.


Table 1.2. Summary of studies in sub-Saharan Africa comparing growth outcomes among HEU and HUU children

<table>
<thead>
<tr>
<th>Physical growth measure</th>
<th>ART for PMTCT</th>
<th>Study design</th>
<th>HEU</th>
<th>HUU</th>
<th>Infant age assessed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birthweight</strong></td>
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</tr>
<tr>
<td>DRC (1988-1989)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>190</td>
<td>256</td>
<td>20 months</td>
<td>Lower mean birthweight among HEU vs HUU†</td>
</tr>
<tr>
<td>Malawi &lt;1996</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>270</td>
<td>686</td>
<td>24 months</td>
<td>Lower mean birthweight among HEU vs HUU (p&lt;0.05)</td>
</tr>
<tr>
<td>Zimbabwe (1997-2002)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>3120</td>
<td>9209</td>
<td>24 months</td>
<td>Lower mean birthweight among HEU vs HUU (p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Height-for-age (Stunting)</strong></td>
<td></td>
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</tr>
<tr>
<td>DRC (1988-1989)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>190</td>
<td>256</td>
<td>20 months</td>
<td>No difference in mean LAZ (20 months)</td>
</tr>
<tr>
<td>Rwanda (1988-1993)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>140</td>
<td>207</td>
<td>48 months</td>
<td>No difference in mean LAZ (24 months) but not after 6 months</td>
</tr>
<tr>
<td>Kenya (1991-1994)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>155</td>
<td>139</td>
<td>18 months</td>
<td>No difference in mean LAZ (24 months)</td>
</tr>
<tr>
<td>Malawi &lt;1996</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>270</td>
<td>686</td>
<td>24 months</td>
<td>No differences in mean LAZ (24 months)</td>
</tr>
<tr>
<td>Zimbabwe (1997-2002)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>3120</td>
<td>9209</td>
<td>24 months</td>
<td>No difference in mean LAZ (24 months)</td>
</tr>
<tr>
<td>Botswana (2001-2003), 2006-2008</td>
<td>ART v. ZDV</td>
<td>2 RCTs</td>
<td>516</td>
<td>303</td>
<td>24 months</td>
<td>Lower LAZ among HEU (12 months, p&lt;0.05)</td>
</tr>
<tr>
<td>Uganda (2010-2011)</td>
<td>ART era‡</td>
<td>c/section</td>
<td>200</td>
<td>400</td>
<td>4-6 months</td>
<td>Lower LAZ among HEU (12 months, p&lt;0.05)</td>
</tr>
<tr>
<td>Botswana (2013-2014)</td>
<td>Plan B</td>
<td>c/section</td>
<td>396</td>
<td>1109</td>
<td>24 months</td>
<td>Lower LAZ among HEU (12-24 months, p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Weight-for-age (Underweight)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRC (1988-1989)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>190</td>
<td>256</td>
<td>20 months</td>
<td>No difference in mean WAZ (20 months)</td>
</tr>
<tr>
<td>Rwanda (1988-1993)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>140</td>
<td>207</td>
<td>48 months</td>
<td>No difference in mean WAZ (24 months)</td>
</tr>
<tr>
<td>Malawi &lt;1996</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>270</td>
<td>686</td>
<td>24 months</td>
<td>No differences in mean WAZ (24 months)</td>
</tr>
<tr>
<td>Zimbabwe (1997-2002)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>3120</td>
<td>9209</td>
<td>24 months</td>
<td>Lower WAZ among HEU (12 months, p&lt;0.05)</td>
</tr>
<tr>
<td>South Africa (2001-2004)</td>
<td>sdNVP</td>
<td>p/cohort</td>
<td>902</td>
<td>1060</td>
<td>24 months</td>
<td>No differences in mean WAZ (24 months)</td>
</tr>
<tr>
<td>Botswana (2001-2003), 2006-2008</td>
<td>ART v. ZDV</td>
<td>2 RCTs</td>
<td>516</td>
<td>303</td>
<td>24 months</td>
<td>Lower LAZ among ART vs ZDV (24 months, p&lt;0.05)</td>
</tr>
<tr>
<td>Uganda (2010-2011)</td>
<td>ART era‡</td>
<td>c/section</td>
<td>200</td>
<td>400</td>
<td>4-6 months</td>
<td>Lower WAZ among HEU (5.2 months, p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Weight-for-age (Wasting)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRC (1988-1989)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>190</td>
<td>256</td>
<td>20 months</td>
<td>No difference in mean WLZ (20 months)</td>
</tr>
<tr>
<td>Kenya (1991-1994)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>155</td>
<td>139</td>
<td>18 months</td>
<td>Higher WLZ among HUU (at 6 &amp; 18 months) p&lt;0.05</td>
</tr>
<tr>
<td>Malawi &lt;1996</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>270</td>
<td>686</td>
<td>24 months</td>
<td>No differences in mean WLZ through (24 months)</td>
</tr>
<tr>
<td>Zimbabwe (1997-2002)</td>
<td>Pre-ART</td>
<td>p/cohort</td>
<td>3120</td>
<td>9209</td>
<td>24 months</td>
<td>Lower WLZ among HEU (12 months, p&lt;0.05)</td>
</tr>
<tr>
<td>Uganda (2010-2011)</td>
<td>ART era‡</td>
<td>c/section</td>
<td>200</td>
<td>400</td>
<td>4-6 months</td>
<td>Lower WLZ among HEU (12 months, p&lt;0.05)</td>
</tr>
</tbody>
</table>

NB: † P values not specified; ‡ ART era although the ARV-based PMTCT regimen was not specified in the article; p/cohort – Prospective Cohort study; RCT – Randomized Controlled Trial; c/section – Cross-sectional study;
Table 1.3. Power and sample size calculation

<table>
<thead>
<tr>
<th>Fixed sample size</th>
<th>Minimum detectable effect size (MDES*)</th>
<th>Length-for-age Z-score detectable difference (SD~1.27)</th>
<th>Head-circumference-for-age Z-score (SD~1.12)</th>
<th>Absolute Neutrophil Count (ANC) age Z-score (SD~1.31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>960</td>
<td>0.18</td>
<td>0.23</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>480</td>
<td>0.26</td>
<td>0.33</td>
<td>0.29</td>
<td>0.34</td>
</tr>
<tr>
<td>240</td>
<td>0.36</td>
<td>0.46</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>120</td>
<td>0.52</td>
<td>0.66</td>
<td>0.58</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Key: SD, standard deviation; MDES, Cohen’s difference between means expressed in standard deviation units (meaningful effect sizes defined as exceeding 1/3 of the standard deviation); MDES estimates for the respective outcomes were based on SD estimates based on the literature; MDES estimates with power ≥ 80%, using two-sided tests with a 0.05 level of significance are in bold.
Figure 1.1. Increased antiretroviral drug coverage for prevention of mother-to-child transmission of HIV, by Global Plan Priority (GPP) country in sub-Saharan Africa (2009 -2015)


All the 22 UNAIDS GPP countries are in sub-Saharan Africa, except India. The presented 2016 estimates do not include Ethiopia. Country-level data were based on proportion of HIV-infected pregnant women who received antiretroviral medicines (excluding single-dose nevirapine only regimens) for PMTCT. Botswana, Mozambique, Namibia, South Africa, Swaziland, and Uganda achieved the UNAIDS 90% coverage by 2015 target set in 2009. To assess impact, MTCT risk in 2015 was compared to baseline in 2009.
Figure 1.2. Determinants of optimum in-utero and early childhood growth and development

↓ childhood morbidity and mortality  ↓ emotional, social skills and neurodevelopment  ↑ learning skills and school achievements  ↑ adult stature and ↓ non-communicable diseases  ↑ economic productivity

Breastfeeding, weaning practices and nutritive value and frequency of meals  Parenting, caregiving, feeding, weaning and child stimulation  Infectious disease burden

Food security (availability, economic access, and consumption)  Caregiving resources (maternal health, household, and community levels)  Environmental (access and utilization of health services, *WASH factors, hygiene)

Global factors (socio-economic, financial resources, politics, and governance)

Figure 1.3. Conceptual framework

Key: ARV, antiretroviral drug; BF, breastfeeding; CTXp, cotrimoxazole prophylaxis; ID, infectious disease; MD, maternal death; WASH, water, sanitation, and hygiene; SES, socio-economic status. The dashed lines depict the potential co-factors operating at the individual and/or population levels.
Figure 1.4. Antepartum and postpartum antiretroviral drug exposures (PROMISE trial randomization schemas)

**ANTEPARTUM RANDOMIZATION**
(14 weeks gestation to term)

- **Arm-A (ZDV; sdNVP; FTC+TDF tail)**
  - Zidovudine-alone prophylaxis
    - ZDV, 300 mg twice daily
    - NVP, 200 mg at labor onset
    - FTC, 200mg and TDF, 300 mg, once daily for 6–14 days after delivery

- **Arm-B (ZDV+3TC+LPV/r)**
  - Zidovudine-based ART prophylaxis
    - ZDV, 300 mg and 3TC, 150 mg, b.d.
    - LPV, 200mg and ritonavir 100 mg b.d.
    - LPV, 600 mg and ritonavir 150 mg b.d
      (third trimester)

- **Arm-C (TDF+FTC+LPV/r);**
  - Tenofovir-based ART prophylaxis
    - FTC, 200mg and TDF, 300 mg, o.d.
    - LPV, 200mg and ritonavir 100 mg b.d.
    - LPV, 600 mg and ritonavir 150 mg b.d
      (third trimester)

**POSTPARTUM RANDOMIZATION**
(6-14 days postdelivery/birth)

- **Arm-A (maternal and infant prophylaxis)**
  - Maternal
    - TRV (FTC 200mg & TDF, 300 mg), o.d.
    - LPV/r, 200mg and ritonavir 100 mg b.d.
  - Infant: NVP, dosed per age-bands, o.d., 6-9 days through 42 days-of-age

- **Arm-B (Infant prophylaxis)**
  - Infant: NVP, dosed per age-bands, o.d.
Key: b.d., twice-daily; o.d., once-daily; 3TC, lamivudine; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; TDF, tenofovir; FTC, emtricitabine; TRV, Truvada, a fixed dose combination of FTC and TDF; ZDV, zidovudine. Antepartum randomization antiretroviral drug regimens were taken through 6–14 days after delivery, and maternal (arm A) and infant (arm B) postpartum regimens taken through breastfeeding cessation or through 18-months-of-age, whichever came first. Infant postpartum arm A nevirapine was taken through 42 days of age. Infant nevirapine dosed per infant age-bands: birth to 6 weeks, 15 mg once daily if birth weight $> 2500$ gm or 10 mg once daily if birth weight 2000 to 2499 gm; > Week 6 to 6 months: 20 mg once daily, >6 months to 9 months: 30 mg once daily; > 9 months to weaning: 40 mg once daily
Figure 1.5. Map of sub-Saharan Africa and the study sites in Malawi and Uganda

Key:
- Sub-Saharan Africa
- Sub-Saharan Africa (GPP country)
- Sub-Saharan Africa, GPP country and study site

GPP, UNAIDS Global Plan Priority country for rapid-scale up of antiretroviral therapy programs.58
Appendices 1.1 - 1.6 obtained with permission from the NEURODEV (ND) study protocol team

Appendix 1.1. ND study maternal schedule of evaluations

<table>
<thead>
<tr>
<th>Activity</th>
<th>Screening</th>
<th>12</th>
<th>24</th>
<th>30</th>
<th>36</th>
<th>42</th>
<th>48</th>
<th>54</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent for study screening&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X</td>
<td></td>
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<tr>
<td>Documentation of HIV status&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>Hopkins Check List (HSCL) Depression/Anxiety (15 min)</td>
<td>X X X X X</td>
<td>X</td>
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<tr>
<td>Social Economic And AFASS Questionnaire&lt;sup&gt;3&lt;/sup&gt;</td>
<td>X X X X X</td>
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<tr>
<td>Home Observation for Measurement of the Environment (HOME)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>X X</td>
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</tbody>
</table>

<sup>1</sup> Applicable for mothers and their babies potentially eligible for the comparison group of the ND study, identified from outside the PROMISE study; screening will be done between 6-15 months of age for the comparison infant to ensure they can be age group matched with the HIV and ARV exposed infants enrolled from the PROMISE study.

<sup>2</sup> For mothers co-enrolled in the PROMISE study, documentation of HIV infection will be obtained from the PROMISE study records.

<sup>3</sup> For mothers co-enrolled in the PROMISE study, documentation of Social Economic and AFASS will be obtained from the PROMISE study Case Report Form (QLW0176) whose date is closest the ND study visit date, whenever available.

<sup>4</sup> HOME Infant/Toddler Form.
Appendix 1.2. ND study infant schedule of evaluations

<table>
<thead>
<tr>
<th>Evaluation Category</th>
<th>Screening</th>
<th>12</th>
<th>24</th>
<th>30</th>
<th>36</th>
<th>42</th>
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<tbody>
<tr>
<td><strong>Clinical Evaluations</strong></td>
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<td>Interval Medical History</td>
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<td>Neurologic Exam (15 min)</td>
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<tr>
<td><strong>Development and Neuropsychological Assessments</strong></td>
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<tr>
<td>Mullen Scales of Early Learning (MSEL) (45 min)</td>
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<td>Kaufman Assessment Battery for Children (2nd Ed) - KABC-II (120 min)</td>
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<td>Bruninks-Oseretsky Test of Motor Proficiency (2nd Ed) - BOT-2 (60 min)</td>
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<tr>
<td><strong>Infant/Child Assessments Administered to the Caregiver</strong></td>
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<tr>
<td>Multiple Indicators Cluster Survey (Round 4) - MICS4 Disability Questionnaire (5 min)</td>
<td></td>
<td>X</td>
<td>X</td>
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<tr>
<td>Multiple Indicators Cluster Survey (Round 4) - MICS4 Early Child Development (5 min)</td>
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<td>Achenbach Child Behavior Checklist (40 min)</td>
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<tr>
<td>Behavior Rating Inventory of Executive Functioning (30 min)</td>
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<td><strong>Laboratory</strong></td>
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<td>Complete Blood Count (CBC)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Infant HIV Screening</td>
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</tbody>
</table>

1. For infants co-enrolled in the PROMISE study, history and physical growth assessment information will be obtained from the PROMISE study records.
2. For infants co-enrolled in the PROMISE study, CBC results at the 12 and 24 months of infant age will be obtained from the PROMISE study records for the 12 and 24 months PROMISE study scheduled visits respectively. A CBC result with documentary evidence obtained within 3 months of a given study visit may be used for study purposes, in which case no blood draw at the study visit is warranted.
3. Infant HIV screening procedures at 2 years for the control infants and at 5 years for both cases and control infants will be performed using a rapid test. If the rapid test is positive, a second rapid test on a separate sample collected at a different time point will be done. A third rapid test will be used as a tie breaker if the first and second rapid tests yield discordant test results. For infants co-enrolled in the PROMISE study, infant HIV screening results for the 2 year point will be obtained from the PROMISE study records.
4. Neurologic exam and medical history prior to enrollment only if a control child screens positive on MICS4 Disability Questionnaire on visual, auditory, motor, or seizure items.
### Appendix 1.3. Child evaluation for malnutrition/failure to thrive

<table>
<thead>
<tr>
<th>Severity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-for-age</td>
<td>Crossing of &lt; 2 percentiles downward on the WHO weight-for-age Growth Standards</td>
<td>Failure to gain weight for &gt; 3 months or weight-for-age measurement crossing 2 major percentiles downward on the WHO Growth Standards</td>
<td>Weight-for-age measurement less than 80% and 70% or more of the median WHO reference (80% &gt; WFA &gt; 70%)</td>
<td>Weight-for-age measurement less than 70% of the median WHO reference (WFA &lt; 70%) AND/OR Bilateral pitting edema of nutritional origin</td>
</tr>
<tr>
<td>Condition according to Pediatric/Maternal Diagnoses</td>
<td>Growth faltering</td>
<td>Failure to Thrive (FTT)</td>
<td>Moderate Acute Malnutrition</td>
<td>Severe Acute Malnutrition</td>
</tr>
</tbody>
</table>
Appendix 1.4. Recruitment of exposed-group participants
Appendix 1.5. Recruitment of control-group participants
Appendix 1.6. ND Study Child Evaluation Form

Makerere University Johns Hopkins University Research Collaboration
NEURODEVELOPMENT STUDY

Child Follow up Form

Participant Number

Place PID Label Here

Nurse Evaluation

1. Date of visit:

   dd / mmm / yyyy

2a. Visit type:

   □ Scheduled Visit (go to 2b)
   □ Interim visit (go to 2c)

2b. Scheduled visit type:

   □ month 12  □ month 24  □ month 36
   □ month 42  □ month 48  □ month 54
   □ month 60

2c. Reason for the interim visit:

   □ Sick visit
   □ Requested review
   □ Other; Specify:

3. Age of the child:

   ________ months (i.e., completed months)

4. Who is the primary caregiver?

   □ Biological parents
   □ Biological mother
   □ Biological father
   □ Adoptive parent
   □ Foster parent
   □ Other relative, specify:
   □ Other, specify:

5. Record the child’s temperature:

   Temp: [ ] [ ] °C Axillary

6. Record the child’s weight:

   b) Weight: [ ] [ ] kg

7. Record Child’s length/height:

   [ ] [ ] cm

8. Record Child’s Head Circumference:

   [ ] [ ] cm

9. Record Mid Upper Arm Circumference: [ ] [ ] cm

10. Comments:

    

    Initials:  

    Date:  

    Empid:

Physician’s Evaluation

11. Medical history: (Including Review of Systems, past medical history including general well being, hospitalizations, seizure history since the last scheduled visit)

    

    

    

    

Child Follow Up Form   Version 2.0, 20 Apr 2014

Page 1 of 5

81
Breastfeeding practices

12a. Has the child stopped breastfeeding since the last visit? (Applicable for children who have not stopped breastfeeding by the last scheduled visit and those who have not resumed breastfeeding if they had previously stopped BF)

☐ yes (indicate age below)
☐ no
☐ N/A

12b. Age in months at which child stopped breastfeeding: [ ] [ ] months

Growth and development history:

13a. Did the child lose any developmental milestones since the last scheduled visit?

☐ yes (comment below)
☐ no (skip to 14)

13b. Comments:
14. Has the child been hospitalized since the last scheduled visit?
   - Yes 
   - No (Skip to 15)

<table>
<thead>
<tr>
<th>Hospitalization</th>
<th>Date of Admission and Date of discharge</th>
<th>Diagnosis during hospitalization or at discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a. 1st hospitalization</td>
<td>Admission: [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]&lt;br&gt;Discharge: [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]</td>
<td></td>
</tr>
<tr>
<td>14b. 2nd hospitalization</td>
<td>Admission: [ ] [ ] [ ] [ ] [ ] [ ] [ ]&lt;br&gt;Discharge: [ ] [ ] [ ] [ ] [ ] [ ] [ ]</td>
<td>N/A</td>
</tr>
<tr>
<td>14c. 3rd hospitalization</td>
<td>Admission: [ ] [ ] [ ] [ ] [ ] [ ] [ ]&lt;br&gt;Discharge: [ ] [ ] [ ] [ ] [ ] [ ] [ ]</td>
<td>N/A</td>
</tr>
<tr>
<td>14d. 4th hospitalization</td>
<td>Admission: [ ] [ ] [ ] [ ] [ ] [ ] [ ]&lt;br&gt;Discharge: [ ] [ ] [ ] [ ] [ ] [ ] [ ]</td>
<td>N/A</td>
</tr>
</tbody>
</table>

15. Review and comment on ongoing concurrent medications, including traditional medicines.
## Physician Examination:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Abnormal</th>
<th>Describe</th>
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</thead>
<tbody>
<tr>
<td>16. General Activity</td>
<td>☐</td>
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<tr>
<td>17. HEENT</td>
<td>☐</td>
<td>☐</td>
<td></td>
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<tr>
<td>18. CVS</td>
<td>☐</td>
<td>☐</td>
<td>Heart rate ________ beats/min</td>
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<tr>
<td>19. RS</td>
<td>☐</td>
<td>☐</td>
<td>RR ________ breaths / min</td>
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<tr>
<td>20. Abdomen</td>
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<tr>
<td>21. Extremities</td>
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<tr>
<td>22. Skin</td>
<td>☐</td>
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<tr>
<td>23. Growth</td>
<td>☐</td>
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</tbody>
</table>

24. Complete the *Neurological Examination Form* (This form should be completed at all scheduled visits) ☐ N/A

## Clinical Investigations:

25. Review child’s laboratory results since the last visit ☐ no

28. Have any unscheduled clinical investigations been performed at this visit? ☐ yes (specify below, including results) ☐ no
**Child Follow up Form**

27. Are there any treatments prescribed at this visit?
   - [ ] yes (list below)
   - [ ] no

28. Next interim visit date:
   - [ ] dd
   - [ ] mmm
   - [ ] yyyy
   - [ ] N/A

29. Next scheduled study visit date:
   - [ ] dd
   - [ ] mmm
   - [ ] yyyy
   - [ ] N/A

30. Additional Comments:

   ![Additional Comments field]

   ![Physician's Initials field]
   ![Date field]
   ![Empid field]

**Specimen collection: (To be completed by Nurse/phlebotomist)**
31. Were all specimens collected as required per protocol?
   - [ ] yes
   - [ ] no
   - [ ] N/A

   If specimens were not collected as specified by the protocol, specify reasons why:

   ![Nurse initials field]
   ![Date field]
   ![Empid field]

   [QC1 Initials field]
   [Date field]
   [Empid field]

   [QC2 Initials field]
   [Date field]
   [Empid field]

   [Transcriber's Initials/Date field]
   [Empid field]
CHAPTER II

PHYSICAL GROWTH AMONG CHILDREN WITH PERINATAL EXPOSURES TO MATERNAL HIV AND ANTIVIRAL DRUGS VERSUS UNEXPOSED CONTROLS IN MALAWI AND UGANDA
In-utero and postpartum exposures to maternal-HIV and antiretroviral therapy and anthropometric outcomes among Malawian and Ugandan children at 12 and 24 months-of-age

Jim Aizire¹, Alla Sikorskii², Lillian Wambuzi Ogwang³, MacPherson Mallewa⁴, Rachel Kawalazira⁵, Itziar Familiar-Lopez⁶, Alex Mutebe³, Sufia Dadabhai¹, Taha Taha¹, Michael J. Boivin⁶,⁷, Mary Glenn Fowler⁸ for the PROMISE-NEURODEV study team

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²Department of Statistics & Probability, Michigan State University;
³Makerere University – Johns Hopkins University;
⁴Malawi College of Medicine, University of Malawi, Blantyre, Malawi;
⁵College of Medicine – Johns Hopkins University Research Project, Blantyre, Malawi;
⁶Department of Psychiatry, Michigan State University;
⁷Department of Psychiatry, University of Michigan;
⁸Department of Pathology, Johns Hopkins University School of Medicine
2.1 ABSTRACT

Objective: To compare anthropometric outcomes among children with in-utero and postpartum exposures to maternal-HIV and antiretroviral therapy (ART) versus unexposed controls.

Design: Prospective cohort study.

Methods: Children exposed to HIV and ART were co-enrolled from the PROMISE randomized-clinical-trial (combination-ART (cART) versus non-cART), and age-and-gender-matched controls separately enrolled from child-well clinics, between 09/2013-10/2014, at two international sites in Malawi, and Uganda. The WHO-population (2006) growth-standards were used to derive weight-for-age (WAZ); length-for-age (LAZ); weight-for-length (WLZ); and head-circumference-for-age (HCAZ) z-scores. Wilcoxon Rank-Sum/Fischer’s exact tests were used to compare variables, and Generalized-Estimating-Equations models used to measure associations.

Results: Overall, 471 (50.5%) exposed and 462 (49.5%) control children were enrolled. Exposed verses control children from Uganda had significantly lower mean-Z-scores: LAZ (p<0.001) and WAZ (p<0.001) at 12 and 24 months-of-age, respectively; and HCAZ (p=0.016) at 24 months. Similar trends in Malawi (p>0.05). In a multivariate analysis adjusted for breastfeeding-duration; maternal-age; household income, electricity/gas use, and water-source, relative-risk (RR), 95% confidence interval (CI) of stunting was higher among exposed-children: 2.11 (1.14, 3.90), p=0.017, at 12-months, and 1.83 (1.03, 3.24), p=0.039, at 24-months-of-age, in Uganda; and 1.57 (1.18, 2.10), p=0.002, at 24-months-of-age, in Malawi. Relative-risk of HCAZ below WHO median was higher among exposed-children at 24-months-of-age, RR (95 CI) = 1.78 (1.10, 2.90), p=0.019, in Malawi; and 1.28 (0.82, 2.01), p=0.279, in Uganda. Risk-estimates were homogeneous across cART and non-cART exposure-groups (p>0.05); and similar in separate exposure (in-utero versus cumulative (in-utero and postpartum)) multivariate-models.
**Conclusion:** *In-utero*, but not postpartum, exposures to maternal-HIV and prophylactic-ART, regardless of cART versus non-cART, are associated with lower LAZ (including stunting), WAZ and HCAZ at 24 months-of-age.
2.2 INTRODUCTION

The efficacy of maternal and infant antiretroviral (ARV) drugs for prevention of mother-to-child transmission of HIV-1 (PMTCT) in prolonged-breastfeeding populations is well established.\(^1\)\(^-\)\(^3\) The WHO currently recommends initiation of life-long combined-antiretroviral therapy (cART) for all HIV-infected pregnant women at diagnosis.\(^4\) While pre-ART era studies that assessed growth-faltering among HIV exposed uninfected (HEU) children were inconclusive,\(^5\)\(^,\)\(^6\) two recent Botswana studies suggest growth impairment persists through 2 years of life and beyond among HEU children with \textit{in-utero} exposures to ART.\(^7\)\(^,\)\(^8\) However, cumulative \textit{in-utero} and prolonged postpartum exposures have not been explored.

Sub-Saharan Africa, a region with more than 90% of the global PMTCT need, and where rapid scale-up of ART-based PMTCT programs is underway, will be home to most of the estimated 1.5 million newborn HEU children per year by 2020.\(^9\) Prolonged breastfeeding is the norm and potentially more than 27 months of cumulative (9 months \textit{in-utero} plus 18-24 months postpartum during extended breastfeeding) ART exposures may occur.\(^4\) In these settings, high background levels of childhood growth faltering due to nutritional deficiencies and infectious diseases are associated with increased morbidity and mortality.\(^10\)\(^-\)\(^12\) Therefore, a receding vertical HIV transmission epidemic because of successful ART-based PMTCT programs may result in a generation of HEU children with long-term exposure to complex ARV drug regimens, with potentially unique long-term sequelae that could impact growth, and ultimately survival.

We compared anthropometric measures among HEU and HIV-unexposed-uninfected (HUU) children at 12 and 24 months-of-age; and conducted sensitivity analyses to explore \textit{in-utero} and cumulative (\textit{in-utero} plus postpartum) exposures; as well as cART versus non-cART exposures.
2.3 METHODS

2.3.1 Study Population

Prospective cohort of exposed (maternal-HIV and prophylactic ARV drugs) and unexposed control-group children, enrolled (2013-2014) at two international clinical research sites: College of Medicine-Johns Hopkins University (CoM-JHU) research project, in Blantyre, Malawi, and Makerere University-Johns Hopkins (MU-JHU) research project, in Kampala, Uganda. A total of 960 mother-child pairs were targeted, 240 exposed-group and 240 age-and gender-matched control-group children at each site, respectively. Eligible exposed-group children were identified from the PROMISE (Promoting Maternal and Infant Survival Everywhere) PMTCT ARV drug trial at the respective sites. We targeted control-group children with similar social-economic backgrounds via child-well/immunization clinics within the medical facilities that served as source populations for the PROMISE trial participants. The eligibility criteria were as follows: 6-12 months-of-age at enrollment (subsequently revised to 6-18 months-of-age) and gender-and-age (+/- 4 months) matched controls; birth weight ≥ 2000 gm; confirmed child HIV-uninfected and maternal HIV-infected status (exposed-group); confirmed maternal HIV-uninfected (control-group); no known serious chronic condition; and obtained written informed consent from the mother. The exclusion criteria included serious pre-existing clinical conditions, lived outside the site catchment area, or the mother/caregiver not willing to be home-visited. The US National Institutes of Health funded the study (HD 073296). Institutional Review Boards (IRBs) and other relevant regulatory bodies in Malawi and Uganda, and the Johns Hopkins Medical Institute IRB, approved study conduct.

The PROMISE clinical trial (number NCT01061151: clinicaltrials.gov registry) described previously,1,13 was a PMTCT open-label randomized controlled ARV drug trial that enrolled HIV-infected pregnant women between 2011 and 2016 across several international sites. Two sequential randomizations were conducted (figure 2.1). The antepartum randomization (first
14 weeks of gestation or later) had 3 arms: ‘antepartum-Arm A’ included antepartum and intrapartum zidovudine (ZDV) followed by intrapartum maternal single-dose-nevirapine (sdNVP) and a combination of tenofovir (TDF) and emtricitabine (FTC); ‘antepartum-Arm B’ comprised of a combination of ZDV, lamivudine (3TC) and ritonavir-boosted lopinavir (LPV/r); and ‘antepartum-Arm C’ had a combination of TDF, FTC and LPV/r. Antepartum ARV prophylaxis per randomization was maintained through the point of postpartum randomization. The postpartum randomization (6-14 days of delivery/birth) included two arms: ‘postpartum-Arm A’ a maternal c-ART regimen based on three or more antiretroviral drug regimens per local standard-of-care guidelines, taken for the duration of breastfeeding, plus daily infant NVP through 6 weeks of infant age; and ‘postpartum-Arm B’, a single infant NVP regimen taken daily through breastfeeding cessation. Breastfeeding cessation was defined as more than 28 days of no milk ingestion. Standardized procedures across sites were used to institute temporary/permanent drug holds (toxicity/adverse reactions, infant HIV infection or medical indications) or study drug switch (toxicity, ART-indication; treatment failure or as deemed by the clinicians).

2.3.2 Clinic Procedures

Demographic and socioeconomic characteristics for the mother-child pairs were collected at study entry. Child medical history, breastfeeding/nutrition status, and physical exam including anthropometric measures (weight, length, and head-circumference) were collected at baseline and updated at the 24 months-of-age study visit. With maternal consent and permission from the PROMISE protocol team, we abstracted local PROMISE trial records for data on maternal HIV-infection, ARV drug exposure, maternal health (HIV viral load), infant birth-weight, and infant HIV-free status at 12 and 24 months-of-age. Infant date-of-birth, birth-weight and immunization history for the control-group children were obtained from the standard-of-care immunization/growth card. Standardized tools and operating procedures across both sites were
implemented by experienced staff who received routine training. Standard calibrated scales were used to assess child-weight, length boards used to assess recumbent child-length and standard tape-measure used to measure head-circumference.

2.3.3 Laboratory Procedures

We used a rapid HIV test to screen control-group mothers at study pre-entry, and control-group children at 24 months-of-age visit. If the test was positive, a second rapid test on a separate sample was performed. A third rapid test was used as a tie breaker if the first and second tests yielded discordant results.

HIV screen results for the exposed-group participants were abstracted from the PROMISE Trial records. PROMISE trial children who had not achieved complete cessation of breastfeeding received a Nucleic-Acid-Test (NAT) using HIV DNA PCR or HIV RNA PCR. If the initial HIV NAT was positive, a confirmatory test was performed as soon as possible with a repeat HIV-NAT on a second sample drawn on a different day. Among children who had achieved complete cessation of breastfeeding, HIV antibody testing was done using an Enzyme Linked Immunosorbent Assay (EIA) or HIV rapid test. If HIV antibody test was negative, no further HIV testing was done. Children with a positive HIV antibody test had a follow-up HIV-NAT test performed as soon as possible on a separate sample on a different day. HIV-NAT test was used to quantify maternal viral load at trial entry, delivery; weeks 6, 26, 50, and thereafter every 12 weeks through 24 months postdelivery.

2.3.4 Statistical Methods

Data were entered and validated using ‘FileMaker 12 and Server 14’, and analyzed using Stata version 14.2 (Stata, College Station TX, USA). This analysis was restricted to children with a negative HIV-NAT or HIV-rapid test at the 24 months-of-age visit.
Primary analyses compared exposed-group (aggregated in-utero and postpartum exposures) versus control-group children. We then performed a 2-factor (antepartum and postpartum randomization) exposure sensitivity analysis, versus control-group references, respectively. To assess the impact of ARV exposures during critical in-utero periods, two separate analyses were performed: 1) exposed-group children (disaggregated by antepartum randomization arms) versus control-group; 2) exposed-group (disaggregated by two levels, combined ART (cART) regimens – composite of ‘antepartum arms B and C’, and the less complex regimen ‘antepartum arm-A’) versus the control-group children, respectively. The impact of cumulative in-utero and postpartum exposures were assessed by comparing exposed-group children disaggregated by cART regimens (composite of ‘antepartum-arms B and C’, plus ‘postpartum-arm A’), and less complex ARV regimens (composite of ‘antepartum-arm A’ plus ‘postpartum-arm B’), versus control-group reference, respectively. We also compared cART versus non-cART regimens. The cART regimens contain 3 or more ARV drug classes, and were presumed to have homogeneous effects regardless of individual ARV drug composition, relative to the simpler/single ARV drug regimens. By study design, the independent associations of maternal HIV infection and prophylactic ARV drug exposures on infant growth could not be isolated in these analyses. In addition, independent ARV drug-type or drug-class/family could not be assessed given the strategy of combination ARV-based strategies for PMTCT.

Anthropometric data were used to calculate age-and gender-based Z scores for length-for-age (LAZ); weight-for-length (WLZ); weight-for-age (WAZ) and head-circumference-for-age (HCAZ) using the WHO Growth standards (2006). Based on the derived Z-scores, anthropometric outcomes defined by Z-scores below the median of the WHO population standards, respectively, were compared across the exposed and control groups. Stunting, wasting and underweight were defined as LAZ, WLZ and WAZ below minus two SDs from the medians of the respective references. A descriptive analysis of mean LAZ with corresponding Confidence
Interval (CI) at birth, 12 and 24 months is provided to depict the longitudinal linear-growth outcomes overtime.

Wilcoxon Rank Sum and Fischer’s exact tests were used to compare continuous and categorical variables, respectively. The Mantel-Haenszel chi-square ($\chi^2$) was used to assess trends within categorical variables. All the p-values were based on a two-sided hypothesis test with a type-1 error ($\alpha = 0.05$). Generalized Estimating Equation (GEE) regression models with a log-link were used to compute the relative risk of binary anthropometric outcomes at 12 and 24 months-of-age visits, with 95% Confidence Intervals (CI) computed with robust variance estimators, while accounting for within individual correlations. Univariate and multivariate models were used to explore covariates defined *apriori*, correlated with exposure, and predictors of infant health. We considered maternal viral load, exposure (HIV and ARV) duration, breastfeeding status (yes/no), breastfeeding duration, infant-age, and gender. Additional covariates with a p-value $\geq 0.10$ in univariate analysis with the anthropometric outcome, were added to the multivariate models to control for potential confounding bias. Exposure duration was computed as the accumulated time between antepartum ARV drug initiation through breastfeeding cessation, when exposure was effectively ceased. Other covariates explored include maternal demographic/socio-economic factors (age, maternal care-giver (yes/no), health-status (standardized Hopkins Symptoms Checklist (HSCL)), household-income, electricity, and water source. Household-income-stability index score was based on a composite of five binary-questions (yes/no) of the standardized AFASS questionnaire: “Is mother currently working?”, “Is mother the primary bread-winner?”, “Is pooled income sufficient?”; “Is pooled income sufficient for 12-months?”, “Is there enough to spend on infant nutrition and transport to healthcare?”. Equal weights (yes=1; no=0) were applied to each question and based on the total score, household-income was considered relatively-stable (5, 4 or 3); fairly-stable (2) or unstable (1 or 0). Product terms of site (Malawi versus Uganda) and exposure variables were added to the multivariate regression models to assess whether site modified the
exposure and outcome associations. Likelihood ratio tests were used to compare null and extended models.

2.4 RESULTS

2.4.1 Study profile

Overall, 933 mother-child pairs (471 (50.5%) exposed and 462 (49.5%) control) were enrolled between August 2013 and December 2014. We accrued 788 (84.5%) pairs within the 12-months-of-age visit window, of these 50 (6.3%) missed or were lost to follow-up by 24-months-of-age visit, non-differentially by exposure (p>0.05). Median (IQR) infant-age in months among exposed and control children was 14.1 (12.3, 16.3), and 14.0 (12.3, 15.8), p= 0.82, at 12-months-age visit; 24.0 (23.9, 25.0), and 24.0 (23.9, 25.5), p= 0.887, at 24-months-age visit, respectively. Sample sizes at both visits are summarized in figure 2.2.

2.4.2 Maternal HIV and ART exposures

Of the 471 exposed children, 254 (53.9%), and 213 (45.2%) had cART and non-cART in-utero exposures, respectively, while four (0.9%) were late-presenters and had no in-utero ART exposures. In terms of cumulative (in-utero plus postpartum) exposures, 237 (50.3%) had cumulative-cART (antepartum Arms B or C followed by postpartum Arm-A); 134 (28.5%) had cumulative-mixed (antepartum-cART followed by postpartum non-cART or vice-versa); and 100 (21.2%) had cumulative-non-cART (antepartum Arm-A followed by postpartum Arm-B) exposures, respectively. Maternal viral load (copies/ml) at labor/delivery; 6, 12, 18 and 24 months post-delivery, was homogeneous across cART versus non-cART categories per in-utero classification, respectively, p>0.05 (figure 2.3). The median (IQR) of maternal viral load (copies/ml) was 409 (39 to 11,780) at 12 months-of-age; and 520 (39 to 15,371) at 24-months-age visits, respectively.
2.4.3 Baseline characteristics

Demographic, socio-economic status (SES), and clinical mother-child characteristics comparing exposed versus control-group individuals at the baseline study visit (or otherwise stated) are summarized by site in Table 1. Exposed versus control-group mothers tended to be older, median (Interquartile Range (IQR)) age in years: 28.0 (25.0-31.0) versus 23.0 (20.0-28.0), p<0.0001 in Malawi; and 27.0 (24.0-30.0) versus 25.0 (22.0-30.0), p=0.053, in Uganda, respectively. The primary care giver was mostly (>95%) the mother, similarly across the exposure groups (p>0.5). Maternal well-being based on the standardized Hopkins Symptoms Checklist (HSCL) score for anxiety and depression domains was homogeneous across the exposure groups. There was a tendency towards higher SES among control-group households based on the proportion that reported electricity-use (p<0.0001) in Malawi, or tap-water use (p≤ 0.015), at the respective sites. Control-group compared to exposed-group individuals in Uganda (p<0.0001) were more likely to have a relatively stable household level income, but not in Malawi (p=0.973). Infant age and sex were homogeneous across the two exposure groups, at both sites. Compared to exposed-group children, controls had higher birth-weights in Malawi (p=0.014) and Uganda (p<0.0001). In addition, Malawian children had lower median-birthweight (IQR), kilograms, compared to their Ugandan counterparts: 3.0 (2.8, 3.3) versus 3.3 (3.0, 3.6), p=0.0038 among the controls, and 3.0 (2.7, 3.3) versus 3.0 (2.8, 3.4), p=0.008 among the exposed-group children. The prevalence of breastfeeding was high at both sites, more predominant in Malawi compared to Uganda: 12 months-of-age visit (88.0% versus 67.4%, p<0.0001) and 24 months-of-age visit (17.9% versus 10.5%, p=0.002), respectively; and consistently higher among the control-group compared to the exposed-group children (p≤ 0.008). Exposed-group children versus controls were more likely to have had a prior hospitalization reported at baseline, in Uganda, p=0.024, but not in Malawi, p=0.061.
2.4.4 Mean anthropometric measures at 12 and 24 months-of-age study visits

Mean LAZ and WAZ scores at 12 and 24-months-of-age visits were consistently below the median of the respective WHO population references, but not with WLZ or HCAZ mean scores (Table 2). In Uganda, at both 12- and 24-months-of-age visits, exposed versus control children had significantly lower mean LAZ (p<0.001), and lower WAZ (p<0.001), respectively. In addition, exposed children had lower HCAZ at the 24-months visit (p=0.016). The lower mean HCAZ among exposed versus control children at 12 months, and lower WLZ at both visits, were not statistically significant. However, in Malawi, while similar trends of lower mean LAZ, WLZ, WAZ or HCAZ scores were observed among exposed versus controls, the differences were not statistically significant. Overall, there was a tendency towards lower mean Z scores among Malawian versus Ugandan control children: LAZ (P<0.001); WAZ (p<0.001), WLZ (p<0.001), at both time points, respectively, although mean HCAZ scores were higher among Malawian versus Ugandan controls, at 12-months (p<0.001) and 24 months, (p=0.002). Mean anthropometric Z-score comparisons by cART versus non-cART groups, at 12 and 24-months-of-age were not significant, p >0.05. The prevalence of stunting was high at both sites, significantly higher among exposed versus control children in Uganda: 29.8% versus 13.3%, p<0.001 at 12 months, and 32.3% versus 18.2%, p=0.001 at 24 months; but not in Malawi: 35.6% versus 40.4%, p=0.341 at 12 months, and 48.4% versus 42.7% at 24 months.

2.4.5 Relative risk of stunting, underweight, wasting and small-HC (HCAZ <WHO median)

The relative risk (RR) and 95% CI estimates of stunting, underweight, wasting, and small head-circumference (HCAZ below the median of the WHO reference) among exposed versus control children are summarized in Table 3. Malawi site multivariate models adjusted for in-utero ART duration; breastfeeding; maternal-age; electricity/gas use; and water-source (home tap-water; community tap-water, borehole/other); and Uganda site multivariate models adjusted for
in-utero ART duration; breastfeeding; and water-source. Exposed versus control children had a higher risk of stunting: adjusted RR (95 CI) = 2.10 (1.18, 3.72), p=0.011, at 12-months-of-age, and 1.81 (1.04, 3.16), p=0.036, at 24-months-of-age, in Uganda; and 1.56 (1.18, 2.10), p=0.002, at 24 months, in Malawi. In addition, Malawian exposed-versus control children had a significantly increased risk of having a head-circumference below the WHO population median at 24-months-of-age, adjusted RR (95% CI) = 1.77 (1.10, 2.90), p=0.023. A similar trend was observed among Ugandan children at 24-months-of-age, which was significant in the univariate, 1.36 (1.10, 1.78), p=0.022, but not the multivariate analysis, 1.26 (0.82, 1.94), p=0.302.

2.4.6 Sensitivity analyses: timing- and type-of-exposure and risk of stunting

To explore the timing-of-exposure (in-utero or cumulative (in-utero plus postpartum)), as well as type-of-exposure (cART, non-cART or mixed (switched from cART to non-cART or vice-versa between the in-utero and postpartum periods, respectively)), we performed the following analyses: model-A, exposed (cumulative aggregated exposures) versus controls; model-B (antepartum cART versus antepartum non-cART versus controls); and model-C (cumulative exposures disaggregated by cART versus non-cART versus mixed versus controls) as summarized in Table 4. The RR (95% CI) of stunting at 12- and 24-months-of-age visits was consistent in models A and B, respectively, but not with model-C. The relative-risk of stunting at both 12- and 24-month-age visits was consistently homogeneous across the antepartum-exposure categories (cART and non-cART), versus controls, respectively, except for Ugandan children at 24-months who had qualitative differences: cART versus controls, adjusted RR (95% CI) =1.56 (0.85, 2.87), p=0.153; and non-cART versus controls, 2.18 (1.21, 3.92), p=0.009. We also made direct comparisons of ART categories (no control references), and consistently observed homogenous risk of stunting across cART versus non-cART exposed children, separately for antepartum, and cumulative (antepartum plus postpartum) exposures, respectively.
2.5 DISCUSSION

In this prospective study, which enrolled participants from two African sites with prolonged breastfeeding, exposed versus control-group children had significantly lower mean-LAZ and lower mean-WAZ at 12- and 24-months-of-age, respectively, and lower mean-HCAZ at 24 months-of-age. Based on multivariate regression analyses both Malawian and Ugandan exposed-group children compared to their respective controls had significantly increased risk of stunting (extreme linear-growth faltering) at 12 and 24 months-of-age, as well as increased risk of smaller head-circumference (HCAZ below the WHO population reference median) at 24-months-of-age. The increased risk of stunting among exposed versus control children was homogeneous across: cART and non-cART (primarily zidovudine); as well as zidovudine-based-cART and non-cART, exposed children, respectively. Lastly, the relative-risk estimates of stunting were similar in separate analyses modeling cumulative (antepartum and postpartum) exposures and antepartum exposure alone, suggestive of in-utero timing of the risk-factor for the observed childhood growth-perturbations.

These findings are crucial additions to the limited knowledge on growth of HEU children in resource-limited breastfeeding populations, in the current roll-out era of WHO-recommended maternal HIV-test and initiation of life-time cART (option B-plus strategy). Previous studies had certain study-design limitations. A recent article by Powis et al, based on two completed randomized clinical trials in Botswana conducted between 2001-2003, and 2006-2008, respectively, suggests that while inferior LAZ and WAZ among HEU children with in-utero exposures to cART versus ZDV-monotherapy (non-cART), respectively, persisted from birth through 24 months-of-age, levels of stunting and wasting at 24 months-of-age were similar across the two groups. However, there was no HUU comparison group, the two trials were non-concurrent with a 3-year lag-period with a potential for secular-trends bias, and breastfeeding cessation was mostly encouraged as early as 6 months-of-age per standard-of-care. Two other
recent cross-sectional studies, a hospital-based survey in Uganda conducted between 2010-2011 during the era of ‘WHO option-A or B’, and a population-based survey in Botswana, conducted between 2013-2014 during the era of ‘WHO option B-plus’, both reported inferior length-for-age among HEU compared to HUU children through 6-12 months, and through 12-24 months, respectively. In addition, the Ugandan study reported inferior weight-for-age and inferior weight-for-length among HEU versus HUU children. Both studies had design limitations in ascertaining type and duration of ARV drug exposures. Also, reliance on standard-of-care infant HIV-testing algorithms used in these settings with wide-intervals between tests (6 weeks-of-age (HIV-NAT-based) and 18 months-of-age (HIV-antibody-based)), during a period of sustained breastmilk transmission risk, may have misclassified infant HEU status. A South African study that reported no differences in weight-for-age through 24 months of age had limited ARV drug exposure (sdNVP-based PMTCT strategies). Other studies that assessed growth among HEU children were conducted prior to the era of using ARV drugs for PMTCT, and reported mixed findings, summarized in two previous reviews. A Zimbabwean trial reported a similar trend towards smaller heads among HEU versus HUU children, although differences were observed during the first but not the second-year-of-life.

Our findings are based on a prospective cohort of HEU and age-and-gender-matched HUU children concurrently evaluated at 12 and 24 months-of-age using standardized procedures across two-international research-experienced sites. Carefully documented, well-characterized exposure data based on the PROMISE randomized clinical trial enabled sensitivity-analyses comparing cART and non-cART exposed children versus HUU controls, respectively, as well as evaluation of timing and duration of maternal HIV and ARV drug exposures on growth outcomes. Standardized maternal and infant HIV-screening algorithms were followed to ensure accurate ascertainment of infant HIV-exposure status through 24-months-of-age.
To minimize heterogeneity of environmental/socio-economic factors across exposure groups, control-group mother/child pairs were recruited via child-well clinics linked with hospital complexes that served as source-populations for the PROMISE trial participants, which in-turn served as the source for the exposed-group participants in our study. At analysis, we adjusted for key environmental/socio-economic variables including water source, household income and electricity/gas use in the multivariate regression analyses. Infectious diseases such as measles, diarrhea, pneumonia, meningitis, and malaria; environmental/tropical enteropathy and socio-economic cofactors such as house-hold level food-security; water hygiene and sanitation (WASH) factors and household level socio-economic stability are key-determinants of early-childhood growth impairment in resource-constrained settings.\textsuperscript{10,18,19} Tropical enteropathy is an acquired syndrome characterized by altered gut immunity and diminished capacity to absorb nutrients, associated with poor sanitary conditions.\textsuperscript{18} Other covariates added to the final multivariate models include \textit{in-utero} ARV exposure duration and breastfeeding duration. Exposed-group versus control-group children were significantly more likely to have stopped breastfeeding by 12, and 24-months-of-age, respectively. Breastfeeding through 24-months-of-age with appropriate weaning practices and nutritious complementary foods is associated with increased childhood length and weight measures.\textsuperscript{20,21} Analyses were adequately powered (≥80%) to minimize type-II error.

In this large cohort, infant birth-weights were significantly lower among exposed-versus-control-group children, suggestive of \textit{in-utero} etiological perturbations. Fetal growth restriction is associated with childhood growth impairment, and about 20% of childhood stunting has been attributed to small-for-gestation-age.\textsuperscript{8,10} However, the lack of linear-growth anthropometric measurements among control-group children at birth precluded a mediation analyses to ascertain the proportion of the observed relative-risk of stunting attributed to fetal growth restriction. Trends of inferior LAZ, including higher risk of stunting (LAZ < 2 SD), were consistently
demonstrated among exposed-versus-control children, but was not the case with WAZ or WLZ. While exposed-group children had lower median WAZ at 12- and 24-months-of-age, the risk of being underweight (WAZ < 2SD), or wasted (WLZ < 2SD) was homogenous across exposure groups. These findings suggest that the relative growth impairment through 24-months-of-age observed among exposed-group children is a consequence of long-term perturbations, since stunting is a biomarker of chronic growth impairment, while underweight and wasting reflect the child’s current nutritional status, and acute or recent nutritional deficits, respectively. The relative-risk of stunting comparing exposed-versus-control-group children was higher among Ugandan versus Malawian children, a heterogeneity-of-effects in part explained by the observed lower baseline-normal LAZ scores, among Malawian versus Ugandan control-group children, p<0.001. This is consistent with the high levels of stunting in Malawi, and Uganda. In addition, the negative impact of the 2014/2015 Malawian floods on child-nutrition, homogenously across the exposed and control-groups, potentially resulted in attenuation of the HIV and ARV exposure associations with stunting. Flood-exposed populations are associated with chronic malnutrition and increased levels of stunting.

There are some limitations to these analyses. We were not able to isolate the independent effects of maternal-HIV and prophylactic-ART exposures on childhood growth. However, the appropriate study-design comparing HEU (with, and without ARV drug exposures), versus HUU-controls, respectively, would have been unethical given the contemporaneous standard-of-care at the respective sites, based on WHO-guidelines, to initiate maternal and/or infant ARV drugs for PMTCT. Suggested biological mechanisms for ARV-drug-induced childhood morbidity include mitochondrial perturbations that could cause end-organ toxicity; hematopoietic stem-cell nuclear DNA toxicity; and dysregulation of bone metabolism and/or mineralization, in the developing fetus and the young infant. Maternal-HIV-exposure has been associated with immunological perturbations such as immune activation, decreased number and function of T-cells, and deranged
humoral immune responses.\textsuperscript{27,28} Also, since disaggregated-exposure (cART versus non-cART) analyses were based on the PROMISE trial randomization schemes, misclassification may have resulted from post-randomization non-compliance; clinician-induced drug switches due to toxicities or maternal indication of cART; malabsorption syndrome; or drug-interactions ultimately affecting bioavailability of the ARV drugs as randomized. Based on maternal report, very high (>95%) drug-adherence was maintained through follow-up.\textsuperscript{1} Due to limited information on TDF safety during pregnancy at that time, randomization to the ‘antenptum Arm-C’ (TDF+FTC+LPV/r) was initially only rationed to HIV and hepatitis B virus co-infected women. Therefore, the limited sample size accrued to this arm precluded comparisons of ZDV-based verses non-ZDV-based cART regimens.\textsuperscript{1}

Nonetheless, these findings are informative and suggest that breastfed HEU children with perinatal exposures to prophylactic ARV drugs remain a vulnerable subgroup that should be prioritized for the overall PMTCT goal of pediatric HIV-free survival to be achieved. Decreased LAZ, WAZ and HCAZ are measurable and modifiable indicators of underlying childhood ill-health. Stunting and underweight are associated with childhood infectious disease severity, and both contribute up to 14% of attributed child deaths.\textsuperscript{10–12} Early childhood growth-faltering even with catch-up later in childhood has been associated with non-communicable diseases later in adulthood.\textsuperscript{29} Poor child development among school-going-age children including cognitive and school-learning outcomes, have been attributed to early-childhood stunting and decreased HCAZ.\textsuperscript{30,31} Head-circumference through 24-months-of-age is correlated with brain size, and inferior HCAZ among exposed-group children may be a reflection of impaired brain function.\textsuperscript{32} We previously reported inferior cognitive ability scores among exposed-group versus control-group children at 24-months-of-age in this cohort,\textsuperscript{33} consistent with findings from a Zimbabwean study.\textsuperscript{34}
Our findings are likely to be generalizable to other breastfeeding HEU pediatric populations with perinatal ART exposures in sub-Saharan Africa, home to more than 80% of the global PMTCT need, with ongoing rapid scale-up of ART programs, and where the majority of the projected 1.5 million new HEU cases per year by 2020, will come from.\textsuperscript{9,35} Anthropometric monitoring is critical for adequate child health care in these settings. Since we observe similar risks of stunting across non-cART (primarily ZDV) and ZDV-based cART exposure groups compared to unexposed children, and sensitivity analyses suggest critical exposures were likely \textit{in-utero}, further research is needed to better understand the role of antepartum exposures to ZDV. Likewise, efforts should be made to assess whether newer cART regimens will also place HEU African children at increased risk of adverse growth outcomes.
2.6 REFERENCES


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Figure 2.1. Antepartum and postpartum antiretroviral drug exposures (PROMISE trial randomization schemas)
Key: b.d., twice-daily; o.d., once-daily; 3TC, lamivudine; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; TDF, tenofovir; FTC, emtricitabine; TRV, Truvada, a fixed dose combination of FTC and TDF; ZDV, zidovudine. Antepartum randomization antiretroviral drug regimens were taken through 6–14 days after delivery, and maternal (arm A) and infant (arm B) postpartum regimens taken through breastfeeding cessation or through 18-months-of-age, whichever came first. Infant postpartum arm A nevirapine was taken through 42 days of age. Infant nevirapine dosed per infant age-bands: birth to 6 weeks, 15 mg once daily if birth weight > 2500 gm or 10 mg once daily if birth weight 2000 to 2499 gm; > Week 6 to 6 months: 20 mg once daily, >6 months to 9 months: 30 mg once daily; > 9 months to weaning: 40 mg once daily.
Figure 2.2. Study flow diagram. Enrolment and study evaluations at the study clinics in Malawi and Uganda, August 2013 – April 2016

933 mother/infant dyads enrolled 788 prior to, and 145 after the 12-month visit (Aug 2013 – Dec 2014)

12-month visit (n=788)

CoM-JHU CRS, Blantyre, Malawi (n=377)

Lost to follow-up (n=50)

MUJHU CRS, Kampala, Uganda (n=411)

Enrolments after 12-months study visit (n=145)

24-month visit (n=883)

CoM-JHU CRS Blantyre, Malawi (n=434)

MUJHU CRS, Kampala, Uganda (n=449)

HEU children (n=194)  HUU children (n=183)

HEU children (n=208)  HUU children (n=203)

HEU children (n=223)  HUU children (n=211)

HEU children (n=229)  HUU children (n=220)
Figure 2.3. Maternal viral load (copies/ml) through 24 months post-delivery by *in-utero* cART versus non-cART exposures

Key: Combination antiretroviral therapy (cART) contains antepartum *Arm-B* (ZDV+3TC+LPV/r) and *Arm-C* (TDF+FTC+LPV/r); and non-cART contains antepartum *Arm-A* (ZDV; sdNVP; FTC+TDF tail). Labor/delivery (L/D) period spurns from 2 months before L/D through 21 days after L/D, sample size (n) = 380 (238 cART, 142 ncART); 6 (4-7) months, n= 406 (219 cART, 187 ncART); 12 (11-15) months, n= 405 (218 cART, 187 ncART); 18 (15-21) months, n= 214 (123 cART, 91 ncART); 24 (21-28) months, n= 402 (226 cART, 176 ncART); P-value based on the Wilcoxon rank-sum (Mann-Whitney) test. Box hinges represent 25th and 75th percentiles respectively, and box midline is the median of the log 10 of maternal viral load, respectively.
Figure 2.4. Risk of stunting by exposure-group and site, at 12 and 24 months-of-age

Key: Sample size at 12 and 24 months of age, was 367 and 417 children in Malawi; and 407 and 434 in Uganda, respectively, with 52% and 51% HEU in Malawi and Uganda, respectively.
Figure 2.5. Risk of head-circumference (HC) less than the WHO population median by exposure-group and site

<table>
<thead>
<tr>
<th></th>
<th>12 MONTHS</th>
<th>24 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malawi</td>
<td>47.7</td>
<td>47.9</td>
</tr>
<tr>
<td>Uganda</td>
<td>52.3</td>
<td>52.1</td>
</tr>
<tr>
<td>Malawi</td>
<td>45.6</td>
<td>58.1</td>
</tr>
<tr>
<td>Uganda</td>
<td>54.4</td>
<td>41.9</td>
</tr>
</tbody>
</table>

Malawi: Controls = 52.3%, Exposed = 47.7%
Uganda: Controls = 54.4%, Exposed = 52.1%

HC < WHO MEDIAN, %
Table 2.1. Baseline maternal/child characteristics by exposure (maternal HIV and prophylactic antiretroviral drugs) status, and by site

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malawi site (N=456)</th>
<th>Uganda site (N=477)</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed-group (N=231)</td>
<td>Control-group (N=225)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (years), median [IQR]</td>
<td>28.0 [25.0-31.0]</td>
<td>23.0 [20.0-28.0]</td>
<td>&lt;0.0001</td>
<td>0.053</td>
</tr>
<tr>
<td>Maternal care (primary care giver), n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-month visit</td>
<td>176 (99.8%)</td>
<td>167 (99.4%)</td>
<td>0.488</td>
<td></td>
</tr>
<tr>
<td>24-month visit</td>
<td>209 (99.5%)</td>
<td>200 (99.5%)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Maternal well-being (Hopkins Symptoms Checklist (HSCL) score, median [IQR])</td>
<td>0.19 [0.06-0.35]</td>
<td>0.23 [0.10-0.45]</td>
<td>0.097</td>
<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Household income stability score, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatively-stable</td>
<td>46 (22.1%)</td>
<td>49 (22.7%)</td>
<td>0.973</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fairly-stable</td>
<td>42 (20.2%)</td>
<td>45 (20.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable</td>
<td>120 (57.7%)</td>
<td>122 (56.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electricity or gas in household, n (%)</td>
<td>93 (42.3%)</td>
<td>130 (59.4%)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Refrigerator in household, n (%)</td>
<td>26 (11.8%)</td>
<td>36 (16.4%)</td>
<td>0.173</td>
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</tr>
<tr>
<td>Water source for household use, n (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tap water (in house)</td>
<td>33 (15.4%)</td>
<td>35 (15.9%)</td>
<td>0.015</td>
<td>0.013</td>
</tr>
<tr>
<td>Tap water (communal)</td>
<td>124 (58.0%)</td>
<td>151 (68.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borehole and other sources</td>
<td>57 (26.6%)</td>
<td>34 (15.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>111 (48.0%)</td>
<td>108 (48.0%)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Age (months), median [IQR]</td>
<td>14.4 [13.1-16.3]</td>
<td>14.7 [13.7-15.9]</td>
<td>0.775</td>
<td>0.777</td>
</tr>
<tr>
<td>Birthweight (kilograms), median [IQR]</td>
<td>3.0 [2.7-3.3]</td>
<td>3.0 [2.8-3.3]</td>
<td>0.014</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Breastfeeding status (yes), n (%)</td>
<td>151 (79.9%)</td>
<td>173 (96.7%)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prior hospitalization at baseline (yes), n (%)</td>
<td>8 (3.6%)</td>
<td>2 (0.9%)</td>
<td>0.061</td>
<td></td>
</tr>
</tbody>
</table>

Notes: a = p < 0.05, b = p < 0.01.
Key: IQR, Interquartile range; \(^a\) p-value from Wilcoxon rank-sum test; \(^b\) p-value from Fisher’s exact test. Highlighted p-values are statistically significant.
Missing data: maternal-age (2.7%); care-giver (3.9%); maternal wellbeing (9.9%); Household income (12.4%); electricity-use (2.1%); refrigerator-use (1.9%); water-source (3.0%); infant gender (0.0%); infant-age (0.0%); birthweight (0.2%); breastfeeding (0.8%); prior hospitalizations (0.3%)
Table 2.2. Mean Anthropometric Z scores at 12 and 24 months-of-age visits by exposure group and by site

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean anthropometric z-score (95% Confidence Interval), p-value</th>
<th>Malawi</th>
<th>Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed-group</td>
<td>Control-group</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td>(n= 189)</td>
<td>(n=179)</td>
<td></td>
</tr>
<tr>
<td>Length-for-age</td>
<td>-1.51 (-1.71, -1.31)</td>
<td>-1.62 (-1.84, -1.40)</td>
<td>0.476</td>
</tr>
<tr>
<td>Weight-for-length</td>
<td>0.04 (0.14, 0.22)</td>
<td>0.17 (0.04, 0.37)</td>
<td>0.372</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.69 (-0.84, -0.54)</td>
<td>-0.66 (-0.83, -0.50)</td>
<td>0.830</td>
</tr>
<tr>
<td>Head circumference</td>
<td>0.81 (0.64, 0.98)</td>
<td>0.85 (0.68, 1.01)</td>
<td>0.783</td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td>(n= 210)</td>
<td>(n=221)</td>
<td></td>
</tr>
<tr>
<td>Length-for-age</td>
<td>-1.96 (-2.12, -1.80)</td>
<td>-1.90 (-2.10, -1.73)</td>
<td>0.568</td>
</tr>
<tr>
<td>Weight-for-length</td>
<td>0.39 (0.23, 0.55)</td>
<td>0.36 (0.18, 0.53)</td>
<td>0.787</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.74 (-0.88, -0.61)</td>
<td>-0.73 (-0.87, -0.59)</td>
<td>0.890</td>
</tr>
<tr>
<td>Head circumference</td>
<td>0.96 (0.81, 1.11)</td>
<td>0.84 (0.69, 0.99)</td>
<td>0.255</td>
</tr>
</tbody>
</table>

Key: P-value based on a two-sample t-test (with un-equal variances) comparing mean Z-scores (exposed versus control group), respectively. Mean anthropometric Z-score comparisons by combined-antiretroviral therapy (cART) versus non-cART exposed-group children, at 12 and 24-months-of-age were not significant, p >0.05 (not presented). Missing data: 9/788 (1.1%) across sites at 12 months; 3/883 (0.3%) across sites at 24 months, independent of exposure, respectively.
Table 2.3. Relative Risk of stunting, underweight, wasting and small-heads at 12 and 24-month-age visits, by exposure-status and site

<table>
<thead>
<tr>
<th>Exposure categories</th>
<th>Relative Risk (RR), 95% Confidence Interval (CI)</th>
<th>Malawi</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Uganda</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>P-value</td>
<td>Multivariate</td>
<td>P-value</td>
<td>Univariate</td>
<td>P-value</td>
<td>Multivariate</td>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Stunting (LAZ &lt; 2 SD below WHO population median)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>0.86 (0.66, 1.11)</td>
<td>0.247</td>
<td>1.20 (0.86, 1.67)</td>
<td>0.284</td>
<td>2.37 (1.59, 3.51)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>2.10 (1.18, 3.72)</td>
<td>&lt;ref-&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>1.13 (0.92, 1.38)</td>
<td>0.264</td>
<td>1.56 (1.18, 2.10)</td>
<td>0.002</td>
<td>1.79 (1.28, 2.52)</td>
<td>0.001</td>
<td></td>
<td></td>
<td>1.81 (1.04, 3.16)</td>
<td>0.036</td>
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</tr>
<tr>
<td>Underweight (WAZ &lt; 2 SD below WHO population median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>0.83 (0.45, 1.54)</td>
<td>0.562</td>
<td>0.71 (0.34, 1.50)</td>
<td>0.374</td>
<td>2.71 (0.78, 2.46)</td>
<td>0.117</td>
<td></td>
<td></td>
<td>1.52 (0.26, 9.10)</td>
<td>0.644</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>0.90 (0.51, 1.57)</td>
<td>0.699</td>
<td>0.77 (0.35, 1.69)</td>
<td>0.520</td>
<td>2.67 (0.85, 8.44)</td>
<td>0.094</td>
<td></td>
<td></td>
<td>2.28 (0.31, 16.9)</td>
<td>0.418</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wasting (WHZ &lt; 2 SD below WHO population median)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>0.45 (0.16, 1.24)</td>
<td>0.122</td>
<td>0.36 (0.10, 1.32)</td>
<td>0.125</td>
<td>1.96 (0.18, 21.63)</td>
<td>0.583</td>
<td></td>
<td></td>
<td>1.46 (0.90, 2.35)</td>
<td>0.129</td>
<td></td>
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<tr>
<td>24 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>1.39 (0.48, 4.03)</td>
<td>0.539</td>
<td>1.32 (0.36, 4.84)</td>
<td>0.671</td>
<td>0.96 (0.10, 15.30)</td>
<td>0.977</td>
<td></td>
<td></td>
<td>1.57 (0.98, 2.51)</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCAZ score below WHO population median</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>1.00 (0.69, 1.45)</td>
<td>0.982</td>
<td>1.58 (0.96, 2.60)</td>
<td>0.071</td>
<td>1.20 (0.92, 1.57)</td>
<td>0.171</td>
<td></td>
<td></td>
<td>0.95 (0.61, 1.49)</td>
<td>0.834</td>
<td></td>
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</tr>
<tr>
<td>24 months</td>
<td>Exposed-group Controls (HUU)</td>
<td>1.10 (0.74, 1.50)</td>
<td>0.787</td>
<td>1.77 (1.10, 2.90)</td>
<td>0.023</td>
<td>1.36 (1.10, 1.78)</td>
<td>0.022</td>
<td></td>
<td></td>
<td>1.26 (0.82, 1.94)</td>
<td>0.302</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Key: HUU, HIV-unexposed uninfected; P-value corresponding to the two-sample t test (with unequal variances) comparing mean scores. Malawi site multivariate models (in-utero ART duration; breastfeeding; maternal-age; electricity/gas use; and water source) and Uganda site multivariate models (in-utero ART duration; breastfeeding; and water source).
Table 2.4. Length-for-age z-score (LAZ) univariate and multivariate analyses at 12 and 24-month-age, by exposure groups and by site

<table>
<thead>
<tr>
<th>Exposure categories</th>
<th>Relative Risk (RR), 95% Confidence Interval (CI) of stunting (LAZ &lt; 2 SDs of the median of WHO reference)</th>
<th>Malawi</th>
<th>Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>12 months</td>
<td></td>
<td>(n= 189)</td>
<td>(n=179)</td>
</tr>
<tr>
<td>Model A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>0.86 (0.66, 1.11)</td>
<td>0.247</td>
<td>1.20 (0.86, 1.67)</td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
<tr>
<td>Model B (antepartum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CART</td>
<td>0.82 (0.59, 1.14)</td>
<td>0.229</td>
<td>1.14 (0.77, 1.69)</td>
</tr>
<tr>
<td>Non-CART</td>
<td>0.88 (0.63, 1.23)</td>
<td>0.432</td>
<td>1.26 (0.86, 1.84)</td>
</tr>
<tr>
<td>Control (HUU)</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
<tr>
<td>Model C (cumulative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CART regimens</td>
<td>0.75 (0.53, 1.10)</td>
<td>0.119</td>
<td>1.10 (0.70, 1.63)</td>
</tr>
<tr>
<td>Mixed regimens</td>
<td>0.94 (0.64, 1.38)</td>
<td>0.749</td>
<td>1.37 (0.91, 2.10)</td>
</tr>
<tr>
<td>Non-CART regimens</td>
<td>0.92 (0.60, 1.41)</td>
<td>0.695</td>
<td>1.32 (0.82, 2.11)</td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
<tr>
<td>24 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>1.13 (0.92, 1.38)</td>
<td>0.264</td>
<td>1.56 (1.18, 2.10)</td>
</tr>
<tr>
<td>Exposed</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model B (antepartum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CART</td>
<td>1.17 (0.92, 1.48)</td>
<td>0.198</td>
<td>1.58 (1.16, 2.16)</td>
</tr>
<tr>
<td>Non-CART</td>
<td>1.02 (0.79, 1.33)</td>
<td>0.862</td>
<td>1.53 (1.11, 2.10)</td>
</tr>
<tr>
<td>Control (HUU)</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
<tr>
<td>Model C (cumulative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CART regimens</td>
<td>1.13 (0.82, 1.56)</td>
<td>0.438</td>
<td>1.61 (1.17, 2.21)</td>
</tr>
<tr>
<td>Mixed regimens</td>
<td>0.95 (0.68, 1.33)</td>
<td>0.766</td>
<td>1.36 (0.94, 1.98)</td>
</tr>
<tr>
<td>Non-CART regimens</td>
<td>0.96 (0.67, 1.38)</td>
<td>0.819</td>
<td>1.78 (1.25, 2.53)</td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td>ref-</td>
<td>ref-</td>
<td>ref-</td>
</tr>
</tbody>
</table>
Key: 1‘Model A’ – aggregated and cumulative (antepartum and postpartum) exposures, versus HUU-controls, respectively; 2‘Model B’ – disaggregated (cART versus non-cART) antepartum exposures (regardless of postpartum exposures) model, versus HUU-controls, respectively; 3‘Model C’ – cumulative (antepartum and postpartum) exposure model disaggregated (cART, mixed and non-cART), versus HUU-controls, respectively. Malawi site multivariate models (in-utero ART duration; breastfeeding; maternal-age; electricity/gas use; and water source) and Uganda site multivariate models (in-utero ART duration; breastfeeding; and water source). HUU, HIV-unexposed uninfected; P-value corresponding to the two-sample t-test (with unequal variances) comparing means.
CHAPTER III

MORBIDITY (HEMATOLOGICAL EVENTS, AND HOSPITALIZATIONS) AND MORTALITY AMONG EXPOSED (MATERNAL HIV AND ANTIRETROVIRAL THERAPY) VERSUS UNEXPOSED CONTROLS IN MALAWI AND UGANDA
Morbidity and mortality at 12 and 24 months-of-age among Malawian and Ugandan HIV-exposed uninfected children with \textit{in-utero} and postpartum exposures to antiretroviral therapy

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\textsuperscript{6}Department of Psychiatry, Michigan State University;
\textsuperscript{7}Department of Psychiatry, University of Michigan;
\textsuperscript{8}Department of Pathology, Johns Hopkins University School of Medicine
3.1 ABSTRACT

Objective: To compare morbidity and mortality outcomes among exposed (in-utero and postpartum maternal-HIV and antiretroviral therapy (ART)), versus unexposed-control children.

Methods: Prospective cohort study of exposed (PROMISE randomized-clinical-trial (combination-ART (cART) versus non-cART)), and age-and-gender-matched controls, enrolled (09/2013-10/2014) in Malawi and Uganda. We used Wilcoxon Rank-Sum or Fischer’s exact tests to compare variables; and Generalized-Estimating-Equations, and Cox proportional-hazards models to measure associations.

Results: We enrolled 471(50.5%) exposed and 462(49.5%) control children. Maternal viral load (copies/ml) was homogeneous across cART and non-cART groups at delivery, 12, and 24 months post-delivery, p>0.05. Child-hospitalization risk since birth reported at 12 months-of-age visit was similar across exposed versus control groups: relative risk (RR), 95% confidence interval (CI), 0.95 (0.64, 1.40), p=0.782, after adjusting for breastfeeding, maternal-age, income-index, water-source, and electricity-use. Adjusted hazard ratio (HR) of hospitalization or death during study follow-up through 24 months-of-age visit was HR (95% CI) = 0.71 (0.40, 1.28), p=0.258. Hematological parameters were similar (p>0.05) across exposure groups, or more favorable among exposed children: hematocrit (p=0.029); and mean corpuscular volume (MCV, (p<0.001)) at 12 months-of-age visit in Uganda; and hemoglobin (p=0.009), hematocrit (p=0.03), and MCV (p<0.001) at 24 months-of-age visit, at both sites. Moderate (grade 2 or higher) anemia risk was lower among exposed versus control children: adjusted RR (95% CI) = 0.33 (0.17, 0.64), p=0.001 in Uganda, and RR (95% CI) = 0.56 (0.26, 1.16), p=0.119, in Malawi. Similar trends were observed with severe anemia (grade 3 or higher) risk: crude RR (95% CI) = 0.36 (0.12, 1.11), p=0.075, and adjusted RR (95% CI) = 0.30 (0.10, 1.40), p=0.125, in Uganda.

Conclusion: Morbidity (hematological events, hospitalizations) or mortality risk through 24 months-of-age among 12 months-of-age survivors, were independent of peripartum exposures to
maternal HIV and ART. Better hematological patterns were observed among exposed versus control children, possibly a reflection of differentially better care among exposed versus control participants prior to study entry.
3.2 INTRODUCTION

Pre-antiretroviral therapy (ART) era studies conducted in breastfeeding African populations were inconclusive regarding the potential for increased morbidity or mortality among HIV-exposed-uninfected (HEU) children compared to HIV-unexposed-uninfected (HUU) controls. Similarly, childhood hematological perturbations attributed to maternal-HIV exposure were reported, albeit inconsistently across studies. Sub-Saharan Africa bears a dual-epidemic burden, with more than 90% of the global need for prevention of mother-to-child transmission of HIV (PMTCT), and home to more than 50% of the child morbidity and mortality worldwide, mostly due to infectious diseases such as malaria, pneumonia and diarrhea.

Currently, combination ART (cART) drug regimens with proven efficacy and relative safety, based on randomized control trials in these settings, are being scaled up across several sub-Saharan Africa countries. The region will contribute majority (>90%) of the projected 1.5 million new ART-exposed HEU children per year by 2020. Overall PMTCT goal is to achieve health-equilibrium and long-term HIV-free survival among HEU versus HUU children, although empirical evidence remains scanty. African studies during the ART-era reported mixed findings, but were based on simple and/or ultra-short duration ART regimens. Moreover, some studies suggest a paradoxical increased risk of HEU child morbidity and mortality. While limited breastfeeding durations, as a PMTCT strategy in these earlier studies, was a likely etiological factor, ART exposures have been implicated, and specific drugs such as zidovudine (ZDV) have been associated with hematological toxicities. Proposed biological mechanisms of ART-induced cell pathology include mitochondrial damage; and toxicity to nuclear DNA of hematopoietic stem cells in a developing fetus and/or young infant.

Since breastfeeding is critical for infant health and survival in these settings, up to 27 to 31 month months of cumulative in-utero (9 months) followed by postpartum (18-24 months) cART exposures may occur, according to current WHO guidelines. This study compared morbidity
(hospitalizations, and severe hematological perturbations) and mortality outcomes at 12, and 24 months-of-age among HEU children with chronic in-utero plus postpartum ART exposures, versus HUU controls.

3.3 METHODS

3.3.1 Study Population

The parent neurodevelopment (ND) study targeted enrollment of 960 mother-child pairs between 2013-2014, including 240 exposed (in-utero and postpartum exposures to maternal HIV and prophylactic ARV drug regimens) and 240 age and gender-matched HIV unexposed uninfected control-group children at two international research sites, in Blantyre, Malawi and Kampala, Uganda, where prospective follow-up is ongoing. Written informed consent was obtained from all mothers prior to study entry. Approvals for initiation and ongoing research conduct were obtained from the Institutional Review Boards (IRBs) in Malawi and Uganda, and the Johns Hopkins Medical Institutions IRB.

Exposed-group source population was the PROMISE (Promoting Maternal and Infant Survival Everywhere) ART randomization PMTCT trial conducted contemporaneously at the respective sites. As a strategy to identify control children with similar socio-economic backgrounds, we targeted healthy children attending child-well/immunization clinics within the same clinics through which PROMISE trial participants were recruited. Mother-child eligibility was based on: written informed consent from the mother; 6-12 months-of-age children at entry (subsequently revised to 6-18 months-of-age) and gender-and-age (+/- 4 months) matched controls; birth weight of 2000 grams or more; documented confirmation of child HIV-uninfected and maternal HIV-infected status (exposed-group); and confirmed maternal HIV-uninfected (control-group). Children with serious pre-existing clinical conditions, or lived outside the site catchment area, or unwillingness by the mother/caregiver to be home visited were excluded.
The PROMISE study was a strategy-based open-label randomized controlled ART trial for prevention of mother-to-child transmission of HIV in breastfeeding populations. Eligible HIV-infected pregnant women were enrolled between 2011 and 2016 across several international sites. This NIH funded study is registered under clinicaltrials.gov (NCT01061151), and was previously described.\textsuperscript{4,22} In brief, two randomizations (antepartum and postpartum) were conducted sequentially as depicted in figure 2.1. Eligible HIV-infected pregnant women beyond 14-weeks of gestation were randomized to one of three arms: ‘antepartum-Arm A’, a single-ARV drug regimen of antepartum and intrapartum ZDV followed by intrapartum maternal single-dose-nevirapine (sdNVP) and a 6-14 day post-delivery tail of tenofovir (TDF) and emtricitabine (FTC); ‘antepartum-Arm B’, a c-ART regimen of ZDV, lamivudine (3TC) and ritonavir-boosted lopinavir (LPV/r); and ‘antepartum-Arm C’, a c-ART regimen of TDF, FTC and LPV/r. Mother-child pairs deemed eligible and within 6-14 days after delivery/birth, were randomized to either of two arms: ‘postpartum-Arm A’, a maternal c-ART regimen based on three or more antiretroviral drug regimens per local standard-of-care guidelines, taken for the duration of breastfeeding, plus daily infant NVP through 6 weeks of infant age; and ‘postpartum-Arm B’, a single infant NVP regimen taken daily through breastfeeding cessation. Cessation of breastfeeding was defined as 28 days or more of no breastmilk ingestion. A standard protocol was followed across both sites, including consultation of the protocol clinical management and adjudication committee, prior to initiation of temporary/permanent drug holds (toxicity/adverse reactions, infant HIV infection or medical indications), or study drug switch (toxicity, ART-indication; treatment failure or as deemed by the clinicians). Toxicities were graded per the NIH, Division of AIDS (DAIDS) ‘Tables for Grading the Severity of Adult and Pediatric Adverse Events’, version 1.0, December 2004, clarified August 2009.\textsuperscript{23}
3.3.2 Clinic Procedures

Experienced ND study staff received routine training on standardized data collection tools and operating procedures. Demographic and socioeconomic characteristics were collected at study entry. Child clinical (medical history, breastfeeding/nutrition, physical exam, and anthropometric) data, and a blood sample were collected at 12 and 24-month-of-age study visits. Additional exposed-group participant data obtained from the PROMISE clinical trial records includes: confirmed maternal HIV-infection, maternal health (HIV viral-load), ART randomizations, infant birth-date, birth-weight, immunization, and hospitalization history, confirmed HIV-free status at 12 and 24 months-of-age, and maternal/infant drug-adherence history. We used the standard-of-care child-immunization cards as the source-records for control-group child date-of-birth, birth-weight, and immunization history.

3.3.2.1 Clinical monitoring of morbidity outcomes

The DAIDS toxicity tables (version 1.0 December 2004, clarified in August 2009), were used to monitor hematological and clinical parameters across the exposure groups as summarized in appendix 3.1. Clinical interventions on a given parameter was at the discretion of the site clinical management team. Oral hematinic medications were routinely prescribed for children with grade 2 or higher anemia defined as decreased hemoglobin ≤ 9.9 g/dl, with closer follow-up review.

Per standard-of-care guidelines, study mothers/caregivers received health education messages on common childhood diseases (prevention, early identification of danger-signs, and appropriate first-aid), and encouraged to seek professional healthcare immediately. During a routine study scheduled visit, we assessed past medical history, verified, and documented any interim out-patient and in-patient hospital/clinic visits. Clinical records presented by the caregiver were used as source documentation for any interim hospitalizations and diagnoses. All hospitalizations were deemed medical interventions necessary to prevent permanent impairment.
or death and therefore considered to be grade 4 events. Study health-visitors tracked participant follow-up and made telephone call reminders of impending or missed study scheduled visits. Follow-up home visits were made when necessary. In case of a child death, the mother/caregiver was encouraged to come to the study clinic at a convenient date for study close-out review. Source documentation of child deaths was based on a death certificate or a verbal autopsy report documented by a study health visitor.

3.3.3 Laboratory Procedures

We used a rapid HIV test to screen control-group mothers at study pre-entry, and control-group children at 24 months-of-age visit. If the test was positive, a second rapid test on a separate sample was performed. A third rapid test was used as a tie breaker if the first and second tests yielded discordant results. HIV screen results for the exposed-group participants were abstracted from the PROMISE Trial records. PROMISE trial children who had not achieved complete cessation of breastfeeding received a Nucleic-Acid-Test (NAT) using HIV DNA PCR or HIV RNA PCR at 38, 50, 62, 74, 86, 98, and 104 weeks-of-age trial visits. If the initial HIV NAT was positive, a confirmatory test was performed as soon as possible with a repeat HIV-NAT on a second sample drawn on a different day. Among children who had achieved complete cessation of breastfeeding, HIV-NAT was used at 14, 26, and 50 weeks-of-age; and HIV antibody testing (Enzyme Linked Immunosorbent Assay (EIA) or HIV rapid test), was used at 74, and 98 weeks-of-age trial visits. If HIV antibody test was negative, no further HIV testing was done. Children with a positive HIV antibody test had a follow-up HIV-NAT test performed as soon as possible on a separate sample on a different day. HIV-NAT test was used to quantify maternal viral load at trial entry, delivery; weeks 6, 26 50, and thereafter every 12 weeks through 24 months postdelivery.

A complete blood count (white blood cell counts and differential, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin
concentration, and platelet counts) were assessed using the Beckman–Coulter AcT5 Part Diff (California, USA).

### 3.3.4 Statistical Methods

Morbidity (hospitalizations, and hematological adverse events) and mortality outcomes were compared across exposure groups. For each outcome, sensitivity analyses exploring different exposure classifications were done to determine the role of timing (in-utero versus postpartum), duration (in-utero versus cumulative (in-utero and postpartum)), or ART-regimen (cART versus non-cART). Primary analyses compared aggregated (regardless of timing, duration, or ARV drug combination) exposures, versus controls. Separate analyses were conducted to assess in-utero ART exposures: 1) exposed (antepartum randomization arms) versus controls; 2) exposed (cART (‘antepartum arms B and C’) versus non-cART (‘antepartum arm-A’)) versus controls, respectively; and 3) duration of antepartum ART-exposure (per-month). We also compared cumulative (in-utero and postpartum) cART (‘antepartum-arms B, and C’, plus ‘postpartum-arm A’), versus non-cART (‘antepartum-arm A’ plus ‘postpartum-arm B’), versus controls, respectively, to explore the impact of cumulative ART exposures.

We used Fischer’s exact and Wilcoxon Rank-Sum tests to compare categorical and continuous variables, respectively, and Mantel-Haenszel chi-square ($\chi^2$) to assess trends within categorical variables. To account for correlations between measures from the same child, Generalized Estimating Equation (GEE) regression models with a log-link were used to compute the relative risk (RR) of binary hematological outcomes at 12 and 24 months-of-age visits, with 95% Confidence Intervals (CI) computed with robust variance estimators. Poisson regression with robust error variance was used to estimate the RR of morbidity (at least one hospitalization since birth) through study entry, by exposure groups. Time-to-event (first-hospitalization or death) after 12 months-of-age was evaluated using Kaplan-Meier risk-curves stratified by exposure. The log-rank test was used to compare risk-curves. We used age as the time-metric.
while accounting for late-entries which were staggered since the study children were enrolled at different ages. Cox-proportional hazards models were used to compute hazard-ratio (HR) of child hospitalization or death after 12 months-of-age through 24-months-of-age.

For the respective regression models, univariate and multivariate analyses were performed to explore and control for potential confounding. Covariates defined a-prior included maternal viral load, breastfeeding; infant-age; and gender. Demographic/socio-economic factors explored included maternal factors (age, health-status (standardized Hopkins Symptoms Checklist (HSCL) score for anxiety and depression domains)); maternal care-giver (yes/no); and household-factors (income, electricity, and water-source). We generated a composite household-income-stability index score based on five binary-questions (yes/no) of the standardized AFASS questionnaire: “Is mother currently working?”, “Is mother the primary bread-winner?”, “Is pooled income sufficient?”, “Is pooled income sufficient for 12-months?”, “Is there enough to spend on infant nutrition and transport to healthcare?”. Equal weights (yes=1; no=0) were applied to each question and based on the total score, household-income was considered relatively-stable (5, 4 or 3); fairly-stable (2) or unstable (1 or 0). A product term between site (Malawi versus Uganda) and main exposure variable, was added to explore ‘effect’-modification. P-values were based on a two-sided hypothesis test with a type-1 error (α = 0.05). Data were entered and validated using ‘FileMaker 12 and Server 14’, and analyzed using Stata version 14.2 (Stata, College Station TX, USA).

3.4 RESULTS

3.4.1 Study profile

Overall, 933 mother-child pairs (471 (50.5%) exposed and 462(49.5%) control) were enrolled between August 2013 and December 2014. We accrued 788 (84.5%) pairs within the 12-months-of-age visit window, of these 50 (6.3%) missed or were lost to follow-up by 24-months-of-age visit, non-differentially by exposure (p>0.05). Median (IQR) infant-age in months among
exposed and control children was 14.1 (12.3, 16.3), and 14.0 (12.3, 15.8), p = 0.82, at 12-months-age visit; 24.0 (23.9, 25.0), and 24.0 (23.9, 25.5), p = 0.887, at 24-months-age visit, respectively. Sample sizes at both visits are summarized in figure 3.2.

3.4.2 Maternal-HIV and ART exposures

Of the 471 exposed children, 254 (53.9%), and 213 (45.2%) had cART and non-cART in-utero exposures, respectively, while four (0.9%) were late-presenters and had no in-utero ART exposures. In terms of cumulative (in-utero plus postpartum) exposures, 237 (50.3%) had cumulative-cART (antepartum Arms B or C followed by postpartum Arm-A); 134 (28.5%) had cumulative-mixed (antepartum-cART followed by postpartum non-cART or vice-versa); and 100 (21.2%) had cumulative-non-cART (antepartum Arm-A followed by postpartum Arm-B) exposures, respectively. Maternal viral load (copies/ml) at labor/delivery; 6, 12, 18 and 24 months post-delivery, was homogeneous across cART versus non-cART categories per in-utero classification, respectively, p > 0.05 (figure 3.3). The median (IQR) of maternal viral load (copies/ml) was 409 (39 to 11,780) at 12 months-of-age; and 520 (39 to 15,371) at 24-months-age visits, respectively.

3.4.3 Baseline characteristics

Mother-child demographic, socio-economic status (SES), and clinical characteristics at study-entry (or otherwise stated) are summarized by exposure and site in table 3.1. Exposed versus control-group mothers were older, median (IQR) age in years: 28.0 (25.0-31.0) versus 23.0 (20.0-28.0), p < 0.0001 in Malawi; and 27.0 (24.0-30.0) versus 25.0 (22.0-30.0), p = 0.053, in Uganda, respectively. The primary care giver was mostly (>95%) the mother, similarly across the exposure groups (p > 0.05). Maternal anxiety and depression domains score was homogeneous across the exposure groups. There was a tendency towards higher SES among control versus exposed-group households: electricity (p < 0.0001) in Malawi; or tap-water (p ≤ 0.015), at both
sites. Control versus exposed-group households in Uganda (p<0.0001) were more likely to have a relatively stable household level income, but not in Malawi (p=0.973). Infant age and sex were homogeneous across exposure groups, at both sites. Control versus exposed children had higher birth-weights in Malawi (p=0.014) and Uganda (p<0.001). In addition, Malawian versus Ugandan children had lower median (IQR) birthweight in kilograms: 3.0 (2.7, 3.3) versus 3.0 (2.8, 3.4), p=0.008 among exposed, and 3.0 (2.8, 3.3) versus 3.3 (3.0, 3.6), p=0.004, among control children. The prevalence of breastfeeding was high at both sites, more predominant in Malawi versus Uganda: 88.0% versus 67.4%, p<0.001, at 12-months-of-age visit; and 17.9% versus 10.5%, p=0.002, at 24 months-of-age visit, respectively; and consistently higher among control versus exposed children (p≤ 0.008). Exposed versus control children were more likely to have had a prior hospitalization since birth reported at baseline, in Uganda, p=0.024, but not in Malawi, p=0.061.

3.4.4 Childhood hospitalizations and deaths

Among 933 children assessed at baseline, 96 (10.3%) had at least one prior hospitalization since birth. Of these, 85 (88.5%) were attributed to infectious disease (ID) diagnoses: 34 (35.4%) pneumonia/respiratory tract infections (RTIs); 21 (21.9%) diarrheal; 15 (15.6%) malaria-related; and 15 (15.6%) due to other infections or involved other anatomical sites, and/or malnutrition. At 12-months-age visit, Ugandan exposed versus control children were more likely to report at least one prior ID-hospitalization since birth, crude relative-risk (RR, 95% confidence interval (95% CI)) = 1.34 (0.97, 1.84), p=0.075, but the association reversed after adjusting for breastfeeding, maternal-age, and household-income index, RR (95% CI) = 0.95 (0.64, 1.40), p=0.782. Stable household-income was independently associated with 44% reduction in hospitalization-risk, RR (95% CI) = 0.56 (0.35, 0.89), p=0.014. In Malawi, exposed versus control children had a lower risk of prior hospitalization since birth, crude RR (95% CI) = 0.35 (0.14, 0.90), p=0.029, which was attenuated after adjusting for breastfeeding and maternal-
Six child-deaths occurred during follow-up between 12.6 and 18.0 months-of-age: malaria (1/6); diarrhea (2/6); fever of unknown origin (1/6); congestive cardiac failure (1/6); and road traffic accident (1/6). Overall, there were 3.1 (95% CI: 0.8, 12.4) deaths per 1000 person-years among exposed, and 6.5 (95% CI: 2.5, 17.4) deaths per 1000 person-years among control-group children, p=0.415. Fifty children were hospitalized at least once during follow-up between 11.3 to 31.1 months-of-age. Of these, 45 (90%) were attributed to ID etiology: 16 (32.0%) malaria events; 13 (26%) pneumonia/RTIs; 8 (16%) diarrheal complications and 8 (16.0%) due to other ID causes. Across sites, the ID-hospitalization incidence rate was 3.4 (95% CI: 2.2, 5.2) per 100 person-years among exposed, and 5.0 (95% CI: 3.5, 7.2) per 100 person-years among control-group children, respectively, p=0.182.

Overall, incidence rates of ID-related events (hospitalizations or deaths) at both sites were lower among exposed versus control children: 3.7 (95% CI: 2.5, 5.6) versus 5.7 (95% CI: 4.1, 7.9), events per 100 person-years, respectively, crude hazard-ratio, (HR (95% CI) = 0.67 (0.40, 1.13)), p=0.134. After adjusting for breastfeeding, maternal-age, and household factors (income-index score, water-source, and electricity use), HR (95% CI) = 0.71 (0.40, 1.28), p=0.258. Hospitalization or death risk-curves across sites are demonstrated in figure 3.4. Among Malawian children, the crude incidence rate of ID-related events among exposed versus control children was 3.9 (95% CI: 2.1, 7.0) versus 5.6 (95% CI: 3.4, 9.3) events per 100 person-years, (HR (95% CI) = 0.70 (0.32, 1.53)), p=0.375, which attenuated after adjusting for breastfeeding, maternal-age, water-source, and electricity-use, HR (95% CI) = 0.90 (0.39, 2.10), p=0.814. Similar trends were observed in Uganda, 3.7 (95% CI: 2.1, 6.3) versus 5.7 (95% CI: 3.7, 9.0) events per 100 person-years, crude HR (95% CI) = 0.65 (0.32, 1.31), p=0.227; and HR (95%
CI) = 0.53 (0.23, 1.19), p=0.124, after adjusted for breastfeeding, household income-index, and water-source.

3.4.5 Hematologic parameters at 12 and 24 months-of-age study visits

The median and interquartile-range (IQR) values of hemoglobin, g/dl; hematocrit, %; mean corpuscular volume (MCV), fL/cell; absolute neutrophil count (ANC), 10^3/UL; and platelet count, 10^3/UL, at 12 and 24-months-of-age study visits comparing exposed versus control-group children at the respective sites are summarized in table 3.2. At 12-months-of-age visit, hematological parameters (median (IQR)) were homogeneous (p>0.05) across exposure groups at both sites, with two exceptions. In Malawi, exposed versus control-group children had lower median (IQR) ANC, 10^3/UL, 2.1 (1.5, 2.6) versus 2.4 (2.0, 3.1), p<0.001. Exposed versus control-group children in Uganda had higher median (IQR) hematocrit, %, 32.2 (30.6, 33.9) versus 31.9 (29.9, 33.5), p=0.029; and higher median (IQR) MCV, fL/cell, 68.0 (63.0, 74.0) versus 66.0 (61.0, 71.0), p<0.001, respectively.

At 24 months-of-age visit, exposed versus control-group children at both sites consistently had higher median (IQR) values of hemoglobin, hematocrit, and MCV. In Malawi, median (IQR) among exposed-versus-controls: hemoglobin, g/dl, 11.3 (10.7, 12.0) versus 11.0 (10.4, 11.6), p=0.004; hematocrit, %, 34.6 (32.6, 36.6) versus 34.0 (32.3, 35.7), p=0.03; and MCV, fL/cell, 75.0 (69.0, 79.0) versus 72.0 (68.0, 77.0), p<0.001. Similarly, in Uganda: hemoglobin, g/dl, 11.2 (10.6, 11.7) versus 11.0 (10.4, 11.5), p=0.009; hematocrit, %, 33.8 (32.1, 35.4) versus 33.3 (31.6, 34.9), p=0.026; and MCV, fL/cell, 73.0 (69.0, 78.0) versus 71.0 (66.0, 75.5), p<0.001. Exposed versus control-group children in Uganda had a marginally lower median (IQR) ANC, 10^3/UL, 2.7 (2.1, 3.4) versus 2.9 (2.3, 3.5), p=0.045; but homogenous across groups in Malawi. The median (IQR) platelet counts, 10^3/UL across exposure groups was similar at both sites (p>0.05).
3.4.6 Anemia, neutropenia, and thrombocytopenia events at 12 and 24 months-of-age visits

Overall, grade-one or higher anemia (decreased hemoglobin (≤ 10.9 g/dl)) was reported in 261 (64.9%) exposed versus 242 (62.7%) control-group children, p=0.515, at 12 months-of-age visit; and 161 (35.6%) versus 202 (46.8%), p=0.001, at 24 months-of-age visit, respectively. Moderate anemia (grade 2 or higher decreased hemoglobin (≤ 9.9 g/dl)), was similar across exposure groups at 12 and 24 months-age visits, at both sites, respectively, except for significantly lower proportion at 24-months-of-age visit among Ugandan exposed versus control-group children: 20 (8.7%) versus 38 (17.3%), p=0.008. Similarly, severe anemia events, defined as grade 3 or higher decreased hemoglobin, (≤ 8.9 g/dl), were homogeneously distributed across the exposure groups at both visits, except for Ugandan exposed versus control-group children at 12 months-age visit: 10 (4.8%) versus 25 (12.3%), p=0.008, respectively (figure 3.3).

Grade-one or higher neutropenia, defined as ≤ 1,300 neutrophils/UL was homogeneous among exposed versus control-group children, at 12 months-of-age visit, 47 (12.2%) versus, 47 (11.7%), p=0.913; and 35 (7.7%) versus 27 (6.3%), p=0.43, at 24 months-of-age visit, respectively. Similarly, severe (grade 3 or higher) neutropenia defined as decreased ANC (≤ 750 neutrophils/UL) was similar across exposure groups (p >0.05), as demonstrated in figure 3.4.

Mild or moderate thrombocytopenia was reported in 2 (1.1%) Malawian controls compared to none of the exposed children at 12-months-of-age, p=0.344; and 7 (3.3%) controls (including one case classified as life-threatening), compared to no none of the exposed children at 24 months, p=0.053. In Uganda, one case of mild thrombocytopenia was reported among exposed children versus none of the controls, at both 12 and 24-months-of-age visits, respectively.
### 3.4.7 Relative risk of severe and clinical anemia by exposure group

The relative risk (RR) and 95% CI estimates of moderate (grade 2 or higher), and severe (grade 3 or higher) anemia by exposure at 12 and 24-months-of-age visits are summarized in tables 3.3, and 3.4, respectively. At both sites, the risk of moderate, and severe anemia at 12 months-of-age visit was homogeneous across exposure groups, \( p > 0.05 \), with a tendency towards lower risk of severe anemia among exposed versus controls. In Uganda, crude RR (95% CI) = 0.40 (0.20, 0.81), \( p=0.011 \), and RR (95% CI) = 0.47 (0.20, 1.19), \( p= 0.112 \), after adjusting for breastfeeding, household income-index, and water-source, while in Malawi, crude RR (95% CI)=0.75 (0.35, 1.62), \( p = 0.466 \), and 0.82 (0.33, 2.10), \( p=0.680 \), after adjusting for breastfeeding, maternal-age, water-source and electricity-use.

At 24 months-of-age visit, exposed versus control children in Uganda had a lower risk of moderate anemia, crude RR (95% CI) = 0.51 (0.31, 0.85), \( p=0.01 \), and RR (95% CI) = 0.33 (0.17, 0.64), \( p=0.001 \) after adjusting for breastfeeding, household income index and water-source. A similar trend was observed in Malawi, crude RR (95% CI) = 0.55 (0.28, 1.10), 0.078, and RR (95% CI) = 0.56 (0.26, 1.16), \( p=0.119 \), after adjusting for breastfeeding, maternal-age, water-source, and electricity-use. Similarly, severe anemia risk was lower among exposed versus control children in Uganda, crude RR (95% CI) = 0.36 (0.12, 1.11), \( p= 0.075 \), and adjusted RR (95% CI) = 0.30 (0.10, 1.40), \( p=0.125 \).

### 3.5 DISCUSSION

We report similarities and differences in childhood morbidity (hospitalizations, and hematological events), and mortality outcomes by exposure at two African sites. Risks of ID hospitalization from birth reported at 12 months-of-age visit, as well as ID hospitalization or death during follow-up through 24 months-of-age visit, were homogeneous across exposure groups. Hematological patterns were similar across exposure groups with a few exceptions.
Median (IQR) values of hemoglobin, hematocrit, and MCV were significantly higher among exposed versus control children at 24 months-of-age visits, at both sites. Similar hematocrit and MCV trends were observed among Ugandan children at 12 months-of-age visit. Moderate anemia was common, trending towards lower risk among exposed versus control children, and a similar pattern was observed with severe anemia. While there was a trend for lower median ANC values among exposed versus control group children at 24 months-of-age, severe neutropenia, or thrombocytopenia were rare and homogeneous across exposure groups. Lastly, there were no outcome differences across cART versus non-cART, or antepartum versus cumulative (antepartum and postpartum), compared to control-group references, respectively.

These child morbidity/mortality outcome findings are contemporary and reassuring since previous African studies conducted during the ART-era are few, and were based on simpler and ultra-short duration ART. Also, breastfeeding a known predictor of infant survival in these settings, was mostly restricted to 6 months-of-infant-age or less in prior research based on WHO infant feeding recommendations for HIV infected women prior to 2010, to shorten breastmilk HIV exposures as part of an overall PMTCT strategy. In addition, by design (longer duration of breastfeeding), exposed children in our study received longer co-trimoxazole prophylaxis coverage, which has been associated with decreased ID morbidity, including common bacterial and protozoal infections (pneumonia, diarrhea, and malaria). In some studies, laboratory evidence of infant HIV-free status was not routinely verified, with a potential for misclassification of HIV-infected children as HEU.

In contrast to our study, a Botswana study (2002-2004) that assessed intrapartum sdNVP plus 6 months of infant ZDV, and a surveillance program in Mozambique (1996-2009) with sdNVP followed by 4 weeks of infant ZDV, each reported increased mortality among HEU versus HUU children. More hospitalizations were reported among HEU versus HUU children throughout infancy in the Botswana study, but no differences were observed at 12 months-of-age
in the Mozambican study. Both studies reported breastfeeding durations through 6-months-of-age for HEU and longer durations up to 12 months-of-age for HUU children. Similarly, in study populations with peripartum sdNVP exposure alone, and where breastfeeding was encouraged through 6 months-of-age, higher mortality among HEU versus HUU children was reported at 12 months-of-age in a multinational study (2001-2003) in Malawi, Tanzania and Zambia; at 20 months-of-age in a Malawian study; and from 1 through 24 months-of-age in a Zambian study.\textsuperscript{9,14,15} The Malawian study reported similar hospital admission rates across HEU versus HUU children.\textsuperscript{14} However, two South African studies with sdNVP or ZDV monotherapy, reported similar mortality rates across HEU and HUU groups, through 9 months-of-age\textsuperscript{10} and 12 months-of-age, respectively.\textsuperscript{11} One of the studies, conducted between 2002 and 2004, reported markedly lower rates of breastfeeding in the HEU versus HUU infant groups, although the deleterious effects might have been minimal since about 30\% of the participants were from wealthier areas where formula feeding is relatively safe.\textsuperscript{10} The larger study on the other hand reported high rates of exclusive breastfeeding across the HEU and HUU groups which might explain the homogenous mortality rates across the two groups.\textsuperscript{11} Another small South African study (2009) suggests that HEU children with maternal and infant short-course ZDV with sdNVP had similar rates of infection as HUU controls, although HEU were more likely to be hospitalised.\textsuperscript{24}

In our study, there was no evidence of detrimental hematological outcomes among exposed versus control children. Moreover, exposed children tended to have lower risk of severe anemia, although no correlations with hospitalizations or mortality were observed. This is consistent with earlier European and North American longitudinal studies which suggested that the early-infancy non-clinically significant anemia and/or neutropenia associated with \textit{in-utero} cART (mostly implicated ZDV), was reversed by 6 to 18 months-of-age.\textsuperscript{20,25–28} Observations in our study of a predominantly microcytic (MCV less than 80 fL) pattern among children with severe anemia,
regardless of exposure, is indicative of iron-deficiency etiology, rather than a macrocytic picture characteristic of ZDV toxicity. More than 50% of childhood anemia in these settings is attributed to iron-deficiency anemia (IDA), which often co-exists with other causes such as malaria, parasitic infestation, nutritional deficiencies or hemoglobinopathies. Previous hematological African studies during the ART era are few, and were limited to simple and/or shorter duration ART, shorter breastfeeding periods, or no HUU comparison. A Malawian RCT (2004-2010) reported no differences in severe anemia at 7 months-of-age among infants with ART exposures since birth (maternal ZDV-based cART versus infant sdNVP), versus un-exposed HEU controls. All children received intrapartum sdNVP followed by a seven-day tail of ZDV+3TC, and cotrimoxazole from 6 weeks-of-age through cessation of breastfeeding. A longitudinal study based on two completed RCTs (2001-2003, and 2006-2008, respectively) in Botswana, reported increased risk of severe anemia at 6 months-of-age among infants with exposures to maternal ZDV-based cART versus infant-ZDV, taken from delivery/birth. Maternal cART group infants also received ZDV for 1 month. However, there was no HUU comparison group, the infant-ZDV group infants were from the earlier trial with a potential for secular-trends bias given the rapidly evolving PMTCT practices then, and some of the children in the earlier trial were randomized to formula-feeding versus breastfeeding (mostly encouraged to stop as early as 6 months-of-age per standard-of-care). In a Kenyan study (2003-2009), breastmilk exposure to maternal ZDV-based cART for the first 6 months of life among HEU infants who received concomitant cotrimoxazole prophylaxis through breastfeeding cessation, was not associated with severe infant anemia, there was no HUU comparison. Earlier RCTs in Malawi demonstrated transient anemia and granulocytopenia among HEU infants exposed to ultra-short perinatal ART regimens (sdNVP versus sdNVP plus daily infant ZDV for 1 week), which normalized by 3 months-of-age. We previously reported no apparent risk of severe anemia or neutropenia among HEU infants taking daily NVP and cotrimoxazole prophylaxis through 6 months-of-age, based on RCT data from Uganda and Zimbabwe.
Overall these findings are suggestive of health-equilibrium across exposure groups, and in some instances exposed versus control children had significantly lower risk of severe iron-deficiency anemia most likely related to the close monitoring and early iron supplementation given as part of the PROMISE clinical trial from which the ND group were co enrolled. We had hypothesized \textit{a-prior} that \textit{in-utero} and breastmilk exposures to maternal HIV and prophylactic ART would be associated with increased morbidity and/or mortality among HEU children based on earlier research from both Europe and in Africa. Postulated biological mechanisms of morbidity induced by exposure to maternal HIV include immunological perturbations such as immune activation, decreased number and function of T-cells, and deranged humoral immune responses;\textsuperscript{37,38} while ART-induced mechanisms include mitochondrial damage, or toxicity to nuclear DNA of hematopoietic stem cells.\textsuperscript{17,27} Therefore, these findings of no evidence of increased morbidity or laboratory toxicity HIV and ART exposed children compared to HIV unexposed uninfected controls are reassuring.

There are a few limitations to consider. In resource-limited settings where standard-of-care may not be routinely available, the controlled-clinical trial environment in the exposed source population potentially resulted in differential medical-care experiences across exposure groups prior to study entry \textit{(in-utero} and early postpartum periods). PROMISE trial mothers received antenatal standard-of-care including vaccinations; malaria intermittent preventive treatment (IPT); treated mosquito-nets; as well as hematinic and anti-helminth prophylaxis. Postpartum care included maternal reproductive-health services, and health education including infant-feeding. Infants received routine immunization; co-trimoxazole prophylaxis; growth monitoring; and anti-helminth prophylaxis. Therefore, selection of potentially healthier exposed children into our study might have resulted in underestimated measures of association. Also, since the majority (67\%) of the under-five child mortality in these settings occurs before the first birthday, and about 45\%
during the neonatal period, mortality differences across HEU and HUU source populations potentially induced a survival-bias in our study.\textsuperscript{39}

Similarly, infant complete blood counts (CBC) were monitored frequently from birth through 24 months-of-age as part of the clinical trial procedures, and iron-supplements administered to children with moderate (grade 2) decreased hemoglobin. In addition, lower diagnostic thresholds per DAIDS toxicity tables (version 1.0, Aug 2009) potentially resulted in unnecessary clinical interventions. DAIDS tables are based on non-African predominantly white populations, and yet lower baseline normal hematological parameters have been described among black-African pediatric populations.\textsuperscript{40,41} Differential cumulative hematinic benefits prior to ND study entry may account for the tendency to more favorable hematological patterns, and lower risk of severe (grade 3 or higher) decreased hemoglobin among exposed versus controls observed in our study. We conducted a sensitivity analyses of hematological outcomes based on recently modified DAIDS toxicity tables (version 2.0, December 2014),\textsuperscript{42} and while lower rates of anemia were observed, similar measures of association were observed.

To minimize the potential bias from study pre-entry differences in medical-care and/or socio-economic status across exposure groups, we targeted healthy unexposed control children attending immunization clinics from the same medical facilities where PROMISE trial participants were recruited. We also ensured uniform clinical, and hematological follow-up procedures across exposure groups during ND study follow-up. To mitigate the potential for survival-bias and ‘immortal person-time’ bias, we performed continuous time-to-event (hospitalization and/or death) analyses using date-of-birth as time-origin, and computed individual study entry and exit times while accounting for late-entries (since infants entered study follow-up at different ages between 9 and 18 months-of-age).
Despite these limitations, our findings based on cART and non-cART in-utero exposures initiated as early as 14-weeks-of-gestation, followed by breast-milk ART exposures through 18 months-of-age, are timely and informative, given the current WHO guidelines to initiate all HIV-infected pregnant women on life-long cART. We used standardized HIV-screening algorithms to ensure accurate classification of infant HIV-exposure status through 24-months-of-age. Exposure data based on a completed RCT, was well characterized, and enabled sensitivity analyses to compare cART and non-cART regimens verses age-and gender matched unexposed controls, respectively; as well as assess timing and duration of exposures on health outcomes. By 2020, more than 90% of the projected 1.5 million new HEU cases per year will be born in the sub-Saharan Africa. However, in these resource-limited settings routine monitoring of ART adverse events including hematological toxicity cannot be guaranteed. Thus, our findings of comparable health among ART-exposed HEU versus HUU children are reassuring, and should be generalizable to breastfeeding populations in sub-Saharan Africa where rapid scale-up of ART programs are underway.
3.6 REFERENCES


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29. Richard H, Gary L, Suran L. Mean cell volume (MCV) changes in HIV-positive patients taking ... 2002;(Mcv).


Figure 3.1. Antepartum and postpartum antiretroviral drug exposures (PROMISE trial randomization schemas)

**ANTEPARTUM RANDOMIZATION**
(14 weeks gestation to term)

- **Arm-A (ZDV; sdNVP; FTC+TDF tail)**
  - **Zidovudine-alone prophylaxis**
    - ZDV, 300 mg twice daily
    - NVP, 200 mg at labor onset
    - FTC, 200mg and TDF, 300 mg, once daily for 6–14 days after delivery

- **Arm-B (ZDV+3TC+LPV/r)**
  - **Zidovudine-based ART prophylaxis**
    - ZDV, 300 mg and 3TC, 150 mg, b.d.
    - LPV, 200mg and ritonavir 100 mg b.d.
    - LPV, 600 mg and ritonavir 150 mg b.d (third trimester)

- **Arm-C (TDF+FTC+LPV/r)**
  - **Tenofovir-based ART prophylaxis**
    - FTC, 200mg and TDF, 300 mg, o.d.
    - LPV, 200mg and ritonavir 100 mg b.d.
    - LPV, 600 mg and ritonavir 150 mg b.d (third trimester)

**POSTPARTUM RANDOMIZATION**
(6-14 days postdelivery/birth)

- **Arm-A (maternal and infant prophylaxis)**
  - **Maternal**
    - TRV (200mg and TDF, 300 mg), o.d.
    - LPV/r, 200mg and ritonavir 100 mg b.d.
  - **Infant**: NVP, dosed per age-bands, o.d., 6-9 days through 42 days-of-age

- **Arm-B (Infant prophylaxis)**
  - **Infant**: NVP, dosed per age-bands, o.d.
Key: b.d., twice-daily; o.d., once-daily; 3TC, lamivudine; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; TDF, tenofovir; FTC, emtricitabine; TRV, Truvada, a fixed dose combination of FTC and TDF; ZDV, zidovudine. Antepartum randomization antiretroviral drug regimens were taken through 6–14 days after delivery, and maternal (arm A) and infant (arm B) postpartum regimens taken through breastfeeding cessation or through 18-months-of-age, whichever came first. Infant postpartum arm A nevirapine was taken through 42 days of age. Infant nevirapine dosed per infant age-bands: birth to 6 weeks, 15 mg once daily if birth weight > 2500 gm or 10 mg once daily if birth weight 2000 to 2499 gm; > Week 6 to 6 months: 20 mg once daily, >6 months to 9 months: 30 mg once daily; > 9 months to weaning: 40 mg once daily
Figure 3.2. Study flow diagram: enrolments (August 2013 – April 2016), and losses to follow-up

933 mother/infant dyads enrolled 788 prior to, and 145 after the 12-month visit (Aug 2013 – Dec 2014)

12-month visit (n=788)

Lost to follow-up (n=50)

CoM-JHU CRS, Blantyre, Malawi (n=377)

HEU children (n=194)

HUU children (n=183)

MUJHU CRS, Kampala, Uganda (n=411)

HEU children (n=208)

HUU children (n=203)

Enrolments after 12-months study visit (n=145)

24-month visit (n=883)

CoM-JHU CRS Blantyre, Malawi (n=434)

HEU children (n=223)

HUU children (n=211)

MUJHU CRS, Kampala, Uganda (n=449)

HEU children (n=229)

HUU children (n=220)
Figure 3.3. Maternal viral load (copies/ml) through 24 months post-delivery by *in-utero* cART versus non-cART exposures

Key: Combination antiretroviral therapy (cART) contains antepartum *Arm-B* (ZDV+3TC+LPV/r) and *Arm-C* (TDF+FTC+LPV/r); and non-cART contains antepartum *Arm-A* (ZDV; sdNVP; FTC+TDF tail). Labor/delivery (L/D) period spurns from 2 months before L/D through 21 days after L/D), sample size (n) = 380 (238 cART, 142 ncART); 6 (4-7) months, n= 406 (219 cART, 187 ncART); 12 (11-15) months, n= 405 (218 cART, 187 ncART); 18 (15-21) months, n= 214 (123 cART, 91 ncART); 24 (21-28) months, n= 402 (226 cART, 176 ncART); P-value based on the Wilcoxon rank-sum (Mann-Whitney) test. Box hinges represent 25th and 75th percentiles respectively, and box midline is the median of the log 10 of maternal viral load, respectively.
Figure 3.4. Kaplan-Meier failure (hospitalization or death) estimates across sites between 12 and 24 months-of-age study visits

Key: * Log-rank tests $p$-values for homogenous failure (hospitalization or deaths) across exposure groups. Log-ranks test $p$-value for the adjusted plot estimated by the Wald-statistic of the `stcox` regression with robust variance estimator.
Figure 3.5. Clinical (≥grade 2) and severe (≥grade 3) anemia, by exposure and site, at 12 and 24 months-of-age study visits

Key: * Classification was based on the US National Institutes of Health, Division of AIDS (DAIDS) toxicity grading (Version 1.0, Dec 2004, modified Aug 2009), accessed at: http://rsc.tech-res.com/docs/default-source/safety/table_for_grading_severity_of_adult_pediatric_adverse_events.pdf; Sample sizes: Malawi: 12-months-of-age visit, n= 377 (194 exposed, 183 controls); 24-months visit, n= 434 (223 exposed, 211 controls); Uganda: 12-months-of-age visit, n= 411 (208 exposed, 203 controls); 24-months-of-age visit, n= 449 (229 exposed, 220 controls). P-values based on the Fisher’s exact 2-sided test. Highlighted p-values are statistically significant.
Figure 3.6. Proportion of neutropenia (≥grade 1) and severe neutropenia (≥grade 2) by exposure and site, at 12 and 24-months-age visits

Key: * Classification was based on the US National Institutes of Health, Division of AIDS (DAIDS) toxicity grading (Version 1.0, Dec 2004, modified Aug 2009), accessed at: http://rsc.tech-res.com/docs/default-source/safety/table_for_grading_severity_of_adult_pediatric_adverse_events.pdf; Sample sizes: Malawi: 12-months-age visit, n= 377 (194 exposed, 183 controls); 24-months visit, n= 434 (223 exposed, 211 controls); Uganda: 12-months-age visit, n= 411 (208 exposed, 203 controls); 24-months-age visit, n= 449 (229 exposed, 220 controls). P-values based on the Fisher’s exact 2-sided test.
Table 3.1. Baseline characteristics by exposure (maternal-HIV and prophylactic antiretroviral drugs) status, and by site

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malawi site (N=456)</th>
<th>Uganda site (N=477)</th>
<th>P-value</th>
<th>Malawi site (N=456)</th>
<th>Uganda site (N=477)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed-group (N=231)</td>
<td>Control-group (N=225)</td>
<td></td>
<td>Exposed-group (N=240)</td>
<td>Control-group (N=237)</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years), median [IQR]</td>
<td>28.0 [25.0-31.0]</td>
<td>23.0 [20.0-28.0]</td>
<td>&lt;0.0001</td>
<td>27.0 [24.0-30.0]</td>
<td>25.0 [22.0-30.0]</td>
<td>0.053a</td>
</tr>
<tr>
<td>Maternal care (primary care giver), n (%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-month visit</td>
<td>176 (99.8%)</td>
<td>167 (99.4%)</td>
<td>0.488</td>
<td>206 (99.5%)</td>
<td>202 (99.8%)</td>
<td>1.000b</td>
</tr>
<tr>
<td>24-month visit</td>
<td>209 (99.5%)</td>
<td>200 (99.5%)</td>
<td>1.000</td>
<td>217 (96.4%)</td>
<td>210 (95.9%)</td>
<td>0.809b</td>
</tr>
<tr>
<td>Maternal well-being (Hopkins Symptoms Checklist (HSCL) score, median [IQR])</td>
<td>0.19 [0.06-0.35]</td>
<td>0.23 [0.10-0.45]</td>
<td>0.097</td>
<td>0.23 [0.13-0.48]</td>
<td>0.23 [0.13-0.39]</td>
<td>0.402a</td>
</tr>
<tr>
<td>House income stability score, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatively-stable</td>
<td>46 (22.1%)</td>
<td>49 (22.7%)</td>
<td>0.973</td>
<td>47 (22.1%)</td>
<td>87 (48.3%)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>Fairly-stable</td>
<td>42 (20.2%)</td>
<td>45 (20.8%)</td>
<td></td>
<td>54 (25.3%)</td>
<td>49 (27.2%)</td>
<td></td>
</tr>
<tr>
<td>Unstable</td>
<td>120 (57.7%)</td>
<td>122 (56.5%)</td>
<td></td>
<td>112 (52.6%)</td>
<td>44 (24.4%)</td>
<td></td>
</tr>
<tr>
<td>Electricity or gas in household, n (%)</td>
<td>93 (42.3%)</td>
<td>130 (59.4%)</td>
<td>&lt;0.0001</td>
<td>98 (41.0%)</td>
<td>118 (50.2%)</td>
<td>0.053b</td>
</tr>
<tr>
<td>Refrigerator in household, n (%)</td>
<td>26 (11.8%)</td>
<td>36 (16.4%)</td>
<td>0.173</td>
<td>36 (15.1%)</td>
<td>52 (22.1%)</td>
<td>0.058b</td>
</tr>
<tr>
<td>Water source for household use, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water (in house)</td>
<td>33 (15.4%)</td>
<td>35 (15.9%)</td>
<td>0.015</td>
<td>74 (31.4%)</td>
<td>51 (21.7%)</td>
<td>0.013b</td>
</tr>
<tr>
<td>Tap water (communal)</td>
<td>124 (58.0%)</td>
<td>151 (68.6%)</td>
<td></td>
<td>105 (44.5%)</td>
<td>135 (57.4%)</td>
<td></td>
</tr>
<tr>
<td>Borehole and other sources</td>
<td>57 (26.6%)</td>
<td>34 (15.5%)</td>
<td></td>
<td>57 (24.1%)</td>
<td>49 (20.9%)</td>
<td></td>
</tr>
<tr>
<td>Infant characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>111 (48.0%)</td>
<td>108 (48.0%)</td>
<td>1.000</td>
<td>117 (48.8%)</td>
<td>115 (48.5%)</td>
<td>1.000b</td>
</tr>
<tr>
<td>Birthweight (kilograms), median [IQR]</td>
<td>3.0 [2.7-3.3]</td>
<td>3.0 [2.7-3.3]</td>
<td>0.014</td>
<td>3.0 [2.8-3.4]</td>
<td>3.3 [3.0-3.6]</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td>Breastfeeding status (yes), n (%)</td>
<td>151 (79.9%)</td>
<td>173 (96.7%)</td>
<td>&lt;0.0001</td>
<td>102 (49.0%)</td>
<td>175 (86.2%)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>Prior hospitalization at baseline (yes), n (%)</td>
<td>8 (3.6%)</td>
<td>2 (0.9%)</td>
<td>0.061</td>
<td>53 (22.1%)</td>
<td>33 (13.9%)</td>
<td>0.024b</td>
</tr>
</tbody>
</table>
Key: IQR, Interquartile range; \(^a\) p-value from Wilcoxon rank-sum test; \(^b\) p-value from Fisher’s exact test. Highlighted p-values are statistically significant. Missing data: maternal-age (2.7%); care-giver (3.9%); maternal wellbeing (9.9%); Household income (12.4%); electricity-use (2.1%); refrigerator-use (1.9%); water-source (3.0%); infant gender (0.0%); infant-age (0.0%); birthweight (0.2%); breastfeeding (0.8%); prior hospitalizations (0.3%)
<table>
<thead>
<tr>
<th>Category</th>
<th>Median, Inter-Quartile Range (IQR), p-value</th>
<th>Malawi</th>
<th>Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exposed-group (n= 182)</td>
<td>Control-group (n=177)</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>10.5 (9.8, 11.2)</td>
<td>10.7 (9.9, 11.4)</td>
<td>0.270</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32.5 (30.8, 34.7)</td>
<td>33.4 (31.1, 35.4)</td>
<td>0.073</td>
</tr>
<tr>
<td>MCV, fl/cell</td>
<td>67.5 (63.0, 73.0)</td>
<td>67.0 (61.0, 71.0)</td>
<td>0.055</td>
</tr>
<tr>
<td>Neutrophils, 10^3/UL</td>
<td>2.1 (1.5, 2.6)</td>
<td>2.4 (2.0, 3.1)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Platelets, 10^3/UL</td>
<td>447 (345, 554)</td>
<td>434 (346, 535)</td>
<td>0.616</td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>11.3 (10.7, 12.0)</td>
<td>11.0 (10.4, 11.6)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>34.6 (32.6, 36.6)</td>
<td>34.0 (32.3, 35.7)</td>
<td><strong>0.030</strong></td>
</tr>
<tr>
<td>MCV, fl/cell</td>
<td>75.0 (69.0, 79.0)</td>
<td>72.0 (68.0, 77.0)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Neutrophils, 10^3/UL</td>
<td>2.6 (2.2, 3.4)</td>
<td>2.8 (2.2, 3.4)</td>
<td>0.363</td>
</tr>
<tr>
<td>Platelets, 10^3/UL</td>
<td>410 (337, 497)</td>
<td>407 (314, 489)</td>
<td>0.220</td>
</tr>
</tbody>
</table>

Key: P-value based on a two-sample (exposed versus control group) Wilcoxon rank-sum (Mann-Whitney) test. Median (IQR) comparisons by combined-antiretroviral therapy (cART) versus non-cART exposed-group children, at 12 and 24-months-of-age were not significant, p >0.05 (not presented). Missing data: overall, 19/788 (2.4%) across sites at 12 months; 11/883 (1.2%) across sites at 24 months, independent of exposure, respectively.
Table 3.3. Moderate anemia (≥ grade 2) univariate and multivariate analyses at 12 and 24-month-age, by exposure groups and by site

<table>
<thead>
<tr>
<th>Exposure categories</th>
<th>Relative Risk (RR), 95% Confidence Interval (CI) of clinical anemia (≥ grade 2)</th>
<th>Malawi</th>
<th>Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate (n)</td>
<td>P-value</td>
<td>Multivariate (n)</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed Controls (HUU)</td>
<td>1.22 (0.86, 1.73)</td>
<td>0.269</td>
<td>1.32 (0.88, 1.96)</td>
</tr>
<tr>
<td>Model B (antepartum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cART</td>
<td>1.12 (0.74, 1.71)</td>
<td>0.594</td>
<td>1.11 (0.61, 2.02)</td>
</tr>
<tr>
<td>Non-cART Controls (HUU)</td>
<td>1.22 (0.80, 1.87)</td>
<td>0.343</td>
<td>1.15 (0.61, 2.20)</td>
</tr>
<tr>
<td>Model C (cumulative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cART regimens</td>
<td>1.17 (0.77, 1.80)</td>
<td>0.452</td>
<td>1.31 (0.83, 2.10)</td>
</tr>
<tr>
<td>Mixed regimens</td>
<td>0.80 (0.43, 1.49)</td>
<td>0.479</td>
<td>0.86 (0.43, 1.71)</td>
</tr>
<tr>
<td>Non-cART regimens</td>
<td><strong>1.61 (1.01, 2.57)</strong></td>
<td><strong>0.043</strong></td>
<td><strong>1.78 (1.10, 2.92)</strong></td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed Controls (HUU)</td>
<td>0.55 (0.28, 1.10)</td>
<td>0.078</td>
<td>0.56 (0.26, 1.16)</td>
</tr>
<tr>
<td>Model B (antepartum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cART</td>
<td><strong>0.32 (0.11, 0.92)</strong></td>
<td><strong>0.034</strong></td>
<td>0.16 (0.02, 1.35)</td>
</tr>
<tr>
<td>non-cART control (HUU)</td>
<td>0.71 (0.33, 1.53)</td>
<td>0.385</td>
<td>0.76 (0.24, 2.39)</td>
</tr>
<tr>
<td>Model C (cumulative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cART regimens</td>
<td><strong>0.25 (0.10, 0.86)</strong></td>
<td><strong>0.028</strong></td>
<td><strong>0.19 (0.10, 0.94)</strong></td>
</tr>
<tr>
<td>Mixed regimens</td>
<td>0.59 (0.21, 1.64)</td>
<td>0.314</td>
<td>0.70 (0.24, 2.03)</td>
</tr>
<tr>
<td>Non-cART regimens</td>
<td>0.93 (0.37, 2.33)</td>
<td>0.874</td>
<td>0.90 (0.32, 2.52)</td>
</tr>
<tr>
<td>Controls (HUU)</td>
<td></td>
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</tbody>
</table>
Key: HUU, HIV-unexposed uninfected; P-value corresponding to the two-sample t-test (with unequal variances) comparing mean Z-scores (exposed versus control group), respectively. 'Model A' – aggregated and cumulative (antepartum and postpartum) exposures model, versus HUU-controls reference category, respectively; 'Model B' – disaggregated (cART versus non-cART) antepartum exposures (regardless of postpartum exposures) model, versus HUU-controls references category, respectively; 'Model C' – disaggregated (cART, mixed and non-cART) and cumulative (antepartum and postpartum) exposures model with exposure variable disaggregated (combined ART (cART) and non-cART regimen exposure levels) versus HUU-controls reference category, respectively. Maternal viral load (copies/ml) was homogeneous across cART versus non-cART, and infant age and gender similar across exposure versus control groups. In addition, breastfeeding duration; maternal age; household income index; electricity/gas use in household (yes/no); and source of water for household use (domestic tap-water; community tap-water, borehole/other) were considered in the multivariate regressions.
Table 3.4. Severe anemia (≥ grade 3) univariate and multivariate analyses at 12 and 24-month-age, by exposure groups and by site

<table>
<thead>
<tr>
<th>Exposure categories</th>
<th>Relative Risk (RR), 95% Confidence Interval (CI) of severe anemia</th>
<th>Malawi</th>
<th>Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate P-value</td>
<td>Multivariate P-value</td>
<td>Univariate P-value</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed Controls (HUU)</td>
<td>0.75 (0.35, 1.62) -ref-</td>
<td>0.466</td>
<td>0.82 (0.33, 2.10) -ref-</td>
</tr>
<tr>
<td><strong>Model B (antepartum)</strong></td>
<td>1.12 (0.74, 1.71) 0.594</td>
<td>0.930</td>
<td>0.34 (0.13, 0.88) 0.030</td>
</tr>
<tr>
<td>cART</td>
<td>1.23 (0.80, 1.87) 0.343</td>
<td>0.469</td>
<td>0.48 (0.20, 1.17) 0.107</td>
</tr>
<tr>
<td>Non-cART Control (HUU)</td>
<td>-- --</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed Controls (HUU)</td>
<td>-- --</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Model B (antepartum)</strong></td>
<td>-- --</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>cART</td>
<td>-- --</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>non-cART control (HUU)</td>
<td>-- --</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Key: HUU, HIV-unexposed uninfected; P-value corresponding to the two-sample t-test (with unequal variances) comparing mean Z-scores (exposed versus control group), respectively. ‘Model A’ – aggregated and cumulative (antepartum and postpartum) exposures model, versus HUU-controls reference category, respectively; ‘Model B’ – disaggregated (cART versus non-cART) antepartum exposures (regardless of postpartum exposures) model, versus HUU-controls references category, respectively. Maternal viral load (copies/ml) was homogeneous across cART versus non-cART, and infant age and gender similar across exposure versus control groups. In addition, breastfeeding duration; maternal age; household income index; electricity/gas use in household (yes/no); and source of water for household use (domestic tap-water; community tap-water, borehole/other) were considered in the multivariate regressions.
### Appendix 3.1. Severity grading of hematological and clinical outcomes DAIDS Hematological toxicity grading

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade 1 (mild)</th>
<th>Grade 2 (Moderate)</th>
<th>Grade 3 (severe)</th>
<th>Grade 4 (potentially life threatening)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemoglobin, g/dl</td>
<td>10.0 – 10.9 g/dL or any decrease 2.5 – 3.4 g/dL</td>
<td>9.0 – 9.9 g/dL or any decrease 3.5 – 4.4 g/dL</td>
<td>7.0 – 8.9 g/dL or any decrease ≥ 4.5 g/dL</td>
<td>&lt; 7.0 g/dL</td>
</tr>
<tr>
<td>2. Neutrophils, /UL</td>
<td>1,000 – 1,300/UL</td>
<td>750 – 999/UL</td>
<td>500 – 749/UL</td>
<td>&lt; 500/UL</td>
</tr>
<tr>
<td>Platelets, /UL</td>
<td>100,000 – 124,999/UL</td>
<td>50,000 – 99,999/UL</td>
<td>25,000 – 49,999/UL</td>
<td>&lt; 25,000/UL</td>
</tr>
<tr>
<td>Medical diagnosis&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Symptoms causing no or minimal interference with usual social and functional activities</td>
<td>Symptoms causing greater than minimal interference with usual social and functional activities</td>
<td>Symptoms causing inability to perform usual social &amp; functional activities</td>
<td>Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death</td>
</tr>
</tbody>
</table>

Key: 1. Hemoglobin parameters among HIV negative adults and children ≥ 57 days old; 2. Absolute neutrophil counts among adults and children older than 7 days of age; 3. Children were considered to have a grade 4 clinical illness if they were hospitalised (medical intervention indicated to prevent permanent impairment or death. Source: Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0 December, 2004, clarification memo, August 2009. 23
CHAPTER IV

DISCUSSION
4.1 DISCUSSION

Our overall research goal was to compare morbidity and mortality outcomes among HIV-exposed-uninfected (HEU) children with peripartum exposures to antiretroviral therapy (ART), versus HIV-unexposed-uninfected (HUU) controls. We hypothesized that exposures to maternal-HIV and ART during critical in-utero and early childhood (breastfeeding) periods, are associated with increased childhood morbidity and mortality. Maternal-HIV exposure has been associated with immunological perturbations (immune activation, decreased number and function of T-cells, and deranged humoral immune response), and ultimately increased vulnerability to infectious disease (ID), among HEU children.1,2 In addition, ART exposure is linked with mitochondrial changes with a potential for end-organ damage, 3–5 and toxicity to nuclear DNA of hematopoietic stem cells. 6–8 We employed a prospective cohort study design. Between August 2013 and December 2014, we targeted enrolment of 240 exposed (in-utero and postpartum exposures to maternal-HIV and prophylactic ART) and 240 age-and-gender-matched control children at two international sites, in Blantyre, Malawi and Kampala, Uganda, where prospective follow-up through 60 months-of-age is ongoing. Exposed-group mother/baby pairs were identified from the contemporaneous PROMISE ART randomized control trial (RCT), which presented a rare opportunity to abstract well-characterized data on in-utero and postpartum maternal-HIV and ART exposures, as well as other key co-factors of HEU child survival such as maternal viral load, breastfeeding, and past medical history since birth. These analyses specifically compared physical-growth (aim 1), hematology (aim 2), and hospitalizations/mortality (aim 3) outcomes among exposed (maternal-HIV and ART) versus control children at 12 and 24 months-of-age, respectively.

Physical growth outcomes were mostly less favorable among exposed versus control children. According to WHO Growth standards (2006),9 age and gender-based Z-scores for length (LAZ), and weight (WAZ), at 12 and 24 months-of-age respectively; and head-circumference
(HCAZ) at 24 months-of-age, were significantly lower among exposed versus control children. There were no weight-for-length (WLZ) differences across exposure groups. Stunting (LAZ < 2 standard deviations (SD) below WHO population median), a measure of extreme linear growth-faltering was common at 12 months-of-age visit: 143 (38.0%) in Malawi, and 89 (21.7%) in Uganda, p<0.0001; and at 24 months-of-age visit: 198 (45.6%) in Malawi, and 114 (25.4%) in Uganda, P<0.0001 respectively. Other forms of extreme growth-faltering including wasting (WLZ < 2SD), and underweight (WAZ <2SD), were relatively rare (<10%) at both study visits, across sites, respectively. After adjusting for key confounding variables, the relative risk of stunting among exposed versus control children was more than double at 12 months-of-age visit (p=0.011), and more than 80% at 24 months-of-age visit (p=0.036), among Ugandan children, respectively; and more than 50% at 24 months-of-age (p=0.002), among Malawian children. In addition, exposed versus control children in Malawi had close to 80% higher risk of having a head-circumference below the WHO population median at 24 months-of-age visit (p=0.023).

Hematological parameters, however, trended towards homogeneity across exposure groups, or more favorable among exposed versus control children, including significantly higher median (IQR) values of hemoglobin, hematocrit, and mean corpuscular volume (MCV) observed at 12 months-of-age visit in Uganda, and at 24 months-of-age visit, at both sites. However, exposed versus control children in Malawi had significantly lower median (IQR) absolute neutrophil counts (ANC), at 12 months-of-age visit. Based on DAIDS toxicity tables for severity grading (version 1.0 December 2004, clarified in August 2009), 10 moderate/ grade 2 or higher anemia (hemoglobin ≤ 9.9 g/dl) was common at 12 months-of-age visit: 95 (25.2%) in Malawi, and 126 (30.7%) in Uganda, p=0.096; but less common at 24 months-of-age visit: 35 (8.1%) and 58 (12.9%), p=0.021, respectively. Severe/ grade 3 or higher anemia (hemoglobin ≤ 8.9 g/dl) was relatively rare, less than 8.0% at 12 months and less than 3.0% at 24 months-of-age visits, respectively. Exposed versus control children at each site, had similar or lower risk of moderate,
and severe anemia at both time points. Specifically, exposed versus control children had more than 60% reduction in moderate-anemia risk at 24 months-of-age visit, at both sites. Severe anemia risk was homogeneous at both visits, across sites. Overall, neutropenia (ANC <1,300/UL), and thrombocytopenia (<125,000 /UL) were rare: 6.1% and < 1.0%, at both study visits, across sites, respectively, and homogeneous across exposed versus control groups (p<0.05), respectively.

Similarly, infectious disease (ID) associated child hospitalizations from birth through 12 months-of-age visit, and ID-hospitalizations/deaths during follow-up through 24 months-of-age, were homogenous across exposure groups, although events were rare. There were 3.1 (95% CI: 0.8, 12.4) deaths per 1000 person-years among exposed, and 6.5 (95% CI: 2.5, 17.4) deaths per 1000 person-years among control children, p=0.415. Incidence rates of ID-related events (hospitalizations or deaths) among exposed versus control children across sites were 3.7 (95% CI: 2.5, 5.6) versus 5.7 (95% CI: 4.1, 7.9), events per 100 person-years, respectively, adjusted hazard ratio (95% CI) = 0.71 (0.40, 1.28), p=0.258. Commonly reported ID-events were pneumonia/respiratory tract infections, diarrheal, malaria-related; other infections/anatomical sites, and/or malnutrition.

Overall, in this study population at two African sites with prolonged breastfeeding, prior exposures to maternal-HIV and prophylactic ART during in-utero and early childhood, compared to unexposed controls, were associated with growth-impairment at 12 and 24-months-of-age study visits, but no evidence of increased hematological-sequelae or increased susceptibility to ID (hospitalizations or death). These findings reflect child-health conditioned on survival through 9-18 months-of-age at study entry. It is likely that early infancy hematological complications and/or ID susceptibility attributable to in-utero or early infancy exposures to maternal-HIV and/or ART reported in previous studies, were already reversed/resolved by study entry. Two Malawian RCTs previously reported transient anemia and granulocytopenia which normalized by
3 months-of-age, although ART exposures were limited to ultra-short perinatal regimens (sdNVP versus sdNVP plus daily infant ZDV for 1 week). Similar findings were reports by earlier European and North American longitudinal studies which suggested that the early-infancy non-clinically significant anemia and/or neutropenia associated with in-utero cART was reversed by 6 to 18 months-of-age.7,11–14

In addition, sensitivity analyses demonstrating similar risks of stunting regardless of in-utero only, or cumulative (in-utero and postpartum) exposures, suggest that the pertinent or critical exposures in our study occurred in-utero. There were other observations to support this idea. First, although we were not able to isolate individual drug effects by study design, ZDV-based ART exposures were common in-utero but not postpartum. Earlier studies in Europe and North America implicated in-utero ZDV exposures as the most likely culprit for fetal and/or infant mitochondrial disease attributed to ART-exposure.7,11–14 Also, infant plasma levels of ZDV following postpartum exposures to ZDV-based-maternal-cART were likely minimal given earlier reports from a Kenyan pediatric cohort, suggestive of low ZDV bioavailability through breastmilk exposures.19 In our study, in-utero ZDV exposures were experienced among participants randomized to antepartum arms A or B, but not arm C. Postpartum randomization arm A (maternal-cART) was primarily based on country-specific standard-of-care with a predilection towards first-line cocktail of tenofovir (TDF) and emtricitabine (FTC); and ritonavir-boosted lopinavir (LPV/r), or nevirapine (NVP)).20 Postpartum randomization arm B entailed daily infant NVP only. We reported a homogenous risk of stunting across antepartum exposures (non-cART (ZDV only), versus cART (arms B and C), and similarly when we restricted the cART group to arm B (ZDV based cART). The sample size in arm C was not adequate to explore ZDV-based versus non-ZDV based ART regimens during the antepartum period. Second, we reported increased risk of stunting but not underweight, or wasting. Since stunting is a biomarker of chronic growth impairment, while underweight and wasting reflect more acute or recent
nutritional deficits, respectively, we hypothesize that the observed stunting risk was likely a result of earlier (in-utero or early infancy) maternal-HIV and/or ART exposures, rather than cumulative or recent postpartum exposures. We also demonstrated that about 20% of the observed stunting risk at 12 and 24 months-of-age, respectively, was mediated by infant birth-weight, consistent with previous studies linking childhood stunting and small-for-gestational-age. However, the lack of birth-length data on control children precluded a more appropriate linear growth mediation assessment. Nonetheless, these observations collectively suggest that the biologically relevant exposure time-period was in-utero, possibly driven primarily by ZDV exposures, with no evidence to suggest that cumulative growth impairment occurred due to additional/prolonged postpartum exposures.

It is also noteworthy that increased risk of stunting among exposed versus control children reported in our study was not correlated with risk of ID hospitalizations or deaths, although stunting and underweight have been associated with childhood ID severity, and deaths. Routine ART safety and clinical monitoring during the PROMISE RCT prior to entry in our study, and strict adherence to standard-of-care in both studies, potentially contributed to the observed comparable health among exposed versus control children. Prior to study entry (during PROMISE RCT follow-up), exposed participants were routinely administered standard-of-care during pregnancy (vaccinations, malaria intermittent preventive treatment (IPT), treated mosquito-nets, as well as hematinic and anti-helminth prophylaxis); and postpartum-care (maternal reproductive-health services, health-education (infant-feeding), infant immunization; co-trimoxazole prophylaxis; growth monitoring; and anti-helminth prophylaxis). Also, as an ART safety monitoring strategy during the PROMISE RCT follow-up, exposed-group mothers and their infants had routine complete blood count monitoring and were administered hematinic medicines as clinically indicated.
However, this study had some limitations. Independent effects of maternal-HIV, or ART exposures on morbidity outcomes could not be isolated in our study. To isolate the effects of ART exposure, the appropriate comparison group that simulates the counterfactual population for the exposed (maternal-HIV and ART) children, entails HEU children with no ART exposures, and with otherwise exchangeable outcomes under homogenous exposures. However, as ART-based standard-of-care in these breastfeeding populations has slowly evolved over the last 10 years to the current recommended cART-for-life initiated among HIV-infected pregnant women, a non-ART PMTCT strategy study-design would be unethical. The ART randomization-scheme used in the PROMISE RCT was a strategic design to reflect the contemporaneous WHO PMTCT guidelines, option-A, and option-B. In any case, our study design addresses the more pertinent question of pediatric HIV-free survival among ART-exposed HEU children, in the current context of maternal-cART for life. Both Malawi and Uganda are part of the 22 UNAIDS global plan priority (GPP) countries targeted for rapid-scale up of ART based programs. All the GPP countries but India are located in sub-Saharan Africa, home to more than 90% of all pregnant women living with HIV globally. By 2015, more than 95% of HIV-infected women in Uganda, and 80% in Malawi, were on cART during the vertical transmission risk period, and beyond, for life. It is projected that more than 90% of 1.5 million incident ART-exposed HEU children worldwide per year, by 2020, will be in sub-Saharan Africa.

Inherent to observational studies as ours is the possibility of residual-confounding. To mitigate this bias, we restricted and matched on strong potential confounders at study design level; and used multivariate regression techniques at analysis to adjust for additional covariates. Specifically, to minimize heterogeneity by socio-economic characteristics, we purposefully and systematically identified eligible controls through child-well clinics within the same hospital complexes that served as the source for the PROMISE RCT participants; and age (+/- 4months) and gender-matched controls were enrolled. We also excluded children with pre-existing severe
clinical conditions. Analyses were stratified by site, and considered the most parsimonious multivariate model that included \textit{a-priori} defined covariates, plus any variables with a \textit{p}-value $\geq 0.10$ in univariate analysis with the outcomes, respectively. All multivariate models included duration of antepartum ART exposure, breastfeeding status, and household water-source. In addition, maternal-age and household electricity-use were included in the Malawi site models; and household income-index in the Ugandan site models. Maternal viral load a strong predictor of HIV-specific immune activation among HEU children, based on a recent Kenyan cohort study,\textsuperscript{29} was homogeneous across cART and non-cART categories in our study, at 12 and 24 months after delivery, respectively.

Other threats to internal validity include potential selection and misclassification biases. Sampling of HEU participants from a controlled clinical trial setting where standard-of-care was always met, and participants had frequent clinical reviews as part of safety monitoring, potentially selected healthier HEU children into our study compared to HEU children in the general population pool in these settings. The differential selection of healthier HEU but not HUU children into the study, potentially resulted in underestimation of association of exposures (maternal-HIV and ART) with study outcomes. In addition, since majority (67\%) of the under-five child mortality in these high ID-burden settings, occurs before the first birthday, and about 45\% during the neonatal period, mortality differences across HEU and HUU source populations potentially induced a survival-bias in our study.\textsuperscript{33} Also, since we classified ART exposures according to the PROMISE trial randomization schemes, misclassification bias may have resulted from post-randomization non-compliance; clinician-induced drug switches due to toxicities, or maternal indication for cART; malabsorption syndrome; or drug-interactions ultimately affecting bioavailability of the ARV drugs as randomized. Apart from maternal indication for switching to cART, the other factors would have operated non-differentially across cART versus non-cART categories, ultimately biasing the results towards the null. We expect the magnitude of the bias if
any was minimal, since according to maternal report, very high (>95%) drug-adherence was maintained through follow-up, across the PROMISE RCT sites. Ecological factors during study follow-up such as the devastating 2014/2015 Malawian floods potentially resulted in biased estimates. Flood-exposed populations are associated with chronic malnutrition and increased levels of stunting, and homogenous effects across exposed and control-groups, potentially led to attenuation of measures of association.

A key strength of our study design is the use of laboratory diagnostic evidence to confirm maternal and child HIV-infection status, which guaranteed appropriate HEU and HUU classification throughout study follow-up. Similarly, we used standardized procedures to measure and classify outcome-status across exposed versus control groups. Extreme growth-faltering (stunting, underweight, or wasting) parameters were classified as 2 or more standard deviations below the median of the WHO Growth standards (2006); and small head-circumference as measures below the WHO population median. However, since the current WHO population standards are based on a pooled sample from six countries (Brazil, Ghana, India, Norway, Oman and the USA) that participated in the multicenter growth reference study (MGRS), there was a potential for misclassification of Malawian or Ugandan children. Also, classification of hematological measures for clinical management during follow-up, and subsequently for these analyses, was based on NIH Division of AIDS (DAIDS) toxicity tables (version 1.0, Aug 2009). However, since the DAIDS tables were based on non-African populations in the United States, we likely overestimated the risk of hematological sequelae in this predominantly black-African study population. Lower baseline normal hematological parameters have been described among pediatric populations of African origin. A standardized questionnaire was used to assess prior hospitalizations or deaths. Overall, any biases due to outcome-misclassification were potentially minimal, and non-differential by exposure category, which likely resulting in attenuated measures of association. In a sensitivity analyses of
hematological outcomes based on recently modified DAIDS toxicity tables (version 2.0, December 2014), while lower rates of anemia were observed, the measure of association were similar to estimates based on the version 1.0 DAIDS tables. Another strength of our study is the high level of data completeness as well as very high retention rates. Missing data ranged from 0.0% to 3.0% for key variables including exposure; outcomes (anthropometric; hematological; hospitalizations/death); and key covariates (infant-age; gender; breastfeeding; birthweight; maternal-age; electricity use and water-source). Missing data was independent of key variables (outcome, exposures, and covariates) in our analyses, and we conducted complete case analyses assuming missing completely at random (MCAR). Collectively, the potential biases were likely minimal, and resulted in underestimated measures of association.

Despite these limitations, our research findings are informative and timely given the current guidelines to initiate all pregnant women on life-long cART, and the ongoing drive for scale-up of cART based PMTCT programs. It is likely that these research findings are generalizable to other breastfeeding populations in Malawi, Uganda, and elsewhere within the GPP sub-Saharan African countries. To the best of our knowledge, no prior study assessed morbidity and mortality outcomes through 24 months-of-age among HEU children with a history of in-utero and prolonged postpartum ART exposures beyond 12-18 months-of-age. These results suggest that breastfed HEU children with perinatal exposures to prophylactic ARV drugs remain a vulnerable subgroup that should be prioritized for the overall PMTCT goal of pediatric HIV-free survival to be achieved. Our findings suggestive of reversal of early infancy complications attributed to in-utero exposures to maternal HIV and ART, in the context of ongoing and prolonged postpartum exposures to maternal-HIV and cART, is reassuring given the current WHO guidelines in these settings to initiate HIV-infected pregnant women on lifelong-ART, coupled with recommendations to breastfeed their children through 24 months-of-age. Ongoing expansion of ART services in these settings should be coupled with effective delivery of pediatric standard-of-
care to achieve and maintain health-equilibrium among HEU and HUU children. In addition, anthropometric monitoring is critical for adequate child health care in these settings. Further research is needed to better understand the role of antepartum exposures to ZDV as well as newer cART regimens on childhood growth, with longer follow-up beyond 24 months-of-age.
4.2 REFERENCES


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CURRICULUM VITAE

Jim Aizire, MD, PhD

A. PERSONAL DATA
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B. EDUCATION AND TRAINING

2013-2017, Doctor of Philosophy (PhD), Johns Hopkins University, MD, USA
Field of study: Epidemiology of Infectious diseases. Thesis: “In-utero and postpartum exposure to antiretroviral drugs: long term complications related to physical growth and hematological outcomes among HIV exposed uninfected children”.

2001-2003, Masters in Health Science (MHS), Johns Hopkins, Baltimore, MD, USA
Field of study: Epidemiology of Infectious diseases, with a focus on HIV/AIDS and other Sexually Transmitted Infections (STIs). Thesis: “Epidemiology of Gonorrhea and Chlamydia in a rural based population in Rakai District, Uganda, with a background of high prevalence of HIV/AIDS”.

1994-1999, Bachelors of Medicine & Surgery (MBChB) Makerere University, Uganda
Field of study: Clinical disciplines: Internal Medicine; Surgery; Pediatrics; Obstetrics & Gynecology; Psychiatry and Public health (epidemiology & biostatistics); and basic sciences: Physiology; Anatomy; Biochemistry; Psychology and Sociology.

Medical Licensure and Certification: Uganda Medical and Dental Practitioners Council

C. PROFESSIONAL EXPERIENCE

Medical Experience

2004 – 2013 Physician, and Clinical Trial Adverse Event reviewer, Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration, Kampala, Uganda

2000 – 2001 Medical Officer Mengo Hospital & CASE Medical Center, Kampala, Uganda. Clinical management (Obstetrics & Gynecology, Pediatrics, Surgery, and medicine), and Continuous Medical Education (CME) coordinator.
1999 – 2000 Medical Intern Mulago National Referral Hospital, Kampala, Uganda
Role: Out-patient and in-patient clinical management including Obstetrics and Gynecology, Pediatrics, Surgery, and Internal medicine.

Research Experience
2013 – Present, Co-Investigator, H²U Consortium, Johns Hopkins Medical Institute
2012 – Present, Co-Investigator, NEURODEV study, Johns Hopkins Medical Institute
2009 – Present, Co-Investigator, PROMISE Trials, Johns Hopkins Medical Institute
2009 – Present, Co-Investigator, P1084s study, Johns Hopkins Medical Institute
2009 – 2013, Site Co-Investigator, HPTN 046 study, MUJHU CRS, Kampala, Uganda
2004 – 2009, Study Coordinator, HPTN 046 Study, MUJHU CRS, Kampala, Uganda
2009 – 2012, Principal Investigator, ‘Nevirapine and Cotrimoxazole Safety’ study
2004 – 2005, Adverse Events Coordinator MU-JHU Research Kampala, Uganda
2009 – 2012, Principal Investigator, ‘Nevirapine and Cotrimoxazole Safety’ study
2004 – 2005, Site Co-Investigator, HPTN 046 study, MUJHU CRS, Kampala, Uganda
2009 – 2013, Study Coordinator, HPTN 046 Study, MUJHU CRS, Kampala, Uganda
2004 – 2009, Site Co-Investigator, HPTN 046 study, MUJHU CRS, Kampala, Uganda

Teaching Experience
2014 – 2016, Teaching Assistant (TA), Johns Hopkins Bloomberg SPH, USA

• Lead TA (2015/16) – As the Dr. Abe Lillenfeld Fellow (2015/16), I was the lead TA for the core epidemiology methods courses (EPI 752, EPI 753 and EPI 754) in the department of epidemiology. I supported the course instructors in leading a team of more than a dozen lab instructors and Teaching Assistants. I was involved with the preparation of teaching, quiz, and exam materials; grading exams, conducting review and lab sessions, and maintaining the online discussion forum. The course enrollments are more than 250 students per quarter.

• TA (2014/15) – I was a TA for the core epidemiology courses (Epi 751-753, each with more than 250 student enrolment per quarter), and the ‘Outbreak Investigation’ course (~80 students enrolled per quarter). I prepared course materials, conducted review lectures, tutorials, and graded transcripts.

2011-to-2013, Lecturer, Makerere University SPH, Kampala, Uganda
2004-to-2005

• Prepared course materials and exams; conducted class review and tutorial sessions; graded transcripts; support supervision of master level students including dissertations.

• I was part of the planning committee for the long-distance Masters of Public Health (MPH) degree program at the School of Public Health.

2002-to-2004 Teaching Assistant, Johns Hopkins Bloomberg SPH, MD, USA

• ‘Infectious Disease Epidemiology’ Nov/Dec 2002.
• ‘Natural History and Epidemiology of Human Viral Infections’ Jan/Feb 2003.
• I conducted review sessions, contributed to exams process including grading.
D. PROFESSIONAL ACTIVITIES

Membership in Professional Associations

2016 – Present  Member, American College of Epidemiologists (ACE)
2016 – Present  Member, International AIDS Society (IAS)
2014 – Present  Member, U.S. Pediatric HIV/AIDS Cohort (PHACS) group
2010 – Present  Member, Scientific Committee, National Pediatric HIV/AIDS Conference
2009 – 2013  Member, PMTCT Scientific Committee, International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network
2004 – Present  Member, International Maternal Pediatric AIDS Clinical Trials (IMPAACT) Group
2004 – Present  Member, HIV Prevention Trials Network (HPTN)
2003 – Present  Member, Uganda Society of Health Scientists (USHS)

E. EDITORIAL ACTIVITIES

Peer Review Activities for selected international peer reviewed journals

1. The AIDS Journal
2. The Journal of General Internal Medicine (JGIM)
3. The Pediatric Infectious Disease Journal (PIDJ)
4. The British Medical Journal (BMJ-Open)
5. The Springer Journal
6. The Annals of Infectious Disease and Epidemiology
7. The Pediatric and Perinatal Epidemiology Journal

Editorial Board Membership/Ad Hoc Review of Proposals

NIH IMPAACT, PMTCT Scientific Committee member

As a member of the PMTCT Scientific committee, I reviewed research proposals by other peers. The IMPAACT network funded by the US National Institutes of Health (NIH) is a multimillion dollar research funding institution through which multidisciplinary collaborative research proposals with the highest scientific merit and potential public health impact, in accordance with the NIH research agenda, are supported. This is a highly selective two-tier review process composed of Scientific Committees followed by the Scientific Leadership Group. New studies may be proposed by IMPAACT investigators, by external investigators or may be commissioned by the Scientific Leadership Group. Scientific Committee members provide initial review and prioritization of proposals as well as input into scientific priorities for IMPAACT research.

Uganda National Pediatric HIV/AIDS Conference (NPHC), Scientific Committee member

I provide leadership to the Scientific Committee in setting priorities for the NPHC. In addition, I have played critical roles in reviewing submitted abstracts as well as a moderating and/or chairing multiple sessions during the conference. The NPHC is an annual meeting in
Kampala, Uganda that brings together both local and international experts engaged in pediatric HIV/AIDS research and care.

F. HONORS AND AWARDS

Research Awards

2015  ‘Dr. Charles Armstrong Award’, Johns Hopkins Bloomberg School of Public Health (JHBSPH), Baltimore, MD

2015  ‘Dr. Charlotte Ferencz Award’ JHBSPH, Baltimore, MD

2014  ‘Dr. Robert Dyar Award’ JHBSPH, Baltimore, MD

2013  ‘International Scholarship Award’, 20th Conference on Retroviruses & Opportunistic Infections (CROI), Atlanta, Georgia

2009  ‘Early Investigator Award’, the NIH, IMPAACT Network, Washington D.C

2008  ‘International Scholarship Award’, 15th CROI, Boston, Massachusetts

2001  ‘NIH Fogarty International Fellow’, AIDS International Training and Research Program, JHBSPH, Baltimore, MD

Academic Awards

2015  ‘Teaching Assistant (TA) Recognition Award’- 2015/16, JHBSPH, Baltimore, MD

2015  ‘Dr. Abraham Lilienfeld TA Fellowship’ - 2015/16, JHBSPH, Baltimore, MD

2008  ‘International Scholarship Award’, 15th CROI, Boston, Massachusetts

2001  ‘Early Investigator Award’, the NIH, IMPAACT Network, Washington D.C

2000  Outstanding Intern-Doctor (Honors), Department of Obstetrics & Gynecology, Mulago National Referral hospital, Kampala, Uganda

1994  Full Government of Uganda scholarship, Makerere University Medical School, Kampala, Uganda

G. PUBLICATIONS

Journal articles (peer reviewed)


7. Gray R; **Aizire J**; Serwadda D; Kiwanuka N; Kigozi G; Kiddugavu M; Nalugoda F; Li X; Wawer M. Male circumcision and the risk of sexually transmitted infections with HIV in Rakai, Uganda. *AIDS*. 2004 Dec 3;18(18):2428-30

**Journal articles (Collaborative Networks)**


H. RESEARCH GRANT PARTICIPATION

**Ongoing research:**

1. **5-U54-CA190165** (Kirk, GD) **09/19/2014 – 08/31/2019**
   - **Funder:** US National Cancer Institute (NCI), and Fogarty International Center (FIC)
   - **Funding so far:** $1,499,799 (Fiscal Years 09/19/2015 – 08/31/2016)
   - **Project title:** ‘HIV and Hepatocellular Carcinoma in Uganda: The H2U CONSORTIUM’.
   - The H2U consortium is a collaborative partnership between Makerere University and Johns Hopkins on HIV and hepatocellular carcinoma (HCC).
   - **specific roles:**

2. **5-R01-HD073296** (Boivin, M and Fowler, MG) **08/01/2012 – 07/31/2017**
   - **Funder:** US Eunice Kennedy Shriver National Institute of Child Health & Human development
   - **Funding so far:** $1,876,322 (Fiscal Years 08/01/2012 – 07/31/2016)
   - **Project title:** ‘Developmental and Growth outcomes for antiretroviral drug exposed HIV uninfected children’
   - **Awardee organization:** Johns Hopkins University
   - **Specific roles:**
     - a. Co-Investigator, 08/2012-to-date
     - b. MUJHU Site Study Investigator-of-Record, 08/2012-08/2013
     - c. Project Coordinator across sites (Malawi and Uganda), 09/2013-to-date

3. **UM1-A1069530** (Fowler, MG; Jackson, JB and Guay AL) **02/05/2007 – 11/30/2020**
   - **Funder:** US National Institute of Allergy & infectious Diseases
   - **Funding so far:** $34,858,088 (Fiscal Years: 02/05/2007 – 11/30/2015)
   - **Project title:** ‘Johns Hopkins University Kampala-Nanning Clinical Trials Unit’. The Johns Hopkins (JHU) Clinical Trial Unit (CTU) has three affiliated Clinical Research sites (CRS), two in Kampala, Uganda, and one in Nanning, China. The CRSs are conducting high priority HIV Prevention and Therapeutic clinical trials within three of the current United States NIH HIV Clinical Trial Networks.
   - **Awardee organization:** Johns Hopkins University
   - **Specific roles:**
     - a. Co-Investigator (data analysis & publication), PROMISE and P1084s protocols: ongoing
     - b. MUJHU CRS Study Co-Investigator-of-Record, PROMISE (P1077BF) protocol: 2009-2013
     - c. MUJHU CRS Study Principal Investigator-of-Record, P1084s protocol: 2009-2013
Completed Research Support

1. **U01-AI048054** (Guay, LA) 07/01/2000 – 12/31/2007
   - **Funder**: US National institute of Allergy & infectious Diseases
   - **Funding total**: $14,448,627 (Fiscal Years: 07/01/2000-12/31/2007)
   - **Project title**: ‘MU-JHU HPTU TRIAL SITE-UGANDA’. The MUJHU CRS was established to conduct high priority HIV Prevention and Therapeutic clinical trials within United States NIH HIV Prevention Trials Network (HPTN).
   - **Awardee organization**: Johns Hopkins University
   - **Specific roles**:
     a. MUJHU CRS Study Co-Investigator-of-Record, HPTN 046 protocol: 2009-2013
     b. MUJHU CRS Study Coordinator, HPTN 046 protocol: 2004-2009
     c. Co-Investigator (data analysis & publication), HIVNET 012 Trial: 2006-2008
     d. Physician Adverse Events Coordinator, SWEN Trial: 2004-2006

2. **U01-AI068632 05** (Jackson BJ) 06/01/2007 – 08/31/2014
   - **Funder**: US National institute of Allergy & infectious Diseases
   - **Project title**: International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Group
   - The IMPAACT Network was established to conduct high priority HIV Prevention and Therapeutic clinical trials within United States and Internationally.
     - **Sub-award** (Aizire J) 04/15/2009 – 06/15/2010
     - **Project title**: ‘IMPAACT New Investigator Award’. The goal was to identify young outstanding researchers within the IMPAACT network, and support them to conduct primary research and support through mentoring (pairing with a senior IMPAACT network researcher)
     - **Role**: Principal Investigator, ‘Safety of extended use of nevirapine and cotrimoxazole among HIV exposed uninfected children’ study.

I. PRESENTATIONS

**Oral presentations**

1. 5th National Pediatric HIV and AIDS Conference, Kampala, Uganda, 2011
2. 18th Conference on Retroviruses and Opportunistic Infections (CROI), Boston, Massachusetts, 2011
3. 4th National Pediatric HIV and AIDS Conference, Kampala, Uganda, 2010
4. The 2nd International Workshop on HIV Pediatrics, Vienna, Austria, 2010
5. The XVIII International AIDS Conference, Vienna, Austria, 2010
6. 8th Annual Scientific Conference, Uganda Society of Health Scientists (USHS), Kampala, Uganda, 2007

**Poster presentations**

1. 7th International Workshop on HIV Pediatrics, Durban, South Africa, 2016 (3 presentations)
2. 21st International AIDS Conference, Durban, South Africa, 2016
3. 20th Conference on Retroviruses and Opportunistic Infections (CROI), Atlanta, Georgia, 2013 (2 presentations)
4. 3rd International Workshop on HIV Pediatrics, Rome, Italy, 2011
5. 6th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention, Rome, Italy, 2011
6. XVIII International AIDS Conference, Vienna, Austria, 2010
7. 17th Conference on Retroviruses and Opportunistic Infections (CROI), San Francisco, California, 2010
8. 15th Conference on Retroviruses and Opportunistic Infections (CROI), Boston, Massachusetts, 2008

J. ADDITIONAL INFORMATION

Personal statement
I am committed to improving public health through advancing medical and infectious diseases research, and teaching epidemiology. I have background training, and accumulated leadership and research experiences necessary to pursue a successful career of innovative and impactful research. I have more than 14 years of conducting high level research internationally. I served as a Project Coordinator, Co-investigator and Principal Investigator on several international Johns Hopkins HIV/AIDS research projects funded by the US National Institutes of Health (NIH). These were mostly multicenter Prevention of Mother-to-Child Transmission (PMTCT) of HIV antiretroviral (ARV) drug clinical trials, conducted at international sites in several countries in Africa, the Americas and in India. My research work has had a major influence on the field, and is widely published in high impact peer reviewed journals. My research has received recognition by international professional peers notably the 'Early Investigator Award' through the NIH funded International Maternal Pediatric Adolescent Clinical Trials (IMPAACT) network. I have contributed extensively to teaching epidemiology at the highest level including at Johns Hopkins University in Baltimore, Maryland, USA, and Makerere University, in Kampala, Uganda. I have been consulted on several occasions, notably, I was invited by the François-Xavier Bagnoud (FXB) as an expert on protocol training and pre-implementation research capacity building: BJ Medical College, in Pune, India (August 2008) and the Kilimanjaro Christian Medical Centre, Moshi, Tanzania (November 2008). My current research is exploring pediatric HIV-free survival by assessing long term complications associated with in-utero and postpartum exposure to antiretroviral drugs among HIV exposed but uninfected children. My other research activities include liver cancer risk among HIV and hepatitis B virus (HBV) co-infected adults.

Key words
PMTCT (prevention of mother-to-child transmission of HIV); antiretroviral therapy; maternal and child health; infectious diseases; HIV