HEU outcomes: population-evaluation and screening strategies (HOPE)

Study protocol:

Principal investigator: Grace John-Stewart
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1. List of abbreviations

ABR	Auditory Brain Responses
AIDS	Acquired Immunodeficiency Syndrome
ASD	Autism Spectrum Disorders
ART	Antiretroviral Therapy
CHW	Community Health Worker
CCC	Comprehensive care clinic
DBS	Dried Blood Spot
EMR	Electronic Medical Records
ERC	Ethical Review Committee
FP	Family Planning
HCW	Health Care Worker
HEU	HIV Exposed Uninfected
HIV	Human Immunodeficiency Virus
HUU	HIV Unexposed Uninfected
IRB	Institutional Review Board
KNH	Kenyatta National Hospital
M-CHAT-RF	Modified Checklist for Autism in Toddlers, Revised and with follow-up
MCH	Maternal and Child Health
MDAT	Malawi Developmental Assessment Tool
MLHIV	Mothers Living with HIV
MoH	Ministry of Health
NASCOP	National AIDS and STI Control Program
OAE	Otoacoustic Emission
US	United States
UW	University of Washington
WHO	World Health Organization

1. **Title:** HEU outcomes: population-evaluation and screening strategies (HOPE)

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6. Executive summary

Design

This study will provide rigorous epidemiologic evidence regarding the influence of fetal exposure to HIV and ART on developmental and mental health outcomes from infancy to adolescence using the following study designs: Longitudinal cohort study, cross sectional assessments and costing and implementation surveys. Accrued data will be disseminated in a stakeholder workshop conducted in the last year of the study.

To do this we will enroll a longitudinal cohort of HIV exposed uninfected (HEU) and HIV uninfected (HUU) infants and follow them up 6 monthly with extended follow-up to 4 years. Assessments will include growth, hearing and neurodevelopment (Aim 1a) and telomere length (Aim 1b).

In Aim 2, we will utilize a cross-sectional study design to pilot test mobile screening strategies to detect neurodevelopmental and mental health outcomes in HEU (Aim 2a). Pilot results will inform the implementation of a larger population based cross-sectional assessment of HEU outcomes (Aim 2b).

In aim 3, we will estimate the cost of the screening strategies and convene a stakeholder workshop to review data regarding programmatic integration of HEU screening.

Population

Aim 1: HEU and HUU infants age 6 weeks and their mothers
Aim 2: HEU and HUU children and adolescents age 3-18 years and their caregivers
Aim 3: Abstracted records of cost information, Stakeholders in pediatric and adolescent health, health care workers (HCW), policy makers

Sample size

Aim 1: 2000 mother-infant pairs, 1000 HEU and 1000 HUU
Aim 2: Pilot phase: 200 HEU and 200 HUU and their caregivers, Population based assessments: 4000 caregiver-child HEU pairs, 400 caregiver-child HUU pairs
Aim 3: 25-50 HCW, policy makers and stakeholders in pediatric health

Study sites:

Aim 1: 5 public sector maternal and child health (MCH) clinics, 2 in urban Nairobi (Mathare, Kenyatta National Hospital) and 3 rural MCH clinic sites (Homa Bay, Kisumu, and Rachuonyo).
Aim 2: Pilot phase: Rachuonyo sub-county hospital. Population based assessments: 100 large HIV care clinics throughout Kenya
Aim 3: Workshop will be concerned in Nairobi/Western Kenya

Study Duration:

5 Years

8. Background

Maternal HIV and antiretroviral exposure may be potential fetal determinants of mental health

Fetal exposure to infection or medications can result in susceptibility to long-term mental illness. Several studies have suggested fetal origins for schizophrenia following influenza in maternal pregnancy[1]. There is evidence from a meta-analysis of 15 studies involving over 40,000 cases of autism spectrum disorder (ASD) that ASD risk is increased in offspring with prenatal exposure to maternal infections, evidence that is supported by similar findings in mouse and macaque models[1]. A recent study from Sweden using medical record data from over 1.5 million individuals observed increased risk of ASD, depression and suicide among adults with whose mother was hospitalized for *any infection* during pregnancy[2]. Mechanisms speculated to explain fetal origins of mental illness include direct neuronal injury, placental inflammation, and dysregulation of placental serotonin secretion (reviewed in Al-Haddad 2019, Figure 1)[1]. In addition to maternal infections, fetal exposure to <u>medications</u> may also influence mental health outcomes. For example, recent evidence suggests that acetaminophen use in pregnancy may be associated with increased risk of autism and ADHD [3,4,5] <u>It is therefore plausible that fetal HIV or ART exposure could similarly affect neurodevelopment and risk of mental illness</u>. HIV infection has prominent effects on the central nervous system (CNS) with infection of CNS macrophages and microglial cells, resulting in HIV-associated neurocognitive disorder (HAND)[6,7]. Early studies detected HIV DNA in fetal neural cells[8] and untreated congenital HIV infection has been associated with severe encephalopathy[9]. There is evidence (Table 1) that fetal HIV exposure influences neurodevelopment in HIV-exposed uninfected (HEU) children this and could compromise long-term mental health outcomes.

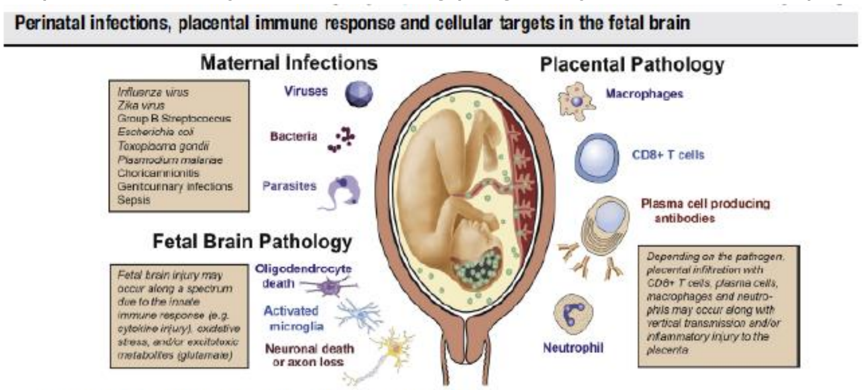

Figure 1 from Al-Haddad Am J Obstet Gyn 2019

Neurodevelopment in HEU may be influenced by maternal viremia, ART, depression, and fetal growth

Several studies both in the pre-ART and post-ART era have observed cognitive and language delays in HEU[27]. A meta-analysis of 11 studies showed lower cognitive and motor scores in HEU compared to HUU. Studies have noted persistent, lower, or no difference in HEU versus HUU neurodevelopmental comparisons in the post-ART than in the pre-ART era[12]. Cumulative maternal viremia in pregnancy was associated with motor and expressive delays but not cognition among HEU in South Africa, whose mothers all received Option B+ regimens [28]. However, in a recent smaller cohort from South Africa, there was no evidence of neurodevelopmental differences between HEU and HUU[29,30]. White matter changes have been seen in HEU infants in brain imaging studies[31,32]. <u>Maternal **stress** and **depression** during pregnancy</u> have also been associated with fetal origins of mental health outcomes in the general population[33] and are prevalent among women living with HIV. In a study of maternal-child dyads in South Africa half of mothers reported depression during pregnancy and maternal prenatal depression predicted delayed gross motor development in HEU[34]. Other studies have noted

Table 1. Selected studies of neurodevelopment in HEU

Author, Year	Location	Findings
LeDoare, 2012[10]	Review	Speech and language delay
Sherr, 2014[11]	Systematic review	Lower cognitive performance
McHenry, 2018[12]	Systematic review and meta-analysis	Lower cognitive and motor skills
Boivin 2019[13]	5 African countries	No difference
Nicholson 2015[14]	Zambia	Poorer academic performance
Kerr 2014[15]	Thai/Cambodia	Lower IQ, language, fine motor
Nozyce 2014[16]	US	No ARV effects on ND outcomes
Rice 2016[17-21]	US	Language impairments
Chaudry 2017[22,23]	Botswana	Expressive language delay
Le Roux 2018[24]	South Africa	Motor and cognitive delay
Chadna 2019[25]	Zimbabwe	Language and motor delay
Wedderburn 2019[26]	South Africa	Expressive language delay

associations between postnatal maternal depression and HEU cognitive and executive function[35,36]. A recent study from Sweden which assessed over 500,000 sibling pairs found that higher **birthweight** was associated with significantly lower risk of depression, PTSD, ADHD, anxiety and ASD, after controlling for familial confounders[37]. There is consistent evidence of lower birthweight and poorer growth in HEU compared to HUU[38,39]. A recent study from Denmark with 485 HEU and 2,495 HUU observed significant growth differences between the groups at birth but differences decreased by 5 years of age[40]. Growth trajectories in HEU may be less compromised among those whose receive Option B+ vs. earlier or no PMTCT regimens, and may transition from underweight to overweight during infancy[41,42, 43]. Using a fetal origins perspective, it is plausible that HEU may have poorer neurodevelopmental and mental health outcomes via fetal exposure to HIV, ART, poor fetal growth, or maternal stress or depression in pregnancy.

Option B+ PMTCT regimen switch from EFZ to DTG may influence HEU outcomes

In 2019, guidelines for ART changed from EFZ-backbone to DTG-backbone regimens, including during pregnancy. Psychiatric symptoms including insomnia, anxiety, depression and suicidality occur with both EFZ and DTG, generally at low prevalence with infrequent discontinuation; since these psychiatric symptoms are markedly increased with HIV infection it is difficult to attribute drug-effects[44]. EFZ was associated with increased suicidal ideation at 12 months postpartum in a cohort of South African women[45]. There is additional evidence that EFZ is associated with mild-moderate neurocognitive impairment, although mechanisms are unclear[46]. Discontinuing EFZ improved sleep quality in a recent randomized trial[47]. DTG administration in pregnancy results in high fetal exposure[48] and periconceptional DTG was associated with a slightly increased risk of neural tube defects in a large study of 119,033 infants in Botswana[49-51]. In a US multi-site study of 3,747 HEU in the SMART (Surveillance Monitoring for ART Toxicity) cohort, fetal EFZ exposure was associated with increased neurologic conditions (microcephaly, seizures, and eye abnormalities) (Crowell *IDWeek* 2018). There have not been studies to determine long-term outcomes of EFZ and DTG on HEU neuropsychiatric outcomes.

Increased mortality and morbidity in HEU suggest impact of fetal HIV/ART exposure in diverse domains

A meta-analysis of 22 studies of 8,840 HEU and 20,372 HUU demonstrated 70% increased mortality in HEU exposed to untreated maternal HIV; mortality risk in HEU versus HUU declined in magnitude but remained elevated following expansion of PMTCT[52]. Another meta-analysis of 12 studies involving 5,074 HEU and 12,881 HUU estimated 20% and 30% increase risk of pneumonia and diarrhea in HEU, respectively[53]. In a US study involving 2,404 HEU children and data from over 3.6 million HUU children in the Medicaid Analytic Extract database, HEU children had ~2-fold increased rate of hospitalization[54]. HEU differ from HUU in gut microbiome, immune activation, and inflammatory markers, which in turn may alter growth or infectious morbidity[55-58]. HEU have recently been shown to have poorer lung function than HUU[59]. These data suggest that common mechanisms following fetal HIV/ART exposure could drive diverse long-term outcomes in HEU, including mental health outcomes[60].

HEU have risk of poor mental health outcomes

There are limited data regarding mental health outcomes in HEU. Pediatric HIV (PHIV) infection has been associated with increased anxiety, attention deficits, impulsivity, hyperactivity and depression in high- and middle-income settings. Some studies have noted high prevalence of behavioral problems, often with similar prevalence in HEU and PHIV[61]. In a comparison of PHIV, HEU and HUU from Uganda, children aged 6-18 years old were assessed for psychosocial adjustment; HEU were noted to have significantly lower positive outlook and self-esteem than HUU[62]. Caregiver depressive symptoms were associated with child behavioral outcomes (poor executive function) among PHIV in a recent study[35]. Similarly, mental health outcomes in HEU may be influenced by caregiver interactions, complicating ascertainment of the effect of fetal HIV/ART exposure.

Implications of fetal origins of mental illness among HEU

Because HIV is an incurable disease with high risk of substantial morbidity and mortality, ART both for maternal health and prevention of infant HIV is required. Recent studies on DTG and neural tube defects and isoniazid prophylaxis in pregnancy illustrate the complexities of optimizing maternal/infant outcomes[49,50,63]. The time delay involved in recognizing mental health outcomes that may take 1-2 decades to be detected complicates investigations further. As ART regimens evolve, monitoring for HEU outcomes will remain important. Maternal HIV prevalence is >20-30% in some regions of sub Saharan Africa, which means that a substantial proportion of *all* children/adolescents have fetal exposure to HIV/ART, which may have long-term impact on developmental outcomes. Ideally, well conducted studies could provide reassuring data of limited risks from fetal HIV/ART exposure. Detecting epidemiologic signals for links between fetal HIV/ART exposure and mental health outcomes is important to inform our understanding of biologic or psychosocial determinants of adolescent mental health both among HEU and in general. If there are risks, regimens and psychosocial interventions could be tailored to decrease these risks as mechanisms are better understood and children at risk could be identified for early intervention.

9. Literature review

Parallel longitudinal cohorts and population-based cross-sectional groups combined in a virtual cohort will provide an innovative and efficient approach to evaluate HEU from infancy to adolescence, assessing short- and long-term outcomes. Our proposed cohorts span urban and rural settings and span HEU/HUU from 6 weeks to 18 years with adequately powered age strata to discern differences in mental health outcomes. This deep and broad assessment is novel and will provide distinct cohorts in which to demonstrate epidemiologic consistency in findings regarding HEU risk and cofactors. Parallel study designs provide complementary strengths – the longitudinal infant cohort with universal Option B+ coverage and homogeneous age at recruitment provides clear ascertainment of perinatal HIV/ART exposure and early outcomes with minimal bias; this cohort will provide timely data as DTG use expands and could be extended past the study period. The older cross-sectional cohort may reveal associations of fetal HIV/ART exposure with mental health outcomes that are not detectable in early childhood. Multiple cohorts strengthen causal inference – by including longitudinal and population-based surveys, short- and long-term outcomes, and nested case-control studies – we can strengthen causal inference by demonstrating *consistency* between studies.

The study will contribute novel data regarding potential fetal origins of mental illness in Africa. Epidemiologic studies exploring fetal origins of disease predominantly derive from Europe and the US, with scant data from Africa. Programmatic expansion of HIV services throughout Africa including electronic medical records can be leveraged to contribute insights to fetal origins and pregnancy surveillance research. The proposal aligns with a long-term national vision for coordination of data between different databases in the health system (ie., HIV EMR, DHS, MCH).

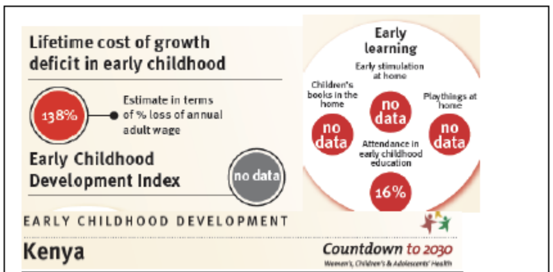

Figure 2. Paucity of data regarding early childhood development in Kenya (Kenya Countdown 2030, WHO)

The study will provide new data on child development and adolescent mental health in Africa. This study will add to the very limited available data on early child development and adolescent mental health outcomes in Kenya (Figure 2).

Engaging women attending HIV care is an efficient novel model to identify older HEU. We have previously screened >69,000 adults in clinics in Western Kenya and Nairobi to access children for pediatric HIV testing. Adults living with HIV have been willing to bring their children back to clinic for evaluation. Using HIV Care clinics to recruit HEU is an efficient and sustainable approach to monitor HEU.

The study will examine biomarkers for fetal HIV and ART exposure. Telomere length is a plausible biomarker for fetal inflammation, can be practically ascertained at scale, and could provide novel data to discern biologic impact of fetal exposure to HIV and ART.

Combining the study with active engagement of stakeholders can accelerate dissemination and impact. Engaging national and county-level policy makers, implementing partners, clinicians, and community-members throughout the study planning, execution, and dissemination process increases the likelihood of evidence being translated into action, with potentially sustained screening and early interventions in HEU, should there be evidence of increased risk or high disease burden.

Figure 3. Selected studies in MTCT and HEU

IDENTIFICATION	INFECTIONS	DEVELOPMENT	MECHANISMS	DATA
Counseling and Testing for Children at Home (CATCH) John-Stewart, Wamalwa, Njuguna, Wagner *(Royalty Research Fund Pilot; NIH R21; NIH F31)*	Maternal-infant viral transmission, Lehman (*Fred Hutchinson Cancer Research Center*); Effect of HIV exposure and infection on immunity to TB in children, John-Stewart (*NIH R01*)	Impact of HIV, immune activation, and ART on child neurodevelopment in Kenya (INK) Benki-Nugent *(NIH K01)*	Effects of human milk oligosaccharides and gut microbiome on growth and morbidity in HEU, McGrath (*NIH R01*)	Transition from pediatric to adult HIV care in Kenya, John-Stewart, Njuguna (*NIH R01*); CHIME-HEI, PHASE, John-Stewart, McGrath (*CDC*)
N>69,000 screened	N=640	N=280	N=400	N>28,000
Accessing HEU children through HIV+ adults in care efficiently	Longitudinal HEU & HUU cohort enrollment & retention	Detailed neurocognitive assessments on HEU & HUU ages 5-12	Growth among HEU in Kenya	Accessing paper-based and electronic medical record data, including VL, in large sample of clinics across Kenya

Preliminary data relevant to this application

Prior MTCT studies (Figure 3) led by our team yielded excellent retention and demonstrated substantial morbidity and growth compromise in HEU. Our collaborative research team has developed numerous longitudinal MTCT studies in Kenya over the past >25 years. While our emphasis was on understanding determinants of vertical HIV transmission, these cohorts have yielded important data suggesting that HEU have considerable risk of morbidity, mortality, poor growth and neurodevelopmental compromise. Our studies have involved follow-up of >8,000 mother-infant pairs, spanning varied PMTCT regimens and demonstrate our expertise in longitudinal studies of mother-infant pairs with high retention[64-103]. Three ongoing UW studies are following HEU and HUU infants in Kenya to compare virome, microbiome, TB infection, and breastmilk components. Our proposed study complements these studies and involves wider recruitment and less intensive research procedures than the molecular epidemiology studies to provide population-based estimates.

<u>Longitudinal studies suggest HEU have neurodevelopmental deficits when compared to HUU and demonstrate the team's expertise conducting a variety of neurodevelopmental assessments</u> Over the past 10 years, our team has conducted detailed studies on neurocognition in school-aged children. **To evaluate neurodevelopment in infants and young children, we trained clinical officers to administer the Malawi Developmental Assessment Tool (MDAT)[104]** at community health clinic and hospital study sites in Kenya. We found that following ART initiation, PHIV <5 years old had significant improvements in gross and fine motor domains, a trend for improvement in language, but no gain in social functioning (Table 2).[105] These data demonstrate our ability to conduct neurodevelopmental assessments in children. **Dr. Benki-Nugent has established capacity for cognitive assessments in Kenya.** As part of her K01 award, Dr. Benki-Nugent received guidance and training from Drs. Michael Boivin (MSU) and Paul Bangirana (Makerere University) to build capacity for cognitive assessments. The assessment battery included the Kauffman Assessment Battery for Children, 2nd ed. (KABC), the Test of Variables of Attention (TOVA), the Behavior Rating

Table 2. Mean baseline and 6-month change in developmental Z-scores in PHIV initiating ART

	Mean at baseline	Mean 6 mo change	P-value*
Gross Motor (N=46)	-1.02	0.36	0.03
Fine Motor (N=47)	-1.02	0.38	0.007
Social (N=46)	-0.55	0.13	0.4
Language (N=48)	-0.89	-0.19	0.06

*Paired T test, Malawian norm

Table 3. Differences in cognitive and motor ability for HEU (N=58) vs HUU (N=61) school-aged children

Neurocognitive domain (Assessment)	Adj. z-score difference
Cognition (KABC)	**-0.3***
Short-term memory (KABC)	**-0.4****
Processing speed (TOVA)	**-0.9****
Visual-spatial (KABC)	-0.2
Attention (TOVA)	-0.2
Executive functioning (BRIEF)	-0.3
Motor (BOT-MP)	**-0.3***

Note, HUU, HIV-unexposed; Raw scores standardized using US norm. *p ≤0.05; ** p≤0.005

Inventory of Executive Function (BRIEF) and the Buterinks Oseretsky Test of Motor Proficiency , 2nd ed. (Brief Form) (BOTMP)[106-108]. **We found significant deficits in HEU children in memory, processing speed and motor scores, compared with HUU children,** in analyses adjusted for socioeconomic indicators including caregiver education, household monthly rent and food security (Table 3). **These studies illustrate our expertise in neurodevelopmental assessments in Kenya, including with HEU and HUU**, **and demonstrate that HEU may have cognitive delays compared to HUU**.

Figure 4. CHIME study teams visited 141 clinics throughout Kenya

<u>Prior nationwide surveys using mobile teams involving HIV+ mothers and their children have been feasible in over 100 geographically dispersed HIV care or MCH clinics, with growth deficits noted in HEU:</u> In collaboration with HIV and MCH clinics, Ministry of Health, University of Nairobi, and CDC, our team has conducted 6 surveys using mobile teams to evaluate mother-infant pairs, women, or to abstract medical data from clinics throughout Kenya[109-117] (Table 4). In our ATTACH and PHASE studies, we obtained EMR data from ~100 clinics and merged this with VL data from the Kenya National Viral Load Database.

Table 4. National HIV/PMTCT surveys in Kenya conducted by our team

Study, publications	# clinics years	# surveyed	In-person survey	Data extracted
CHIME[109-112,114,115]	141 clinics 2013	2521 mat/inf pairs	Yes	No
CHIME-FP Chen (*submitted*)	108 clinics 2016	4805 WLHIV	Yes	No
Option B+	51 clinics 2018-19	1488 mat/inf pairs	Yes	Yes, EID
CHIME-HEI[116]	62 clinics 2014	9284 data	No	Yes, Registers
PHASE	102 clinics 2017	1492 data	No	Yes EMR, VL
ATTACH Njuguna (*submitted*)	99 clinics 2018	9921 data	No	Yes EMR, VL

A recent analysis from the CHIME cohort demonstrated significantly lower LAZ among HEU compared to HUU (Neary, *in preparation*). **These studies demonstrate that our teams are able to link with diverse clinics throughout Kenya, navigate regulatory permissions to access EMR and VL data, and use well-trained mobile nurses to examine women and children in efficient surveys (a week or month per clinic).**

<u>We have successfully identified HEU from HIV Comprehensive Care Clinics</u>: Counseling and testing for children at home (CATCH) (R21 John-Stewart, F31 Wagner). The CATCH study was conducted between 2013-2016 at 7 sites in Nairobi and western Kenya. We employed a systematic, efficient waiting room-based screening approach to identify HIV-exposed children and tested children either at a clinic or home[118]; overall, 5.8% of children tested were HIV positive, meaning most were HEU or born before maternal HIV infection (*Mugo, under review*). This index case testing approach was scaled up nationally and family tree cards were integrated into adult medical records, *enabling efficient identification of HEU* using standardized EMR tools. In a subsequent study in 19 clinics in 2017-18, 62% of adults in HIV care had all their children tested (*Njuguna & Wagner, under review*).

10. Rationale

This study will provide rigorous epidemiologic evidence regarding the influence of fetal exposure to HIV and ART on developmental and mental health outcomes from infancy to adolescence, including obtaining a national population-based estimate of HEU burden of developmental and mental health outcomes and strategies for programmatic surveillance and referral.

11. Specific aims and hypotheses

Aim 1: Within a longitudinal cohort of 1000 HEU and 1000 HUU infants enrolled at 6 weeks of age from 5 MCH clinics and followed 6-monthly:

Aim 1a. To compare HEU and HUU infants and children for growth, hearing, and neurodevelopmental outcomes; and determine influence of ART timing/regimen on HEU outcomes. To determine whether fetal HIV/ART exposure impacts 4-year neurodevelopmental outcomes; and assess impact of peripartum ART regimen and timing.

Aim 1b: To compare telomere length in HEU and HUU and determine associations with neurodevelopmental outcomes.

Aim 2a: To determine the impact of HEU and HUU status, as well as maternal peripartum ART, on neurodevelopment and mental health (ASD, ADHD, depression, and anxiety). We will pilot a mobile screening strategy to detect adverse neurodevelopmental and mental health outcomes in HEU for use in large-scale screening in Aim 2b. Mothers attending HIV care will be invited to bring their HEU (aged 3-18) (200 HEU, 50 per age-stratum: 3-6, 7-10, 11-14, 15-18) for screening; age- matched HUU will be recruited from the community.

Aim 2b. To estimate population-burden and cofactors of adverse neurodevelopmental and mental health outcomes in HEU using mobile teams to screen HEU in ~100 large HIV Care Clinics throughout Kenya (4000 HEU; age-stratified, 3-6, 7-10, 11-14, 15-18) and 400 age-stratum matched community HUU.

Aim 3. To estimate the cost of this screening strategy and convene stakeholders to review data regarding programmatic integration of HEU screening.

12. Study design and methodology
    a. Study area description

Kenyatta National Hospital & University of Nairobi: Study participants for aim 1 will be recruited, and followed up clinics in Nairobi and Western Kenya. Cross-sectional surveys (Aim 2) will be done in various sites across the country. Kenyatta National Hospital and University of Nairobi collaborators will participate in study design, data analysis and interpretation, and manuscript preparation. Specimens will be processed and stored at the University of Nairobi Pediatric Research Laboratory.

Population Justification: This study will be conducted in Kenyan children and adolescents age 6 weeks-18 years. HIV-exposed uninfected (HEU) populations are growing in Kenya, and there is need to understand health differences and the effect of maternal HIV status and treatment on long term outcomes.

b. Summary table

	Aim 1	**Aim 2**	**Aim 3**
Study design	Longitudinal cohort study	Cross-sectional assessments	Implementation surveys, stakeholder workshop
N	Aim 1: 2000 mother-infant pairs: 1000 HEU and 1000 HUU	Aim 2: 4800 children and adolescents and their caregivers	Aim 3: 25-50 HCW, policy makers, stakeholders in pediatric health
Intervention	None	None	None

Outcomes	Neurodevelopment assessment scores, hearing, growth, hospitalization, morbidity and mortality	Neurodevelopment assessment scores, mental health, hearing	Cost, feasibility of screening, referral options for HEU
Enrollment age range	Infants age 6 weeks	3-18 years	>18 years
Health status	HEU, HUU infants	HEU, HUU children and adolescents	NA
Clinical Site	Nairobi, Western Kenya	Multiple sites in the country	NA
Study follow-up	6 weeks to age 4	NA	NA
Specimens	Dried blood spot	Dried blood spot	NA
Specimen collection timeline	Enrollment, yearly	Enrollment	NA
Use of specimens	Telomere length	Telomere length	NA

c. Aim 1

i. **Study design**

Longitudinal cohort study

ii. **Study populations**

2000 HEU and HUU infant-mother pairs enrolled at 6 weeks of age

iii. **Recruitment procedures**

Recruitment of participants

1000 mothers living with HIV (MLHIV) and 1000 HIV-uninfected mothers will be recruited with their infants (HEU and HUU) at 6 weeks during routine postnatal care at 5 MCH clinics (2 in Nairobi, 3 in Western Kenya). Clinic staff will be aware of the study and asked to identify potentially eligible mother-infant pairs and refer them to study nurse. A standard recruitment script will be used for recruitment. A recruitment log will be used to document reasons for declining to participate.

For all aims, the study nurse will provide an in-depth explanation of the purpose and procedures of the study, in the language of the woman's choosing, answer any questions the woman may have, and invite the woman to participate in the study. Mothers will be informed that their participation is voluntary and that participation or nonparticipation in the study will in no way alter the nature of health care services that they receive. If interested in participating, the study nurse will screen HEU and HUU mother-infant pairs for eligibility, and, if eligible, woman will provide written consent for enrollment. If the participant is not literate, a witness unrelated to the study will be present and the consent will be read aloud. The participant will provide oral consent with a witnessed thumbprint documented as consent. This system of recruitment has been highly successful in past and ongoing studies conducted by the research team.

Eligibility criteria
Inclusion criteria:
Infant age 6 weeks (+/- 2 weeks)
Healthy infant as determined by study nurse
Mother age $\geq$18
Planning to remain in study catchment area for 4 years

Exclusion criteria:
Infant HIV positive
Mother HIV status unknown (determined using mother-baby maternal and child health booklet)
Caregiver planning to relocate

iv. Enrollment and study procedures

Data: At enrollment, contact information, maternal sociodemographic characteristics, adverse childhood events (ACEs), depression (maternal PHQ9), obstetric and medical history, and infant birth history and anthropometrics will be assessed. Medical records from MCH/CCC and viral load records will be abstracted from existing data sources. Maternal HIV status will be determined using mother-baby maternal and child health booklet; if the mother has not been tested for HIV, she will be offered HIV testing.

At every visit, study staff will perform anthropometric measurements (height/weight/head circumference, MUAC), neurodevelopmental and mental health assessments, and collect data on past and current child comorbidities and nutrition.

Summary of procedures for recruitment and enrollment

1. MCH nurse reads recruitment script to potential participant
2. MCH nurse refers interested participant
3. MCH nurse documents reason for declining to participate
4. Study nurse assesses eligibility
5. Study nurse gives more information on the study
6. Interested mothers give informed consent to participate
7. Study staff conduct HIV testing for mothers if HIV status not available in mother-baby maternal and child health booklet
8. Study staff collect contact and locator information
9. Study staff collect enrollment data - demographics, HIV status, pregnancy history, ART use and history
10. Study staff conduct anthropometric measures, neurodevelopmental and mental assessments, hearing tests on infant and collect DBS
11. Study staff reimburse client and give appointment for the next visit

v. Laboratory methods

Blood samples: Dried blood spots (DBS) for assessment of telomere length will be collected at enrollment and 12 monthly either using finger prick or venous blood draw (0.5ml).

vi. Data collection instruments

Socio-demographic, pregnancy and post-partum data: Socio-demographic information, family characteristics, medical history (including HIV status), mental health history and substance use history will be collected from caregivers. We will assess for caregiver depression at every visit using PHQ-9.

Growth assessments: Standard anthropometric measures including weight, height/length, head circumference and mid-upper arm circumference will be assessed by trained study staff. These measures will be converted to standard Z-scores using the WHO growth standards.

Neurodevelopmental assessments: Neurodevelopmental assessments will be conducted by trained study staff to assess developmental progress. The Malawi Developmental Assessment Tool (MDAT) will be used as a measure of infant neurodevelopment. The MDAT assesses gross motor, fine motor, social, and language domains, with 34 pass/fail items in each. Tests will be administered in the preferred language of the child and mother. Scripts for each assessment will be available in English, Kiswahili or Dholuo. Prior to conducting assessments, study staff will undergo several role-playing and practice test sessions, and will have satisfactory performance a monitored practice session. Caregivers will be asked about the child's well-being on the day of testing in order to determine if testing should be rescheduled. During the assessment, children will be given regular breaks as needed. Cognitive skills are assessed within the language and fine motor domains. Most gross and fine motor and language items must be directly observed, and some may be caregiver reported. All social items may be caregiver reported. Items will be administered until a child has 6 consecutive passes and 6 consecutive fails. We will measure interobserver reliability by assessing the same child independently on the same occasion by two observers in a subset.

We will use the Modified Checklist for Autism in Toddlers, Revised and with follow-up (M-CHAT-R/F) to screen for Autism Spectrum Disorders (ASD) at 18 and 24 months of age. Children screening positive will be referred for further assessment and evaluation.

Screening for child mental health conditions. The Strengths and Difficulties Questionnaire[131] is a 25-item scale widely used as a screening tool for mental health in numerous LMIC settings[132], including SSA[133]. The SDQ will be used beginning at age 3. The SDQ includes 4 difficulty scales (emotional symptoms, conduct problems, hyperactivity/inattention, and peer relationship problems) and a scale for prosocial behavior. The Strengths and Difficulties Questionnaire[131] is a 25-item scale widely used as a screening tool for mental health in numerous LMIC settings[132], including SSA[133]. The SDQ will be used beginning at age 3. The SDQ includes 4 difficulty scales (emotional symptoms, conduct problems, hyperactivity/inattention, and peer relationship problems) and a scale for prosocial behavior. SDQ scales had good internal consistency in African studies [135-139], although the hyperactivity/inattention scale has performed less well [138]. Poor SDQ scores correlated with expected demographic characteristics in South Africa and Uganda[137]. A z-score corresponding to the 80^{th}-90^{th} and >90^{th} percentiles have been considered borderline and indicative of 'caseness'[136], and will prompt further screening.

Hearing: We will use combined otoacoustic emission (OAE) and auditory brainstem response (ABR) screening tests to assess hearing. Infants will be screened at 6 weeks using OAE and referred for ABR if they screen positive. At age 3 and 4 years, we will use a smart phone based hearing screen (Hearscreen TM) to assess hearing. These tests are non-invasive and does not involve input of energy into the body.

Summary table for aim 1 data collection

	Enrollment	Month 6	Month 12	Month 18	Month 24	Month 30	Month 36	Month 42	Month 48
Consent	X								
Locator data	X	X	X	X	X	X	X	X	X
Medical history	X	X	X	X	X	X	X	X	X
Anthropometry	X	X	X	X	X	X	X	X	X
MDAT	X	X	X	X	X	X	X	X	X
SDQ							X	X	X
ASD screen				X	X				X
Hearing screen	X						X		X
DBS	X		X		X		X		X

vii. Sample size determination & data analysis

For **all analyses,** *a priori* cofactors of interest in the multivariate analysis include household sociodemographic factors, birthweight, preterm birth, maternal depression, infant sex, maternal education, and infant/child LAZ. Collinearity will be examined using standard error assessments and multivariate models will include non-collinear variables associated with growth in univariate model ($p<0.05$).

Neurodevelopmental outcomes: **MDAT scores** (social, fine motor, gross motor, and language) will be analyzed as z-scores and descriptive statistics for each domain will be summarized and compared between HEU and HUU using linear regression, with clustering for site (Table 6). MDAT scores at baseline (6 weeks) and changes in MDAT scores during follow-up will be compared between HEU and HUU and among HEU by maternal VL detection in pregnancy, and by pre-conception versus later ART, and ART regimen (EFZ versus DTG-backbone) using univariate and multivariate linear regression. Prevalence of high risk for **ASD** as assessed by M-CHAT R/F and **hearing loss** will be compared for HEU and HUU using multivariate generalized linear models (GLM) with log link and binomial family for relative risk estimation, overall and stratified in HEU and HUU.

Table 6. Outcomes and analytic approach for longitudinal cohort

Outcome variable	Comparisons	Analytic approach
Primary outcome: Neurodevelopmental outcomes MDAT ASD prevalence or hearing loss	HEU vs. HUU Maternal VL Pre-conception ART EFZ vs. DTG	Linear regression for social, gross motor, fine motor, and language z-score GLM
Secondary outcomes		
Growth (WAZ, WHZ, HAZ, HC at 12, 24, 48 months) Prevalence of underweight, wasting and stunting Growth trajectory (change in HAZ)	HEU vs. HUU Pre-conception ART EFZ vs. DTG	Linear mixed effects models
Hospitalization	HEU vs. HUU	Cox regression
Severe infections	HEU vs. HUU	Cox regression
Mortality	HEU vs. HUU	Cox regression

Growth outcomes: We will compare growth outcomes between HEU and HUU and among HEU by maternal VL detection in pregnancy, maternal pre-conception ART, and by ART regimen. Growth will be converted to z-scores using WHO Anthro software and 12- 24- and 48-month median WAZ, WHZ, and LAZ and prevalence of underweight, stunting and wasting will be compared. In addition, growth trajectory will be compared. Missing values will be imputed using Markov chain Monte Carlo procedures. Loess curves will be created to plot growth trajectory over time for WAZ, WHZ, and LAZ. Linear mixed-effects models will be used to determine correlates of growth (separately for WAZ, WHZ, and LAZ) in univariate and multivariate models.

Mortality, infectious morbidity, and hospitalizations: Cox regression will be used to compare survival of the HEU and HUU cohorts and to determine predictive factors for mortality overall and stratified by HEU/HUU. During the 4-year follow-up period for the aim 1 cohort, incidence of hospitalization, severe pneumonia, and diarrhea will be calculated and compared between HEU and HUU cohorts as number of events over person-time of follow- up with censoring for mortality and loss to follow-up. Predictors of first morbidity (separate analyses for pneumonia, severe diarrhea, and hospitalizations) will be identified using Cox proportional hazards regression with censoring for mortality and loss to follow-up.

Analysis: We will compare MDAT social, motor and language scores and prevalence of ASD between HEU and HUU and among HEU by maternal VL detection in pregnancy, maternal pre-conception ART, and by ART regimen. The percentage of normal, borderline and high scores on the SDQ will be calculated for the domains: emotional problems, conduct problems, hyperactivity, peer problems, and prosocial behavior and for total difficulty. Univariate and multivariate logistic regression will be used to determine whether HEU vs. HUU is associated with higher likelihood of high scores on SDQ domains overall. Among HEU, we will assess influence of maternal VL, ART, and regimen. Multivariate analyses will be adjusted for maternal sociodemographic factors and depression and infant birthweight and growth.

Telomere length analyses Telomere length (TL) may reflect prior fetal exposure to inflammation. In one study PHIV had significantly shorter TL than HEU and HUU children; untreated PHIV had shorter TL than those on ART[160]. Among HEU infants, TL was significantly shorter among those whose mothers were untreated in pregnancy than those whose mothers received zidovudine monotherapy and maternal viremia was inversely associated with infant TL[161]. TL was significantly shorter in South African PHIV (ART-treated) and HEU children than in HUU (mean age 6.4 years)[162]. However, not all studies have demonstrated associations between HEU and TL. One study found no difference in TL between PHIV, HEU, and HUU children, although TL shortening was associated with viremia in PHIV[163]. Similarly, there were no significant differences in TL between HEU and HUU children in a recent Canadian study[164]. There is also evidence that maternal IPV or adverse childhood experiences (ACEs) influence infant TL[165,166]. This evidence is limited by differences in exposure and outcome measurements [167-169]. TL shortening has been associated with emotional processing on fMRI[170]and with ADHD and depression[171-174]. **A molecular signal such as TL shortening could reflect 'archived' history of fetal HIV exposure to further support associations with HEU.** *Telomere length assessment*: DNA will be extracted from DBS in the University of Nairobi Pediatric Research laboratory prior to shipping to the Risque lab for TL assessment; the Risque lab has extensive experience conducting TL assays. Methods for TL assessment using DBS have been recently developed by Rej et al (*in press*) in the UW Eisenburg lab, who has shared these methods with the Risque lab. Analysis: We will compare TL from 500 HEU and 500 HUU in Aim 1 cohort at 6 weeks, 1, and 4 years of age and compare TL between infants with neurodevelopmental compromise (or with ASD) to those without; overall, and stratified by HEU/HUU status. DBS will be collected in cross-sectional survey of older HEU from 20 sites for future studies, conditional on evidence from Aim 1 results. We will use linear regression to compare TL in HEU to HUU and conditional linear regression for comparisons of neurodevelopmental outcomes; with multivariate adjustment for cofactors as previously described.

d. Aim 2
    i. Study design

Cross-sectional surveys. These will be done in 2 phases 1) Pilot phase and 2) Population phase. The pilot phase will be conducted in one facility (Rachounyo sub-County hospital) after which we will begin population based assessments in 100 large HIV clinics. Procedures for enrollment and assessment are similar for 2 phases.

ii. Study populations

HEU and HUU caregiver-child pairs

iii. Recruitment procedures

MLHIV attending HIV Care clinics will be asked about potentially eligible HEU children aged 3-18 by study nurse. MLHIV who are interested in participating will be invited to return with their child/adolescent for participation in the study. Study nurses will also liaise with clinic community health workers (CHWs) to recruit eligible HUU in the relevant age strata (3-6, 7-10, 11-14, 15-18 years) in the community. CHWs will invite mothers of eligible HUU children to participate in the study for evaluation and mother-child/adolescent pairs will be enrolled at a neutral site (typically MCH or family planning [FP] clinic) or at home. Study nurses will be accompanied by the community health worker for home enrollments. A standard recruitment form will be used.

The target enrollment is 400 children and adolescents (200 HEU and 200 HUU) in the pilot phase and 4400 (4000 HEU and 400HUU) age 3-18 years (stratified to the following groups: 3-6, 7-10, 11-14, 15-18 and by HIV status (HEU or HUU)) in the population phase. The pilot phase will be conducted in one facility in rural Kenya (Rachuonyo Hospital HIV clinic), and the population phase in 100 large HIV care clinics with at least 1000 adult women in HIV care.

For HEU, caregivers attending HIV clinics who have children in the proposed age-groups will be recruited by the clinic staff. Caregivers who are interested will meet with the study nurse and be provided with details on the study and study procedures and invited to bring children for enrollment. All study procedures will be completed at enrollment with no follow-up procedures.

HUU will be recruited from the neighboring community around the HIV Care Clinic from where the HEU are recruited. Nurses and Community health workers (CHW) will visit households to recruit interested caregivers of children age 3-18 and enroll those who meet eligibility criteria and are willing to confirm HIV status (using HIV self-test kits) and enroll their children in the study.

Eligibility criteria
Child or adolescent age 3-18 years
Child/adolescent is healthy and HIV negative
Mother HIV status known
Mother HIV diagnosis and ART timing known (for HEU)
Caregiver >=18 years willing to take children to clinic for evaluation

iv. Enrollment and study procedures

Enrollment will be conducted at a neutral location (typically public MCH clinic) or at home. No additional visits will be conducted after enrollment.

<u>Determination of HIV status for HEU and HUU</u>: A combination of medical records and HIV testing will be used to determine HIV status of mothers and children. Maternal HIV-positive status during the pregnancy with each child will be determined first using the mother-baby maternal and child health booklet; in the event that this

booklet is not available, records from the HIV care clinic documenting the date when the mother was diagnosed with HIV or started ART will be used (only for HIV positive mothers). In the event of no records are available, maternal report of being diagnosed with HIV either during or prior to the pregnancy with each child will be used (only for HIV positive mothers). For mothers who report not being HIV positive at the time of pregnancy and for whom mother-baby maternal and child health booklets are not available, we will offer maternal HIV testing; mothers who do not consent to HIV testing will not be enrolled.

For HEU dyads, children's HIV negative status will be determined using the same set of procedures (first reviewing mother-baby maternal and child health booklet, then infant HIV testing history records); if the child has not been tested for HIV after the cessation of breastfeeding, HIV testing will be offered for the child. Children ages 7-14 years will be asked to provide assent without mention of HIV; children ages 15-18 will be asked to provide assent (or consent for 18 year olds) with explicit mention of HIV testing and will be told that they can choose whether they wish to share their results with their caregivers. If children decline HIV testing, they will not be enrolled in the study.

For HUU dyads, children will be assumed to be HIV negative if their mother is negative and if they are <15 years of age. Children ages 15-18 will be asked to provide assent (or consent for 18 year olds) with explicit mention of HIV testing and will be told that they can choose whether they wish to share their results with their caregivers. If children decline HIV testing, they will not be enrolled in the study.

At enrollment, growth, neurodevelopmental and mental health outcomes will be collected by interviews with caregivers/adolescents/children. For HIV positive mothers, additional details on HIV treatment history, ART regimen, perinatal illnesses and general health will be obtained from medical records.

Caregivers and adolescents age 18 will give informed consent to participate in the study. Children and adolescents age 7-17 will give assent to participate.

At enrollment, study staff will perform anthropometric measurements (height/weight/head circumference, MUAC), neuro-developmental, mental health and hearing screening and assessments and collect data on past and current child comorbidities and nutrition. Children and adolescents who fail screening tests will be referred for further evaluation as needed.

Summary of procedures for recruitment and enrollment

1. MCH nurse or CHW reads recruitment script to potential participant
2. MCH nurse or CHW refers interested participant
3. MCH nurse or CHW documents reason for declining to participate
4. Study nurse assesses eligibility
5. Study nurse gives more information on the study
6. Interested mothers give informed consent to participate
7. Children age 7 and above give assent to participate
8. Determine maternal and/or child HIV status using medical records
9. Conduct maternal and/or child HIV testing, if not determined using medical records
10. Study staff collect enrollment data - demographics, pregnancy history, ART use and history
11. Study staff conduct anthropometric measures, neurodevelopmental and mental assessments, hearing tests and collect DBS
12. Study staff reimburse client

v. Laboratory methods

Blood samples: Dried blood spots (DBS) for assessment of telomere length will be collected either using finger prick or venous blood draw (0.5ml) at enrollment in 20 selected sites.

HIV testing: HIV testing will be conducted using saliva-based HIV tests, administered by a health care worker.

vi. Data collection instruments

Socio-demographic, pregnancy and post-partum data: Socio-demographic information, family characteristics, medical history, mental health history and substance use history will be collected from caregivers. We will assess for caregiver depression using PHQ-9.

Medical record abstraction: Medical records will be reviewed for the HEU cohort to determine timing of maternal HIV diagnosis in relation to child's birth date to ascertain documentation of fetal HIV and ART exposure.

Growth assessments: Standard anthropometric measures including weight, height, head circumference and mid-upper arm circumference will be assessed by trained study staff. These measures will be converted to standard Z-scores using the WHO growth standards.

Neurodevelopmental assessments: A battery of neurodevelopment and mental health assessments will be conducted by trained staff. Selected tools are amenable to use by nurses or community health workers without specialized training and are validated as diagnostic screening tools. The table below summarizes the tools, age-group they are used in, screening time and domains or disorders they test. All screening tools will first be used and then where the child fails the screening tool, diagnostic tests will be conducted either at the same visit or soon after. School performance will be assessed from caregiver reports of national examination reports or other school reports.

Hearing: We will use a smart phone based hearing screen (Hearscreen TM) to assess hearing. This test is non-invasive and does not involve input of energy into the body.

Child mental health screening. The SDQ (described above) will be used to screen for mental health conditions in children through age 16 years. In 7-11 year old orphans in South Africa, the Parent SDQ had good construct validity with the Computerized Diagnostic Interview Schedule for Children -4th Ed. (CDISC-IV), with area under the curve (AUC) ranging from 0.73-0.82[138]. In pre-school aged children the Total Difficulties Score (TDS; an aggregate) had 0.79 sensitivity and 0.93 sensitivity versus the Child Behavior Checklist[140]. For older adolescents (>16 yrs), the PHQ-9 is a widely used screening test for depression validated in Ethiopia, Nigeria, Kenya and Uganda[141-143]. In 15-40 year olds in Nigeria, the PHQ-9 had $\geq$0.85 sensitivity and 0.99 specificity for combined major and minor depressive disorders and major depression[144]. We will administer SDQ to the child and with the parent (will respond to questions about their children).

Screening for ADHD. Because the SDQ may not optimally screen for ADHD, the SNAP-IV (revision of the Swanson, Nolan, and Pelham – SNAP) questionnaire will be administered to screen for ADHD[145,146]. The Multimodal Treatment Study (MTA) version includes 26-items, has robust psychometric properties[145] and a caregiver/parent only approach has been used in South Africa[147]. As in this South African study a score at the 95th percentile will be considered positive and will prompt further evaluation with the MINI-KID.

Diagnosis of neuropsychiatric disorders. The Mini International Neuropsychiatric Interview for children and adolescents (MINI-KID)[148,149] is a diagnostic tool that included diagnosis of over 30 common pediatric psychiatric morbidities among children and adolescents age 6-17 years, including depressive disorders, suicidality, anxiety disorders and ADHD.[150] The MINI-KID and the corresponding adult form (MINI) will be used to evaluate children and adolescents who screen positive on the SDQ or PHQ-9. Administration can take as little as 10 minutes by nurses and social workers[151]. The MINI-KID has been used in Nigerian and Ugandan adolescents age 10-19[152,153]. Diagnosis of major depressive episodes using MINI-KID in Rwanda in 10-17 year olds aligned with local expression of depression-like symptoms[154].

Cognitive outcomes. Cognitive assessments will be done using NIH Toolbox. The battery will include the tests Dimensional Change Card Sort Test, Flanker Inhibitory Control and Attention Test, List Sorting Working Memory Test, Pattern Comparison Processing Speed Test, and Picture Sequence Memory Test, which evaluate working memory, attention, processing speed, and other aspects of executive functioning. The NIH Toolbox has been validated for US children and was recently adapted through a rigorous process involving translation and backtranslation for Kenya, including translation to Dholuo and Kiswahili.

The table below summarizes the tools, age-group they are used in, screening time and domains or disorders they test.

Table 7: Neurodevelopment and mental health assessment tools

Tool	**Age-group**	**Time (minutes)**	**Types**	**Domains/Disorder**
MDAT	0.6-5	30	Screening	Gross motor, fine motor, language, social
HearScreen TM	3+	5	Screening	Hearing
NIH Toolbox	6-17	25-30	Screening	working memory, attention, processing speed, executive functioning
Strengths and difficulties questionnaire (SDQ)	3-16 & caregivers	10	Screening	Emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems
SNAP-IV	6-12	5-10	Screening	ADHD
PHQ-9	>16 & caregivers	3	Screening	Depression
MINI-KID	6-17	10	Diagnostic	Common psychiatric conditions
MINI	18	10	Diagnostic	Common psychiatric conditions
School performance	>10	1	NA	National exam results Parental report

vii. Sample size determination & data analysis

<u>Effect size calculations</u>: For comparisons of z-scores in MDAT or NIH-toolkit scores, we will be able to detect differences of 0.13-0.18 assuming a standard deviation of 1.0 or 1.4, 80% power and alpha of 0.05 in Aim 1 cohort (Table 10). In the cross-sectional cohort, 2- to 3-fold differences will be detectable, in diagnoses such as depression and ADHD. To detect smaller differences, more HUU could be enrolled in some age-strata based on pilot feasibility.

Table 10. Sample size estimates

R61/R33 Aim 1 cohort	**Effect detectable (no attrition)**		**Effect detectable (20% attrition)**	**Assumptions**
Difference in z-score for MDAT	0.13 (SD=1.0) 0.18 (SD=1.4) 0.18 (SD=1.0) 0.25 (SD=1.4)	1000 HEU:1000 HUU 500 DTG: 500 EFZ	0.14 (SD=1.0) 0.20 (SD=1.4) 0.20 (SD=1.0) 0.28 (SD=1.4)	Standard deviation = 1.0-1.4[105]
Difference in ASD prevalence	2.2% 3.3%	1000 HEU:1000 HUU 500 DTG: 500 EFZ	2.5% 3.8%	HUU ASD 2%
Difference in SDQ+ prev.	5.2% 7.5%	1000 HEU:1000 HUU 500 DTG: 500 EFZ	5.9% 8.5%	HUU SDQ+ 20%
Difference in hearing loss	1.7% 2.7%	1000 HEU:1000 HUU 500 DTG: 500 EFZ	2.0% 3.1%	HUU 1%
Difference in TL	0.18	500 HEU: 500 HUU		SD=1.0
R33 Aim 2				
Difference in BENCI scores	0.29	1000 HEU: 100 HUU age stratum (7-11yo)		SD=1.0
Difference in ADHD prev.	7.7%	1000 HEU: 100 HUU		2.7% in HUU[175]
Difference in depression prev	10.1%	1000 HEU: 100 HUU per age stratum		HUU 10%

Analyses: For binary outcomes such as ADHD and depression, multivariate generalized linear models (GLM) with log link and binomial family for relative risk estimation clustered by site will be used. For continuous variables such as NIH-Toolkit, we will use multivariate GLM with Gaussian family and identity link. As previously noted in Aim 1, we will include *a priori* potential confounders - maternal depression, preterm birth, and infant birthweight - in multivariate analysis, after assessment of collinearity.

Table 11. Analyses for outcomes assessed	
MDAT (social, fine motor, gross motor, language)	GLM
Hearing	GLM
BENCI (processing speed, visual-motor coordination, attention, language, memory, executive function)	GLM
SDQ total domain score	GLM
ADHD screen (analyze positive SNAP-IV and subset validated on MINI-Kid) PHQ-9 (prevalence of mod-severe symptoms)	GLM
Neuropsychiatric diagnoses	GLM

<u>Training procedures for aim 1 and 2</u>

The study team will develop protocols and training materials for clinic staff. A 5-day training workshop will be designed. The package will include the basics of early childhood development, pediatric mental health, screening and use of the study screening and diagnostic tools. The training will cover neurodevelopmental assessments, psychometric testing, and administration, scoring and interpretation of the MDAT and NIH Toolbox. The 5-day nurse training workshop will cover general sensitization to the testing situation, including ensuring that the child follows the instructions, methods of cross-checking to determine whether a child understood instructions, learning when to give breaks, how to re-engage the child and how to react to distress. The training will cover how to recognize need for referrals and provide motivational interviewing and culturally sensitive assessment skills. To ensure quality control, the study coordinator will conduct refresher training for staff to ensure operating procedures are followed consistently. We will also develop checklists for tests requiring multiple tests as well as the use of tablet based tests to ensure quality assessments.

e. Aim 3

i. Study design

This Aim of the study involves 2 cross-sectional components:

We will conduct a micro-costing study to determine the total cost of screening HEU in Kenya for growth, cognitive delays, and mental health. To collect costs, detailed cost information will be obtained from local, county and national level; these do not include human subjects and are not further described in this protocol. In addition to the non-human subjects cost collection elements, we will conduct time and motion studies with health care workers to assess the time spent delivering and receiving services; this set of activities is described below as time-and-motion data collection.

Additionally, we will convene a 2-day stakeholder workshop at the end of the study period to disseminate results and envision a screening program.

ii. Study populations

Caregivers: caregiver to a child receiving screening in Aims 1 or 2, >=18 years of age

Health care workers (for costing): >=18 years, delivering screening services within Aims 1 or 2; may be study staff or non-study staff

Health care workers (for stakeholder workshop): >=18 years, has experience working in a health facility in Kenya

Stakeholders: >=18 years, involved in informing decision-making for ministries of health or related organizations

iii. Recruitment procedures

Time-and-motion: We will invite caregivers and health care workers within Aims 1 & 2 to learn about additional elements of this study at the end of their study visits. They will be invited to participate in time-and-motion data collection, assessing the duration of time they spend delivering and receiving screening assessments, and their salaries (to estimate costs). For HCW we will also invite them to provide information on the hypothetical impact of additional screening activities on their ability to deliver other routine services.

Stakeholder workshop: Health care workers, policy makers and stakeholders in pediatric health in Kenya will be purposively recruited to participate in a 2 day workshop on child/adolescent mental health screening in program setting. They will be informed that during the workshop, the study team will present data from Aims 1 and 2, and brainstorm on screening tools, structure and components of an integrated HEU screening program in Kenya.

iv. Enrollment and study procedures

Time-and-motion: Interested caregivers and HCW will complete an oral informed consent to participate in time-and-motion data collection. They will note the time when services began and ended, their salaries, and the HCW will report on the hypothetical impact on service delivery.

Stakeholder convening and dissemination: We will convene a 2-day stakeholder workshop in the last 3 months of the study period. Invited participants will include policy makers in PMTCT, pediatric and adolescent HIV (drawn from the Kenya National AIDS and STI Control Program [NASCOP]), members of the National Pediatric HIV Technical Working Group, HIV care partners), policy makers in child development including ECD and from the Ministry of Education, child health partners (UNICEF, World Bank), health care workers in MCH and HIV care settings in Kenya, caregivers of HEU (drawn from existing networks of PLHIV) and HEU adolescent representatives. The guest list will be reviewed with Ministry of Health partners to ensure relevant stakeholders are included. We have conducted similar meetings for the development of a national disclosure and transition framework and tools and therefore have experience convening these meetings. The purpose of the meeting will be to: 1) present results of the study regarding burden of neurodevelopmental and mental health outcomes in HEU and HUU, and evidence for elevated risk in HEU or subgroups of HEU at-risk, 2) discuss experience recruiting HEU cohorts and potential for scale-up, 3) discuss experience with, and cost of, screening tools and potential integration within existing health services, 4) brainstorm on structure, components and outcomes of an integrated HEU screening program in Kenya. The discussions will be audio recorded and information on the process written up into a publishable manuscript.

v. Laboratory methods

N/A

vi. Data collection instruments

Time-and-motion survey including time spent, salary, and hypothetical impact on clinical duties

Stakeholder meeting notes about brainstorm on structure, components and outcomes of an integrated HEU screening program in Kenya.

vii. Data analysis

Cost data collection and analysis methods: We will estimate the costs of conducting screening for growth deficits, hearing deficits, cognitive delays, and mental health for children in each age stratum defined in Aim 2. In addition to the cost per child screened in each age stratum for each condition, we will quantify the cost per child who screens positive and is referred for subsequent either diagnostic or management services. Costs to be collected and data sources are noted in Table 12. We will conduct a detailed, in-country micro-costing using primary data from study budget records, expense reports, and interviews with program staff to understand resource utilization. We will also utilize secondary cost data from literature, health facilities, and county health expenditure reports. We will conduct time-and-motion observation to estimate time spent by health care providers and clients on conducting each of the screening assessments and assess how these tasks impact their regular clinical duties. We will follow the principles outlined in the Global Health Cost Consortium Reference Case [176] and the International Society for Pharmaceutical and Outcomes Research's guidance on costing analyses [177]. We will take a payer perspective for this analysis.

Table 12: Micro-costing components and estimation approach

Cost	Cost type	Estimation approach
Start up costs		
Micro-planning	Fixed	Project budget abstraction
Screening test licensing fees	Fixed	Project budget abstraction
Training	Fixed	Project budget abstraction
Tablets	Fixed	Project budget abstraction
Recurrent costs		
Personnel		
HCW time (service provision)	Variable	Quantity: Time-and-motion studies at select facilities
HCW time (record keeping)	Variable	
HCW time (supervision)	Fixed	Unit cost: HCW salaries from records
Caregiver time during screening	Variable	Quantity: Time-and-motion studies at select facilities Unit cost: Caregiver salaries from surveys
Transportation		
HCW transportation	Variable	Transportation costs from surveys
Caregiver transportation	Variable	
Facilities & overhead		
Space costs	Fixed	Estimated based on square footage at nearby commercial property value
Airtime & data	Variable	Project budget abstraction

viii. Training procedures

Staff will be trained on data collection for time in motion surveys. A senior study staff or co-investigator will summarize discussions at the stakeholder meeting.

13. Quality assurance procedures

Adherence to protocol: Weekly reporting of enrollment and data collection will enable us to monitor that the study is running according to approved protocols. Frequent reporting will also enable us to respond quickly to any problems that arise during the study.

Data Quality: A dedicated data team will be responsible for data collection using an electronic data collection platform, RedCAP. The data team will communicate weekly with the operations team and leadership including reporting on data cleaning, study monitoring, and interim analyses.

14. Ethical considerations

Direct benefits: Direct benefits to study participants will include developmental, mental health and hearing screening.
Indirect benefits: The study may produce data that will lead to improved screening and interventions for HEU, thus a benefit to society.

Disclosure of HIV status: There is a non-negligible risk of disclosure of caregiver HIV status to others including the risk that children/adolescents may accidentally learn their caregiver HIV status. The study comparison groups require the study team to know caregiver and child HIV status. The study team will be trained on protection of human subjects and will make every effort not to disclose HIV status of enrollees to others outside the study team. Caregivers may not have disclosed their HIV status to their children/adolescents. The study team will make every effort to ensure confidentiality of maternal HIV status. This will be done by: 1) Enrollment visits for HEU/HUU children will be conducted in the general clinic settings and not in HIV clinics. 2) Assent forms and consent forms used for children and adolescents will not mention HIV status of caregivers. Screenings will be described as general health screenings that will include HIV testing. Caregivers who are willing to disclose their HIV status to their children will be supported through services available in the clinic. In case of accidental disclosure to children or adolescents, the study team will refer the caregiver-child pair to counselling services available in clinics.

Family/Caregiver/adolescent distress: Results of screening tests may be uncomfortable and cause family distress. Questions used in screening may cause caregiver/child/adolescent discomfort. Participants can refuse to respond to any question at any time. Caregivers will be informed that the tests are only screening tests and will need additional tests for confirmation of diagnosis. All children/adolescents will be referred for additional diagnostic services through established referral systems.

Quality of care: The study procedures do not interfere with routine care. However, it is possible that participating in a study could have unforeseen effects that could interfere with care. Children will continue with regular care at MCH clinics. The study procedures will be conducted outside of regular MCH procedure, however, visits will be aligned to reduce multiple clinic visits.

Sample collection: Risks to study participants include discomfort during specimen collection which can be painful. Application of heat packs (heel/finger prick) or Tetracaine gel (phlebotomy) will be used to reduce pain associated with blood collection.

Confidentiality: In all research studies there is a non-negligible risk of loss of confidentiality of medical information. All data are kept in password-protected, encrypted databases, in a locked study office, accessible only to study personnel. Study identifiers are linked to coded data; clinical staff have access to patient identifiers, but analysts receive only coded data. Links between patient identifiers and study codes will be kept until the end of the study.

Non-coercion: In order to minimize the risk of coercion, there are no financial incentives to study participation. However, study participants will receive a reimbursement in their travel fees for each visit of 600 KES (about $USD 6); this amount is periodically reviewed according to cost-of-living increases and is approved by the local Ethics and Research Committee of KNH.

Incidental findings: Results from mental health and developmental screening will be provided to caregivers. All children/adolescents who screen positive will be linked to appropriate services. Prior to study start, the study team will assess available mental health, neurodevelopmental and hearing services in the community, and nearest referral centers around the community and establish a referral plan.

Alternatives to study participation:
Declining study participation will have no impact on the ability to receive routine care at the facility, or to participate in other studies that might be ongoing at the site.

15. Data management

Collection: Data will be managed at the study clinic/study office in Nairobi. All data will be entered into a RedCap study database for storage and management.

Management: A dedicated data team will be responsible for the entry, management, and monitoring of study data. The Nairobi data team will communicate frequently with the PIs for periodic data cleaning, study monitoring, and interim analyses.

Protection: All Clinical data and study files will be kept in a locked Study Office, accessible only to Study Staff. All Study Staff will be trained in data protection and privacy. RedCap databases are encrypted, and enabled with 2-step verification to restrict user access. Only Clinical files will contain patient identifiers; Study analysts will receive only coded data. The link between study identifiers and patient IDs will be kept under lock and key. The link between patient identifiers and Study ID codes will be retained until completion of the study.

Data Ownership: The proposed project is a collaborative effort between investigators at the UW, KNH and UoN. The aforementioned institutions will jointly share ownership of the data. Study investigators at the UW, KNH and UoN will have full access to the data. Authorship on publications, conference presentations, abstracts and other materials generated from this study will reflect contribution to design, execution and analysis of the study.

Data Release/Sharing Policy: All quantitative data collected as part of this proposed research project will be made available to access or download files on a study related website (URL to be determined) following ERC/IRB approval for data sharing and agreement to the data sharing agreement. The data sharing agreement will ensure commitments to:

1. Using the data only for research purposes and without attempting to identify study participants (if applicable);
2. Securing the data using appropriate computer technology;
3. Destroying or returning the data after analyses are completed;
4. Restrictions on redistribution of the data to third parties; and
5. Proper acknowledgement of the data resource.

Data Monitoring Committee: This study will not involve a data safety monitoring board or committee.

16. Study limitations and how to minimize them

<u>Pitfalls and alternative strategies</u>: The Aim 1 cohort is designed to be homogeneous – with all HEU having initiated ART either before or during pregnancy and all enrolled contemporaneously at 6 weeks of age, which accommodates secular trends in comparisons with the HUU cohort. HUU will be recruited from the same MCH clinic as HEU, which ensures that mothers reside in the same community, and thus have similar sociodemographic characteristics; this attenuates risk for confounding in comparisons between HEU and HUU. We will not enroll during pregnancy, which will under-ascertain perinatal morbidity and preterm birth. However, we have access to 3 ongoing birth cohorts that are accruing pregnancy-enrolled HEU and HUU. Data from those cohorts complement this study, which is designed to be a population-based multi-site urban/rural cohort.

17. Timeline

Timeline: In the first 6 months we will obtain study approval, recruit and train staff and establish study sites. We anticipate to complete enrollment for the longitudinal cohort in the second half of year 1. Aim 2 pilot will begin in the second half of year 1 and be complete in 10 months. Aim 2 population surveys will begin after the pilot in the second half of year 2. Aim 3 activities will be conducted in year 4-5.

Study Timeline					
	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Study approvals, training					
Aim 1 Enroll longitudinal cohort					
Follow-up longitudinal cohort					
Aim 2 Pilot cross-sectional cohort					
Aim 2 Population based assessments					
Aim 3 Cost analysis					
Aim 3 Stakeholder meeting					

18. Human subjects

a) Ethical Approval

We will obtain ethical approval from the University of Washington (UW) Human Subjects Division (IRB) and Kenyatta National Hospital-University of Nairobi (KNH-UoN) Ethics and Research Committee (ERC). There will be minimal risk to the participants taking part in this study. Any changes to the protocol will be submitted to the UW IRB and KNH-UoN ERC.

b) Collaborating sites

The study will be conducted in collaboration with the UW, KNH, and UoN. The study will be reviewed by the KNH ERC and UW IRB and will not be started before approvals are obtained from all two organizational review boards.

c) Informed Consent

A Study Nurse will meet with caregivers to assess eligibility criteria and describe the study procedures in detail. If eligibility criteria are met and the caregivers wish to proceed with enrollment, the study nurse will re-assess caregiver(s)' understanding of the study risks/benefits, review the detailed study procedures and obtain written

informed consent. Caregivers will be required to provide written consent for enrolment of their children. Consent will be administered in English, Luo or Kiswahili, as per the participant's preference. All children age 7-17 will provide written assent in addition to caregiver written consent to participate in the study. Adolescents age 18 will provide their own consent for participation.
NCIBE

19. Budget

Salaries and wages	$	709,086
Service contracts	$	133,200
Other contractual services	$	967,333
Travel	$	127,000
Supplies and materials	$	21,000
Equipment	$	-
Retirement and benefits	$	197,783
Total budget (5 years)	**$**	**2,155,402**

20. Roles and responsibilities

- The Principal Investigator Dr. John-Stewart is responsible for overall leadership and will provide input for scientific, implementation and dissemination aspects of the study.
- Drs. Wamalwa and Njuguna will provide leadership and oversight of the research team in Kenya and guidance on HIV research in children and adolescents, hearing screens and clinical impact and interpretation of results.
- Drs. Dorsey and Kumar will provide input on mental health and neurocognitive assessments.
- Drs. Kumar, Benki-Nugent, Wagner and Njuguna will develop protocols and training materials for clinic staff and will be responsible for training staff.
- Dr. McGrath will provide support for conduct of national survey and growth assessments.
- Dr. Wagner will provide support for costing activities and implementation research agenda at the stakeholders' workshop.

21. List of appendices:

a) Consents and Assents:

- Aim 1 Consent for enrollment
- Aim 2 Caregiver consent for enrollment (age 3-17)
- Aim 2 Caregiver consent for enrollment (age 18)
- Aim 2 Youth consent for enrollment (age 18)
- Aim 2 Assent for enrollment age 7-17
- Aim 3 HCW consent for time and motion studies
- Aim 3 Consent for stakeholder's workshop

b) Recruitment scripts

- Aim 1 and 2 caregiver recruitment script
- Aim 2 Youth recruitment script
- Aim 3 Caregiver recruitment script for time and motion studies
- Aim 3 HCW recruitment script for time and motion studies
- Aim 3 Stakeholders workshop recruitment script

c) List of question topics