Statistical Analysis Plan

Post Exposure Prophylaxis for Leprosy in the Comoros and Madagascar

PEOPLE

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1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main, pre-planned analyses for the study “Post Exposure Prophylaxis for Leprosy in the Comoros and Madagascar", including interim analyses. The purpose of this study is to document effectiveness of three alternative ways of implementing post exposure prophylaxis (PEP) at village level, by comparing to villages in which no PEP is provided. The study conduct is described in the Protocol (Clinicaltrials.Gov. NCT03662022).

These planned analyses will be performed by the statistician(s) at the Clinical Trials Unit of the Institute of Tropical Medicine (Antwerp) and the research consortium. The analysis results will be described in a statistical analysis report, to be used as the basis of the primary research publications. This document describes statistical methods for the primary and secondary objectives of the study as defined by protocol. Additional analyses may be performed but are not covered by the current analysis plan.

Two interim analyses will be conducted upon completing the second and third door-to-door surveys in which incidence rates of leprosy to date in study arms 2, 3 and 4 will be compared to the incidence rate in arm 1, just as in the final analysis. These interim analyses will allow us to assess whether the a priori assumptions on annual incidence made have been realistic or whether an extended study duration should be considered.

This analysis plan should be finalized and approved before the first interim analysis that will be performed by the study statistician. Major changes in statistical methodology used for the main and pre-planned analyses from this SAP, 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

The PEOPLE study is a cluster randomized trial. There will be four door-to-door surveys of which the first one will allow us to calculate a baseline leprosy prevalence

per island. On the Comoros villages have been randomized after having been grouped by island into 'Blocks' of four in decreasing order of incidence over the period 2013-2017. Within each block villages were randomly and mutually exclusively allocated to the four study arms. For the study district in Madagascar reliable incidence estimates were not available. For that reason randomization for Madagascar is foreseen only for the end of the first study year, once the first screening round has been completed.

There will be four study arms to which villages on Madagascar and the Comoros islands Anjouan and Mohéli will be randomly allocated. These villages have been preselected based on reported leprosy incidence over the period 2013-2017. In all four study arms, annual door-to-door surveys will be conducted covering entire villages. All permanent residents will be invited for screening for leprosy. Leprosy patients (further referred to as 'index patients') detected will be offered free treatment according to the international and country guidelines. Depending on the study arm, contacts of leprosy patients will be offered PEP. In arm 1 no PEP will be provided, which is the current routine care on Madagascar and the Comoros. In arms 2, 3 and 4 a single dose of 20 mg per kg bodyweight of Rifampicin (hereafter called 'SDDR-PEP') will be offered to all eligible participants who have not received Rifampicin in the preceding 2-year period. In arm 2 only household contacts of an index patient will be provided with SDDR-PEP, which target group is considered the standard approach when PEP is provided for leprosy. In arms 3 and 4 SDDR-PEP will also be provided to neighbourhood contacts living within a radius of 100 meter of an index patient. In arm 3 all such contacts (including household contacts) will be provided SDDR-PEP, in arm 4 SDDR-PEP will be provided to all household contacts and to all those within the 100-meter perimeter testing positive to a serological screening test. If in arms 3 or 4 more than 50% (arm 3) or 75% (arm 4) of the population live within 100 meters of an index case, the entire village will in principle be eligible for PEP.

2.2. Study objectives

Primary objective:
To compare effectiveness in curbing transmission of leprosy of three different approaches of post exposure prophylaxis

Secondary objectives:

1. To assess cost and feasibility of SDDR-PEP under program conditions
2. To identify patterns of clustering in transmission of leprosy, allowing better targeting of control measures
3. To monitor rifampicin resistance among leprosy patients
4. To estimate incidence and prevalence of smear positive pulmonary tuberculosis in the study villages

3. Deviations from protocol

In the protocol one of the secondary objectives was to estimate incidence and prevalence of smear positive pulmonary tuberculosis (see section above). The criterion to collect sputum was reported cough of 2 weeks duration or more. On a total population screened of just over 102,000 during the first year, only 115 (0.11%) reported such

cough. Either the question is not properly understood or cough is not a good screening criterion in the context of this study population. It was therefore decided to drop this secondary objective.

4. Description of study population

The study population will be described overall, and in each island (Anjouan, Mohéli, Madagascar) separately.

4.1. Patient accounting

Details of subjects screened, those who meet the study inclusion criteria, those who are eligible and enrolled, those who are eligible but not enrolled, those who withdraw from the study after randomization and those who are lost to follow-up will be summarized in a CONSORT flow diagram. The number of participants attending each annual survey will be reported. Since people were informed about their assigned study arm before providing consent and enrollment, the proportion of eligible subjects who are not enrolled will also be summarized by study arm.

4.2. Description of study population

Participants in each study arm, overall and by island, will be described with respect to baseline characteristics (i.e. at time of randomization). The description will be in terms of medians and quartiles for continuous characteristics and using counts and percentages for categorical characteristics. The clinical importance of any imbalance will be noted but statistical tests of significance of baseline imbalance will not be undertaken.

5. Description of efficacy patient populations and outcomes

5.1. Patient populations

Note that in Madagascar enrolment/randomization will only take place at the second survey. Hence for Madagascar only data from the second survey onwards will be used for the primary objective. For secondary objectives 2 and 3 data from the first survey will also be used.

We will analyse the efficacy data using Intention-to-Treat and Per-Protocol approaches, with Intention-to-Treat as primary approach.

5.1.1. Intention to treat (ITT) analysis

In the Intention-to-Treat analysis, all participants will be analysed according to their randomized allocation, even in case they do not receive SDDR-PEP as allocated, show protocol violations prior to or during the study.

5.1.2. Per protocol (PP) analysis

In the per-protocol analysis only participants who receive SDDR-PEP as planned, have complete follow-up, and follow the protocol as planned are included.
In Table 1 the protocol violations are classified as minor and major where minor violations can be included in the PP analysis population and major violations are excluded.

Table 1: The protocol violations classified as minor or major violation

Protocol Violation	Major/Minor Violation	Comments
Inclusion criteria		
1. Living in one of the study villages	Major	
2. Ages 2 years and above (only applicable for those receiving SDDR-PEP)	Major	
3. Able and willing to provide informed consent for leprosy and tuberculosis screening, and SDDR-PEP administration	Major	
4. For leprosy index cases only: able and willing to provide informed consent for participation and sampling	Major	
Exclusion criteria (only applicable for those receiving SDDR-PEP)		
1. Signs of active leprosy	Major	
2. Signs of active pulmonary tuberculosis	Major	
3. Having received Rifampicin within the last 24 months	Major	
Treatment violations		
1. Missing SDDR-PEP	Major	
2. Not following the randomized intervention	Major	
Follow-up violations		
1. Incomplete follow-up	Minor	

5.2. Efficacy study endpoints

Primary endpoint
The primary efficacy objective is to compare leprosy incidence rate. The health worker will perform a physical examination to look for signs of leprosy. Note that for this study, the diagnosis of leprosy will be made based on the so-called cardinal signs, i.e. a patch with loss of sensation, enlarged peripheral nerves and/or a slit skin smear positive for

acid fact bacilli. Results of qPCR and anti-PGLI testing will be not be used as diagnostic criteria.

Secondary endpoints
Genotyping will be done by a novel sequencing technique called the Deeplex® MycLep assay.

Rifampicin resistance will be tested with molecular markers.

6. Interim analysis

Two interim analyses will be conducted upon completing the second and third door-to-door surveys in which leprosy incidence rates to date in study arms 2, 3 and 4 will be compared to the leprosy incidence rate in arm 1, just as in the final analysis. These interim analyses will allow us to assess whether the a priori assumptions on annual incidence made have been realistic or whether an extended study duration should be considered.
The analysis performed will be similar to the analysis of the main efficacy outcome (Section 7) but restricted to data up to the second resp. third survey. Hence the interim analysis after completing the second survey will only include data from the Comoros.

7. Analysis of main efficacy outcomes: incidence rate of leprosy

Each diagnosis of leprosy made after the survey in which the participant was enrolled will be counted as an incident case. Note that not only diagnoses made at the annual surveys but also diagnoses made in between the surveys will be counted. For each individual we will calculate the exact time at risk. For individuals residing in intervention villages (arms 2, 3 and 4) we will use the number of days from the median date of the first SDDR-PEP administration in the village until end of follow-up or until diagnosis of leprosy.

We will fit a random effects Poisson model with incident case (0/1) as dependent variable, study arm as explanatory variable, using the non-intervention group (arm 1) as baseline level, log(time at risk) as offset, and with 'island', 'block' (as described above, eight on Anjouan, four on Mohéli, four to six on Madagascar) and 'village' as nested random effects. As study outcome we will report incidence rate ratios between arm 1 and each of the other arms with 95% confidence interval, considering a p-value of < 0.017 as statistically significant to account for the fact that we make three comparisons.

7.1. Subgroup analyses

No subgroup analyses are planned.

7.2. Other aspects

a. Multiplicity

A p-value of < 0.017 will be considered as statistically significant to account for the fact that we make three comparisons. Interim analyses will be performed, but these analyses will not influence the continuation of the study.

b. Missing data

Data will be used until time point at which participant is last seen. If there are intermediate missing results, these will be considered to be negative for leprosy.

8. Analysis of secondary efficacy objectives

8.1. To assess cost and feasibility of SDDR-PEP under program conditions

Costs will be calculated per person screened, per person treated with SDDR-PEP and per leprosy case averted.

8.2. To identify patterns of clustering in transmission of leprosy, allowing better targeting of control measures

Results of DNA finger printing will be used to assess the degree of clustering at household and at village level. We will calculate proportions of patients belonging to a same cluster by household and by village. The space-time K-function will be used to assess clustering in time and space, to be triangulated with results of DNA finger printing. For this purpose, we will use results of all leprosy patients identified, including those found at baseline.

To establish the transmission networks in the Comoros islands and in the study area in Madagascar, we will overlay the molecular network with the spatial- and social networks. This will determine whether the 100m high risk zones around each index patient, applied in this study, capture most transmission events, or whether additional risk factors, beyond spatial, need to be taken into account in future PEP administration efforts in the study areas and elsewhere. Phylogenetic clustering of the *M. leprae* isolates allows us to identify risk factors for leprosy due to recent transmission. The findings of the spatial and social network analysis can be mapped onto a phylogenetic tree and molecular clusters based on the VNTRs, which can reveal risk factors for transmission of leprosy.

Mixed effects Poisson model (same model as for the main analysis) with study arm (only when comparing 2019 to 2020 data) and distance to nearest index case as fixed covariates will be used to test for an association between distance to index case and leprosy incidence rate. We will compare six distance categories: same household, neighbors at < 25 meters and neighborhood contacts at 25-<50 meters, at 50 - < 75 meters, at 75 - < 100 meters and at 100 meters or beyond. Two models will be fitted. The first model will use leprosy diagnosis at the time of the survey in 2019 (either a new diagnosis or a patient still on treatment) as outcome and distance to nearest index case from 2019 as independent variable, the second model will use incident leprosy cases in 2020 as outcome and distance to nearest index case from 2019 as independent variable. In addition we will use Kulldorff's spatial scan statistic to fit a purely spatial Poisson model to identify clusters of high leprosy prevalence.

8.3. To monitor rifampicin resistance among leprosy patients

All leprosy patients with a positive/negative test result for Rifampicin resistance will be included in a random effects logistic regression model with rifampicin resistance (0/1) as dependent variable, island as explanatory variable, and with 'block' (as described above, eight on Anjouan, four on Mohéli, four to six on Madagascar) and 'village' as nested random effects. Based on this model, prevalence of Rifampicin resistance per island will be estimated with 95% confidence interval.

9. Safety analyses

9.1. Safety analysis population

For the analysis of safety outcomes, all participants who effectively received a SDDR-PEP dose are included in the safety analysis (all-patients-treated approach).

9.2. Safety outcomes and analyses

9.2.1. Adverse events

General aspects: 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 participants with a specific category and not on counts of individual adverse events. The relationship between AEs and treatment is determined by the local investigator and categorized as "drug-related" if possibly, probably or definitely related to treatment. Only adverse events that occur between the administration of the drug and three days after administration will be recorded for study purposes.

The following summaries will be presented:

a. description of all deaths of participants up to three days after SDDR-PEP administration;

b. the total number and percentage of participants with any AE by preferred term and body system up to three days after SDDR-PEP administration;

c. the total number of participants and percentage with any drug-related AE by preferred term and body system up to three days after SDDR-PEP administration;

d. the total number of participants and percentage with any SAE by preferred term and body system up to three days after SDDR-PEP administration;

e. the total number of participants and percentage with any drug-related SAE by preferred term and body system up to three days after SDDR-PEP administration;

95% confidence intervals will be calculated for the following statistics:

a. percentage of participants with any AE, any serious AE, any drug-related AE, any drug-related serious AE;

b. percentage of participants with the most common AEs, drug-related AEs, SAEs, drug-related SAEs (most common: with an incidence of 10% or more).

These confidence intervals will be calculated based on a random effects logistic regression model with ‘island’, ‘block’ (as described above, eight on Anjouan, four on Mohéli, four to six on Madagascar) and ‘village’ as nested random effects.