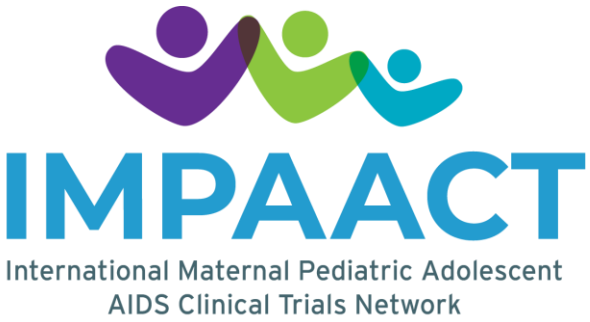

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Non-IND Study
DAIDS ES # 38693

This file contains the current IMPAACT 2028 protocol, which is comprised of the following documents, presented in reverse chronological order:

- Letter of Amendment #1, dated 10 February 2023
- Clarification Memorandum #1, dated 26 April 2021
- Protocol Version 1.0, dated 23 December 2020

Letter of Amendment #1 for:

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Version 1.0, dated 23 December 2020

DAIDS Study ID #38693

Letter of Amendment Date: 10 February 2023

Information/Instructions to Study Sites from the Division of AIDS

The information contained in this Letter of Amendment (LoA) affects the IMPAACT 2028 study and must be submitted to site Institutional Review Boards and/or Ethics Committees (IRBs/ECs) as soon as possible for their review and approval. Approval must also be obtained from other site regulatory entities if applicable per the policies and procedures of the regulatory entities. All applicable IRB/EC and regulatory entity requirements must be followed.

Upon obtaining all required IRB/EC approvals and any other applicable regulatory entity approvals, each site should immediately begin implementing this LoA. Sites are required to submit an LoA registration packet to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center. Sites will receive a registration notification for the LoA after the DAIDS PRO verifies that all required registration documents have been received and are complete. Sites should not await this notification before implementing this LoA.

Please file this LoA, all associated IRB/EC and regulatory entity correspondence, and all correspondence with the DAIDS PRO in your essential document files for IMPAACT 2028. If the IMPAACT 2028 protocol is amended in the future, the contents of this LoA will be incorporated into the next version of the protocol.

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

DAIDS Study ID #38693

Version 1.0, Letter of Amendment #1
Letter of Amendment Signature Page

I will conduct this study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Council for Harmonisation Guideline for Good Clinical Practice (ICH E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

_____ _____

Signature of Investigator of Record Date

_____

Name of Investigator of Record
(printed)

Summary of Modifications and Rationale

The purpose of this LoA is to permit an exemption from storage of peripheral blood mononuclear cells (PBMCs) at sites where the local laboratory does not participate in the DAIDS immunology quality assurance (IQA) program. Updates of the protocol team and study site rosters are also included in this LoA.

Implementation

A. Exemption from Storage of PBMCs

Protocol Section 5.8.2 specifies that PBMCs stored for this study must be processed and cryopreserved by an IQA-approved laboratory. In the event that DAIDS determines a site laboratory cannot be added to the IQA program, on a case-by-case basis, the protocol team may exempt the site from PBMC storage. If this option is implemented, this will be documented in a memorandum from the protocol team that should be filed in the relevant site's essential document files for IMPAACT 2028.

B. Protocol Team Roster Updates

To reflect current Protocol Team membership, Jason Rippe is removed (deletions not shown); Jared Kneebone and Christina Reding are replaced by Andi Ace; and Colleen Foley is replaced by Laura Hovind. Contact details are shown below.

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C. Study Site Roster Updates

The investigator at Clinical Research Site 5048 has determined that the site is not expected to enroll any participants in this study; as such, this site is removed from the roster (deletions not shown).

To reflect recent staffing changes, LaTeshia Thomas Seaton is replaced by Sierra Jordan-Thompson as the Study Coordinator at Site 5030; Kathleen Graham is replaced by Paulino Four Castro as the Study Coordinator at Site 5055; Jullapong Achalapong is replaced by Pradthana Ounchanum as the Investigator of Record at site 5116; Nastassja Ramsagar is replaced by Deirdre Josipovic as the Study Coordinator at Site 8052; Portia Kamthunzi is replaced by Tisungane Mvalo as the Investigator of Record at site 12001; and Barbara Pahud is replaced by James Christopher Day as the Investigator of Record at Site 32017. Contact details are shown below.

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12001, Malawi Clinical Research Site
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32017, Children's Mercy Hospital Clinical Research Site
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Clarification Memorandum #1 for:

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Version 1.0, dated 23 December 2020

DAIDS Study ID #38693

Clarification Memorandum Date: 26 April 2021

Summary of Clarifications and Rationale

This Clarification Memorandum (CM) updates protocol specifications to reflect current policies of the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), and National Institutes of Health (NIH). It also updates the protocol team and study site rosters and adds guidance for collection of nasopharyngeal and rectal swabs in the event that supplies required for collection of these specimens are not available.

Implementation

Institutional Review Board/Ethics Committee (IRB/EC) approval of this CM is not required by the study sponsor prior to implementation; however, sites may submit it to IRBs/ECs for their information or, if required by the IRBs/ECs, for their approval prior to implementation.

IRBs/ECs may have acknowledged and/or approved remote site monitoring strategies prior to the issuance of this CM. If so, documentation of the acknowledgement and/or approval should be filed in your essential document files for IMPAACT 2028. This CM and any applicable IRB/EC correspondence should also be filed in your essential document files for IMPAACT 2028.

The information included in this memorandum will be incorporated into the next protocol amendment.

A. DAIDS Policy Updates

1. Protocol Section 9 is updated to reflect current DAIDS policies for clinical site monitoring, which do not require monitoring for non-IND studies and allow for monitoring, when performed, to be performed on-site or remotely. The prior contents of this section are replaced with the following:

   Under contract to DAIDS or NICHD, site monitors may inspect study site facilities and review participant study records — including informed consent and assent forms, paper-based CRFs (if used), eCRFs, medical records, and laboratory records — to ensure protection of study participants, compliance with the IRB/EC approved protocol, and accuracy and completeness of records. Monitors also may review essential document files to ensure compliance with all applicable regulatory requirements. Site investigators will make study facilities and documents available for inspection by monitors.

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by DAIDS or NICHD. Remote monitoring visits may be performed in place of, or in addition to, onsite visits to ensure the safety of study participants and data integrity (1). Site investigators will make study documents available for site monitors to review utilizing a secure platform that is HIPAA compliant. Potential platform options include: Veeva SiteVault, Medidata Rave Imaging Solution, Medidata Remote Source Review, site-controlled SharePoint or cloud-based portal, and direct access to electronic medical records. Other secure platforms that are HIPAA compliant may be utilized, as allowed by DAIDS or NICHD.

Reference:

1. **FDA Guidance on Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency: Guidance for Industry, Investigators, and Institutional Review Boards, March 2020, Updated on January 27, 2021. Accessed at: https://www.fda.gov/media/136238/download**

2. Protocol Section 11.5 refers to the DAIDS policy on identification and classification of critical events. This policy has been retired. Section 11.5 is removed from the protocol and Section 10.1 has been updated to refer to the reporting requirements that still apply for sites conducting this study. The prior contents of the first paragraph in Section 10.1 are replaced with the following:

   Prior to study initiation, site investigators must obtain IRB/EC review and approval of this protocol and site-specific informed consent and assent forms in accordance with 45 CFR 46; subsequent to initial review and approval, IRBs/ECs must review the study at least annually. Site investigators must promptly report to the IRBs/ECs any changes in the study and must comply with the requirements of 45 CFR 46.108(a)(4) for promptly reporting the following: unanticipated problems involving risks to participants or others; serious or continuing noncompliance with applicable regulations or the requirements or determinations of their IRBs/ECs; and any suspension or termination of IRB approval.

3. Protocol Sections 8.1, 8.2, 8.3, 10.2, 10.7, 11.3, and 11.4 refer to the following DAIDS policies:

   - Requirements for Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials
   - Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials
   - Requirements for Clinical Quality Management Plans
   - Requirements for Manual of Operational Procedures
   - Enrolling Children (including Adolescents) in Clinical Research: Clinical Site Requirements

   These policies have been retired and replaced with instructions for sites that are now contained in the DAIDS Site Clinical Operations and Research Essentials (SCORE) Manual. Throughout the protocol, references to the above-listed policies are replaced with requirements specified in the DAIDS SCORE Manual (edits not shown here). The SCORE Manual is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

B. Protocol Team Roster Updates

To reflect current protocol team membership, Michael Whitton is added as a Clinical Trials Specialist; Yvonne Woolwine-Cunningham is replaced by Rebecca Dirschberger as a Protocol Data Manager; Scott Watson is replaced by Aundria Charles as the Westat Representative; and address information is updated for Diane Costello. Contact details are shown below. Michael Whitton is also added as a Clinical Trials Specialist on the protocol cover page.

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C. Study Site Roster Updates

The investigator at Clinical Research Site 4001 has determined that the site is not expected to enroll any participants in this study; as such, this site is removed from the roster (deletions not shown).

To reflect recent administrative changes, the site number assigned to the Texas Children's Hospital Clinical Research Site is changed from 3801 to 5128; the site number assigned to the Pediatric Perinatal HIV Clinical Trials Unit Clinical Research Site is changed from 4201 to 5127; and Feiona Heaven is replaced by Kathleen Graham as the Study Coordinator at Site 5055 (contact details are shown below).

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In addition, two sites are added:

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D. Additional Guidance for Specimen Collection During the COVID-19 Pandemic

Protocol Appendix VI provides operational guidance for study implementation at sites experiencing operational disruptions due to COVID-19. Clinical and laboratory supplies required for specimen collection and storage are in short supply globally; as such, procurement delays and stock-outs may occur. The following guidance is added as the fourth bullet point under Prioritization of Study Visit Procedures:

- **In the event that required specimen collection supplies are not available at a visit when nasopharyngeal and rectal swabs are specified to be collected and stored, this specimen collection may be deferred until the next visit occurring after the required supplies are available.**

IMPAACT 2028

Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

A Study of the International Maternal Pediatric Adolescent AIDS Clinical Trials Network

Sponsored by:
National Institute of Allergy and Infectious Diseases
Eunice Kennedy Shriver
National Institute of Child Health and Human Development
National Institute of Mental Health

DAIDS Study ID #38693
Non-IND Study

Protocol Chair:	Shaun Barnabas, MD, PhD
NIAID Medical Officers:	Hans Spiegel, MD, PhD Dwight Yin, MD, PhD
NICHD Program Officers:	Eric Lorenzo, PhD Sai Majji, PhD
Clinical Trials Specialist:	Anne Coletti, MS

FINAL Version 1.0
23 December 2020

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

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PROTOCOL SIGNATURE PAGE

I will conduct this study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Council for Harmonisation Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

_____ _____

Signature of Investigator of Record Date

_____

Name of Investigator of Record
(printed)

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
bNAb	Broadly neutralizing monoclonal antibody
CFR	Code of Federal Regulations
CI	Confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case report form
CRS	Clinical research site
DAIDS	Division of AIDS
DAIDS PRO	Division of AIDS Protocol Registration Office
ddPCR	Droplet digital polymerase chain reaction
DMC	Data Management Center
DNA	Deoxyribonucleic acid
DTaP	Diphtheria, tetanus, and pertussis vaccine
EC	Ethics committee
eCRF	Electronic case report form
GLP	Good Laboratory Practice
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICF	Informed consent form
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
IQA	DAIDS Immunology Quality Assurance program
IRB	Institutional Review Board
LDMS	Laboratory Data Management System
LPC	Laboratory Processing Chart
mAb	Monoclonal antibody
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NIMH	National Institute of Mental Health
NIH	United States National Institutes of Health
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PID	Participant Identification Number
RNA	Ribonucleic acid
RR	Risk ratio
RSC	Regulatory Support Center
SD	Standard deviation
SDMC	Statistical and Data Management Center
SES	Subject Enrollment System
SID	Study Identification Number
sIRB	Single Institutional Review Board
SMC	Study Monitoring Committee

SOP	Standard operating procedure
TB	Tuberculosis
US	United States
VQA	DAIDS Virology Quality Assurance program
WHO	World Health Organization

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

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IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

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IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

SCHEMA

Purpose:	To characterize a cohort of early treated children who may participate in future research related to HIV remission or cure
Design:	Observational prospective cohort study
Study Population:	Children living with perinatally-acquired HIV who received early treatment in IMPAACT network studies or other research studies sponsored by the United States National Institutes of Health. Early treatment is defined as treatment with at least three antiretroviral agents from at least two classes of antiretroviral therapy initiated within 12 weeks of birth. Early treatment regimens may also include broadly neutralizing antibodies (in addition to at least three antiretroviral agents). Within the overall study population, children who initiated treatment within 48 hours of birth will be classified as having received very early treatment.
Sample Size:	Up to approximately 250 participants
Study Intervention:	No intervention is provided in this study
Study Duration:	Approximately seven years
Objective:	To characterize the long-term clinical, immunologic, and virologic profiles of children who received early treatment for perinatally-acquired HIV

1 INTRODUCTION

1.1 Background

In 2013, the World Health Organization (WHO) recommended antenatal HIV testing and immediate initiation of antiretroviral therapy (ART) for all pregnant women living with HIV, resulting in a dramatic decline in perinatal HIV transmission (1). Nevertheless, there were still 160,000 new pediatric HIV infections in 2018, with only 50% of these children accessing ART (2), thus emphasizing the need for HIV remission and cure research in this age group.

During acute infection, HIV establishes a latent reservoir in long-lived resting memory CD4+ T cells (3). These latent cells harboring integrated, intact provirus are susceptible to reactivation and virus production during ART administration. Treatment interruption results in rebound viremia (4) and presents one of the main barriers to cure. Untreated perinatal HIV infection is characterized by high levels of viremia that takes years to reach steady-state levels (5) compared to the rapid reduction, within weeks, seen in adult infection (6) and that reflects the delayed development of HIV-specific CD8+ T cell responses in perinatal infection (7). Early and very early ART (defined as treatment within 12 weeks or 48 hours of birth, respectively) is lifesaving as 50% of untreated infants living with HIV in Africa die by age two (8). Early and very early ART also limits latent reservoir size and reduces the persistence of long-lived HIV- infected cells (9). Additionally, virologically suppressed adolescents with controlled HIV replication from infancy due to effective ART have a lower reservoir size than adolescents in whom HIV replication was poorly controlled until later in childhood (10). Achieving low HIV reservoir size through early or very early ART provides a permissive state for HIV ART-free remission or cure.

With effective early or very early treatment of perinatal HIV infection, there is a lack of HIV-specific immune responses and very low reservoir size (11-13). The “Mississippi baby” was the first case of perinatal ART-free remission in which persistent viral suppression was seen for 27.6 months after ART interruption. This child initiated very early ART, by 30 hours of life, with viral suppression achieved by day 28. After unplanned treatment interruption at 18 months of age, a prolonged period of HIV remission was observed. The rebounding virus was confirmed by phylogenetic analysis to be maternal transmitted. This delayed re-emergence of transmitted virus supports the idea that prolonged viral remission is achievable through time-limited very early ART initiation after perinatal infection, but does not signify cure. This case formed the basis for IMPAACT P1115, Very Early Treatment of HIV-Infected Infants to Achieve Remission, the Treating Infants Early Study (TIES), the Latency and Early Neonatal Provision of Antiretroviral Drugs (LEOPARD) (all described below), and the Early Infant Treatment study in Botswana (14).

Following the case description of the “Mississippi baby,” long-term remission was also reported in the “South African child,” identified at 9.5 years of age, who initiated early ART. This child’s viral load was less than 50 copies/mL by week 24 of treatment and less than 20 copies/mL by week 50. He underwent planned ART interruption at week 50 of treatment and had sustained viral suppression through 9.5 years of age, with no HIV-specific antibody responses, apart from weak CD4+ T cell responses to Gag and gp120 and a very strong anti-CD16 induced natural killer cell response (15). Similarly, a French teenager who initiated early ART at three months of age and underwent unplanned treatment interruption between 5.8 and 6.8 years of age has maintained viral suppression more than 12 years off treatment with a viral load less than 50 copies/mL, in association with weak HIV-specific CD8+ T-cell responses and low level T-cell activation (16). None of these three cases exhibited the HLA-alleles associated with the elite controller status

seen in adults (15), confirming that in a subset of children with perinatally-acquired HIV, early ART may promote ART-free remission.

Early and continued treatment provides immunologic and virologic benefits to infants living with perinatally-acquired HIV. ART initiated within eight days of life can produce viral suppression as early as six days after initiation, with continued viral decay of cellular reservoirs over twelve months (17). Viral decay in early and continuously treated infants occurs more rapidly than with delayed treatment or among those who initiated treatment early but underwent interruption and then recommenced treatment (18). Notably, very early treatment demonstrated a more rapid decline of intact HIV DNA sequences compared to the decline of defective virus (14), potentially decreasing the likelihood of continued effective viral replication after treatment interruption. Altogether, these findings indicate that early treatment of perinatally-acquired HIV alters the establishment and dynamics of HIV reservoir in infancy. Further, there is evidence that early ART preserves immunity and vaccine responses to routine childhood vaccines, supporting the notion that these children may be ideal candidates for cure or remission-targeted trials.

The number of ARV drugs available for treating HIV in infancy is extremely limited; however, treatment options may be expanded through the use of injectable, long-acting broadly neutralizing monoclonal antibodies (bNAbs). The antiviral properties of bNAbs are largely mediated through virus neutralization, although immune-enhancing effects have been seen in non-human primates (with enhanced endogenous neutralizing antibody responses and decreased disease progression) and in adults living with HIV (with increased antibody breadth and/or potency and an increase in heterologous neutralizing activity), raising the possibility of a "vaccinal effect" with enhancement of HIV-specific immune responses (19-22). The effects of combining early ART with bNAbs on HIV reservoir size and HIV-specific immune responses are being studied in IMPAACT P1115 and IMPAACT 2008. The long-term immune effects of early ART or bNAbs in the treatment of HIV infection through childhood have not yet been described and will be examined in this study.

IMPAACT P1115

This study is currently ongoing. Enrollment under protocol Version 1.0 occurred between January 2015 and December 2017 (54 infants with in utero HIV infection were enrolled). Enrollment under protocol Version 2.0 is expected to continue through 2023 (45 infants with in utero HIV infection are expected to be enrolled). Follow-up of participants enrolled under Version 1.0 and Version 2.0 is expected to continue through 2027. All infants with in utero infection receive very early treatment in this study.

The primary objective of IMPAACT P1115 (NCT02140255) is to assess HIV remission among neonates with *in utero* HIV infection who initiate very early intensive ART within 48 hours of birth. In Version 1.0 of this study, infants at high risk for *in utero* infection, i.e., those born to mothers with presumed or confirmed HIV who did not receive ARVs during pregnancy, were enrolled in Cohort 1 within 48 hours of birth and started nevirapine-based ART while being evaluated for *in utero* HIV transmission. If *in utero* transmission was excluded, these infants were switched to standard of care perinatal prophylaxis and exited the study at four weeks of age. Infants who had initiated an ART regimen within 48 hours of life outside of the study but were confirmed to have *in utero* transmission were enrolled in Cohort 2 within the first 10 days of life.

Cohort 1 and Cohort 2 infants with confirmed *in utero* HIV infection entered Step 2 of the study and continued nevirapine-based ART. Lopinavir/ritonavir was added after 42 weeks postmenstrual age for a four-drug ART regimen. Nevirapine was withdrawn once virologic suppression for at least 12 consecutive weeks was confirmed. If virologic suppression was achieved by Week 24 and maintained to Week 48 and beyond, evaluation for potential treatment

cessation began. If strict protocol-specified criteria are met at or after Week 96 (e.g., no detectable HIV DNA by droplet digital PCR (ddPCR), negative HIV antibody by fourth generation ELISA), infants enter Step 3 of the study for ART cessation. In this study, remission is defined as having no confirmed plasma HIV RNA greater than or equal to the limit of detection (LOD) of the assay through 48 weeks of ART cessation. If viral rebound occurs in Step 3, participants enter Step 4, resume ART, and are followed through five years of age. IMPAACT P1115 participants who do not achieve undetectable HIV RNA between Week 48 and Week 192 are not eligible for treatment cessation and exit the study. Participants who maintain undetectable HIV RNA but do not enter Step 3 by Week 192 also exit the study. All participants with confirmed *in utero* HIV infection who have exited from Version 1.0 will be eligible for IMPAACT 2028 (23).

In P1115 Version 2.0, participants are treated with raltegravir, nevirapine, and two nucleoside reverse transcriptase inhibitors, with or without the bNAb VRC01. Infants in whom *in utero* HIV infection is not confirmed transition to standard of care prophylaxis by two weeks of age and are followed for 12 weeks before exiting the study; any of these infants identified with HIV infection acquired through the 12-week follow-up period will be eligible for IMPAACT 2028 if they initiated treatment, with at least three ARV agents from at least two classes of ART, within 12 weeks of life.

Infants in P1115 Version 2.0 with confirmed *in utero* transmission progress through Steps 2-4 as described above for Version 1.0. However, regardless of viral RNA suppression status, these infants will be followed on P1115 for at least 192 weeks (approximately four years); after exiting P1115, they will be eligible for IMPAACT 2028.

IMPAACT 2008

This study is currently ongoing. Enrollment occurred between August 2018 and March 2020 (61 infants were enrolled, all received early treatment). Follow-up is expected to continue through March 2021.

IMPAACT 2008 (NCT03208231) is evaluating the safety and antiviral activity of the bNAb VRC01 in combination with ART to promote HIV clearance in infants. The study population includes 61 infants who began ART within 12 weeks of birth at IMPAACT sites in Botswana, Brazil, Malawi, and Zimbabwe. Approximately half of the enrolled infants were randomly assigned to receive VRC01 in addition to ART at study entry and weeks 2, 6, and 10. All infants are followed for 48 weeks.

Pharmacokinetics, safety, development of anti-VRC01 antibodies, time to viral suppression, biomarkers of viral persistence and immune responses are also evaluated in this study. Given the 48-week follow-up period, capacity to examine the long-term virologic and immunologic effects of this novel therapeutic approach is limited; long-term evaluation will be facilitated in IMPAACT 2028.

The participants in both IMPAACT P1115 and IMPAACT 2008 are unique with respect to their treatment intervention and anticipated low reservoir diversity and size. They will have well-characterized early virologic and immune measurements collected and stored. It is important to better understand the longer-term effects of these novel interventions on HIV reservoirs and on immune and viral profiles to inform future interventions.

Latency and Early Neonatal Provision of Antiretroviral Drugs (LEOPARD)
This study was conducted between August 2015 and April 2020. Seventy-three infants were enrolled; 46 received very early treatment, 27 received early treatment.

The LEOPARD study (NCT02431975) identified infants in South Africa with *in utero* transmission of HIV and initiated nevirapine-based ART within 14 days of life. At 42 weeks postmenstrual age, nevirapine was changed to lopinavir/ritonavir per the local standard of care. The study examined changes to the viral reservoir and detection of HIV-specific antibody responses over time, with a primary outcome of identifying the percent of participants with viral suppression (plasma HIV RNA less than 400 copies/mL by 24 weeks after ART and less than 50 copies/mL by 48 weeks of age and no confirmed viral load greater than 50 copies/mL after suppression was attained). The study also aimed to determine the relationship between markers of neonatal immune quiescence, the extent of seeding, and rate of decline of the viral reservoir in early treated infants. During the first year of treatment, ART initiated within 48 hours after birth (i.e., very early treatment as defined for IMPAACT 2028) was observed as a significant contributory predictor of proviral DNA reservoir size, when compared to later ART initiation (24). Data published from this cohort in early 2020 examined the effects of early and very early treatment (as defined for IMPAACT 2028) on the potential for HIV remission (25). The study team concluded that decisions regarding ART initiation should be based on the optimization of clinical outcomes rather than the achievement of disease remission as few infants met the defined laboratory criteria for treatment interruption. LEOPARD study participants will be eligible for IMPAACT 2028.

Treating Infants Early Study (TIES)
This study is currently ongoing. Enrollment occurred between December 2015 and September 2020 (15 infants were enrolled, 13 received early treatment, 2 received very early treatment). Follow-up is expected to continue through December 2020.

The Treating Infants Early Study is an observational study of infants living with HIV in the US who initiated early ART with at least three ARVs within the first six weeks of life. The primary objective is to assess the safety of initiating ART and characterize the decline in HIV RNA over time. In addition, biological samples have been collected and stored for future studies of HIV reservoirs and HIV immune responses. These children will be eligible for IMPAACT 2028.

Children with HIV Early Antiretroviral (CHER) Trial
This study was conducted between July 2005 and January 2013. Three hundred and seventy-seven infants were enrolled; all received early treatment.

In the CHER trial (NCT00102960), South African infants living with HIV 6-12 weeks of age with a CD4+ cell percentage greater than 25% were randomly assigned to receive ART when the CD4+ cell percentage decreased to less than 20% (or less than 25% if the child was younger than one year) or when clinical criteria were met (the deferred therapy group) or to immediate ART initiation until either one or two years of age (the early ART groups). Two hundred and fifty-two infants received early therapy at a median of 7.4 weeks of age, which reduced early infant mortality by 76% and HIV progression by 75% when compared with infants in the deferred therapy group (26, 27). An additional 34 infants were randomized to early limited ART for either one or two years but were not included in the primary analysis. The CHER children are now 12-14 years of age and are eligible for IMPAACT 2028.

In the remainder of this protocol, the term "parent study" is used to refer to the IMPAACT P1115, IMPAACT 2008, CHER, LEOPARD, and TIES studies, from which participants in IMPAACT 2028 will be drawn. Table 1 presents the number of participants enrolled in each parent study (projected for IMPAACT P1115) and the Protocol Team's estimate of the approximate number of children who may be enrolled in IMPAACT 2028 from each parent study. These estimates reflect allowances for loss-to-follow-up during the parent studies and estimates of the likelihood of re-contact following the last contact for the parent study. Because the likelihood of re-contact cannot be estimated with certainty, the total of 250 reflects that the maximum number that may be enrolled rather than a target number to be enrolled.

Table 1
Potential Enrollment from Parent Studies

Parent Study *dates of study implementation*	Number Enrolled in Parent Study	Number Who May Enroll in IMPAACT 2028	Number Very Early Treated	Number Received bNAb
IMPAACT P1115 *January 2015 to present (ongoing)*	99	64	64	15
IMPAACT 2008 *August 2018 to present (ongoing)*	61	61	0	30
CHER *July 2005 to January 2013*	377	70	0	0
LEOPARD *August 2015 to April 2020*	73	40	25	0
TIES *December 2015 to December 2020*	15	15	2	0
Total	625	250	91	45

1.2 Rationale

Early treatment of infants living with HIV offers a unique population in which to investigate long-term clinical, immunologic, and virologic outcomes and to better characterize how early ART impacts HIV infection over time. The overarching goals of IMPAACT 2028 are to establish long-term follow-up of participants who received early treatment for future interventional cure and/or remission trials and to establish a unique biorepository from which additional scientific questions can be answered. In lower and middle-income settings, where the majority of these trial participants live, the significant mobility of enrolled participants may reduce access for future studies, particularly if they are currently not receiving care at a clinical research site. The durability of early treatment, the effect of acquired co-infections on reservoir dynamics, the long-term effects of ART and/or bNAbs on a maturing immune system and on the evolution of HIV-specific neutralizing antibodies, cell-mediated immunity, systemic immune activation, inflammation, and the predictive value of biomarkers associated with increased morbidity and mortality in adults are poorly understood in children. It is thus anticipated that IMPAACT 2028 will longitudinally track these participants while simultaneously advancing our understanding of the long-term effects of early ART and/or bNAb initiation through establishing a biorepository to address scientific questions and promote innovation in this field.

Study Design and Population

This is an observational prospective cohort study characterizing and establishing a biorepository of early treated children who may participate in future research related to HIV remission or cure. Early treatment is defined as treatment with at least three ARV agents from at least two classes of ART initiated within 12 weeks of birth. Within the overall study population, for descriptive and analysis purposes, children who initiated treatment within 48 hours of birth will be classified as having received very early treatment. A subset of children will have received bNAbs as part of their treatment regimens.

This study design was chosen to facilitate rollover from US and international studies of early treatment to gain a longer-term perspective on clinical, virologic, and immunologic trajectories, as well as to identify participants for inclusion in future cure and/or remission studies. Although it is anticipated that many participants will have achieved viral suppression owing to their participation in prior studies, current detectable viremia is not exclusionary. This will allow for examination of differences in trajectories based on the presence or absence of viral suppression and permit identification of comparison groups within the cohort. Likewise, participants may receive any ART or bNAbs prior to or following enrollment, potentially permitting inquiry into whether particular drug combinations influence clinical, virologic, or immunologic responses.

Up to approximately 250 children living with HIV who received early treatment in IMPAACT network studies or other research studies sponsored by the US National Institutes of Health (NIH) will be enrolled and followed semi-annually for up to seven years. Clinical, virologic, and immunologic evaluations will be performed semi-annually at each study visit, and specimens will be collected for the study's biorepository for future investigations. This sample size is based on ongoing or completed studies of infants who received early treatment and feasibility of enrolling these infants into a long-term study. Given the lack of longitudinal data on this group, the development of a biorepository will address scientific questions and contribute to the literature on early ART and its impact on HIV reservoir size in this unique population.

Real Time and Batched Laboratory Evaluations

CD4+ and HIV-1 RNA assays will be performed in real time at each study visit. ARV resistance assays will also be run in real time when clinically indicated. The results of these clinically relevant assays will be provided to participants/parents/guardians and to participants' primary care providers.

HIV-1 DNA and HIV-1 antibody assays will be performed in batches approximately every six months. The results of these assays will establish basic virologic and immunologic biomarker profiles for the study cohort and provide snapshots of the state of HIV persistence in the cohort over time. The results will also inform hypotheses to be investigated with use of specimens stored in the study repository. For researchers developing future studies of interventions for HIV-1 remission and cure, results may serve to guide study eligibility criteria.

Selection and optimization of assays to quantify the size of the HIV reservoir is an active area of HIV-1 cure-related research. The single-amplicon HIV-1 DNA ddPCR assay was selected for use in this study because it offers a feasible approach to rapidly characterize large numbers of participants with different HIV-1 subtypes for whom reservoir size may be quite small due to receipt of early or very early ART. The assay is fully validated and CLIA-certified, and can be performed with non-viable PBMCs that do not require liquid nitrogen for transport. Although this assay may overestimate the size of the replication competent reservoir due to the preponderance of defective HIV-1 genomes (28), it provides a benchmark biomarker to which the results of other measures of replication competence can be referenced.

Other HIV-1 DNA assays, for example the intact proviral DNA assay, which allows quantitation of intact and defective genomes, may be performed with specimens stored in the study repository. As assay development and validation for different HIV-1 subtypes progresses over time, the Protocol Team may choose to replace the single amplicon HIV-1 DNA ddPCR assay with an alternative assay. When such a choice is made, a protocol clarification memorandum will be issued to specify the new assay to be performed.

Specimen Repository

Specimens for biorepository storage will be collected throughout the study follow-up period. Plasma, PBMCs, and serum will be collected at each visit; nasopharyngeal and rectal swabs will be collected once, at the first visit occurring after the participant has reached three years of age (28). These specimens will be used in investigations related to the clinical, immunologic, and virologic effects of early treatment. Specimens may be accessed by investigators via a competitive proposal-based process. For illustrative purposes, a listing of potential investigations is provided in Table 2.

Table 2
Potential Investigations using Specimens Collected and Stored in IMPAACT 2028

Illustrative Hypotheses	Illustrative Outcome Measures
Immunologic	
Very early compared to early ART will lead to improved immune health and lower levels of immune activation and exhaustion	• CD4+ and CD8+ cell counts and percentages • Expanded T cell immunophenotyping • HIV-1 antibody profiles
Addition of a bNAb to very early and early ART will lead to improved immune outcomes	• CD4+ cell counts and percentages • CD8+ cell counts and percentages • Expanded B and T cell immunophenotyping
Very early compared to early ART will lead to more durable responses to routine childhood vaccines	• Durable antibody responses to routine childhood vaccines
Addition of a bNAb to very early and early ART will induce a vaccinal effect and manifest as durable HIV-1-specific responses	• Breadth of HIV-specific humoral and cellular immune responses
Very early compared to early ART will lead to less gut dysbiosis	• Microbiome differences in very early versus early ART
A healthy microbiome is correlated with immune health in perinatal HIV infection	• Expanded B and T cell immunophenotyping • Vaccine responses
Intermittent episodes of viremia may boost autologous HIV-1 neutralization antibody responses and HIV-1 cellular immune responses	• In-depth profiling of functional HIV-1 specific immune responses
Virologic	
Very early compared to early treatment will lead to sustained lower reservoir size including lower intact proviral reservoir size	• Reservoir size as measured by total HIV-1 DNA • Composition of HIV-1 reservoir by intact and defective genomes

Addition of a bNAb to very early and early ART will lead to lower intact proviral reservoir size	• Composition of HIV reservoir by intact and defective genomes
Addition of a bNAb to very early and early ART will modify the reservoir composition	• HIV-1 env analyses for sequence diversity and neutralization
Intermittent episodes of viremia may lead to clonal amplification or depletion of HIV-1 reservoir cells	• Changes in HIV-1 reservoir size and composition with near full-length sequencing • Changes in the proportion of intact and defective genomes, degree of clonal expansion, and contribution of reservoir cells to rebound virus

As part of the informed consent and assent (if applicable) process, participants/parents/guardians will be informed that specimens collected for the repository may be used for testing related to HIV or other diseases with opt in or opt out choices for genetic testing of these specimens.

Collection of Nasopharyngeal and Rectal Swab Specimens

Gut mucosal damage, associated with HIV infection, is characterized by CD4+ T cell depletion, immune activation and translocation of microbial products (30). HIV infection influences the composition of the gut microbiome with recent HIV infection associated with transient loss of bacterial taxonomic richness, while chronic infection showed a depletion of *Akkermansia*, *Anaerovibrio*, *Bifidobacterium*, and *Clostridium* (30). Further, children with HIV have an increased alpha-diversity and higher inflammatory plasma factors than children without HIV, which is associated with specific microbiome profiles (32). Cotrimoxazole therapy alters the gut microbiome of children living with HIV and decreases inflammation (32). The interaction between the gut microbiome, immune system, and inflammation in children living with HIV is complex and has not yet been fully characterized.

The composition of the gut microbiome influences the development of the immune system (33, 34). Some of the individual variation in vaccination response is thought to be secondary to gut microbial changes. In 2018, a systematic review of one adult and three infant studies examined the association between the composition of the gut microbiome and vaccine responses. A higher relative abundance of *Actinobacteria* and *Firmicutes* was associated with increased humoral and cellular vaccine responses, while increased *Proteobacteria* and *Bacteroidetes* decreased responses (35-37). The lipopolysaccharide derived from several *Bacteroidetes* species is less immunogenic or possibly immune-inhibitory and may cause the decreased responses seen (38). A relative abundance of *Bifidobacterium* in early infancy was associated with increased CD4 T-cell responses to BCG, tetanus toxoid, and hepatitis B vaccines (39). Moreover, interventions that changed the gut microbiome composition were associated with altered immune responses to the influenza vaccine (40). There is a paucity of data on the impact of ART on the microbiome of early treated children that warrants further research (41). In adult studies, ART administration was associated with decreased alpha diversity when compared to people living with HIV who are not on ART.

Much less is known about the interactions of the respiratory microbiome and the immune system. However, there is growing evidence of a bidirectional interaction between the two (41-44).

Murine studies have shown that bacterial load in the lungs increases during the first two weeks of life with changes in the microbiota associated with accumulation of a PD-L1-dependent T regulatory cell population (45). The acquisition of a normal lung microbiome has been shown as important in protecting the lung from harmful responses to inhaled antigens (45).

In asymptomatic humans an increased abundance of *Prevotella* and *Veillonella* species has been associated with higher levels of lymphocyte and neutrophil inflammation in bronchoalveolar lavage fluid (47), while in chronic lung disease, the ability of lung microbiota to modulate local inflammatory responses may influence disease progression (47). Moreover, a recent study utilizing PET-MR technology scan with HIV-env specific monoclonal antibody VRC01 demonstrated a significant presence of HIV within nasal turbinates suggestive of an HIV reservoir site, which increases interest in evaluating a respiratory microbiome that may be an HIV reservoir site (49).

The gut microbiome may also influence respiratory health via the gut-lung axis with oral probiotics shown to be beneficial in preventing ventilator-assisted pneumonia and upper respiratory tract infections in adults (49, 50). There appears to be a complex interplay between the development of the gastrointestinal and respiratory microbiota and the regulation of immune function with bacterial metabolites, such as short-chain fatty acids, influencing respiratory health (52). This lung-gut axis may be bi-directional, with mouse studies showing that exposure of the lungs to lipopolysaccharide results in changes to the bowel flora (52).

There is a growing interest in understanding the role of the human microbiome in child health, as dysbiotic microbial communities have been implicated in a variety of adverse outcomes. HIV infection is known to influence both microbial composition and systemic immunity, while immunity in both the gut and the lung is affected by changes in the local microbiome. Thus, a better understanding of these interactions could help explain some of the alterations in immunity that are seen in children living with HIV.

Summary

This study aims to characterize the long-term clinical, immunologic, and virologic profiles of children who received early treatment for HIV. Secondarily, it will establish a pediatric cohort who received early treatment for HIV and may be eligible for future studies of interventions for HIV remission or cure. Lastly, it will establish a biorepository for further investigation of the long-term effects of early ART and the developing immune system. This study is crucial to the evolution of the scientific community's understanding of the longitudinal effects of early treatment on the progression of HIV. This unique population will add to the body of literature supporting early infant interventions and has the potential to provide novel and innovative insights into HIV cure and remission.

2 OBJECTIVE

The objective of this study is to characterize the long-term clinical, immunologic, and virologic profiles of children who received early treatment for perinatally-acquired HIV.

3 STUDY DESIGN

This is an observational prospective cohort study to characterize the long-term clinical, immunologic, and virologic profiles of early treated children who may participate in future research related to HIV remission or cure. Up to approximately 250 children who received early treatment for perinatally-acquired HIV in IMPAACT network studies or other research studies sponsored by the US NIH will be enrolled and followed semi-annually for up to seven years. As shown in Appendix I and detailed in Section 5, clinical, virologic, and immunologic evaluations will be performed at each study visit; specimens will be collected for the study's biorepository at each visit.

Early treatment is defined as treatment with at least three ARV agents from at least two classes of ART initiated within 12 weeks of birth. Early treatment regimens may also include bNAbs. Within the overall study population, for descriptive and analysis purposes, children who initiated treatment within 48 hours of birth will be classified as having received very early treatment. Data analyses planned to fulfill the primary study objective are described in Section 7.

Data and specimens collected in this study will be utilized for additional research related to HIV remission or cure via a competitive proposal-based process. Proposals for use of data and/or specimens will be solicited and reviewed on a routine basis following a process described in the study-specific manual of procedures. Proposals received from early career investigators will be prioritized for approval.

4 STUDY POPULATION

Children will be selected for this study according to the criteria in Sections 4.1 and 4.2 and the guidelines in Section 4.3. The study-specific approach to recruitment, screening, and enrollment is described in Section 4.4. Considerations related to participant retention and withdrawal from the study are provided in Sections 4.5 and 4.6, respectively.

4.1 Inclusion Criteria

4.1.1 Participated in one of the parent studies listed in Table 1 on page 26 (documentation should be obtained from parent study records and should minimally include the date of enrollment and date of final visit in the parent study).

4.1.2 Confirmed HIV-1 infection based on documented nucleic acid testing of two blood samples collected at different time points (documentation should be obtained from parent study records and should minimally include dates of specimen collection, tests performed, and test results)

4.1.3 Received early treatment for HIV-1 infection, defined as treatment with at least three ARV agents from at least two classes of ART, initiated within 12 weeks of birth (documentation should be obtained from parent study records and should minimally include the date of initiation of the early treatment regimen and each agent in the regimen)

4.1.4 Based on parent or guardian report at entry, child is expected to be available for at least 24 months of follow-up

4.1.5 Parent or guardian is willing and able to provide written informed consent for child's study participation and, when applicable per institutional review board/ethics committee (IRB/EC) policies and procedures, child is willing and able to provide written assent for study participation

Note: All sites must follow all applicable IRB/EC policies and procedures; for US sites, this includes single IRB (sIRB) policies and procedures.

4.2 Exclusion Criteria

Potential participants who meet the following criterion will be excluded from the study:

4.2.1 Has any documented or suspected clinically significant medical condition or any other condition that, in the opinion of the site investigator, would make participation in the study unsafe or otherwise interfere with completing study procedures

Note: Periods of treatment interruption are not exclusionary.

4.3 Co-Enrollment Considerations

Co-enrollment in other studies, concurrent with participation in IMPAACT 2028, is not precluded, although careful consideration must be given to visit burden, blood draw volumes, and interpretation of outcome data across studies.

Participants enrolled in this study will ideally be eligible for future studies of interventions for HIV remission or cure. For participants who become eligible for interventional studies, every effort will be made to accommodate co-enrollment in the interventional study and IMPAACT 2028. For example, study sites would be encouraged to abstract clinical findings and laboratory test results from interventional study records for entry into IMPAACT 2028 electronic case report forms (eCRFs), rather than duplicate these evaluations for IMPAACT 2028. Further operational guidance on managing periods of co-enrollment will be provided in the study-specific manual of procedures.

Regardless of the type of studies in which participants may be co-enrolled, co-enrollment must be approved in advance by the Protocol Teams of both studies; requests for approval should be emailed to the IMPAACT 2028 Protocol Team (impaact.team2028@fstrf.org).

4.4 Recruitment, Screening, and Enrollment Process

Participant recruitment methods for this study may vary across sites but are generally expected to rely on referrals to study staff from the parent studies listed in Section 1.

Upon identification of a potentially eligible child, study staff will provide information about the study to the potential participant and/or the potential participant's parent or guardian as appropriate based on the age and maturity of the potential participant, guided by site SOPs and applicable IRB/EC policies and procedures. Potential participants/parents/guardians who expresses interest in learning more about the study will be provided additional information, education, and counseling as part of the study informed consent and assent processes. The informed consent process will include detailed review of the study informed consent form (ICF),

time to address any questions or concerns the participant/parent/guardian may have, and an assessment of understanding before proceeding to the informed consent decision. Potential participants who meet applicable IRB/EC criteria for provision of assent will undergo an age-appropriate assent process. Informed consent and assent processes will be fully documented, consistent with the Division of AIDS (DAIDS) policies referenced in Section 8.2. Refer to Section 10.3 for further information on informed consent and assent procedures for this study.

Eligibility screening will be initiated after written informed consent, and assent if applicable, are obtained (i.e., informed consent, and assent if applicable, must be obtained before any study-specific screening procedures are performed). For potential participants who are found to be eligible, it is generally expected that screening and enrollment procedures will be completed at a single visit. However, multiple visits may be conducted. When more than one visit is required, all procedures must be performed within 60 days, counted from the date of informed consent. If the 60-day period is exceeded, the screening process may be repeated; in this case, most but not all screening evaluations must be repeated, as specified in Section 5.1.

Each site must establish standard operating procedures (SOPs) for eligibility determination that describe where and when screening procedures will be performed; roles and responsibilities for performing the required procedures; roles and responsibilities for assessing and confirming eligibility; and procedures for documenting the process, taking into consideration the required timing of enrollment.

The IMPAACT Data Management Center (DMC) Subject Enrollment System (SES) will be used to enroll participants in this study. When informed consent is obtained, a participant identification number (PID) will be assigned to the participant by study staff. For children found to be eligible, enrollment will occur upon successful entry of required eligibility data into the SES. Successful entry into the SES will generate a study identification number (SID). Refer to Section 7.5 for more information on monitoring participant accrual in this study.

4.5 Participant Retention

Study staff will make every effort to retain each enrolled child in follow-up for the protocol-specified duration of follow-up, thereby minimizing potential biases and loss of precision associated with loss-to-follow-up. Each site must establish SOPs for participant retention that describe procedures for retention-related activities such as collecting locator information, providing visit reminders, and taking action on missed visits in a timely manner. Given that study visits will occur relatively infrequently, procedures for between-visit contacts to verify locator information and other strategies to avoid loss-to-follow-up between visits should also be described. Refer to Section 7.5 for more information on monitoring participant retention in this study.

4.6 Participant Withdrawal or Termination from the Study

Regardless of the participant retention procedures referenced above, participants may be voluntarily withdrawn from the study by their parents or guardians; participants who meet IRB/EC criteria for providing assent may also withdraw from the study themselves. Participants may be terminated from the study by the site investigator or designee under the following circumstances:

- Participant re-locates away from the study site (with no options to transfer to another site), is otherwise unable to attend study visits, or is determined to be lost-to-follow-up
- Investigator or designee determines that continued participation in the study would be unsafe or otherwise not in the best interest of the participant, after consultation with the Protocol Team
- The study is stopped or canceled by the sponsors or government or regulatory authorities
- Site participation in the study is canceled by the sponsors, government or regulatory authorities, the sIRB (for US sites), or site IRBs/ECs (for non-US sites)

For any participant who is withdrawn or terminated from the study prior to scheduled completion of follow-up, study staff will document the reason for the withdrawal or termination in detail and will make every effort to complete final evaluations for the study, following the procedures shown in Section 5.2. If the circumstances that led to a participant's withdrawal or termination change (e.g., he or she returns to the study site area after having re-located previously), the site investigator or designee should contact the Protocol Team to discuss options for resuming follow-up.

5 STUDY VISITS AND PROCEDURES

An overview of the study visit and evaluation schedule is provided in Appendix I; blood draw volumes for each visit are also detailed in Appendix I. Presented in this section is additional information on visit-specific study procedures.

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with site SOPs, including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent and assent; scheduling visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. Locator contacts are encouraged between scheduled visits to maximize participant retention. All such tasks should be documented consistent with site SOPs.

Study staff should inform participants/parents/guardians of clinically meaningful physical exam findings and laboratory test results when available; given the semi-annual study visit schedule, site SOPs should specify expectations and procedures for providing results between scheduled visits when applicable. Study staff should provide results to participants' primary care providers when applicable and provide referrals to non-study sources of medical care or other services when clinically indicated.

Follow-up visits should be conducted as close as possible to target visit dates and within targeted visit windows (±4 weeks) whenever possible. When visits cannot be conducted within the targeted windows, care should be taken to conduct sequential visits at least 12 weeks apart. Visits may be split, with required procedures performed on more than one day within the allowable visit window if necessary.

All visits and procedures must be documented in accordance with the DAIDS policies for source documentation; refer to Section 8 for more information on documentation requirements and entry of eCRFs.

Note: For sites that may experience operational disruptions due to COVID-19, guidance for study implementation during periods of disruption is provided in Appendix VI.

5.1 Screening and Entry Visit

It is generally expected that all screening (eligibility determination) and enrollment procedures required for this study will be completed at a single visit. However, multiple visits may be conducted. When more than one visit is required, all procedures must be performed within 60 days, counted from the date of informed consent.

Informed consent must be obtained before any study-specific procedures are performed. Procedures that may provide information relevant to eligibility — including all administrative and regulatory procedures and the physical examination shown below — should be performed prior to enrollment. Clinical procedures may be performed before or after enrollment. Blood should be collected for laboratory evaluations and specimen storage after enrollment. Nasopharyngeal and rectal swabs should be collected after enrollment.

Note: Participants are considered enrolled in the study upon successful entry of required eligibility data into the SES.

Screening and Entry Visit Procedures	
Administrative and Regulatory	• Obtain written informed consent for child study participation; additionally obtain written assent if applicable per IRB/EC policies and procedures • Assign PID • Review parent study records and available medical records as needed in relation to study eligibility criteria • Assess expected availability for study participation in relation to study eligibility criteria • Complete final eligibility determination and confirmation • Complete paper-based eligibility checklist, enter checklist data into SES to enroll the participant, print and file a copy of the confirmation file
Clinical	• Collect baseline medical and medications history (see Section 5.4.1); includes ascertainment of gender identity if applicable) • Collect feeding history (see Section 5.5) • Perform physical examination (see Section 5.6)
Laboratory	• Collect blood for: – HIV-1 RNA PCR – HIV-1 DNA ddPCR – CD4+ and CD8+ cell counts and percentages – HIV-1 antibody – Serum, plasma, and peripheral blood mononuclear cell (PBMC) storage – ARV resistance testing if clinically indicated (following consultation with the Protocol Team) • *If child is three years of age or older,* collect nasopharyngeal and rectal swabs for storage

Note: Gender identity should be ascertained for participants who are 12 years of age and older as part of the medical and medications history obtained at this visit. However, this procedure should not be performed at sites that have formally opted out of collection of gender identity data, in accordance with policies specified in the IMPAACT Manual of Procedures. Gender identity data should be collected from participants in private, without a parent or guardian present.

If the 60-day screening period is exceeded before an eligible participant can be enrolled, the screening process may be repeated. In this case, all screening procedures listed above must be repeated, except that a new PID should not be assigned. Previously documented medical and medications history information should be reviewed and updated through the date of re-screening (it is not necessary to re-record history information that was previously documented).

5.2 Semi-Annual Follow-Up Visits

Follow-up visits are targeted to occur every 24 weeks, counted from the date of enrollment as Day 0, with a targeted window of ±4 weeks and an allowable window of ±12 weeks. Every effort should be made to conduct each visit within the targeted window; however, visits may be conducted on any day within the allowable window. When visits cannot be conducted within the targeted windows, care should be taken to conduct sequential visits at least 12 weeks apart. There is no required sequencing of procedures at these visits.

Note: For any participant who is withdrawn or terminated from the study prior to scheduled completion of follow-up, a final study visit should be performed, if possible, following the procedures described in this section. However, this visit need not be performed if a scheduled visit was completed within four weeks prior to the participant's withdrawal or termination.

Semi-Annual Visit Procedures ***(Q24 Weeks ±4 weeks targeted, ±12 weeks allowed)***	
Clinical	• Collect interval medical and medications history (see Section 5.4.2; includes ascertainment of gender identity if applicable) • *If applicable,* collect feeding history (see Section 5.5) • Perform physical examination (see Section 5.6)
Laboratory	• Collect blood for: – HIV-1 RNA PCR – HIV-1 DNA ddPCR – CD4+ and CD8+ cell counts and percentages – HIV-1 antibody *(Q48 weeks)* – Serum, plasma, and PBMC storage – ARV resistance testing if clinically indicated (following consultation with the Protocol Team) • *If child has reached three years of age since the last visit, and has not previously had swabs collected,* collect nasopharyngeal and rectal swabs for storage

Note: Gender identity should be ascertained for participants who were younger than 12 years of age at entry who subsequently reach 12 years of age while on study; this ascertainment should occur at the first follow-up visit after the participant reaches 12 years of age and should not be repeated thereafter. However, this procedure should not be performed at sites that have formally opted out of collection of gender identity data, in accordance with policies specified in the IMPAACT Manual of Procedures. Gender identity data should be collected from participants in private, without a parent or guardian present.

5.3 Interim Clinical Care Visits

Participants may return to the study site between scheduled follow-up visits for a variety of reasons. Participants needing clinical care are generally expected to be managed consistent with local clinical practice standards. When clinical care visits occur between scheduled study visits, study procedures are not expected to be performed and study data are not expected to be collected. Rather, the study visit and data collection schedule should be maintained, with clinical information of interest for the study (as indicated in Section 5.4.2) ascertained as part of the interval medical and medications history performed at the next scheduled visit.

5.4 Medical and Medications Histories

Medical and medication history information is collected at each scheduled visit. A baseline history is established at the Screening and Entry Visit and interval (since the last visit) histories are obtained at follow-up visits.

Note: The term "written records" is used in this section to refer to parent study records and/or non-study medical records. If a discrepancy is identified between data recorded in parent study records and data recorded in non-study medical records, data recorded in parent study records will be used.

5.4.1 Baseline Medical and Medications History

At the Screening and Entry Visit, the medical and medications history elements listed below should be source documented; all should also be entered into eCRFs.

Baseline History Element	Data Collection Details
Name of parent study, date of enrollment in parent study, identification number assigned in parent study, date of final visit in parent study, whether early or very early treatment was received	Must be abstracted from parent study records
Date of birth and sex at birth	Should be based on written records to the extent possible; may be based on participant or caregiver report
Gender identity (if applicable)	Must be based on participant report (if 12 years of age and older at entry)
Mode of delivery (vaginal delivery or Cesarean section)	Must be based on written records
Gestational age at birth and method of determination if available	Must be based on written records
Weight, length, and head circumference at birth	Must be based on written records
Weight and length/height at approximately two, four, and six months after birth and every six months thereafter	Must be based on written records; record documented measurements dated as close as possible to the targeted timepoints
Head circumference approximately two, four, and six months after birth and every six months thereafter through 24 months	Must be based on written records; record documented measurements dated as close as possible to the targeted timepoints

Baseline History Element	Data Collection Details
Diagnostic HIV testing details (specimen collection date, specimen collection time if available, test type, and result of initial positive test and of confirmatory test)	Must be based on written records
Date of early treatment initiation	Must be based on written records
Receipt of ARVs (all approved/licensed and investigational agents received for prophylaxis and/or treatment with start and stop dates since birth and reason for each ARV change)	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Any periods of planned ARV treatment interruption (relevant dates since birth)	Must be based on written records
Receipt of other HIV treatment agents (all approved/licensed and investigational agents received with start and stop dates since birth)	Must be based on written records
Receipt of immunizations (agents received with administration dates since birth)	Must be based on written records
Receipt of investigational vaccines or investigational agents for indications other than HIV	Must be based on written records
Receipt of TB prophylaxis or treatment (agents received with start and stop dates since birth)	Must be based on written records
Receipt of blood products (e.g., blood or plasma transfusions, immune globulins) (agents received with start and stop dates since birth)	Must be based on written records
Receipt of any antibiotic or antiprotozoal within the 30 days prior to microbiome swab collection (agents received and dates of receipt)	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Date and result of all documented CD4+ and CD8+ counts and percentages since birth	Must be based on written records
Date and result of all documented HIV-1 RNA tests since birth	Must be based on written records
Any documented ARV resistance (since birth)	Must be based on written records
WHO clinical stage at entry in IMPAACT 2028	Should be based on site investigator review of all available clinical and laboratory information from all sources
Any WHO clinical Stage 3 or Stage 4 condition or other medical condition of interest as listed in Appendix II (since birth)	Should be based on site investigator review of all available clinical and laboratory information from all sources
Any medical condition that required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, or was life-threatening (since birth)	Should be based on site investigator review of all available clinical and laboratory information from all sources

5.4.2 Interval Medical and Medications History

At Semi-Annual Follow-Up Visits, the medical and medications history elements listed below should be source documented; all should also be entered into eCRFs.

Interval History Element	Data Collection Details
If co-enrolled in an interventional study, name of interventional study, date of enrollment in interventional study, and date of final visit in interventional study	Should be abstracted from interventional study records if possible; may be based on communications from interventional study staff and/or participant or caregiver report
Gender identity (if applicable)	Must be based on participant report (if 12 years of age and older and not previously collected)
Receipt of ARVs (all approved/licensed and investigational agents received with start and stop dates and reason for each ARV change)	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Any periods of non-adherence when no ARVs were taken for seven consecutive days or longer (relevant dates)	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Any periods of planned ARV treatment interruption (relevant dates)	Must be based on written records
Receipt of other HIV treatment agents (all approved/licensed and investigational agents received with start and stop dates)	Must be based on written records
Receipt of immunizations (agents received with administration dates)	Must be based on written records
Receipt of investigational vaccines or investigational agents for indications other than HIV	Must be based on written records
Receipt of TB prophylaxis or treatment (agents received with start and stop dates)	Must be based on written records
Receipt of blood products (e.g., blood or plasma transfusions, immune globulins) (agents received with start and stop dates)	Must be based on written records
Receipt of any antibiotic or antiprotozoal within the 30 days prior to microbiome swab collection (agents received and dates of receipt)	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Receipt of blood products (e.g., blood or plasma transfusions, immune globulins) (agents received with start and stop dates)	Must be based on written records
Use of contraceptive methods	Should be based on written records to the extent possible; may be based on or supplemented with participant or caregiver report
Date and result of any documented non-study CD4+ and CD8+ cell counts and/or percentages	Must be based on written records
Date and result of any documented non-study HIV-1 RNA tests	Must be based on written records

Interval History Element	Data Collection Details
Any documented ARV resistance (based on non-study testing)	Must be based on written records
Any WHO clinical Stage 3 or Stage 4 condition or other medical condition of interest as listed in Appendix II	Should be based on site investigator review of all available clinical and laboratory information from all sources
Any medical condition that required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, was life-threatening, or resulted in death	Should be based on site investigator review of all available clinical and laboratory information from all sources
For participants who die, date of death, cause of death, and other narrative details pertaining to death	Should be based on site investigator review of all available clinical and laboratory information from all sources
For participants who become pregnant, Date of first day of last menstrual period prior to pregnancy, pregnancy outcome, date of pregnancy outcome	Should be based on site investigator review of all available clinical and laboratory information from all sources

5.5 Feeding History

At the Screening and Entry Visit, the following should be source documented and entered into eCRFs: whether the child was ever formula fed, date of first and last exposure to formula if applicable, whether the child was ever breastfed, date of first and last exposure to breast milk if applicable, and date of first exposure to complementary foods. For children who are formula feeding or breastfeeding at the Screening and Entry Visit, date of last exposure to formula or breast milk will be recorded (source documented and entered into eCRFs) at a subsequent Semi-Annual Follow-up Visit. These data should be collected based on written records to the extent possible and may be supplemented with caregiver report.

5.6 Physical Examinations

A physical examination is required at each scheduled visit. At each visit, examinations should include the following:

- Assessment of vital signs: temperature, heart rate, respiratory rate, and blood pressure
- Measurement of height and weight
- *If two years of age or younger*, measurement of head circumference
- Examination of:
  - General appearance
  - Head, eyes, ears, nose, neck, mouth, and throat
  - Lymph nodes
  - Lungs
  - Heart
  - Abdomen
  - Extremities
  - Skin
  - Neurologic
  - Sexual maturity
  - Other body systems if clinically indicated

At all visits, additional assessments may be performed at the discretion of the examining clinician.

All exam findings should be source documented; weight, height, head circumference, and sexual maturity rating will be entered into eCRFs. Findings may also contribute to identification of clinical events of interest, as listed in Section 5.4.

5.7 Laboratory Evaluations

HIV-1 RNA PCR assays and CD4+ and CD8+ cell counts and percentages will be performed in real time for this study locally; results of these study tests will be entered into eCRFs or transferred to the DMC via the Laboratory Data Management System (LDMS). ARV resistance tests will be performed in real time for this study locally, regionally, or centrally; results will be entered into eCRFs.

Note: HIV-1 RNA PCR assays, CD4+ and CD8+ cell counts and percentages, and ARV resistance tests may also be performed outside of the study; results of adequately documented non-study tests will be noted as part of the medical and medications histories described in Section 5.4 and will be entered into eCRFs.

HIV-1 DNA ddPCR and HIV-1 antibody (fourth generation or higher) assays will be performed in batches at designated testing laboratories. Results will be electronically transferred to the DMC.

5.8 Additional Considerations for Laboratory Procedures

Each study site and laboratory involved in this study will comply with the DAIDS policy on Requirements for DAIDS Funded and/or Sponsored Laboratories in Clinical Trials Policy, which is available at:
https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimens-management

5.8.1 Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Evaluations and per detailed guidance provided in the Laboratory Processing Chart (LPC), which will be available on the study-specific website: https://impaactnetwork.org/studies/IMPAACT2028.asp

In accordance with NIH recommendations, pediatric blood collection will not exceed 5 mL/kg in a single day or 9.5 mL/kg in any eight-week period. In the event that blood collection must be limited, available specimens should be prioritized for use in the following order: (1) HIV-1 RNA PCR, (2) CD4+ and CD8+ cell counts and percentages, (3) ARV resistance testing, (4) HIV-1 DNA ddPCR, (5) HIV-1 antibody, (6) stored plasma, serum, and PBMCs.

5.8.2 Specimen Preparation, Testing, Storage, and Shipping

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with the DAIDS policy referenced in Section 5.8, site and local laboratory SOPs, and the LPC. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations. The LDMS will be used to document specimen collection, testing, storage, and shipping as specified in LPC.

HIV-1 RNA PCR assays must be performed in a CLIA-certified or equivalent laboratory (for US sites) or a VQA-certified laboratory (for non-US sites). CD4+ and CD8+ cell counts and percentages must be performed in a CLIA-certified or equivalent laboratory (for US sites) or an IQA-certified laboratory (for non-US sites). PBMCs must be processed and cryopreserved by an IQA-approved laboratory (for all sites) consistent with the HIV/AIDS Network Coordination Cross-Network PBMC Processing SOP, which is available at: https://www.hanc.info/labs/labresources/procedures/Pages/pbmcSop.aspx

Specimens collected, processed, and stored at site laboratories are expected to be shipped to designated repositories approximately every six months. Specimens collected for HIV-1 DNA and HIV-1 antibody assays are expected to be shipped to the designated testing laboratories approximately every six months, either from the study sites or from the repositories. Alternative specimen shipping arrangements may be specified by the Protocol Team as needed. Refer to the LPC for detailed shipping instructions. Sites based in locations where local policies or regulations do not allow specimen storage in repositories will retain specimens locally until requested for testing.

5.8.3 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood, collection of nasopharyngeal and rectal swabs, and shipping and handling of all specimens for this study as currently recommended by the US Centers for Disease Control and Prevention, NIH, and other applicable agencies. All specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

5.9 Maternal Data Collection

Most but not all children enrolled in this study are expected to be in the care of their biological mothers and in many cases informed consent for child participation in the study will be provided by the biological mother. When biological mothers are available, they will also be asked to provide written informed consent for collection of limited maternal data for study purposes. For consenting mothers, the following will be source documented and entered into eCRFs:

- ARV use during pregnancy and breastfeeding (may be based on maternal report or written records)
- HIV viral load at delivery and during breastfeeding (must be based on written records)
- CD4+ cell count at delivery (must be based on written records)

These data should ideally be collected concurrent with the child's entry into the study but may be collected thereafter if applicable. For example, additional time may be needed to contact and complete the informed consent process with the biological mother and/or to obtain maternal medical records. It may also be necessary to collect maternal data at multiple timepoints to capture all applicable information through cessation of breastfeeding.

6 PARTICIPANT MONITORING, REPORTING, AND MANAGEMENT

This observational study does not involve the use of any investigational agents or interventions. It also does not involve any investigational procedures; all procedures are common minimal risk procedures. As such, requirements for safety monitoring and reporting are limited. Adverse event and expedited adverse event reporting are not required for this study. Participant management will be guided by local clinical practice standards.

Site investigators are responsible for monitoring the safety of study participants and alerting the Protocol Team if unexpected concerns arise. Site investigators are also responsible for prompt reporting of any unanticipated problems involving risks to participants or others to all applicable IRBs/ECs and other applicable review bodies, per the procedures of each applicable review body.

Data collection for this study will include source documentation and entry into eCRFs of the clinical events of interest specified in Section 5.4. Management of any such events will be at the direction of participants' primary care providers. Depending on the local context, participants may receive their primary care at the study site or from non-study care providers; for participants who receive their primary care from non-study providers, study staff will actively communicate and coordinate with the non-study providers when clinically indicated. Study staff will also refer participants to non-study sources of further evaluation and/or management when applicable. Study staff will similarly provide counseling and support to participants/parents/guardians when applicable.

Site investigators will monitor participants' HIV-1 RNA results and take action as needed in response to these results. When ARV resistance is suspected, the site investigator will coordinate with the participant's primary care providers and consult with the Protocol Team to review the participant's history and determine whether resistance testing should be performed through the study. When resistance testing is performed, the site investigator will provide results to the participant's primary care providers, who will ultimately be responsible for any ARV regimen management decisions.

Site investigators may also consult with the Protocol Team regarding any other participant management issues, questions, or concerns that may arise during follow-up.

7 STATISTICAL CONSIDERATIONS

7.1 General Design Issues

This is an observational prospective cohort study to characterize the long-term clinical, immunologic, and virologic profiles of early treated children who may participate in future research related to HIV remission or cure. Early treatment is defined as treatment with at least three ART agents from at least two classes of ART initiated within 12 weeks of birth. Early treatment regimens may also include bNAbs. Within the overall study population, for descriptive and analysis purposes, children who initiated treatment within 48 hours of birth will be classified as having received very early treatment. Enrolled participants will complete semi-annual follow-up for up to seven years. Clinical, virologic, and immunologic evaluations will be performed at each study visit; specimens will be collected for the study's biorepository at each visit.

The cohort of participants enrolled in this study may represent a specific subset of the parent study populations, which raises the potential for selection bias in IMPAACT 2028 analyses. For example, long-term survivors may be overrepresented, particularly among CHER participants who are now 12-14 years of age. Infants in IMPAACT P1115 with sustained virological suppression will remain in that study and not enroll in IMPAACT 2028. The direction and magnitude of potential selection bias will depend on where these participants fall within exposure groups and their likelihood of outcomes of interest. Adjusting for this type of selection bias at enrollment would depend on having access to data from all participants in the parent studies to estimate the probability of being enrolled in IMPAACT 2028. As the IMPAACT 2028 Protocol Team is unlikely to have full access to these data, analytic methods will be used to estimate the direction of potential selection bias and put bounds around the magnitude of the bias (54). To inform these sensitivity analyses, the Protocol Team will compare the characteristics of participants enrolled in IMPAACT 2028 with those reported in parent study publications. This will allow evaluation of the robustness of the effect estimates observed in IMPAACT 2028 analyses and the strength of conclusions drawn from these analyses. Efforts to minimize the magnitude of potential selection bias will also be explored. This may include, for example, collaborating with parent study teams to include all parent study participants in analyses.

Because this is an observational study without any exposure assignment, comparisons should be carefully interpreted for confounding effects. Additionally, analyses within participant subgroups will have smaller sample sizes and will therefore provide less precise estimates.

Although this study is observational, study participation itself, with access to the expertise of study investigators, may result in stricter ARV adherence and more frequent monitoring of HIV disease parameters and complications than would occur outside the study. This may impact the generalizability of study findings.

All potential biases and limitations, including selection bias, unmeasured confounding, and limited generalizability as described above, will be acknowledged in study publications. Discussion will include the impact of such potential biases and limitations on the reported conclusions.

7.2 Outcome Measures

Corresponding to the primary objective of the study, the clinical, immunologic, and virologic outcome measures listed below will be analyzed to characterize participants at study entry and during follow-up. The timeframe for ascertainment of all primary outcome measures is through the end of scheduled follow-up.

Clinical Outcome Measures
• Somatic growth indicators: – Weight, height, and weight-for-height Z score – Head circumference and head circumference-for-age Z score (through 2 years of age) • WHO clinical Stage 3 or Stage 4 conditions (see Appendix II) • Other medical conditions of interest (see Appendix II) • Medical conditions that required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, were life-threatening, or resulted in death • Mortality

Immunologic Outcome Measures
• CD4+ cell counts and percentages • CD8+ cell counts and percentages • HIV-1 antibody status
Virologic Outcome Measures
• HIV-1 RNA in plasma • HIV-1 DNA concentration in PBMCs • HIV-1 ARV resistance mutations

7.3 Randomization and Stratification

This is not a randomized study. To monitor accrual from each of the parent studies, participants will be stratified at entry by parent study.

7.4 Sample Size and Accrual

7.4.1 Sample Size

Up to approximately 250 children will be enrolled in this study. Refer to Table 1 on page 26 for estimated numbers of participants who may be enrolled from each parent study.

Data and specimens collected in this study will be used for additional research related to HIV remission or cure via a competitive proposal-based process. Most analyses performed for this study are expected to be based on proposals approved through the process noted in Section 3. More detailed power calculations relevant to specific analyses may be conducted within individual proposals. In proposed analyses, outcome measure data may be analyzed categorically or continuously.

Table 3, Table 4, and Table 5 present general calculations for a binary or continuous outcome measure under varying assumptions of underlying event rates and distributions across a range of two-group comparison sample sizes (e.g., exposure, no exposure) to inform feasibility of future analysis proposals. For all calculations, the maximum sample size of 250 participants is assumed, with a two-sided α=0.05. With this sample size, the calculations represent the highest power and precision achievable in this study. Depending on analysis objectives, sample sizes for some outcome measures may be smaller, with corresponding lower precision.

Categorical Outcome Measures

For simplicity, it is assumed that for a given exposure, a comparison of interest is the proportion of exposed participants with an event versus the proportion of unexposed participants with the event measured in terms of a risk ratio (RR). For example, there may be interest in comparing the RR of a WHO Stage 3 or Stage 4 event in very early versus early treated infants. If 30% of the study population is very early treated and 70% is early treated with a 5% event probability among the very early treated children, then the study has 80% power to detect a RR of 3.28 (Table 3), which implies early treated children have 3.28 times the risk of a WHO Stage 3 or Stage 4 event compared to very early treated children (corresponding to a 16.4% event probability in early treated).

Table 3 presents the minimum detectable RR at 80% power assuming that exposure increases the risk of an event (RR>1). The RRs range from 1.57 to 19.61 depending on the underlying event probability and the percent of the population in the unexposed group.

A similar summary is presented in Table 4, but under the assumption that exposure is protective (RR<1). Table 4 presents the maximum detectable RR at 80% power assuming that exposure decreases the risk of an event. The RRs range from 0.04 to 0.51 depending on the underlying event probability and the percent of the population in the unexposed group.

With a maximum sample size of 250, rare events are unlikely to be observed in this study. If exposure increases the risk of an event, reasonably small effect sizes (RR between 1.5 and 3.5) may be detected if the probability of the event in the unexposed group is 10% or greater. For rare events (≤5% probability in the unexposed group), only very large associations would be detectable (Table 3). If exposure is protective, reasonable effect sizes (RR between 0.46 and 0.51) would be detectable if the probability of the event is greater than 30% with 30-70% of the population in the unexposed group. For less frequent events (<30%), only quite large associations would be detectable (Table 4).

Continuous Outcome Measures

Additional primary outcome measures of interest may be continuous, such as weight-for-height Z-score. For example, if the comparison of interest is having received bNAbs versus not having received bNAbs, and 20% of participants have received bNAbs, the study has 80% power to detect a mean difference of 0.40 standard deviations (Table 5).

Table 5 presents the minimum detectable mean differences comparing two groups of with a t-test (unequal variances) at 80% power. These differences range from 0.40 to 0.60 standard deviations depending on the percent of the population in the unexposed group.

Table 3
Detectable Effects at 80% Power
Assuming Exposure is Associated with Increased Risk and a Sample Size of 250

Event Probability in Unexposed	Percent of Population in Unexposed Group	Minimum Detectable RR for for Exposed vs Unexposed*
1%	90%	12.52
	70%	8.65
	50%	8.53
	30%	10.32
	10%	19.61
5%	90%	4.51
	70%	3.28
	50%	3.16
	30%	3.51
	10%	5.48
10%	90%	3.15
	70%	2.40
	50%	2.31
	30%	2.50
	10%	3.51
20%	90%	2.30
	70%	1.86
	50%	1.79
	30%	1.88
	10%	2.40
30%	90%	1.94
	70%	1.62
	50%	1.57
	30%	1.63
	10%	1.96

*Based on Mantel-Haenszel test. Calculations performed using PASS 15 (NCSS, 2017).

Table 4
Detectable Effects at 80% Power
Assuming Exposure is Associated with Decreased Risk and a Sample Size of 250

Event Probability (in Unexposed)	Percent of Population in Unexposed Group	Maximum Detectable RR for Exposed vs Unexposed*
10%	90%	N/A
	70%	0.09
	50%	0.17
	30%	0.16
	10%	0.04
20%	90%	0.06
	70%	0.33
	50%	0.39
	30%	0.36
	10%	0.19
30%	90%	0.22
	70%	0.46
	50%	0.51
	30%	0.48
	10%	0.30

*Based on Mantel-Haenszel test. Calculations performed using PASS 15 (NCSS, 2017).

Table 5
Detectable Mean Differences Between Exposed and Unexposed
Assuming Unequal Variances and a Sample Size of 250

Percent of Population in Unexposed Group	Minimum Detectable Mean Difference In Standard Deviations Between Exposed and Unexposed*
50%	0.40
40% or 60%	0.40
30% or 70%	0.40
20% or 80%	0.40
10% or 90%	0.60

*Based on two-sample t-test allowing for unequal variances. Calculations performed using PASS 15 (NCSS, 2017).

7.4.2 Accrual

Accrual into this study will remain open for the duration of follow-up (or until the maximum sample size of 250 is met). Accrual of participants recruited from the IMPAACT P1115 (Version 1.0), IMPAACT 2008, CHER, LEOPARD, and TIES study populations is expected to take approximately 6-12 months at each site. Because IMPAACT P1115 will be conducted concurrently with IMPACT 2028, accrual of participants from IMPAACT P1115 is expected throughout the duration of IMPAACT 2028.

7.5 Monitoring

Implementation of this study will be monitored at multiple levels, consistent with standard IMPAACT procedures. A study monitoring plan that details monitoring roles and responsibilities and data to be reviewed at each level will be prepared before the study opens to accrual. Sections 8 and 9 provide for more information on on-site monitoring and quality management at the site level; see also Section 6. Further information on monitoring of study progress and the quality of study conduct across sites is provided below.

7.5.1 Monitoring by the Protocol Team

The Protocol Team is responsible for continuous monitoring of study progress, including timely achievement of key milestones, and the quality of study conduct.

The Protocol Team will closely monitor participant accrual based on reports that will be generated by the SDMC. The pace and completeness of accrual will be monitored relative to the projections specified in Section 7.4.2; however, no minimum accrual target is applicable, and accrual will continue until the maximum sample size is enrolled or through the duration of the study. The Protocol Team will actively communicate with site investigators regarding the progress of accrual at each site and determine when accrual should be discontinued at each site.

In addition to participant accrual, the Protocol Team will monitor participant retention and other key indicators of the quality of study conduct (e.g., data quality, data and specimen completeness) based on reports generated by the SDMC. The team will take action with study sites as needed to ensure high quality study conduct throughout the period of study implementation. The Protocol Team will also monitor aggregate clinical, immunologic, and virologic parameters over time based on reports generated by the SDMC.

Data reports required for the team's monitoring will be generated at least monthly during the early period of study implementation. Thereafter, reports will be generated on a frequency specified in the study monitoring plan.

7.5.2 Monitoring by the Study Monitoring Committee (SMC)

An independent IMPAACT SMC will review this study regularly, following policies described in the IMPAACT Manual of Procedures.

SMC reviews will occur at least annually or on a more frequent or *ad hoc* basis if any issues or concerns arise. Based on any of its reviews, the SMC may recommend that the study proceed as currently designed, proceed with design modifications, or be discontinued. The SMC may also provide operational recommendations to help address any study implementation challenges that may be identified during their reviews.

The SMC will monitor study progress and the quality of study conduct, and will review summary clinical, immunologic, and virologic data. The SMC will generally review the same types of data reports as the Protocol Team (e.g., participant accrual, participant retention, data quality, data and specimen completeness). For *ad hoc* reviews, more limited data may be reviewed, focusing on the issues or concerns that necessitated the reviews. Beginning approximately two years after study initiation (first enrollment), the SMC will also review information pertaining to the productivity of the proposal-based process for use of study data and stored specimens.

7.6 Analyses

The primary analysis to characterize the IMPAACT 2028 cohort will describe clinical, immunologic, and virologic characteristics at enrollment, and from birth (as available), as well as at early follow-up, by very early (within 48 hours of age) versus early (between 48 hours and 12 weeks of age) treatment and by receipt of bNAbs as part of treatment. This analysis will be descriptive and will present point estimates of summary statistics with associated confidence intervals or selected quantiles as appropriate. This analysis is expected to be performed once the Protocol Team determines that all participants expected to be enrolled from the parent studies, except IMPAACT P1115 Version 2.0 participants, have been enrolled. This analysis will be described in the reference publication for the study and will inform subsequent analysis proposals, along with study monitoring reports that summarize additional data during the study follow-up period.

Additional analyses will be performed after the primary analysis, based on approved proposals, during follow-up and after follow-up has been completed. Such analyses may be cross-sectional or longitudinal depending on available data and may explore exposures other than very early versus early treatment or receipt of bNAbs, such as:

- Male versus female
- *In utero* versus intrapartum or postpartum HIV transmission
- Breastfed versus formula fed
- Have versus have not had exposure to HIV during breastfeeding (as indicated by maternal ARV use and maternal viral load during breastfeeding)
- Have versus have not maintained virologic suppression

The analysis approach will be tailored to the type of outcome measure of scientific interest. For categorical data, RRs will be estimated using modified Poisson models or log-binomial models. For continuous measures (e.g., CD8+ percentage), linear regression will be used. For longitudinal outcomes, mixed effects models and generalized estimating equations may be used to evaluate associations of interest. Time-to-event outcome measures will be analyzed using survival methods for failure time data such as Kaplan-Meier, log rank tests, and proportional hazards regression models, with consideration for competing events, time-varying covariates, and the non-informative censoring assumption, as appropriate. For count data, Poisson regression may be used.

A detailed statistical analysis plan will be developed for the primary analysis and separately for each approved proposal. As needed, analysis plans will specify the covariates for regression models. Covariate data may include those collected as history and at study entry.

8 DATA HANDLING AND RECORD KEEPING

8.1 Data Management Responsibilities

As described in Section 4.4, eligibility and enrollment data will be collected using the DMC SES.

Study sites must maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including paper-based CRFs (if used), eCRFs, and supporting source data. In maintaining these records, sites must comply with the standards of source documentation specified in the DAIDS policy on Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (available on the website referenced in Section 8.2).

eCRFs and an eCRF completion guide will be made available to study sites by the DMC. Study site staff will enter required data into eCRFs, with system checks applied and data queries generated immediately upon saving the entered data. Data must be entered within timeframes specified by the DMC; queries must also be resolved in a timely manner. Selected laboratory data will be transferred electronically to the DMC through the LDMS.

Further information on eCRFs and IMPAACT data management procedures will be provided by the DMC. A User Manual for the SES is available on the DMC portal at: https://www.frontierscience.org

8.2 Essential and Source Documents and Access to Source Data

All DAIDS policies referenced in this section are available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

Study sites must comply with DAIDS policies on Requirements for Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials and Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. In its policy on Requirements for Manual of Operational Procedures, DAIDS requires sites to establish SOPs for maintaining essential and source documents in compliance with these policies. Site SOPs should be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study, and site SOPs should be followed throughout the study.

Per the DAIDS policy on Storage and Retention of Clinical Research Records, study records must be stored in a manner that ensures privacy, confidentiality, security, and accessibility during the conduct of the study and after the study is completed. Records must be retained for a minimum of three years after the completion of the study.

All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, IMPAACT, site IRBs/ECs, the sIRB (for US sites), the Office for Human Research Protections, and other US, local, and international regulatory entities. Records must be kept on-site throughout the period of study implementation; thereafter, instructions for off-site storage may be provided by the National Institute of Allergy and Infectious Diseases (NIAID) or National Institute of Child Health and Human Development (NICHD). No study records may be removed to an off-site location or destroyed prior to receiving approval from NIAID or NICHD.

8.3 Quality Control and Quality Assurance

Study sites must ensure that essential documents and participant research records are subject to continuous quality control and quality assurance procedures consistent with the DAIDS policy on Requirements for Clinical Quality Management Plans, which is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

9 CLINICAL SITE MONITORING

Site monitors under contract to NIAID or NICHD will visit study sites to inspect study facilities and review participant study records — including informed consent and assent forms, paper-based CRFs (if used), eCRFs, medical records, and laboratory records — to ensure protection of study participants, compliance with the IRB/EC approved protocol, and accuracy and completeness of records. The monitors also will review essential document files to ensure compliance with all applicable regulatory requirements. Site investigators will make study facilities and documents available for inspection by the monitors.

10 HUMAN SUBJECTS PROTECTIONS

10.1 Institutional Review Board/Ethics Committee Review and Approval

Prior to study initiation, site investigators must obtain IRB/EC review and approval of this protocol and site-specific informed consent and assent forms in accordance with 45 CFR 46; subsequent to initial review and approval, IRBs/ECs must review the study at least annually. Site investigators must also promptly report to the IRBs/ECs any changes in the study and any unanticipated problems involving risks to participants or others.

Non-US sites are frequently overseen by more than one IRB/EC. US sites are overseen by an sIRB, with additional review by local IRBs if required per their agreements with the sIRB. Site investigators are responsible for awareness of and adherence to the policies and procedures of all applicable IRBs/ECs. All such policies and procedures must be followed and complete documentation of all correspondence to and from all applicable IRBs/ECs must be maintained in site essential document files. Sites must submit documentation of both initial review and approval

and continuing review to the DAIDS Protocol Registration Office (PRO) in accordance with the DAIDS Protocol Registration Manual (see also Section 11.2).

10.2 Vulnerable Participants

The NIH is mandated by law to ensure that children are included in clinical research when appropriate (55). This study responds to that mandate.

Nonetheless, the children enrolled in this study are considered vulnerable participants per 45 CFR 46 Subpart D. IRBs/ECs must consider the potential benefits, risks, and discomforts of the study children and assess the justification for their inclusion in this study. As part of this assessment, IRBs/ECs must determine the level of risk to children in the categories specified in 45 CFR 46.404-407. Documentation of this determination is required to complete the DAIDS protocol registration process described in Section 11.2, and the risk category assigned by the IRB/EC determines the parental informed consent requirements for the study at each site. Per 45 CFR 46.408 (b), the IRB/EC may find that the consent of one parent is sufficient for research to be conducted under 46.404 or 46.405. If the IRB/EC finds that the research is covered by 46.406 or 46.407, both parents must give their consent, unless one parent is deceased, unknown, incompetent, or not reasonably available or when only one parent has legal responsibility for the care and custody of the child (as determined locally). IRBs/ECs must document their risk determination, and study sites should adapt the signature pages of their site-specific ICF as needed to accommodate the parental consent requirements associated with the IRB/EC determination.

Study sites must comply with the requirements of the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research: Clinical Site Requirements, which is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

In addition to the US regulations cited above, sites in countries other than the US must also comply with all applicable local and national guidelines and regulations.

10.3 Informed Consent and Assent

Refer to Section 4.4 and the study-specific manual of procedures for further information on informed consent and assent procedures for this study. Refer to Appendix III and Appendix IV for sample informed consent and assent forms, respectively, for child study participation. Refer to Appendix V for the sample ICF for maternal data collection.

10.3.1 Informed Consent and Assent for Child Study Participation

Written informed consent for study participation will be obtained from each child's parent or guardian before any study-specific procedures are performed. It is generally expected that the consent of one parent (or guardian) will be sufficient for child participation in this study. However, consenting requirements at each site will depend on the IRB/EC risk determination as described in Section 10.2; all applicable IRB/EC requirements must be followed. When applicable per IRB/EC policies and procedures, written assent will also be obtained from each child before any study-specific procedures are performed. For participants who do not meet IRB/EC criteria for providing assent at the time of screening and enrollment, if such criteria are met during follow-up, assent should be obtained at the next study visit after the criteria are met.

Likewise, informed consent should be obtained from participants who reach the legal age or circumstance of consent.

The informed consent process will include information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent, including the potential risks, benefits, and alternatives to study participation. The process will describe expectations for abstraction of information from parent study records and recording of information from non-study medical records. The assent process will include a similar but age-appropriate discussion. The amount of information and level of detail provided as part of the assent process should be tailored to the age and maturity of the potential participant, guided by applicable IRB/EC policies and procedures. The extent to which HIV status is disclosed as part of the assent process should also be guided by applicable IRB/EC policies and procedures. When preparing site-specific assent forms, sites may remove or modify the wording included in the sample form (see Appendix IV) in order to provide the most appropriate information and level of detail, consistent with IRB/EC policies and procedures. This may include preparing site-specific form versions that do not disclose HIV status.

Participants/parents/guardians will be asked whether they agree to collection, storage, and future research use of biological specimens (serum, plasma, PBMCs, nasopharyngeal swabs, rectal swabs) as applicable. This is optional and may be declined with no impact on other aspects of study participation. Consent for genetic testing of these specimens is also optional.

Should the consenting parent or guardian of an enrolled child die or no longer be available for any reason, all applicable IRB/EC policies and procedures should be followed. Study-specific evaluations should not be performed until informed consent for continued study participation is obtained from the child's authorized guardian, as defined locally. If an authorized guardian cannot be identified, or if the guardian does not consent to continued study participation, the child must be withdrawn from the study. In accordance with the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research: Clinical Site Requirements, all sites must establish and maintain written procedures describing the standards that will be followed to identify who may serve as guardian for an enrolled child, reflective of applicable IRB/EC guidance for conduct of human subjects research within the context of available local law, regulation, or government policy.

10.3.2 Informed Consent for Maternal Data Collection

See also Section 5.9. When available, the biological mothers of enrolled children will be asked to provide written informed consent for collection of limited maternal data for study purposes. This informed consent process should be conducted in a manner similar to the process for child study participation, including information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent. The process should ideally be conducted such that, for consenting mothers, maternal data can be collected concurrent with the child's entry into the study. However, this is not required; the process may be conducted any time after informed consent for child study participation is obtained. In addition, consent for maternal data collection is optional; this consent may be declined with no impact on child study participation.

10.4 Potential Benefits

There may be no direct benefit to participants in this study. However, it is possible that information learned in this study may be of benefit to participants and others in the future. Participants and their families may appreciate the opportunity to contribute to HIV-related research. Evaluations such as HIV-1 RNA assays and CD4+ and CD8+ cell counts and percentages may be performed more frequently in this study than is standard in some settings and the results of these and other evaluations (e.g., physical examinations) may provide information to guide aspects of participants' clinical care and management.

10.5 Potential Risks

The potential risks of participation in this study include the minimal risks associated with common medical procedures. Blood collection may cause pain, bruising, swelling, and fainting. There is a very small risk of infection where the needle is inserted. Nasopharyngeal and rectal swab collection may cause discomfort.

Refer to Section 10.7 for further information on privacy and confidentiality. Despite efforts to maintain confidentiality, children's involvement in this study could become known to others, possibly leading to unfair treatment, discrimination, or other social impacts (e.g., because participants could become known as having HIV). For example, children could be treated unfairly or discriminated against or could have problems being accepted by their families and/or communities. Every effort will be made to protect children's information, but this cannot be guaranteed.

10.6 Reimbursement/Compensation

Pending IRB/EC approval, participants will be reimbursed for costs associated with completing study visits (e.g., transport costs). Reimbursement amounts will be specified in site-specific ICFs and/or other materials per applicable IRB/EC policies and procedures.

10.7 Privacy and Confidentiality

Study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. Site staff will follow applicable institutional and IRB/EC policies with respect to information-sharing with the parents or guardians of study participants. Participant information will not be released without written permission to do so except as necessary for review, monitoring, and/or auditing as described in Section 8.2.

All study-related information will be stored securely. Participant research records will be stored in locked areas with access limited to study staff. All laboratory specimens, CRFs, and other documents that may be transmitted off-site will be identified by PID only. Likewise, communications between study staff and Protocol Team members regarding individual participants will identify participants by PID only.

Study sites are encouraged but not required by DAIDS policies to store study records that bear participant names or other personal identifiers separately from records identified by PID. All local databases must be secured with password-protected access systems. Lists, logbooks, appointment books, and any other documents that link PID numbers to personal identifying information should be stored in a separate, locked location in an area with limited access.

In addition to the above, a Certificate of Confidentiality has been deemed issued for the IMPAACT Network by the US Department of Health and Human Services. This certificate protects study staff from being compelled to disclose study-related information by any US Federal, state, or local civil, criminal, administrative, legislative, or other proceedings. It thus serves to protect the identity and privacy of study participants. Because the certificate cannot be enforced outside of the US, however, it applies only to US sites and participants.

10.8 Communicable Disease Reporting

Study staff will comply with local requirements to report communicable diseases identified among study participants to health authorities. Participants/parents/guardians will be made aware of applicable reporting requirements as part of the study informed consent process.

10.9 Management of Incidental Findings

Site clinicians will inform participants/parents/guardians of all clinically meaningful physical exam findings and laboratory test results, including CD4+ and CD8+ cell counts and percentages, HIV-1 RNA PCR results, and ARV resistance test results. Site clinicians will provide results to participants' primary care providers when applicable and will provide referrals to non-study sources of medical care for further evaluation and/or treatment of study-related findings when clinically indicated.

The results of other tests that may be performed using specimens collected in this study are not planned to be provided to participants/parents/guardians, as these are considered research tests that are not expected to be relevant to clinical care and management. If, however, new information becomes available indicating that the results of any tests are of clinical relevance, the results will be provided.

10.10 Management of New Information Pertinent to Study Participation

Study staff will provide participants/parents/guardians with any new information learned over the course of the study that may affect their willingness to continue study participation.

11 ADMINISTRATIVE PROCEDURES

11.1 Regulatory Oversight

This study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), and National Institute of Mental Health (NIMH), which are part of the US National Institutes of Health (NIH).

Within the NIAID, DAIDS is responsible for regulatory oversight of this study.

NIAID and NICHD provide funding to the clinical research sites at which this study will be conducted. Each institute contracts with independent clinical site monitors who will perform monitoring visits as described in Section 9. As part of these visits, monitors will inspect study-related documentation to ensure compliance with all applicable US, local, and international regulatory requirements.

11.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the study informed consent and assent forms approved, as appropriate, by applicable IRBs/ECs, and any other applicable regulatory entities; for US sites, this includes the sIRB. Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received.

Site-specific ICFs will be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

For any future protocol amendments, upon receiving final IRB/EC and any other applicable regulatory entity approvals, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received. Site-specific ICFs will not be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, which is available at:
https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

11.3 Study Implementation

This study will be conducted in accordance with the protocol, international good clinical practice guidelines, and all applicable US and local regulations. Study implementation will also be guided by the IMPAACT Manual of Procedures, study-specific manual of procedures, LPC, and other study implementation materials, which will be available on the study-specific website:
https://impaactnetwork.org/studies/IMPAACT2028.asp

Study implementation at each site will also be guided by site-specific SOPs. The DAIDS policy on Requirements for Manual of Operational Procedures specifies the minimum set of SOPs that must be established at sites conducting DAIDS funded and/or sponsored clinical trials (available on the website referenced in Section 8.2). These SOPs should be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study.

11.4 Protocol Deviation Reporting

Per the policy for Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (available at the website referenced in Section 8.2), all protocol deviations must be documented in participant research records. Reasons for the deviations and corrective and preventive actions taken in response to the deviations should also be documented.

Deviations should be reported to applicable IRBs/ECs and other applicable review bodies in accordance with the policies and procedures of these review bodies; for US sites, this includes the sIRB, per the requirements available at the website cited in Section 6. Serious deviations that are associated with increased risk to one or more study participants and/or significant impacts on the integrity of study data must also be reported within IMPAACT, following procedures specified in the IMPAACT Manual of Procedures.

11.5 Critical Event Reporting

Per the DAIDS policy on Identification and Classification of Critical Events, a critical event is defined as an unanticipated study-related incident that is likely to cause harm or increase the risk of harm to participants or others or has a significant adverse impact on study outcomes or integrity. All such events must be reported following procedures specified in the DAIDS Critical Events Manual, which is available at:
https://www.niaid.nih.gov/research/daids-clinical-research-event-reporting-safety-monitoring

11.6 ClinicalTrials.gov

This observational study is not subject to the Food and Drug Administration Amendments Act of 2007 or the NIH Policy on Dissemination of NIH-funded Clinical Trial Information. However, it will be registered in ClinicalTrials.gov to meet International Committee of Medical Journal Editors requirements.

12 PUBLICATIONS

All presentations and publications of data collected in this study are governed by IMPAACT policies, which are available in the IMPAACT Manual of Procedures.

13 REFERENCES

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Appendix I: Schedule of Evaluations

	Screening and Entry Day 0	Semi-Annual Follow-Up Q24 weeks
ADMINISTRATIVE, REGULATORY, AND CLINICAL EVALUATIONS		
Assess expected availability for study participation for eligibility determination	X	
Review parent study records and available medical records for eligibility determination	X	
Collect baseline medical and medications history	X	
Collect interval medical and medications history		X
Collect feeding history[1]	X	[X]
Perform physical examination	X	X
LABORATORY EVALUATIONS		
HIV-1 RNA PCR[2] (quantitative, performed locally)	3 mL	3 mL
HIV-1 DNA ddPCR[2] (performed centrally)	3 mL	3 mL
CD4+ and CD8+ cell counts and percentages[3] (performed locally)	1-2 mL	1-2 mL
HIV-1 antibody[2] (performed centrally)	1.5 mL	1.5 mL Q48 only
Plasma and PBMC storage (stored locally)[2]		
– Less than 5 kg	5 mL	5 mL
– 5 to less than 10 kg	7 mL	7 mL
– 10 to less than 15 kg	12 mL	12 mL
– 15 kg or greater	17 mL	17 mL
Serum storage (stored locally)	3 mL	3 mL
ARV resistance testing if clinically indicated[2] (performed locally, regionally, or centrally)	3 mL	3 mL
If child is three years of age, nasal and rectal swab storage (stored locally)[4]	[X][4]	[X][4]
Total Blood Volume	**19.5-32.5 mL**	**18.0-32.5 mL**

[1]Collect for all participants at Screening and Entry Visit. Collect at Semi-Annual Follow-up Visit(s) only if needed to record date of last exposure to formula and/or date of last exposure to breast milk if applicable.
[2]To maximize specimen yields, whenever possible, blood volumes collected for HIV-1 RNA PCR, HIV-1 DNA ddPCR, HIV-1 antibody, and/or ARV resistance testing should be processed for PBMC storage; all residual plasma should also be stored. Refer to the LPC for detailed guidance on specimen collection, processing, and storage.
[3]At sites where a complete blood count with white blood cell differential is needed to obtain CD4+ and CD8+ cell counts and percentages, up to 2 mL of blood may be collected; otherwise, 1 mL should be collected.
[4]One nasopharyngeal swab and one rectal swab should be collected from each participant once he or she has reached three years of age. For participants who are three years of age or older at study entry, the swabs should be collected at study entry. Otherwise, the swabs should be collected at the first follow-up visit occurring after the participant has reached three years of age.

Appendix II: WHO Clinical Stage 3 and Stage 4 Conditions and Other Medical Conditions of Interest for IMPAACT 2028

WHO Stage 3 Conditions for Participants 15 Years of Age and Older	**WHO Stage 3 Conditions for Participants Less than 15 Years of Age**
• Unexplained severe weight loss (>10% of presumed or measured body weight) • Unexplained chronic diarrhoea for longer than 1 month • Unexplained persistent fever (intermittent or constant for longer than 1 month) • Persistent oral candidiasis • Oral hairy leukoplakia • Pulmonary tuberculosis • Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) • Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis • Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 x 10^9/l) and/or chronic thrombocytopaenia (<50 x 10^9/l)	• Unexplained moderate malnutrition[b] not adequately responding to standard therapy • Unexplained persistent diarrhoea (14 days or more) • Unexplained persistent fever (above 37.5°C, intermittent or constant, for longer than one 1 month) • Persistent oral candidiasis (after first 6 weeks of life) • Oral hairy leukoplakia • Lymph node tuberculosis • Pulmonary tuberculosis • Severe recurrent bacterial pneumonia • Acute necrotizing ulcerative gingivitis or periodontitis • Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 x 10^9/l) or chronic thrombocytopaenia (<50 x 10^9/l) • Symptomatic lymphoid interstitial pneumonitis • Chronic HIV-associated lung disease, including bronchiectasis
WHO Stage 4 Conditions[c] for Participants 15 Years of Age and Older	**WHO Stage 4 Conditions[c] for Participants Less than 15 Years of Age**
• HIV wasting syndrome • *Pneumocystis (jirovecii) pneumonia* • Recurrent severe bacterial pneumonia • Chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site) • Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) • Extrapulmonary tuberculosis • Kaposi sarcoma • Cytomegalovirus infection (retinitis or infection of other organs) • Central nervous system toxoplasmosis • HIV encephalopathy • Extrapulmonary cryptococcosis, including meningitis • Disseminated nontuberculous mycobacterial infection • Progressive multifocal leukoencephalopathy • Chronic cryptosporidiosis • Chronic isosporiasis • Disseminated mycosis (extrapulmonary histoplasmosis, coccidioidomycosis) • Lymphoma (cerebral or B-cell non-Hodgkin) • Symptomatic HIV-associated nephropathy or cardiomyopathy • Recurrent septicaemia (including nontyphoidal *Salmonella*) • Invasive cervical carcinoma • Atypical disseminated leishmaniasis	• Unexplained severe wasting, stunting or severe malnutrition[d] not responding to standard therapy • *Pneumocystis (jirovecii) pneumonia* • Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection, • meningitis, but excluding pneumonia) • Chronic herpes simplex infection (orolabial or cutaneous of more than 1 month's duration or • visceral at any site) • Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) • Extrapulmonary tuberculosis • Kaposi sarcoma • Cytomegalovirus infection (retinitis or infection of other organs with onset at age more than 1 month) • Central nervous system toxoplasmosis (after the neonatal period) • HIV encephalopathy • Extrapulmonary cryptococcosis, including meningitis • Disseminated nontuberculous mycobacterial infection • Progressive multifocal leukoencephalopathy • Chronic cryptosporidiosis (with diarrhoea) • Chronic isosporiasis • Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis)

Other Medical Conditions of Interest

- Asthma
- Hepatitis (includes viral hepatitis as well as clinical hepatitis)
- Congenital cytomegalovirus
- Measles
- Chickenpox
- Insulin resistance
- Diabetes
- Lipodystrophy
- Lipoatrophy
- Hyperlipidemia
- Other metabolic syndrome
- Anemia
- Leukopenia
- Thrombocytopenia
- Decreased bone mineral density
- Bone fracture
- Allergy or hypersensitivity to medication (specify medication; at follow-up visits, also specify type of reaction)
- Autoimmune disorder
- Malignancy

Source: Adapted from Annex 1, WHO clinical staging of HIV disease in adults, adolescents and children, of the Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, recommendations for a public health approach; World Health Organization; June 2013.

[b] For children younger than 5 years, moderate malnutrition is defined as weight-for-height <–2 z-score or mid-upper arm circumference ≥115 mm to <125 mm.

[c] Some additional specific conditions can be included in regional classifications, such as penicilliosis in Asia, HIV-associated rectovaginal fistula in southern Africa and reactivation of trypanosomiasis in Latin America.

[d] For children younger than 5 years of age, severe wasting is defined as weight-for-height <–3 z-score; stunting is defined as length-for-age/height-for-age <–2 z-score; and severe acute malnutrition is defined as either weight for height <–3 z-score or mid-upper arm circumference <115 mm or the presence of oedema.

Appendix III: Sample Informed Consent Form for Study Participation

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Version 1.0, 23 December 2020

Introduction

This form is for the parent or guardian of a child who is being asked to participate in the research study named above.

Participants in this study may be young babies, older children, or adolescents. In this form, participants are referred to as "children" regardless of their age. For most participants, it is expected that a parent or guardian will provide informed consent for participation in the study. This form refers to "your child" with the expectation that parents and guardians will be reading the form. However, some adolescents may qualify to provide informed consent for themselves. For these adolescents, when reading this form, "your child" refers to the adolescent who is providing informed consent.

This form gives information about the study. Please read it or have it read to you. Ask any questions you may have. We will take as much time as needed for you to fully understand the study. We will ask you questions to see if we have explained the study clearly.

Here is a summary of important information about the study:

- The study is to learn more about children who started treatment for HIV soon after birth.
- Children will be in the study for up to 7 years. During this time, children will have clinic visits every 6 months (2 times per year). Clinic visits will include physical examinations and blood collection for tests.
- There are possible risks for children in the study. However, these risks are minimal.
- There may be no benefits for children in the study. However, information learned in this study may benefit other children with HIV in the future.
- Your decision on your child's participation in the study will have no effect on the medical care your child receives. Your child's access to services, and the benefits and rights he or she normally has, will not be affected.

More information about the study is given in this form. You should feel that you understand the study before deciding whether your child will participate. If you decide that your child will participate, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

About the study

The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) and *<<site name>>* are doing this study. The person in charge of the study at *<<site name>>* is *<<Investigator of Record name>>*.

The study is for children who have been in other studies of treatment for HIV started within 12 weeks of birth. The study will include up to 250 children from many countries, including Brazil, Botswana, Haiti, Malawi, South Africa, Thailand, Uganda, Zimbabwe, and the United States.

The United States National Institutes of Health is paying for the study.

1. The study is to learn more about children who started treatment for HIV soon after birth.

Many studies are being done to learn about treatments for children with HIV. Starting treatment early (as soon as possible after birth) can help children stay healthy. Starting treatment early may also help control the amount of HIV in a child's body.

There is still much to be learned about treatment for children with HIV. For example, how well do children who started treatment early grow? What health problems do they have or not have as they get older?

There is also much to be learned about how the immune system works in children who started treatment early. The immune system is the part of the body that fights infections. HIV attacks the immune system. Learning more about the immune system may help researchers understand what is needed for better treatments for children with HIV.

2. Only children who qualify can participate in the study.

If you decide to have your child join this study, we will first check whether your child qualifies. More information about this is given in #4. If your child qualifies, he or she will be entered in the study. If your child does not qualify, he or she cannot be entered in the study.

3. It is your decision whether to have your child participate in the study.

Deciding to have your child to join the study is voluntary (your choice). You are free to have your child join or not join. If you decide to have your child join, you can change your mind and take your child off the study. Your decisions will have no effect on the medical care your child receives. Your child's access to services, and the benefits and rights he or she normally has, will not be affected.

Take your time and consider your decision carefully. If you wish, you can talk to other people about the study before you decide. You can bring other people here to learn about the study with you.

Finding out if your child qualifies

4. We will review your child's records, ask questions, and examine your child.

To find out if your child qualifies, we will:

- Review your child's records. This includes records from the study your child was in before this one. It also includes other medical records such as from the clinic where your child receives treatment for HIV. In order for your child to be in this study, you will need to give us permission to review these records. If information needed for this study is available in your child's records, we will record that information.
- Ask questions about your child's health and medications.
- Give your child a physical examination.
- Talk with you about the study requirements and if your child will be able to meet the requirements.

These procedures will take about 2 hours. After the procedures are done, we will tell you if your child qualifies for the study.

Entering the study

5. If your child qualifies, he or she will enter the study.

If your child qualifies for the study, we will collect additional information from your child's records.

<<Sites may remove assessment of gender identity if there are any significant concerns about participant safety or may insert locally appropriate language as needed>> If your child is 12 years of age or older, we will ask your child about gender identity. Gender identity is the internal sense people have of whether they are male, female, or something in between. A person's gender identity may be different from their sex at birth. We will ask your child about gender identity in private, without you being present.

We will also draw your child's blood for tests. About 13 mL or 3 teaspoons will be drawn for tests of:

- Your child's CD4 and CD8 cells. These cells are part of the immune system. They help fight HIV and other infections. The number of CD4 and CD8 cells should go up and stay up when your child is taking anti-HIV medicines (ARVs).
- The amount of HIV in your child's blood. This is called your child's "viral load." The viral load should go down and stay down when your child is taking ARVs.
- The amount of HIV in your child's cells. Researchers have learned that HIV can hide inside cells in the body. The cells that HIV can hide in are inactive. However, these cells can later become active. When these cells become active, the HIV inside the cells also becomes active and starts making more copies of itself. The inactive cells that HIV hides in are called the "reservoir." Little is known about the reservoir in children. Tests done in this study will help researchers learn more about this.
- Whether your child has antibodies to HIV. Antibodies are substances made by the immune system to help fight infection. Researchers have learned that antibodies may help control the amount of HIV in the body. However, little is known about the effects of antibodies in children. Tests done in this study will help researchers learn more about this.

We may also do tests for resistance to ARVs. If your child has resistance, some ARVs may not work well against the HIV in his or her body. As a result, your child's viral load may go up. If we think your child may have resistance to ARVs, we will test for this.

We would also like to draw blood from your child to be stored for future tests. If you agree to this, the amount drawn will depend on your child's weight. Less blood is drawn while children are younger and smaller. More blood is drawn as children get older and bigger. The amount drawn will be between 8 mL (less than 2 teaspoons) and 20 mL (about 4 teaspoons). More information on blood stored for future tests is given in #8.

During the study

6. Your child will have clinic visits every 6 months.

Your child will have visits here every 6 months (2 times per year) while in the study. The study is expected to go on for 7 years. Depending on when your child joins the study, he or she may have up to 14 visits.

Each visit will take about 2 hours. At these visits we will:

- Review your child's records. If information needed for this study is available in your child's records, we will record that information.
- Ask questions about your child's health and medicines. If your child becomes pregnant, this will include asking questions about the pregnancy.
- Ask questions about any new studies your child has joined.
- *<<Sites may remove assessment of gender identity if there are any significant concerns about participant safety or may insert locally appropriate language as needed>>* If your child reaches 12 years of age while in the study, we will ask your child about gender identity. This will be done in private, without you being present.
- Give your child a physical examination.
- Draw your child's blood for tests. About 8 mL or 2 teaspoons will be drawn to for tests of:
  - Your child's CD4 and CD8 cells.
  - Your child's viral load.
  - The amount of HIV in your child's cells
  - Whether your child has antibodies to HIV

  If we think your child may have resistance to ARVs, we will also test for this.

If you agree, we will also collect blood (up to 20 mL or about 4 teaspoons) for future tests. More information on blood stored for future tests is given in #8.

7. Other types of samples will be collected.

We would like to collect a sample from your child's nose and a sample from your child's rectum to be stored for future tests. These samples are collected with a swab. One swab is inserted in the nose. Another swab is inserted in the rectum. Each swab is inserted for about 5-10 seconds. If you agree, these samples will be collected only once, after your child has reached 3 years of age.

These samples will be used for tests of the bacteria living in your child's body. All people have bacteria living on their skin and in the mouth, nose, and gut. These bacteria can have helpful effects. For example, they help with digesting food. They may affect body weight, functioning of the brain, and mood. Researchers have learned that these bacteria may also affect the immune system. However, little is known about this in children. Researchers would especially like to learn more about whether changes in these bacteria make it more or less likely for children with HIV to have certain illnesses. Tests done in this study will help researchers learn more about this.

More information on samples stored for future tests is given in #8.

8. Different tests will be done at different laboratories at different times.

Testing During the Study

We will do tests of your child's viral load and CD4 and CD8 counts here at our laboratory. Tests for resistance to ARVs may be done here or at laboratories in other locations. We will give you the results of these tests and explain the results to you. We will also give the results to your child's medical care providers. This study cannot provide ARVs or medical care to your child. However, if test results show that your child may need different ARVs or other medical care, we will tell you where you can go for this care.

Tests of HIV in cells and of antibodies to HIV will be done at laboratories in the United States or South Africa. These tests are for research purposes only. The results are not expected to give information relevant to your child's health. Therefore, the results will not be given to you.

Future Testing

Permission for different types of sample collection and future testing is requested at the end of this form.

<<Sites insert one of the following two paragraph options. Non-US sites should select the option that applies based on whether local regulations do or do not permit specimen storage for future research in the US; if local regulations do not permit specimen storage for future research, this section of the form should be deleted.>>

If you agree to have samples collected for future testing, the samples will be collected during your child's visits and kept in a repository. A repository is a secure facility used to store samples. The IMPAACT Network repository is in the United States. If you agree to have your child's extra samples stored, the samples will be kept in this repository. There is no limit on how long the samples will be kept. *<<Sites may modify the preceding sentence to specify time limits or additional site-specific requirements if required by local authorities.>>*

If you agree to have samples collected for future testing, the samples will be collected during your child's visits and kept in a repository. A repository is a secure facility used to store samples. The IMPAACT Network repository is in the United States. However, our regulations require that samples be stored in our country. Therefore, if you agree to have your child's specimens stored, they will be kept here at our laboratory. There is no limit on how long the samples will be kept. *<<Sites may modify the preceding sentence to specify time limits or additional site-specific requirements if required by local authorities.>>*

The samples may be used for future testing related to HIV or other diseases. Because children in this study started treatment for HIV early, testing of these samples may help researchers learn more about treatment for HIV and control of HIV in the body. The testing may be done in laboratories in the United States or in other countries.

The samples may also be used for tests of your child's genes. Genes are passed to children from their birth parents. They affect how people look and how their bodies work. Differences in people's genes can help explain why some people get a disease while others do not. Your child's samples may be tested for specific genes, such as genes related to HIV. Samples may also be tested for genes related to other diseases. Testing of all your child's genes, which is sometimes called whole genome sequencing, may also be done.

Future testing may not give information that is directly relevant to your child's health. For this reason, the results of future tests may not be given to you. If any results that are relevant to your child's health become available while your child is in the study, those results will be given to you. Results that become available after your child is no longer in the study are not expected to be given to you.

9. We may take your child off the study.

Children are expected to stay in the study for up to 7 years. However, we may take your child off the study early if:

- The study is stopped for any reason.
- We determine that your child cannot meet the study requirements.
- We determine that staying in the study might harm your child.

10. Please tell us if you want your child to leave the study.

You are free to take your child off the study at any time for any reason. You are also free to change your mind about collection and future testing of your child's samples. The medical care your child receives will not be affected by your decisions, but it is important that we know your decisions. We will record your decisions and ensure they are followed.

If your child leaves the study early, we will ask your child to complete one last visit. At this visit, we will perform the same procedures described in #6. We will answer any questions you may have and tell you how to contact us in the future, if you wish.

If your child leaves the study early, we will still use the information and samples already collected from your child. However, if you do not agree to this, you can tell us and your decisions will be followed.

Risks of the study

11. There is little risk from the study procedures.

Most procedures done in this study are routine medical procedures, with little risk to your child. Drawing blood can cause fainting, pain, swelling, bruising, or bleeding where the needle is inserted. Rarely, drawing blood can cause fainting or infection. Collection of samples from your child's nose and rectum can cause discomfort. Your child may feel nervous or embarrassed when answering questions for the study.

12. There may be risks of disclosure of your child's information.

We will make every effort to keep your child's information private and confidential. Study records and samples will be kept in secure locations. All samples and most records will be labeled only with a code number. However, your and your child's name will be written on some records.

<<US sites insert this paragraph>> Your child's privacy may also be protected by a Certificate of Confidentiality that helps us avoid being forced to release information that may identify your child, such as by the courts or police. The certificate cannot be used in all situations, but it can be used to resist demands for information that would identify your child. The certificate does not protect against requests for information from the United States federal government. Regardless of the certificate, you can release information about your child's participation in the study to others, if you wish.

The information we collect about your child for this study will be combined with information collected from all other children in the study. This will be done at an organization called a statistical and data management center. The IMPAACT Network statistical and data management center is in the United States. We will send your child's information to this center. The information will be sent securely, following applicable laws and policies. Your child's name and other information that could personally identify your child will not be sent.

Information and samples collected for this study may be used for other research in the future. Any such research must be approved by the IMPAACT Network. Information and samples used for approved research will be labeled with a code number only. The only link between the code number and your child's name will be kept here at this clinic. Your child's name and other information that could personally identify your child will not be given to other researchers.

Despite our best efforts to keep your child's information private, it is possible that your child's information could be obtained by someone who should not have it. If this were to happen, your child could be treated badly or unfairly.

Benefits of the study

13. There may be no benefit from being in the study.

By joining the study, your child will be part of research for children with HIV. Being in this study is not expected to benefit your child directly. However, information learned may benefit other children with HIV in the future.

Your child will have regular visits here, with physical examinations and laboratory tests. This study cannot provide medical care to your child. However, if study procedures show that your child may need medical care, we will tell you where you can go for the care your child needs. This may help your child stay healthy.

Other information about the study

14. There are no costs to you for your child being in the study.

There are no costs to you or your child for study visits or procedures.

If you agree to have blood, nose, and rectal samples collected for future research, there is no cost to you or your child for this. The samples will not be sold, and you will not be paid for the samples. It is possible that research done with the samples could lead to a new discovery or a new product. If this happens, there is no plan to share any money with you or your child.

<<Sites insert information about compensation/reimbursement, for example>> You will be reimbursed for the cost of transport to study visits. For each visit, you will be given *[specify amount].>>*

15. Your child's records may be reviewed by study staff and groups that oversee the study.

Groups that oversee the study include:

- *<<US sites insert single IRB>>*
- *<<All sites insert applicable local IRBs/ECs>>*
- *<<All sites insert other applicable regulatory entities>>*
- The United States National Institutes of Health and its study monitors
- The United States Office for Human Research Protections
- Other United States, local, and international regulatory entities
- The IMPAACT Network that is coordinating the study

Like the study staff, these groups are required to make efforts to keep study records private and confidential.

The results of the study may be presented publicly or published. However, no presentation or publication will use your child's name or identify your child personally. The same is true for research done in the future with stored samples.

A description of this study will be available on ClinicalTrials.gov. This website will not include information that can identify your child. At most, the website will include a summary of the results. You can search this website at any time.

Your child's study information may be disclosed to other authorities if required by law. *<<Sites may add other applicable local reporting requirements in this section.>>*

16. If your child gets sick or injured, please contact us.

Your child's health is important to us. If your child gets sick or injured, contact us immediately. We will make every effort to protect your child's well-being and minimize risks. It is possible, however, that your child could have an illness or injury that is study-related. This means that the illness or injury occurred as a direct result of being in the study.

<<Sites may modify this paragraph to reflect local institutional policies; information regarding coverage available through clinical trial insurance obtained by the site should be included if applicable; the statement regarding no program for compensation through the NIH may not be removed.>> If a study-related illness or injury occurs, we will treat your child or tell you where you can get the treatment your child needs. The cost for this treatment may be charged to you or your health insurance. There is no program to pay money or give other forms of compensation for study-related illness or injury through *<<site name>>* or the United States National Institutes of Health.

Who to contact

If you have questions, concerns, or problems at any time, use these contacts.

- If you have questions about the study:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you have questions about future use of blood, nose, or rectal samples:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you have questions about your child's rights as a study participant, or problems or concerns about how your child is being treated in the study:
  <<Name, phone number, and other relevant contact details of IRB/EC contact person or other appropriate person or organization>>

- If your child has any health or other problems that may be related to study participation:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you want your child to leave the study:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

Signatures

If you decide to have your child join this study, please sign or make your mark below.

Before deciding whether to have your child join this study, make sure you have read this form or had it read to you. Make sure all your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your child.

We will tell you any new information from this study or other studies that may affect your willingness to keep your child in the study. You are welcome to ask questions or request more information at any time.

You do not give up any rights by signing this form.

<<Sites insert initial and signature blocks as required by IRB/EC and institutional policies. Separate consent decisions must be documented for genetic testing of stored samples.>>

For participation in the study (all three should be initialed/marked for participants to be enrolled)

_____ I agree to have my child join this study.

_____ I agree that my child's previous study records may be reviewed and that information from these records can be recorded for this study.

_____ I agree that my child's medical records may be reviewed and that information from these records can be recorded for this study.

For collection of blood samples for future research (one of the three choices should be initialed/ marked)

_____ I allow blood to be collected from my child for future research. I also allow my child's blood to be used for tests of his or her genes.

_____ I allow blood to be collected from my child for future research. I do not allow my child's blood to be used for tests of his or her genes.

_____ I do not allow blood to be collected from my child for future research.

For collection of nose samples for future research (one of the two choices should be initialed/marked)

_____ I allow a sample to be collected from my child's nose for future research.

_____ I do not allow a sample to be collected from my child's nose for future research.

For collection of rectal samples for future research (one of the two choices should be initialed/marked)

_____ I allow a sample to be collected from my child's rectum for future research.

_____ I do not allow a sample to be collected from my child's rectum for future research.

Name of Participant (print)	Participant Signature *(only for those who reach the legal age or circumstance to provide independent consent)*	Date
Name of Parent or Guardian (print)	Parent or Guardian Signature	Date
Name of Witness (if applicable, print)	Witness Signature	Date
Study Staff Conducting Consent Process Name (print)	Study Staff Signature	Date

Appendix IV: Sample Assent Form for Study Participation

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Version 1.0, 23 December 2020

<<The amount of information and level of detail provided in site-specific assent forms should be tailored to the age and maturity of study participants, guided by applicable IRB/EC policies and procedures. Sites may develop multiple assent forms, if desired, in anticipation of different information needs across the study age range. When preparing site-specific assent forms, the wording included in this sample form may be modified, added to, or removed in order to provide the most appropriate information and level of detail for participants, consistent with IRB/EC policies and procedures. This may include preparing site-specific form versions that do not disclose HIV status.>>

Introduction

You are being asked to take part in a research study. For you to take part, you must give your permission. Your parent or guardian must also give permission.

This form gives information about the study. Please read it or have it read to you. Ask any questions you may have. After we talk about the study, you will record your decisions at the end of this form. You will be offered a copy to keep.

Your rights

It is up to you and your parent or guardian to decide if you will take part in the study. You can say yes or no. If you say yes now, you can change your mind later. Your decision will have no effect on your medical care.

About the study

The study is to learn about children who started taking medicines for HIV soon after being born. Studies of these medicines have been done in the past, but there is still more to learn. For example, we would like to learn more about how well children who take these medicines grow. We would also like to learn about health problems children may have as they get older.

What happens in the study

We will first ask some questions, review your records, and examine you (check your body) to see if you qualify for the study.

If you qualify, you will enter the study and have visits here every 6 months for up to 7 years. The visits will take about 2 hours. We will ask questions about your health and the medicines you take. We will examine you and see how much you have grown. We will draw blood for tests. This will be done with a needle in your arm.

We would also like to take sample from your nose and a sample from your rectum for tests. If you agree, we will take the samples with a cotton swab that is placed in the nose or rectum for 5-10 seconds. If you do not agree, these samples will not be taken.

Some tests will check on your health. These will be done right away after each visit. We will tell you and your parent or guardian the results of these tests. Other tests will be done in the future. These tests are not expected to give information that is important for your health. The results will not be given to you or your parent or guardian.

We will tell you as much information as you want about the study. Please ask any questions you may have. Please tell us if anything bothers you or scares you. We will do our best to explain the study and help you feel more comfortable.

What good and bad effects could happen

By taking part in the study, you will be helping to learn more about medicines for children with HIV. This may not have any benefits (good effects) for you. However, information learned in the study may have good effects for other children with HIV in the future.

Having your blood drawn may hurt. This may also cause fainting, bleeding, bruising, swelling, or infection where the needle goes in your arm. Having samples collected from your nose or rectum may hurt. You may feel nervous or embarrassed when answering questions for the study.

There could be other bad effects. For example, other people could find out that you are in the study or learn other information about you. We will do all we can to keep this from happening. For example, most of the records we keep here for the study will be labeled with a code number (not your name). We will share information about you, including information that you tell us, with your parent or guardian. We will not share your information with other people unless you or your parent or guardian ask us to.

Who to contact

You and your parent or guardian can contact us at any time. Please talk to your parent or guardian and to us about any questions or problems you may have.

- If you have questions about the study:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you have problems related to being in the study:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you have questions about your rights or how you are treated in the study:
  <<Name, phone number, and other relevant contact details of IRB/EC contact person or other appropriate person or organization>>

- If you want to leave the study:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

Signatures

Before deciding whether to take part in the study, make sure you have read this form or had it read to you. Make sure all your questions have been answered.

<<Sites insert initial and signature blocks as required by IRB/EC and institutional policies.>>

Please write your initials or make your mark next to your choices.

_____ I agree to take part in the study.

_____ I do not agree to take part in the study.

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Assent Process Name (print)	Signature of Study Staff	Date

Appendix V: Sample Informed Consent Form for Maternal Data Collection

IMPAACT 2028
Long-Term Clinical, Immunologic, and Virologic Profiles of Children who Received Early Treatment for HIV

Version 1.0, 23 December 2020

Introduction

Your child is taking part in the research study named above. The study is to learn more about children who started treatment for HIV soon after birth. You are being asked for permission to collect information about you to be combined with information about your child for the study. You are not being asked to join the study.

Here is a summary of important information:

- If you agree, information about you will be collected for the study by asking you questions and reviewing your medical records.
- There is little risk to you from having information collected for the study.
- There may be no benefit to you from having information collected for this study.
- Your decision on having your information collected will have no effect on your child's participation in the study. Your decision will have no effect on the medical care you or your child receive. Your and your child's access to services, and the benefits and rights you normally have, will not be affected.

More information is given in the rest of this form. Please read it or have it read to you. Ask any questions you may have. We will take as much time as needed for you to fully understand the information. We will ask you questions to see if we have explained the information clearly.

If you decide to have information about you collected for the study, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

About the study

The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) and *<<site name>>* are doing this study. The person in charge of the study at *<<site name>>* is *<<Investigator of Record name>>*. The United States National Institutes of Health is paying for the study.

The study will include up to 250 children from many countries, including Brazil, Botswana, Haiti, Malawi, South Africa, Thailand, Uganda, Zimbabwe, and the United States.

To learn more about children who started treatment for HIV soon after birth, we would like to collect some information about the biological mothers of children taking part in this study. If you agree, we will ask you questions and review your medical records to collect information on:

- The anti-HIV medications (ARVs) you took while pregnant and breastfeeding (if you breastfed)
- The amount of HIV in your blood when you gave birth and while breastfeeding (if you breastfed)
- Your CD4+ cell count when you gave birth

Deciding to have your information collected for the study is voluntary (your choice). You are free to say yes or no. If you say yes now, you can change your mind later. Your decisions will have no effect on your child's participation in the study. Your decisions will have no effect on the medical care you or your child receive. Your and your child's access to services, and the benefits and rights you normally have, will not be affected.

Possible benefits

There may be no benefit to you from having information collected for this study. By having your information collected, you will be part of research for children with HIV. Although this is not expected to benefit you directly, information learned in the study may benefit children with HIV in the future.

Possible risks

There is little risk to you from having information collected for the study. We will make every effort to keep your information private and confidential. Study records will be kept in secure locations. Most records will be labeled only with a code number. However, your name will be written on some records.

<<US sites insert this paragraph>> Your privacy may also be protected by a Certificate of Confidentiality that helps us avoid being forced to release information that may identify you, such as by the courts or police. The certificate cannot be used in all situations, but it can be used to resist demands for information that would identify you. The certificate does not protect against requests for information from the United States federal government. Regardless of the certificate, you can release your information to others, if you wish.

The information we collect about you for the study will be combined with information collected about all other children in the study, and their mothers. This will be done at an organization called a statistical and data management center. The IMPAACT Network statistical and data management center is in the United States. We will send your information to this center. The information will be sent securely, following applicable laws and policies. Your name and other information that could personally identify you will not be sent.

Information collected for this study may be used for other research in the future. Any such research must be approved by the IMPAACT Network. Information used for approved research will be labeled with a code number only. The only link between the code number and your name will be kept here at this clinic. Your name will not be given to other researchers.

Despite our best efforts to keep your information private, it is possible that your information could be obtained by someone who should not have it. If this were to happen, you could be treated badly or unfairly.

Costs to you

There are no costs to you for having your information collected for the study.

<<Sites insert information about compensation/reimbursement, for example>> You will be reimbursed for the cost of transport to study visits. For each visit, you will be given *[specify amount].>>*

Review of study records

The records that contain the information collected from you for the study may be reviewed by study staff and groups that oversee the study. Groups that oversee the study include:

- *<<US sites insert single IRB>>*
- *<<All sites insert applicable local IRBs/ECs>>*
- *<<All sites insert other applicable regulatory entities>>*
- The United States National Institutes of Health and its study monitors
- The United States Office for Human Research Protections
- Other United States, local, and international regulatory entities
- The IMPAACT Network that is coordinating the study

Like the study staff, these groups are required to make efforts to keep study records private and confidential.

The results of the study may be presented publicly or published. However, no presentation or publication will use your name or identify you personally.

A description of this study will be available on ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Your information may be disclosed to other authorities if required by law. *<<Sites may add other applicable local reporting requirements in this section.>>*

Who to contact

- If you have questions about the study or the information collected from you for the study, contact:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you change your mind about having information collected for the study, contact:
  <<Name, phone number, and other relevant contact details of investigator or other study staff>>

- If you have questions about your rights or problems or concerns about how your information is being used in the study:
  <<Name, phone number, and other relevant contact details of IRB/EC contact person or other appropriate person or organization>>

Signatures

If you decide to have your information collected for the study, please sign or make your mark below.

Before deciding whether to have your information collected, make sure you have read this form or had it read to you. Make sure all your questions have been answered.

We will tell you any new information from this study or other studies that may affect your willingness to have your information used for the study. You are welcome to ask questions or request more information at any time.

You do not give up any rights by signing this form.

<<Sites insert signature blocks as required by IRB/EC and institutional policies.>>

Name of Child Participating in Study (print)		
Name of Mother (print)	Mother Signature	Date
Name of Witness (if applicable, print)	Witness Signature	Date
Name Study Staff Conducting Consent Process With Mother (print)	Study Staff Signature	Date

Appendix VI: Operational Guidance for Study Implementation at Sites Experiencing Operational Disruptions Due to COVID-19

To safeguard the health and well-being of study participants in the context of circulating SARS-CoV-2 and the associated coronavirus disease 2019 (COVID-19), the guidance provided in this appendix may be implemented at sites experiencing operational disruptions due to COVID-19.

The extent to which site operations may be disrupted by COVID-19 may vary across sites and over time. **All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.** All sites must also comply with any directives received from the study sponsor, the IMPAACT Network, and/or the IMPAACT 2028 Protocol Team. Should a determination be made in the future that the guidance provided in this appendix is no longer applicable, sites will be formally notified and instructed to inform their IRBs/ECs and other applicable regulatory entities.

Visit Scheduling

- Sites are advised to make use of the allowable visit windows specified in Section 5 when scheduling study visits during periods of operational disruption. For example, sites that are able to anticipate operational disruptions are advised to conduct study visits early in the allowable visit window before the disruption occurs. Visits conducted outside of allowable windows are also preferred to missed visits. In the event of a missed visit (i.e., visit not conducted before the allowable window closes) or in situations that may result in conducting more than one visit within a given window, the Protocol Team should be contacted for guidance on visit completion on a case-by-case basis.

Prioritization of Study Visit Procedures

- If it is not possible to conduct study visits in-person at the study site, visit procedures may be performed off-site or remotely (e.g., by telephone) as described below. Site investigators must ensure that standard operating procedures are in place for off-site and remote procedures.
- Sites may conduct study visits — in full or in part — off-site if permitted by applicable government, health authority, and institutional policies. Where this option is permitted, site staff should communicate with participant families to determine in advance where and when such visits will take place, with adequate protections for safety, privacy, and confidentiality. Off-site visit procedures should be conducted by site staff who are adequately qualified and trained to conduct the procedures, as determined by the site investigator, with attention paid to occupational health, biohazard containment, and specimen and data chain of custody. These staff should also be adequately qualified and trained to immediately assess and/or manage any clinical events or social impacts that may occur during the visits. If clinical events requiring further evaluation or management are identified during an off-site visit, staff conducting the visit should arrange for appropriate clinical management, in consultation with the site investigator as needed.
- Sites with limited capacity to conduct in-person visits should refer to the prioritization provided in Section 5.8.1. HIV-1 RNA and CD4+ and CD8+ assays are of highest priority. If these assays cannot be performed consistent with a site's Protocol Analyte List, the tests may be performed in alternate laboratories using alternate assays (alternate laboratories must adhere to local regulations for clinical laboratory testing). Specimens should also be collected for storage whenever possible, assuming laboratory staff and facilities are available to process and store specimens consistent with the Laboratory Processing Chart. When necessary, medical and medication histories as well as feeding histories can be obtained from participants or caregivers remotely. Written records that supplement participant and caregiver report may be collected at a later date (when it is feasible to do so).

Documentation

- Site-specific contingency plans, and the implementation thereof, should be documented in essential document files for IMPAACT 2028.
- Documentation should be entered in participant study charts in real-time (or close to real-time) if any of the following occur:
  - Missed visits
  - Out-of-window visits
  - Off-site visits (document the location of the visit)
  - Incomplete or partial visits (document which procedures were performed and which were not)
  - Remote contacts performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)
  - Any other participant contacts
  - Use of alternate laboratories or alternate laboratory assays
- In consultation with the Division of AIDS, the IMPAACT Network has developed and disseminated guidance for documenting and/or reporting protocol deviations that may occur due to limited site capacity to conduct study visits or procedures due to COVID-19. Please contact the IMPAACT Operations Center Clinical Trials Specialists with any questions related to documentation and reporting requirements.