Testing NEW STRATegies for patients hospitalized with HIV-associated disseminated TB (NewStrat-TB) trial

STATISTICAL ANALYSIS PLAN:

Plan for analysis of the comparison between the prednisone versus placebo arms

Version: 30 December 2025

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Testing NEW STRATegies for patients hospitalized with HIV-associated disseminated TB (NewStrat-TB) trial

Objectives:

The main objectives of the trial are to assess safety and efficacy of both intensified TB treatment (high dose rifampicin [35 mg/kg/day] plus levofloxacin) and immunomodulation with prednisone with the objective of reducing mortality in patients hospitalised with disseminated HIV-associated tuberculosis (HIV-TB) compared to standard anti-tuberculosis therapy. We plan to include 732 adults hospitalized with a new diagnosis of disseminated HIV-associated TB at Mitchells Plain, Khayelitsha, and New Somerset Hospitals in Cape Town, South Africa.

Trial Design:

Phase III, 2x2 factorial, randomised controlled superiority trial of high dose rifampicin plus levofloxacin versus rifampicin at 10mg/kg as part of standard antituberculosis therapy and prednisone versus placebo to assess safety and efficacy with the objective of reducing early mortality in patients hospitalized with disseminated HIV-associated TB. At enrolment participants will be randomized to two interventions:

1. First intervention: TB treatment (open label):

   a) Experimental arm: Standard first line anti-tuberculosis therapy plus additional rifampicin to reach 35 mg/kg/day for 14 days plus levofloxacin 750 mg/day for weight <46kg and 1g/day for weight ≥46kg for 14 days (*Intensified TB treatment*).

   b) Control arm: Standard TB therapy containing rifampicin 10 mg/kg for 14 days (*Standard TB treatment*).

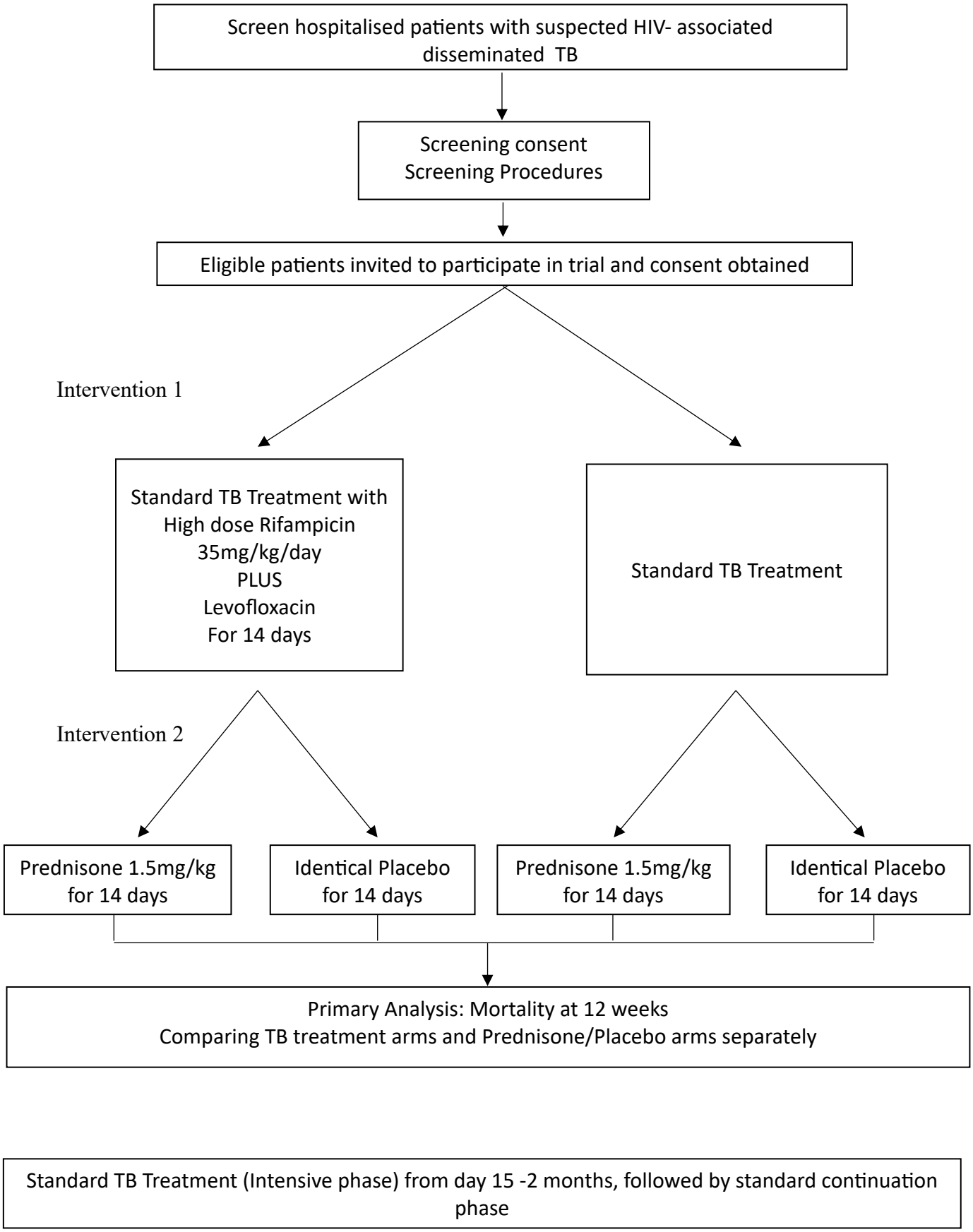
   After 14 days both study arms will continue with standard TB therapy with rifampicin at 10mg/kg to complete 2 months of intensive phase in total. This will be followed by standard continuation phase TB therapy.
2. Second intervention: Prednisone or placebo (double blind):
   a) Experimental arm: Prednisone 1.5 mg/kg per day for 14 days
   b) Control arm: Identical placebo for 14 days

This will yield four treatment arms and two primary analyses.

All randomised participants will be followed up until death or 24 weeks post-randomisation (whichever occurs sooner). Follow-up will include scheduled study visits (in-person and/or

telephonic); study staff will also use routine health care systems and databases to determine treatment progress and vital status if a participant is not contactable.

Overall trial schema:



Trial interventions:

	Intervention 1	**Intervention 2**
Experimental arm	Standard weight-based TB therapy with fixed combination tablets RHEZ (rifampicin, isoniazid, ethambutol, pyrazinamide) plus additional rifampicin to reach 35mg/kg/day for 14 days and levofloxacin 750mg/day for weight <46kg and 1g/day for weight ≥46kg for 14 days.	Prednisone 1.5mg/kg for 14 days
Control arm	Standard weight-based TB therapy with fixed combination tablets RHEZ, with rifampicin at 10mg/kg.	Identical placebo for 14 days

After 14 days all study arms will continue with standard weight-based TB therapy with rifampicin at 10mg/kg to complete 2 months intensive phase TB therapy and then switch to continuation phase RH for an additional 4 months.

Study population:

HIV-positive adults admitted to hospital with untreated, disseminated HIV-associated TB, diagnosed with positive urine lipoarabinomannan (Alere LAM), positive urine Xpert Ultra or positive blood Xpert Ultra test.

Primary and secondary outcomes:

Primary outcome:

1) All-cause mortality at 12 weeks

Secondary outcomes:

1) All-cause mortality at 2 weeks
2) All-cause mortality at 24 weeks
3) Change in venous lactate over 14 days of study medication administration
4) Change in C-reactive protein over 14 days of study medication administration
5) Change in hemoglobin over 14 days of study medication administration

The safety and tolerability of both interventions will also be assessed.

Scope of this Statistical Analysis Plan:

This analysis plan (SAP), based on that described in the protocol, has been developed by the investigators for analysis of the unblinded data for the prednisone versus placebo arms (intervention 2). Randomization to the first factorial intervention (intensive versus standard TB treatment) was prematurely discontinued following a DSMB recommendation due to evidence of harm, while randomization to prednisone versus placebo continued until the target sample size was reached. The SAP for the TB treatment intervention has been presented previously; this document therefore focuses on the analysis of the second intervention and any interaction between the two interventions. This analysis plan has been developed prior to investigators having access to the unblinded data.

This analysis will be conducted after the last participant randomized reaches the 12-week endpoint. The analysis will be conducted once data up to the 12-week timepoint for these participants is available for analysis, having been entered on the database and after the data is cleaned.

General analysis considerations:

This statistical analysis plan is an update to the plan outlined in the study protocol. Results will be reported following CONSORT guidelines. Baseline characteristics and study outcomes will be described by treatment arm. P-values < 0.05 will be regarded as statistically significant. There will be no correction for multiple comparisons.

Analysis populations:

Modified intention to treat (mITT) population: All participants who were randomized and received at least one dose of study drug, prednisone or placebo, including those meeting an exclusion criterion after enrolment but having taken study drug. Participants who were randomized, but excluded before receiving prednisone/placebo will be excluded from the mITT population. Reasons for these exclusions will be presented (eg. exclusion criterion found between randomization and first dose of study drug). The mITT population will be used for both efficacy and safety analyses.

Per protocol (PP) population: mITT population but excluding participants who stopped protocol defined treatment within the 14 day intervention period for >= 7 days for any reason (except in cases where reason for stopping was death or drug intolerance). Participants in whom a diagnosis of rifampicin-resistant TB (specimen taken +/- 30 days from enrolment date) or an alternative diagnosis of non-tuberculous mycobacterial infection (specimen taken +/- 30 days from enrolment date) was made after enrolment will be excluded from per protocol analysis, regardless of whether they completed study intervention or not.

Modified intention to treat interaction (mITT-int) population: All participants who were randomized to both factorial interventions and received at least one dose of study drug from each intervention (ie, intensified TB or standard TB treatment and prednisone or placebo), and who were enrolled prior to the premature discontinuation of randomization intervention 1 on 6 June 2024 (ie, participants randomized to both interventions in parallel).

Definitions:

Time points for survival analyses:

Week 2 defined as 14 days

Week 12 defined as 84 days

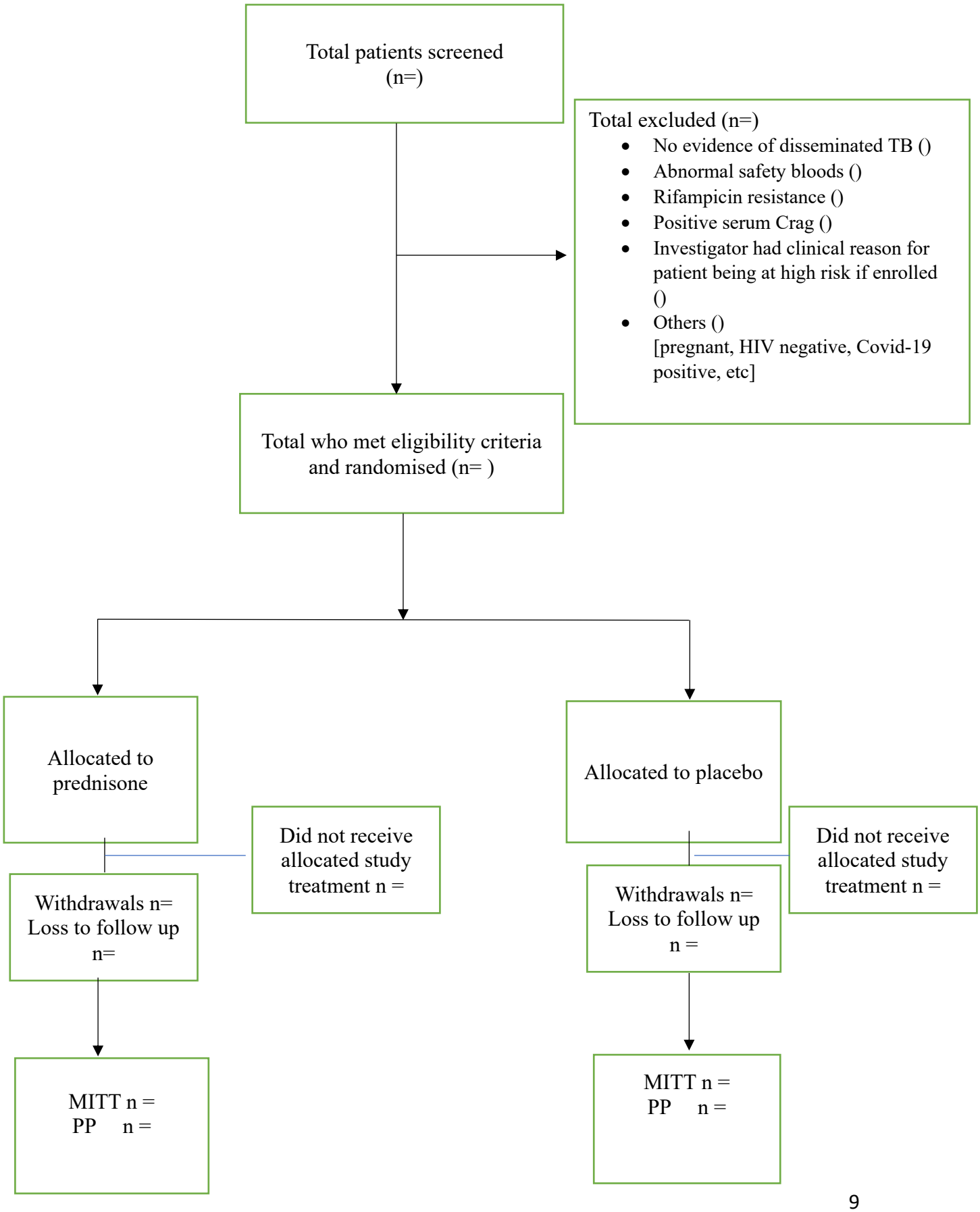
Week 24 defined as 168 days

Descriptive analysis:

The flow of participants through the trial will be summarised for each arm using a CONSORT diagram. The diagram will describe the numbers screened, randomised, received study medication, withdrawals, lost to follow-up, and included in the mITT and PP populations by treatment arm for intervention 2 (ie. prednisone versus placebo) (see Figure 1).

Baseline characteristics will be described for participants by randomisation and by arm. The characteristics to be described are shown in the Table 1. The number and percentage will be presented for binary and categorical variables. The median and interquartile range will be presented for continuous variables.

Figure 1: Consort diagram



Participants lost to follow-up and who withdrew will be included in the mITT and PP populations according to the definitions described above.

Table 1: Baseline characteristics (mITT population)

This table will be presented in the final publication with the following columns according to randomisation and arm:

Intervention 1: Standard TB treatment during randomisation period; Intensified TB treatment during randomisation period; received Standard TB treatment after randomisation to Intensified TB treatment arm was closed

Intervention 2: Placebo; Prednisone

A column describing all participants included in any of the mITT analyses will also be included

(Similar table for PP population will be presented in supplement)

	By arm with N(%) or median (IQR)
Age (years)	
Sex	
Hospital site	
Previous TB treatment	
Duration of TB symptoms (days)	
Duration of TB treatment prior to enrolment (doses)	
Measured/estimated BMI	
ECOG performance status	
Respiratory rate	
Pulse rate	
Mean arterial blood pressure	
AVPU score	
Glasgow Coma Scale score	
qSOFA score	
CD4 count (cells/mm^3)	
HIV VL (Log 10)	
HIV VL <50 copies/mL	
Hepatitis B surface Ag positive *	
Haemoglobin (*g/dl)* All Female Male	
Glucose (*mmol/l)*	
ALT (IU*/l)*	
AST (IU*/l)*	
ALP (IU*/l)*	
Total bilirubin (µmol*/l)*	
Creatinine (µmol*/l)*	
Urea (*mmol/l*)	

Sodium (*mmol/l)*	
Potassium *(mmol/l)*	
White cell count ($x10^9$)	
Absolute neutrophil count ($x10^9$)	
Absolute lymphocyte count ($x10^9$)	
Platelets ($x10^9$)	
Lactate (mmol/L)	
CRP (mg/L)	
eGFR (mL/min/1.73m2) eGFR categories: <10 10-29 30-49 50-59 ≥60	
HIV status (n, %) Previously diagnosed New HIV diagnosis Unknown if previous or new	
ART status (n, %) Currently on ART Previously on ART ART naïve	
Disseminated TB diagnosis: positive (n, %) Urine LAM Urine Xpert[1] Blood Xpert[1]	
Baseline TB blood culture (n, %) Positive Negative Contaminated Not available	
INH drug resistance (where result available)	
TB treatment Randomised to intensified TB treatment Randomised to standard TB treatment Not randomised: Standard TB treatment (after intensified TB treatment randomisation was closed)	

[1]Positive & trace positive results
*Testing introduced after trial start

Resistance to rifampicin (where result became available after enrolment will also be reported) in footnote
Number of missing variables for each baseline characteristic will be presented within or below the table.

Comparative analyses:

For all outcomes, the primary analysis will be conducted on the mITT population. Comparisons will be made between the two treatment arms: prednisone and placebo. Sensitivity analyses of the primary and secondary outcomes will be performed using the PP population.

Primary endpoint analysis: Modified Intention-to-Treat (mITT) population

The primary efficacy endpoint of 12-week mortality will be presented as the proportion of all participants who died by week 12, comparing the difference between the two arms. This will be estimated using the Kaplan-Meier method, with censoring on the date those lost to follow-up or who withdrew were last known to be alive, and at 12 weeks.

To estimate the absolute difference in proportion of participants who died between the two arms and to obtain the 95% confidence interval and associated p-value, bootstrap methodology will be used on the Kaplan-Meier estimates at 12 weeks using 1,000 repetitions.

Kaplan Meier curves will be used to visually represent survival in each treatment arm.

Cox proportional hazards model secondary analyses for death within 12 weeks (mITT population):

The initial Cox proportional hazards model analysis will be adjusted for the site (randomization stratification factor) and unadjusted for other baseline characteristics. Hazard rate estimates will be reported with 95% confidence intervals.

An additional adjusted analysis will adjust for pre-specified baseline clinical variables (known to be associated with poor outcome) and the randomisation stratification factor (study site) to assess their impact on time to death within 12 weeks. These variables will include age, sex, CD4 count, HIV viral load, haemoglobin, creatinine, CRP, lactate, Glasgow Coma Scale, functional status (AVPU and ECOG scores), measured/estimated BMI, positive screening TB tests (urine and blood Xpert Ultra, urine LAM) and TB blood culture at screening. Continuous variables will be tested for non-linearity using natural cubic splines during development of the model. If variables are collinear, one or more will be removed from the model. Other variables may be adjusted for after exploration and these will be highlighted as not having been pre-specified. Adjusted estimates will be presented with 95% confidence intervals.

As in the primary analysis, participants who are alive at the end of the follow-up period (week 12/day 84) will be censored at week 12. Participants who are lost to follow up or withdrew from the study will be censored at the date they were last known to be alive.

Per-protocol analyses:

The primary outcome analysis and the Cox proportional hazards models will be performed again but participants will be analysed according to the PP population.

Secondary efficacy endpoints:

1. All-cause mortality at 2 weeks (14 days)
2. All-cause mortality at 24 weeks
3. Change in venous lactate over 14 days of study medication administration.
4. Change in C-reactive protein over 14 days of study medication administration.
5. Change in haemoglobin over 14 days of study medication administration.

All-cause 2 week and 24 week mortality by trial arm will be compared using using Kaplan-Meier method with censoring on the date those lost to follow-up or who withdrew were last known to be alive, and at 2 or 24 weeks. To estimate the absolute difference in percentage of participants who died between the two arms and 95% CI for this, and associated p-value, bootstrap methods will be used on the Kaplan-Meier estimates using 1,000 repetitions.

Cox proportional hazards models equivalent to that for the 12-week timepoint described above will be completed but with censoring at 2 weeks and 24 weeks respectively.

For longitudinal continuous biomarker outcomes (serial measure data, secondary endpoints 3-5 above), only observed data will be used (i.e. the number with measures at each timepoint – days 2, 4, 7 and 14 or the closest measurement within equally spaced windows to each nominal point to ensure no observed data is excluded). Results will be presented as descriptive statistics by measurement day (screening, day 2, day 4, day 7, and day 14) and treatment arm. Haemoglobin results will be presented overall and by gender. Generalized estimating equation (GEE) modelling with robust standard errors and independent correlation matrices (adjusting for baseline values) will be used to account for the nesting of observations within-participants for these longitudinal outcomes when assessing intervention group by time interaction effects.

Analyses within a GEE model will include an interaction between baseline and outcome week to allow the effect of baseline to vary across timepoints.

Secondary efficacy analyses will be performed in both the mITT and PP populations.

Safety and tolerability endpoints (within 12 weeks, unless stated)

1. Occurrence of hepatotoxicity using the American Thoracic Society (ATS) hepatotoxicity criteria: alanine aminotransferase (ALT) elevation of more than three times the upper limit of normal (ULN) in the presence of hepatitis symptoms and/or jaundice or five times the upper limit of normal in the absence of symptoms.
2. Laboratory safety data (Grade 3 and 4 abnormalities using the ACTG grading system): liver function tests (alanine and aspartate aminotransferase [ALT, AST], alkaline phosphatase [ALP], conjugated and total bilirubin [CBR, TBR]), glucose, full blood counts (including white cell, neutrophil and platelet counts plus haemoglobin) and electrolytes (sodium, potassium), and creatinine. The change in values for these tests over 14 days will also be reported.
3. Occurrence of paradoxical tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) in participants starting antiretroviral therapy
4. All grade 3 and 4 clinical adverse events (using the ACTG grading system)
5. Serious adverse events (summarised as i) non-fatal SAEs and ii) deaths)
6. Adverse events requiring study drug interruption and/or withdrawal
7. Adverse drug reactions attributed to study drug
8. Non-infective corticosteroid adverse events within 4 weeks (28 days) of enrolment (namely new hypertension, new poor blood pressure control in a known hypertensive, hyperglycaemia, hypomania or mania, depression, acne, gastritis symptoms, upper GIT bleed, and avascular bone necrosis)
9. Severe infections defined as invasive bacterial or fungal infections or other WHO stage 3 or 4 defining condition
10. Malignancies, including Kaposi's sarcoma

Safety endpoints will be presented as number and proportion of participants experiencing at least one event, by treatment arm (i.e. assuming that those not observed for the full follow-up period to 12 weeks did not experience the event). Any events occurring after the 12 week visit window (12 weeks + 1 week) closes will be excluded. We will compare categorical data using Chi squared or Fisher's exact test (as appropriate) without correction for multiple comparisons. For safety data involving longitudinal continuous biomarker outcomes, as in the previous section, generalized estimating equation (GEE) modelling with robust standard

errors and independent correlation matrices (adjusting for baseline values) will be used to account for the nesting of observations within-participants for these longitudinal outcomes when assessing intervention group by time interaction effects.

The safety population will include any participant who received at least one dose of study medication.

An endpoint review committee will not be established for review of safety endpoints.

Subgroup analyses of the primary endpoint and the 2 and 24 week mortality endpoints in mITT population:

Prespecified subgroups, based on baseline variables, for subgroup analyses of the primary endpoint (12 week), 2 week and 24 week mortality endpoint are as follows:

- TB treatment (randomised to standard, randomised to intensified, not randomised (recruited after intensified randomisation stopped))
- *Mycobacterium tuberculosis* blood stream infection diagnosed by blood Xpert
- *Mycobacterium tuberculosis* blood stream infection diagnosed by blood culture
- CD4 cell count ≤100 cells/mm^3 or > 100 cells/mm^3
- Haemoglobin ≤7 g/dL or >7 g/dL
- eGFR =<50 or > 50
- Estimated BMI =< 18.5 or > 18.5
- Age > 35 years or =< 35years
- Lactate >=2.5 or < 2.5 mmol/l
- ECOG performance status 0-2 vs 3-4
- Hospital trial site

Analyses will be conducted using the unadjusted Cox proportional hazards model. Analysis of subgroups will be based on heterogeneity tests to evaluate whether the difference between randomised arms varies by subgroup (using an interaction term between treatment group and the subgroup variable).

Analysis of interaction between trial interventions in factorially randomised population testing for heterogeneity

In a supplementary table we will present baseline characteristics stratified into 4 groups depending on what combination of interventions the participant was randomised to: standard versus intensified TB treatment, and prednisone versus placebo (ie, Intensified TB + Prednisone; Intensified TB + Placebo; Standard TB + Prednisone; Standard TB + Placebo). No statistical comparisons will be performed on these variables. These analyses will be restricted to participants who were randomised in both parts of the factorial, ie will exclude participants randomised to prednisone/placebo after the intensified TB treatment randomisation was stopped.

As specified in the protocol, an analysis for interaction between the interventions will be performed. This analysis will use an interaction term in a Cox proportional hazards model* for time to death up to 12 weeks, performed on the mITT-int population (for this analysis, all of whom received at least one dose of each intervention prior to the intensified TB treatment randomisation being stopped). This model will include main effects for the two randomisations (standard vs intensified, prednisone vs placebo) and their interaction term. Study site (the only randomisation stratification factor) will be adjusted for, but no other variables will be adjusted for. A p-value < 0.05 for the interaction term will be regarded as demonstrating evidence of interaction.

This same interaction analysis (using an interaction term in a Cox proportional hazards model*) will also be performed in relation to the secondary endpoint of death up to 2 weeks.

As a supplementary analysis to evaluate the robustness of the assumption of no interaction, 12-week mortality will be presented as the proportion of all participants who died by week 12, for each of the 4 arms in the mITT-int population. This will be estimated using the Kaplan-Meier method, with censoring on the date those lost to follow-up or who withdrew were last known to be alive, and at 12 weeks. Kaplan-Meier curves will be used to visually represent survival in each treatment arm. Simple (stratified) comparisons will be estimated and reported:

1) Intensified TB vs Standard TB, given Prednisone
2) Intensified TB vs Standard TB, given Placebo
3) Prednisone vs Placebo, given Intensified TB

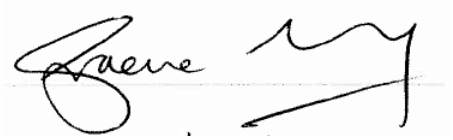
4) Prednisone vs Placebo, given Standard TB

For each of the four stratified comparisons, we will report the absolute difference in proportion of participants who died by 12 weeks (Kaplan-Meier-based estimate), with the 95% confidence interval. CIs will be obtained using bootstrap methodology on the Kaplan-Meier estimates at 12 weeks using 1,000 repetitions.

* A pre-specified test for non-proportionality using Schoenfeld residuals (Grambsch-Therneau, with a criteria $p<0.01$) will be applied to the Cox proportional hazards models. In the scenario that there is non-proportionality, the hazard will be modelled using flexible parametric models (Parmar and Royston, Stat Med).

Signed date …31 Dec 2025…….by Principal Investigators:

Dr Charlotte Schutz



Prof Graeme Meintjes

Prof Thomas Harrison, Chair, and on behalf of, NewStrat-TB Trial Steering Committee