Rapid Urine-Based Screening for Tuberculosis to Reduce AIDS-Related Mortality in Hospitalized Patients in Africa (STAMP) Trial

Short title:	STAMP trial
Research Organisation Ref:	LOI:13.016 / pFACT6451 / ITCRZE64
Ethics Reference:	LSHTM REC 9360, COMREC P.06/15/1743, UKZN BREC BFC215/15
Trial Registration Number:	ISRCTN71603869
Date and Version No:	Version 1.5, 14th July 2016

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Funder:	Joint Global Health Trials funded jointly by the Medical Research Council, the UK Department for International Development (DfID) and the Wellcome Trust.
Conflict of interests:	We declare no conflicts of interest

Compliance:	The trial will be conducted in compliance with the protocol, ICH GCP Guidelines and other relevant regulatory requirements applying in the countries in which the trial will be conducted.

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2. SYNOPSIS

Trial Title	Rapid Urine-Based Screening for Tuberculosis to Reduce AIDS-Related Mortality in Hospitalized Patients in Africa (STAMP) Trial	
Short title	STAMP Trial	
Trial Design	A multi-country (Malawi and South Africa) individually randomised clinical trial with two study arms (1:1 recruitment)	
Trial Participants	HIV-infected adult patients requiring acute admission to hospital medical wards at clinical trial sites: a) Zomba Central Hospital, Zomba, Malawi. b) Edendale Regional Hospital, Pietermaritzburg, KwaZulu-Natal, South Africa.	
Planned Sample Size	1,300 patients per arm	
Follow up duration	56 days post-randomisation	
Planned recruitment period	August 2015-July 2017	
	Objectives	Outcome Measures/Endpoints
1. Primary	To determine the impact on all-cause mortality of the addition of rapid, high sensitivity urine-based TB screening to the standard of care TB screening in HIV-infected patients requiring admission to medical wards in hospitals in southern Africa	Risk of death at 56 days after randomisation from any cause, compared between arms.
2. Secondary	To determine the impact of additional urine-based TB screening versus standard of care on: 2.1 time to death from any cause 2.2 the yield of TB diagnoses that are (a) microbiologically confirmed disease or (b) clinically diagnosed disease (see definitions in section 8.6). 2.3 time to (a) TB diagnosis and (b) start of TB treatment (see definitions in section 8.6) 2.4 use of other medical interventions, including (a) antibacterial treatment (b) antiretroviral therapy (ART)	The secondary outcomes will compare between arms the following endpoints occurring within 56 days of randomisation: 2.1 time to all-cause mortality 2.2 proportions of patients with (a) a microbiologically confirmed diagnosis of TB and (b) clinically diagnosed TB disease 2.3 time from randomisation to (a) TB diagnosis and (b) start of TB treatment in days 2.4 proportion of patients receiving (a) antibacterial treatment (b) in ART naïve patients, proportion starting ART and time to ART initiation in days

	2.5 other clinically relevant outcomes, including (a) duration of hospital admission, (b) the need for hospital readmission and (c) loss to follow-up.	2.5 (a) duration of hospital stay in days (b) cumulative incidence of hospital readmission (c) cumulative incidence of loss to follow-up.
3. Economic Analysis	3.1 to determine the incremental cost-effectiveness of high sensitivity urine-based TB screening in HIV-infected medical in-patients in South Africa (a middle-income country) and Malawi (a low-income country). 3.2 to determine the 2-year and 5-year budgetary impact of implementation and scale-up of this intervention from a Ministry of Health perspective for both South Africa and Malawi.	3.1 economic analysis will require measurement of trial-related efficacy endpoints in addition to data on: (a) length of hospital stay and subsequent healthcare utilisation (b) hospital resource utilisation (eg drugs administered, investigations undertaken, procedures undertaken) (c) hospital resource costs (eg drug costs, investigations, laboratory costs, hospitalisation costs) (d) patient quality of life data These will be modelled and compared between current standard of care for TB detection and intensified TB screening protocol which includes urine-based TB screening. 3.2 outcomes, data from economic analysis and data on local and national Ministry of Health budgets.
Investigational Trial Intervention(s)	Testing of urine using: (a) Determine TB-LAM (Alere, Walthem, MA, USA; a rapid lateral-flow assay), (b) Xpert MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA) following concentration of urine by centrifugation. Results will be provided to the responsible medical team as soon as available to inform decisions regarding TB treatment (intervention arm only).	

3. ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
BMI	Body Mass Index
CI	Chief Investigator
CRF	Case Report Form
CRP	C-reactive Protein
CTRG	Clinical Trials and Research Governance
CXR	Chest X-Ray
DMC/DSMB	Data Monitoring Committee / Data Safety and Monitoring Board
FBC	Full Blood Count
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IRB	Independent Review Board
IUATLD	International Union Against Tuberculosis and Lung Disease
LAM	Lipoarabinomannan
LSHTM	London School of Hygiene & Tropical Medicine
LTFU	Loss to follow-up
MLW	Malawi-Liverpool-Wellcome Trust Centre
PI	Principal Investigator
PITC	Provider initiated testing and counselling
REC	Research Ethics Committee
PLHIV	People living with HIV infection
SAE	Severe Adverse Event
SAR	Severe Adverse reaction
SFTP	Secure File Transfer Protocol
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
WHO	World Health Organisation

4. BACKGROUND AND RATIONALE

4.1. HIV-associated tuberculosis (TB)

Burden of disease: Tuberculosis (TB) and TB/HIV co-infection remain enormous challenges to international public health even in regions with high coverage of ART. The HIV/AIDS epidemic has had a particularly devastating impact on TB control in sub-Saharan Africa which accounts for 78% of the global burden of HIV-associated TB and a majority of the resulting 360,000 deaths each year [1]. The countries of southern Africa are hardest hit and here more than half of TB patients are HIV co-infected. TB is the leading cause of death in people dying with HIV/AIDS [2]. This high mortality burden has become a high-level strategic priority with calls spurring for action from WHO, UNAIDS, STOP TB, PEPFAR and other agencies.

Current failure of diagnosis: Post-mortem studies of patients with HIV/AIDS dying in hospitals in countries in west, east and southern Africa uniformly report that between 32% and 64% of cadavers have evidence of TB (mostly disseminated) [3–7] even in patients receiving ART [3,4]. Multi-drug resistant disease is another important factor in South Africa [3,8]. Much of the TB found in these post-mortem studies remained undiagnosed and unsuspected at the time of death, presenting a strong rationale for routine systematic TB screening of all HIV-infected admissions, irrespective of clinical presentation.

We believe that mortality is fuelled by under-diagnosis, delays in diagnosis due to selective investigation of patients, overreliance on sputum-testing, inability to diagnose much extrapulmonary disease, lack of effective diagnostic tools, weak laboratory infrastructure and failure to screen for rifampicin resistance. Recent key developments in the field of TB diagnostics provide new opportunities to address these deficiencies. Based on the CI's recent studies in Cape Town, South Africa, this study will evaluate a fundamentally new, rapid, high sensitivity approach to screening for HIV-associated TB in HIV-infected medical in-patients using urine-based diagnostics [9].

New diagnostic tools: The **Xpert MTB/RIF** rapid molecular assay has been endorsed by WHO for diagnosis of HIV-associated pulmonary TB and rifampicin resistance using sputum and it is being implemented in many African countries [6]. An increasing evidence base shows good diagnostic accuracy when testing a wide range of extrapulmonary samples [10,11]. Of note, assay specificity has been uniformly high (approximately 98%) regardless of sample type tested [10,11], indicating that the test can reliably be used on non-respiratory samples as a rule-in test to inform TB treatment decisions. In 2014, the WHO released guidelines for the use of Xpert to test non-respiratory samples for diagnosis of EPTB [12,13]. Studies (including those of the CI) have reported useful diagnostic accuracy when used to screen urine samples from HIV-infected inpatients and out-patients [9,14,15].

The **Determine TB-LAM** assay is a simple lateral-flow (strip-test) antigen detection assay for urine lipoarabinomannan (LAM) that is now commercially available [16–18]. It can be used to diagnose TB within 30 minutes. In the most comprehensive assessment of specificity to date among in-patients in South Africa, specificity exceeded 99%, indicating that the test can reliably be used as a rule-in test to inform TB treatment decisions [9].

Relevant systematic reviews: One systematic review and three comprehensive narrative reviews summarize the current state of urine-based TB diagnosis. No studies have yet

reported on the impact of urine-based diagnostics on clinical outcomes when used for routine, systematic TB screening of HIV-infected hospital in-patients.

- Peter J. et al. Urine for the diagnosis of tuberculosis: current approaches, clinical applicability, and new developments. Curr Opin Pulm Med 2010 May; 16(3):262-70. [19]
- Minion J. et al. Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis. Eur Respir J 2011 Dec; 38(6):1398-405. [20]
- Lawn SD. Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infect Dis 2012 Apr 26; 12(1):103. [16]
- Lawn SD, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis 2013 Apr; 13(4):349-61. [21]

Please see appendix F for a summary of diagnostic accuracy of Xpert MTB/RIF and Determine TB LAM assays.

4.2. Study Rationale

Background studies informing the study intervention: Background studies conducted by the CI in South Africa (see Figure 1 below and ref [9]) have demonstrated that:

- The overall prevalence of TB (defined using comprehensive microbiological sampling and investigation) among unselected HIV-infected medical in-patients at the time of hospital admission was very high (33% overall; 40% in ART-naïve patients; 27% in those on ART). Symptoms were neither sufficiently sensitive nor sufficiently specific to be a useful component of TB screening strategies applied to this patient group.

- The diagnostic yield from testing sputum samples with Xpert MTB/RIF in the initial diagnostic screen was low, at 27% of the total microbiological diagnoses (Figure). This, in part, reflects the difficulty in obtaining sputum samples from debilitated in-patients: only one half of patients screened were able to produce a sputum sample during their total in-patient stay. Of all patients with a confirmed diagnosis of TB, just 54% had microbiological evidence of TB from respiratory samples compared to 83% from non-respiratory samples ($P<0.001$).

- In contrast, urine samples were obtained from almost all patients screened and yielded a majority (69%) of total TB diagnoses. Rapid urine testing using Determine TB-LAM lateral-flow test for lipoarabinomannan (LAM) antigen had high specificity (98.9%) and provided results within 30 minutes using a 60µL aliquot of unprocessed urine. A second urine test used a 40 ml urine sample which was first concentrated by centrifugation and then tested using the semi-automated rapid molecular assay, Xpert MTB/RIF, providing results within 2 hours. Xpert MTB/RIF testing of concentrated urine provided the greatest yield (59%) of any single test, and the yield was further increased when combined with other tests. The comparative yields of sputum- and urine-based tests are outlined in Figure 1.

- Use of these rapid assays on both sputum and urine samples obtained in the first 24 hours rapidly diagnosed a large majority (81%) of total microbiologically confirmed TB disease (Figure 1).

- Urine-based testing also diagnoses TB in patients with the highest mortality risk [14,22], thus rapidly identifying those who have most potential gain from rapid initiation of TB treatment.

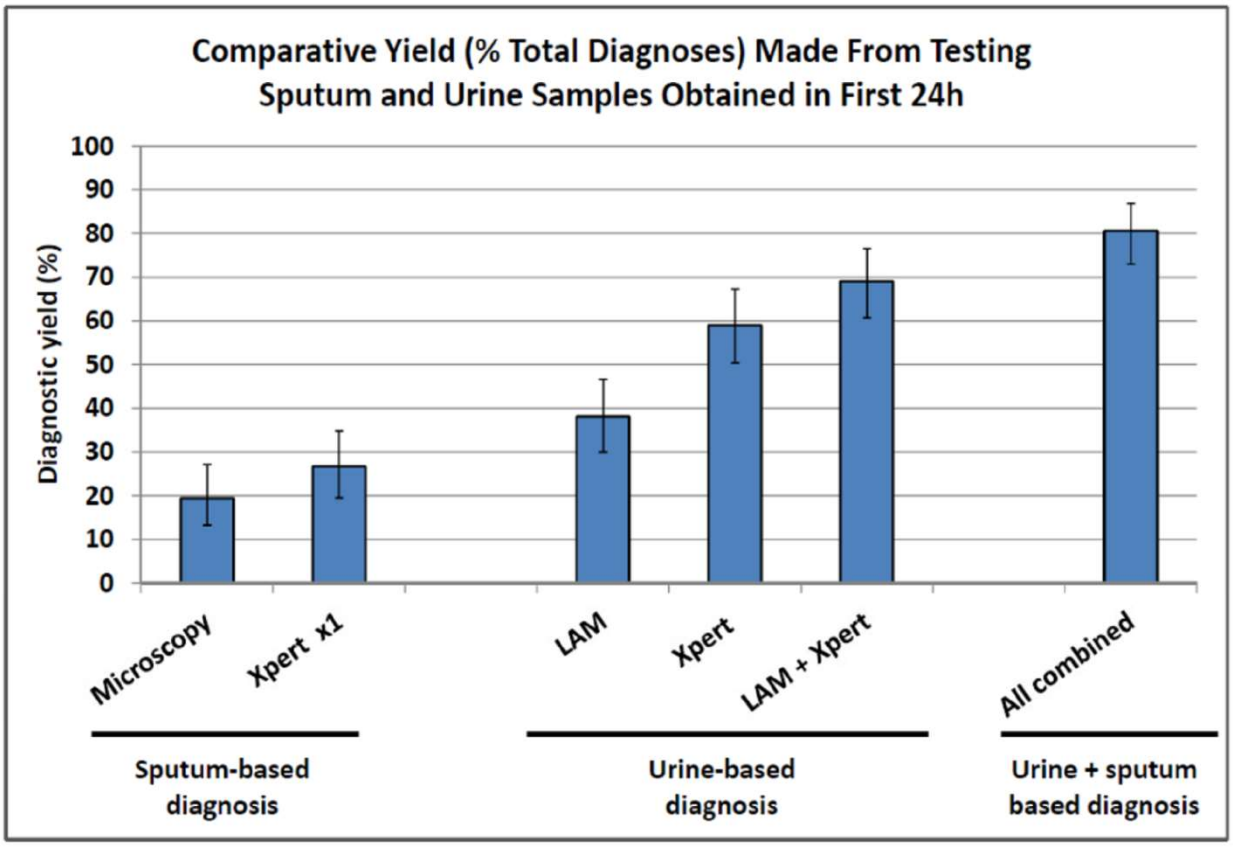

Figure 1. *Data from background study in Cape Town showing the comparative diagnostic yield of sputum-based, urine-based and combined approaches in unselected HIV+ medical in-patients. The reference standard was a composite of total microbiologically confirmed TB following intensive investigation* [9]***)***

The need for studies of clinical impact: Although the use of urine-based screening for TB increases the sensitivity of TB diagnosis in this clinical population, there is no evidence that urine-based testing will have any impact on clinical outcomes.

Replacement of sputum smear microscopy with sputum Xpert MTB/RIF has not been found to improve clinical outcomes when investigating patients suspected of having TB [23–27]. This may be due to high rates of empirical TB treatment which may be increased as a result of changes in prescribing behaviour in studies with unblinded study design [25–27].

However, a critical difference in the design of the present study is the strategy of investigating ALL patients regardless of whether TB is suspected or not: post-mortem studies and

background data from Cape Town [9] show that a huge burden of TB remains clinically unsuspected and is not empirically treated. Moreover this study uses masking of clinical teams, thereby reducing the risk that empirical TB treatment or changes in prescribing practice during the trial will undermine this study [28].

Update 5th February 2016: The LAMRCT trial released its results in December 2015.[Abstract 41. The Union/CDC Late-Breaker Session on TB, 46th Conference of the International Union against Tuberculosis and Lung Disease, Cape Town, December 2015] It randomised adult, HIV-positive hospitalised patients 'with TB symptoms' to either 'routine diagnostics' or 'routine diagnostics plus adjunctive urine LAM testing'. The study was conducted at 4 sites in sub-Saharan Africa (South Africa, Zimbabwe, Zambia and Tanzania). The primary outcome was mortality at 8 weeks, and the trial reported a 4% reduction in mortality in the urine LAM arm (20.8% mortality in no LAM arm, compared to 24.9% mortality in the LAM arm). [Peters J, Zijenah LS , Chanda D et al, The mortality impact of point-of-care urine lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-infected hospitalised patients: a multi-country randomised controlled trial. The Lancet 2016, In press]

Potential harms from screening: High diagnostic sensitivity alone does not justify the implementation of urine-based diagnosis. Rapid TB diagnosis might benefit this clinical population, but there is no evidence demonstrating that this is the case. Urine-based testing could be associated with a range of adverse consequences. These include the following:

- Rapid TB diagnosis may reduce the likelihood that patients receive an empirical course of simple antibiotics as part of the diagnostic work-up such that concurrent sepsis is not treated and may divert clinical attention from the presence of other co-pathologies.
- As with most diagnostic assays, the specificity of these urine-based approaches to diagnosis is unlikely to be consistently 100%, giving rise to the potential for some false-positives.
- Use of Xpert MTB/RIF to test urine samples may be associated with false-positive rifampicin resistance results in some patients. In populations with low prevalence of MDRTB, the positive predictive value of this result is low and may lead to the inappropriate use of more toxic and expensive MDR-TB treatment.
- If this strategy were adopted as policy, use of limited health service resources for purchasing costly diagnostics may divert resources from other important health system / programmatic needs which may have greater impact on health.

In the presence of unproven benefits but also the potential risk for harm (both individual and programmatic), a randomised controlled trial is essential to assess overall clinical impact and cost-effectiveness to provide the essential evidence base to formulate policy.

Generalisability of research: Autopsy studies of HIV-infected in-patients conducted across different regions of Africa (west, east and southern) and in India [3–7], show that undiagnosed TB is common in all settings. This study will build on the CI's previous work in Cape Town by including a new site in South Africa, and a site in Malawi (a resource-limited setting) with an anticipated lower prior probability of TB than South Africa. This will ensure the results of this trial will be broadly generalisable to other HIV-infected medical in-patient populations across sub-Saharan Africa, and also in other resource-limited settings with a high burden of TB. However, the results should not be generalised to HIV-infected medical out-

patients in whom the frequency of disseminated disease and renal involvement with TB is likely to be lower.

4.3. Principal Research Question

Does implementation of a novel, rapid, high sensitivity urine-based screening strategy for TB, used in combination with routine sputum-based standard of care diagnosis, reduce all-cause mortality among HIV-infected medical in-patients newly admitted to hospitals in southern African countries?

5. OBJECTIVES AND OUTCOME MEASURES / END-POINTS

Objectives	Outcome Measures/Endpoints
1. Primary Objective To determine whether the addition of rapid, high sensitivity urine-based TB screening to the diagnostic standard of care for HIV-infected patients requiring admission to medical wards in hospitals in southern Africa reduces all-cause mortality	Risk of death at 56 days after randomisation from any cause, compared between arms.
2. Secondary Objectives To determine the impact of additional urine-based TB screening versus standard of care on: 2.1 time to death from any cause 2.2 the yield of TB diagnoses that are (a) microbiologically confirmed disease or (b) clinically diagnosed disease (see definitions in section 8.6) 2.3 time to (a) TB diagnosis, (b) start of TB treatment and (c) time between TB diagnosis and start of TB treatment (see definitions in section 8.6) 2.4 use of other medical interventions, including (a) antibacterial medication (b) antiretroviral therapy (ART) 2.5 other clinically relevant outcomes, including (a) duration of hospital admission, (b) the need for hospital readmission and (c) loss to follow-up.	The secondary outcomes will compare between arms the following endpoints occurring within 56 days of randomisation: 2.1 time to all-cause mortality 2.2 proportions of patients with (a) a microbiologically confirmed diagnosis of TB and (b) clinically diagnosed TB disease 2.3 time from randomisation to (a) TB diagnosis, (b) start of TB treatment in days and (c) time between TB diagnosis and start of TB treatment 2.4 proportion of patients receiving (a) antibacterial medication (b) in ART naïve patients, proportion starting ART and time to ART initiation in days 2.5 (a) duration of hospital stay in days, (b) cumulative incidence of hospital readmission, and (c) cumulative incidence of loss to follow-up.
3. Economic Analysis Objectives 3.1 to determine the incremental cost-effectiveness of high sensitivity urine-based TB screening in HIV-infected medical in-patients in South Africa (a middle-income country) and Malawi (a low-income country).	3.1 economic analysis will require measurement of trial-related efficacy endpoints in addition to data on: (a) length of hospital stay and subsequent healthcare utilisation

	(b) hospital resource utilisation (eg drugs administered, investigations undertaken, procedures undertaken) (c) hospital resource costs (eg drug costs, investigations, laboratory costs, hospitalisation costs) (d) patient quality of life data These will be modelled and compared between current standard of care for TB detection and intensified TB screening protocol which includes urine-based TB screening.
3.2 to determine the 2-year and 5-year budgetary impact of implementation and scale-up of this intervention from a Ministry of Health perspective for both South Africa and Malawi.	3.2 outcomes, data from economic analysis and data on local and national Ministry of Health budgets.

6. TRIAL DESIGN

A pragmatic, multi-country (Malawi and South Africa), individually randomized, clinical trial with two study arms (1:1 recruitment). Patients, research nurses, investigator team and the responsible medical team (other than trial statistician and laboratory technicians) will be masked to the randomised screening allocation. Patients randomised to the standard of care arm will be screened by testing sputum with Xpert MTB/RIF. Those in the intervention arm will, in addition to standard of care sputum testing, undergo rapid urine-based screening with Determine TB-LAM lateral flow assay and Xpert MTB/RIF assay testing of 40 ml urine following concentration by centrifugation. Laboratory-based diagnoses of TB will be communicated to the responsible medical team to inform TB treatment (while maintaining masking to screening arm allocation), but the clinical management by the medical team will not be altered. Outcomes will be compared between arms 56 days after randomisation.

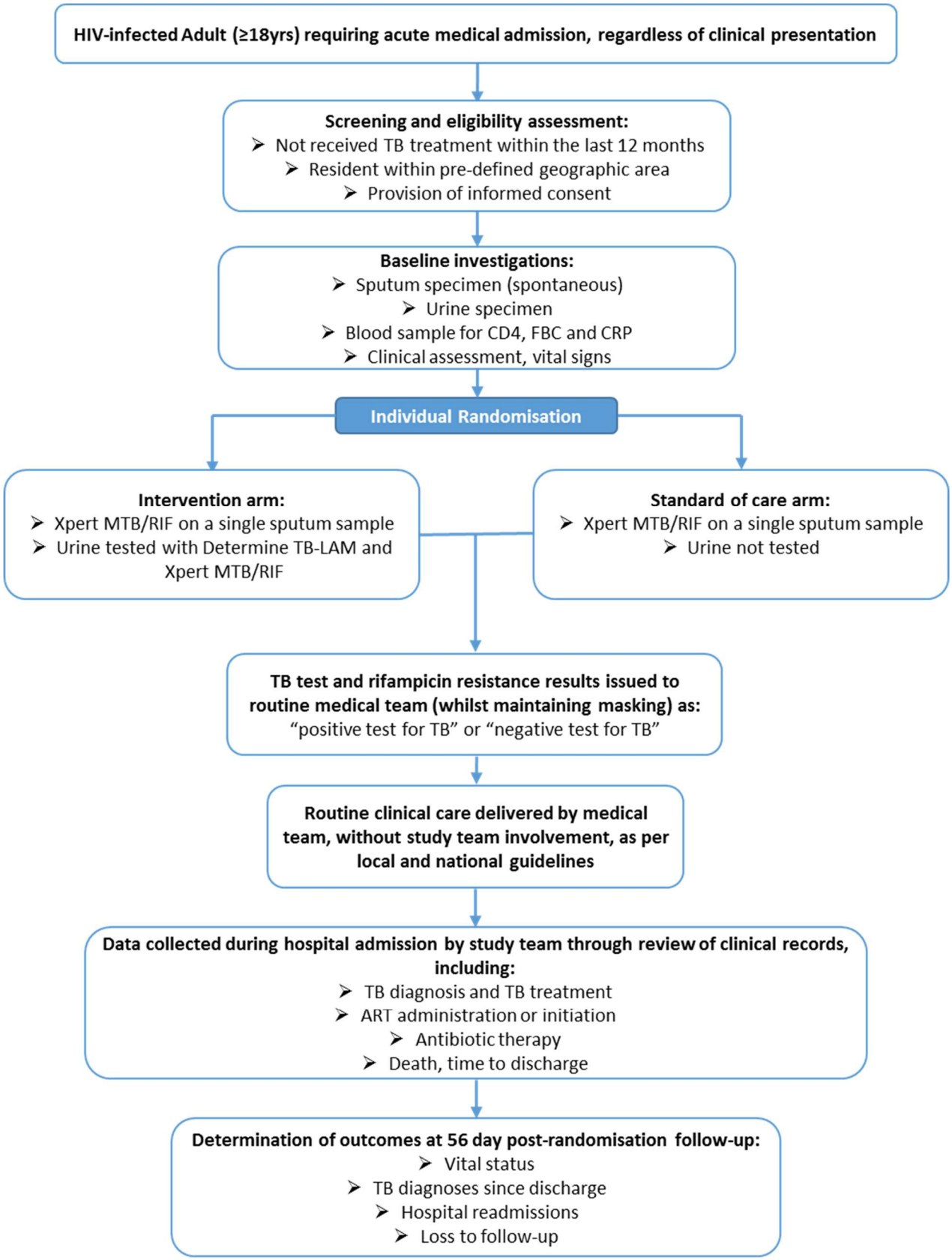

Figure 2. *Trial flow chart*

7. PARTICIPANT IDENTIFICATION

7.1. Trial Participants

People (aged ≥18 years) living with HIV infection (PLHIV) who require acute admission to hospital medical wards and who are willing and able to provide informed consent will be enrolled, regardless of clinical presentation and whether or not TB is clinically suspected, to the following clinical trial sites:

- Zomba Central Hospital, Zomba, Malawi
- Edendale Regional Hospital, Edendale, Pietermaritzburg, KwaZulu-Natal, South Africa

7.2. Inclusion Criteria

All medical admissions will be screened for eligibility, regardless of the admitting complaint.

- Requires acute admission to a hospital medical ward at either clinical trial site for any reason
- Have confirmed HIV-infection (existing or new diagnosis, irrespective of ART status)
- Willing and able to provide informed consent

7.3. Exclusion Criteria

- Aged <18 years
- Has been admitted to a medical ward for longer than 48 hours
- Has received treatment for TB within the preceding 12 months, or has received isoniazid preventative therapy (IPT) within the last 6 months
- Residence does not lie within a pre-defined geographic area (with regard to the feasibility of follow-up) or plans to leave this area during the period of trial follow-up.
- Unable or unwilling to provide informed consent

8. TRIAL PROCEDURES

8.1. Recruitment

All adult PLHIV (either a known diagnosis or a new diagnosis) referred for admission to medical wards (irrespective of clinical presentation and the reason for medical admission) will be referred by health service staff, with the patient's prior agreement, for explanation of the study and assessment of eligibility. All adult patients who are not known to be HIV-infected are offered HIV testing (provider-initiated testing and counselling) on admission as standard clinical practice at both study sites.

8.2. Screening and Eligibility Assessment

Potential participants will be approached by the study team and informed about the study. Those willing to be screened for eligibility will be assigned a screening ID number and assessed by the study team as per the above criteria. A recruitment log will be kept of all potentially eligible patients admitted to each study site and screened patients who either are or are not enrolled, including the reasons for not being enrolled when available.

If patients are HIV-positive, confirmation will be sought either in the form of confirmatory documentation (eg health passport or viral load results) or confirmatory HIV testing offered by the study team. Participants who meet all criteria but do not know their HIV-status will be offered rapid HIV testing by the study team. HIV testing will be undertaken by the recruiting study nurse who will be fully trained in local provider-initiated testing and counselling (PITC) practices, and using a locally approved HIV rapid testing algorithm. Results will be provided to the patient with appropriate counselling, and to responsible medical team for ongoing management, irrespective of whether the patient enrols in the trial.

8.3. Informed Consent

Written informed consent will be sought from participants before any trial-specific procedures are undertaken at screening, with witnessed thumbprint used for patients unable to read and/or write. A copy of the signed informed consent will be given to the participant. The assistance of a translator will be used where necessary and all patient information sheets and consent forms will be translated in to local languages. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

8.4. Baseline Assessments and Procedures

Following enrolment the study team will be responsible for obtaining, where possible:

- one sputum specimen (spontaneously expectorated; no induction of sputum will be done unless this is standard of care at the trial site at the time of the study) to be tested using the Xpert MTB/RIF assay (if patients are unable to produce a sputum sample initially, they

will be instructed on how to do so and left with a sputum specimen container- one further attempt to obtain sputum will also be made by the study team within 4-6 hours). Patients who are not able to produce a sputum sample during this time will remain in the study but, thereafter, no further sputum TB investigations will be arranged by the study team, although the responsible clinical team will remain at liberty to arrange further TB tests as appropriate.

- a urine specimen of >50 mls to be tested using the (i) Determine TB-LAM and (ii) Xpert MTB/RIF assays (testing will only be done on samples obtained from participants randomized to the intervention arm).

15-20 mls of blood will be taken for the following tests:

- CD4 cell count
- Haemoglobin
- C-reactive protein (CRP)
- Serum save

The study team will also collect information on:

- Mobile phone numbers of the patient and/or designated relatives/next-of-kin
- Demographic information including precise geographic/home locator information (to aid follow-up)
- Clinical history including past history of TB treatment, HIV care, ART duration and regimen, and other illnesses such as opportunistic infections
- Vital signs (including danger signs)
- TB symptoms (including the WHO symptom screen)
- Height and weight (to calculate BMI)
- Mid-upper arm circumference
- Quality of life questionnaire (EQ5-D)
- Karnofsky score

8.5. Randomisation procedure

A randomisation list stratified by study site will be generated in advance by the study statistician with random block size to ensure equal numbers of participants are enrolled into each arm and each study site. Paper slips with randomisation number will be printed and inserted into envelopes for each site. The envelopes will be held by the study coordinator at each site and distributed to the nurse on enrolment of a participant. The randomisation number will act as unique study ID number. The envelope will not contain the intervention allocation.

The study laboratory will be provided with a list of randomisation numbers and their study arm allocation. When samples from enrolled patients arrive at the laboratory, the TB tests to be carried out will be identified by checking the randomisation number with the list of study arm allocation. Sputum samples from all patients will be tested using the Xpert MTB/RIF assay, but only urine samples from patients allocated to the intervention arm will be tested using

rapid diagnostic tests. Code-breaking will not be required for this study given the nature of the intervention.

The study team (other than trial statisticians and laboratory technicians) and the routine clinical team responsible for the patient will be masked as to study arm allocation. Unblinding of randomisation is not required, as treatment is not given as part of the study intervention (it is the responsibility of the routine clinical team) and all study results are issued to the routine clinical team.

If patients are deemed to have an SAE related to TB treatment, treatment will simply be stopped at the discretion of the responsible clinical team. Any rare occasion where the clinical team consider that unblinding is necessary will it be considered by members of the trial steering committee on a case by case basis.

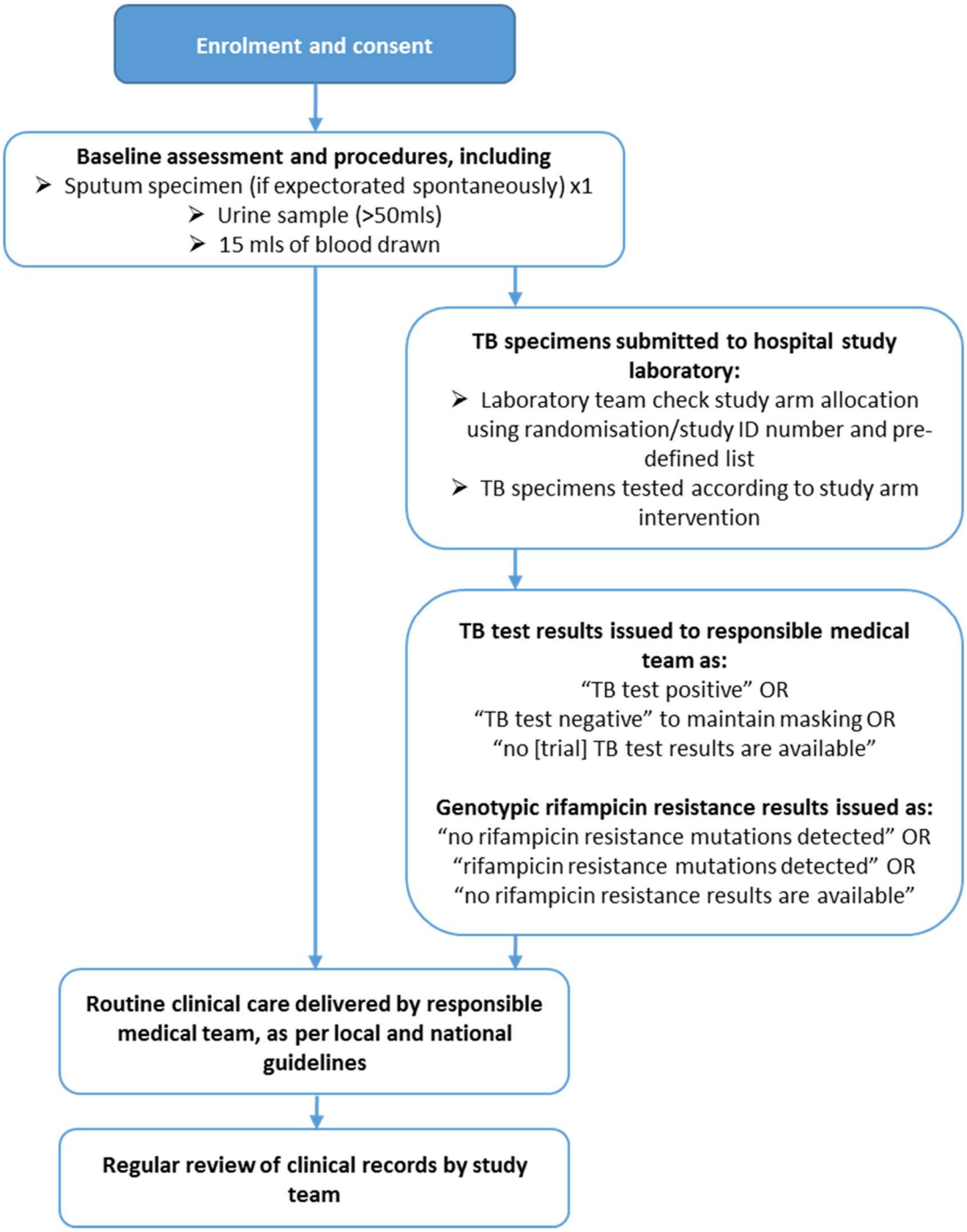

Figure 3. *Flow chart of early trial procedures*

8.6. TB Definitions

TB events will be defined as follows for the purpose of this study:

Microbiological diagnosis of TB (based both on study samples and on any samples requested by the routine medical team) will have at least one smear-microscopy, Xpert and/or culture positive result(s) on any specimen OR a positive urine Determine TB-LAM result.

Clinical diagnosis of TB will have a compatible clinical illness or radiological disease and/or the decision of the responsible clinical team to commence TB treatment in the absence of any positive microbiological tests for TB.

Date of TB diagnosis for microbiological TB diagnoses this will be the date when the positive microbiological test result for TB was communicated to the responsible medical team (eg written in the hospital record), and for clinical diagnoses it will be the date of commencement of TB treatment. Date of commencement of TB treatment will be the first date that anti-TB medication was prescribed.

8.7. Training for responsible medical team

In addition to extensive training in the trial protocols and procedures, staff responsible for the routine delivery of care for patients in this trial will be educated with regards to the TB diagnostic tests being trialled. This will include information about the sensitivity and specificity of the individual tests and estimated setting-specific positive and negative predictive values for TB diagnosis and detection of rifampicin resistance, allowing them to make informed decisions on how to interpret test results.

8.8. Subsequent procedures

TB specimens and results (see figure 3): Samples will be immediately submitted to the hospital laboratory. The study nurse and responsible medical team (independent from the study team) will remain blinded to subsequent randomisation. In the laboratory, patients will have TB specimens tested according to whether randomised to the intervention or standard-of-care arms.

For both standard and intervention arms, Xpert MTB/RIF testing will be carried out on a single sputum sample where available. In the intervention arm only, urine will be tested using: (i) Determine TB-LAM and (ii) 40 ml urine will be concentrated by centrifugation and the pellet tested using Xpert MTB/RIF.

If there is an error or “indeterminate” result with the Xpert MTB/RIF assay, the test will be repeated on the same sample if possible (as per local and WHO guidelines). If an Xpert MTB/RIF test reports an isolate as rifampicin resistant, the test will be repeated as per local and WHO guidelines.[12,13] Similarly, if the Xpert MTB/RIF test gives an inconclusive rifampicin resistance result, the test will be repeated.[12,13]

Results of TB tests will be issued as soon as available and communicated to the responsible medical team to inform decisions regarding TB treatment (anticipated to be within 24-48 hours). These will be issued as “positive test for TB” or “negative test for TB” without specifying sample type so as to maintain masking of the responsible medical team. The results of Xpert MTB/RIF testing for genotypic rifampicin resistance will also be provided as either “no rifampicin resistance mutations detected” or “rifampicin resistance mutations detected”. Discordant rifampicin resistance results will be managed as per local and WHO guidance.[12,13] If only the urine TB-LAM assay is positive, no rifampicin resistance results will be issued to the medical team. If no samples are provided to the study team for TB

testing, or submitted samples are unable to be tested for any reason, it will be communicated to the responsible medical team that “no [trial] TB test results are available”.

The routine investigation and management of patients according to local protocols will not otherwise be altered. Participation in the trial will not prevent the responsible medical team from requesting additional TB tests if clinically indicated. However, the study team will not be involved in the collection or processing of any such additional TB tests.

In addition to TB test results, CD4 cell count and haemoglobin results will be provided to the responsible medical team as soon as they are available for patients in both study arms.

Follow-up during hospital admission: Following the initial TB screen, the study team will have no role in routine patient investigation, care and management.

Patients will remain under the care of their responsible medical teams who will be responsible for all clinical decisions including initiation of TB treatment, ART, co-trimoxazole prophylaxis and treatment for co-morbidities according to local and national guidelines. The research team will supply the results of TB screening assays and CD4 cell counts, but will otherwise have no role in medical management.

However, the study team will extract records of all medical interventions received and clinical outcomes during the hospital admission by regular review of clinical records and/or discussion with medical team. Information will be collected about key medical interventions and clinical events, including:

- Timing of study TB test report
- Any further TB tests, including nature of specimens and results
- TB diagnosis, including date of diagnosis
- Commencement of TB treatment, date of starting treatment, reasons for commencement (e.g. microbiological results, chest x-ray or clinical symptoms in the absence of positive microbiological tests)
- Initiation or administration of ART, including regimen and timing
- Initiation or administration of co-trimoxazole therapy
- Initiation of isoniazid preventative therapy
- Concomitant bacterial infection or prescription of antimicrobials (other than TB treatment)
- Other opportunistic infections based on tests or treatment given
- Vital status, including time of death
- Time to discharge and length of hospital stay
- Side-effects of TB treatment
- Serious or severe adverse events (see section 9)

Storage of specimens: Urine samples from patients in both study arms will be stored for up to 5 years. These will be tested with repeat urine TB LAM assays, Xpert MTB/RIF assay and other antigen capture and/or nucleic acid amplification tests that have been developed in the interim for retrospective case verification and test diagnostic accuracy of new TB diagnostics. The stored urine samples will also be used to investigate options for pre-treatment of urine that could potentially increase the sensitivity of antigen capture diagnostic tests such as TB-

LAM. Repeat testing of urine from patients who test positive with urine TB LAM but negative with Xpert MTB/RIF will also be useful to further understand this patient population.

Similarly, 2-5ml of serum from all patients will also be stored for up to 5 years. This will be used for batch testing for CRP, retrospective Cryptococcal antigen testing and immunological profiling in a supplementary nested case-control study exploring risk factors for mortality (sub-study planned for Malawi trial site only). The immunological tests will include testing functional killing (using *mCherry* labelled *M.smegmatis*) and measuring cytokine responses to help understand the mechanisms underpinning any associations between positive urine-diagnostic tests and mortality. Serum will also tested with other serological, antigen capture and/or nucleic acid amplification tests that have been developed in the interim for retrospective case verification and testing diagnostic accuracy.

8.9. Follow-up procedures and ascertainment of outcomes

Patients enrolled in the study will have subsequent management and follow-up as arranged by the medical team responsible for their care (including HIV care and ART if eligible). In addition, all patients in the study will be followed-up by the study team at 56 days post-randomisation, wherever possible by face-to-face interview. There will be a provision of finance to patients to cover time and local transport costs. During the single follow-up visit, the following information will be collected:

- Vital status
- TB investigations or diagnosis made since discharge, including timing of TB diagnosis and commencement on or discontinuation of TB treatment
- For those patients in whom TB has been diagnosed, linkage to out-patient TB care, progress on TB treatment, TB-treatment related toxicity requiring discontinuation and/or change in course, details of TB clinic attendance, whether they have a supply of TB medication and adherence to treatment
- Health-service use including number and type of clinic visits, new antimicrobials prescribed, site, duration and nature of any hospital admissions (patients will be asked to take note of this upon discharge)
- For patients who have had a subsequent hospitalisation, hospital records will be reviewed to determine duration of hospitalization and diagnoses when possible
- For patients who were diagnosed with TB during their hospital admission and were enrolled in the strain diversity sub-study (Edendale, South Africa site only), a further sputum and urine sample will be requested for mycobacterial culture

In the event that a patient does not attend follow-up, contact will be made by mobile phone with the patient or their designated contact/relative/next-of-kin. Careful documentation during hospital admission of precise home location will also enable home visits to be made, when required, by dedicated community-based study team workers to collect follow-up outcome data. By these means, losses to follow-up will be minimized. Patients who cannot be contacted or traced by phone or home visits will be classified as lost to follow-up after three tracing attempts. Date last seen alive will also be ascertained from next-of-kin when possible. To minimize ascertainment bias, registers (eg ART registers, TB registers) and records will be reviewed for patients lost to follow-up to document vital status and secondary outcomes.

Patients will also be asked to provide informed consent to be contacted by telephone at 6 months after enrolment to ascertain vital status, and/or for death registers to be searched using South African identification numbers to ascertain vital status at 6 months after enrolment (Edendale, South African site only). 6 month mortality will be ascertained for those patients that agree to this.

8.10. Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the trial at any time. In addition, the Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including ineligibility (either arising during the trial or retrospectively having been overlooked at screening), significant protocol deviation or withdrawal of consent. The reason for withdrawal will be recorded in the CRF.

8.11. Definition of End of Trial

The trial will be considered closed following the completion of follow up of the last participant, and once all follow-up and laboratory reports have been received. The trial may be terminated early by the trial steering committee, data safety monitoring board, regulatory authorities or the funders, for example if interim analysis shows an unacceptably high mortality in the intervention arm, as defined in the analysis plan.

8.12. Understanding risk-factors and mechanisms for mortality in patients with HIV-associated TB diagnosed by rapid urine-based tests: a sub-study (Zomba, Malawi site only)

Studies performed by the CI in South Africa, as well as other urine LAM diagnostic accuracy studies, have demonstrated that hospitalised patients who have positive urine-based diagnostic tests (especially using the Determine TB-LAM lateral flow assay) have between two and four-times increased risk of early mortality, even after adjustment for CD4 cell count [9]. The exact mechanisms underlying this increased mortality risk compared to other patients with HIV-associated TB remain unclear. Possible causes of this association include: (i) positive urine-based diagnostic tests may simply be a marker for more severe, disseminated TB disease; or (ii) this effect may be due to high concentrations of LAM systemically, which itself has direct and profound immune-suppressive effects, undermining the host immune response to *Mycobacterium tuberculosis* and/or other opportunistic pathogens. Understanding the mechanisms behind this association may inform adjunctive inventions (for example immunomodulation, broad-spectrum antibiotics or greater intensity of supportive care) aimed at reducing the high mortality in this patient population. This is especially important if the STAMP trial demonstrates TB screening with rapid diagnostics alone are not enough to improve clinical outcomes, in which case adjunctive interventions will be vital.

To address these questions, we will undertake a sub-study amongst participants enrolled in the STAMP trial to assess risk-factors for mortality and compare functional (innate and acquired) immune responses between those with and without positive urine-based diagnostic tests. We will enrol only those patients with microbiologically confirmed HIV-associated TB

(defined as any positive STAMP or non-STAMP related TB investigation including the TB-LAM, Xpert MTB/RIF or mycobacterial culture). The anticipated sample size is 100 patients, although the sample size may be altered in response to initial results.

Prior to commencement of TB therapy, potential participants enrolled in the STAMP trial will be informed of the sub-study and written informed consent will be sought from using standardised information sheets and consent forms (provided as a separate document). Following provision of informed consent and enrolment in the sub-study, a further blood sample of 5-10mls will be taken for functional killing immunological assays to be done entirely in vitro (using *mCherry* labelled *Mycobacterium smegmatis*) and intracellular cytokine staining assays to assess the innate and acquired immune system (these assays require a fresh whole blood sample).

Serum/plasma levels of cytokines (markers of immune activation and anti- and pro-inflammatory cytokines, including TNF-alpha, interferon-gamma, interleukin-6, interleukin-12, and interleukin-10 as well as sCD14 – a marker of macrophage activation) will be measured by enzyme-linked immunoassays using stored serum samples already taken upon enrolment to the STAMP trial. No other additional specimens or data will need be collected as part of this sub-study, only the existing data collected and stored for the STAMP study will be used. No additional samples taken for this sub-study will be stored, any blood that is taken but not used will be safely disposed of immediately. All immunological assays will be run in Malawi. This sub-study will comply with all other aspects of the STAMP study protocol with regard to the acquisition and storage of personal data and maintenance of patient confidentiality. None of the additional immunological parameters to be measured in the sub-study has any immediate medical significance that would warrant the patient or medical team to be informed. Thus, this sub-study will in no way alter patient management.

In the analysis, median levels of cytokines will be compared between patients with and without positive urine-based TB diagnostic tests. Similar analyses will be performed comparing patients who die and those who survive. Associations between cytokine levels and mortality will also be assessed using linear regression. A similar approach will be employed to determine the impact of urine-based diagnostic test status on functional immune response and intracellular cytokine analysis. Logistic regression modelling will also be used to determine clinical and immunological risk factors for mortality, including positive urine-based TB diagnostic tests.

8.13. *Mycobacterium tuberculosis* strain diversity among HIV-infected adults hospitalized in South Africa: pilot cohort sub-study (Edendale, South Africa site only)

Background and research questions

Sub-Saharan Africa (SSA) accounts for almost 80% of the global tuberculosis (TB) burden, and most of the 400 000 TB deaths each year [1]. Mortality due to TB remains the leading cause of death in HIV-infected patients despite of increasing coverage of ART and rollout of a new nucleic amplification diagnostic test [1]. Studies from various geographical settings have reported on the ability of individuals with active TB to simultaneously harbour a diverse range of distinct *Mycobacterium tuberculosis* (MTB) strains. Strain diversity, or within-host

heterogeneity alludes to ‘complex MTB infections’ that arise through either multiple infection events or within-host mutations [29]. In a recent outpatient in the Edendale Hospital catchment area investigating within-host heterogeneity among 500 outpatients initiated on TB treatment, 21.1% of patient had evidence of harbouring mixed MTB infections at treatment initiation; which was also associated with increased failure to culture convert at 8 weeks after treatment initiation (adjusted OR 1.90; 95% CI, 1.03-3.50) [29]. In addition, recent post-mortem studies at Edendale Hospital using new whole genome sequencing (WGS) techniques have shown that mixed MTB infections not only move from one lung site to another, but also to disseminate to extrapulmonary sites [30].

In life, most body compartments cannot be easily sampled to detect MTB stain diversity. However, recent work suggests that urine-based TB diagnostics may offer additional diagnostic yield to more conventional sputum based tests, offering the opportunity to compare MTB strain diversity in the respiratory and renal compartments [31,32]. Yet, it is not known what the implications would be of strain diversity detected on WGS in these compartments, for example on culture conversion in hospitalized adults with newly diagnosed TB. The understanding of such preferential compartmentalisation could inform alternative diagnostic and treatment strategies, and potentially impact HIV-associated TB mortality. To address these questions, we will undertake a pilot cohort sub-study (embedded in the STAMP trial) amongst all patients proven to have microbiologically confirmed HIV-associated TB on Xpert MTB/RIF and/or urine Determine TB-LAM at Edendale Hospital in order to:

1. Determine what is the prevalence of complex MTB infections among HIV-positive individuals hospitalized in a regional hospital KwaZulu Natal
2. Determine whether TB strains show diversity between sputum and urine compartments in hospitalized HIV-infected individuals
3. Explore associations between mixed MTB infections and clinical outcomes (including culture conversion) at 8 weeks after treatment initiation

Study design

We will obtain written informed consent from all patients enrolled in the STAMP trial to get extra samples, at two time points, of (a) sputum (3ml) and (b) urine (40 ml) from all those who are diagnosed with tuberculosis during hospital admission in the STAMP study (either based on STAMP study TB investigations or routine hospital TB investigations). These samples will be taken directly after the diagnosis of TB (this will not delay TB treatment initiation) and at 56-day follow up. All samples will be immediately and securely transported to the KwaZulu Natal Research Institute for TB and HIV (K-RITH), Durban, KZN. Here, specimens will be pooled and grown in liquid culture medium (BACTEC MGIT 960; Becton-Dickinson, NJ). Positive cultures will be identified by the niacin-nitrate test. Mycobacterial DNA will then be extracted from the liquid medium from all positive cultures and be batched processed and analyzed for strain diversity/heterogeneity using next generation whole genome sequencing (WGS) [33]. The results will not be reported to patients or medical teams unless drug resistance genes are identified. WGS results will not be available within the 56-day follow up period and therefore will not impact or alter the STAMP study design.

Definitive mixed strain/complex infection will be defined by the presence of more than one allele at more than one MIRU-VNTR locus and as definite clonal population infections when more than one allele at a single locus will be present. In depth bio-informatics analysis will be done to identify strain diversity within (i.e. sputum compartment) and between anatomical sites (i.e. urinary and sputum compartments). Analysis will also be undertaken to calculate (a) the prevalence of complex infection overall and amongst urinary and sputum compartments at baseline. The results of MTB WGS testing will not be clinically meaningful and therefore will not be reported to patients or medical teams.

Sample Size

Assuming that 20% of 'STAMP" patients will be diagnosed with TB, 20% of the 800 consecutive patients (n=160) to be recruited as part of the STAMP trial (as of from 1 August 2016) will be included in the sample size for further genomic analysis. For an overall sample size of 160 and assuming the prevalence of mixed infections ranges from 10-20%, then the following table shows precision for estimates of prevalence of mixed infections and power for the exploratory objective of comparing culture conversion in the mixed infections and non-mixed infection groups:

Prevalence of mixed infection (number)	95% CI for prevalence	Power assuming 30% culture convert in mixed infections group & 60% culture convert in non-mixed infections group
10% (16)	5.8-15.7%	53%
15% (24)	9.9-21.5%	72%
20% (32)	14.1-27.0%	85%

This sub study will comply with all other aspects of the STAMP trial protocol with regard to the acquisition and storage of personal data and maintenance of patient confidentiality.

8.14. Ultrasonic evidence of extra-pulmonary TB using focused assessment with sonography for HIV/TB (FASH) in patients with HIV-associated TB diagnosed by rapid urine-based tests- a sub-study (Zomba, Malawi site only)

Background information and introduction

There is strong evidence to support that HIV-associated TB diagnosed by rapid urine-based tests is indicative of haematogenously disseminated renal TB.[34] The diagnosis of extra-pulmonary TB (EPTB) remains challenging, especially in poorly resourced settings. However, ultrasonography can be used to aid diagnosis of EPTB through detection of characteristic features such as pleural effusions, pericardial effusions, abdominal lymph node and/or splenic micro abscesses.[35–37] A simple point-of-care ultrasound protocol for focused assessment with sonography for HIV/TB (FASH) has been developed and successfully used

to identify EPTB following training of non-radiologist health care workers in resource-limited settings.[38–41]

Rationale/justification for the research project

It remains unknown what proportion of patients diagnosed with HIV-TB who are admitted to hospital have ultrasonic evidence of EPTB using FASH, and whether this is more common in patients with positive rapid urine-based TB tests (and therefore have disseminated TB). It is also unknown what proportion of HIV-positive hospital in-patients with no evidence of TB have ultrasound signs suggestive of EPTB. Understanding the prevalence of these ultrasound changes can inform the use of FASH for identification of EPTB amongst HIV-positive hospitalised patients. To address these questions we will undertake a sub-study amongst patients enrolled in the STAMP trial to describe the prevalence of ultrasonic evidence of EPTB using FASH.

Objectives of the study

1. To describe the prevalence of ultrasound evidence of EPTB using FASH amongst patients admitted to hospital with HIV-associated TB
2. To compare the prevalence of EPTB using FASH between patients with and without positive urine-based diagnostic tests
3. To describe the prevalence of ultrasound evidence of EPTB using FASH in HIV-positive patients with no microbiological or clinical evidence of TB disease

Study Population

To establish the prevalence of EPTB as described by FASH and sensitivity of FASH (based on microbiologically confirmed TB as the gold standard) we will recruit only those patients enrolled to the STAMP trial with HIV-associated TB who have been commenced on TB treatment (defined as any positive STAMP or non-STAMP related TB investigation including the TB-LAM, Xpert MTB/RIF mycobacterial culture or sputum smear microscopy or the decision to commence TB treatment by a clinician). Following diagnosis of HIV-associated TB, potential participants enrolled in the STAMP trial will be approached for enrolment in this sub-study.

To establish the prevalence of ultrasound evidence of EPTB using FASH in HIV-positive patients with no microbiological or clinical evidence of TB disease we will recruit only those patients enrolled to the STAMP study that have negative microbiological tests for TB (STAMP or non-STAMP related TB investigations), have no clinical evidence of TB infection during the STAMP study follow-up. Potentially eligible patients will be approached for enrolment at their STAMP trial 56-day follow-up visit.

Sample Size

We aim to recruit all eligible patients enrolled in the STAMP trial for the remaining duration of the trial. If TB prevalence is 15-30% amongst hospitalised HIV-positive patients enrolled into the STAMP trial, we would expect to recruit between 105-210 patients in this sub-study. Assuming recruitment of 150 patients to this sub-study, and 50% prevalence of EPTB, we

would be able to report EPTB prevalence with an accuracy of +/-8% based on 95% confidence intervals. Assuming a prevalence of FASH abnormalities of 8%,[36] recruiting 100 patients without HIV-TB would allow us to calculate the prevalence of FASH abnormalities with an accuracy of +/-5.3% based on 95% confidence intervals.

Study Procedures, Data Collection and Analysis

Following informed consent and enrolment in the sub-study, a focused assessment with sonography for HIV/TB (FASH) will be undertaken by a member of the STAMP study team (doctor or clinical officer) trained in FASH for the diagnosis of EPTB.[41] The ultrasound operator will be blinded to the STAMP study arm allocation, therefore will not know if the patient has a positive-urine test for TB. The FASH protocol will take fewer than 15 minutes, and can be done after initiation of TB treatment or those patients with TB disease. Results will be recorded as the presence or absence of pleural effusions, pericardial effusions, abdominal lymphadenopathy and splenic micro abscesses. Data will be recorded onto case report forms and also documented in the patients' routine medical notes. Patients with at least one of: pleural effusions, pericardial effusions, abdominal lymphadenopathy and splenic micro abscesses will be defined as having evidence of EPTB using FASH.

The study procedures during this study will in no way alter the main STAMP trial study intervention.

Analysis will include the proportion of HIV-TB patients (any diagnosis) with ultrasonic evidence of EPTB using FASH and stratification by microbiologically confirmed TB disease and positive urine-based diagnostics. Sensitivity of FASH will also be calculated using microbiologically confirmed TB (defined as any positive STAMP or non-STAMP related TB investigation including TB-LAM, Xpert MTB/RIF mycobacterial culture or sputum smear microscopy) as the reference standard. Prevalence of EPTB using FASH will also be calculated amongst patients with no microbiological or clinical evidence of HIV-TB, and specificity will be calculated using a microbiological and clinical reference standard. This sub-study will comply with all other aspects of the STAMP study protocol with regard to the acquisition and storage of personal data and maintenance of patient confidentiality.

Findings will be presented to the department of medicine at Zomba Central Hospital and Queen Elizabeth Central Hospitals, to the National TB Programme at annual research dissemination meetings, and to the broader scientific community at relevant regional and international conferences. Findings will also be submitted for publication in relevant peer-reviewed journals.

Ethical Considerations

All patients recruited into the STAMP study will have been informed of the sub-study and written informed consent sought using standardised information sheets and consent forms. The FASH protocol will take fewer than 15 minutes, and can be done after initiation of TB treatment, therefore it will not delay initiation of TB treatment or have any other potential risks or side-effects for participants. There will be no radiation exposure to patients or staff from ultrasound imaging. Any patients who have no microbiological or clinical evidence of TB but have signs of EPTB on FASH will be referred to HIV and/or TB clinics for further assessment and management as appropriate.

9. SAFETY REPORTING

The main study-related procedures in this trial are systematic screening of hospitalised PLHIV for TB. In countries with generalised HIV epidemics, systematic symptoms-based screening for TB in PLHIV at each medical contact is internationally recommended standard of care, and part of national policy in both Malawi and South Africa. Because of the limited sensitivity of all available TB screening tools in PLHIV, there is no single recommended screening algorithm.

Potential harms specifically attributable to the intervention arm of the trial, therefore, relate to the use of more intensive urine-based diagnostic tests. Unwarranted initiation of TB treatment as a result of a false-positive screening result is a potential concern (although evidence to date indicates high specificity of this screening approach).

It will not, however, be possible to distinguish true-positive results from false-positive results on an individual basis, owing to the lack of a highly sensitive and specific gold-standard confirmatory test. Moreover, given the high mortality from undiagnosed TB in this patient group, combined with the high frequency with which empirical TB treatment is used, and the established good safety profile of standard tuberculosis treatment, the overall potential for harm to participants in the intervention arm is very low.

Instead, risks will be minimised by:

- Clearly communicating the limitations of all screening approaches and specifically relating to positive-predictive value of rifampicin resistance (Xpert MTB/RIF) to responsible medical teams through on-site training that will be repeated periodically.
- Repeating the Xpert MTB/RIF before reporting any MTB isolate as rifampicin resistant (as per 2014 WHO recommendations)[12]
- Conducting regular proficiency testing and quality assurance (QA) for the trial laboratories.

As standard Serious Adverse Event (SAE) reporting will not be possible or appropriate for the reasons outlined above, the trial team will instead investigate and report periodically to the TSC, DSMB and Ethics Committees all events of:

a) Death on TB treatment within 2 months of enrolment: the circumstances leading to decision by the clinical team to start TB treatment will be documented, including the results of screening tests allowing an excessive number of single-test positives to be identified promptly.
b) Initiation of second-line TB treatment for suspected MDR TB

All deaths (irrespective of cause) and readmissions to hospital will already be recorded and analysed as primary and secondary study outcomes.

9.1. Definitions

Standard definitions of serious adverse events as outlined by the ICH Guidelines for Good Clinical Practice are:

Serious Adverse Event (SAE): a serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening (at risk of death at the time of the event, not an event that hypothetically may have caused death if event was more severe)
- requires inpatient hospitalisation (regardless of length of stay, even if hospitalisation is a precautionary measure for observation, but excluding hospitalisation for pre-existing conditions) or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

9.2. Causality

The relationship of each serious adverse event to the trial intervention will be determined by a medically qualified investigator, including causality, strength of the causal relationship and whether it was expected. Definitions are as follows:

- **Not related**: the adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant; there is no evidence of causal relationship with the trial intervention
- **Possibly related**: there is some evidence to suggest a causal relationship with the trial intervention, for example a plausible temporal relationship, but other factors or therapies may explain the event.
- **Probably related**: there is evidence to suggest a causal relationship, and more likely explained by the trial intervention than other factors or therapies
- **Definitely related**: there is clear evidence to suggest a causal relationship, with no other possible contributing factors or therapies

9.3. Procedures for Recording Serious Adverse Events

Adverse events will be ascertained from:

- patient medical records kept and reviewed during hospital admissions (including readmissions)
- patient follow-up visit at 56 days post-randomisation, or reports from relatives/next-of-kin if patient cannot be contacted at follow-up

The following information will be recorded in the CRF: description, date of onset, severity, assessment of relatedness to trial interventions, the circumstances leading to decision by the clinical team to start TB treatment/second line TB treatment (including the results of screening tests) and action taken. Only serious adverse events as described above and occurring during the study follow-up (56 days post-randomisation) will be recorded. SAEs will be recorded in CRFs and periodically reported to the DSMB and ethics committees.

9.4. Management of Serious Adverse Events

Since all drugs used in this study are licensed drugs being used for licensed indications and being prescribed by the responsible clinical team, management of SAEs will be undertaken by the clinical team as per national and local guidelines and practice. As soon as the study team are aware of SAEs, as much information as possible will be documented in the CRF as per the relevant SOP.

9.5. Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will be responsible for independently reviewing study conduct, progress and interim findings, and making recommendations to the Trial Steering Committee (TSC), including any concerns that may warrant early termination of the trial. The DSMB will consist of at least 3 members, including an independent statistician and at least 2 clinicians, with at least 1 having clinical trials experience, and at least 1 having clinical expertise in the management of HIV and TB co-infection. The DSMB will receive 6-monthly reports, including changes to protocols, study progress, enrolment statistics, SAEs and withdrawals. The DSMB will meet via teleconference following this report, and will provide written feedback to the PI/TSC. An interim analysis is planned and will be agreed upon with the DSMB before trial starts.

10. ECONOMIC ANALYSIS

Demonstrating the economic feasibility and cost-effectiveness of this rapid urine-based TB screening strategy, compared to the current standard of care, will be essential before policy implementation should it prove effective in reducing mortality or improving clinical outcomes. As this intervention is focussed at a hospital level, the economic evaluation will take a health service provider perspective. The comparatively small personal costs to patients and broader societal costs are highly unlikely to impact on the feasibility and cost-effectiveness, therefore will not be included in this analysis. All costs of TB investigations, TB treatment and associated toxicities, other investigations and treatment and HIV care and treatment during the study period will be estimated.

Considering the complexities of diagnosis and treatment of HIV-associated TB for patients requiring hospital admission, we will estimate health service costs by combining the number of diagnostic tests, interventions and treatments with estimated unit-costs for each of the tests, procedures or interventions. Costs of hospital admission are likely to account for a large proportion of health service provider expenditure, and this will be calculated based on length of stay, and estimated unit-costs per day of hospitalisation.

Once healthcare resource utilisation has been calculated, this will be combined with health service costs, which will be calculated using a predominately top-down approach. Resource utilization will be multiplied by the country-specific unit costs that including professional time, supplies, non-ART medications, as well as capital costs. We will derive ART and TB drug costs from the WHO Global Price Reporting Mechanism database. Quality of life measures will be recorded at enrolment and follow-up visits.

In addition to conducting cost-effectiveness analyses, we will determine economic feasibility by estimating the 2-year and 5-year budgetary impact of implementation and scale-up of this intervention from a Ministry of Health perspective. Costing approaches will be the same for both study sites, however cost-effectiveness and budgetary impact will be calculated independently for South Africa (a middle-income country) and Malawi (a lower income country); professional costs in this countries will be different whereas drug costs will have less variation. Longer term health and cost-effectiveness will be estimated based on the Cost-effectiveness of Prevention of AIDS Complications International (CEPAC-I) computer simulation model.

For the purpose of this economic analysis, the following data will be collected during the trial.

For all patients:

- length of hospital stay and subsequent health service use after discharge
- number of HIV diagnostic tests required for enrolment
- number of TB diagnostic tests and prescription of TB medications and resulting toxicities
- details of HIV care, such as ART and CD4 cell count.

For a subsample of the first 100 patients recruited at each trial site:

- details of antibiotic and other prescription medicines during in-patient stay and at discharge
- details of other (non-TB) diagnostic tests and procedures undertaken

Data will then be analysed, and if necessary repeated in an additional sample of patients to obtain sufficient precision around the cost estimates.

For each trial site

- The costs associated with each day of hospital admission
- The costs associated with both TB and non-TB tests, procedures and treatments

11. STATISTICS

11.1. Sample size justification

Mortality risk: Recent unpublished data from the trial sites show mortality of HIV-infected medical in-patients during the hospital stay is 23% in Zomba and 21% in Edendale. We assume that the 56-day all-cause mortality (accruing between the date of admission and 56 days follow-up) will lie in the range 25%-30% in the control arm. Despite using limited sampling techniques, recent post-mortem studies from South Africa (including at one of the trial sites), reported finding active TB in 50%-67% of medical HIV-infected in-patients who died [3,4]. Disease burdens are less well characterised in Malawi, but TB has consistently been identified as the leading cause of HIV related death throughout Africa accounting for 32%-50% of deaths outside South Africa [3–7]. Thus, we estimate that between the two sites, approximately 40%-50% of total deaths in the control arm will be TB-related.

Intervention: The addition of urine-based TB screening to the standard of care diagnostic screening increases the early diagnostic yield 3.0-fold (see Section 4.2) and sensitivity is highest among those with the lowest CD4 counts and poorest prognosis [42]. In background studies in South Africa, retrospective urine based testing was positive in all HIV-related TB deaths among both in-patients and out-patients [9,42]. Thus, we assume that all patients in the intervention arm at risk of TB-related death beyond the first day of admission will have rapid TB diagnosis and treatment initiation. However, it remains unknown what proportions of these deaths are preventable. In addition to the major effect of reducing TB-related mortality by increasing the yield and speed of TB diagnosis, early TB treatment might also directly increase survival via antimicrobial activity against gram-positive sepsis and indirectly by accelerating medical management and progression to start of ART.

Effect size: Holtz et al.[43] reported on a study in South African hospitals with a before-after evaluation of implementation of the WHO algorithm for suspected smear-negative TB [44]. This complex intervention involved early initiation of empirical TB treatment among HIV-infected in-patients in whom TB was suspected but unconfirmed. The observed reduction in mortality at 8 weeks was 47% (from 32% to 17%) and this was strongly associated with increased use of TB treatment [43]. The current trial is powered to detect a 20%-25% reduction in all-cause mortality. Assuming that half of the deaths are TB-related as shown by post-mortem studies, this equates to a 40%-50% reduction in TB-related mortality among those patients who do indeed have TB (consistent with the 47% mortality reduction observed by Holtz et al.).

11.2. The Number of Participants

The sample size calculation is based on the primary endpoint of mortality risk by 56 days from randomisation. Assuming an all-cause mortality of 25% in the control arm after 56 days of follow-up and a 2-sided type I error of 5%, inclusion of 1,300 patients per arm would provide 90% power to detect a 25% reduction in mortality and 80% power to detect a 20% reduction, allowing for loss to follow-up (LTFU) of 10%-15% by 56 days (see table 11.1). If the 56 day mortality risk in the control arm were unexpectedly lower (eg 20%), then this sample size would still be sufficient to provide 80% power to detect a 25% reduction in all-

cause mortality with 15% LTFU. The study will have greater power for the secondary endpoint of time to death, measured up to 56 days from randomisation.

All-Cause Mortality: Control Arm	All-Cause Mortality Reduction	All-Cause Mortality: Intervention Arm	Number of patients per arm			
			Power 80% 10% LTFU	Power 80% 15% LTFU	Power 90% 10% LTFU	Power 90% 15% LTFU
20%	20%	16.0%	1663	1761	2208	2338
20%	25%	15.0%	**1050**	**1112**	1391	1473
20%	30%	14.0%	**719**	**761**	**950**	**1006**
25%	20%	20.0%	**1260**	**1334**	1671	1769
25%	25%	18.75%	**798**	**845**	**1056**	**1118**
25%	30%	17.5%	**548**	**580**	**722**	**765**
30%	20%	24.0%	**991**	**1049**	**1313**	**1391**
30%	25%	22.5%	**629**	**666**	**831**	**880**
30%	30%	21.0%	**432**	**458**	**570**	**604**

Table 11.1. Sample-size calculations

11.3. Data analysis plan

A detailed statistical analysis plan will be written and approved by the DSMB prior to completion of enrolment. Analyses will be carried out under intention-to-treat practices and will be adjusted for site as a design factor. The primary outcome risk of all-cause mortality from randomisation to follow-up at 56 days will be analysed as a risk difference and odds ratio. Imbalances in baseline covariates will not be tested statistically but if there appear to be any large imbalances then additional adjustment will be made for these in the analysis.

The secondary outcome time from randomisation to all-cause mortality (follow-up to 56 days) will be compared between treatment arms using survival analysis with Kaplan-Meier curves and the Cox proportional hazards model where the intervention effects are summarised by rate ratio. Additionally the following secondary outcomes will be compared between intervention groups using risk differences and odds ratios: the proportion of patients with a diagnosis of TB, the proportion of patients receiving antibiotic treatment, the proportion of patients starting antiretroviral therapy (ART) among those not on ART at enrolment. The time from randomisation to the following outcomes will be compared between intervention groups using Cox regression: TB diagnosis, start of TB treatment, start of ART treatment for those not on ART at enrolment.

Treatment decisions and outcomes will also be analysed by calendar period to assess for any changes in clinical practice over time. Differences in treatment effects by site will be investigated by considering interactions between randomisation group and site. The study is not powered for this so it would be an exploratory analysis.

12. DATA MANAGEMENT

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical charts, laboratory and pharmacy records, diaries, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12.3. Data Recording and Record Keeping

Case report forms (CRFs) will be completed in separate sections for each patient as follows, with data collected directly from the patient unless stated otherwise:

- Eligibility and screening
- Locator information
- Baseline demographic and clinical information (collected from patients where possible, supplemented by information from hospital records)
- Specimen collection
- In-patient hospital follow-up (data collected from hospital records and/or clinical team)
- Hospital discharge or in-patient death (data collected from hospital records and/or clinical team)
- Out-patient follow-up, loss-to-follow-up or outpatient death (data from next-of-kin, relative or official register).

The completeness and accuracy of each form will be checked by a second member of the study team and then countersigned following any amendments. CRFs will be completed as TeleForms (TeleForms Inc.) to allow direct entry of scanned data into a SQL Server Express database pre-programmed with logical data consistency checks. Once the TeleForm data have been scanned and imported into the database, validation will be performed through a visual inspection, with potential problematic values flagged for verification. Further validation will be performed to check for duplicates, validation, range checks and consistency. This will be overseen by the Data Coordinator at each site who will send encrypted data-files via secure internet connection to the study data-hub at the Malawi-Liverpool-Wellcome Trust (MLW) centre in Blantyre, Malawi.

Here the data from the two study sites will be merged into a single master database. The data manager at MLW will monitor data-completeness and quality and generate lists of data queries on a weekly basis which will be issued to each study team for review, clarification and feedback. At each study site, hard copies and electronic copies of all CRFs will be stored together with copies of all completed data query forms. All electronic databases will have daily scheduled backups, and a password will be required to gain access to data. The password-protected databases at the trial sites will be sent to LSHTM by Secure File Transfer Protocol (SFTP) on a weekly basis. Only trained staff will be granted access on a need-to-see basis.

Data will be stored using a unique study identifier allocated to each patient at enrolment into the study, name and any other identifying detail will NOT be included in any trial data electronic file. The code to anonymisation will be kept in a secure locked filing cabinet in a locked room, and access to this will be restricted to the PI and lead investigator at each site. All staff will be trained with respect to data management issues.

13. MONITORING AND QUALITY ASSURANCE

The trial will be conducted in accordance with the current approved protocol. The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki, with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996, MRC Guidelines for GCP and relevant regulations and standard operating procedures (SOPs).

Prospective verification of data collected on case report forms will be done by local trial coordinators and data mangers. Furthermore, all data collected will be subject to random sampling for further verification of accuracy in relation to source documents, in addition to data quality checks inbuilt to the data recording systems. Any problems with data quality will be reported to the TSC and appropriate action taken, including increasing frequency of checks. Laboratory testing at both study sites will undergo periodic quality assurance procedures including proficiency testing of study diagnostics, for example testing of known smear-positive sputum samples and culture-negative sputum samples using Xpert MTB/RIF assay. All data monitoring and quality assurance processes will be outlined in detail in a separate SOP, and will be compliant with ICH GCP and MRC guidance.

Regular monitoring will be performed according to ICH GCP. The London School of Hygiene & Tropical Medicine (LSHTM) will act as the main sponsor for the study and the study may be subject to audit by LSHTM under their remit as sponsor, or assessment by the regulatory authorities, to ensure compliance with protocols, GCP and applicable regulatory requirements.

13.1. Trial Steering Committee

The trial steering committee (TSC) will oversee the trial, monitor progress, advise the PI and investigator team, receive reports from the DSMB and report to the trial funders and sponsor. The TSC will include (as of March 2015):

- Independent Chairman: Prof Anthony Harries (International Union Against TB & Lung Disease)
- Independent members: Prof Andrew Ramsay (WHO / TDR) and Prof Frank Cobelens (University of Amsterdam, The Netherlands).
- Investigators: Dr. Stephen D. Lawn (International PI); Dr. Douglas Wilson (Local PI at Edendale site); Dr. Joep van Oosterhout (Local PI at Zomba site).
- Funder's representative

13.2. Trial Investigator Team

This investigational team draws together an extremely strong team of local and international partners. This includes a wealth of clinical expertise in diagnosis and management of HIV-associated TB; research expertise in clinical evaluation of TB diagnostics; the design and/or conduct of large randomised controlled trials; expertise in laboratory TB diagnostics; data management and analysis and strong relationships with local, regional and national partners such as National TB Programmes, National AIDS Commission, Ministries of Health and

National Health Laboratory Service. The structure of the investigator and operations study teams are outlined in appendix F, the investigator team and main responsibilities are as follows:

- Dr. Stephen D. Lawn: Chief Investigator. Will oversee protocol development and all aspects of the international coordination and conduct of the trial, governance and data dissemination in close collaboration with Dr Ankur Gupta-Wright (International Study Coordinator).
- Dr. Katherine Fielding (based at LSHTM) will be responsible for data management systems, data quality procedures and conduct the statistical analyses.
- Prof Elizabeth Corbett (based at MLW, Malawi) will oversee for the central data-hub serving both of the study sites and provide technical support and QA of laboratories to the Zomba field-site.
- Dr. Doug Wilson: Local PI (Edendale Hospital, South Africa) will take overall responsibility for the conduct of the study at this site.
- Dr. Joep van Oosterhout (Dignitas International, Zomba Central Hospital, Malawi) will give input to protocol development and will take overall responsibility for the conduct of the study at this site.
- Prof Mark Nicol Will design and coordinate the laboratory-based R&D component of the work
  at the University of Cape Town and provide technical advisory support regarding the laboratory diagnostics.
- Prof Rochelle Walensky and Prof Ken Freedberg will plan and oversee all the economic analyses (cost-effectiveness and budgetary impact analyses).

13.3. Ethical Considerations and Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to Research Ethics Committees (REC) of the London School of Hygiene & Tropical Medicine, University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) and the College Of Medicine Research and Ethics Committee (COMREC) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Reporting: The investigators shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, host organisations and Sponsor. In addition, an End of Trial notification and final report will be submitted to the REC, host organisation and Sponsor.

Participant Confidentiality: The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a unique study identifier on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

Expenses and Benefits: Participants will receive a small nutritional food parcel during their in-patient stay in acknowledgement of the fact that some patients lack sufficient nutritional

input as in-patients and also as a good will gesture. Costs for time and travel expenses incurred when attending the 56 day post-randomisation follow-up visit will be reimbursed as appropriate.

Risks to patients: Risk of harm to patients in this trial is low. The study requirement for samples of blood (for CD4count measurement), sputum and urine to be obtained from patients is not associated with risk to patient safety. We anticipate that patients in the intervention arm will benefit from urine-based screening due to increased diagnostic yield and acceleration of TB diagnosis. However, it is possible that such screening may inadvertently be harmful in the following ways:

- A very small proportion of screening tests may yield false-positive results, leading to inappropriate treatment for TB (background studies report specificity >98% for urine diagnostic tests).
- Rapid TB diagnosis may reduce the likelihood that patients receive an empirical course of simple antibiotics as part of the diagnostic work-up and therefore concurrent sepsis, if present, may not be treated
- Rapid TB diagnosis may result in other concurrent pathologies being overlooked
- Use of Xpert MTB/RIF may be associated with false-positive rifampicin resistance results in a very small proportion of patients, leading to inappropriate initiation of treatment for MDR-TB

14. FINANCE AND INSURANCE

Funding: The trial is funded by Joint Global Health Trials (a jointly funded initiative by Medical Research Council, the UK Department for International Development and the Wellcome Trust).

Insurance: The London School of Hygiene and Tropical Medicine (LSHTM) will act as the main sponsor for the study and holds public liability and clinical trial insurance policies which apply to this study which would operate in the event of any participant suffering harm as a result of their involvement in the research.

15. PUBLICATION POLICY

Academic dissemination: Data will be disseminated to local institutions as well as academic bodies within South Africa and Malawi with which the members of the investigational team already have links. Data will also be rapidly made available through presentations at relevant leading international conferences and regional conferences in southern Africa Primary data will be published in a timely manner in high impact peer reviewed journals. Findings will be summarised and made readily accessible on the institutional web-sites of the PI (LSHTM) and co-investigators.

Engagement with Policy Makers: Policy-makers both nationally and internationally will be presented the findings of this study. These will include WHO, the STOP TB Partnership, the WHO Strategic and Technical Advisory Group for TB (STAG-TB), the WHO Strategic and Technical Advisory Group for HIV, the WHO TB/HIV taskforce and the WHO TB/HIV Working Group; the International Union Against TB and Lung Disease (IUATLD); National Ministries of Health of Malawi and South Africa, including their National TB Programmes and National AIDS Commissions / Councils. Malawi has established an innovative Knowledge Translation Platform (KTP Malawi) within the Malawi Ministry of Health (MoH) and with support from Dignitas International. KTP Malawi engages national-level policymakers, researchers and implementers in a coordinated approach to enhance the generation and utilization of the findings from health-sector research in Malawi. KTP Malawi has received official endorsement from the Malawi Ministry of Health's Senior Management Team. The trial would utilize this novel knowledge translation forum along with the more traditional methods of engaging with national level policymakers such as technical working groups to maximize the dissemination and policy impact of the trial.

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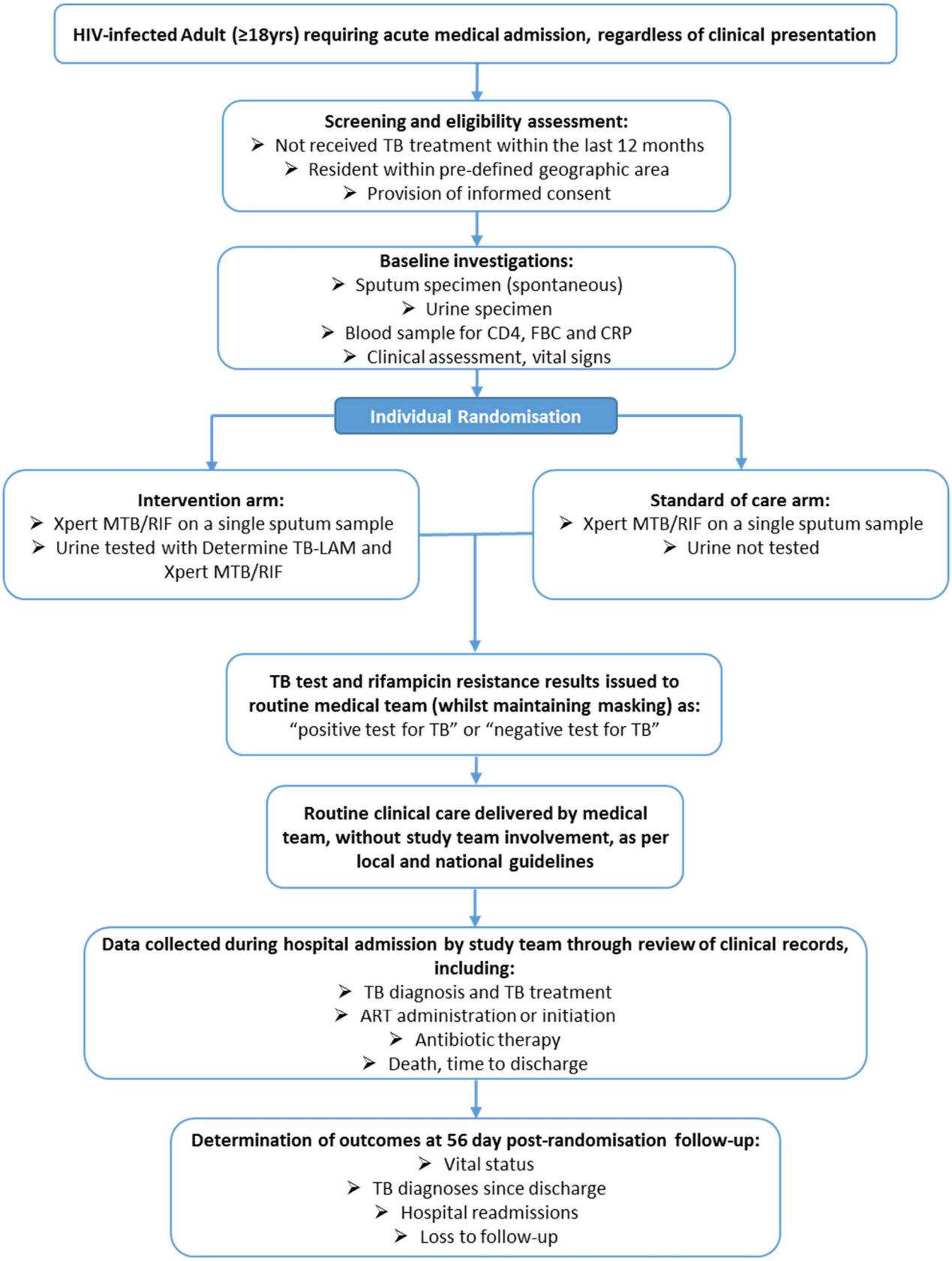
17. APPENDIX A: TRIAL FLOW CHART



18. APPENDIX B: PATIENT INFORMATION LEAFLET AND CONSENT FORM

INFORMATION ABOUT THE RESEARCH

What you should know about this research study:

- This information sheet is so that you can read about the purpose, risks and benefits of this research study.
- Taking part is voluntary, and you have the right to refuse.
- If you agree to take part now, you can change your mind at any time in the future.
- Please read this information sheet carefully, and ask any questions before you make a decision.

What is the study for?

HIV is the virus that causes AIDS, and we know HIV increases the chances of you developing tuberculosis (TB). TB can be very difficult to diagnose in people living with HIV. We are doing a research study to see if using a new test for TB (done on a urine sample) on all people admitted to hospital who are living with HIV can help us diagnose and treat TB more quickly and help improve patients' health. A study has shown one urine TB test (called TB-LAM) improved health when it was used in patients with HIV admitted to hospital and who were suspected of having TB.

The research is taking place in South Africa and Malawi. The research team is from Malawi-Liverpool-Wellcome Trust Research Centre in Blantyre, Dignitas International NGO in Zomba, Malawi, Edendale Hospital in KwaZulu-Natal, South Africa and London School of Hygiene & Tropical Medicine.

Why have I been asked to take part?

You have been asked to take part because you need admission to hospital, and are HIV-positive. If you do take part, you will be one of 2,600 people in this study.

Who can't take part?

If you are less than 18 years old, if you have taken TB treatment in the last year or you live outside the study area you will not be able to take part.

What do I have to do if I take part?

If you do take part, you will be asked to provide a urine sample, a sample of sputum (if possible), and about 3-4 teaspoons (15-20mls) of blood. It will be decided by chance (like

tossing a coin) whether your urine is tested for TB or not. Half of the patients will have urine tests done and half will not.

Sputum and urine test results will be given to the doctors and nurses looking after you. They will decide whether to treat you for TB or not, and will provide all your medical care. We will need to see you again in 2 months to find out about your health.

Part of the urine and blood samples you give will be tested immediately, with the rest stored for a period of up to 5 years in a secure laboratory storage facility to be tested later as part of this study. After this time they will be destroyed.

What are the benefits?

If you do have TB, then by participating in this research you may be able to start treatment more quickly, but we cannot guarantee that you will receive any benefits from this study. Taking part will not cost you anything and all the tests will be done free of charge. There is no payment for you to take part in the study but we will provide a small, healthy food parcel for you and we will refund your costs for attending the follow-up appointment in 2 months' time.

What are the risks?

The blood test may cause discomfort or a small bruise. The study is unlikely to lead to any harm. Occasionally laboratory tests for TB can give a wrong positive results (less than 1 in 100 tests). This could lead you to starting TB treatment that is not needed, or a combination of drugs that is not the ideal choice for you.

What information will be collected, and will it be kept private?

Details about your health, your home situation and your hospital stay will be written down.

All information collected is kept private and the records will not include your name. Only approved study staff or people regulating the study will have access to your information, which will be stored electronically. The study results may be published in a medical journal so that others can learn from them, but your personal information will not be included and there is no way you can be identified from it. The study data may be made available to other researchers so that it can be used to improve care, but your personal information will not be included and there is no way you can be identified form it.

Do I have to take part?

You do not have to take part, it is up to you to decide. You can change your mind about taking part at any time, you just need to let your doctor know. If you don't want to take part, or decide

to pull out of the study, your doctors and nurses will still care for you and give you the best treatment they can.

Who has approved the study?

The organisations conducting the studies and at least 2 independent groups of people looking after your interests (Research Ethics Committees) have checked the study and agreed it is safe for you to participate.

Who to contact with questions or problems?

You can talk to your study nurse or doctor about the study. You can also speak to _____ who is in charge of the study at your hospital. You are encouraged to ask any questions you wish before, during or after the study. You may also use the following telephone numbers if you still need more information.

Research Nurse _____

Tel no _____

Study doctor _____

Tel no _____

What if something goes wrong?

The London School of Hygiene and Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that the School is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of the study then you should immediately inform the local Investigator, _____.

CONSENT FORM FOR PATIENT/REPRESENTATIVE

Local PI and contact details:

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE, HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND HAVE DECIDED TO PARTICIPATE.

- I have read and understood the participant information sheet [or have understood the verbal explanation] and had the chance to consider the information, ask questions and have had these answered
- I understand that it is my choice to take part in this study, and that I am free to pull out at any time, without giving reason, and without my medical care or rights being affected
- I understand my medical notes may be looked at by responsible individuals involved in the study, from the London School of Hygiene & Tropical Medicine or from regulatory authorities. I give permission for this.
- I give my permission for data collected about me in this study to be used for future research, subject to further ethical approval (with all my personal information removed)
- I agree to participate in the STAMP trial, and for data and samples to be stored and tested later as part of this study.

Participant Consent:

Name of Participant (Print) _____

Signature (or thumbprint/mark) of Participant _____

Date_____

Name of Person Obtaining Consent (Print) _____

Signature of Person Obtaining Consent _____

Date_____

If the participant is unable to sign.

As an impartial witness, I confirm that all the information about the study was given and the participant consented to taking part.

Impartial Witness Name: _____

Impartial Witness Signature: _____

Date_____

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP

19. APPENDIX C: SCHEDULE OF PROCEDURES

Procedures	Screening	Enrollment	During hospital admission[a]	56-day follow-up
Confirm HIV-status and age	✓			
Confirm no history of TB treatment in preceding 12 months	✓			
Informed consent		✓		
Demographic information		✓		
Clinical history		✓		
TB symptoms		✓		
Vital signs		✓		
Height, weight and MUAC		✓		
Quality of Life questionnaire		✓		
Karnofsky score		✓		
Blood sample 15mls (FBC, CD4, CRP)[b]		✓		
Sputum sample[c]		✓		
Urine sample (>50mls)[d]		✓		
Issue TB results to responsible medical team[e]			✓	
Clinical record review			✓	
Follow-up visit questionnaire				✓

[a] Clinical record review will occur during inpatient admission on at least days 1,2,3,4,5,7, 10, 14, and every 4 days thereafter

[b] Sample also to be stored, total volume of blood approx. 15mls

[c] Spontaneously expectorated only

[d] tested with (i) Determine TB-LAM and (ii) Xpert MTB/RIF assays (in the intervention arm only)

[e] Within 24 hours of randomiastion when possible

20. APPENDIX D: CLASSIFICATION OF ADVERSE EVENTS

Below are the relevant sections of the Division of AIDS table for grading the severity of adverse events (published December 2004, clarification August 2009) ULN = Upper Limit of Normal ; LLN = Lower Limit of Normal. Only Grade 3 or 4 will be reported for this study.

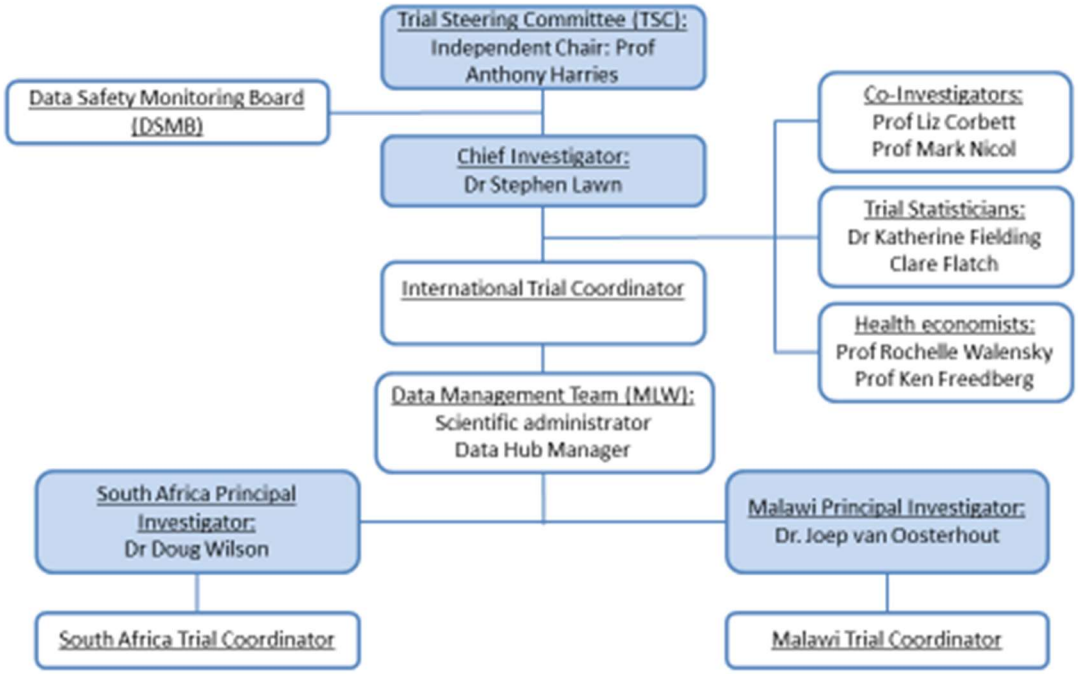
Definitions:
Basic Self-care Functions: activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.
Usual Social & Functional Activities: adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
SYSTEMIC				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)
SKIN – DERMATOLOGICAL				
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)

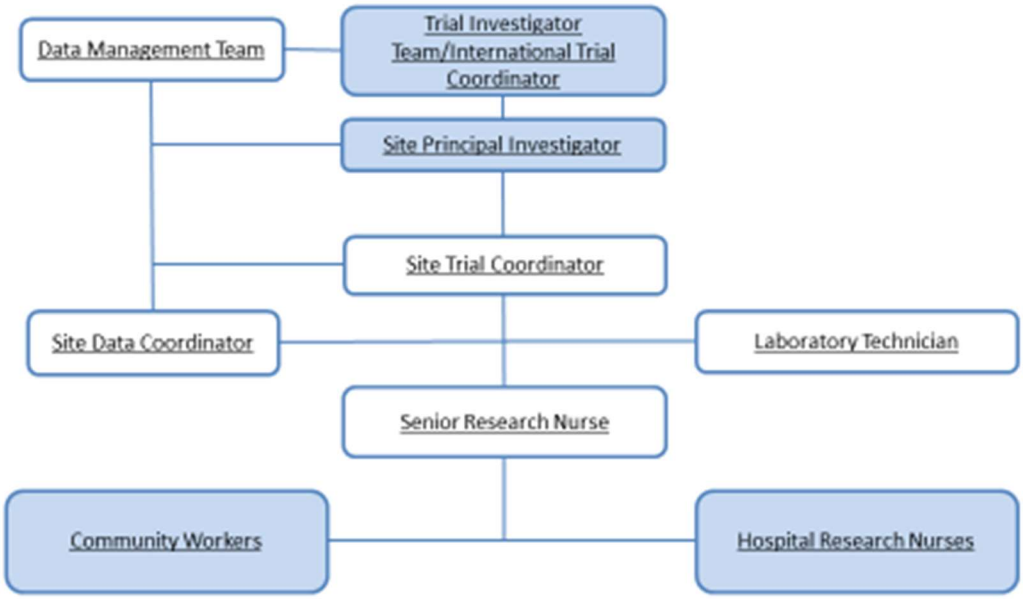
CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GASTROINTESTINAL				
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
NEUROLOGIC				
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
OCULAR/VISUAL				
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
Laboratory testing				
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bilirubin (Total)	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Creatinine	1.1 – 1.3 x ULN†	1.4 – 1.8 x ULN†	1.9 – 3.4 x ULN†	≥ 3.5 x ULN†
Other clinical event not specified above				
Clinical adverse event NOT identified elsewhere in this DAIDS AE grading table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

21. APPENDIX E: STRUCTURE OF THE STUDY TEAM

STAMP organisation flow chart: senior management and governance structures



STAMP organisation flow chart: trial operations team (at each site)



22. APPENDIX F: DIAGNOSTIC ACCURACY OF RAPID TESTS

Summary of diagnostic accuracy for Xpert MTB/RIF assay (pulmonary specimens, extrapulmonary and urine) and Determine TB LAM.

Source	Assay	Description/population tested	Sensitivity (95% CI)	Specificity (95% CI)	Reference standard/notes
Steingart et al 2014 [45]	Xpert MTB/RIF	Pooled estimates from systematic review for pulmonary TB Pooled estimates from systematic review for pulmonary TB in **HIV-positive patients**	median pooled 0.89 (0.85-0.92) median pooled 0.97 (0.70-0.86)	median pooled 0.99 (0.98-0.99) median pooled 0.98 (0.96-0.99)	solid or liquid culture of sputum
Denkinger et al 2014 [10]	Xpert MTB/RIF	Pooled estimates from systematic review for extrapulmonary TB	Lymph node 0.81 (0.72-0.88) Pleural fluid 0.21 (0.09–30.4) Cerebrospinal fluid 0.63 (0.48–0.76)	Lymph node 0.99 (0.95-1.00) Pleural fluid 1.00 (0.99-1.00) Cerebrospinal fluid 0.99 (0.96–1.00)	composite reference standard
Maynard-Smith et al 2015 [11]	Xpert MTB/RIF	Pooled estimates from systematic review for extrapulmonary TB	median pooled 0.83 (IQR 0.68–0.94)	median pooled 0.98 (IQR 0.89–1.00)	solid or liquid culture
Minion et al 2011 [20]	Determine TB LAM	Pooled estimates from systematic review in **HIV-positive patients**	pooled 0.56 (0.40-0.71)	pooled 0.95 (0.77-0.99)	solid or liquid culture
Lawn et al 2012 [17]	Determine TB LAM Xpert MTB/RIF	Active screening of **HIV-positive out-patients** using **urine**	0.28 (0.19-0.39) 0.19 (0.11-0.29)	0.99 (0.97-1.00) 1.00	liquid culture, using grade-2 cut-off
Peter et al 2012 [18]	Determine TB LAM	Active screening of **HIV-positive in-patients** who were TB suspects	0.45 (0.39–0.53)	0.96 (0.89–1.00)	composite reference standard, using grade-2 cut-off
Peter et al 2012 [15]	Xpert MTB/RIF	Active screening of **HIV-positive in-patients** who were TB suspects using **urine**	0.48 (0.40-0.57)	0.98 (0.95-1.00)	liquid culture
Lawn et al 2014 [9]	Determine TB LAM Xpert MTB/RIF	Active screening of **HIV-positive in-patients** Active screening of **HIV-positive in-patients** using **concentrated urine**	0.35 (0.26-0.44) 0.56 (0.47-0.66)	0.99 (0.97-1.00) -	liquid culture or Xpert positive

23. APPENDIX G: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V1.1	29/07/2015	Ankur Gupta-Wright	1.Ethics reference numbers and trial registration updated 2. Section 8.3 informed consent minor edit of wording in response to BREC request 3. Calcification that immunology sub-study will only take place at Malawi site (section 8.8) in response to BREC request Version approved by BREC on 09/09/2015
2	V1.2	19/11/2015	Ankur Gupta-Wright	1. Addition of section 8.12, details of immunology sub-study for Zomba site
3	V1.3	05/02/2015	Ankur Gupta-Wright	1. Addition of information in the background about the LAMRCT results 2. Change to patient information sheet in light of results of LAMRCT
4	V1.4	27/06/2016	Ankur Gupta-Wright	1. Addition of Dr Katherine Fielding as co-Chief Investigator 2. Additional of six month follow-up for vital status at Edendale site (section 8.9) 3. Addition of section 8.13, details of the strain diversity sub-study for the Edendale site
5	V1.5	14/07/2016	Ankur Gupta-Wright	1. Addition of section 8.14, details of the FASH ultrasound sub-study for the Zomba, Malawi Site

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee.