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ISGlobal - Barcelona Institute for Global Health

"Expanding Xpert Ultra for TB diagnosis among HIV-positive patients admitted to hospitals in Tanzania and Mozambique (EXULTANT)"

STATISTICAL ANALYSIS PLAN

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Approval Page

The signatures below confirm that the signatories have reviewed and approved this document which shall govern the stipulated statistical analysis tasks of the specified research study.

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DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Table of Contents

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

1 Abbreviations and Definitions

AE	*Adverse Event*
AlereLAM	*Alere Determine TB LAM Ag*
CRF	*Case Report Form*
DMP	*Data Management Plan*
FujiLAM	*Fujifilm SILVAMP TB LAM*
HIV	*Human immunodeficiency virus*
IMP	*Investigational Medical Product*
ITT	*Intention to treat*
*KPS**	*Karnofsky Performance Status Scale definitions rating (%) criteria*
LF-LAM	*Lateral flow urine lipoarabinomannan assay*
PP	*Per Protocol*
SAP	*Statistical Analysis Plan*
TB	*Tuberculosis*
Ultra	*Xpert MTB/RIF Ultra*

2 Introduction

2.1 Preface

Tuberculosis (TB) is a leading cause of death worldwide and ranks above HIV/AIDS as the leading cause of mortality from a single infectious agent. The World Health Organization (WHO) estimated that there were around 10 million new cases and 1.5 million deaths in 2021. Eight and 14% of the total number of TB cases and deaths respectively occurred in people living with HIV (PLHIV), with 70% of these occurring in the African region. PLHIV are at increased risk of acquiring Mycobacterium tuberculosis (Mtb) infection and have a >20-fold increased risk of TB disease progression compared to HIV-uninfected peers. In addition, mortality during TB treatment remains as high as 11% globally, although fatal outcomes of PLHIV under TB treatment are even higher in Sub-Saharan African countries

Among people living with HIV, microbiological confirmation of TB is suboptimal due to sputum collection challenges and paucibacillary sputum resulting in poor diagnostic yield. Options that have been recommended by WHO to ensure earlier diagnosis and reduce mortality include strategic placement of rapid molecular TB diagnostics such as Xpert or Ultra within HIV care settings (which could enable availability of results within 24 hours), and uptake of the urine lipoarabinomannan assay (LF-LAM) strip-test, the Alere Determine™ TB LAM Ag (AlereLAM), for seriously ill or immunosuppressed PLHIV. However, despite AlereLAM being a cheap and fast test, only 9 of the 30 high TB and HIV burden countries had in 2020 a national policy and algorithm indicating the routine use of urine LF-LAM (with various levels of implementation). In addition, PLHIV often are sputum paucibacillary and a considerable proportion of them cannot provide a good quality sputum specimen for molecular testing.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

The limitations of existing sputum-based tests for M. tuberculosis (Mtb) diagnosis among (PLHIV) admitted to hospital create imperatives to explore the usefulness of easy to collect, non-respiratory specimens which could improve TB diagnosis, while supporting and accelerating treatment guidance. Previous studies have shown that Xpert testing of urine and stool can confirm Mtb in participants who would otherwise test negative with traditional sputum-based methods. Evidence shows that these diagnostics may detect additional cases that are not detected by AlereLAM. Thus, an algorithm using the different tests (sputum Ultra, urine Ultra and stool Ultra) offered as a screening intervention to admitted HIV positive participants should increase the Mtb bacteriological yield (compared to current WHO recommended screening and testing strategies**) and contribute to earlier initiation of TB treatment. Given that some detected cases may not present with classical TB symptoms at the time of hospitalization, we believe that this strategy will increase the overall number of participants with diagnosed TB and started on treatment and ultimately reducing mortality.

3 Study Objectives and Endpoints

3.1 Main Study Objectives

The primary objective of the study is to investigate the effect of an expanded TB screening strategy performed in HIV-positive participants admitted to hospital on the proportion of bacteriologically confirmed TB cases starting treatment within 72 hours of enrolment

INTERVENTION ARM (expanded TB screening strategy): Xpert Ultra on sputum, stool/rectal swab and AlereLAM on urine, performed regardless of presence of TB symptoms

CONTROL ARM: Ultra testing (on sputum/any tissue) if TB-compatible symptoms and AlereLAM (on urine) only in those fulfilling WHO testing recommendations.

3.2 Secondary and Exploratory Study Objectives

The study has the following secondary objectives.

Secondary objectives

1. To assess the impact of this screening strategy on 2-month all-cause mortality.
2. To assess the feasibility of multiple specimen collection for TB diagnosis within 72 hours of enrolment.

**to the main protocol includes additional exploratory objectives, which will be specified in a separate SAP*

3.3 Study design

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

This is a multicentre, parallel, individually randomized, controlled diagnostic trial in consecutively enrolled HIV positive adults admitted to hospital in selected sites in Mozambique and Tanzania.

This trial has two study arms, the intervention and control arm. The intervention arm for this study consists of HIV participants with TB testing performed regardless of presence of TB symptoms. Testing will be done on expectorated sputum, stool/rectal swab and concentrated urine with Ultra and urine with AlereLAM.

The **control arm** for this trial will consist of participants managed according to the current WHO recommended TB testing practices for HIV positive in participants (as of Q1 2022). TB testing will be done as follows:

Sputum Ultra performed whenever the patient has cough, fever, weight loss overnight sweats and/or

Ultra performed on any tissue (including lymph nodes) from participants with clinical suspicion of extrapulmonary TB.

and/or:

Urine Alere TB-LAM performed if participants have signs and symptoms of TB (pulmonary and/or extrapulmonary), or with advanced HIV disease, or who are seriously ill, or else irrespective of signs and symptoms of TB, but combined with a CD4 cell count of less than 200 cells/mm^3.

3.4 Endpoints

All study endpoints will be compared between control and intervention arm. Also, the final results will be presented disaggregated by sex.

3.4.1 The **primary endpoint** is the proportion of participants diagnosed with microbiologically confirmed TB and started on TB treatment within 72 hours of enrolment* in whom TB is microbiologically confirmed*/**

* The numerator of the primary endpoint includes participants per study arm who are diagnosed with microbiologically confirmed TB and initiated TB treatment within 72 hrs of enrolment. The denominator is the number of enrolled participants in each study arm.

** Participants are defined to have microbiologically confirmed TB if at least one confirmatory TB test (Ultra, AlereLAM) gives a positive result.

Secondary endpoints are:

Related to main objective

3.4.2.1 Time-to-TB-treatment-initiation among microbiologically confirmed cases and among all TB cases since enrolment. We will estimate Kaplan Meier curves and hazard ratios at day 7, 14, 28 and 56. Additionally we will compare the time to related estimates (median and IQR) of intervention and control group.

3.4.2.2 Proportion of cases re-admitted to hospital. The numerator will be the number of admissions for any reason (not included in the exclusion criteria) after initial discharge and within 8 weeks from enrolment. The denominator will be the number of participants enrolled.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

3.4.2.3 Proportion of participants who are diagnosed with TB (irrespective of bacteriological confirmation) and have started on TB treatment within 72 hours of enrolment.

3.4.2.4 Proportion of participants who are diagnosed with microbiologically confirmed TB and are started on treatment at 7, 14 days and 8 weeks of enrolment.

3.4.2.5 Time to laboratory confirmed TB diagnosis and any TB diagnosis since enrolment.

3.4.2.6 Proportion of microbiologically confirmed TB participants started on treatment without Rif resistance information (only AlereLAM positive)).

Related to secondary objectives.

3.4.2.7 Eight-week all-cause mortality. The numerator will be the number of deaths during eight weeks after enrolment, the denominator the number of participants enrolled.

3.4.2.8 In hospital and 4-week all-cause mortality among all participants enrolled (assessed as the eight-week all-cause mortality).

3.4.2.9 Proportion of study participants who are able to provide the different specimens (sputum and urine samples) within 24, 48 and 72 hours of enrolment in the control and treatment arms.

4 Study Methods

4.1 General Study Design and Plan

The study design is a multicentre, parallel, individually randomized, controlled diagnostic trial in consecutive HIV positive adults admitted to hospital in selected sites of Mozambique and Tanzania.

Schedule of Visits.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Control arm	**Day 0 (recruitment)**	**Day 0 (recruitment)** *Intervention arm*	**Day 0-3** *(<72h)*	**Daily**	**At discharge** *If discharged before day 28/56*	**Week 4** *+/- 3 days*	**Week 8** *+/- 3 days*
Baseline Evaluation							
Informed consent	X	X					
Eligibility criteria verified	X	X					
CD4 count / Viral Load[&]	X	X					
Locator information	X	X					
Brief medical history	X	X					
Proof of HIV status stored	X	X					
WHO Symptom screening	X	X			X		
TB Investigations							
Samples collected and tests	**Urine AlereLAM#** **Sputum Ultra*** Blood CD4	**Sputum Ultra** **Stool/rectal swab Ultra** **Urine Ultra** **Urine AlereLAM** 2x Oral Swabs Urine FujiLAM Blood CD4 Blood for CRP					
Results issued			X				
Further contacts							
Verification TB diagnosis / ad hoc TB investigations				X	X	X	X
Verification TB treatment initiation				X	X	X	X
Verification vital status				X	X	X	X
HIV care / ART				X	X	X	X

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

4.2 Inclusion-Exclusion Criteria and General Study Population

This study has the following inclusion and exclusion criteria.

Inclusion criteria:

1. Adults 18 years or older.
2. Confirmed HIV infection (including both antiretroviral (ART)-naïve and experienced).
3. Admitted to the hospital (adult medical wards) at the time of enrolment.

Exclusion criteria:

1. Unable to provide informed consent (if no authorized relatives are in the position to provide the consent).
2. Living outside the catchment area of the participating hospital(s).
3. With plans to migrate outside the catchment area within 2 months after recruitment.
4. Currently receiving anti-TB therapy or having received anti-TB therapy in the last 6 months prior to enrolment.
5. Receiving preventive TB treatment in the preceding 6 months
6. Participants admitted for traumatic reasons, acute abdomen, delivery or pregnancy (maternal conditions), or for planned/scheduled surgery.
7. Referred from other hospital
8. Positive result to a SARS-CoV-2 test (RT-PCR, antigen test, or other similar test that might define COVID-19 case in the study site)*

Study population

The study population will be adult (≥18 years) PLHIV participants admitted to hospital and living in the catchment areas within the 4 district hospitals in Tanzania and Mozambique, regardless of presence of TB compatible symptoms.

4.3 Randomisation and Blinding

This is an individually randomized clinical trial. Eligible participants will be randomized to either the intervention or control arm, with 50% of participants in each of the arms. Randomization will be conducted using a web-based application https://odk-central.finddx.org/-/x/6RPyVJQoEjzOPUd3qHIRQYX6MtFnnwq that can be used on any smartphone device with internet connection. The day of randomization for each participant will be the enrolment day (study Day 0). At the time a participant is randomized, the study staff enters in the application several key data (patient study number and date of birth) and in reply, the allocated randomized arm is provided a few seconds later in the same application interface. Thus, internet connection is needed for randomizing participants. Pre-registered users of the study team will be allowed to use the randomization application.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Developing a reliable training plan by which study teams (at the clinic and at the lab) are fully aware of the diagnostic allocation is critical so that arm-specific testing can be implemented without mistakes in a timely manner. This includes careful strategic measures (posters, signs, weekly meetings, etc) as part of the site internal planning, which also need to consider staff shifts, holidays (both in the hospital and at the laboratory). A screening and randomization log need to be daily updated. Day 0 CRF needs to be data entered in a timely manner to allow the central monitoring of number of recruited subjects per arm, site and week and overall mapping of participants included in the study.

This is an unblinded trial. Neither participants, nor study staff will be blinded to tests practiced or test results.

4.4 Study Variables

The Baseline participants variables we will collected from TB-CAPT-HIV EXULTANT-BaselineCharacteristics-v3, and the following versions of this document. From this CRF the important variables for the analysis are:

Variable		Used in
Label	name	
Date of enrolment	ENROL_D	To calculate endpoints related to time.
Time of enrolment	ENROL_T	To calculate endpoints related to time.
Country	COUNTRY	To adjust some regression models. For previous analysis about at random lost to follow up.
Sex	SEX	To describe the participant's profile. For previous analysis about at random lost to follow up.
Ethnic group	ETHNIC ETHNIC_SPEC	To describe the participant's profile. For previous analysis about at random lost to follow up.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Level of education	EDUCATION EDUCATION_SPEC	To describe the participant's profile. For previous analysis about at random lost to follow up.
Employment Status	EMPLOYMENT EMPLOYMENT_SPEC	To describe the participant's profile. For previous analysis about at random lost to follow up.
Type of Employment: 2.6 Is the patient employed as a health care worker? 2.7 Is the patient employed as a prison warder/work at a correctional facility? 2.8 Is the patient employed as a miner?	EMPL_HLTH_YN EMPL_PRSN_YN EMPL_MINE_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.
Early HIV positive result 3.1.1 Date of earliest HIV positive result:	HIV_POS_DATE	To describe the participant's profile. For previous analysis about at random lost to follow up.
Current taking antiretroviral	ART_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.
Previous TB episodes	TB_TX_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.
Received BCG vaccination	BCG_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Participant live alone?		To describe the participant's profile. For previous analysis about at random lost to follow up.
Patient taking cotrimoxazole?	CTX_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.
High blood pressure	HIGH_RR_YN	To describe the participant's profile.
Diabetes	DIABETES_YN	To describe the participant's profile.
Cardiac disease	CARDIAC_YN	To describe the participant's profile.
Epilepsy	EPILEPSY_YN	To describe the participant's profile.
Karnofsky Performance Status Scale	KARNOFSKY	To describe the participant's profile. For previous analysis about at random lost to follow up.
Patient's WHO clinical Stage	WHO_STAGES	To describe the participant's profile. For previous analysis about at random lost to follow up.
TB treatment start	TB_TX_START_YN	To describe the participant's profile. For previous analysis about at random lost to follow up.
Random group	RANDOM_ASSIGN	For previous analysis about at random lost to follow up.
Date of randomization	RANDOM_D	To calculate endpoints related to time.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

The Follow-up week4 participants variables will be collected from TB-CAPT-HIV EXULTANT-Week-4-v3 CRF, and the following versions of this document. From this CRF the important variables for the analysis are:

Variable		Used in
label	name	
Is the visit taking place? (y/n)	W4_VISIT_YN	To calculate the number of people who come to visit at week 8
Is the visit taking place?, date	W4_VISIT_D	To calculate the time in the study, to draw the study K-M graphs in group of people who come to this visit.
If the patient has died, date of death	W4_DEATH_D	To use in the K-M graphs as an endpoint for these participants.
Has the patient started TB treatment since discharge?	W4_TB_TX_YN	To calculate the proportion of main study objective
Has the patient started TB treatment since discharge?, date	W4_TB_TX_START_D	To calculate the proportion of people started on treatment.
Has the patient abandoned TB treatment since last visit?	W4_TB_TX_END_YN	To calculate the failure TB treatment

The Follow-up week8 participants variables will be collected from TB-CAPT-HIV EXULTANT-Week-8-v3 CRF, and the following versions of this document. From this CRF the important variables for the analysis are:

Variable		Used in
label	name	
Is the visit taking place? (y/n)	W8_VISIT_YN	To calculate the number of people who come to visit at week 8

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Is the visit taking place? (date)	W8_VISIT_D	To calculate the time in the study, to draw the study K-M graphs in group of people who come to this visit.
If the patient has died, date of death	W8_DEATH_D	To use in the K-M graphs as an endpoint for these participants.
Has the patient started TB treatment since discharge?	W8_TB_TX_YN	To calculate the proportion of main study objective
Has the patient started TB treatment since discharge?, date	W8_TB_TX_START_D	To calculate time at risk
Has the patient abandoned TB treatment since last visit?	W8_TB_TX_END_YN	To calculate the failure TB treatment

From Hospitalization Assessment TB-CAPT-HIV EXULTANT-Hospitalization assessment-v3, and the following versions of this document we will use for the analysis the next variables:

Variable		Used in
label	name	
Date of enrolment	ENROL_D	To calculate the participant's start time
Time of enrolment (24h format)	ENROL_T	
Date of discharge	HOSP_END_D	To calculate the participant's hospitalization time
Time of discharge (24h format)	HOSP_END_T	
Date of lab result: Sputum Ultra	SP_XP_RES_RECEPT_D	To calculate the time to obtain the laboratory results and classify the primary outcome <= 72h or > 72h for Sputum Ultra sample
Time of lab result: Sputum Ultra	SP_XP_RES_RECEPT_T	

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Date of lab result: Urine Ultra	URINE_XP_RES_RECEPT_D	To calculate the time to obtain the laboratory results and classify the primary outcome <= 72h or > 72h for Urine Ultra sample
Time of lab result: Urine Ultra	URINE_XP_RES_RECEPT_T	
Did the patient have cough during hospitalization?	HOSP_COUGH_YN	We will use to describe symptoms. We will use as a factor variable in modelling
Did the patient have fever (>38ºC) during hospitalization?	HOSP_FEVER_YN	We will use to describe symptoms. We will use as a factor variable in modelling
Did the patient have night sweats during hospitalization?	HOSP_NIGHT_SWEATS_YN	We will use to describe symptoms. We will use as a factor variable in modelling
Did the patient lose weight during hospitalization?	HOSP_WEIGHT_LOSS_YN	We will use to describe symptoms. We will use as a factor variable in modelling
Was TB treatment initiated during hospitalization?	HOSP_TB_TX_YN	To calculate the primary objective variable, start treatment within 72h of enrolment
TB treatment initiated, Date and Time	HOSP_TB_TX_START_D HOSP_TB_TX_START_T	
If Yes, AND the patient has a microbiologically confirmed TB diagnosis (question 2.3), was the treatment initiated within 72hrs of enrolment?	HOSP_TB_TX_72H_YN	To determine the proportion of microbiologically confirmed TB cases starting treatment within 72 hours

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

If wasn't the treatment initiated within 72hrs of enrolment, reason	HOSP_TB_TX_72H_RSN HOSP_TB_TX_72H_RSN_SPEC	To determine the reasons behind starting treatment failure

The laboratory results we will be collected from (15) TB-CAPT-HIV EXULTANT-LabResults-v3- CRF, and the following version of this document. From this source we will select to study the following variables:

Variable		Used in
Label	name	
Date of sample collection	SAMP_COL_D	To check the range of dates in context of our research objectives
CD4 results	CD4_RES	To analyse the primary objective stratified by this variable
Viral load results	VL_RES	To analyse the primary objective stratified by this variable
Sputum Xpert Ultra	SP_XP_RES	To determine whether a patient is microbiologically confirmed
Urine Xpert Ultra	URINE_XP_RES	To determine whether a patient is microbiologically confirmed
Stool Xpert Ultra	STOOL_XP_RES	To determine whether a patient is microbiologically confirmed
Xpert Ultra in another specimen I	S1_XP_RES	To determine whether a patient is microbiologically confirmed
Xpert Ultra in another specimen II	S2_XP_RES	To determine whether a patient is microbiologically confirmed

The AlereLAM Results will come from the TB-CAPT-HIV EXULTANT-AlereLAM & CRP v3 form, and the update versions of this document. From this CRF will be get:

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Variable		Used in
Label	name	
AlereLAM result	ALERE_RES	To determine whether a patient is microbiologically confirmed To do the sensibility analysis of test
If results available, date	ALERE_RES_D	To calculate the lab results time
If results available, time	ALERE_RES_T	
If results not available, reason	ALERE_RES_YN_RSN	To calculate the proportion and reasons of no AlereLAM result

The Study Completion participants variables we will collected from TB-CAPT-HIV EXULTANT-StudyCompletion-v3 form, and the following versions of this document. From this CRF the important variables for the analysis are:

Variable		Used in
Label	name	
Did participant complete the study procedures at study visit week 8?	VISIT_WK8_D	To calculate the proportion of endpoints at week 8
Did participant complete the study procedures at study visit week 8? date	VISIT_WK8_D	To calculate the time in the study, to draw the study K-M graphs
If not, and the participant withdrew, was withdrawn, dropped out or died during the study, indicate the primary reason	END_RSN	To calculate the proportion deaths, lost to follow-up, withdraws and complete study

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

Date participant completed, withdrew, was withdrawn/excluded or dropped out from study, date	END_D	To calculate the time in the study, to draw the study K-M graphs

5 Sample Size

We expect the absolute difference in proportions of bacteriological confirmation between the two arms to be 7% (from 19% to 26% from the control to the intervention arm). Sample size was chosen to be sufficient to detect a 36.8% relative increase in the primary outcome in the intervention arm (as compared to the control arm).

Under this premise and the following assumptions:

- Alpha (two sided): 0.05
- Beta (power 80%): 0.2
- Estimated proportions of participants with microbiologically confirmed TB in the control arm 19% and int the intervention arm 26%
- Lost to follow up:5% of recruited participants.

The study will recruit 586 PLHIV participants per trial arm, then we will include in the analysis 1172 participants in total. This number of participants will come from 4 sites (293 participants per arm at each site).

For this calculation we have used the following formulas based on Normal asymptotic approximation for proportions:

Sample Size formula

$$n_1 = \frac{\left(Z_{1-\alpha/2}\sqrt{(1+\varphi)\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{\varphi p_1(1-p_1) + p_2(1-p_2)}\right)^2}{\varphi(p_2 - p_1)^2}$$

Power formula

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

$$\varphi = \frac{1 - w_1}{w_1}$$

$$\bar{p} = \frac{p_1 + \varphi p_2}{1 + \varphi}$$

$$1 - \beta = \Phi\left(\frac{|p_2 - p_1|\sqrt{\varphi n_1} - Z_{1-\alpha/2}\sqrt{(1+\varphi)\bar{p}(1-\bar{p})}}{\sqrt{\varphi p_1(1-p_1) + p_2(1-p_2)}}\right)$$

6 General Considerations

6.1 Timing of Analyses

The analysis will be done after database lock and the data cleaning process is finalized.

We will analyse the data using primarily an intention to treat (ITT) analysis approach. This dataset will be composed for all randomized participants by their assigned intervention independent of participation time of this participants in the study. Also, we will analyse the results using the Per Protocol Population approach.

6.1.1 Per Protocol Population

For the primary and some secondary endpoints, we will be using the set of data defined as “per-protocol” (PP). This dataset we will only include participants with all required samples collected and results available within 72 hours from enrolment.

This can shed light on a potential effect of poor adherence to study protocol procedures on the primary and secondary endpoints.

6.2 Missing Data

Given the characteristics and design of the trial (including data monitoring activities) we expect a low proportion of missing variables.

We will analyse the mechanisms that cause missing data. We plan to identify the three typical patterns for missing data: missing completely at random (MCAR); missing at random (MAR); and missing not at random (MNAR).

To assess the potential influence of missing values in intervention related variables or visit related variables (due to withdraws or death), we will compare the results of the ITT and per protocol analyses. To assess the potential influence of missing values in endpoint related

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

variables, we will perform a complete case analysis when the proportion of missing data is below, approximately, 5% and it is implausible that certain patient groups specifically are lost to follow-up in one of the compared arms. In this case we will analyse previously if the missing data are random or follow some pattern.

For missing data over 5%, we will do a worst/best scenario sensitivity analysis for the main endpoint related variables (related TB treatment initiation, time from enrolment and microbiological results). This consists of assigning the best plausible outcome to the above endpoint-related missing values in both arms (i-e TB treatment started, TB treatment started before 72 hours from enrolment, or TB microbiological confirmation) and the other assigning the worst possible outcome to all missing values in both groups (TB treatment not initiated, TB treatment started after 72 hours from enrolment, non-TB microbiological confirmation).

We will also compare the results of one assigning the best possible outcome to missing values in the intervention arm and the worst possible to those of the intervention arm, and vice-versa.

If this sensitivity analyses raise inconsistent results on the intervention effect, their repercussions will be discussed between the statisticians involved in trial analysis.

6.3 Multiple Testing

We consider unnecessary to apply a general adjustment of significance level by multiple comparisons test. We will use the all the statistical power for inference only in the primary study objective. To evaluate the other study endpoints, we use the p-value threshold at 0.05 to find the differences in our study without the objective to extrapolate the results to the global community. However, we will establish a p-value of 0.01 as a threshold for statistical significance in the comparisons related to a) selected secondary efficacy analyses (section 8.2, analyses 8.2.5, 8.2.6, 8.2.7) and subgroup analysis (see section 9.0)

We will adjust the results by site, demographic variables, and other baseline variables if we will detect differences or if these variables have a confounder characteristics profile. To decide this, we will do a preliminary analysis of these variables between sites.

7 Summary of Study Data

7.1 Subject Disposition

We will use the ***Study Completion, Withdrawal and Drop-out Form (TB-CAPT-HIV EXULTANT-StudyCompletion-v3.1)*** to show how many participants reached the study stages. Data from this form, ***from question 1 to 4***, will allow us to know the number of dropouts and their reasons and how many participants will arrive to week 8 or not.

DocuSign Envelope ID: 9E4E8B7F-E077-4685-93BB-EDA3E7D493DD

7.2 Derived variables

We will create some derived variables according to solve the study hypothesis, some of them we will describe in the following SAP versions.

The variables that we will create are:

New Variable	Type	Values
Type of Employment	Categorical	0: Unemployed for any reason 1: Health care worker 2: Prison warder or work at a correctional facility 3: Miner
Time from last HIV+ result	Continuous	0 to randomization date
Time of follow-up	Continuous	From randomization date to end study completion date
Time to TB treatment initiation (in hours)	Continuous	From randomization date to start TB treatment date
ITT cohort	Categorical	0: Not included in ITT 1: Included in ITT
ATP cohort	Categorical	0: Not included in ATP 1: Included in ATP
Participants diagnosed with microbiologically confirmed TB	Categorical	0: No 1: Yes
Participants diagnosed with microbiologically confirmed TB and started on TB treatment within 72 hours of enrolment	Categorical	0: No 1: Yes
CD4 (binary)	Categorical	0: CD4 count <100

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		1: CD4 count >=100

8 Efficacy Analyses

8.1 Primary Efficacy Analysis

The primary efficacy is defined as the difference in the proportion of microbiologically confirmed TB and therapy initiated within 72 hours from enrolment in the intervention and control arm. (the comparison by estimating the difference of proportions and see whether is significantly differs from zero)

We will compare the difference of proportions, defined above, between both study arms and see if the results are significantly different from zero. We will test this differences using the test statistics z has an asymptotic standard normal distribution, and the p-value will be computed as:

$p = 2 \{1- \Phi (|z|)\}$ where $\Phi(\cdot)$ is the cdf of a standard normal distribution and $|z|$ is an absolute value of z. We will calculate logit-transformed 95% confidence intervals

We will compare this proportion as a risk difference and we will stratify by study countries, baseline CD4 counts (under the hypothesis the highest difference in % of bac confirmed participants between control and intervention arm will occur in those immunosuppressed ones, or in countries with highest % of immunosuppressed admitted participants).

We will evaluate the primary efficacy analysis by ITT and Per protocol populations. We will show the both results in the final analysis document with the correspondent tables and graphs.

8.2 Secondary Efficacy Analyses

All secondary efficacy analyses will be calculated on ITT and Per protocol populations. The Secondary Efficacy Analyses are:

8.2.1 We will compare the proportion of deaths during eight weeks after enrolment between all participants enrolled in two study groups. We will test this differences using the test statistics z has an asymptotic standard normal distribution These groups are microbiologically confirmed TB and therapy initiated within 72 hours from admission (numerator/success) and non-lab-confirmed TB or therapy not initiated within 72 hours (denominator/failure). We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

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8.2.2 We will estimate the Kaplan Meier curves to assess time to TB treatment initiation among those with microbiologically confirmed TB and among all TB (also including clinically diagnosed). We will compare both arms using the log rank test. We will use the KM procedure to calculate the survival rate from the survival function. If deemed necessary, to study the effects of some covariates and start of treatment over time we will use the Cox regression.

8.2.3 We will compare the proportion of cases re-admitted to hospital, to test this proportion we calculate the number of admissions after initial discharge and within 8 weeks from enrolment from all participants enrolled. We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

8.2.4 We will compare the proportion of participants who are diagnosed with TB (irrespective of bacteriological confirmation and are started on TB treatment within 72 hours of enrolment). We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

8.2.5 We will calculate the proportion of participants who are diagnosed with microbiologically confirmed TB and are started on TB treatment at 7d, 14d, 4 and 8 weeks of enrolment. We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

8.2.6 We will calculate the proportion of participants who are able to provide the different samples (sputum and urine) at 24, 48 and 72 hours (or later) from enrolment. We will describe the participants profile for every previous point and participants who are unable to provide these samples. We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

8.2.7 We will analyse the time to laboratory confirmed TB diagnosis and any TB diagnosis since enrolment. For this we will calculate the Kaplan Meier estimator and curves. Additionally, we will do proportional hazards models and calculate the hazard ratios at days 7, 14, 28 and 56 and compare the time related estimates between intervention and control group. The models will use the following covariates from Baseline CRF: Site, Sex, CD4 counts.

8.2.8 We will compare the proportion of deaths during first four weeks after enrolment between all participants enrolled in two study groups. We will compare these proportions using Chi-square test or Fisher Test, according to the application condition. These groups are microbiologically confirmed TB and therapy initiated within 72 hours from admission (numerator/success) and non lab-confirmed TB or therapy not initiated within 72 hours (denominator/failure).

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8.2.9 We will calculate the proportion of participants started on treatment without Rif resistance information (only AlereLAM positive). We will compare, using Chi-square test or Fisher Test, these proportion values between intervention and control group.

9. Subgroup analysis

The analysis of all endpoints will be done for all patients of both countries as sample size did not take into consideration country-specific analyses of the specified primary endpoint. Thus, we will merge the data from the four sites we will analyse the resulting database as unique source of data.

However, a subgroup analysis of the primary endpoint and main secondary endpoint (mortality at month 2) will be done by study site (Mozambique and Tanzania, CD4 counts and viral load).

A separate analysis of the primary and secondary endpoints will also be performed by sex of participants, in order to asses if differences arise in any of the outcomes.

10. Other Analyses

We will do a comparison of baseline characteristics between participants who can complete the study and participants who we will lose during their follow-up period. We will do this analysis to test if the participant loss is by chance. We will use Chi2 statistical test. The variables that we will tested are: Country (COUNTRY), Sex (SEX), Ethnic group (ETHNIC), Level of education (EDUCATION), Employment Status (EMPLOYMENT), Early HIV positive result (EARLY_HIV), Current taking antiretroviral (ART_YN), Previous TB episodes (TB_TX_YN), Received BCG vaccination (BCG_YN), Participant live alone? (ALONE), Patient taking cotrimoxazole (CTX_YN), Karnofsky Performance Status Scale (KARNOFSKY), Patient's WHO clinical Stage (WHO_STAGES) and TB treatment start (TB_TX_START_YN).

11 Reporting Conventions

We will use two decimals in numeric results. We will round to up the value of second decimal when the third decimal value will be equal or higher than 5, in other cases we will do nothing.

All confidence intervals (CI) will be calculated at 95% of confidence. These will be calculated using the logit-transformed confidence interval method. We will use the Haybittle-Peto approach for the evaluation of the p-value, under this approach we will consider statistically significant when the p-value's test is lower than 0.05 in the final analysis

Where necessary, variables will be categorized using the cut-off described in previous works and research publish documents. For example, CD4 counts will be categorized in 3: less than 100 CD4 counts per ml (as in STAMP trial), 100-200 CD4 counts per ml, and above 200.

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However, depending on the frequencies observed, these categories could be refined. When we don't have a reference cut-off value for a continuous variable, we will calculate these values using the terciles, quartiles or quintiles of the variable and cut it using this calculated cut-off values. (For example, if we want to divide the participants age in three categories, we will calculate the terciles values and later we calculate a new categorical variable using these points. Then we will have a new age group with three categories: from minimum value to first tercile, from first to second tercile and finally from second tercile to maximum value).

12 Technical Details

The statistical analysis we will performed with the Stata software. We will use the Stata 17 version or the most up to date available version when analysis takes place. Scripts used in the analysis can be published as supplementary material of the main publication or uploaded into a public repository.

13 Listing of Tables, Listings and Figures

We will show the results using Tables and Figures. The following is a description of these tables. Some of these tables will summarize the results for ITT and per protocol analysis and always we will make it by study arm. According to the figures we will draw the Kaplan-Meier curves to show the proportion of participants initiating TB treatment over time, stratified by lab confirmation, CD4 count category, viral load and country. Venn diagrams showing the distribution of participants starting TB treatment by positivity to each test will be displayed.

Table 1. Baseline Characteristics of participants by study arm for ITT and per protocol analysis

	ITT Analysis		Per Protocol Analysis	
	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)
Age (years)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Gender	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Country Site Mozambique	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)

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Tanzania	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Employment Status				
Employed	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Unemployed	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Unable to work	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Student	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
House man/wife	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Retired	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Other	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Previous TB episodes				
Yes	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
No	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
BCG vaccination				
Yes	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
No	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Unknown	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Cotrimoxazol treatment				
Yes	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
No	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Unknown	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Antiretroviral treatment				
Yes	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
No	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Unknown	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
CD4 count <200cells/mm^3				
Yes	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)

No	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Who clinical stage				
Stage I	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Stage II	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Stage III	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)
Stage IV	XXX (XX%)	XXX (XX%)	XXX (XX%)	XXX (XX%)

Table 2. Microbiological results by study arm for both the ITT and per protocol analyses

	ITT Analysis			Per Protocol Analysis		
	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)	p-value	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)	p-value
Samples available	XXX (XX.X)	XX.X (XX.X)	X.XXX	XX.X (XX.X)	XX.X (XX.X)	X.XXX
Sputum Ultra available	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX
Sputum Ultra results						
Negative	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX
Indeterminate	XXX (XX%)	XXX (XX%)		XXX (XX%)	XXX (XX%)	
Positive	XXX (XX%)			XXX (XX%)		
Urine Ultra available		XXX (XX%)			XXX (XX%)	
Urine Ultra results						
Negative		XXX (XX%)			XXX (XX%)	
Indeterminate		XXX (XX%)			XXX (XX%)	

Positive		XXX (XX%)			XXX (XX%)	
Stool Ultra available		XXX (XX%)			XXX (XX%)	
Stool Ultra results Negative Indeterminate Positive		 XXX (XX%) XXX (XX%) XXX (XX%)			 XXX (XX%) XXX (XX%) XXX (XX%)	
AlereLAM available	XXX (XX%)			XXX (XX%)		
AlereLAM results Negative Indeterminate Positive	 XXX (XX%) XXX (XX%) XXX (XX%)			 XXX (XX%) XXX (XX%) XXX (XX%)	 XXX (XX%) XXX (XX%) XXX (XX%)	
Microbiologically confirmed	XXX (XX.X)	XX.X (XX.X)	X.XXX	XX.X (XX.X)	XX.X (XX.X)	X.XXX

Table 3. TB treatment initiation within 72 hours

	ITT Analysis			Per Protocol Analysis		
	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)	p-value	Standard-of-care group (n=XXXX)	Intervention group (n=XXXX)	p-value
Start treatment within 72 hours	XXX (XX.X%)	XXX (XX.X%)	X.XXX	XXX (XX.X%)	XXX (XX.X%)	X.XXX
Hours to start treatment (treatments <= 72h)	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX

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Days to start treatment (treatments > 72h)	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX
Start treatment within 72 hours						
Confirmed TB	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX
Unconfirmed TB	XXX (XX%)	XXX (XX%)	X.XXX	XXX (XX%)	XXX (XX%)	X.XXX

Table 4 TB outcomes at 8 weeks by arm (ITT analysis)

	Standard-of-care group (n=XXXX, %)	Intervention group (n=XXXX, %)	Risk Difference % (95% CI)	p-value
Start treatment within 72 hours	XXX (XX.X)	XX.X (XX.X)	X.X (X.X, X.X)	X.XXX
Microbiologically confirmed TB	XXX (XX%)	XXX (XX%)	X.X (X.X, X.X)	X.XXX
Clinically diagnosed TB	XXX (XX%)	XXX (XX%)	X.X (X.X, X.X)	X.XXX
Deaths at week 8	XXX (XX%)	XXX (XX%)	X.X (X.X, X.X)	X.XXX

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Figure 1. Trial flowchart