IMPAACT P1092

IMPAACT P1092: PHASE IV EVALUATION OF THE STEADY STATE PHARMACOKINETICS OF ZIDOVUDINE, LAMIVUDINE, AND LOPINAVIR/RITONAVIR IN SEVERELY MALNOURISHED HIV-1-INFECTED CHILDREN

Statistical Analysis Plan

Version 3.0

August 20, 2018

ClinicalTrials.gov ID: NCT01818258

This is IMPAACT P1092 SAP Version 3.0 with names of authors, names of publication writing team members and analysis timeline redacted.

Table of Contents:

1. Introduction

This document describes the proposed content for the primary statistical analysis of study P1092, focusing on analyses that address the primary objectives and secondary objectives that will contribute to the planned primary manuscript. This version 2.0 of the plan focuses on the final analysis and updates version 1.0 which described the analyses in reports for the Study Monitoring Committee (SMC) while the study was ongoing. This plan forms the core of any presentation or publication used to disseminate the primary conclusions of the study.

2. Study Overview

2.1 Study Design

P1092 (version 2.0) is a Phase IV open label study to evaluate the pharmacokinetics (PK), safety, and tolerability of antiretroviral drugs zidovudine (ZDV), lamivudine (3TC), and ritonavir boosted lopinavir (LPV/r) in children infected with HIV and children aged ≥6 months (≥180 days) to <36 months who were grouped by their nutritional status. The overall goals for P1092 are to characterize the PK and safety of ARVs in severely malnourished children (SAM) following the initiation of nutritional rehabilitation to compare results to normal-mildly malnourished children in order to determine if current recommended doses are optimum in severely malnourished children.

Two cohorts of children were targeted: cohort 1: children living with HIV who were severely malnourished and eligible for HAART as defined by WHO 2013 pediatric guidelines (described in protocol section 4.14 and below) and cohort 2: a control group of children living with HIV and mild malnutrition-normal nutrition who were also eligible for HAART. Children with severe malnutrition underwent an approximate two week nutrition rehabilitation program before entering the study.

The 2013 WHO pediatric guidelines for severe acute malnutrition (non-edematous), mild nutrition, and normal nutrition were used to classify the two cohorts (protocol section 4.14). The classification was as follows:
- Severe non-edematous malnutrition: weight for height z-score (WHZ) < - 3 or mid-upper arm circumference (MUAC) < 115 mm (Cohort 1)
- Mild nutrition: -2 < weight for height z-score (WHZ) ≤ -1 (Cohort 2)
- Normal nutrition: weight for height z-score (WHZ) > -1   (Cohort 2)

The study involved two stages. Stage 1 was the pre-entry/screening stage for both cohorts.   Children with severe malnutrition underwent a 10 to 18 day nutrition rehabilitation program in Stage 1 before entering the study. The WHO (World Health Organization) approach to the management of SAM was used, which involves a stabilization and rehabilitation phase. Stage 2 began with the study participant's registration to the study and initiation of the study antiretroviral (ARV) regimen. All participants were to receive ZDV+3TC+LPV/r upon study entry/registration (Stage 2).   For children with severe malnutrition, pre-entry/screening and enrollment occurred during an inpatient stay in the nutritional rehabilitation unit, with the entry/registration visit conducted 10-18 days after the day of admission to the nutritional rehabilitation unit. Children with mild malnutrition-normal nutrition were enrolled from HIV treatment centers and were scheduled to have the entry/registration visit within 14 days after pre-entry/screening. Children in both cohorts were judged by their clinician to be stable before study entry/registration. Participants needed to clinically improve on nutrition rehabilitation in order to be registered/enrolled to the study after Stage 1 (see protocol section 4.17 for the definition of nutrition rehabilitation). The study regimen was ideally initiated on the day of enrollment but was permitted to be initiated up to 3 days after the day of enrollment. ARVs were dosed based on WHO weight band dosing (see protocol section 5.1). Participants were allowed to have a receipt of ARVs prior to study entry, including during the rehabilitation period.

Each enrolled participant was to be followed for 48 weeks, with scheduled visits at weeks 1, 2, 4, 8, 12, 16, 20, 24, 36, and 48 of follow-up. At weeks 1, 12, and 24, intensive PK sampling was to be carried out following a morning dose of ARVs; for children weighing at least 5 kg, an additional sample generating 1 mL of plasma was collected at the 2 hour time point to measure free fraction of lopinavir at the time of peak concentration at weeks 1, 12, and 24. At weeks 4, 8, 16, 36, and 48 a trough PK sample was collected just prior to the morning dose of ARVs. PK trough samples were collected also just prior to dosing as part of intensive sampling at weeks 1, 12, and 24. Steady state was expected to be reached by week 1.

It was expected that 25 children needed to be enrolled in each cohort to achieve 17 evaluable in each cohort, with evaluable defined as having results available for the week 1 intensive PK evaluations and either the week 12 or the week 24 intensive PK evaluations. Fifty-two participants were enrolled: 25 in cohort 1 and 27 in cohort 2. The study accrued for a total of 13 months with cohort 2 closing to accrual in March 2016 and cohort 1 closing to accrual in November 2016. Each nutrition cohort was stratified by two age groups of <18 months and ≥18 months. Because it was desired to achieve balanced age distribution within and between cohorts, an initial upper bound of 13 participants was established for each cohort age group. Eleven and 13 participants under 18 months were enrolled in cohort 1 and cohort 2, respectively. Fourteen participants aged 18 months or older were enrolled in cohort 1, and 14 were enrolled in cohort 2.

2.2 Hypotheses

It is hypothesized that children with severe acute malnutrition will have reduced absorption of antiretroviral drugs compared to those with mild malnutrition-normal nutrition (protocol section 1.12).
A secondary hypothesis is the change from baseline for CD4 percent at 12 and 24 weeks will be smaller for the cohort with severe malnutrition at baseline (protocol section 1.23).

2.3 Study Objectives

Objectives and outcomes are per protocol version 2.0, sections 2 and 8, except where indicated with text in italics.

2.3.1   Primary Objectives and Outcome Measures

1. **Objective:** To compare the PK exposure (as estimated by the area under the plasma concentration versus time curve, AUC) and clearance of ZDV, 3TC, and LPV/r under steady-state conditions between severely malnourished children and children with mild malnutrition-normal nutrition at weeks 1, 12, and 24 following study entry.

   Outcome Measure: Steady-state AUC and plasma clearance (CL/F) for ZDV, 3TC, and LPV/r

2. **Objective:** To evaluate the safety and tolerability of ZDV, 3TC, and LPV/r in severely malnourished children and children with mild malnutrition-normal nutrition at 24 weeks following study entry.

   Outcome Measure: Safety and tolerability measures, which include the number and percent of participants with at least Grade 3 adverse events related to study drugs and at least Grade 3 adverse events regardless of the relationship to study drugs *through week 24*.

2.3.2   Secondary Objectives and Outcome Measures

1. **Objective**: To compare the minimum concentration (C_{trough}) of LPV/r between severely malnourished children and children with mild malnutrition-normal nutrition at 1, 4, 8, 12,16, 24, 36, and 48 weeks following study entry.

   Outcome Measure: The minimum concentration ("trough") of LPV/r in the two cohorts at weeks 1, 4, 8, 12, 16, 36, and 48

2. **Objective:** To investigate the impact of malnutrition on LPV protein binding by comparing the free fraction of LPV in severely malnourished children and children with mild malnutrition-normal nutrition at 1, 12, and 24 weeks following study entry.

   Outcome Measure: The measures of LPV protein binding *(free fraction)* in the two cohorts at weeks 1, 12, and 24

3. **Objective:** To compare the viral loads between severely malnourished children and children with mild malnutrition-normal nutrition at baseline, 12, 24, 36, and 48 weeks following study entry.

   Outcome Measure: The *plasma* HIV-RNA load in the two cohorts at study entry and Weeks 12, 24, 36, and 48

4. **Objective:** To compare CD4 cell percent between severely malnourished children and children with mild malnutrition-normal nutrition at baseline, 12, 24, 36, and 48 weeks following study entry.

   Outcome Measure: The CD4 percent in the two cohorts at study entry and Weeks 12, 24, 36, and 48

5. **Objective:** To describe the recovery of lean body mass and linear growth in severe acute malnutrition at 24 and 48 weeks following study entry.

   Outcome Measures:
   - Description of the recovery of the lean body mass by assessment of weight to height z-score (WHZ) and mid-upper arm circumference (MUAC) in children in severe acute malnutrition at weeks 24 and 48
   - Assessment of height and change in height over time in severe acute malnutrition.

Analyses of all primary and secondary outcome measures, except secondary outcome measure 2 investigating LPV protein binding, will contribute to the planned primary manuscript. The LPV protein binding analysis and additional exploratory analyses, including the investigation of micronutrient data, will be evaluated in future analyses.

2.4 Study Sample Size

From protocol version 2.0 section 8.4: "The primary objective is to compare severely malnourished and normal–mildly malnourished children on HAART with respect to pharmacokinetic exposures quantified as concentration AUCs. The coefficient of variation of AUC for LPV was estimated to be approximately 0.40 in similarly aged children (50). The protocol team considers a 40% difference between groups with respect to LPV AUC to be clinically important. A parallel-arm study with two groups would require N=17 patients per group to detect a 40% difference between groups in the LPV AUC with 80% power and a two-sided Type I error of 5% for a CV of 0.4. In addition, a 10% LTFU and 20% mortality rate (current mortality rate in the malnutrition unit) is considered for the estimate. This yields a necessary sample size of 17 children + 30% for a total of 23 children required per treatment group. Because of uncertainties in the estimation of AUC CV and possible heterogeneity in variance associated with differential malnutrition status, we suggest a further 10% inflation to 25 children per nutrition status stratified by age."

2.5 Protocol Visit and Evaluation Schedule

2.5.1   Stage 1

The Stage 1 visits begin after consent and include screening/pre-entry visit data. Stage 1 visits must have been performed within 18 days prior to entry/registration for cohort 1 and within 14 days prior to entry/registration for cohort 2.

2.5.2   Stage 2

Clinic study visits for Stage 2 were scheduled at Stage 2 registration (entry/registration visit), week 1 (+3 days), week 2 (+/- 2 days), week 4 (+/- 7 days), then every 4 weeks (± 7 days) until week 24, then every 12 weeks (+/- 14 days) until week 48 (+/- 14 days).

2.6 Major Changes in Protocol History

Protocol version 1.0 was finalized on February 24, 2012. Letter of Amendment (LoA) #1 was issued on September 13, 2012 and included a number of protocol modifications. While waiting for the site institutional review board (IRB) approval, IMPAACT leadership paused the protocol. After IMPAACT leadership gave approval for further development of the protocol in 2014, protocol version 2.0 was finalized on February 11, 2015. No participants were enrolled under version 1.0 or LoA #1 of the protocol. All participants were enrolled under protocol version 2.0.

LoA #1 for protocol version 2.0 was issued on April 12, 2016. Initially, the SMC was notified when hematologic grade 3 or 4 events occurred and accrual was temporarily suspended. Instead of stopping accrual for every hematologic grade 3 or 4 event, the LoA #1 allowed the DAIDS/NICHD Medical Officers to review grade 3 and 4 hematologic events to determine if accrual needed to be temporarily suspend and if an SMC review was required.

2.7 Monitoring

The P1092 Core Team (the protocol chair, vice chair, DAIDS and NICHD medical officers, clinical trials specialists, data and laboratory data managers, statisticians, pharmacologists, immunologist, nutritionist, laboratory technologist) was responsible for ongoing safety monitoring. The Core Team focused on the clinical management of individual participants. Conference calls were scheduled monthly to review protocol activation, accrual, barriers to any issues arising with study conduct, and new adverse events as well as assessment of relationship to study treatment by the Chairs and Medical Officers recorded in the database by the data manager. In addition, serious adverse events (SAEs) were reported to the DAIDS Safety Office during the protocol-defined expedited adverse event (EAE) reporting period which was the entire study duration for an individual participant (from study enrollment until study completion or discontinuation).

At any time the P1092 Core Team could have requested an external review by the SMC to review any study treatment-related events that may have compromised participant safety.

2.7.1   SMC Review History

The SMC reviewed study conduct, including accrual, retention, and safety. A full review was to occur every 12 months or on a schedule specified by the SMC. An *ad-hoc* SMC review was required if:

- Any participant had a life-threatening adverse event that was judged to be probably or definitely attributable to study drug or any Grade 4 event that may not be judged to be life-threatening but was judged to be probably or definitely attributable to the study drug.
- Or if more than 6 out of the first 20 participants or at least 30% of participants thereafter have Grade 3 or greater toxicity that was judged to be definitely related to study drug.

The Core Team monitored participant events and assessed relationship with study treatment within 48 hours of notification. If events were assessed as probably or definitely related to study drug by the Core Team, accrual was required to immediately pause and the SMC to be urgently convened to review the events and determine whether the study should be suspended or modified. Immediately following review by the SMC, the Core Team provided the study sites with documentation of the events that occurred, the team and SMC review of the events, and the recommendations of the SMC, for submission to their ethical and drug regulatory review bodies.

The IMPAACT SMC conducted 4 interim reviews to monitor accrual, safety, participant growth and viral load, PK sample evaluability, and study implementation quality approximately every six months. After the second interim review, the SMC suggested to no longer require pausing accrual for grade 3 or 4 hematologic events.

2.8 General Statistical Considerations

Sample
   a. Participants' data through their study follow-up discontinuation (off study) date will be summarized.
   b. PK evaluability: the goal of the study was to have all participants have intensive PK parameters available for weeks 1, 12, and 24 following initiation. One criterion for evaluability was the participant must have had results for the week 1 intense PK evaluations and either week 12 or 24 intense PK results. Additional exclusions for PK evaluability will be summarized along with reasons for the exclusion.
   c. Participants will be excluded from the week AUC calculation if 3 or more concentration samples are missing from the intensive PK evaluation.
   d. Drug concentration levels: the lower limit of detection (LLD) of the assays was the following for the study ARVs: 24 ng/mL for ZDV, 24 ng/mL for 3TC, 0.02 ng/mL for LPV, and 0.005 ng/mL for RTV. Drug concentrations below the limit of quantification (BLQ, the equivalent to LLD) will be handled using standard pharmacokinetic methods to assure all data are handled in a consistent manner. Pre-dose concentrations BLQ will be assigned a concentration of 0. The first concentration value post-dose BLQ will be assigned 50% BLQ then a concentration of 0 thereafter.
   e. Participants eligible for safety and tolerability analyses received at least one dose of ZDV, 3TC, and LPV/r.

Definitions
   f. <u>Participant level PK parameters</u> will be calculated by the study pharmacologists and data will be submitted to the study database (see protocol section 9).
       1. Per protocol, for PK AUCs a non-compartmental analysis will be performed for individual PK estimates via the linear up-log down trapezoidal rule in conjunction with an oral input model using WinNonlin 5.0.1 (Pharsight Corporation, Mountain View, USA) from intensive PK sampling visits. PK AUC is based on a 0-12 hour dosing cycle.
       2. Per protocol, plasma PK data from intensive sampling visit will be analyzed using non-compartmental analysis to determine the following PK measures for ZDV, 3TC, LPV and RTV. C_{trough}, C_{max}, and T_{max} will be determined by inspection of the results.
   g. Per protocol section 9.3, C_{trough} is the pre-dose sample concentration, C_{max} is the peak plasma concentration, and T_{max} is the time to reach C_{max} after drug ingestion. C_{trough} samples will be included in analyses if drawn within 2 hours before dosing (post-protocol definition). If there is more than one sample within 2 hours before dosing, the minimum concentration value will be used as the C_{trough} measurement. <u>Safety outcome measures</u> related events were graded according to The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0.
   h. <u>Weight-for-height z-scores</u> were calculated from the SAS macro for Z-score calculations based on WHO standards for children aged 0-≤ 5 years (< 61 months), assuming length was measured. More information on the SAS macro is located at www.who.int/childgrowth/software/en.
       For each clinical visit, weight and height were collected and weight-for-height z-scores will be calculated from these measurements with the SAS macro.
   i. <u>Early ARV treatment discontinuation</u> is permanently discontinuing all components the study drugs of ZDV, 3TC, and LPV/r before completing 48 weeks of study follow-up as defined by the protocol for any reason (participant must have a study discontinuation date within or after the window for the week 48 visit date). Switches to abacavir (ABC) due to ZDV toxicity will be summarized with early treatment discontinuation reasons, if applicable.

Statistical Analyses
   j. Unless otherwise stated, summaries will be shown by cohort group.

k. Unless otherwise stated, tests will be two-sided; continuous outcome measures will be compared between cohorts with a t-test with unequal variance; categorical outcome measures will be compared by Fisher's exact test.

l. Repeated measure analyses will use a linear mixed effects model to evaluate intensive PK parameters and of growth over time within and between cohorts. The average difference and difference in trajectories of PK and growth parameters will be evaluated.

m. Summaries for PK parameters will provide the following: mean, standard deviation, and quartiles on the original scale, geometric mean and geometric mean ratio. All comparisons will be performed on the (natural) logarithmic scale.

n. Per protocol section 8, pre-specified null hypotheses will be tested individually at the 0.05 level of significance. Ad hoc analysis of data within subgroups will be presented with caveats.

o. This is a phase IV study and not subject to NIH requirements that primary analyses of treatment comparisons be summarized by sex and race.

p. Statistical software SAS 9.4 will be used to generate analysis summaries.

3. Key Elements of the Statistical Report

3.1 Screening

Summary of inclusion and exclusion screening failures

3.2 Accrual

a. Summary of participants enrolled over time by country, site, cohort, and age group.
b. Summary of the time between screening and entry/registration

3.3 Eligibility Violations

Summary of the types of reported eligibility violations by cohort and age group

3.4 Selected Characteristics at Screening and Baseline

Baseline values are the values measured on the Stage 2 study entry/registration visit (≤ 0 days from entry/registration).
Screening/pre-entry values are the values measured on the Stage 1 screening visit (date of consent). If screening/pre-entry measurements were taken at multiple times before entry/registration, the measurement closest to the entry/registration date will be used if within 18 days of the entry/registration date for cohort 1 or 14 days for cohort 2 (maximum days between screening/pre-entry and entry/registration visits are defined in protocol version 2.0).
No statistical comparison will be done comparing cohorts.

The following characteristics will be summarized at either baseline or screening, as specified:
- Age at enrollment (months) and categorized age groups(<18, ≥18 months)
- Gender
- Race/ethnicity
- Country
- Nutrition category (normal, mild malnutrition, and severe malnutrition)
- Baseline HIV-1 RNA (copies/mL) (< 400, 2,000-< 10,000, 10,000-<20,000, ≥ 20,000)
- WHO clinical stage

- Categorized screening/pre-entry absolute CD4+ counts (cells/mm^3) (< 400, 400-<1,100, 1,000-< 1,500, 1,500-<2,000, 2,000-<2,500, 2,500-<3,000, ≥ 3,000)
- Categorized screening/pre-entry CD4% (< 15, 15-<25, 25-<50, ≥ 50)
- Screening/pre-entry absolute CD4+ counts (cells/mm^3), CD4%
- Baseline $\log_{10}$ HIV-1 plasma RNA (copies/mL)
- Baseline weight (kg)
- Baseline height (cm)
- Baseline head circumference (cm)
- Baseline middle upper arm circumference (cm)
- Baseline weight-for-height WHO Z-score
- ARV regimens previously taken by participants
- Most common diagnoses before or ongoing at study entry/registration
- Baseline electrolyte abnormalities and lipid profiles

3.5 Study Follow-up Status

a. CONSORT diagram statistics summarizing the number of participants screened, enrolled, started treatment, completed treatment, completed study, and reasons for the early discontinuation of treatment or the study
b. Total summary of participant off study week with reason for discontinuing
c. Summary of participant off study week with reason for discontinuing by cohort and age group
d. Summary of types of reported protocol deviations

3.6 ARV Initiation and Modification

a. Summary of days from entry/registration to study ARV initiation
b. Summary of early permanent study ARV treatment discontinuations with reasons
c. Summary of switches from ZDV to Abacavir (ABC)

3.7 Descriptive Summaries

3.7.1   Pharmacokinetics

Summary PK measures will be provided for study drugs ZDV, 3TC, LPV, and RTV. Intensive PK measurements were scheduled for sampling at pre-dose and hours 1, 2, 4, 8, and 12 post-dose.

a. Summary of exclusions from PK analyses and reasons for exclusions
b. Summary of imputations for concentration values below the lower limit
c. Concentration-time plots for each participant at weeks 1, 12, and 24 by study drug
d. Summary of C_{max}, T_{max}, C_{trough}, and AUC of study drugs at weeks 1, 12, and 24
e. Summary of time between past dose, C_{trough} sample measurement, and next dosing

3.7.2   Safety

a. Descriptions and narratives of study deaths
b. Summary of grade 3 or higher adverse events regardless of relationship to study treatment

Notes for safety outcome measure definitions:

1. The protocol required grading of events according to The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, Nov 2014, which is available on the RCC website: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_Grading_Table_v2_NOV2014_Highlighted_Changes.pdf

2. The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, was used for study event reporting. In addition, other events that sites were required to report in an expedited fashion included all malignancies, seizures, and grade 3 and 4 hepatotoxicities whether or not symptomatic or related to study drug, and all other grade 3 or 4 related toxicities for which a relationship to study drug could not be ruled out.

3. The drugs that were assessed for relatedness included ZDV, 3TC, and LPV/r. If participants switched from ZDV to ABC, this was noted and relatedness to ABC was also assessed.

4. The following are examples of details which clarify the events to be included or excluded:
    *   Events with date of onset or specimen date prior to or on the first study treatment dose will be excluded
    *   All events afterwards (on or off study treatment) will be included
    *   Signs and symptoms with an onset date the same as the date of the first study treatment dose will be considered a sign and symptom at baseline and will be excluded.
    *   Laboratory abnormalities with a specimen date the same as the date of the first study treatment dose will be excluded on the assumption that the specimen was drawn before study treatment was administered.
    *   Deaths will be included with grade assigned 5 and will be included in analyses

5. During the study, the Core Team assigned their assessment of treatment relationship to the following targeted study events:
    *   Grade ≥ 3 signs/symptoms, laboratory values
    *   All diagnoses at entry/registration and thereafter
    *   For Triglycerides/Total Cholesterol, if fasting, we will report all grade ≥ 3 events. Since we do not have DAIDS grading criteria for non-fasting Triglycerides/Total Cholesterol, we will use the grade 2 cut off values for non-fasting participants for Triglycerides/Total Cholesterol. It is specified as follows: Triglycerides is > 300mg/dL or Triglycerides > 3.42mmol/L, Total Cholesterol ≥ 200mg/dL or Total Cholesterol ≥ 5.15mmol/L

3.7.3   HIV Plasma Viral Load

Longitudinal summary of the $\log_{10}$ HIV-1 plasma RNA copies/mL for each participant

3.7.4   Growth

a. Longitudinal summary of the by-participant weight-for-height WHO Z-score
b. Longitudinal summary of the by-participant growth data including: height, weight, head circumference, skin fold thickness, and MUAC

4. Primary Analysis

1. **Objective:** To compare the PK exposure (as estimated by the area under the plasma concentration versus time curve, AUC) and clearance of ZDV, 3TC, and LPV/r under steady-state conditions between severely

malnourished children and children with mild malnutrition-normal nutrition at 1, 12, and 24 weeks following study entry/registration.

Outcome Measure: Steady-state AUC_{0-12} and plasma clearance (CL/F) for ZDV, 3TC, and LPV/r

Analysis: Two-sided t-tests with 95% CIs will be applied at weeks 1, 12, and 24 to compare the geometric mean of AUC and clearance using the log (ln) transformed values between the two cohorts.

A sensitivity analysis linear regression adjusting for the age stratification factor will be conducted to compare the cohort differences of AUC and clearance.

A supplementary repeated measures analysis using a mixed effects model will be applied to evaluate AUC and plasma clearance over time with indicator (class) week variables. The average AUC (clearance) over time will be estimated within and compared between cohorts. Pairwise differences between weeks in PK parameters will be assessed within and between cohorts.

2. **Objective:** To evaluate the safety and tolerability of ZDV, 3TC, and LPV/r in severely malnourished children and children with mild malnutrition-normal nutrition at 24 weeks following study entry/registration.

   Outcome Measure: Grade 3 or higher adverse events at least related to the study treatment and grade 3 or higher adverse events regardless of the relationship to the study treatment through week 24 in stage 2 follow-up.

   Two-sided Fisher exact tests (presenting both 90% and 95% confidence intervals, as specified in the sample size calculation in protocol section 8) will be applied to compare the frequencies of the grade 3 or higher adverse events through week 24 between cohorts. One test will be restricted to adverse events assessed by the Core Team as related to the study treatment. Summaries of other toxicity measures such as proportion of participants experiencing vomiting and diarrhea, and frequency of serious of adverse events through week 24 will also be provided.

   A subset analysis will be completed to compare the proportion of adverse events experienced among participants evaluable for the intensive PK analysis.

5. Secondary Analyses

1. **Objective:** To compare the minimum concentration (C_{trough}) of LPV/r between severely malnourished children and children with mild malnutrition-normal nutrition at 1, 4, 8, 12, 16, 24, 36, and 48 weeks following study entry.

   Analysis: Summaries of the C_{trough} of LPV and RTV will be provided at weeks 1, 4, 8, 12, 16, 24, 36, and 48 between the two cohorts.

   Target PK—The trough measurements collected in children will be compared to published measurements for lopinavir trough of >1 ng/mL. The proportion (with 95% CI) of children with >1 ng/mL of LPV trough will be summarized for each week by cohort. The proportion of children with C_{trough} of LPV above 1 ng/mL across all weeks will also be summarized by cohort. A repeated measures analysis will be applied to evaluate C_{trough} of LPV over time.

   A sensitivity analysis adjusting for age stratification factors will be conducted to compare the cohort differences of the C_{trough} of LPV and RTV.

2. **Objective:** To investigate the impact of malnutrition on LPV protein binding by comparing the free fraction of LPV in severely malnourished children and children with mild malnutrition-normal nutrition at 1, 12, and 24 weeks following study entry. **[This objective will not be included in the primary manuscript]**

   Analysis: Summaries of the free fraction of LPV will be provided for weeks 1, 12, and 24 following study entry/registration between the two cohorts. The average and 95% CI for each week by cohort will be calculated. T-tests with 95% CIs will be applied at weeks 1, 12, and 24 to compare the free fraction of LPV between the two cohorts. A repeated measures analysis will be applied to evaluate the free fraction of LPV over time.

   A sensitivity analysis adjusting for age stratification factors will be conducted to compare the cohort differences of the free fraction of LPV.

3. **Objective:** To compare the viral loads between severely malnourished children and children with mild malnutrition-normal nutrition at baseline, 12, 24, 36, and 48 weeks following study entry.

   Analysis:

   a. T-test on the mean change of $\log_{10}$ HIV plasma viral load from study entry/registration at weeks 12, 24, 36, and 48 between cohorts
   b. Number of participants (%) with a HIV viral load ≤ 400 copies/mL at study entry/registration, weeks 24 and 48 by cohort. Two-sided Fisher exact tests will be applied to compare frequencies between cohorts. A sensitivity analysis including all participants on study, counting those who do not have data as not suppressed, will also be conducted.

4. **Objective:** To compare CD4 cell percent between severely malnourished children and children with mild malnutrition-normal nutrition at baseline, 12, 24, 36, and 48 weeks following study entry.

   Analysis: T-test between cohorts on the mean change of CD4% from baseline to weeks 12, 24, 36, and 48.

   Note: CD4 was observed at the screening visit. The CD4% measurement closest to the entry/registration visit will be used for this analysis.

5. **Objective:** To describe the recovery of lean body mass and linear growth in severe acute malnutrition at 24 and 48 weeks following study entry.

   Analysis: The following analyses will be carried within the cohort 1 (severely malnourished) and will employ a two sided one-sample t-test at a 5% type I error level for the change in WHO weight-for-height Z-score from baseline at weeks 24 and 48. A repeated measures analysis will be applied to evaluate the growth of participants over time with mixed effects model, with week as a continuous covariate.

   The recovery of the lean body mass will be assessed by WHO weight-for-height z-score (WHZ) and mid-upper arm circumference (MUAC) in children in severe acute malnutrition (protocol section 8.2). Linear growth will be assessed by height and change in height over time in severe acute malnutrition (protocol section 8.2).

   Summaries of WHZ, MUAC, height, and MUAC Z-Score will provided for cohort 1 at study entry/registration, weeks 24 and 48.

6. Appendix

Schedule of Evaluations

	Screening	Entry	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 36	Week 48
Physical Exam[1]	X	X	X	X	X	X	X	X	X	X	X	X
Nutritional Assessment[2]	X	X	X	X	X	X	X	X	X	X	X	X
Hematology[3]	X		X		X	X	X			X		X
Chemistries[4]	X		X		X	X				X		X
Total Protein/ Albumin		X				X		X				X
Glucose					X							X
Lipid Profile[5]		X						X				X
Micronutrients[6]		X										X
HIV-1 RNA[7]	X	X					X			X	X	X
Lymphocyte Subsets[8]	X						X			X	X	X
Trough PK					X	X		X			X	X
Intensive PK			X				X			X		

1. Physical exam includes length, weight, head circumference, and vital signs (temperature, heart rate, and respiratory rate). Skin fold thickness (triceps) will be at entry, weeks 24 and 48
2. Nutritional assessment includes mid upper arm circumference
3. Hematology include CBC with differential and platelet count
4. Chemistries include AST, ALT, creatinine, sodium, potassium, chloride, and bicarbonate
5. Lipid profile includes triglycerides and total cholesterol
6. Micronutrients include zinc and selenium to be processed and stored until shipping to the central laboratory for testing
7. Must be performed at DAIDS VQA-certified laboratory
8. Lymphocyte subsets include absolute counts and percentages of CD4 and CD8

SAP Version History

Version	Changes Made	Date
1.0	First Version	February 22, 2016
2.0	• Expanded on SMC Review History • Clarification added to Sections 1 and 2 • Letters a-k added to General Statistical Considerations Section • Summaries (including tables and figures) used only for monitoring reports were omitted • Sections 3.4-3.7 were added or updated for final report summaries • Planned statistical analyses were updated for clarification or summaries were added to both primary outcomes and all secondary outcomes • Core manuscript writing team roster updated • Schedule of evaluations added per protocol • Timeline for data analysis and manuscript preparation updated	February 1, 2018
3.0	• Clarified what ARVs could be taken prior to study entry • Provided updates in section 2.8 for clarification • Provided further clarification on longitudinal analysis in section 2.8 and in section 4 for analysis of primary outcomes • Table summary (c) in 3.7.2 was deleted • Both confidence intervals (90% and 95%) will be presented for the safety primary analysis	August 20, 2018