Statistical Analysis Plan

Isala study: Analytical treatment interruption in HIV positive patients with low viral reservoir to evaluate the potential of a functional cure

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1. Introduction

This Statistical Analysis Plan (SAP) provides a description of the main, pre-planned analyses for the Isala study. The purpose of this study is to demonstrate that treatment interruption of longstanding combined antiretroviral therapy (cART) in participants with very low proviral reservoir results in "functional cure". The study conduct is described in Protocol ITM0714.

These planned analyses will be performed by the statistician at the Clinical Trials Unit of the Institute of Tropical Medicine (Antwerp) in collaboration with the coordinating investigator. The analysis results will be described in a statistical analysis report, to be used as the basis of the primary research. This document describes statistical methods for the primary, secondary and tertiary outcomes of the study as defined by protocol. Additional analyses may be performed but are not covered by the current analysis plan. Statistical methods for these additional analyses will be described together with the results.

This analysis plan will be finalized and approved before final database lock. Major changes in statistical methodology used for the main and pre-planned analyses from this SAP, which is finalized before database lock, will require detailed description and justification in the statistical analysis report. The final analysis datasets, programs, and outputs are archived following good clinical practice guidelines (ICH E9).

2. Study design and objectives

2.1. Study design

This is a single arm multi-centric non-randomized (prospective) clinical trial. The study will be divided in two parts. The first one will aim at quantifying the HIV reservoir in a large group of patients under ART with strictly predefined inclusion and exclusion criteria. The second step encompasses an analytical treatment interruption among participants with the lowest viral reservoir.

2.2. Study objectives and Hypotheses

Primary Objective

The primary objective is to determine the proportion of post treatment controllers (PTC) (i.e. patients under cART with low baseline peripheral blood proviral DNA that will show sustained viral suppression at 48 weeks post treatment interruption (TI).

Secondary Objectives

a. To confirm the safety of a TI strategy in selected HIV patients

b. To assess the viral reservoir magnitude prior, during and after TI

c. To assess the viral and host dynamics in those participants with viral rebound post TI.

Exploratory Objective

a. The identification of predictors (clinical and/or laboratory) of PTC phenotype.

b. The identification of predictors for viral rebound over time.

2.3. Variables of interest

Primary: The proportion of participants with plasma viral load (pVL) below the lower limit of detection (< 50 HIV RNA copies/ml plasma) 48 weeks following interruption of ART.

Secondary:

- The numbers and percentages of AE's and SAE's during the whole study period.
- Viral reservoir (total HIV-1 DNA) and viral transcription (total HIV-1 cell-associated RNA) measurements at every visit between baseline (just before TI) until viral rebound and 12 weeks after viral rebound.
- Repeated pVL measurements.

Exploratory: Potential clinical and/or laboratory variables, such as demographic and epidemiological data, medical history (past and current), vital parameters, ultra-sensitive HIV viral load, CD4 T cell count and laboratory test data (e.g. hemoglobin, ANC, platelets, creatinine, AST & ALT), viral reservoir (quantitative viral outgrowth measurement) and viral release measurements (spontaneous and stimulated) at baseline.

3. Description of study population

3.1. Analysis populations

The statistical analyses will be performed on all participants enrolled in the study and who met all inclusion and exclusion criteria. Complete case analysis will be performed for all statistical analyses.

3.2. Patient accounting

The numbers of participants screened, enrolled and excluded will be presented. Excluded participants will be summarized by reason for exclusion in a CONSORT flow diagram by the PI.

All patients who conform to all inclusion and exclusion criteria and are enrolled into the study will be tabulated. The number of patients discontinued will be tabulated by reason for study discontinuation (Completed the study, Lost to Follow Up, Withdrawal of Consent, Discontinuation of drug for Safety or Other Reasons, Death,

Other) (Example Table 1). This information will also be described separately in a CONSORT flow diagram.

3.3. Description of study population

Patients will be described with respect to baseline characteristics for each of the two stages of the study separately. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics (Example Tables 2). The clinical importance of any imbalance will be noted, though statistical tests of significance will not be undertaken.

4. Statistical Methods

4.1. Primary Objective

The primary objective will be assessed by estimating the proportion of participants with undetectable viral load (<50 HIV RNA copies/ml plasma) 48 weeks after interruption of ART. A 95% CI for the proportion will be calculated using Wilson's score method (Example Table 3). Respective proportions at different time points (week 2, 4, 6 and 8) will be also calculated for descriptive purposes.

4.2. Secondary Objectives

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MEDDRA) and will be reported based on MEDDRA preferred terms and body systems. All AEs will be analyzed based on counts of patients with a specific category and not on counts of individual adverse events. The relationship between AEs and ARV interruption is determined by the investigator and categorized as "drug-related" if unlikely, possibly, likely or definitely related to treatment interruption. All AEs definitely unrelated to intervention will also be used to examine the relationship between AEs and intervention.

All AEs and all serious AEs from the beginning of treatment interruption until the end of the study (end of follow-up period) are reported. AE counts will be summarized for the whole study period (treatment interruption and follow-up period). Counts (%) of patients with AEs, drug-related AEs and serious AEs will be presented by Meddra body system and preferred term together with a 95% Wilson confidence interval (Example Table 4 and 5).

Reservoir replenishment and HIV viral load kinetics following ART interruption will be summarized in terms of medians and interquartile ranges (Example Table 3). No formal statistical testing will be done in these results. If present, pVL rebound will be represented graphically with a spaghetti plot. Furthermore, the measurements over time of viral load rebound and viral reservoir will be presented graphically for every patient included in the study (one line for every patient over time).

4.3. Exploratory Objective

Potential predictors of post-treatment control (end of second part of the study) will be assessed by Fisher's exact test at 5% significance level (Example Table 6). The time to viral rebound will be assessed by survival analysis methodology, e.g. Kaplan-Meier estimates and curves (Example table 7). Potential predictors of viral rebound will be examined by single Cox regression models (Example Table 8).

4.4. Subgroup Analysis

No subgroup analyses will be performed in this study due to the small sample size .

5. Example Tables and Figures

Example Table 1: Patient Accounting

	n (%)
Screened	xx
Excluded	xx (xx %)
[add reason for exclusion]	xx (xx %)
…	xx (xx %)
Enrolled to the first stage	xx (xx %)
- Completed the study	xx (xx %)
- Early withdrawals	xx (xx %)
- Lost to follow-up	xx (xx %)
- Consent withdrawal	xx (xx %)
Excluded from second stage	
Enrolled to the second stage	xx (xx %)
- Completed the study	xx (xx %)
- Early withdrawals	xx (xx %)
- Lost to follow-up	xx (xx %)
- Consent withdrawal	xx (xx %)

Example Table 2: Baseline Characteristics at Recruitment

	n (%)
N	xx
Epidemiological findings	
Age (yr): median (IQR)	xx (xx - xx)
Gender: male (%)	xx (xx)
female (%)	xx (xx)
Ethnicity: [add ethnic categories]	
Transmission route: [add categories]	
Duration of HIV infection (months/years): median (IQR)	
Total duration of antiviral treatment (months): median (IQR)	
Clinical findings	
Last antiviral treatment: PI+ NRTI	xx (xx)
NNRTI+NRTI	xx (xx)
II+ NRTI	xx (xx)
other	xx (xx)

Laboratory findings	
- Total WBC count: median (IQR)	xx (xx - xx)
- Neutrophil percent: median (IQR)	xx (xx - xx)
- Lymphocyte percent: median (IQR)	xx (xx - xx)
- Hemoglobin: median (IQR)	xx (xx - xx)
- Platelet count: median (IQR)	xx (xx - xx)
- Current CD4 count: median (IQR)	xx (xx - xx)
HIV subtype [add categories]	
Nadir CD4 count: median (IQR)	

Example Table 3: Summary of Primary and Secondary Analysis Results

	n/N (%, 95% CI)
Primary analysis	
Number of participants with undetectable viral load (<50 HIV RNA copies/ml plasma) 48 weeks after interruption of ART	xx/xxx (xx; xx - xx)
Number of participants with undetectable viral load (<50 HIV RNA copies/ml plasma) at week:	
- 2	xx/xxx (xx; xx - xx)
- 4	xx/xxx (xx; xx - xx)
- 6	xx/xxx (xx; xx - xx)
- 8	xx/xxx (xx; xx - xx)
Secondary analysis	
Viral Reservoir at baseline (/10*6 PBMC's): median (IQR)	xx (xx - xx)
Viral Reservoir after TI (/10*6 PBMC's): median (IQR)	xx (xx - xx)
Viral load rebound by week (in copies/ml plasma): median (IQR)	
- **0 (baseline)**	xx (xx - xx)
- **2**	xx (xx - xx)
- **4**	xx (xx - xx)
- **8**	xx (xx - xx)

Example Table 4: Counts of patients (%) with Adverse Events by Body System and Preferred Term (Meddra)

	(N=xx) n(%)
Blood and Lymphatic System	xx (xx)
Anaemia	xx (xx)
Gastrointestinal Disorders	xx (xx)
Diarrhoea	xx (xx)
...	xx (xx)

Note: similar tables will be presented for drug-related AEs, serious AEs, and serious drug-related AEs.

Example Table 5: Counts of patients (%) with Adverse Events by causality classification

Number of patients (%; 95% CI) with:	(N=xx)
- any adverse event	xx (xx; xx - xx)
- any intervention-related adverse event	xx (xx; xx - xx)
- any serious adverse event	xx (xx; xx - xx)
- any intervention-related serious adverse event	xx (xx; xx - xx)

Example Table 6: Exploratory analysis of potential predictors of post-treatment control (PTC)

Characteristic	Levels	PTC = Yes n(%)	PTC = No n(%)	OR (95% CI)	p - value
Age (years)	< 30	xx (xx)	xx (xx)	1 (ref)	xx
	30 – 40	xx (xx)	xx (xx)	xx (xx - xx)	
	> 40	xx (xx)	xx (xx)	xx (xx - xx)	
Gender	Male	xx (xx)	xx (xx)	1 (ref)	xx
	Female	xx (xx)	xx (xx)	xx (xx - xx)	xx
HIV viral load		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
CD4 T cell count		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
Hemoglobin		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
ACN		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
platelets		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
Creatinine		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
AST		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
ALT		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
Viral reservoir at baseline		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
Viral outgrowth at baseline		xx (xx - xx)	xx (xx - xx)	xx (xx - xx)	xx
…..					

Example Table 7: Kaplan-Meier estimates for time until viral load rebound

	Week 2		Week 4		Week 6		Week 8	
	n failed/ n censored	Probability viral suppression (95 % CI)	n failed/ n censored	Probability viral suppression (95 % CI)	n failed/ n censored	Probability viral suppression (95 % CI)	n failed/ n censored	Probability viral suppression (95 % CI)
All	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)
Subset analysis								
…	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)
…	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)	xx/xx	xx (xx - xx)

Example Table 8: Exploratory analysis of potential predictors of viral rebound

Predictor	Levels	Hazard ratio (95% CI)	p-value
Age (years)	< 30	1 (ref)	xx
	30 – 40	xx (xx - xx)	xx
	> 40	xx (xx - xx)	xx
Gender	Male	1 (ref)	xx
	Female	xx (xx - xx)	xx
HIV viral load		xx (xx - xx)	xx
CD4 T cell count		xx (xx - xx)	xx
Hemoglobin		xx (xx - xx)	xx
ACN		xx (xx - xx)	xx
platelets		xx (xx - xx)	xx
Creatinine		xx (xx - xx)	xx
AST		xx (xx - xx)	xx
ALT		xx (xx - xx)	xx
Viral reservoir at baseline		xx (xx - xx)	xx
Viral outgrowth at baseline		xx (xx - xx)	xx
…..			