

Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Meintjes G, Stek C, Blumenthal L, et al. Prednisone for the prevention of paradoxical tuberculosis-associated IRIS. *N Engl J Med* 2018;379:1915-25. DOI: 10.1056/NEJMoa1800762

This supplement contains the following items:

1. Original protocol, version 1.3, dated 07 August 2013 *
2. Final protocol, version 1.4, dated 03 February 2014
3. Addendum to protocol version 1.4, dated on 28 January 2015
4. Summary of changes
5. Statistical analysis plan, dated 14 December 2016 **

* Versions 1.1 and 1.2 of the protocol were submitted for ethical and regulatory review. Changes were made to these versions based on recommendations made during the review processes. Protocol version 1.3 was the active protocol at the time of enrollment of the first participant and therefore has been included as the original protocol.

** No changes were made to this original statistical analysis plan.

Original protocol, version 1.3, dated 07 August 2013

CLINICAL TRIAL PROTOCOL

Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomized placebo-controlled trial of prednisone (Pred-ART trial)

Funder: European and Developing Countries Clinical Trials Partnership (EDCTP)

Sponsor: University of Cape Town

Partner organizations: Institute of Tropical Medicine (Antwerp) and Imperial College London

Principal Investigator: Graeme Meintjes (University of Cape Town)

Co-investigators: Lut Lynen, Robert J. Wilkinson, Robert Colebunders, Gary Maartens

Clinicaltrials.gov number: To be obtained

Date: 7 August 2013

Version: 1.3



**Imperial College
London**

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SPONSOR REPRESENTATIVE:

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Date: _____
Signature: _____

STATEMENT OF COMPLIANCE & CONFIDENTIALITY

By signing this protocol, the Principal Investigator acknowledges and agrees:

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the most recent version of the Declaration of Helsinki, WHO and ICH Good Clinical Practice (GCP) and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses, laboratory staff and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

This document contains information that is privileged and confidential. As such, it may not be disclosed to any other persons than involved research staff and the concerned Ethics Committees, unless specific permission is granted in writing by the University of Cape Town, or such disclosure is required by national or other laws or regulations. In any event, persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future study-related information supplied that is regarded as privileged or confidential.

The Sponsor of this study will at any time have access to the source documents from which Case Report Form information may have been generated. The Case Report Forms and any other data pertinent to this study are the property of the Sponsor. The data may only be utilized upon review and after discussion with the trial steering committee and/or the Sponsor. All study material will be maintained according to regulatory requirements and until the Sponsor advises that it is no longer necessary.

I, the Principal Investigator; agree to conduct the present study in full accordance with the most recent approved version of the protocol, within applicable timelines, according to the relevant standard operating procedures and in full agreement with all applicable regulations and the international guidelines regarding the conduct of clinical research.

I, the Principal Investigator; by signing this protocol declare that I will permit trial-related monitoring, audits, independent Ethics Committee review, and regulatory inspections, providing direct access to source data/documents during and after the course of the trial.

I, the Principal Investigator; by signing this protocol declare that I will make the protocol and all relevant related information available to all physicians, nurses, laboratory staff and other personnel who participate in conducting this study. I will also ensure that the study team at my site receive adequate training so that they are fully informed and qualified for the conduct of the study.

I also acknowledge the paragraph relevant to study confidentiality.

PRINCIPAL INVESTIGATOR:

Title, Name:

Site:

Date:

Signed:

SCHEMA

Title: Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomised placebo-controlled trial of prednisone

Short title: Prednisone with early ART in HIV-TB (Pred-ART trial)

Phase: III

Objective: To determine whether the addition of prednisone to the first 4 weeks of antiretroviral therapy (ART) reduces the risk of paradoxical TB-IRIS in HIV-infected patients being treated for TB who are at high risk of developing TB-IRIS (CD4 <100 cells/ μ l and starting ART within 30 days of TB treatment).

Design: A randomized double-blind placebo-controlled trial to evaluate the incidence of paradoxical TB-IRIS over the first 12 weeks of ART in participants who receive a 4 week course of prednisone versus participants who receive a 4 week course of placebo.

Primary efficacy endpoint:

The development of paradoxical TB-IRIS within 12 weeks of starting ART (defined using the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition).

Secondary efficacy endpoints:

- 1) Time to IRIS event
- 2) Severity of IRIS events (defined by the following: need for hospitalisation for IRIS, C-reactive protein, and neurological involvement)
- 3) Duration of TB-IRIS event (from onset of symptoms/signs to resolution of TB-IRIS symptoms/signs)
- 4) Mortality attributed to TB and TB-IRIS
- 5) All-cause mortality
- 6) Composite endpoint of death, hospitalization, or hepatotoxicity (using the protocol-specified definition of Grade 3 or 4 increase in ALT or bilirubin).
- 7) Other (non-TB) IRIS events
- 8) Quality of life assessment (measured using PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score)
- 9) Adverse events and severe adverse events ascribed to TB treatment, ART or co-trimoxazole. This will include a pre-specified analysis of drug-induced liver injury and drug rash. This assessment will include the number of treatment interruptions for drug adverse events.
- 10) Discontinuation of either ART or TB treatment for > 5 days due to adverse events
- 11) Number of hospitalizations and total days hospitalized

Safety and tolerability endpoints:

- 1) Corticosteroid-associated adverse events, classified by severity and relation to study drug. These will include hypertension, hyperglycaemia, hypomania/mania, depression, acne, epigastric pain, upper gastro-intestinal bleeding, Cushingoid features, new oedema and

avascular bone necrosis.

2) Laboratory safety data: glucose, full blood count and electrolytes

3) Other infections (AIDS-related, bacterial, fungal and viral) and malignancies (Kaposi's sarcoma)

4) All grade 1, 2, 3 and 4 adverse events (clinical and laboratory using the ACTG grading system)

Sample size: 240 participants will be enrolled over 13 months. Each participant will be followed for 12 weeks.

Population: HIV-infected, ART-naïve adult (≥ 18 years) patients diagnosed with active tuberculosis who have a CD4 < 100 cells/ μ L and who start ART within 30 days of starting TB treatment. Other inclusion criteria include: diagnosis of TB (smear, culture, Xpert MTB/RIF test, histology or strong clinical and radiological evidence of TB with symptomatic response to TB treatment), eligible for and consent to starting ART and written informed consent for trial. Exclusion criteria include: Kaposi's sarcoma, pregnancy, TB meningitis or tuberculoma at TB diagnosis (because these patients receive corticosteroids), known rifampicin-resistant TB, being on corticosteroids for another indication within the past 7 days, on other immunosuppressive medication within the past 7 days and uncontrolled diabetes mellitus.

TB treatment and ART: TB treatment will be prescribed and monitored by the clinical staff in the local HIV-TB clinic. TB treatment will be given according to South African Department of Health guidelines. This involves rifampicin (R), isoniazid (H), ethambutol (E) and pyrazinamide (Z) for 2 months followed by RH for 4 months. ART will be prescribed by the clinical staff at the HIV-TB clinic according to South African Department of Health guidelines. Standard first line ART in TB patients is tenofovir, emtricitabine (or lamivudine) and efavirenz. Co-trimoxazole prophylaxis will be prescribed to all patients unless a contra-indication exists.

Intervention: Oral prednisone 40mg daily for 14 doses started on the first day that ART is taken, followed by 20mg daily for 14 doses (or identical placebo). A total of 28 days of study medication will thus be prescribed.

Follow-up: Patients will be screened once established on TB treatment, but before starting ART. If the patient is eligible, written informed consent will be taken. There will be six planned study visits that will be in relation to the start of ART: week 0 (the day ART is initiated), week 1, week 2, week 4, week 8 and week 12. Patients will be seen at unscheduled visits if clinical deterioration occurs. If paradoxical TB-IRIS is diagnosed this will be treated with open label prednisone at clinician discretion if symptoms are moderate or severe. If patients experience clinical complications (eg. TB-IRIS) follow-up will be prolonged beyond week 12 in order to stabilize their condition before referral back to the general TB-HIV clinical service for ongoing management.

Data monitoring: The trial will be monitored by an independent Data and Safety Monitoring Board (DSMB) comprising 3 independent researchers and an independent statistician. After an initial meeting for agreeing on their Charter, the DSMB will meet twice (after 80 and 160

participants have completed follow-up) to review data quality and data with respect to safety and trial endpoints. If there is evidence of harm related to study medication or trial conduct the DSMB may advise the sponsor that trial enrollment should be stopped.

Clinical trial site: Khayelitsha Site B HIV-TB clinic (Ubuntu clinic)

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References

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2. EXECUTIVE SUMMARY

Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomised placebo-controlled trial of prednisone

Tuberculosis (TB) is the most common opportunistic infection amongst HIV-infected patients starting antiretroviral therapy (ART) in developing countries and thus the most frequent form of immune reconstitution inflammatory syndrome (IRIS). Paradoxical TB-IRIS occurs in 8- 43% of patients starting ART while on TB treatment and results in morbidity, hospitalisation, consumes health care resources and TB-IRIS may be fatal. We have previously demonstrated in a clinical trial that prednisone reduces morbidity when used for treatment of paradoxical TB-IRIS. This trial is a double-blind placebo-controlled trial of prophylactic prednisone (40mg/day for 2 weeks followed by 20mg/day for 2 weeks, started on the same day as ART) in patients with TB who are identified as being at high risk for paradoxical TB-IRIS (starting ART within 30 days of initiating TB treatment and $CD4 \leq 100/\mu L$). The trial will enroll 240 participants, randomised 1:1 (prednisone:placebo). The primary endpoint is development of paradoxical TB-IRIS, defined using international consensus case definitions. Secondary endpoints include time to IRIS event, severity of IRIS, quality of life assessment, mortality and corticosteroids adverse events. The trial is powered to determine a reduction in TB-IRIS events. If results of this trial demonstrate benefit and safety, our intention is to take this intervention forward into a larger phase 3 clinical trial with mortality as the primary endpoint.

3. INTRODUCTION

3.1. Primary study objective

To determine whether a 4-week course of prednisone in patients starting antiretroviral therapy (ART) within 30 days of starting treatment for tuberculosis (TB) and a CD4 count \leq 100/ μ L reduces the incidence of paradoxical TB-IRIS, without an excess of adverse events. The trial is powered to determine a reduction in TB-IRIS events. If the clinical trial proposed here demonstrates benefit and safety, our intention is to take this intervention forward into a larger phase 3 clinical trial with a sufficiently large sample size to determine a mortality reduction.

3.2 Paradoxical TB-IRIS

TB is the commonest opportunistic disease in HIV-infected patients in low- and middle-income countries, and is therefore a common indication for starting ART. A substantial proportion of patients starting ART in sub-Saharan Africa are thus on treatment for active TB (up to 42% [1]), and are therefore at risk of paradoxical TB-associated immune reconstitution inflammatory syndrome (TB-IRIS). When ART is commenced in patients on TB treatment an immunopathological reaction, known as paradoxical TB-IRIS, commonly occurs (in 8-43% of TB patients starting ART [2]) resulting in new or recurrent TB signs and symptoms. TB-IRIS causes considerable morbidity; with 30% requiring hospitalization in a prospective study we conducted [3].

One study from the US reported that 44% of patients who developed TB-IRIS required hospitalization for a median of 7 days (range 3-66) and many patients in this study required therapeutic procedures [4]. In a recent report of TB-IRIS cases that occurred during the SAPIt trial in Durban, South Africa (discussed below) among those patients who started ART within 4 weeks of TB treatment who developed TB-IRIS 42% required hospitalization and among those who started ART 8-12 weeks after TB treatment and developed TB-IRIS 22% required hospitalization [5].

Although the mortality of TB-IRIS is relatively low (estimated at 3.2% in a meta-analysis [6]), our group has shown that mortality is about ten times higher than this in patients with neurological manifestations [7, 8]. Importantly, over two-thirds of patients who develop neurological TB-IRIS do not have clinical features of neurological involvement at initial diagnosis of TB [7]. The diagnosis of TB-IRIS is clinical, and it is important to exclude other opportunistic diseases. In resource-constrained settings this puts a strain on over-burdened health services by utilizing diagnostic tests and empiric treatment of suspected alternative diagnoses. In the majority of cases the onset of TB-IRIS is within the first 4 weeks of ART: in our cohort median onset was 14 days (IQR 7-25) [9]. Therefore prophylactic interventions may only be required for one month.

3.3. Corticosteroids in the treatment of TB

Corticosteroids are known to exert anti-inflammatory effects on most types of immune cells through direct effects on transcription of inflammatory mediators via the Glucocorticoid Responsive Element, indirect genomic effects via interference with other transcriptional factors such as NF- κ B and AP-1, and non-genomic effects on anti-inflammatory proteins [10, 11]. These effects result in increased transcription of a number of anti-inflammatory

mediators and decreased transcription of pro-inflammatory cytokines, chemokines, enzymes, receptors and adhesion molecules [12, 13]. In addition, corticosteroids have been shown to reduce T cell survival by enhancing apoptosis [12]. Corticosteroids have been used as adjunctive treatment in TB for several decades [14]. Because the host immune response plays an important part in the pathology caused by TB, corticosteroids have been used in all forms of TB with the intention of improving outcomes and reducing complications such as pericardial constriction, hydrocephalus, focal neurological deficits, pleural adhesions and intestinal strictures. However, evidence of benefit from controlled clinical trials exists only for TB meningitis and pericardial TB as well as paradoxical TB-IRIS [3, 15].

TB Meningitis: Thwaites and colleagues [16] conducted a randomized, double-blind, placebo-controlled trial of dexamethasone for the treatment of TB meningitis in Vietnam amongst patients older than 14 years of age (n=545). The initial dose of intravenous dexamethasone used was 0.4mg/kg/d for patients with Grade 2 and 3 TBM disease and 0.3mg/kg/d in patients with Grade 1 disease. The total duration of dexamethasone (initially intravenous followed by oral) was 8 weeks in those with Grade 2 and 3 disease and 6 weeks in those with Grade 1 disease. At 9 months follow-up, dexamethasone was associated with a reduced risk of death (relative risk = 0.69, 95% CI= 0.52 – 0.92), but no reduction in the proportion of patients with severe disability. Eighteen percent of participants were HIV seropositive and in a pre-specified subgroup analysis of these patients dexamethasone was associated with a non-significant trend towards reduced mortality (relative risk = 0.78, 95%CI = 0.59 -1.04). Significantly fewer severe adverse events occurred in patients who received dexamethasone. In particular, 8 cases of severe hepatitis (one was fatal) occurred in the placebo group and none in the dexamethasone group. This may be an additional benefit of corticosteroids: by reducing drug reactions they prevent treatment interruptions that adversely impact survival. A Cochrane systematic review of corticosteroids as an adjunct to TB treatment in TB meningitis published in 2008 included seven trials and a total of 1140 participants (with 411 deaths). Dexamethasone or prednisolone was the corticosteroid used in all studies. Corticosteroids reduced the risk of death (relative risk = 0.78, 95% CI = 0.67 - 0.91). The survival benefit occurred irrespective of the severity of TB meningitis. Adverse events that occurred across studies included gastro-intestinal bleeding, bacterial and fungal infections and hyperglycaemia, but were mild and treatable [17].

Pericardial TB: A randomized, double blind, placebo-controlled trial of prednisolone (for 11 weeks) for the treatment of TB pericardial effusion was conducted in Transkei, South Africa, in the 1980's prior to the HIV epidemic by Strang and colleagues. Patients in this study were also randomized to open pericardial biopsy and complete drainage of pericardial fluid on admission or percutaneous aspiration when required. Among patients who did not have open drainage on admission, 3% given prednisolone compared with 14% given placebo died of pericarditis (p <0.05) [18]. Patients who received prednisolone required repeat pericardiocentesis less frequently. A Cochrane review [19] of controlled trials evaluating the role of adjuvant corticosteroids for TB pericarditis included 2 trials from the pre-HIV era with a total of 383 participants (the 2 trials included were the study published by Strang and colleagues in 1988 [18] and a prior trial of patients with TB constrictive pericarditis conducted by the same group [20]). There was a non-significant trend towards reduced death in the intervention group (relative risk = 0.65, 95% CI = 0.36–1.16). There was a significant reduction in the combined endpoint of death or disability at 2 years although there

was substantial heterogeneity in the trials. In a small randomised controlled trial of prednisolone for TB pericarditis in HIV-infected patients conducted in Zimbabwe (n=58) [21] there were 10 deaths among those who received placebo compared with 5 deaths among those who received prednisolone. This represented significantly lower mortality in the prednisolone group using the log-rank test to compare Kaplan Meier survival curves [21]. However, when cumulative mortality was compared the difference was not significant (RR=0.50, 95% CI = 0.19 - 1.28) [19]. The Cochrane review concluded that corticosteroids could have clinical benefit, but the trials published to date are too small to demonstrate an effect [19]. In particular, the efficacy and safety of corticosteroids in HIV-infected patients needs to be evaluated in a larger clinical trial.

Corticosteroids are used in other forms of TB. Tuberculomas involving the brain parenchyma or spinal cord may develop despite effective TB treatment resulting in focal neurological deficits or seizures. The host immune response is thought to play an important role in such paradoxical TB reactions. Corticosteroids have been used with anecdotal reports of symptomatic benefit [22]. In miliary and pulmonary TB initiation of TB treatment may be complicated by the development of adult respiratory distress syndrome with acute respiratory failure [23]. Corticosteroids are frequently used in this situation, but efficacy has not been determined in an adequately powered clinical trial. One study of 55 patients with miliary TB showed a non-significant trend towards improved survival with corticosteroids [24]. A review of the literature of corticosteroids used for all forms of TB concluded that corticosteroids do not diminish the efficacy of TB treatment [14].

A recently published meta-analysis of all trials in which corticosteroids were prescribed as adjunctive treatment for TB patients regardless of which organ system was involved, reported that corticosteroids significantly reduced mortality by 17% (relative risk = 0.83, 95% CI=0.74-0.92). This finding was consistent across all organ systems [25].

The above findings mainly apply to HIV-negative patients and there are concerns regarding the use of adjuvant corticosteroids in HIV-infected patients. In a Ugandan trial that evaluated prednisolone in HIV-infected patients with TB pleural effusions, prednisolone was associated with more rapid clinical and radiological improvement, but an excess of Kaposi's sarcoma [26]. A study was conducted in Uganda specifically in HIV-infected patients with pulmonary TB, but prior to ART availability. In this phase 2 trial of short-term prednisolone in HIV-infected TB patients with CD4 count ≥ 200 cells/mm³, prednisolone was associated with more rapid clearance of MTB from sputum, reduced immune activation and resulted in a non-significant increase in CD4 count, but caused a transient increase in HIV viral load and worsened underlying hypertension and caused fluid retention and hyperglycaemia. High dose prednisolone was used in this trial (initially 2.75mg/kg/day for 4 weeks) [27]. Other corticosteroid trials in HIV-infected patients have raised concerns related to reactivation of herpes infections [28]. All patients on our proposed trial will be on ART, and ART is likely to be at least partially protective against certain of these corticosteroid side effects, such as the development of Kaposi's sarcoma.

Corticosteroids have drug-drug interactions with rifampicin, a potent inducer of cytochrome P450 enzymes. Rifampicin increases the clearance of prednisolone by 45% resulting in reduction in the area under the plasma concentration time curve of prednisolone by 66% [29].

We factored this in our decision regarding prednisone dose for this trial.

Corticosteroids in the treatment of TB-IRIS: We have previously conducted a randomised double-blind placebo-controlled trial of prednisone for the treatment of paradoxical TB-IRIS [3]. The trial was conducted at GF Jooste Hospital in Cape Town between 2005 and 2008. 110 patients referred to our hospital and diagnosed with paradoxical TB-IRIS based on a clinical case definition [2] and after diagnostic work-up, were enrolled. Patients with immediately life threatening TB-IRIS (mainly neurological involvement) were excluded and received treatment with corticosteroids in the clinical service. Participants received a 4-week course of prednisone or identical placebo (1.5 mg/kg/day for 2 weeks followed by 0.75 mg/kg/day for 2 weeks). Follow-up was for 12 weeks. The primary endpoint was days of hospitalisation and outpatient therapeutic procedures (counted as an additional hospital day) and this was significantly lower in those who received prednisone (median of 0 days vs 3 days ($p=0.04$)). Prednisone also resulted in significantly more rapid improvement in symptoms, MOS-HIV quality of life score, chest radiology score and reduction in C-reactive protein. There was no excess of corticosteroid metabolic side effects or severe infections in the prednisone arm. There were more mild infections (oral candida and uncomplicated herpes simplex) among those who received prednisone [3].

We have subsequently demonstrated in samples taken from participants on this trial that prednisone, but not placebo, resulted in significant decreases, after correcting for multiple comparisons, in the serum concentrations of IL-6, IL-10, IL-12p40, TNF- α , IFN- γ and IP-10 suggesting that the beneficial effect of corticosteroids in TB-IRIS is mediated, at least in part, through the attenuation of pro-inflammatory cytokine responses [30]. We have previously demonstrated that these same cytokines are differentially increased in the serum of HIV-TB patients who develop TB-IRIS compared to control subjects who start ART and do not [31]. We, therefore, hypothesise that prednisone given from the start of ART may prevent the development of TB-IRIS or at least attenuate its clinical severity through suppression of pro-inflammatory cytokine responses.

Our group also recently reported from an observational study [32] that patients who were on corticosteroids at the time of starting ART (the indication was mainly TBM) had lower interferon γ (IFN- γ), IP-10, tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, IL-10, IL-12p40, and IL-18 concentrations prior to ART when compared with HIV-TB patients starting ART who were not on corticosteroids ($p \leq 0.02$). In this same study, fewer patients amongst those receiving corticosteroids developed TB-IRIS (38.1% versus 51.7%), but the difference was not statistically significant ($p = 0.287$). We also noted a trend for patients not on corticosteroids to present with more severe TB-IRIS clinical presentations, compared with patients who were on corticosteroids (10/31, 32% vs 0/8, 0% ($p = 0.082$)). The study was relatively small, non-randomised and very likely confounded by indication for prescribing corticosteroids and thus no firm conclusions can be drawn. However, these results suggest there is an important effect of corticosteroids on the immune response in HIV-TB patients starting ART and provide further rationale for this RCT.

Neither corticosteroids, nor any other medication, are currently prescribed for preventing paradoxical TB-IRIS in the current standard of care making a placebo-controlled trial ethically acceptable because it reflects current standard of care.

3.4. Corticosteroids for other HIV-related conditions

Corticosteroids are used in the treatment of pneumocystis pneumonia (PCP) in HIV-infected patients. A meta-analysis of six randomised trials of adjuvant corticosteroids in the treatment of PCP showed a survival benefit [33]. A 21-day course of corticosteroids used for PCP treatment does not increase the risk of mortality or common HIV-related opportunistic conditions, apart from oesophageal candidiasis [34].

In the ACTG A5164 trial that compared ART timing strategies in patients with non-TB opportunistic and bacterial infections, receipt of corticosteroids during OI management (mainly for PCP) and the timing of ART were not associated with the development of IRIS [35]. However, no cases of IRIS developed while patients were still receiving corticosteroids [36]. In a report of the immunological profile of participants enrolled in this study, amongst patients who had received corticosteroids IFN- γ , IL-6, IL-8, IL-17 and TNF- α and sTNFrII levels were attenuated at time of IRIS [37]. In addition, patients who received corticosteroids, after controlling for the development of IRIS, had lower levels of TNF, sTNFrII and IL8 at baseline compared to those who did not receive corticosteroids [36]. The investigators concluded: “It ... appears that corticosteroids do suppress the same inflammatory markers that seem to identify patients at higher risk for IRIS, lending some support for their empirical use for treatment and potential prevention of IRIS events” [36].

3.5. Rationale for this study

Three recent RCTs that investigated the optimal time to start ART in HIV-TB patients [38-40] demonstrated that starting ART ~2 weeks into TB treatment in patients with low CD4 counts ($CD4 \leq 50/\mu L$) reduced mortality and AIDS progression compared with starting at ~8 weeks. Given these RCT findings, there will now be a major impetus at a programme level to start HIV-TB patients with low CD4 counts on ART after 2 weeks of TB treatment. However, low CD4 count and shorter interval between TB treatment and ART are the two most important factors increasing the risk of TB-IRIS [41]. In these same RCTs there was a 2 to 3-fold increase in risk of TB-IRIS in those who started ART ~2 weeks. In a recent paper the SApiT investigators concluded: “Furthermore, a randomized, placebo-controlled trial that would investigate whether corticosteroids in patients with a CD4 count less than 0.050×10^9 cells/L initiating highly active ART early in tuberculosis treatment reduce frequency and severity of IRIS events and need for hospitalization is warranted [5].”

In these 3 RCTs, mortality in the group of patients who started at ~2 weeks was lower than in the group who started later, but mortality in the early arms was still 6-14% over the first year of ART. It is likely that certain of these deaths were attributable to TB-IRIS particularly if the central nervous system was affected. TB-IRIS will continue to be a major complicating factor in ART programmes in sub-Saharan Africa, causing considerable morbidity. Interventions to reduce the incidence and/or severity of TB-IRIS are urgently needed. Given the clinical trials evidence regarding the use of corticosteroids as adjuvant treatment in TB, for the treatment of TB-IRIS and to treat immunopathology in other HIV related conditions such as PCP, there is a compelling rationale for performing a trial of corticosteroids for TB-IRIS prevention.

IRIS incidence in SAPiT trial
IRIS cases/100 person-years

| Group | All | CD4 < 50 | CD ≥ 50 |
|--------------------------------------|------|----------|---------|
| Early integrated (Within 4 weeks) | 19.5 | 45.5 | 15.3 |
| Late integrated (8 -12 weeks) | 7.5 | 9.7 | 7.1 |

Naidoo, Ann Intern Med 2012;157:313

Figure 1. Incidence of paradoxical IRIS stratified by baseline CD4 count and ART timing in the SAPiT trial [5, 38]

Another important consideration is that corticosteroids have been shown to reduce the incidence of adverse drug reactions when used as adjunctive treatment in TB meningitis [16] and in HIV-infected patients with PCP [42]. Adverse drug reactions are a major complicating factor in the management of HIV-TB patients who are typically taking multiple drugs that are associated with drug hypersensitivity reactions and drug induced liver injury (TB treatment, ART and co-trimoxazole). In HIV-TB patients these drug reactions result in hospitalisations, drug interruptions that may result in resistance and disease progression and in some cases reactions are fatal. At our own hospital 3-month mortality among patients who were seen with TB drug or ART-induced liver injury was 35% [43]. By potentially reducing the incidence of these hypersensitivity reactions this is another way in which corticosteroids could potentially reduce morbidity, mortality and burden on limited health care resources.

In summary, we propose a proof-of-concept clinical trial of prednisone versus placebo for TB-IRIS prevention to assess efficacy and safety. The findings will influence clinical practice and will be used to design a larger phase 3 clinical trial to assess the impact of corticosteroids on mortality. Paradoxical TB-IRIS will remain common because many patients with HIV-TB still start ART with advanced immunosuppression in sub-Saharan Africa as a result of difficulties accessing HIV testing and treatment services. Even though international ART guidelines advocate for earlier start of ART during the course of HIV disease (WHO adult ART guidelines, 2010) the reality is that patients still present late. Moreover, recent reduction in pledging to the Global Fund and budget cuts by PEPFAR will likely cause longer ART waiting lists in some countries (personal communication, Anja De Weggheleire MSF in DRC).

Clinical services struggle to deal with this complex condition (TB-IRIS). The role of corticosteroids in preventing TB-IRIS is unknown, but there are several lines of evidence that suggest corticosteroids may have the potential to prevent or ameliorate the severity of TB-IRIS. In the proposed clinical trial equipoise exists because corticosteroids also have potential risks in the setting of advanced HIV.

3.6. Timelines and study site

Recruitment of the 240 patients to the study will take 13 months, with a further 3 months follow-up time after the last participant is enrolled. Initial analysis of results will be performed in the 3 months after the database has been locked and further analysis will take an additional 6 months. We have demonstrated capacity to enroll large numbers of patients with HIV-TB in prior studies. The clinical site chosen for the study is in Khayelitsha in Cape Town (single centre trial), where large numbers of patients start ART annually (over 20 000 patients have started ART in Khayelitsha since 2002), and up to 40% of patients starting ART are on treatment for TB [1] thus we are confident that there will be sufficient eligible patients to complete enrolment for the study in 12 months. Participant follow-up in this study is relatively short (12 weeks).

3.7. Expected impact of the study

We hypothesise that corticosteroids for the first 4 weeks of ART will reduce the incidence and severity of TB-IRIS and delay onset of TB-IRIS. Furthermore we hypothesise that TB-IRIS is an important contributor to early deaths in patients with low CD4 counts receiving early ART, and that this mortality could be reduced by prophylactic corticosteroids. In this proposed proof-of-concept phase 3 clinical trial we aim to assess the efficacy of steroids in reducing TB-IRIS and the safety of this intervention. If results of this trial demonstrate efficacy and safety we will take this intervention forward into a larger RCT with mortality as the primary endpoint. The findings of this trial will inform design and sample size calculation for the larger phase 3 trial.

We anticipate that the findings of this proposed clinical trial will themselves inform clinical practice and guidelines in sub-Saharan Africa. If corticosteroids are shown to reduce the risk of TB-IRIS, without safety concerns, clinicians will have an evidenced-based therapeutic option in patients at high risk for TB-IRIS provided no contra-indications to corticosteroid use exist.

3.8. Risks and dependencies

One risk is that we will not recruit sufficient numbers of eligible patients at the Sit B HIV-TB clinic in Khayelitsha during the study period. To overcome this we will raise awareness of the trial among all doctors and clinical nurse practitioners in Khayelitsha (visiting other clinics and distributing information pamphlets and posters that will be submitted to and pre-approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee) and ask them to refer potentially eligible and agreeable patients to the clinical trial site for screening. Transport costs for the clinic visit (R150) will be paid to all patients at the screening visit whether they are enrolled or not. In this way we are confident that we will meet the enrolment target based on ART figures obtained for 2011. Data regarding patients initiating ART in Khayelitsha clinics in 2011 was obtained from Andrew Boule (Centre for Infectious Disease Epidemiology and Research, UCT, and Public Health Specialist responsible for Program Evaluation at Western Cape Provincial Government) who curates the IeDEA database at UCT. Across all 11 Khayelitsha ART clinics, 5000 adult patients initiated ART in 2011: 2000 in clinics administered by the City of Cape Town and 3000 in clinics administered by the provincial government. Detailed data currently available for the City clinics from 2011 show that 23% of the patients had a CD4 count < 100/ μ L and

35% were on TB treatment at the time of ART initiation. It is likely that the profile of adult patients in the provincial government clinics was similar.

There is the risk that corticosteroids may do harm in this patient group due to immunosuppression and metabolic side effects. Prednisone used for 4 weeks for the treatment of TB-IRIS at higher doses in our previous clinical trial did not result in an excess of severe infections or metabolic side effects [3]. Nonetheless the potential for harm, in addition to potential benefit, defines the equipoise in the proposed study. We will ascertain all potential harms from corticosteroids (infections and metabolic side effects) by close clinical monitoring during the trial and documenting and analysing all potential side effects (metabolic and infectious) as pre-defined safety endpoints. The DSMB will review this data at the interim review and if there is evidence of significant harm from prednisone the trial would be stopped according to DSMB stopping rules that will be determined before trial initiation. The trial protocol will undergo ethical review at the Institutional Review Board of the ITM, the Ethics Committee of the Antwerp University Hospital in Belgium, and at the University of Cape Town, which involves rigorous peer review of the protocol.

4. Study Objectives and Design

4.1. Objectives

- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the risk of paradoxical TB-IRIS in patients with HIV-associated TB at high risk for TB-IRIS.
- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the severity and deleterious consequences of paradoxical TB-IRIS in patients with HIV-associated TB at high risk of TB-IRIS.
- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the risk of hypersensitivity drug reactions and consequent drug interruptions in patients with HIV-associated TB starting ART.
- To determine if such a course of prednisone is safe when used in this setting for this indication.

4.2. Design

This is a proof-of-concept phase III, randomised double-blind placebo-controlled trial of prednisone to assess efficacy and safety in preventing paradoxical TB-IRIS in high risk patients starting ART. The intervention will be oral prednisone 40mg daily for 14 doses started on the first day that ART is taken, followed by 20mg daily for 14 doses (or identical placebo) (Total 28 days of study medication).

Method of allocation, masking and concealment of allocation: Blinded treatments will be packaged at an independent off-site pharmacy, authorized by the MCC for this specific activity, in consecutively numbered identical packages. The packages will contain either prednisone or identical placebo tablets packaged according to a blocked (block size 8), 1:1 randomisation sequence prepared before the study start by a statistician not involved in the study conduct. Each package will have a number from 1 to 240. The numbered packages will be transported to the study pharmacy. Participants will be enrolled sequentially and will receive the next study number from 1 to 240 and the corresponding medication package. The medication packages will have identical appearance, be of equal weight and be tamper-proof. Participants, clinical site staff, investigators, data management personnel and the study statistician will remain blinded to the treatment allocation throughout the course of the trial. The study statistician will only have access to the randomisation sequence when the trial follow-up is complete and the database has been locked. The correctness of assignment and of recording of this in the CRF will be checked for each participant by the external monitor. The external monitor will specifically check to see that medication packages are assigned in consecutive order.

The treatment allocation for each participant will be kept off-site by the study statistician in a locked cupboard to allow unblinding of an individual patient's treatment allocation under exceptional circumstances where a patient deteriorates clinically and it is deemed essential for optimal clinical care by the treating physician to know the actual treatment that he/she received. Any instances of unblinding will be recorded and reported on the case report form, in the database, and in the study report and publication.

In order that unblinding could occur if the independent statistician was unavailable, a set of sealed opaque numbered envelopes with the random allocation assignment of each participant in will be kept at the Groote Schuur Hospital pharmacy in a locked box. It will be visible if an envelope has been opened and this will be checked by the external monitor.

5. Selection and enrollment of participants

5.1. Approaching potential participants, information and informed consent

Patients who could fulfill the enrollment criteria will be approached by the study team about screening for the trial at the clinic where they are receiving TB treatment and asked to attend a screening visit. They will be provided with a screening informed consent form in the language they choose (Xhosa, Afrikaans or English). They will be given time to read the form or it will be read to them and they will have time to ask questions and have them answered by the study team. If they are agreeable to being screened then they will be asked to sign the screening informed consent form. All patients will be re-imbursed transport money (R150) for the screening visit.

Those patients who fulfill inclusion criteria upon screening will be invited to enroll in the clinical trial. This will involve an information sheet and an enrollment informed consent form. A member of the study team will spend at least 1 hour explaining the contents of the information sheet and answering questions the patient has. The information sheet will explain:

- the nature of their condition (HIV-associated TB)
- the risk that they may develop TB-IRIS when starting ART and what TB-IRIS is
- that we are conducting a clinical trial of prednisone versus placebo to prevent TB-IRIS
- the potential medical risks (specific side effects) and benefits of prednisone
- the design of the trial and that participants would randomly receive prednisone or placebo and that neither participant nor trial team will know allocation
- that the trial will involve close clinical follow-up and that every effort will be made to diagnose adverse events and manage them immediately
- the follow-up schedule of the clinical trial and exactly what treatment and investigations are involved
- that participants need to continue to take their TB treatment and will start standard antiretroviral therapy during the trial
- that the information from participants will be anonymised and confidentiality will be protected
- that the sponsor has set up mechanisms to provide indemnity for any harms due to the participation in the study and details of this are included in the informed consent form
- it will be made clear to them that they are free to decline participation in the trial without affecting the treatment of either their HIV or TB and that they similarly can withdraw at any time during the trial without affecting their treatment

Patients will then be invited to participate in the clinical trial, provided that they fulfill inclusion criteria and have no exclusion criteria. Patients will be given time to consider

participation and return on another day if they wish. They will also be given an opportunity to bring a family member to find out about the trial and assist them with their decision (we have found that many patients in our setting request this, from our experience during previous clinical trials). Patients will not be rushed into making a decision regarding participation and it will be emphasized throughout that participation is voluntary and that there are potential risks and potential benefits involved in participation, and that every effort will be made by the clinical trial team to manage any medical complications rapidly and effectively. Patients who agree to participate will be asked to sign the enrollment informed consent form and will only be enrolled once this is done. Both the patient information sheet and all the informed consent forms will be submitted to the University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee and to the other concerned ethics committees (in Belgium) and will not be used until approved by these committees. The informed consent process will be conducted in line with ICH-Good Clinical Practice guidelines and the Helsinki Declaration 2008. All involved staff will be GCP trained and accredited and the consent process will be done according to a written Standard Operating Procedure that will include all the points above. Training on this SOP will be done before the trial is started and ongoing training will occur throughout the study. Any issues that arise during the consenting process will be discussed with the PI in real time and with the chair of the Human Research Ethics Committee at UCT if necessary. In patients who are unable to write, informed consent will be taken in the presence of an independent witness. If the patient agrees to participation they will provide a thumbprint on the informed consent document and the witness will sign to confirm that informed consent has been taken. Patients who are not cognitively competent to provide informed consent will not be enrolled in the trial.

The following members of the study will be responsible for taking informed consent: the principal investigator, the study medical officer, the study nurse and/or the study counselor.

5.2. Study enrollment criteria

5.2.1. Inclusion criteria

1) HIV–infected

HIV infection will be confirmed by two different rapid tests (as per South African national Department of Health guidelines) and an HIV viral load test.

2) CD4 count $\leq 100/\mu\text{L}$

One CD4 count taken within 3 months prior to enrolment less than $100/\mu\text{L}$ will qualify, even if other CD4 counts are greater than $100/\mu\text{L}$

3) ART-naïve

Patients who report having been treated with triple drug or dual drug ART previously will be excluded. Single dose nevirapine or short term AZT monotherapy for PMTCT is not an exclusion.

4) Confirmed diagnosis of TB (smear, culture, Xpert MTB/RIF test or compatible histology) or strong clinical and radiological evidence of TB with symptomatic response to TB treatment

- 5) On TB treatment for less than 30 days prior to study entry.
- 6) Eligible for ART and patient consents to starting ART within 30 days of starting TB treatment.
- 7) Written informed consent for trial

5.2.2. Exclusion criteria

- 1) Kaposi's sarcoma (KS)

A thorough examination for KS lesions will be performed and any suspicious lesion will be biopsied. Any history of treatment for KS will also be an exclusion.

- 2) Pregnant

All female participants of child-bearing potential will have a pregnancy test performed prior to enrollment and will be counseled to use two reliable methods of contraception for the duration of the trial.

- 3) <18 years old

- 4) TB meningitis or tuberculoma at TB diagnosis

- 5) **Clinical** syndrome of pericardial TB at TB diagnosis (a pericardial effusion noted on ultrasound scan alone is not an exclusion criterion)

- 6) Rifampicin-resistant TB diagnosed by Xpert MTB/RIF test or a drug susceptibility test performed on a culture isolate.

- 7) On corticosteroids for another indication or on any other immunosuppressive medication within the past 7 days.

- 8) Uncontrolled diabetes mellitus

- 9) The following abnormal laboratory values:

Alanine aminotransferase > 200 IU/l

Absolute neutrophil count < 500/mm³

- 10) Not on standard intensive phase TB treatment (Rifampicin, isoniazid, pyrazinamide and ethambutol)

- 11) Poor clinical response to TB treatment prior to ART as judged by the clinical investigators.

- 12) Hepatitis B surface antigen positive

6. Study Endpoints

6.1. Primary efficacy endpoint

The development of paradoxical TB-IRIS within 12 weeks of starting ART (defined using the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition [2]).

Panel 2: Case definition for paradoxical tuberculosis-associated IRIS

There are three components to this case definition:

(A) Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of tuberculosis: the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴
- Initial response to tuberculosis treatment: the patient's condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinitiation, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss
- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART—antiretroviral therapy. IRIS—immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as "probable paradoxical tuberculosis-associated IRIS". In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as "paradoxical tuberculosis-associated IRIS" cases.

INSHI consensus case definition for paradoxical TB-IRIS

6.2. Secondary efficacy endpoints

- 1) Time to TB-IRIS event (from start of ART to onset of IRIS symptoms in days)
- 2) Severity of TB-IRIS events (defined by the following: need for hospitalisation for IRIS, C-reactive protein, neurological involvement)
- 3) Duration of TB-IRIS event (from onset of symptoms/signs to resolution of TB-IRIS)

symptoms and signs)

4) Mortality attributed to TB and TB-IRIS

5) All-cause mortality

6) Composite endpoint of death, hospitalization, or hepatotoxicity (using the protocol-specified definition of Grade 3 or 4 increase in ALT or bilirubin).

7) Other (non-TB) IRIS events

8) Quality of life assessment (measured using PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score)

9) Adverse events and severe adverse events ascribed to TB treatment, ART or co-trimoxazole. This will include a pre-specified analysis of drug-induced liver injury and drug rash. This assessment will include the number of treatment interruptions for drug adverse events.

10) Discontinuation of either ART or TB treatment for > 5 days due to adverse events.

11) Number of hospitalizations and total days hospitalized

6.3. Secondary safety and tolerability endpoints

1) Corticosteroid-associated adverse events, classified by severity and relation to study drug. These will include hypertension, hyperglycaemia, hypomania/mania (diagnosed by psychiatrist), depression (diagnosed by psychiatrist), acne, epigastric pain, upper gastrointestinal bleeding, Cushingoid features, new oedema and avascular bone necrosis.

2) Laboratory safety data: glucose, full blood count and electrolytes

3) Other infections (AIDS-related, bacterial, fungal and viral) and malignancies (Kaposi's sarcoma)

4) All grade 1, 2, 3 and 4 adverse events (clinical and laboratory using the ACTG grading system)

6.4. Quality of Life (QoL) assessments

As a secondary outcome measure we will perform health-related quality of life measures of the participants included in the trial at week 0, 4 and 12. The following measurements will be performed: PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score. See section 21 for details of this QoL substudy.

7. Study medication

7.1. Prednisone and identical placebo

Study medication will be prednisone tablets (5mg) or identical placebo tablets. The study medication will be prepared by the Gulf Drug Company, Durban, South Africa. This generic pharmaceutical company supplies prednisone to the government hospital pharmacies in South Africa (marketed as Trolic 5mg tablets). They manufactured prednisone and placebo tablets for our previous trial of prednisone for TB-IRIS treatment. We have estimated a requirement of 21 000 placebo tablets and 21 000 prednisone tablets.

Participants will receive 40mg (8 tablets) daily of 5mg prednisone (or identical placebo) for 14 days followed immediately by 20mg daily (4 tablets) daily of 5mg prednisone (or identical

placebo) for 14 days. Participants will start the study medication on the same day that they take their first dose of antiretroviral therapy. The total duration of study medication will be 28 days.

7.2. Manufacture and delivery of study medication

The study medication will be manufactured by the Gulf Drug Company in Durban. The University of Cape Town will be charged for the manufacture of the study medication and a quote has been obtained. The Gulf Drug Company will deliver the medication to the Groote Schuur Hospital pharmacy (an independent pharmacy) as one container of prednisone and one container of placebo. The Groote Schuur Hospital pharmacist will sign for receipt of the medication.

7.3. Packaging of study medication

The randomization sequence for the trial will be generated by an independent statistician according to a blocked (block size 8), 1:1 randomisation sequence. This will be sent to the independent pharmacist at Groote Schuur Hospital. This pharmacist will pack the prednisone and placebo tablets into sequentially labeled medicine containers labeled from participant 1 to 240. There will be two medicine packages for each participant:

- 1) **Day 0-14:** 8 x 14 tablets = 112 tablets
- 2) **Day 15-28:** 4 x 14 tablets = 56 tablets

This process will be undertaken independent of any investigators or study team members who will remain blinded with respect the randomization sequence and the packaging of the study medication. Once all the study medication has been packaged it will be transferred to the clinical trial site pharmacy and the on-site pharmacist will sign for receipt of the study medication.

7.4. Storage at the clinic

The study medication will be stored in the clinic research pharmacy which is locked by the pharmacist on site when she is not present. It will be stored at room temperature (at or below 25 degrees Celsius in adherence with manufacturers' instructions) and a temperature log will be kept in the pharmacy. Study drug management and accountability will be described in a specific SOP.

7.5. Dispensing of medication

Medication will be dispensed according to patient enrollment number by the study pharmacist from patient 1 to 240. The pharmacist will keep a log of all study medication dispensed recording date, patient number and initials and packet number.

Study medication will be dispensed on two occasions:

- At Week 0 visit, the first 14 days medications will be dispensed
- At Week 2 visit, the 15-28 days medication will be dispensed

At the week 1, 2 and 4 visit participants' adherence to study medication will be checked by means of pill counts and the number of tablets returned will be recorded on the CRF. Participants will be asked to bring back their medication packet for this purpose.

7.6. Maintenance of randomization concealment

Participants, clinical site staff, investigators, data management personnel and the study statistician will remain blinded to the treatment allocation throughout the course of the trial. The randomization sequence will be kept away from the trial site securely by the independent statistician. This statistician will store an electronic copy and a hard copy. A printed and sealed copy of this randomization sequence will also be given to the Sponsor representative (Deputy Dean of Research, Faculty of Health Sciences) for storage to ensure a back-up copy is available. At the trial site the placebo and prednisone tablets and packaging will be identical with no markings or labeling to differentiate which medication containers contain prednisone or placebo. Only once the trial database is locked will the randomization sequence be made available to the trial team.

In the event of unblinding of an individual participant being required in the opinion of the attending clinician (see section 13.10.) the independent statistician will be contacted for this information. In order that unblinding could occur if the independent statistician was unavailable, a set of sealed opaque numbered envelopes with the random allocation assignment of each participant in will be kept at the Groote Schuur Hospital pharmacy in a locked box. It will be visible when an envelope has been opened and this will be checked by the external study monitor.

7.7. Expiration and re-ordering of medication

The expiration date of the study medication will be noted upon delivery. It is planned that the product will have a shelf life that covers the entire study duration. The manufacturers have confirmed a shelf-life of 24 months for prednisone. This will be specified in the order. However, we will have a plan to cover the contingency that product reaches its expiry date. Six months prior to the expiration date, additional prednisone and placebo tablets will be ordered from the Gulf Drug Company. The same process will be followed with respect to delivery, blinding procedures, packaging and storage.

7.8. Destruction of medication

At the conclusion of the trial or when study medication has expired, study medication will be destroyed by the study pharmacist, according to the local regulations, and a record of study medication final accountability and destruction will be made and filed.

7.9. Prohibited concomitant medication

The following concomitant medications are prohibited during the 4 weeks that participants are dispensed study medication: any non-steroidal anti-inflammatory drug (NSAID), any systemic corticosteroid medication or any other immunosuppressive medication or chemotherapy. Clinical investigators will not prescribe these medications and we will

communicate with the participants' primary care clinics that these are prohibited medications during this 4 week period.

8. Antiretroviral therapy (ART)

8.1. ART regimen

ART will be provided according to South African Department of Health guidelines. All of the patients who fulfill criteria for entering this trial are eligible to start ART according to these guidelines and willingness to initiate ART is an inclusion criterion for the trial. The trial site is a Department of Health accredited ART site and patients who are eligible receive ART free of charge at this clinic. Participants will thus be provided with ART from the clinic ART stock and will also be assessed and managed in their ART clinic according to DOH guidelines.

First line ART in Department of Health clinics in South Africa is tenofovir 300mg daily plus emtricitabine 200mg daily (or lamivudine 300mg daily) plus an NNRTI (nevirapine or efavirenz). It is advised that patients on rifampicin-based treatment for TB (as participants in this trial will be) receive efavirenz rather than nevirapine because of pharmacokinetic drug interactions.

The ART regimen for most participants in this trial will thus be:

Tenofovir (TDF) 300mg daily + Emtricitabine (FTC) 200mg daily (or Lamivudine (3TC) 300mg daily) + Efavirenz 600mg daily (all taken at night).

When available in the clinic the single tablet fixed dose combination of TDF/FTC/Efavirenz will be prescribed, when not available then the individual tablets of TDF, 3TC and Efavirenz will be prescribed. During the period that the FDC is introduced in South Africa it is likely that supply issues will not permit that it is always available to be prescribed.

No dose adjustment of the efavirenz is advised in patients on rifampicin-based TB treatment because virological outcomes have been shown to be unaffected by concomitant rifampicin with ART containing efavirenz 600mg daily in our setting [44, 45].

There may however be circumstances in which either one of the tenofovir or efavirenz is contra-indicated and is substituted by an alternative that is available in government clinics. Such substitutions will be discussed between the ART clinic doctor or nurse and the study team to arrive at a consensus decision. The scenarios in which these substitutions may occur and the alternatives are outlined in Table below:

| Clinical circumstance | Drug to be substituted | Alternative |
|---|------------------------|--|
| Renal impairment (Creatinine clearance < 50ml/min) | Tenofovir | Zidovudine (provided haemoglobin > 9g/dl and neutrophil count > 1.0 x 10 ⁹ /ml) OR |

| | | |
|--|-----------|---|
| | | stavudine (relatively safe for < 6 months) |
| Active psychiatric disease or shift work | Efavirenz | Nevirapine (provided CD4 < 250 cells/ μ l in women or < 400 cells/ μ l in men) OR Lopinavir/ritonavir (needs to be double-dosed if on rifampicin) |

8.2. ART substitutions for toxicity

For the acute management of drug reactions see section 12.4. In the event that a drug reaction or toxicity is attributed to one of the ART drugs then a single drug substitution will be made. Toxicities or reactions that may result in substitutions are listed in the Table below.

| Drug reaction/toxicity | ART drug implicated | Alternative ART drug for substitution |
|-------------------------------|----------------------------|---|
| Renal impairment | Tenofovir | Zidovudine (or stavudine) |
| Red cell aplasia | Lamivudine | Stavudine |
| Neuropsychiatric side effects | Efavirenz | Nevirapine (or double-dose lopinavir/ritonavir if nevirapine is contra-indicated) |
| Hepatitis | Efavirenz | Double-dose lopinavir/ritonavir |

8.3. ART switches for virological failure

No switches for virological failure will be made in the first 3 months of ART (ie. during the study follow-up period). In the Department of Health ART guidelines it is advised that the first HIV viral load is measured at 4-6 months after starting ART. The indication for switching to second line ART is an HIV viral load > 1000 copies/ml on two separate measurements performed 3 months apart. The viral load testing done during this trial is thus before HIV viral load testing is advised in the national guidelines and is before virological suppression would be expected in patients with high baseline HIV viral loads. Because of this, patients detected with primary virological failure* by our HIV viral load testing at 4 weeks would not be eligible to switch to second line ART based on Department of Health guidelines. We will, however, make all HIV viral load results available to the ART clinic clinician and alert the clinician to the concern if a patient has primary virological failure so that it is ensured that they have a repeat HIV viral load at 4 months on ART.

*Primary virological failure defined as the failure to achieve a > 1 log drop of the viral load from baseline by 1 month on ART.

9. TB treatment

Patients with HIV infection and suspected TB in the South African public sector TB clinics are investigated with sputum for MTB/RIF Xpert and sputum smear initially, and if these are negative then culture and chest radiographs are performed. Investigations for extrapulmonary TB include needle aspirations of lymph nodes or pleural effusions or further investigations at a referral hospital.

TB treatment will be prescribed and monitored by the clinical staff in the local HIV-TB clinic. TB treatment will be prescribed according to South African Department of Health guidelines.

The TB treatment regimen for cases presumed or known to have drug susceptible TB over 8 years of age is:

| Pre-treatment body weight | Intensive phase 2 months Given 7 days a week | Continuation phase 4 months Given 7 days a week | |
|---------------------------|---|--|-----------------|
| | RHZE 150/75/400/275mg | RH 150/75mg | RH 300/150mg |
| 30-37kg | 2 tablets | 2 tablets | - |
| 38-54kg | 3 tablets | 3 tablets | - |
| 55-70kg | 4 tablets | - | 2 tablets |
| ≥71kg | 5 tablets | - | 2 tablets |

R=rifampicin, H=isoniazid, Z=pyrazinamide, E=ethambutol

Source: National Tuberculosis Control Programme of the Department of Health, 2009

For management of TB drug reactions and toxicities see section 12.4.

10. Study visits and evaluations

10.1. Schedule of events

| Study visit | Screening | Enrollment | Entry Week 0 | Week 1 | Week 2 | Week 4 | Week 8 | Week 12 | Un-scheduled visit |
|-----------------------------|---------------|-----------------|--------------|---------|----------|----------|----------|----------|--------------------|
| ART day | Not specified | Aim for -7 to 0 | 0 | 7 +/- 4 | 14 +/- 4 | 28 +/- 4 | 56 +/- 4 | 84 +/- 7 | Not specified |
| Document HIV status | X | | | | | | | | |
| Assess eligibility criteria | X | X | X | | | | | | |
| Screening ICF | X | | | | | | | | |
| Enrollment ICF | | X | | | | | | | |
| Study drug administration | | | X | | X | | | | |
| Past medical history | X | | | | | | | | |
| Current symptoms | X | X | X | X | X | X | X | X | X |

| | | | | | | | | | |
|--------------------------------------|---|---|---|---|---|---|---|---|-----------------|
| IRIS questions | | | | X | X | X | X | X | X |
| Adverse events assessment | X | X | X | X | X | X | X | X | X |
| Medication review | X | I | X | X | X | X | X | X | X |
| Karnofsky score | X | | X | X | X | X | X | X | X |
| Pill count | | | | X | X | X | | | |
| QOL assessments | | | X | | | X | | X | |
| Examination | X | I | X | X | X | X | X | X | X |
| Na,K,Cr,glucose | X | I | X | | X | X | | X | I |
| ALT, Alk Phos | X | I | X | | X | X | | X | I |
| FBC, diff | X | I | X | | X | X | | X | I |
| CRP | | I | X | | X | X | | X | I |
| CD4, HIV viral load | X | | | | | | | X | |
| HepBsAg | X | | | | | | | | |
| Serum CrAg | X | | | | | | | | |
| Urinary pregnancy test | X | I | I | I | I | I | I | I | I |
| Storage bloods and immunology assays | | | X | | X | X | | X | If IRIS suspect |
| Storage urine | | | X | | | | | X | If IRIS suspect |
| MTB/RIF Xpert, culture and DST | X | | | | | X | | X | |
| Initiate ART | | | X | | | | | | |

I = If clinically indicated

ICF = Informed consent form

Study visits will occur at screening for the study, at an enrollment visit, week 0 (the day the patient starts ART medication), week 1, week 2, week 4, week 8 and week 12. A window period of +/- 4 days will be allowed for all visits apart from the week 0 visit (no window) and week 12 (where a window of +/- 7 days will be allowed). If visits do not occur in these windows then a “missed visit” will be recorded.

Patients will be telephoned before each visit by the nurse or counselor to remind them of the visit. Patients will be re-imbursed R150 for each scheduled visit towards transport and meal costs. Patients who are screened but not recruited will also be re-imbursed for this visit. This will be stated in the informed consent forms. If patients are lost to follow up then an attempt to ascertain their outcome at 12 weeks will be made telephonically. At each study visit the research team will check that the patient has sufficient supply of TB medication (or is receiving daily from TB clinic) and ART and other prescribed medication.

A maximum of 40ml blood will be drawn at any study visit. Only trained personnel will perform phlebotomy. Patients who have symptomatic anaemia (Hb<8g/dL with symptoms attributable to anaemia) will not have more than 30ml of blood drawn at any given visit.

Screening visit

Patients identified in the HIV-TB clinic (or referred there specifically for screening for this study, as mentioned in 3.8) who potentially meet enrollment criteria will be referred to the study team. Patients should have been on TB treatment for ≤ 21 days at this screening visit. Patients will be briefly informed about the study and written informed consent will be taken from the patient to screen them for eligibility for the clinical trial with a screening visit informed consent form. If patients sign screening consent they will be assessed with the inclusion and exclusion criteria for the trial. The HIV status of the patient will also be documented by the study team at this visit. In all patients being screened for the trial, regardless of whether they have had a previous positive HIV test, HIV testing will be performed with appropriate counseling using two different rapid HIV tests. This is the standard of care in the clinic. If the results are discrepant or indeterminate blood will be sent to the National Health Laboratory Services laboratory for ELISA confirmation. In addition, as stated below an HIV viral load is performed at this visit, that will serve as confirmatory of the HIV diagnosis. The following study procedures will be performed (blood and urine will only be taken if patients fulfill inclusion criteria and have no exclusion criteria after notes review, history and examination):

- 1) Past medical history
- 2) Current drugs and drug allergies
- 3) Current symptoms
- 4) Physical examination
- 5) Chest radiograph (Only if there is no recent chest radiograph available for the clinical team to review and a chest radiograph is deemed necessary by clinical team. Note that all participants will have a chest radiograph performed at study entry visit)
- 6) HIV test as described above
- 7) Na, K, creatinine, glucose
- 8) ALT, alkaline phosphatase
- 9) Full blood count and differential
- 10) Serum cryptococcal antigen test (CrAg)
- 11) Hepatitis B surface antigen
- 12) CD4 count and HIV viral load
- 13) Sputum (and other biological samples where appropriate) for MTB/RIF Xpert, culture and DST (the main reason is to exclude rifampicin-resistant TB)
- 14) In female patients, urinary pregnancy test
- 15) All results done during TB diagnostic work-up and during TB treatment will be obtained from the National Health Laboratory Services laboratory database (including CD4 count and all TB diagnostic tests)
- 16) Karnofsky score
- 17) Assess adverse events

Patients will receive an ART counseling session at this visit and arrangements will be made for two further sessions prior to the potential Week 0 visit. Standard ART counseling practices will be followed. Patients will be invited to bring a relative or “treatment buddy” to ART counseling sessions. In particular ART side effects, need for 100% adherence, lifelong need for treatment and TB-IRIS will be discussed in these sessions. Patients will be asked to return for laboratory results and enrollment visit if eligible and provided a visit date (they will

receive re-imburement for transport of R150 for the screening visit and this follow-up visit whether they are eligible for the trial or not). They will be invited to bring a relative along to the enrollment visit. At this screening visit a target date for starting ART will be set in liaison with the ART clinic staff. The participant will be entered in the screening log at this visit. If the participant fulfills clinical criteria for enrollment they will be given a patient information sheet with information about the trial to read (or it will be read to them) in the language of their choice. They will be able to take this sheet home with them if they wish.

Enrollment visit

The timing of this visit will occur between the Screening visit and the Study Entry (Week 0) visit and the proximity to either will depend on how long the patients has been on TB treatment and when the clinic plan to start ART. The aim is for this visit to be no more than 7 days before the Study Entry visit. Under exceptional circumstances, this visit may be on the same day as the Study Entry visit if patient is to start ART on this day. The main purpose of this visit is to review the laboratory results from the Screening visit to ensure all laboratory inclusion criteria are met and no laboratory exclusion criteria are present. Clinical inclusion and exclusion criteria will also be reviewed. The enrollment CRF will be completed. If patients fulfill all inclusion criteria and have no exclusion criteria they will be invited to participate in the clinical trial.

The patient information sheet will be reviewed with the patient. If they are unable to read then the sheet will be read to them by a counselor if this was not yet done. They will be given an opportunity to ask questions. If they have brought a relative along, the relative will be included in the discussion if the patient requests this. This process will not take less than one hour. If they are willing to provide informed consent and in the judgement of the study doctor they have full capacity to make an informed and voluntary decision, they will sign the enrollment informed consent document. If they are unable to write, they will provide a fingerprint and an independent witness will witness the informed consent process and the fingerprinting of the informed consent document and sign and date as witness.

A full clinical assessment is not planned at this visit, but if patients report new symptoms then they will be examined and investigated and managed appropriately.

The participant will be entered in the enrollment log at this visit.

Study Entry / Week 0 visit (Day 0)

This visit must be when the patient has been on TB treatment for ≤ 28 days.

All trial inclusion and exclusion criteria will be rechecked and this will be documented on the CRF, prior to the patient formally entering the study. If a patient is fully eligible, he/she will be given the next sequential enrollment number and receive the corresponding blinded study medication. Procedures to be performed:

- 1) Current symptoms
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase

- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) Chest radiograph (for all participants)
- 9) Quality of life assessments
- 10) Karnofsky score
- 11) Assess adverse events
- 12) Issue Day 1-14 study medication

A randomization number (corresponding to the next sequential study medication container) will be assigned at this visit and documented on the enrollment log.

Week 1 visit (Day 7 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) ART and study drug pill count to assess adherence

Week 2 visit (Day 14 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) ART and study drug pill count to assess adherence
- 9) Karnofsky score
- 10) Assess adverse events
- 11) Issue Day 15-28 study medication

Week 4 visit (Day 28 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) ART and study drug pill count to assess adherence
- 9) Sputum for MTB/RIF Xpert, culture and DST (to assess the effect of corticosteroids on sputum conversion)
- 10) Quality of life assessments
- 11) Karnofsky score
- 12) Assess adverse events

Week 8 visit (Day 56 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen

- 2) Physical examination
- 3) ART and study drug pill count to assess adherence
- 4) Karnofsky score
- 5) Assess adverse events

Week 12 visit (Day 84 +/- 7 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) CD4 and HIV viral load
- 8) Bloods for immunological assays
- 9) ART and study drug pill count to assess adherence
- 10) Sputum for MTB/RIF Xpert, culture and DST (to assess the effect of corticosteroids on sputum conversion)
- 11) Quality of life assessments
- 12) Karnofsky score
- 13) Assess adverse events
- 14) Refer back to HIV-TB clinical service if clinically stable

Unscheduled visits/sick visits

Participants will be encouraged to attend the clinic for unscheduled visits if they experience symptomatic deterioration. They will also be provided with the mobile number of the study team that will be answered 7 days a week. They will be assessed by the study doctor. If the reason for deterioration is suspected TB-IRIS they will be investigated and managed as described in section 13.2. If drug reaction, such as drug-induced liver injury, is suspected they will be managed as described in section 13.4. Other clinical problems will be managed as appropriate. If patients are sick enough to require hospital admission for investigation and management they will be referred to the local district hospital (Khayelitsha District Hospital) and their clinical condition will be closely monitored there by the study team while they are managed by the medical staff on the inpatient service.

Post-trial visits

For patients who have ongoing TB-IRIS or whose clinical condition is not stable at the 12 week visit further visits will occur for clinical management reasons and to ascertain the end date for TB-IRIS. These will be recorded as unscheduled (post-trial) visits.

Research blood and urine samples for storage

In the enrollment informed consent form patients will be specifically asked to agree to or decline permission for storage of blood and urine samples for later research into HIV, TB and TB-IRIS biomarkers and pathogenesis. They will be able to decline permission for storage without affecting participation in the trial. Any use of stored samples for future research will require specific permission from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee. A separate consent form will be used to obtain consent for taking a blood sample for genetic testing.

The blood stored will be plasma and PBMC (sodium heparin tube, for cytokine analysis and flow cytometry), DNA (one time point at week 0, citrate tube) and RNA samples (Tempus tube). The collection of the DNA will be deferred if the patient has had a recent blood transfusion. The timing of the other bloods will be: Week 0, Week 2, Week 4, Week 12 and suspected TB-IRIS sick visit.

For trial participants that decline storage consent, their study folder will be marked at study entry with a sticker stating “No storage” and storage bloods will not be collected on these participants.

Participants who do not start ART

It is possible that certain patients will be seen at the Week 0 visit will be prescribed ART but will not start the ART medication. In this event the study medication will be stopped as soon as investigators becomes aware, but follow-up will continue and patients will be included in the intent to treat analysis.

Participants who do not attend Study Entry visit within 28 days of starting TB treatment

If a patient signs the enrollment informed consent but fails to attend their Study Entry visit on the planned date, they will be phoned to reschedule this visit. However, if they do not attend for the Study Entry visit within 30 days of starting TB treatment then they will not receive study medication and they will not receive a randomization number, but clinical follow-up may occur as per protocol Addendum 22.5. They will not be included in the intent to treat analysis. The same applies to patients who sign enrollment consent form and are then found to have an exclusion criterion prior to randomization.

Patient withdrawal from trial

Patients are free to withdraw from the trial at any point as stated in the informed consent form. Those patients withdrawing from the trial will be encouraged to continue ART and TB treatment at their local clinic.

11. Definitions

11.1. Paradoxical TB-IRIS will be defined using the International Network for the Study of HIV-associated IRIS (INSHI) case definition. See section 6.1.

11.2. WHO Stage 4 conditions (WHO 2006 revision)

- HIV wasting syndrome
- pneumocystis pneumonia
- recurrent severe bacterial pneumonia
- chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)
- oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- extrapulmonary TB
- Kaposi's sarcoma
- cytomegalovirus infection (retinitis or infection of other organs)

- central nervous system toxoplasmosis
- HIV encephalopathy
- extrapulmonary cryptococcosis including meningitis
- disseminated non-tuberculous mycobacteria infection
- progressive multifocal leukoencephalopathy
- chronic cryptosporidiosis
- chronic isosporiasis
- disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis)
- recurrent septicaemia (including non-typhoidal salmonella)
- lymphoma (cerebral or B cell non-Hodgkin)
- invasive cervical carcinoma
- atypical disseminated leishmaniasis
- symptomatic HIV-associated nephropathy
- symptomatic HIV-associated cardiomyopathy.

11.3. Drug-induced liver injury (DILI)

DILI will be defined using the ACTG grading system (see section 22.2: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009). We will specifically classify Grade 2, 3 or 4 elevations of ALT and total bilirubin as DILI: ALT elevation 2.6-5 x upper limit of normal is grade 2, 5.1-10 x upper limit of normal is grade 3 and > 10 x upper limit of normal is grade 4. Total bilirubin elevation 1.6-2.5 x upper limit of normal is grade 2, 2.6-5 x upper limit of normal is grade 3 and > 5 x upper limit of normal is grade 4.

We will further differentiate clinical hepatitis (these elevations together with symptoms of hepatitis: nausea, vomiting, right upper quadrant pain or lethargy) from all biochemical hepatitis (any elevation above these levels regardless of whether symptomatic or asymptomatic).

11.4. Drug rash

A drug rash will be defined as any rash the onset of which is after the initiation of a drug or drugs, the morphology and natural history of which is compatible with a drug rash. Cases in which the aetiology is unclear will be reviewed by a Dermatologist (either seeing the patient or digital photographs taken of the rash).

11.5. Corticosteroid-associated adverse events

Clinical symptoms and signs that could possibly be attributed to corticosteroids side effects will be documented and recorded as corticosteroid-associated adverse events. This will include: new hypertension (resting BP: systolic > 160 mmHg or diastolic >100mmHg), new poor BP control in known hypertensive (who reports adherence to anti-hypertensive therapy, using same thresholds for systolic and diastolic BP), hyperglycaemia (random glucose > 11.1 mmol/l), new oedema, hypomania/mania (diagnosed by psychiatrist), depression (diagnosed by psychiatrist), Cushingoid features or habitus (in the opinion of the clinical investigator),

acne, gastritis symptoms (epigastric pain), upper gastro-intestinal bleeding or avascular bone necrosis. Avascular necrosis will be investigated if patients present with symptoms of bone pain.

Only features that develop while patients are on the study drug or for the 4 weeks afterwards will be classified as corticosteroid side effects.

Infections will not be recorded under corticosteroid side effects but will be recorded and analysed separately.

12. Laboratory evaluation

All laboratory tests (blood and sputum) will be performed at the National Health Laboratory Services laboratories at Green Point or Groote Schuur Hospital. These laboratories are SANAS (South African National Accreditation System) accredited and experienced in performing investigations for clinical research. Hard copy reports of all results will be forwarded to the clinical trial team as soon as the investigation result is available. These will be signed and dated by the trial team. If there is an abnormality then appropriate action will be taken (eg. recalling patient) and this will be documented on the hard copy result. All abnormal investigation results will be communicated to the TB and ART clinic staff.

13. Clinical Management

13.1. Co-trimoxazole prophylaxis

All patients on the clinical trial are eligible for co-trimoxazole primary prophylaxis, unless they are known to have a sulphonamide allergy. This is because all eligible study patients are HIV-infected with CD4 < 100 cells/ μ l and have WHO stage 3 or 4 disease. The co-trimoxazole dose used for primary prophylaxis will be 960mg daily (2 tablets). In those patients who cannot take co-trimoxazole or who develop an allergic reaction while on co-trimoxazole then dapsone 100mg daily will be considered as an alternative. In cases where patients have had life-threatening co-trimoxazole reactions (eg. Stevens Johnson syndrome) then dapsone will not be used as there a small risk of cross-reactivity.

13.2. Management of suspected TB-IRIS

In patients who deteriorate with clinical or radiographic features of TB after starting ART paradoxical TB-IRIS will be a consideration. This will include patients who develop new or recurrent TB symptoms or fever, enlarging lymph nodes or worsening chest radiograph pulmonary infiltrates, enlarging effusions or TB neurological features. At any such deterioration suggestive of paradoxical TB-IRIS (at a scheduled or unscheduled sick visit) the following investigations will be performed:

- 1) Bacterial blood culture
- 2) Creatinine/electrolytes/glucose, liver functions, full blood count/differential, CRP, serum CrAg (cryptococcal antigen test)
- 3) Sputum and other biological samples for MTB/RIF Xpert, culture and DST

- 5) Chest radiograph
- 6) Other investigations according to clinical presentation (eg. abdominal ultrasound, lumbar puncture, CT head)

The principal aim of these investigations is to exclude alternative causes for clinical deterioration as the diagnosis of paradoxical TB-IRIS is a diagnosis of exclusion. In addition, the adherence of patients to ART and TB medication will be checked by means of an interview with the patient and treatment buddy, and counting pills in the pill container and checking with the TB clinic staff.

If paradoxical TB-IRIS is diagnosed (fulfilling INSHI case definition for paradoxical TB-IRIS), open label corticosteroids will be prescribed for IRIS treatment at physician discretion. Study drug will be stopped on the day the decision is made to start open label corticosteroids. The decision to commence corticosteroids to treat TB-IRIS and exact timing is an individualized one that is based on physician discretion and takes into account the severity of symptoms and the confidence with which alternative causes for clinical deterioration have been excluded.

The prednisone starting dose to treat TB-IRIS will be 1.5mg/kg/day and this will be reduced according to clinical response. In patients with central nervous system TB-IRIS the initial corticosteroid used may be dexamethasone intravenously.

13.3. Management of new opportunistic infections or malignancies

If patients are diagnosed with a new opportunistic infection or malignancy while on the trial they will be referred to the appropriate level of care and management will be according to national Department of Health guidelines for the treatment of opportunistic infections. For example, patients with cryptococcal meningitis will be treated with amphotericin B followed by fluconazole.

When a new WHO stage 4 opportunistic infection or malignancy is diagnosed while patients are on study medication this will be an indication for stopping study medication. For certain opportunistic infections it may be indicated to start corticosteroid therapy (eg. pneumocystis pneumonia with respiratory failure). This decision will be taken by the attending clinician and open label corticosteroid therapy will be documented.

13.4. Management of drug reactions

If TB drug-induced liver injury occurs during follow-up cases will be managed according to the local clinical guidelines (Western Cape Academic Hospitals Antimicrobial Recommendations). These guidelines are based on the American Thoracic Society guidelines for management of TB drug hepatotoxicity [46]:

Hepatotoxicity

TB drug-induced hepatitis is over-diagnosed: the case definition is transaminases more than 5-fold elevated or more than 3-fold elevated with symptoms/jaundice. Antituberculous therapy should be discontinued. The basis for the TB diagnosis should be reviewed. If the grounds for diagnosing TB were reasonable then commence three antituberculous drugs with low/no hepatotoxic potential (see background therapy below). Selected patients may then be rechallenged once symptoms of hepatitis have resolved, bilirubin levels return to normal and transaminases have decreased to <100. Rechallenge is **NOT** recommended for those who have had fulminant hepatitis (defined as hepatic encephalopathy with coagulopathy).

The rechallenge regimen of the American Thoracic Society (Am J Respir Crit Care Med 2006;174:935–52) have been followed as these are simple and quick. Rechallenge with PZA was previously not recommended, but a recent trial has shown that most patients tolerate it. PZA rechallenge should be considered in patients with severe TB (e.g. miliary, meningitis) or with drug resistance. Transaminase levels, especially ALT, should be monitored frequently (e.g. three times weekly) during rechallenge and every two weeks for a month following rechallenge.

If possible all patients with a drug induced liver injury should have their TB isolates sent for drug susceptibility testing. Do not rechallenge with an agent to which the isolate is resistant.

| | | |
|----------------------|--------------------|--|
| rechallenge regimen: | Background therapy | Ethambutol, streptomycin and moxifloxacin. |
| | day 1 | Rifampicin 450 or 600 mg daily depending on weight |
| | day 3 | Check ALT |
| | day 4 | Add INH 300mg daily |
| | day 7 | Check ALT |
| | day 8 | Consider PZA rechallenge (see text) |

NB: Duration of therapy should be individualised after rechallenge – consult ID for advice. The following are guidelines:

- Pyrazinamide not rechallenged/not tolerated: stop moxifloxacin and streptomycin, continue isoniazid, rifampicin and ethambutol for total duration 9 months
- Rifampicin not tolerated: continue streptomycin (for 2 months) and moxifloxacin, isoniazid, and ethambutol for total duration of 18 months
- Isoniazid not tolerated: stop moxifloxacin and streptomycin, add ethionamide (if tolerated – otherwise use moxifloxacin) to rifampicin and ethambutol for total duration 12 months

In addition, if significant liver impairment occurs or patients have symptoms of hepatitis with

liver function derangements then adjustments will be made to the ART regimen (omitting efavirenz and considering replacing it with an alternative ART agent, or stopping all ART if acute liver failure develops). In there is significant DILI co-trimoxazole and other potential hepatotoxic medication will also be stopped. Rechallenge of ART and other potential hepatotoxins will be considered and rechallenge will generally be done after rechallenge of TB medication. Management of each case will be individualized and discussed with the PI.

If patients develop drug rash on the trial this could be potentially related to co-trimoxazole (a frequent cause of drug rashes), efavirenz or one of the TB drugs. For very mild rashes they will be monitored with regular review and symptomatic therapy. In patients with more significant symptoms at presentation or who develop more severe clinical features (extensive rash, fever, systemic symptoms, blistering or desquamation, angio-oedema or mucosal involvement) all potential culprit drugs will be stopped immediately. Patients will be managed in consultation with the Groote Schuur Hospital Division of Dermatology either by sending digital photographs for an opinion or by referral of the patients for outpatient or inpatient management. Rechallenge of drugs after a drug rash will follow local guidelines.

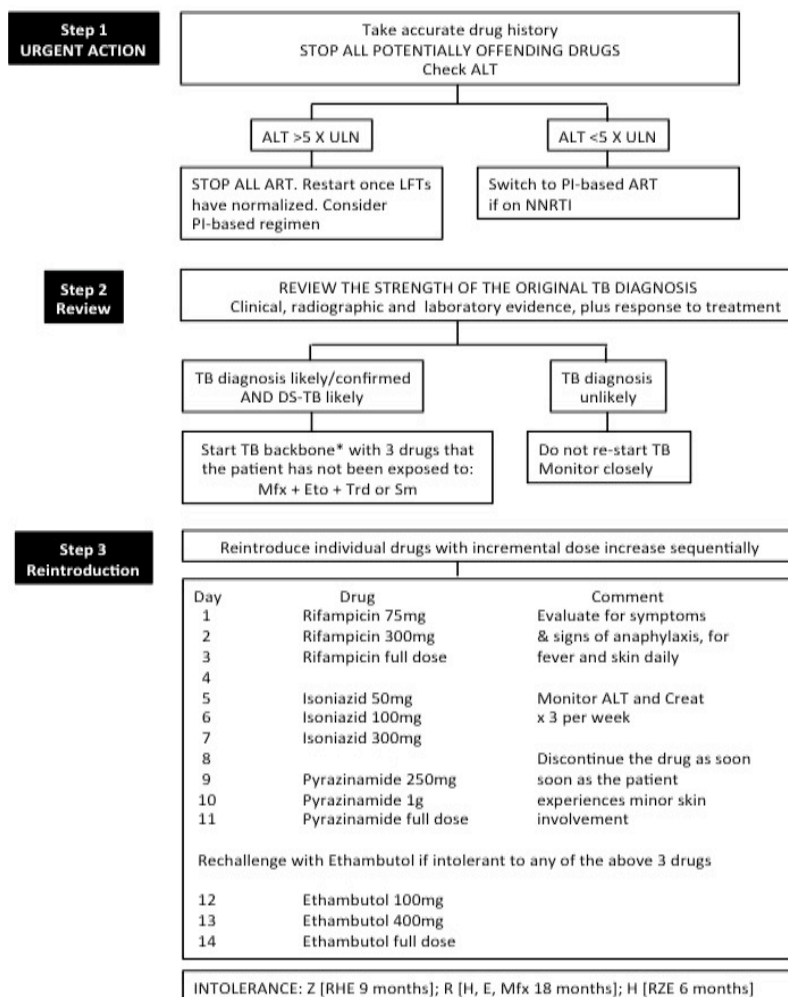
The Groote Schuur Hospital guidelines for the management of cutaneous drug reactions (CDRs) while on TB treatment will be followed:

Mild rash in isolation without systemic symptoms, mucosal involvement or abnormal LFTs can be treated with oral antihistamines and skin moisturizing agents, whilst continuing the drug under close observation.

Severe rashes involve mucosal membranes and are associated with systemic syndromes
Stevens-Johnson Syndrome - <10% skin detachment
Toxic Epidermal Necrolysis - >30% skin detachment
DRESS syndrome – Drug rash eosinophilia and systemic symptoms

The following algorithm will be used for management of severe CDR. If ART also needs to be stopped, then re-start after TB drug rechallenge is complete and consider a PI-based regimen should the patient have previously been on an NNRTI.

Management of severe CDR:



13.5. Management of drug-resistant TB

Any patient diagnosed with drug resistant TB (mono-resistance or multidrug resistant) will be managed according to South African National Drug-Resistant TB guidelines and referred for appropriate treatment and monitoring. Rifampicin-resistant TB is an exclusion criteria for the trial. If rifampicin-resistant TB is diagnosed only after a patient is enrolled on the trial then study medication will be stopped as soon as investigators become aware of the result, but follow-up will continue as planned to 12 weeks as per schedule of events.

13.6. Management of other medical co-morbidities

Participants who have medical co-morbidities or develop these while on the trial will be managed according to South African national Department of Health guidelines for these conditions.

13.7. Contraception and safe sex advice

Pregnancy is an exclusion criterion for this trial. Women of child-bearing potential will be encouraged to use two reliable methods of contraception (one being the use of condoms and the other a form of hormonal contraception) to avoid pregnancy for at least the duration of the trial because they have advanced HIV and are on treatment for active tuberculosis. Those who decline hormonal contraception will not be excluded from the trial. All patients will receive counseling regarding condoms to avoid HIV transmission.

If a female participant falls pregnant during the study, study medication will be stopped. Study follow-up will continue for the 12 weeks as planned and she will be immediately referred for appropriate antenatal care.

13.8. Interruptions in ART

When participants interrupt ART for drug toxicity, study drug will also be stopped on the same day. If participants interrupt ART due to non-adherence and this is reported then if the interruption is for five days or less then study drug will be continued. However, if ART interruption is more than 5 days then study drug will be stopped. Documentation of stops in study drug will be made in such instances.

13.9. Interruptions in study drug (by the participant)

If the participant interrupts the study drug then a discussion will occur regarding how long the interruption was for and the reasons the patient had for the interruption. If the patient interrupted because of side effects and does not want to restart then they will not be restarted and this will be documented. If the participant is agreeable to restarting study medication, then study medication will be restarted if the interruption was 5 days or less. If it was more than 5 days study medication will not be restarted. All interruptions will be fully documented.

In all cases participant follow-up will occur to week 12 unless consent for the trial is withdrawn by the participant.

13.10. Criteria for discontinuation of study medication (study follow-up will continue)

- Kaposi's sarcoma diagnosed
- Other new WHO stage 4 opportunistic condition diagnosed
- Rifampicin-resistant TB diagnosed
- Requirement for prohibited concomitant medication
- Development of TB-IRIS requiring open-label corticosteroid treatment (see section 13.2.)
- Pregnancy
- Request by participant to terminate
- Completion of 4 weeks of treatment as specified in protocol
- Clinical reasons believed life threatening by the clinical investigators, even if not addressed in the toxicity section of the protocol
- Patient interrupted ART for > 5 days
- Patient interrupts study medication > 5 days

The date of stopping study medication and reasons will be fully documented.

Unblinding of the randomization allocation of the participant would only occur under exceptional circumstances after a decision has been taken to stop study medication and when this information is deemed essential for ongoing clinical management by the attending clinician (see section 4).

13.11. Criteria for study discontinuation

- Request by participant to withdraw
- Completion of 12 weeks of trial as specified in the protocol
- At the discretion of the ethics committee or sponsor (in consultation with the investigators)

14. Statistical Methods

14.1. Sample size calculation

Assuming 35% cumulative incidence of TB-IRIS in the placebo arm and a 50% reduction in TB-IRIS in the corticosteroid arm (ie. to 17.5%) and requiring 80% power to test for the difference in TB-IRIS incidence at a two-sided significance level of 5%, the sample size required would be 110 in each arm. We will aim to recruit 240 patients assuming loss to follow-up of 10%. The estimate of 35% incidence of TB-IRIS in the placebo arm corresponds to the findings of Blanc et al (110/332 (33%) of participants who started ART in the early arm in this trial had TB-IRIS events (32)) and a study we conducted at Brooklyn Chest TB Hospital in Cape Town (42% IRIS incidence [47]) and reflects that these will be patients at high risk for TB-IRIS due to low CD4 counts and short interval between TB treatment and ART start. Lawn and colleagues demonstrated in a retrospective study conducted in Cape Town that low CD4 count and shorter interval between TB treatment and ART initiation were the strongest predictors of TB-IRIS. In their study 70% of patients with a CD4 count < 100/μl who started ART within 30 days of TB treatment developed TB-IRIS [48].

14.2. Statistical methods for analysis

The statistical analysis of the clinical trial will be performed according to a Statistical Analysis Plan which will be approved before database lock and study unblinding.

14.2.1 Primary Hypothesis

The primary hypothesis of the study is that the proportion of patients developing of paradoxical TB-IRIS within 12 weeks of starting ART, as determined by the INSHI case definition and confirmed by the endpoint review committee, will be lower in patients randomized to prednisone compared to patients randomized to placebo.

14.2.2. Analysis populations

The primary analysis and secondary efficacy analyses will be performed using an intention-to-treat approach. All patients randomized will be included under the treatment arm they were randomized to, irrespective of the fact that they actually received prednisone or started ART. In addition, an all-patients-treated approach will be performed excluding those participants who were randomized but never started study medication or ART. Safety analyses will be performed using the all-patients-treated approach.

14.2.3. Baseline characteristics

The number of patients screened and enrolled or excluded will be summarized by reason for exclusion. For the patients enrolled in the study, the number of patients discontinued or lost-to follow-up will be tabulated by reason and visit of study discontinuation. This information will be summarized in a CONSORT flow diagram.

Patients in each treatment group, overall and by site, will be described with respect to baseline characteristics. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics. The clinical importance of any imbalance will be noted but statistical tests of significance of imbalance in baseline characteristics will not be undertaken.

14.2.4. Primary analysis

The primary hypothesis will be tested comparing the proportion of patients with paradoxical IRIS among treatment groups using Fisher's exact test. In addition the relative risk of TB-IRIS will be estimated together with a 95% confidence interval.

A pre-specified subgroup analysis of those patients with a baseline CD4 count ≤ 50 cells/ μ L with respect to the primary and secondary outcomes will be performed.

14.2.5. Secondary and tertiary analyses

Secondary endpoints will be analyzed comparing study arms with the Fisher's exact test (for categorical data) or Wilcoxon rank sum test (for continuous data). Time to event analysis (constructing Kaplan Meier survival curves and comparing using the rank sum tests) will be used to compare time to IRIS event from start of ART by study arm.

As a tertiary analysis, we will assess possible risk factors for developing IRIS (such as baseline CD4 count, baseline HIV viral load, duration from TB treatment to ART initiation) in addition to treatment arm in a multiple logistic or Cox (time-to-event) regression model.

If we demonstrate that prednisone results in a reduction in the risk of developing paradoxical TB-IRIS we will perform a secondary cost-benefit analysis considering the cost of prednisone in relation to days of hospitalization, procedures and co-medication prescriptions averted.

14.2.6. Safety analyses

Adverse events will be coded following a standard dictionary (WHO-ART or Meddra) and the procedures will be clarified in a standard operating procedure. Safety endpoints, such as corticosteroid side effects, will be analyzed individually (eg. analysis comparing development of hyperglycaemia in each arm) and collectively (eg. analysis comparing all corticosteroid side effects that occurred in each arm).

15. Evaluation

15.1. Endpoint review committee

An endpoint review committee will be established to review the following endpoints

- Paradoxical TB-IRIS. The committee will adjudicate using the INSHI paradoxical TB-IRIS case definition as this will be used to define the primary endpoint.
- Severity of IRIS
- Drug toxicity (drug rashes and drug-induced liver injury)
- Cause of death
- New opportunistic infections

This committee will comprise investigators not active at the clinical site (Bob Colebunders, Lut Lynen) as well as William Burman an independent member of the TSC, and external reviewers. Two committees of three members will review endpoints. One committee will focus on IRIS endpoints and the other toxicity and deaths. The three reviewers in each committee will each independently adjudicate each event submitted to them. They will be supplied with the relevant CRFs and source document clinical notes relevant to the event and laboratory results. If they require further information this can be requested. Each committee member will decide independently regarding the classification of the event reported. If there is disagreement then consensus will be sought by email consultation. In the event that consensus cannot be reached then the committee members will vote and 2-1 vote will decide. This process will be recorded. Reviewers will be blinded to treatment arm allocation of the case they are adjudicating. The adjudication committee decision will be documented in the meeting minutes and each adjudication member will sign the final list of decisions. The data will be entered in a separate database using double-data entry.

16. Data handling and record keeping

16.1. Confidentiality

Information about trial participants will be kept confidential. All trial data will be de-identified and coded with a study number. The trial enrollment log and all signed ICFs and CRFs will be stored in a locked cabinet that is ICH-GCP compliant.

16.2. Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. For this trial, clinical data will be entered onto clinic visit sheets to provide a narrative record of the history and examination of the patient at each

visit. These sheets will constitute the clinical source document. Data from these sheets will be entered onto the CRFs at the time of that patients clinic visit and the clinician entering will check that the data on the source document and the CRF correspond before signing off and dating the CRF. For this trial, the laboratory result report will constitute the laboratory source document. When the laboratory result is received the study doctor will sign and date it and make a note of actions to be taken for any abnormal result. The laboratory results will be entered onto a corresponding laboratory CRF. The study team member entering this will check that the data on the source document (the laboratory result report form) and the CRF correspond before signing off and dating the CRF. Radiology reports will also constitute source documents and will be entered onto radiology CRFs and checked in the same way.

16.3. Case Report Forms (CRFs)

The case report forms (CRFs) are the primary data collection instruments for the trial. All data requested on the CRF will be recorded. If the data is missing this will be explained. All entries will be printed legibly in black ink. For corrections or to clarify illegible or uncertain entries, the original entry will be crossed out with a single line, signed and dated and the clarification or correction printed above the item. Clinical, radiological and laboratory data will be entered onto hard-copy case report forms (CRFs) from the source documents as described above. The clinical CRFs will be completed by the study doctor or nurse and the laboratory CRFs will be completed by the doctor, nurse or co-ordinator.

The following CRFs will be used

- Screening CRF
- Pre-enrollment CRF (optional)
- Informed consent CRF
- Enrollment CRF
- Week 0 visit clinical CRF
- Week 0 visit labs CRF
- Week 0 QOL assessment CRF
- Week 1 visit clinical CRF
- Week 1 visit labs CRF
- Week 2 visit clinical CRF
- Week 2 visit labs CRF
- Week 4 visit clinical CRF
- Week 4 visit labs CRF
- Week 4 QOL assessment CRF
- Week 8 visit clinical CRF
- Week 8 visit labs CRF
- Week 12 visit clinical CRF
- Week 12 visit labs CRF
- Week 12 QOL assessment CRF
- Unscheduled visit clinical CRF
- Unscheduled visit labs CRF
- IRIS assessment CRF
- IRIS management and outcome CRF
- Medications CRF

- Drug induced liver injury or rash CRF
- Adverse event CRF (will include documentation of opportunistic infections, ADR's and co-morbidities)
- Hospitalisation CRF
- Radiology CRF
- CRF for documentation of drug interruptions or stopping (ART, TB drugs or study drug), unblinding and open label corticosteroids

The CRFs will not contain the patient's name but will be identified using the study number and initials. There will be 2 study numbers: 1) a screening number for all patients screened and entered in the screening log; and 2) an enrollment number only for those enrolled in the trial and allocated a random assignment.

16.4. The electronic database (integerafrica)

For the purpose of monitoring and auditing the study, source documentation will consist of the laboratory report and the hard-copy CRFs developed and maintained by the investigator. Data recorded on source documents will be entered using electronic forms using an electronic data capture and management system provided by integerafrica. The hard-copy CRFs will be entered onto the electronic database by data-enterers at the clinic. integerafrica will design a tailor-made database with electronic forms that mirror the hard-copy CRFs. The electronic database will be a web-based research platform developed and maintained by integerafrica (www.integerafrica.org). The database will be ICH-GCP compliant. A subcontract will be awarded to integerafrica for the purpose of database design and maintenance. The portal is an easy-to-use web-enabled tool that assists medical researchers to track, analyze, and report on an individual case level. The platform allows researchers to enter data from multiple sites using one centralized database and provides tools for real-time data analysis. The architecture is developed according to clinical workflows and chronological procedures. Data are validated at the point of data entry. Multimedia data formats (chest radiographs, CT/MRI scans, ultrasound images or photographs) can be uploaded on the platform, allowing storage of complete clinical records. Training tools and support pathways are installed within the web-portal. The section Frequently Asked Questions (FAQ) serves as a guidance for the use of the platform. After login onto the TBIRIS website, the researchers have access to a variety of information regarding the study. In the download center, documents such as paper CRFs, informed consent forms, clinical guidelines, the study protocol, SOPs, study information sheets for patients, and patient education sheets can be made available. All data can be exported for further analysis in Excel format. The platform is available 24/7. The platform implements appropriate administrative, physical and technical safeguards to ensure the confidentiality, integrity and security of electronic health information. This database is being used in the EDCTP-funded eKhayaVac trial of the novel TB vaccine MVA85A being conducted in Khayelitsha (www.ekhayaovac.org), the Pan African Pulmonary Hypertension Cohort study (www.papuco.org) and the Registro Latinoamericano de Hipertensión Pulmonar (www.relahp.org).

integerafrica technology architecture

The main information technology design philosophy is an end-to-end solution approach, with specific focus on security, scalability, performance, and ease-of-use. The system is designed

to utilize the very best technology available to provide for a cost-effective and sustainable research environment in an African context. This is made possible by the extensive use of open-source technology. The system is built with hundreds of open-source components consisting of thousands of programs and millions of lines of code. The system architecture integrates five main components. 1) Devices: The system supports a wide variety of devices including mobile phones, tablet computers, laptops and desktop computers. Any computer with a browser and internet connection can use the system. 2) Browsers: The development of modern browsers have made it possible to develop and deliver much more powerful and secure web-based solutions for clients. The browser in many cases has replaced the reliance on propriety software and hardware. 3) Interfaces: The system supports any connection to the internet. It is designed for low bandwidth use without sacrificing a rich user experience. A connection speed as low as 512kbps is sufficient. The extensive use of caching technology throughout the system makes this possible. 4) User Experience: The user has access to a host of tools to manage research data with ease, securely. 5) Data Capture: The system allows for the secure capture of research data, geo-location data, imaging, videos, audio files and patient appointments. 6) Support: Training and support is provided in to study personnel. This is supplemented by online support in the form of training videos, frequently asked questions pages as well as a 24/7 online support request framework. 7) Security: Strict password policies, user access control logic, data encryption and SSL connection encryption are all in place to provide a secure user environment. 8) Data Storage: All data storage, as well as on- and off-site backup are provided. 9) Visualization: Real time project summary data is provided and well as basic analysis. All data can be visualized various formats including maps, charts and graphs. 10) Data Export: Data can be searched and selected from and exported in XLS or CSV format for further analysis, or sharing of results. 11) Reports: Custom reports can be generated and exported as PDF files or printed. 12) System Backend Firewall: As security is paramount, the first line of defense on web based systems is the use of various layers of firewall technology to guard against unauthorized intrusion. Both hardware and software firewalls are employed. 13) Server Security: Various industry standard, enterprise security policies are implemented and maintained across the various servers and server software components. 14) Cache and Proxy Servers: The advanced caching and proxy technology used for back-end optimization allows for fast, powerful and scalable solutions. Caching of various server components allows for the exponential increase in users, and fast responsive user experience managing research data online. 15) Web Server: The system utilizes Apache web-server technology that in 2009 was the first web-server to surpass the 100 million website milestone. 16) Application Server: On the application server side the system uses PHP and the Drupal framework to create fast and secure customized research solutions. The Drupal framework is a worldwide open source collaboration of more than 16000 developers in 228 countries. 17) Database Server: The system relies on the world's most popular database MySQL. 18) Backups: The backup policy extends to real-time RAID backups, On- and Off- Site data backups as well as database replication where needed.

The platform is accessible at www.TBIRIS.org and hosted at one of the world's most reliable hosting companies Hetzner online in Germany. Maintenance of the platform includes: daily server quick checks, daily security audits, and implementation of Drupal update releases, Drupal minor security updates, server security updates, server backups, annual SSL renewals. The main lines of our support services are: user and centre registration process guidance, user

personal data maintenance, online training for users on individual level using Skype, and online support requests for all the how-to items.

16.5. Data quality and completeness checking

Automated data checking mechanisms will be installed into the electronic database. Certain variables will be validated at the point of data entry, while data with reference values will be displayed in color codes according to reference range. Different field types (integer, decimal, range integer, range decimal, date, text field, text select list (drop down menu, multi-select, radio button for polar variables) will also be implemented to reduce errors at entry level.

The database is aware of the participant's position in the trial and will only allow certain procedures as per study protocol. Double electronic data entry will occur (hard-copy CRFs will be entered independently by two data enterers) and any discrepant entry will be flagged and/or the electronic record cannot be saved. Various list views allow for visual validation of data at entry and upon saving of the record. Every patient record is in a human readable format which allows the electronic record to be used as a electronic patient card. The database includes a complete check that gives a percentage of completeness of each record and the names of the missing fields. This information is available at the list view modules after login.

Additional data quality checking will be done (and documented) on every CRF by the study team. Any data errors or missing data detected will be corrected on both the hard-copy CRF and on the electronic database by the study doctor, who will sign and date changes on the hard-copy CRF and a system will be put in place to ensure all changes to the hard-copy CRFs are made onto the electronic database within one week of the change being made. Data entry and review will be co-ordinated by the Data Management Team at UCT with advice from the team at ITM (see section 20.1). Further details of the Data Management plan will be detailed in a specific standard operating procedure (SOP).

16.6. Records retention

The sponsor and the investigator will retain trial essential documents for at least 15 years after trial completion. The electronic database will be retained for an indefinite period.

17. Study monitoring, audit and inspection

17.1. Trial monitor

The trial will be monitored by a monitor that is subcontracted by the sponsor UCT to undertake monitoring. The monitor will be Jenny Henderson. She will perform a study initiation visit and thereafter monitoring visits every two months with an average of 3 hours per patient for source document verification as well as monitoring the regulatory binder, ethics committee communication, SAE reporting and informed consent documents.

17.2. Data and safety monitoring board (DSMB)

The trial will be monitored by an independent DSMB. This DSMB will be comprised of 3

independent internationally-recognized HIV-TB researchers and an independent statistician (subcontracted by UCT). The DSMB will be appointed by the sponsor before trial recruitment starts and will also have its first meeting with the investigators (via conference call) to make necessary modifications to the draft DSMB charter prepared by the investigators and approve the final charter before recruitment starts. The charter will specify the stopping rules for the trial that will be based on DSMB review of the blinded and/or unblinded data. It will also specify the specific data to be reviewed by the DSMB and whether or not these data needs to be presented to the DSMB in a blinded or unblinded manner or that the data summaries may be partly concealed.

Once the trial is underway, the DSMB will meet twice during the trial. The first meeting will occur after 80 participants (~33%) have been enrolled and completed follow-up and the second after 160 participants (~66%) have completed follow-up. The DSMB may also decide to convene an unscheduled DSMB review if warranted by safety or data quality concerns. The data for review will be prepared by the independent statistician.

Data including the following will be prepared for DSMB review:

- Study recruitment by month
- Eligibility violations
- Baseline characteristics
- Protocol violations
- Data completeness report
- Primary and certain secondary endpoint summaries
- Summary of adverse events (ACTG grade 3 and 4)
- Deaths on the trial

The task of the DSMB will thus be to review study recruitment, data quality and drug safety and advise the sponsor of major safety issues and data quality issues. The DSMB may advise that trial enrollment should be stopped based on significant safety concerns. However, although the DSMB will have access to the primary endpoint data (paradoxical TB-IRIS events) decisions regarding whether to stop the trial will not be based on this data. The DSMB may choose to review the efficacy data in a blinded fashion (following the DSMB charter). The DSMB meetings during the trial will take place via conference call and will include an open part when the investigators are present and update the DSMB members on unblinded aspects of the study. This will be followed by a closed part after the investigators have left the meeting when blinded data is reviewed and discussed and the DSMB recommendations are made.

The DSMB report and recommendations will be sent to the sponsor (the University of Cape Town represented by the Deputy Dean of the Faculty of Health Sciences at University of Cape Town). The sponsor will then make the decision based on the DSMB recommendations to continue the trial or to prematurely stop enrollment or to implement any alternative recommendation of the DSMB.

18. Adverse event grading, recording and reporting

18.1. Adverse event grading and recording

The ACTG adverse event grading system will be used (see addendum 2: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009).

All grade 1, 2, 3 and 4 clinical and laboratory adverse events will be recorded on the CRF and the adverse event log in each patients study folder.

18.2. Serious adverse event reporting

All adverse drug reactions, serious adverse events and deaths will be reported to the UCT Human Research Ethics Committee and the Medicines Control Council according to these bodies' guidelines on reporting as detailed below. Reporting to the sponsor's representative (UCT Faculty of Health Sciences Deputy Dean for Research) will be done at the same time that reporting is done to the Ethics Committee and to the DSMB. A cumulative report of all serious adverse events and deaths will be presented at each Trial Steering Committee meeting.

18.2.1. Reporting to UCT Faculty of Health Sciences Human Research Ethics Committee (UCT FHS HREC)

The study medical officer, study co-ordinator or principal investigator will report to the UCT FHS HREC using a cover letter and the UCT FHS HREC standard form (fhs008):

Definitions

- Unanticipated event: Must be all three of the following: a) unexpected, b) related to the research and c) harmful to participants or others
- Unexpected adverse event: Any of the following applies: a) Inconsistent with current information given to participants, b) inconsistent with current risk information in the protocol application, c) occurring more frequently than anticipated
- Serious Adverse Event: Results in any of the following: a) death, b) life-threatening incident, c) hospitalization, d) disability, e) congenital abnormality, f) requires medical or surgical intervention, g) inadvertent disclosure of confidential information.

Reporting

- Unanticipated problems will be reported within seven calendar days of being aware the occurrence.
- Adverse Drug Reactions (Fatal and life-threatening, Unexpected): As soon as possible, but no later than 7 calendar days after first learning about the event.
- Adverse Drug Reactions (Non-fatal, Serious, Unexpected): As soon as possible but no later than 15 calendar days.

- Adverse Drug Reactions (Expected): Only if occurring at significantly higher frequency or severity than expected and then within 15 calendar days.
- New Information that might impact the Conduct of a Clinical Trial: 3 Calendar days of first learning of its occurrence.

We will mail all Ethics Committee reports to:

The Chair, University of Cape Town Human Research Ethics Committee, Room 23, Floor E52, Old Main Building Groote Schuur Hospital, Anzio Road, Observatory, 7925.

We will at the same time mail these reports to the two ethics committees in Belgium, unless they request for a different reporting schedule:

Prof dr Anne Buvé, chairperson
 Institutional Review Board, ITM Antwerp
 Nationalestraat 155, 2000 Antwerp
 Tel: +32 3 247 65 33
 E-mail: abuve@itg.be

Prof dr Patrick Cras, chairperson
 Comité voor medische ethiek, University Hospital Antwerp
 Wilrijkstraat 10, 2650 Edegem
 Tel: +32 3 821 35 44
 E-mail: ethisch.comite@uza.be

18.2.2. Reporting to the Medicine Control Council of South Africa

Reporting of adverse events to the MCC will be done according to the document entitled Medicines Control Council: Reporting Adverse Drug Reactions in South Africa (May 2003). Section 5 refers to reporting of adverse events in clinical trials (Appendix C). The events will be reported using a cover letter and a copy of the form submitted to the ethics committee that provides a detailed narrative explaining the event. Expedited reporting is not appropriate if the event is not related to the study product. A named investigator will sign the report and submit a hard copy by mail as well as fax a copy to the MCC.

Key timelines for reporting:

a) Deaths:

All deaths will be reported within 7 days of becoming aware of the event, unless the death is unexpected and due to a serious adverse event, when it will be reported within 3 days as per the study protocol.

b) Adverse Drug Reactions: Fatal or life-threatening, unexpected

MCC requirement: Report as soon as possible but within 7 calendar days.

c) Adverse Drug Reactions: Not fatal or life-threatening, unexpected

Report as soon as possible, but within 15 calendar days.

d) Adverse Drug Reactions: Expected, but change in nature, severity or frequency, or new risk factor identified

Report within 15 calendar days.

e) Information which changes the risk-benefit assessment of trial:

Report within 3 calendar days.

f) Serious adverse events:

Report as part of the 6-monthly progress report in line listing format.

g) Non-serious adverse events:

Report as part of the 6-monthly progress report in line listing format.

We will mail all MCC reports to:

Medicines Control Council Clinical Trials Unit

41 Andries Street

Civitas Building

Pretoria

0001

19. Ethical considerations

Ethical and regulatory approval

Ethical permission for the clinical trial will be obtained from the University of Cape Town Human Research Ethical Committee,

(<http://www.health.uct.ac.za/research/humanethics/adminteam/>) as well as from Institute of Tropical Medicine IRB and the Ethics Committee of Antwerp University Hospital, Belgium, before the trial is initiated. Written informed consent will be obtained from eligible patients prior to any study procedure being undertaken. The trial will adhere to ICH Good Clinical Practice guidelines, South African GCP guidelines of 2006 and the Declaration of Helsinki of 2008. All unexpected serious adverse events and deaths on the trial regardless of cause will be reported to the University of Cape Town Human Research Ethics Committee and the other 2 ethics committees in Belgium as per their guidelines. Regulatory approval for the trial will be obtained from the Medicines Control Council of South Africa (in Pretoria). All unexpected serious adverse events and all deaths on the trial regardless of cause will be reported to the MCC. Annual (UCT ethics committee and 2 ethics committees in Belgium) and six-monthly (MCC) reports will be submitted as per requirements.

Participant confidentiality

Documentation, data and all other information that relates to individual patients will be held in strict confidence during the trial and after trial completion. No information concerning the patient will be released to an unauthorized third party, without written approval of the participant except as necessary for trial monitoring or regulatory review.

The patient's name will be entered in a study log next to the trial number. Thereafter, all trial documentation will be with the study number and initials. This study log and all study documentation will be stored in a GCP compliant locked cabinet. The study database will be

fully anonymised. All data transported off the clinical site and all data sent to the sponsor and to ITM, Belgium, will be fully anonymised.

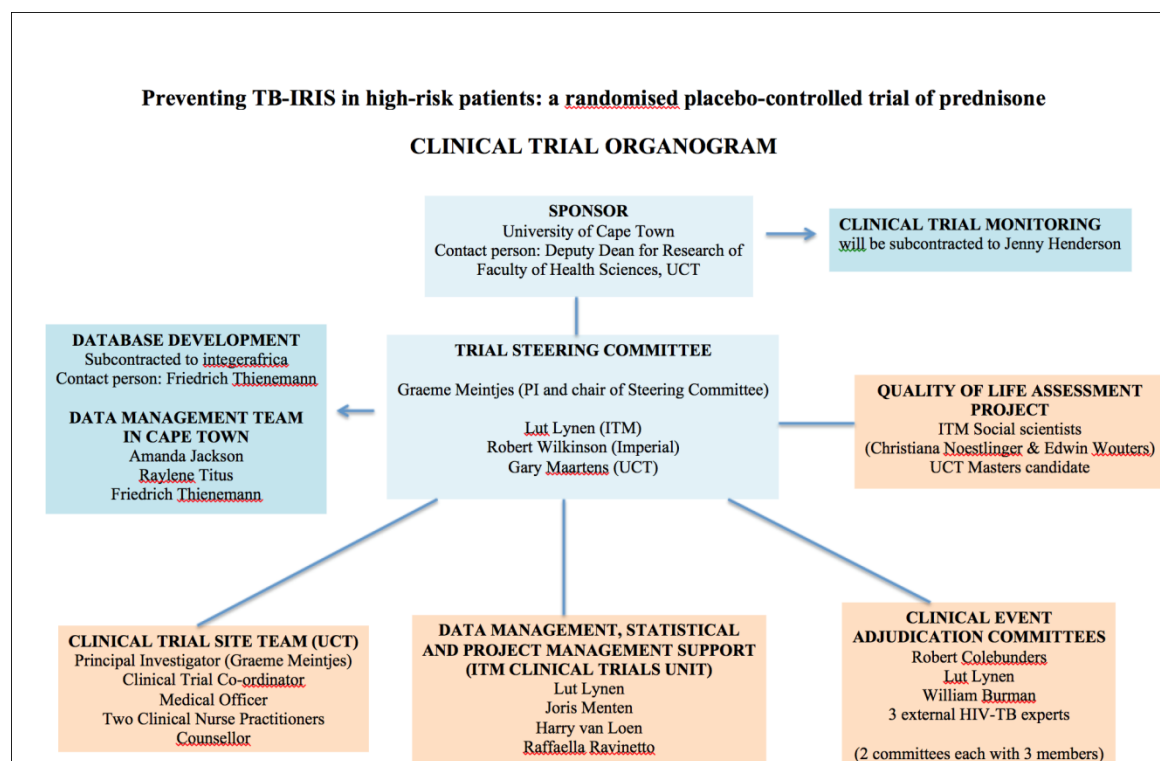
In this study, the database will be constructed, maintained and kept within South Africa, so no identifiable personal data will be transferred to ITM, Belgium. Only the final clinical and laboratory database will be shared with the Belgian partner, with no identifiable personal data. Any data that will be transmitted to Belgium (that is needed for statistical analysis) will be fully anonymized before transmission. This will involve removal of patient identifiers including initials, place of birth, place of residence, day and month of birth. The quality of life database will be shared with Belgian investigators prior to it being finalized as they will be involved in earlier phases of analysis of this database. However, all data that leaves the clinical site from the quality of life substudy and that is transmitted to Belgium will be de-identified in the same way described.

Standard of care

Neither corticosteroids nor any other medication are currently prescribed for preventing paradoxical TB-IRIS in the current standard of care with the implication that a placebo-controlled trial is ethically acceptable because placebo reflects current standard of care. Diagnosis and treatment of TB, antiretroviral therapy prescribed and the timing of antiretroviral therapy in this trial protocol all reflect standard of care in South Africa.

20. Administration, trial management and monitoring

20.1. Clinical Trial Organogram



*Additional members of TSC: Prof William Burman and representative from Ubuntu Clinic

20.2. Governance of the study

The governance and management plan for the trial is summarised in the organogram. The conduct of the clinical trial will be overseen by the Trial Steering Committee (TSC), which will include the PI and one representative from each participating institution (Graeme Meintjes (chair and PI), Lut Lynen, Robert Wilkinson and Gary Maartens). There will be one additional independent member of the TSC (Professor William Burman (University of Colorado, US)) and a member who represents the Ubuntu HIV-TB clinic in Khayelitsha. The TSC will have a meeting every 2 months with Skype conference call link-up with those members not in Cape Town. The TSC will review preparation for the trial, clinical enrolment and events on the study as well as all other aspects such as analysis and sub-studies. For each meeting of the TSC a progress report will be prepared that will include: participant accrual, study primary endpoint and deaths (blinded to study arm), all serious adverse events with details of each individual serious adverse event. This 2-monthly report will simultaneously be submitted to the sponsor's representative (Deputy Dean for Research, Faculty of Health Sciences, UCT).

Graeme Meintjes (PI) will be responsible for day-to-day management at the Clinical Trial site and for human resources issues. He will receive assistance in this regard from the HR Department at UCT who have very well established conditions of employment, contracts of employment and require clear job descriptions for staff (the PI will draft these job descriptions). The Core Office at the Institute of Infectious Disease and Molecular Medicine at UCT will manage the study finances and the financial report will be reviewed at the TSC meetings.

20.3. Sponsor and monitoring

The University of Cape Town is the Trial Sponsor. The official representative of the Sponsor is the Deputy Dean of Research, Faculty of Health Sciences, UCT. The sponsor will subcontract monitoring of the trial to a Clinical Trials Monitor with extensive experience of clinical trials monitoring in South Africa (Jenny Henderson, who previously monitored a study of adjunctive interferon- γ immunotherapy for cryptococcal meningitis that was conducted at our hospital [49]) (see section 17.1.). A copy of all monitoring reports and DSMB reports will be sent to the Deputy Dean for Research at the Faculty of Health Sciences at UCT. The Sponsor will also be informed of all unexpected serious adverse events and deaths occurring on the study at the same time as the ethics committees.

The trial will be conducted according to ICH GCP standards. GCP training and accreditation for all clinical research staff will take place prior to trial initiation. The laboratories used for this trial will be the National Health Laboratory Services (NHLS) in Green Point and at Groote Schuur Hospital. These laboratories are SANAS (South African National Accreditation System) accredited.

21. Quality of life substudy

21.1. Background

In our previous trial of prednisone for TB-IRIS treatment [3], we used the Medical Outcomes Study-HIV (MOS-HIV) Health Survey [50] and the Karnofsky performance score, in parallel. However, when using these tools we experienced practical problems with use in patients who are very ill as many of the questions were not relevant to their current state. Many of the validated HR-QOL measures, like the MOS-HIV, were developed prior to the ART era, and for a UK and US context. Many studies in developing countries have used these instruments often without proper validation and reliability testing [51, 52]. A review of HR-QOL measures for use in HIV/AIDS clinical trials found that the Functional Assessment of HIV-infection (FAHI) and MOS-HIV were the two most appropriate HIV-targeted measurement tools. Both can be self-administered and can be completed in 5-10 minutes. However, they would not be optimal in all HIV subgroups (e.g. adolescents versus adults, women versus men, ART naïve vs. treatment experienced) [53]. Recently a new HIV/AIDS-specific HR-QOL questionnaire, the Patient Reported Outcomes Quality of Life-HIV (PROQOL-HIV) was developed and has undergone psychometric validation in 8 countries. The instrument was developed simultaneously across several continents and accounts for ART and side effects of ART [54, 55]. It is a 38-item questionnaire covering 8 dimensions. Patients rate the different items over the past 2 weeks, on a 5-point scale ranging from 0="never" to 4="always". Although this questionnaire performed well in the 11 countries, it may not be the best questionnaire to assess HR-QOL in patients with an acute illness, as part of the questions deal with long-term side effects. Neither MOS-HIV nor PROQOL-HIV contain questions specific for TB patients.

Another more generic QOL tool, is the EuroQol generic health index, the EQ-5D-3L (<http://www.euroqol.org/home.html>). EQ-5D essentially consists of 2 pages - the EQ-5D-3L descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D-3L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, severe problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box corresponding to the most appropriate statement in each of the 5 dimensions. The EQ VAS records the respondent's self-rated health on a vertical, visual analogue scale where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state'. This information can be used as a quantitative measure of health outcome as judged by the individual respondents.

We aim to define a HR-QOL during this trial that is more suitable for the evaluation of a treatment directed at short-term impact on health outcome. If a novel HIV specific HR-QOL measure is demonstrated to work well in our setting, it can be used in future clinical trials. In order to explain the differences in HR-QOL measures between patient groups it is important to link it with a symptom list. The best known and validated list is the HIV symptom index developed by Justice [56]. This 20-item self-completed symptom index assesses whether symptoms are present or absent and their severity on a 4-point scale (0 = "does not bother me at all" to 4 = "bothers me a lot"). However, the 20-item list does not contain symptoms that are targeted to patients with possible side effects of steroids, or with TB-IRIS specific symptoms.

21.2. Methodology

Measures and their adaptation

1. Existing and validated HR-QOL measures will be used as originally designed: **PROQOL-HIV and EQ-5D-3L**, in combination with **Karnofsky score** and an adapted **HIV-symptom index**. As far as possible the tools will be self-administered by the patient. In case the patient is illiterate a trained study counsellor will read the questions to them. A brief review for completeness by the site investigators will be done. The PROQOL-HIV will be adapted by asking specifically for HIV and TB-related symptoms. The number of questions will not change, but for example the sentence "I have been satisfied with my HIV medicine" would become "I have been satisfied with my HIV and TB medicines".
2. The 20-item HIV symptom index will be complemented with symptoms targeted to patients with IRIS and side effects of steroids. This will be prepared by the investigators and presented and discussed with experts in TB-IRIS from the INSHI network before implementation.
3. The tools (PROQOL-HIV, EQ-5D and HIV symptom index) will be translated in Xhosa. This will include two forward translations and one reconciled version: one backward translation of the reconciled version. For the PROQOL-HIV a Zulu translation is already available, which will be used to facilitate the translation process, as these two language share similarities.
4. Initial validation of the comprehensiveness and the comprehensibility (cognitive interview) will be performed on a sample of 10 patients (including at least 5 with TB-IRIS). At the end of the surveys open-ended questions will be asked such as:
 - a. Did you have trouble understanding what we meant by any of the symptoms (items) listed? If so, please list them and explain why it was confusing to you.
 - b. Do you think that these symptoms are the most common and the most bothersome for patients with TB-HIV who start ART?
 - c. Are there any other symptoms that you have found particularly bothersome? If so, please describe the symptom and how much it has bothered you.
 - d. Are there any other symptoms that you hear about that bother people you know who have TB and HIV? Please describe the symptom and how much it seems to bother them.

These patients will be personally debriefed by trained interviewers, to make sure that the written responses as documented at the end of the survey accurately represent their impressions.

5. A social scientist from ITM, together with a master student from UCT and a local counsellor will select, based on Cape Town's patients' and providers' feedback, the final version of the two HR-QOL measures and the HIV/TB symptom index.
6. Cognitive interviews (see point 4 above) to pretest questionnaires (as described by for instance by DeMaio & Rothgeb [57] and Willis et al [58]) have some limitations, such as using only a very small number of purposively chosen respondents. Hence the results are not generalizable to a larger population.

Unlike the cognitive interview, respondent debriefing is often employed at a later stage in the questionnaire design. It offers the potential of larger and more representative data, and to assess whether findings from cognitive interview research can be re-replicated in the actual survey setting ([58, 59]). Therefore, we suggest to employ respondent debriefing with a random sample of patients with and without IRIS enrolled in the trial, followed by in-depth interviews with purposively selected patients to make sure that the written responses adequately reflect the patients' impressions. Ideally, one should try to achieve data saturation

when conducting in-depth interviews, but we assume that 10-15 interviews should yield sufficient results. In case of discrepancies that cannot be solved and/or constantly new issues coming up the number of qualitative interviews would be increased.

Statistical methods

First, the statistical analyses aim to assess the validity and the reliability of the different scales in our population. Subsequently, the study aims to construct the optimal factor scores to measure the different latent concepts.

The Xhosa versions of the EQ-5D-3L and the Karnofsky Performance score have been applied and validated in HIV-positive populations on ART. The validity of the Xhosa translation of the recently developed PROQOL-HIV and the adapted version of the HIV Symptoms Index will be assessed in a series of tests. Firstly, we aim to test the *construct and concurrent validity* of the translated version of the PROQOL-HIV instrument in this population. In particular, we will explore whether the PROQOL-HIV score has the relationships with other variables (EQ-5D and EQ-VAS) in the expected direction. Similarly, we will test the *predictive validity* of the translated instrument by assessing the correlation with other QoL-measures at follow-up. *Convergent validity* of the QoL-scale will be assessed by employing the Karnofsky Performance score, which should be influenced by the same underlying concept (i.e. health). The *criterion validity* – the relationship with an objective measure – of the PROQOL-HIV will be assessed by calculating its correlation with objective clinical markers (eg. baseline CD4 cell count and viral load). The adapted HIV Symptoms Index will be validated in a similar manner testing its construct, convergent and criterion validity by exploring its association with the Karnofsky Performance score, EQ-5D and objective clinical markers.

The reliability of the different (sub)scales will be assessed by using a measure of internal consistency, Cronbach's alpha (> 0.7). In addition, we will also apply the test-retest method and the split-half method to further assess the external and internal reliability of the scales. The current study aims to optimally utilize the information gathered in the above-mentioned scales. For this reason the current study will employ confirmatory factor analysis (CFA) to test the a priori assumptions which are made in the often used sum scores and indices: we will explore (1) the weight of the different items, (2) the measurement errors, and (3) the number of dimensions measured by the scale. In practice and based on conceptual theory, the selected scales will be subjected to CFA using Mplus to assess whether the proposed factor structure fits with the gathered data and to compute factor scores which can be employed in future analyses containing the latent concepts. CFA provides two important analytic possibilities necessary to optimally address a number of challenges of our research. Firstly, our sample will contain a high number of respondents from vulnerable patient groups, which means that special attention will have to be devoted to the potentially disturbing influence of acquiescence (the tendency to agree) and method effects (negative and positive wording). If needed, the CFAs will include a method factor to control for these sources of response bias.

Secondly, it is important to note that the current study population contains several sub-groups (e.g. IRIS vs. non-IRIS patients, women vs men, patients at time 1 vs time 2, placebo vs prednisone) The majority of studies – including almost all previous QoL-studies on HIV/AIDS – have assumed equivalence of the structure of the measures they compare across

different respondent groups. However, legitimate comparison of means or structural relations across groups requires equivalence of the measurement structures underlying the indicators. The manifest means in a comparison does not only depend on the latent means but on the whole underlying measurement model (i.e., item intercepts and factor loadings). In other words, in order to make sure that the different patient groups (e.g. men vs women, placebo vs prednisone) identically interpret the different items and thus that the constructed factor scores of these different patient groups are measuring the same concept, multi-group confirmatory factor analysis – including measurement invariance testing – will have to be employed. At least partial measurement invariance (configural – metric – scalar invariance) is a prerequisite for cross-cultural or cross-group comparisons.

Using this procedure, we aim to produce refined factor scores, which represent the methodologically optimal measurement of the intended latent concepts and will thus be applicable in subsequent analyses incorporating the concepts measured by the selected scales. The qualitative data assessed will serve as important contextual information to interpret the quantitative results, for instance to explain a specific factor structure that could potentially be different compared to in the original instruments. All qualitative data (cognitive interviews) will be analyzed using an inductive approach. Data from the open-ended questions will first be transcribed verbatim and then translated into English, if conducted in another language (Xhosa). Systematic contextual data analysis methods will be applied using electronic software (NVivo8), by identifying recurrent patterns and themes [60]. A codebook will be developed by at least two independent coders and an iterative coding approach will be taken. Brief data analysis reports will summarize identified themes and rely on narrative text to illustrate and explain any newly emerging concepts in relation to how study participants perceive their health-related quality of life.

Thirdly, on the condition that multi-group scalar invariance is proven (see above), the QOL measures will be used as one of the outcome measures in the clinical trial comparing the use of prednisone with placebo in patients at high risk of TB-IRIS. Comparison of means (SD) and medians (IQR) of the scores (total and domain scores) obtained by the different tools in the placebo and the intervention group will be done using Wilcoxon rank sum, chi-square and Fisher's exact tests, as appropriate.

Table: Summary of Quality of Life assessments to be performed within the clinical trial

| Instrument | Method | Interviewees |
|---|--|---|
| PROQOL-HIV (including “TB” in the questions, next to “HIV”) | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| EQ-5D-3L | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| HIV-symptom index (complemented with specific symptoms related to IRIS and steroid side effects) | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| Karnofsky score | Score assigned by investigator in discussion with participant | All participants at week 0, 4 and 12 visit |
| Respondent debriefing interviews | In-depth interview conducted by an investigator specifically involved in QoL substudy with assistance of counselor for translation | 10-15 interviews with a random sample of participants |

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Final protocol, version 1.4, dated 03 February 2014

CLINICAL TRIAL PROTOCOL

Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomized placebo-controlled trial of prednisone (Pred-ART trial)

Funder: European and Developing Countries Clinical Trials Partnership (EDCTP)

Sponsor: University of Cape Town

Partner organizations: Institute of Tropical Medicine (Antwerp) and Imperial College London

Principal Investigator: Graeme Meintjes (University of Cape Town)

Co-investigators: Lut Lynen, Robert J. Wilkinson, Robert Colebunders, Gary Maartens

Clinicaltrials.gov number: To be obtained

Date: 3 February 2014

Version: 1.4



**Imperial College
London**

PROTOCOL APPROVAL

AUTHOR (1):

Title, Name: _____
Affiliation: _____
Date: _____
Signature: _____

AUTHOR (2):

Title, Name: _____
Affiliation: _____
Date: _____
Signature: _____

AUTHOR (N):

Title, Name: _____
Affiliation: _____
Date: _____
Signature: _____

SPONSOR REPRESENTATIVE:

Title, Name: _____
Date: _____
Signature: _____

STATEMENT OF COMPLIANCE & CONFIDENTIALITY

By signing this protocol, the Principal Investigator acknowledges and agrees:

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the most recent version of the Declaration of Helsinki, WHO and ICH Good Clinical Practice (GCP) and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses, laboratory staff and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

This document contains information that is privileged and confidential. As such, it may not be disclosed to any other persons than involved research staff and the concerned Ethics Committees, unless specific permission is granted in writing by the University of Cape Town, or such disclosure is required by national or other laws or regulations. In any event, persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future study-related information supplied that is regarded as privileged or confidential.

The Sponsor of this study will at any time have access to the source documents from which Case Report Form information may have been generated. The Case Report Forms and any other data pertinent to this study are the property of the Sponsor. The data may only be utilized upon review and after discussion with the trial steering committee and/or the Sponsor. All study material will be maintained according to regulatory requirements and until the Sponsor advises that it is no longer necessary.

I, the Principal Investigator; agree to conduct the present study in full accordance with the most recent approved version of the protocol, within applicable timelines, according to the relevant standard operating procedures and in full agreement with all applicable regulations and the international guidelines regarding the conduct of clinical research.

I, the Principal Investigator; by signing this protocol declare that I will permit trial-related monitoring, audits, independent Ethics Committee review, and regulatory inspections, providing direct access to source data/documents during and after the course of the trial.

I, the Principal Investigator; by signing this protocol declare that I will make the protocol and all relevant related information available to all physicians, nurses, laboratory staff and other personnel who participate in conducting this study. I will also ensure that the study team at my site receive adequate training so that they are fully informed and qualified for the conduct of the study.

I also acknowledge the paragraph relevant to study confidentiality.

PRINCIPAL INVESTIGATOR:

Title, Name:

Site:

Date:

Signed:

SCHEMA

Title: Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomised placebo-controlled trial of prednisone

Short title: Prednisone with early ART in HIV-TB (Pred-ART trial)

Phase: III

Objective: To determine whether the addition of prednisone to the first 4 weeks of antiretroviral therapy (ART) reduces the risk of paradoxical TB-IRIS in HIV-infected patients being treated for TB who are at high risk of developing TB-IRIS (CD4 <100 cells/ μ l and starting ART within 30 days of TB treatment).

Design: A randomized double-blind placebo-controlled trial to evaluate the incidence of paradoxical TB-IRIS over the first 12 weeks of ART in participants who receive a 4 week course of prednisone versus participants who receive a 4 week course of placebo.

Primary efficacy endpoint:

The development of paradoxical TB-IRIS within 12 weeks of starting ART (defined using the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition).

Secondary efficacy endpoints:

- 1) Time to IRIS event
- 2) Severity of IRIS events (defined by the following: need for hospitalisation for IRIS, C-reactive protein, and neurological involvement)
- 3) Duration of TB-IRIS event (from onset of symptoms/signs to resolution of TB-IRIS symptoms/signs)
- 4) Mortality attributed to TB and TB-IRIS
- 5) All-cause mortality
- 6) Composite endpoint of death, hospitalization, or hepatotoxicity (using the protocol-specified definition of Grade 3 or 4 increase in ALT or bilirubin).
- 7) Other (non-TB) IRIS events
- 8) Quality of life assessment (measured using PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score)
- 9) Adverse events and severe adverse events ascribed to TB treatment, ART or co-trimoxazole. This will include a pre-specified analysis of drug-induced liver injury and drug rash. This assessment will include the number of treatment interruptions for drug adverse events.
- 10) Discontinuation of either ART or TB treatment for > 5 days due to adverse events
- 11) Number of hospitalizations and total days hospitalized

Safety and tolerability endpoints:

- 1) Corticosteroid-associated adverse events, classified by severity and relation to study drug. These will include hypertension, hyperglycaemia, hypomania/mania, depression, acne, epigastric pain, upper gastro-intestinal bleeding, Cushingoid features, new oedema and

avascular bone necrosis.

2) Laboratory safety data: glucose, full blood count and electrolytes

3) Other infections (AIDS-related, bacterial, fungal and viral) and malignancies (Kaposi's sarcoma)

4) All grade 1, 2, 3 and 4 adverse events (clinical and laboratory using the ACTG grading system)

Sample size: 240 participants will be enrolled over 13 months. Each participant will be followed for 12 weeks.

Population: HIV-infected, ART-naïve adult (≥ 18 years) patients diagnosed with active tuberculosis who have a CD4 < 100 cells/ μ L and who start ART within 30 days of starting TB treatment. Other inclusion criteria include: diagnosis of TB (smear, culture, Xpert MTB/RIF test, histology or strong clinical and radiological evidence of TB with symptomatic response to TB treatment), eligible for and consent to starting ART and written informed consent for trial. Exclusion criteria include: Kaposi's sarcoma, pregnancy, TB meningitis or tuberculoma at TB diagnosis (because these patients receive corticosteroids), known rifampicin-resistant TB, being on corticosteroids for another indication within the past 7 days, on other immunosuppressive medication within the past 7 days and uncontrolled diabetes mellitus.

TB treatment and ART: TB treatment will be prescribed and monitored by the clinical staff in the local HIV-TB clinic. TB treatment will be given according to South African Department of Health guidelines. This involves rifampicin (R), isoniazid (H), ethambutol (E) and pyrazinamide (Z) for 2 months followed by RH for 4 months. ART will be prescribed by the clinical staff at the HIV-TB clinic according to South African Department of Health guidelines. Standard first line ART in TB patients is tenofovir, emtricitabine (or lamivudine) and efavirenz. Co-trimoxazole prophylaxis will be prescribed to all patients unless a contra-indication exists.

Intervention: Oral prednisone 40mg daily for 14 doses started within 48 hours of initiating ART, followed by 20mg daily for 14 doses (or identical placebo). A total of 28 days of study medication will thus be prescribed.

Follow-up: Patients will be screened once established on TB treatment, but before starting ART. If the patient is eligible, written informed consent will be taken. There will be six planned study visits that will be in relation to the start of ART: week 0 (the day ART is initiated), week 1, week 2, week 4, week 8 and week 12. Patients will be seen at unscheduled visits if clinical deterioration occurs. If paradoxical TB-IRIS is diagnosed this will be treated with open label prednisone at clinician discretion if symptoms are moderate or severe. If patients experience clinical complications (eg. TB-IRIS) follow-up will be prolonged beyond week 12 in order to stabilize their condition before referral back to the general TB-HIV clinical service for ongoing management.

Data monitoring: The trial will be monitored by an independent Data and Safety Monitoring Board (DSMB) comprising 3 independent researchers and an independent statistician. After an initial meeting for agreeing on their Charter, the DSMB will meet twice (after 80 and 160

participants have completed follow-up) to review data quality and data with respect to safety and trial endpoints. If there is evidence of harm related to study medication or trial conduct the DSMB may advise the sponsor that trial enrollment should be stopped.

Clinical trial site: Khayelitsha Site B HIV-TB clinic (Ubuntu clinic)

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 - 22.3. WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI. Ethical Principles for Medical Research Involving Human Subjects, 2008.
 - 22.4. Quality of life questionnaires
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References

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2. EXECUTIVE SUMMARY

Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomised placebo-controlled trial of prednisone

Tuberculosis (TB) is the most common opportunistic infection amongst HIV-infected patients starting antiretroviral therapy (ART) in developing countries and thus the most frequent form of immune reconstitution inflammatory syndrome (IRIS). Paradoxical TB-IRIS occurs in 8- 43% of patients starting ART while on TB treatment and results in morbidity, hospitalisation, consumes health care resources and TB-IRIS may be fatal. We have previously demonstrated in a clinical trial that prednisone reduces morbidity when used for treatment of paradoxical TB-IRIS. This trial is a double-blind placebo-controlled trial of prophylactic prednisone (40mg/day for 2 weeks followed by 20mg/day for 2 weeks, started within 48 hours of initiating ART) in patients with TB who are identified as being at high risk for paradoxical TB-IRIS (starting ART within 30 days of initiating TB treatment and $CD4 \leq 100/\mu L$). The trial will enroll 240 participants, randomised 1:1 (prednisone:placebo). The primary endpoint is development of paradoxical TB-IRIS, defined using international consensus case definitions. Secondary endpoints include time to IRIS event, severity of IRIS, quality of life assessment, mortality and corticosteroids adverse events. The trial is powered to determine a reduction in TB-IRIS events. If results of this trial demonstrate benefit and safety, our intention is to take this intervention forward into a larger phase 3 clinical trial with mortality as the primary endpoint.

3. INTRODUCTION

3.1. Primary study objective

To determine whether a 4-week course of prednisone in patients starting antiretroviral therapy (ART) within 30 days of starting treatment for tuberculosis (TB) and a CD4 count \leq 100/ μ L reduces the incidence of paradoxical TB-IRIS, without an excess of adverse events. The trial is powered to determine a reduction in TB-IRIS events. If the clinical trial proposed here demonstrates benefit and safety, our intention is to take this intervention forward into a larger phase 3 clinical trial with a sufficiently large sample size to determine a mortality reduction.

3.2 Paradoxical TB-IRIS

TB is the commonest opportunistic disease in HIV-infected patients in low- and middle-income countries, and is therefore a common indication for starting ART. A substantial proportion of patients starting ART in sub-Saharan Africa are thus on treatment for active TB (up to 42% [1]), and are therefore at risk of paradoxical TB-associated immune reconstitution inflammatory syndrome (TB-IRIS). When ART is commenced in patients on TB treatment an immunopathological reaction, known as paradoxical TB-IRIS, commonly occurs (in 8-43% of TB patients starting ART [2]) resulting in new or recurrent TB signs and symptoms. TB-IRIS causes considerable morbidity; with 30% requiring hospitalization in a prospective study we conducted [3].

One study from the US reported that 44% of patients who developed TB-IRIS required hospitalization for a median of 7 days (range 3-66) and many patients in this study required therapeutic procedures [4]. In a recent report of TB-IRIS cases that occurred during the SAPIt trial in Durban, South Africa (discussed below) among those patients who started ART within 4 weeks of TB treatment who developed TB-IRIS 42% required hospitalization and among those who started ART 8-12 weeks after TB treatment and developed TB-IRIS 22% required hospitalization [5].

Although the mortality of TB-IRIS is relatively low (estimated at 3.2% in a meta-analysis [6]), our group has shown that mortality is about ten times higher than this in patients with neurological manifestations [7, 8]. Importantly, over two-thirds of patients who develop neurological TB-IRIS do not have clinical features of neurological involvement at initial diagnosis of TB [7]. The diagnosis of TB-IRIS is clinical, and it is important to exclude other opportunistic diseases. In resource-constrained settings this puts a strain on over-burdened health services by utilizing diagnostic tests and empiric treatment of suspected alternative diagnoses. In the majority of cases the onset of TB-IRIS is within the first 4 weeks of ART: in our cohort median onset was 14 days (IQR 7-25) [9]. Therefore prophylactic interventions may only be required for one month.

3.3. Corticosteroids in the treatment of TB

Corticosteroids are known to exert anti-inflammatory effects on most types of immune cells through direct effects on transcription of inflammatory mediators via the Glucocorticoid Responsive Element, indirect genomic effects via interference with other transcriptional factors such as NF- κ B and AP-1, and non-genomic effects on anti-inflammatory proteins [10, 11]. These effects result in increased transcription of a number of anti-inflammatory

mediators and decreased transcription of pro-inflammatory cytokines, chemokines, enzymes, receptors and adhesion molecules [12, 13]. In addition, corticosteroids have been shown to reduce T cell survival by enhancing apoptosis [12]. Corticosteroids have been used as adjunctive treatment in TB for several decades [14]. Because the host immune response plays an important part in the pathology caused by TB, corticosteroids have been used in all forms of TB with the intention of improving outcomes and reducing complications such as pericardial constriction, hydrocephalus, focal neurological deficits, pleural adhesions and intestinal strictures. However, evidence of benefit from controlled clinical trials exists only for TB meningitis and pericardial TB as well as paradoxical TB-IRIS [3, 15].

TB Meningitis: Thwaites and colleagues [16] conducted a randomized, double-blind, placebo-controlled trial of dexamethasone for the treatment of TB meningitis in Vietnam amongst patients older than 14 years of age (n=545). The initial dose of intravenous dexamethasone used was 0.4mg/kg/d for patients with Grade 2 and 3 TBM disease and 0.3mg/kg/d in patients with Grade 1 disease. The total duration of dexamethasone (initially intravenous followed by oral) was 8 weeks in those with Grade 2 and 3 disease and 6 weeks in those with Grade 1 disease. At 9 months follow-up, dexamethasone was associated with a reduced risk of death (relative risk = 0.69, 95% CI= 0.52 – 0.92), but no reduction in the proportion of patients with severe disability. Eighteen percent of participants were HIV seropositive and in a pre-specified subgroup analysis of these patients dexamethasone was associated with a non-significant trend towards reduced mortality (relative risk = 0.78, 95%CI = 0.59 -1.04). Significantly fewer severe adverse events occurred in patients who received dexamethasone. In particular, 8 cases of severe hepatitis (one was fatal) occurred in the placebo group and none in the dexamethasone group. This may be an additional benefit of corticosteroids: by reducing drug reactions they prevent treatment interruptions that adversely impact survival. A Cochrane systematic review of corticosteroids as an adjunct to TB treatment in TB meningitis published in 2008 included seven trials and a total of 1140 participants (with 411 deaths). Dexamethasone or prednisolone was the corticosteroid used in all studies. Corticosteroids reduced the risk of death (relative risk = 0.78, 95% CI = 0.67 - 0.91). The survival benefit occurred irrespective of the severity of TB meningitis. Adverse events that occurred across studies included gastro-intestinal bleeding, bacterial and fungal infections and hyperglycaemia, but were mild and treatable [17].

Pericardial TB: A randomized, double blind, placebo-controlled trial of prednisolone (for 11 weeks) for the treatment of TB pericardial effusion was conducted in Transkei, South Africa, in the 1980's prior to the HIV epidemic by Strang and colleagues. Patients in this study were also randomized to open pericardial biopsy and complete drainage of pericardial fluid on admission or percutaneous aspiration when required. Among patients who did not have open drainage on admission, 3% given prednisolone compared with 14% given placebo died of pericarditis (p <0.05) [18]. Patients who received prednisolone required repeat pericardiocentesis less frequently. A Cochrane review [19] of controlled trials evaluating the role of adjuvant corticosteroids for TB pericarditis included 2 trials from the pre-HIV era with a total of 383 participants (the 2 trials included were the study published by Strang and colleagues in 1988 [18] and a prior trial of patients with TB constrictive pericarditis conducted by the same group [20]). There was a non-significant trend towards reduced death in the intervention group (relative risk = 0.65, 95% CI = 0.36–1.16). There was a significant reduction in the combined endpoint of death or disability at 2 years although there

was substantial heterogeneity in the trials. In a small randomised controlled trial of prednisolone for TB pericarditis in HIV-infected patients conducted in Zimbabwe (n=58) [21] there were 10 deaths among those who received placebo compared with 5 deaths among those who received prednisolone. This represented significantly lower mortality in the prednisolone group using the log-rank test to compare Kaplan Meier survival curves [21]. However, when cumulative mortality was compared the difference was not significant (RR=0.50, 95% CI = 0.19 - 1.28) [19]. The Cochrane review concluded that corticosteroids could have clinical benefit, but the trials published to date are too small to demonstrate an effect [19]. In particular, the efficacy and safety of corticosteroids in HIV-infected patients needs to be evaluated in a larger clinical trial.

Corticosteroids are used in other forms of TB. Tuberculomas involving the brain parenchyma or spinal cord may develop despite effective TB treatment resulting in focal neurological deficits or seizures. The host immune response is thought to play an important role in such paradoxical TB reactions. Corticosteroids have been used with anecdotal reports of symptomatic benefit [22]. In miliary and pulmonary TB initiation of TB treatment may be complicated by the development of adult respiratory distress syndrome with acute respiratory failure [23]. Corticosteroids are frequently used in this situation, but efficacy has not been determined in an adequately powered clinical trial. One study of 55 patients with miliary TB showed a non-significant trend towards improved survival with corticosteroids [24]. A review of the literature of corticosteroids used for all forms of TB concluded that corticosteroids do not diminish the efficacy of TB treatment [14].

A recently published meta-analysis of all trials in which corticosteroids were prescribed as adjunctive treatment for TB patients regardless of which organ system was involved, reported that corticosteroids significantly reduced mortality by 17% (relative risk = 0.83, 95% CI=0.74-0.92). This finding was consistent across all organ systems [25].

The above findings mainly apply to HIV-negative patients and there are concerns regarding the use of adjuvant corticosteroids in HIV-infected patients. In a Ugandan trial that evaluated prednisolone in HIV-infected patients with TB pleural effusions, prednisolone was associated with more rapid clinical and radiological improvement, but an excess of Kaposi's sarcoma [26]. A study was conducted in Uganda specifically in HIV-infected patients with pulmonary TB, but prior to ART availability. In this phase 2 trial of short-term prednisolone in HIV-infected TB patients with CD4 count ≥ 200 cells/mm³, prednisolone was associated with more rapid clearance of MTB from sputum, reduced immune activation and resulted in a non-significant increase in CD4 count, but caused a transient increase in HIV viral load and worsened underlying hypertension and caused fluid retention and hyperglycaemia. High dose prednisolone was used in this trial (initially 2.75mg/kg/day for 4 weeks) [27]. Other corticosteroid trials in HIV-infected patients have raised concerns related to reactivation of herpes infections [28]. All patients on our proposed trial will be on ART, and ART is likely to be at least partially protective against certain of these corticosteroid side effects, such as the development of Kaposi's sarcoma.

Corticosteroids have drug-drug interactions with rifampicin, a potent inducer of cytochrome P450 enzymes. Rifampicin increases the clearance of prednisolone by 45% resulting in reduction in the area under the plasma concentration time curve of prednisolone by 66% [29].

We factored this in our decision regarding prednisone dose for this trial.

Corticosteroids in the treatment of TB-IRIS: We have previously conducted a randomised double-blind placebo-controlled trial of prednisone for the treatment of paradoxical TB-IRIS [3]. The trial was conducted at GF Jooste Hospital in Cape Town between 2005 and 2008. 110 patients referred to our hospital and diagnosed with paradoxical TB-IRIS based on a clinical case definition [2] and after diagnostic work-up, were enrolled. Patients with immediately life threatening TB-IRIS (mainly neurological involvement) were excluded and received treatment with corticosteroids in the clinical service. Participants received a 4-week course of prednisone or identical placebo (1.5 mg/kg/day for 2 weeks followed by 0.75 mg/kg/day for 2 weeks). Follow-up was for 12 weeks. The primary endpoint was days of hospitalisation and outpatient therapeutic procedures (counted as an additional hospital day) and this was significantly lower in those who received prednisone (median of 0 days vs 3 days ($p=0.04$)). Prednisone also resulted in significantly more rapid improvement in symptoms, MOS-HIV quality of life score, chest radiology score and reduction in C-reactive protein. There was no excess of corticosteroid metabolic side effects or severe infections in the prednisone arm. There were more mild infections (oral candida and uncomplicated herpes simplex) among those who received prednisone [3].

We have subsequently demonstrated in samples taken from participants on this trial that prednisone, but not placebo, resulted in significant decreases, after correcting for multiple comparisons, in the serum concentrations of IL-6, IL-10, IL-12p40, TNF- α , IFN- γ and IP-10 suggesting that the beneficial effect of corticosteroids in TB-IRIS is mediated, at least in part, through the attenuation of pro-inflammatory cytokine responses [30]. We have previously demonstrated that these same cytokines are differentially increased in the serum of HIV-TB patients who develop TB-IRIS compared to control subjects who start ART and do not [31]. We, therefore, hypothesise that prednisone given from the start of ART may prevent the development of TB-IRIS or at least attenuate its clinical severity through suppression of pro-inflammatory cytokine responses.

Our group also recently reported from an observational study [32] that patients who were on corticosteroids at the time of starting ART (the indication was mainly TBM) had lower interferon γ (IFN- γ), IP-10, tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, IL-10, IL-12p40, and IL-18 concentrations prior to ART when compared with HIV-TB patients starting ART who were not on corticosteroids ($p \leq 0.02$). In this same study, fewer patients amongst those receiving corticosteroids developed TB-IRIS (38.1% versus 51.7%), but the difference was not statistically significant ($p = 0.287$). We also noted a trend for patients not on corticosteroids to present with more severe TB-IRIS clinical presentations, compared with patients who were on corticosteroids (10/31, 32% vs 0/8, 0% ($p = 0.082$)). The study was relatively small, non-randomised and very likely confounded by indication for prescribing corticosteroids and thus no firm conclusions can be drawn. However, these results suggest there is an important effect of corticosteroids on the immune response in HIV-TB patients starting ART and provide further rationale for this RCT.

Neither corticosteroids, nor any other medication, are currently prescribed for preventing paradoxical TB-IRIS in the current standard of care making a placebo-controlled trial ethically acceptable because it reflects current standard of care.

3.4. Corticosteroids for other HIV-related conditions

Corticosteroids are used in the treatment of pneumocystis pneumonia (PCP) in HIV-infected patients. A meta-analysis of six randomised trials of adjuvant corticosteroids in the treatment of PCP showed a survival benefit [33]. A 21-day course of corticosteroids used for PCP treatment does not increase the risk of mortality or common HIV-related opportunistic conditions, apart from oesophageal candidiasis [34].

In the ACTG A5164 trial that compared ART timing strategies in patients with non-TB opportunistic and bacterial infections, receipt of corticosteroids during OI management (mainly for PCP) and the timing of ART were not associated with the development of IRIS [35]. However, no cases of IRIS developed while patients were still receiving corticosteroids [36]. In a report of the immunological profile of participants enrolled in this study, amongst patients who had received corticosteroids IFN- γ , IL-6, IL-8, IL-17 and TNF- α and sTNFrII levels were attenuated at time of IRIS [37]. In addition, patients who received corticosteroids, after controlling for the development of IRIS, had lower levels of TNF, sTNFrII and IL8 at baseline compared to those who did not receive corticosteroids [36]. The investigators concluded: “It ... appears that corticosteroids do suppress the same inflammatory markers that seem to identify patients at higher risk for IRIS, lending some support for their empirical use for treatment and potential prevention of IRIS events” [36].

3.5. Rationale for this study

Three recent RCTs that investigated the optimal time to start ART in HIV-TB patients [38-40] demonstrated that starting ART ~2 weeks into TB treatment in patients with low CD4 counts ($CD4 \leq 50/\mu L$) reduced mortality and AIDS progression compared with starting at ~8 weeks. Given these RCT findings, there will now be a major impetus at a programme level to start HIV-TB patients with low CD4 counts on ART after 2 weeks of TB treatment. However, low CD4 count and shorter interval between TB treatment and ART are the two most important factors increasing the risk of TB-IRIS [41]. In these same RCTs there was a 2 to 3-fold increase in risk of TB-IRIS in those who started ART ~2 weeks. In a recent paper the SAPIt investigators concluded: “Furthermore, a randomized, placebo-controlled trial that would investigate whether corticosteroids in patients with a CD4 count less than 0.050×10^9 cells/L initiating highly active ART early in tuberculosis treatment reduce frequency and severity of IRIS events and need for hospitalization is warranted [5].”

In these 3 RCTs, mortality in the group of patients who started at ~2 weeks was lower than in the group who started later, but mortality in the early arms was still 6-14% over the first year of ART. It is likely that certain of these deaths were attributable to TB-IRIS particularly if the central nervous system was affected. TB-IRIS will continue to be a major complicating factor in ART programmes in sub-Saharan Africa, causing considerable morbidity. Interventions to reduce the incidence and/or severity of TB-IRIS are urgently needed. Given the clinical trials evidence regarding the use of corticosteroids as adjuvant treatment in TB, for the treatment of TB-IRIS and to treat immunopathology in other HIV related conditions such as PCP, there is a compelling rationale for performing a trial of corticosteroids for TB-IRIS prevention.

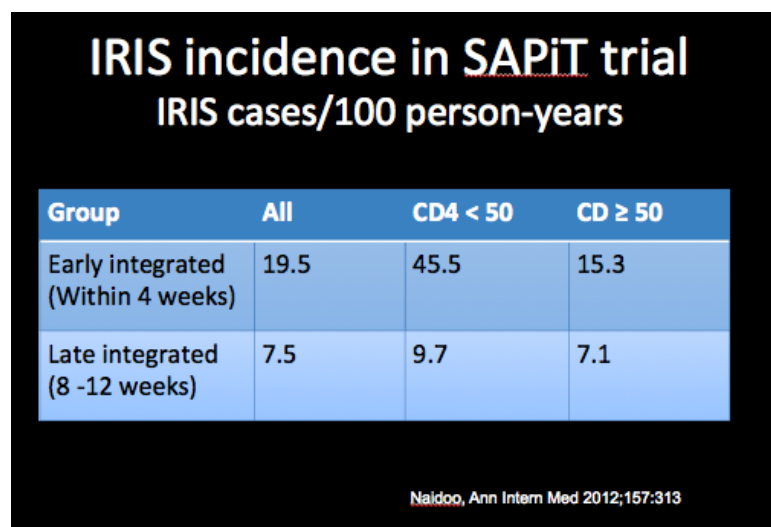


Figure 1. Incidence of paradoxical IRIS stratified by baseline CD4 count and ART timing in the SAPiT trial [5, 38]

Another important consideration is that corticosteroids have been shown to reduce the incidence of adverse drug reactions when used as adjunctive treatment in TB meningitis [16] and in HIV-infected patients with PCP [42]. Adverse drug reactions are a major complicating factor in the management of HIV-TB patients who are typically taking multiple drugs that are associated with drug hypersensitivity reactions and drug induced liver injury (TB treatment, ART and co-trimoxazole). In HIV-TB patients these drug reactions result in hospitalisations, drug interruptions that may result in resistance and disease progression and in some cases reactions are fatal. At our own hospital 3-month mortality among patients who were seen with TB drug or ART-induced liver injury was 35% [43]. By potentially reducing the incidence of these hypersensitivity reactions this is another way in which corticosteroids could potentially reduce morbidity, mortality and burden on limited health care resources.

In summary, we propose a proof-of-concept clinical trial of prednisone versus placebo for TB-IRIS prevention to assess efficacy and safety. The findings will influence clinical practice and will be used to design a larger phase 3 clinical trial to assess the impact of corticosteroids on mortality. Paradoxical TB-IRIS will remain common because many patients with HIV-TB still start ART with advanced immunosuppression in sub-Saharan Africa as a result of difficulties accessing HIV testing and treatment services. Even though international ART guidelines advocate for earlier start of ART during the course of HIV disease (WHO adult ART guidelines, 2010) the reality is that patients still present late. Moreover, recent reduction in pledging to the Global Fund and budget cuts by PEPFAR will likely cause longer ART waiting lists in some countries (personal communication, Anja De Wegheleire MSF in DRC).

Clinical services struggle to deal with this complex condition (TB-IRIS). The role of corticosteroids in preventing TB-IRIS is unknown, but there are several lines of evidence that suggest corticosteroids may have the potential to prevent or ameliorate the severity of TB-IRIS. In the proposed clinical trial equipoise exists because corticosteroids also have potential risks in the setting of advanced HIV.

3.6. Timelines and study site

Recruitment of the 240 patients to the study will take 13 months, with a further 3 months follow-up time after the last participant is enrolled. Initial analysis of results will be performed in the 3 months after the database has been locked and further analysis will take an additional 6 months. We have demonstrated capacity to enroll large numbers of patients with HIV-TB in prior studies. The clinical site chosen for the study is in Khayelitsha in Cape Town (single centre trial), where large numbers of patients start ART annually (over 20 000 patients have started ART in Khayelitsha since 2002), and up to 40% of patients starting ART are on treatment for TB [1] thus we are confident that there will be sufficient eligible patients to complete enrolment for the study in 12 months. Participant follow-up in this study is relatively short (12 weeks).

3.7. Expected impact of the study

We hypothesise that corticosteroids for the first 4 weeks of ART will reduce the incidence and severity of TB-IRIS and delay onset of TB-IRIS. Furthermore we hypothesise that TB-IRIS is an important contributor to early deaths in patients with low CD4 counts receiving early ART, and that this mortality could be reduced by prophylactic corticosteroids. In this proposed proof-of-concept phase 3 clinical trial we aim to assess the efficacy of steroids in reducing TB-IRIS and the safety of this intervention. If results of this trial demonstrate efficacy and safety we will take this intervention forward into a larger RCT with mortality as the primary endpoint. The findings of this trial will inform design and sample size calculation for the larger phase 3 trial.

We anticipate that the findings of this proposed clinical trial will themselves inform clinical practice and guidelines in sub-Saharan Africa. If corticosteroids are shown to reduce the risk of TB-IRIS, without safety concerns, clinicians will have an evidenced-based therapeutic option in patients at high risk for TB-IRIS provided no contra-indications to corticosteroid use exist.

3.8. Risks and dependencies

One risk is that we will not recruit sufficient numbers of eligible patients at the Sit B HIV-TB clinic in Khayelitsha during the study period. To overcome this we will raise awareness of the trial among all doctors and clinical nurse practitioners in Khayelitsha (visiting other clinics and distributing information pamphlets and posters that will be submitted to and pre-approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee) and ask them to refer potentially eligible and agreeable patients to the clinical trial site for screening. Transport costs for the clinic visit (R150) will be paid to all patients at the screening visit whether they are enrolled or not. In this way we are confident that we will meet the enrolment target based on ART figures obtained for 2011. Data regarding patients initiating ART in Khayelitsha clinics in 2011 was obtained from Andrew Boule (Centre for Infectious Disease Epidemiology and Research, UCT, and Public Health Specialist responsible for Program Evaluation at Western Cape Provincial Government) who curates the IeDEA database at UCT. Across all 11 Khayelitsha ART clinics, 5000 adult patients initiated ART in 2011: 2000 in clinics administered by the City of Cape Town and 3000 in clinics administered by the provincial government. Detailed data currently available for the City clinics from 2011 show that 23% of the patients had a CD4 count < 100/ μ L and

35% were on TB treatment at the time of ART initiation. It is likely that the profile of adult patients in the provincial government clinics was similar.

There is the risk that corticosteroids may do harm in this patient group due to immunosuppression and metabolic side effects. Prednisone used for 4 weeks for the treatment of TB-IRIS at higher doses in our previous clinical trial did not result in an excess of severe infections or metabolic side effects [3]. Nonetheless the potential for harm, in addition to potential benefit, defines the equipoise in the proposed study. We will ascertain all potential harms from corticosteroids (infections and metabolic side effects) by close clinical monitoring during the trial and documenting and analysing all potential side effects (metabolic and infectious) as pre-defined safety endpoints. The DSMB will review this data at the interim review and if there is evidence of significant harm from prednisone the trial would be stopped according to DSMB stopping rules that will be determined before trial initiation. The trial protocol will undergo ethical review at the Institutional Review Board of the ITM, the Ethics Committee of the Antwerp University Hospital in Belgium, and at the University of Cape Town, which involves rigorous peer review of the protocol.

4. Study Objectives and Design

4.1. Objectives

- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the risk of paradoxical TB-IRIS in patients with HIV-associated TB at high risk for TB-IRIS.
- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the severity and deleterious consequences of paradoxical TB-IRIS in patients with HIV-associated TB at high risk of TB-IRIS.
- To determine if prednisone given concomitantly with the first 4 weeks of ART reduces the risk of hypersensitivity drug reactions and consequent drug interruptions in patients with HIV-associated TB starting ART.
- To determine if such a course of prednisone is safe when used in this setting for this indication.

4.2. Design

This is a proof-of-concept phase III, randomised double-blind placebo-controlled trial of prednisone to assess efficacy and safety in preventing paradoxical TB-IRIS in high risk patients starting ART. The intervention will be oral prednisone 40mg daily for 14 doses started within 48 hours of initiating ART, followed by 20mg daily for 14 doses (or identical placebo) (Total 28 days of study medication).

Method of allocation, masking and concealment of allocation: Blinded treatments will be packaged at an independent off-site pharmacy, authorized by the MCC for this specific activity, in consecutively numbered identical packages. The packages will contain either prednisone or identical placebo tablets packaged according to a blocked (block size 8), 1:1 randomisation sequence prepared before the study start by a statistician not involved in the study conduct. Each package will have a number from 1 to 240. The numbered packages will be transported to the study pharmacy. Participants will be enrolled sequentially and will receive the next study number from 1 to 240 and the corresponding medication package. The medication packages will have identical appearance, be of equal weight and be tamper-proof. Participants, clinical site staff, investigators, data management personnel and the study statistician will remain blinded to the treatment allocation throughout the course of the trial. The study statistician will only have access to the randomisation sequence when the trial follow-up is complete and the database has been locked. The correctness of assignment and of recording of this in the CRF will be checked for each participant by the external monitor. The external monitor will specifically check to see that medication packages are assigned in consecutive order.

The treatment allocation for each participant will be kept off-site by the study statistician in a locked cupboard to allow unblinding of an individual patient's treatment allocation under exceptional circumstances where a patient deteriorates clinically and it is deemed essential for optimal clinical care by the treating physician to know the actual treatment that he/she received. Any instances of unblinding will be recorded and reported on the case report form, in the database, and in the study report and publication.

In order that unblinding could occur if the independent statistician was unavailable, a set of sealed opaque numbered envelopes with the random allocation assignment of each participant will be kept at the Groote Schuur Hospital pharmacy in a locked box. It will be visible if an envelope has been opened and this will be checked by the external monitor.

5. Selection and enrollment of participants

5.1. Approaching potential participants, information and informed consent

Patients who could fulfill the enrollment criteria will be approached by the study team about screening for the trial at the clinic where they are receiving TB treatment and asked to attend a screening visit. They will be provided with a screening informed consent form in the language they choose (Xhosa, Afrikaans or English). They will be given time to read the form or it will be read to them and they will have time to ask questions and have them answered by the study team. If they are agreeable to being screened then they will be asked to sign the screening informed consent form. All patients will be re-imbursed transport money (R150) for the screening visit.

Those patients who fulfill inclusion criteria upon screening will be invited to enroll in the clinical trial. This will involve an information sheet and an enrollment informed consent form. A member of the study team will explain the contents of the information sheet and answer all questions the patient has. The information sheet will explain:

- the nature of their condition (HIV-associated TB)
- the risk that they may develop TB-IRIS when starting ART and what TB-IRIS is
- that we are conducting a clinical trial of prednisone versus placebo to prevent TB-IRIS
- the potential medical risks (specific side effects) and benefits of prednisone
- the design of the trial and that participants would randomly receive prednisone or placebo and that neither participant nor trial team will know allocation
- that the trial will involve close clinical follow-up and that every effort will be made to diagnose adverse events and manage them immediately
- the follow-up schedule of the clinical trial and exactly what treatment and investigations are involved
- that participants need to continue to take their TB treatment and will start standard antiretroviral therapy during the trial
- that the information from participants will be anonymised and confidentiality will be protected
- that the sponsor has set up mechanisms to provide indemnity for any harms due to the participation in the study and details of this are included in the informed consent form
- it will be made clear to them that they are free to decline participation in the trial without affecting the treatment of either their HIV or TB and that they similarly can withdraw at any time during the trial without affecting their treatment

Patients will then be invited to participate in the clinical trial, provided that they fulfill inclusion criteria and have no exclusion criteria. Patients will be given time to consider participation and return on another day if they wish. They will also be given an opportunity to

bring a family member to find out about the trial and assist them with their decision (we have found that many patients in our setting request this, from our experience during previous clinical trials). Patients will not be rushed into making a decision regarding participation and it will be emphasized throughout that participation is voluntary and that there are potential risks and potential benefits involved in participation, and that every effort will be made by the clinical trial team to manage any medical complications rapidly and effectively. Patients who agree to participate will be asked to sign the enrollment informed consent form and will only be enrolled once this is done. Both the patient information sheet and all the informed consent forms will be submitted to the University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee and to the other concerned ethics committees (in Belgium) and will not be used until approved by these committees. The informed consent process will be conducted in line with ICH-Good Clinical Practice guidelines and the Helsinki Declaration 2008. All involved staff will be GCP trained and accredited and the consent process will be done according to a written Standard Operating Procedure that will include all the points above. Training on this SOP will be done before the trial is started and ongoing training will occur throughout the study. Any issues that arise during the consenting process will be discussed with the PI in real time and with the chair of the Human Research Ethics Committee at UCT if necessary. In patients who are unable to write, informed consent will be taken in the presence of an independent witness. If the patient agrees to participation they will provide a thumbprint on the informed consent document and the witness will sign to confirm that informed consent has been taken. Patients who are not cognitively competent to provide informed consent will not be enrolled in the trial.

The following members of the study will be responsible for taking informed consent: the principal investigator, the study medical officer, the study nurse and/or the study counselor.

5.2. Study enrollment criteria

5.2.1. Inclusion criteria

1) HIV–infected

HIV infection will be confirmed by two different rapid tests (as per South African national Department of Health guidelines) and an HIV viral load test.

2) CD4 count $\leq 100/\mu\text{L}$

One CD4 count taken within 3 months prior to enrolment less than $100/\mu\text{L}$ will qualify, even if other CD4 counts are greater than $100/\mu\text{L}$

3) ART-naïve (or initiated ART less than 48 hours prior to initiation of study drug)

Patients who report having been treated with triple drug or dual drug ART previously will be excluded. Single dose nevirapine or short term AZT monotherapy for PMTCT is not an exclusion.

4) Confirmed diagnosis of TB (smear, culture, Xpert MTB/RIF test or compatible histology) or strong clinical and radiological evidence of TB with symptomatic response to TB treatment

- 5) On TB treatment for less than 30 days prior to study entry.
- 6) Eligible for ART and patient consents to starting ART within 30 days of starting TB treatment.
- 7) Written informed consent for trial

5.2.2. Exclusion criteria

- 1) Kaposi's sarcoma (KS)

A thorough examination for KS lesions will be performed and any suspicious lesion will be biopsied. Any history of treatment for KS will also be an exclusion.

- 2) Pregnant

All female participants of child-bearing potential will have a pregnancy test performed prior to enrollment and will be counseled to use two reliable methods of contraception for the duration of the trial.

- 3) <18 years old

- 4) TB meningitis or tuberculoma at TB diagnosis

- 5) **Clinical** syndrome of pericardial TB at TB diagnosis (a pericardial effusion noted on ultrasound scan alone is not an exclusion criterion)

- 6) Rifampicin-resistant TB diagnosed by Xpert MTB/RIF test or a drug susceptibility test performed on a culture isolate.

- 7) On corticosteroids for another indication or on any other immunosuppressive medication within the past 7 days.

- 8) Uncontrolled diabetes mellitus

- 9) The following abnormal laboratory values:

Alanine aminotransferase > 200 IU/l

Absolute neutrophil count < 500/mm³

- 10) Not on standard intensive phase TB treatment (Rifampicin, isoniazid, pyrazinamide and ethambutol)

- 11) Poor clinical response to TB treatment prior to ART as judged by the clinical investigators.

- 12) Hepatitis B surface antigen positive

6. Study Endpoints

6.1. Primary efficacy endpoint

The development of paradoxical TB-IRIS within 12 weeks of starting ART (defined using the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition [2]).

Panel 2: Case definition for paradoxical tuberculosis-associated IRIS

There are three components to this case definition:

(A) Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of tuberculosis: the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴
- Initial response to tuberculosis treatment: the patient's condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinitiation, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss
- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as "probable paradoxical tuberculosis-associated IRIS". In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as "paradoxical tuberculosis-associated IRIS" cases.

INSHI consensus case definition for paradoxical TB-IRIS

6.2. Secondary efficacy endpoints

- 1) Time to TB-IRIS event (from start of ART to onset of IRIS symptoms in days)
- 2) Severity of TB-IRIS events (defined by the following: need for hospitalisation for IRIS, C-reactive protein, neurological involvement)
- 3) Duration of TB-IRIS event (from onset of symptoms/signs to resolution of TB-IRIS symptoms and signs)

- 4) Mortality attributed to TB and TB-IRIS
- 5) All-cause mortality
- 6) Composite endpoint of death, hospitalization, or hepatotoxicity (using the protocol-specified definition of Grade 3 or 4 increase in ALT or bilirubin).
- 7) Other (non-TB) IRIS events
- 8) Quality of life assessment (measured using PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score)
- 9) Adverse events and severe adverse events ascribed to TB treatment, ART or co-trimoxazole. This will include a pre-specified analysis of drug-induced liver injury and drug rash. This assessment will include the number of treatment interruptions for drug adverse events.
- 10) Discontinuation of either ART or TB treatment for > 5 days due to adverse events.
- 11) Number of hospitalizations and total days hospitalized

6.3. Secondary safety and tolerability endpoints

- 1) Corticosteroid-associated adverse events, classified by severity and relation to study drug. These will include hypertension, hyperglycaemia, hypomania/mania (diagnosed by psychiatrist), depression (diagnosed by psychiatrist), acne, epigastric pain, upper gastrointestinal bleeding, Cushingoid features, new oedema and avascular bone necrosis.
- 2) Laboratory safety data: glucose, full blood count and electrolytes
- 3) Other infections (AIDS-related, bacterial, fungal and viral) and malignancies (Kaposi's sarcoma)
- 4) All grade 1, 2, 3 and 4 adverse events (clinical and laboratory using the ACTG grading system)

6.4. Quality of Life (QoL) assessments

As a secondary outcome measure we will perform health-related quality of life measures of the participants included in the trial at week 0, 4 and 12. The following measurements will be performed: PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score. See section 21 for details of this QoL substudy.

7. Study medication

7.1. Prednisone and identical placebo

Study medication will be prednisone tablets (5mg) or identical placebo tablets. The study medication will be prepared by the Gulf Drug Company, Durban, South Africa. This generic pharmaceutical company supplies prednisone to the government hospital pharmacies in South Africa (marketed as Trolic 5mg tablets). They manufactured prednisone and placebo tablets for our previous trial of prednisone for TB-IRIS treatment. We have estimated a requirement of 21 000 placebo tablets and 21 000 prednisone tablets.

Participants will receive 40mg (8 tablets) daily of 5mg prednisone (or identical placebo) for 14 days followed immediately by 20mg daily (4 tablets) daily of 5mg prednisone (or identical placebo) for 14 days. Participants will start the study medication on the same day or within

48 hours of initiating antiretroviral therapy. The total duration of study medication will be 28 days.

7.2. Manufacture and delivery of study medication

The study medication will be manufactured by the Gulf Drug Company in Durban. The University of Cape Town will be charged for the manufacture of the study medication and a quote has been obtained. The Gulf Drug Company will deliver the medication to the Grootte Schuur Hospital pharmacy (an independent pharmacy) as one container of prednisone and one container of placebo. The Grootte Schuur Hospital pharmacist will sign for receipt of the medication.

7.3. Packaging of study medication

The randomization sequence for the trial will be generated by an independent statistician according to a blocked (block size 8), 1:1 randomisation sequence. This will be sent to the independent pharmacist at Grootte Schuur Hospital. This pharmacist will pack the prednisone and placebo tablets into sequentially labeled medicine containers labeled from participant 1 to 240. There will be two medicine packages for each participant:

- 1) **Day 0-14:** 8 x 14 tablets = 112 tablets
- 2) **Day 15-28:** 4 x 14 tablets = 56 tablets

This process will be undertaken independent of any investigators or study team members who will remain blinded with respect the randomization sequence and the packaging of the study medication. Once all the study medication has been packaged it will be transferred to the clinical trial site pharmacy and the on-site pharmacist will sign for receipt of the study medication.

7.4. Storage at the clinic

The study medication will be stored in the clinic research pharmacy which is locked by the pharmacist on site when she is not present. It will be stored at room temperature (at or below 25 degrees Celsius in adherence with manufacturers' instructions) and a temperature log will be kept in the pharmacy. Study drug management and accountability will be described in a specific SOP.

7.5. Dispensing of medication

Medication will be dispensed according to patient enrollment number by the study pharmacist from patient 1 to 240. The pharmacist will keep a log of all study medication dispensed recording date, patient number and initials and packet number.

Study medication will be dispensed on two occasions:

At Week 0 visit, the first 14 days medications will be dispensed

At Week 2 visit, the 15-28 days medication will be dispensed

At the week 1, 2 and 4 visit participants' adherence to study medication will be checked by means of pill counts and the number of tablets returned will be recorded on the CRF. Participants will be asked to bring back their medication packet for this purpose.

7.6. Maintenance of randomization concealment

Participants, clinical site staff, investigators, data management personnel and the study statistician will remain blinded to the treatment allocation throughout the course of the trial. The randomization sequence will be kept away from the trial site securely by the independent statistician. This statistician will store an electronic copy and a hard copy. A printed and sealed copy of this randomization sequence will also be given to the Sponsor representative (Deputy Dean of Research, Faculty of Health Sciences) for storage to ensure a back-up copy is available. At the trial site the placebo and prednisone tablets and packaging will be identical with no markings or labeling to differentiate which medication containers contain prednisone or placebo. Only once the trial database is locked will the randomization sequence be made available to the trial team.

In the event of unblinding of an individual participant being required in the opinion of the attending clinician (see section 13.10.) the independent statistician will be contacted for this information. In order that unblinding could occur if the independent statistician was unavailable, a set of sealed opaque numbered envelopes with the random allocation assignment of each participant in will be kept at the Groote Schuur Hospital pharmacy in a locked box. It will be visible when an envelope has been opened and this will be checked by the external study monitor.

7.7. Expiration and re-ordering of medication

The expiration date of the study medication will be noted upon delivery. It is planned that the product will have a shelf life that covers the entire study duration. The manufacturers have confirmed a shelf-life of 24 months for prednisone. This will be specified in the order. However, we will have a plan to cover the contingency that product reaches its expiry date. Six months prior to the expiration date, additional prednisone and placebo tablets will be ordered from the Gulf Drug Company. The same process will be followed with respect to delivery, blinding procedures, packaging and storage.

7.8. Destruction of medication

At the conclusion of the trial or when study medication has expired, study medication will be destroyed by the study pharmacist, according to the local regulations, and a record of study medication final accountability and destruction will be made and filed.

7.9. Prohibited concomitant medication

The following concomitant medications are prohibited during the 4 weeks that participants are dispensed study medication: any non-steroidal anti-inflammatory drug (NSAID), any systemic corticosteroid medication or any other immunosuppressive medication or chemotherapy. Clinical investigators will not prescribe these medications and we will communicate with the participants' primary care clinics that these are prohibited medications

during this 4 week period.

8. Antiretroviral therapy (ART)

8.1. ART regimen

ART will be provided according to South African Department of Health guidelines. All of the patients who fulfill criteria for entering this trial are eligible to start ART according to these guidelines and willingness to initiate ART is an inclusion criterion for the trial. The trial site is a Department of Health accredited ART site and patients who are eligible receive ART free of charge at this clinic. Participants will thus be provided with ART from the clinic ART stock and will also be assessed and managed in their ART clinic according to DOH guidelines.

First line ART in Department of Health clinics in South Africa is tenofovir 300mg daily plus emtricitabine 200mg daily (or lamivudine 300mg daily) plus an NNRTI (nevirapine or efavirenz). It is advised that patients on rifampicin-based treatment for TB (as participants in this trial will be) receive efavirenz rather than nevirapine because of pharmacokinetic drug interactions.

The ART regimen for most participants in this trial will thus be:

Tenofovir (TDF) 300mg daily + Emtricitabine (FTC) 200mg daily (or Lamivudine (3TC) 300mg daily) + Efavirenz 600mg daily (all taken at night).

When available in the clinic the single tablet fixed dose combination of TDF/FTC/Efavirenz will be prescribed, when not available then the individual tablets of TDF, 3TC and Efavirenz will be prescribed. During the period that the FDC is introduced in South Africa it is likely that supply issues will not permit that it is always available to be prescribed.

No dose adjustment of the efavirenz is advised in patients on rifampicin-based TB treatment because virological outcomes have been shown to be unaffected by concomitant rifampicin with ART containing efavirenz 600mg daily in our setting [44, 45].

There may however be circumstances in which either one of the tenofovir or efavirenz is contra-indicated and is substituted by an alternative that is available in government clinics. Such substitutions will be discussed between the ART clinic doctor or nurse and the study team to arrive at a consensus decision. The scenarios in which these substitutions may occur and the alternatives are outlined in Table below:

| Clinical circumstance | Drug to be substituted | Alternative |
|---|-------------------------------|--|
| Renal impairment (Creatinine clearance < 50ml/min) | Tenofovir | Zidovudine (provided haemoglobin > 9g/dl and neutrophil count > 1.0 x 10 ⁹ /ml) OR stavudine (relatively safe for |

| | | |
|--|-----------|---|
| | | < 6 months) |
| Active psychiatric disease or shift work | Efavirenz | Nevirapine (provided CD4 < 250 cells/μl in women or < 400 cells/μl in men) OR Lopinavir/ritonavir (needs to be double-dosed if on rifampicin) |

8.2. ART substitutions for toxicity

For the acute management of drug reactions see section 12.4. In the event that a drug reaction or toxicity is attributed to one of the ART drugs then a single drug substitution will be made. Toxicities or reactions that may result in substitutions are listed in the Table below.

| Drug reaction/toxicity | ART drug implicated | Alternative ART drug for substitution |
|-------------------------------|---------------------|---|
| Renal impairment | Tenofovir | Zidovudine (or stavudine) |
| Red cell aplasia | Lamivudine | Stavudine |
| Neuropsychiatric side effects | Efavirenz | Nevirapine (or double-dose lopinavir/ritonavir if nevirapine is contra-indicated) |
| Hepatitis | Efavirenz | Double-dose lopinavir/ritonavir |

8.3. ART switches for virological failure

No switches for virological failure will be made in the first 3 months of ART (ie. during the study follow-up period). In the Department of Health ART guidelines it is advised that the first HIV viral load is measured at 4-6 months after starting ART. The indication for switching to second line ART is an HIV viral load > 1000 copies/ml on two separate measurements performed 3 months apart. The viral load testing done during this trial is thus before HIV viral load testing is advised in the national guidelines and is before virological suppression would be expected in patients with high baseline HIV viral loads. Because of this, patients detected with primary virological failure* by our HIV viral load testing at 4 weeks would not be eligible to switch to second line ART based on Department of Health guidelines. We will, however, make all HIV viral load results available to the ART clinic clinician and alert the clinician to the concern if a patient has primary virological failure so that it is ensured that they have a repeat HIV viral load at 4 months on ART.

*Primary virological failure defined as the failure to achieve a > 1 log drop of the viral load from baseline by 1 month on ART.

9. TB treatment

Patients with HIV infection and suspected TB in the South African public sector TB clinics are investigated with sputum for MTB/RIF Xpert and sputum smear initially, and if these are negative then culture and chest radiographs are performed. Investigations for extrapulmonary TB include needle aspirations of lymph nodes or pleural effusions or further investigations at a referral hospital.

TB treatment will be prescribed and monitored by the clinical staff in the local HIV-TB clinic. TB treatment will be prescribed according to South African Department of Health guidelines.

The TB treatment regimen for cases presumed or known to have drug susceptible TB over 8 years of age is:

| Pre-treatment body weight | Intensive phase 2 months Given 7 days a week | Continuation phase 4 months Given 7 days a week | |
|---------------------------|---|--|-----------------|
| | RHZE 150/75/400/275mg | RH 150/75mg | RH 300/150mg |
| 30-37kg | 2 tablets | 2 tablets | - |
| 38-54kg | 3 tablets | 3 tablets | - |
| 55-70kg | 4 tablets | - | 2 tablets |
| ≥71kg | 5 tablets | - | 2 tablets |

R=rifampicin, H=isoniazid, Z=pyrazinamide, E=ethambutol

Source: National Tuberculosis Control Programme of the Department of Health, 2009

For management of TB drug reactions and toxicities see section 12.4.

10. Study visits and evaluations

10.1. Schedule of events

| Study visit | Screening | Enrollment | Entry Week 0 | Week 1 | Week 2 | Week 4 | Week 8 | Week 12 | Un-scheduled visit |
|-----------------------------|---------------|-----------------|--------------|---------|----------|----------|----------|----------|--------------------|
| ART day | Not specified | Aim for -7 to 0 | 0-2 | 7 +/- 4 | 14 +/- 4 | 28 +/- 4 | 56 +/- 4 | 84 +/- 7 | Not specified |
| Document HIV status | X | | | | | | | | |
| Assess eligibility criteria | X | X | X | | | | | | |
| Screening ICF | X | | | | | | | | |
| Enrollment ICF | | X | | | | | | | |
| Study drug administration | | | X | | X | | | | |
| Past medical history | X | | | | | | | | |
| Current symptoms | X | X | X | X | X | X | X | X | X |
| IRIS questions | | | | X | X | X | X | X | X |

| | | | | | | | | | |
|--------------------------------------|---|---|---|---|---|---|---|---|-----------------|
| Adverse events assessment | X | X | X | X | X | X | X | X | X |
| Medication review | X | I | X | X | X | X | X | X | X |
| Karnofsky score | X | | X | X | X | X | X | X | X |
| Pill count | | | | X | X | X | | | |
| QOL assessments | | | X | | | X | | X | |
| Examination | X | I | X | X | X | X | X | X | X |
| Na,K,Cr,glucose | X | I | X | | X | X | | X | I |
| ALT, Alk Phos | X | I | X | | X | X | | X | I |
| FBC, diff | X | I | X | | X | X | | X | I |
| CRP | | I | X | | X | X | | X | I |
| CD4, HIV viral load | X | | | | | | | X | |
| HepBsAg | X | | | | | | | | |
| Serum CrAg | X | | | | | | | | |
| Urinary pregnancy test | X | I | I | I | I | I | I | I | I |
| Storage bloods and immunology assays | | | X | | X | X | | X | If IRIS suspect |
| Storage urine | | | X | | | | | X | If IRIS suspect |
| MTB/RIF Xpert, culture and DST | X | | | | | X | | X | |
| Initiate ART | | | X | | | | | | |

I = If clinically indicated

ICF = Informed consent form

Study visits will occur at screening for the study, at an enrollment visit, week 0 (the day the patient starts study drug), week 1, week 2, week 4, week 8 and week 12. A window period of +/- 4 days will be allowed for all visits apart from the week 0 visit (no window) and week 12 (where a window of +/- 7 days will be allowed). If visits do not occur in these windows then a “missed visit” will be recorded.

Patients will be telephoned before each visit by the nurse or counselor to remind them of the visit. Patients will be re-imbursed R150 for each scheduled visit towards transport and meal costs. Patients who are screened but not recruited will also be re-imbursed for this visit. This will be stated in the informed consent forms. If patients are lost to follow up then an attempt to ascertain their outcome at 12 weeks will be made telephonically. At each study visit the research team will check that the patient has sufficient supply of TB medication (or is receiving daily from TB clinic) and ART and other prescribed medication.

A maximum of 40ml blood will be drawn at any study visit. Only trained personnel will perform phlebotomy. Patients who have symptomatic anaemia (Hb<8g/dL with symptoms attributable to anaemia) will not have more than 30ml of blood drawn at any given visit.

Screening visit

Patients identified in the HIV-TB clinic (or referred there specifically for screening for this study, as mentioned in 3.8) who potentially meet enrollment criteria will be referred to the study team. Patients should have been on TB treatment for ≤ 21 days at this screening visit. Patients will be briefly informed about the study and written informed consent will be taken from the patient to screen them for eligibility for the clinical trial with a screening visit informed consent form. If patients sign screening consent they will be assessed with the inclusion and exclusion criteria for the trial. The HIV status of the patient will also be documented by the study team at this visit. In all patients being screened for the trial, regardless of whether they have had a previous positive HIV test, HIV testing will be performed with appropriate counseling using two different rapid HIV tests. This is the standard of care in the clinic. If the results are discrepant or indeterminate blood will be sent to the National Health Laboratory Services laboratory for ELISA confirmation. In addition, as stated below an HIV viral load is performed at this visit, that will serve as confirmatory of the HIV diagnosis. The following study procedures will be performed (blood and urine will only be taken if patients fulfill inclusion criteria and have no exclusion criteria after notes review, history and examination):

- 1) Past medical history
- 2) Current drugs and drug allergies
- 3) Current symptoms
- 4) Physical examination
- 5) Chest radiograph (Only if there is no recent chest radiograph available for the clinical team to review and a chest radiograph is deemed necessary by clinical team. Note that all participants will have a chest radiograph performed at study entry visit)
- 6) HIV test as described above
- 7) Na, K, creatinine, glucose
- 8) ALT, alkaline phosphatase
- 9) Full blood count and differential
- 10) Serum cryptococcal antigen test (CrAg)
- 11) Hepatitis B surface antigen
- 12) CD4 count and HIV viral load
- 13) Sputum (and other biological samples where appropriate) for MTB/RIF Xpert, culture and DST (the main reason is to exclude rifampicin-resistant TB)
- 14) In female patients, urinary pregnancy test
- 15) All results done during TB diagnostic work-up and during TB treatment will be obtained from the National Health Laboratory Services laboratory database (including CD4 count and all TB diagnostic tests)
- 16) Karnofsky score
- 17) Assess adverse events

Patients will receive an ART counseling session at this visit and arrangements will be made for two further sessions prior to the potential Week 0 visit. Standard ART counseling practices will be followed. Patients will be invited to bring a relative or “treatment buddy” to ART counseling sessions. In particular ART side effects, need for 100% adherence, lifelong need for treatment and TB-IRIS will be discussed in these sessions. Patients will be asked to return for laboratory results and enrollment visit if eligible and provided a visit date (they will receive re-imburement for transport of R150 for the screening visit and this follow-up visit

whether they are eligible for the trial or not). They will be invited to bring a relative along to the enrollment visit. At this screening visit a target date for starting ART will be set in liaison with the ART clinic staff. The participant will be entered in the screening log at this visit. If the participant fulfills clinical criteria for enrollment they will be given a patient information sheet with information about the trial to read (or it will be read to them) in the language of their choice. They will be able to take this sheet home with them if they wish.

Enrollment visit

The timing of this visit will occur between the Screening visit and the Study Entry (Week 0) visit and the proximity to either will depend on how long the patients has been on TB treatment and when the clinic plan to start ART. The aim is for this visit to be no more than 7 days before the Study Entry visit. Under exceptional circumstances, this visit may be on the same day as the Study Entry visit if patient is to start ART on this day. The main purpose of this visit is to review the laboratory results from the Screening visit to ensure all laboratory inclusion criteria are met and no laboratory exclusion criteria are present. Clinical inclusion and exclusion criteria will also be reviewed. The enrollment CRF will be completed. If patients fulfill all inclusion criteria and have no exclusion criteria they will be invited to participate in the clinical trial.

The patient information sheet will be reviewed with the patient. If they are unable to read then the sheet will be read to them by a counselor if this was not yet done. They will be given an opportunity to ask questions. If they have brought a relative along, the relative will be included in the discussion if the patient requests this. If they are willing to provide informed consent and in the judgement of the study doctor they have full capacity to make an informed and voluntary decision, they will sign the enrollment informed consent document. If they are unable to write, they will provide a fingerprint and an independent witness will witness the informed consent process and the fingerprinting of the informed consent document and sign and date as witness.

A full clinical assessment is not planned at this visit, but if patients report new symptoms then they will be examined and investigated and managed appropriately.

The participant will be entered in the enrollment log at this visit.

Study Entry / Week 0 visit (Day 0)

This visit must be when the patient has been on TB treatment for ≤ 28 days and on ART for <48 hours if ART was already initiated.

All trial inclusion and exclusion criteria will be rechecked and this will be documented on the CRF, prior to the patient formally entering the study. If a patient is fully eligible, he/she will be given the next sequential enrollment number and receive the corresponding blinded study medication. Procedures to be performed:

- 1) Current symptoms
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase

- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) Chest radiograph (for all participants)
- 9) Quality of life assessments
- 10) Karnofsky score
- 11) Assess adverse events
- 12) Issue Day 1-14 study medication

A randomization number (corresponding to the next sequential study medication container) will be assigned at this visit and documented on the enrollment log.

Week 1 visit (Day 7 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) ART and study drug pill count to assess adherence

Week 2 visit (Day 14 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) ART and study drug pill count to assess adherence
- 9) Karnofsky score
- 10) Assess adverse events
- 11) Issue Day 15-28 study medication

Week 4 visit (Day 28 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) Bloods for immunological assays
- 8) ART and study drug pill count to assess adherence
- 9) Sputum for MTB/RIF Xpert, culture and DST (to assess the effect of corticosteroids on sputum conversion)
- 10) Quality of life assessments
- 11) Karnofsky score
- 12) Assess adverse events

Week 8 visit (Day 56 +/- 4 days)

- 1) Current symptoms and specific TB-IRIS symptom screen

- 2) Physical examination
- 3) ART and study drug pill count to assess adherence
- 4) Karnofsky score
- 5) Assess adverse events

Week 12 visit (Day 84 +/- 7 days)

- 1) Current symptoms and specific TB-IRIS symptom screen
- 2) Physical examination
- 3) Na, K, creatinine, glucose
- 4) ALT, alkaline phosphatase
- 5) Full blood count and differential
- 6) C-reactive protein (CRP)
- 7) CD4 and HIV viral load
- 8) Bloods for immunological assays
- 9) ART and study drug pill count to assess adherence
- 10) Sputum for MTB/RIF Xpert, culture and DST (to assess the effect of corticosteroids on sputum conversion)
- 11) Quality of life assessments
- 12) Karnofsky score
- 13) Assess adverse events
- 14) Refer back to HIV-TB clinical service if clinically stable

Unscheduled visits/sick visits

Participants will be encouraged to attend the clinic for unscheduled visits if they experience symptomatic deterioration. They will also be provided with the mobile number of the study team that will be answered 7 days a week. They will be assessed by the study doctor. If the reason for deterioration is suspected TB-IRIS they will be investigated and managed as described in section 13.2. If drug reaction, such as drug-induced liver injury, is suspected they will be managed as described in section 13.4. Other clinical problems will be managed as appropriate. If patients are sick enough to require hospital admission for investigation and management they will be referred to the local district hospital (Khayelitsha District Hospital) and their clinical condition will be closely monitored there by the study team while they are managed by the medical staff on the inpatient service.

Post-trial visits

For patients who have ongoing TB-IRIS or whose clinical condition is not stable at the 12 week visit further visits will occur for clinical management reasons and to ascertain the end date for TB-IRIS. These will be recorded as unscheduled (post-trial) visits.

Research blood and urine samples for storage

In the enrollment informed consent form patients will be specifically asked to agree to or decline permission for storage of blood and urine samples for later research into HIV, TB and TB-IRIS biomarkers and pathogenesis. They will be able to decline permission for storage without affecting participation in the trial. Any use of stored samples for future research will require specific permission from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee. A separate consent form will be used to obtain consent for taking a blood sample for genetic testing.

The blood stored will be plasma and PBMC (sodium heparin tube, for cytokine analysis and flow cytometry), DNA (one time point at week 0, citrate tube) and RNA samples (Tempus tube). The collection of the DNA will be deferred if the patient has had a recent blood transfusion. The timing of the other bloods will be: Week 0, Week 2, Week 4, Week 12 and suspected TB-IRIS sick visit.

For trial participants that decline storage consent, their study folder will be marked at study entry with a sticker stating “No storage” and storage bloods will not be collected on these participants.

Participants who do not start ART

It is possible that certain patients will be seen at the Week 0 visit will be prescribed ART but will not start the ART medication. In this event the study medication will be stopped as soon as investigators becomes aware, but follow-up will continue and patients will be included in the intent to treat analysis.

Participants who do not attend Study Entry visit within 28 days of starting TB treatment

If a patient signs the enrollment informed consent but fails to attend their Study Entry visit on the planned date, they will be phoned to reschedule this visit. However, if they do not attend for the Study Entry visit within 30 days of starting TB treatment then they will not receive study medication and they will not receive a randomization number, but clinical follow-up may occur as per protocol Addendum 22.5. They will not be included in the intent to treat analysis. The same applies to patients who sign enrollment consent form and are then found to have an exclusion criterion prior to randomization.

Patient withdrawal from trial

Patients are free to withdraw from the trial at any point as stated in the informed consent form. Those patients withdrawing from the trial will be encouraged to continue ART and TB treatment at their local clinic.

11. Definitions

11.1. Paradoxical TB-IRIS will be defined using the International Network for the Study of HIV-associated IRIS (INSHI) case definition. See section 6.1.

11.2. WHO Stage 4 conditions (WHO 2006 revision)

- HIV wasting syndrome
- pneumocystis pneumonia
- recurrent severe bacterial pneumonia
- chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)
- oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- extrapulmonary TB
- Kaposi's sarcoma
- cytomegalovirus infection (retinitis or infection of other organs)

- central nervous system toxoplasmosis
- HIV encephalopathy
- extrapulmonary cryptococcosis including meningitis
- disseminated non-tuberculous mycobacteria infection
- progressive multifocal leukoencephalopathy
- chronic cryptosporidiosis
- chronic isosporiasis
- disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis)
- recurrent septicaemia (including non-typhoidal salmonella)
- lymphoma (cerebral or B cell non-Hodgkin)
- invasive cervical carcinoma
- atypical disseminated leishmaniasis
- symptomatic HIV-associated nephropathy
- symptomatic HIV-associated cardiomyopathy.

11.3. Drug-induced liver injury (DILI)

DILI will be defined using the ACTG grading system (see section 22.2: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009). We will specifically classify Grade 2, 3 or 4 elevations of ALT and total bilirubin as DILI: ALT elevation 2.6-5 x upper limit of normal is grade 2, 5.1-10 x upper limit of normal is grade 3 and > 10 x upper limit of normal is grade 4. Total bilirubin elevation 1.6-2.5 x upper limit of normal is grade 2, 2.6-5 x upper limit of normal is grade 3 and > 5 x upper limit of normal is grade 4.

We will further differentiate clinical hepatitis (these elevations together with symptoms of hepatitis: nausea, vomiting, right upper quadrant pain or lethargy) from all biochemical hepatitis (any elevation above these levels regardless of whether symptomatic or asymptomatic).

11.4. Drug rash

A drug rash will be defined as any rash the onset of which is after the initiation of a drug or drugs, the morphology and natural history of which is compatible with a drug rash. Cases in which the aetiology is unclear will be reviewed by a Dermatologist (either seeing the patient or digital photographs taken of the rash).

11.5. Corticosteroid-associated adverse events

Clinical symptoms and signs that could possibly be attributed to corticosteroids side effects will be documented and recorded as corticosteroid-associated adverse events. This will include: new hypertension (resting BP: systolic > 160 mmHg or diastolic >100mmHg), new poor BP control in known hypertensive (who reports adherence to anti-hypertensive therapy, using same thresholds for systolic and diastolic BP), hyperglycaemia (random glucose > 11.1 mmol/l), new oedema, hypomania/mania (diagnosed by psychiatrist), depression (diagnosed by psychiatrist), Cushingoid features or habitus (in the opinion of the clinical investigator),

acne, gastritis symptoms (epigastric pain), upper gastro-intestinal bleeding or avascular bone necrosis. Avascular necrosis will be investigated if patients present with symptoms of bone pain.

Only features that develop while patients are on the study drug or for the 4 weeks afterwards will be classified as corticosteroid side effects.

Infections will not be recorded under corticosteroid side effects but will be recorded and analysed separately.

12. Laboratory evaluation

All laboratory tests (blood and sputum) will be performed at the National Health Laboratory Services laboratories at Green Point or Groote Schuur Hospital. These laboratories are SANAS (South African National Accreditation System) accredited and experienced in performing investigations for clinical research. Hard copy reports of all results will be forwarded to the clinical trial team as soon as the investigation result is available. These will be signed and dated by the trial team. If there is an abnormality then appropriate action will be taken (eg. recalling patient) and this will be documented on the hard copy result. All abnormal investigation results will be communicated to the TB and ART clinic staff.

13. Clinical Management

13.1. Co-trimoxazole prophylaxis

All patients on the clinical trial are eligible for co-trimoxazole primary prophylaxis, unless they are known to have a sulphonamide allergy. This is because all eligible study patients are HIV-infected with CD4 < 100 cells/ μ l and have WHO stage 3 or 4 disease. The co-trimoxazole dose used for primary prophylaxis will be 960mg daily (2 tablets). In those patients who cannot take co-trimoxazole or who develop an allergic reaction while on co-trimoxazole then dapson 100mg daily will be considered as an alternative. In cases where patients have had life-threatening co-trimoxazole reactions (eg. Stevens Johnson syndrome) then dapson will not be used as there a small risk of cross-reactivity.

13.2. Management of suspected TB-IRIS

In patients who deteriorate with clinical or radiographic features of TB after starting ART paradoxical TB-IRIS will be a consideration. This will include patients who develop new or recurrent TB symptoms or fever, enlarging lymph nodes or worsening chest radiograph pulmonary infiltrates, enlarging effusions or TB neurological features. At any such deterioration suggestive of paradoxical TB-IRIS (at a scheduled or unscheduled sick visit) the following investigations will be performed:

- 1) Bacterial blood culture
- 2) Creatinine/electrolytes/glucose, liver functions, full blood count/differential, CRP, serum CrAg (cryptococcal antigen test)
- 3) Sputum and other biological samples for MTB/RIF Xpert, culture and DST

- 5) Chest radiograph
- 6) Other investigations according to clinical presentation (eg. abdominal ultrasound, lumbar puncture, CT head)

The principal aim of these investigations is to exclude alternative causes for clinical deterioration as the diagnosis of paradoxical TB-IRIS is a diagnosis of exclusion. In addition, the adherence of patients to ART and TB medication will be checked by means of an interview with the patient and treatment buddy, and counting pills in the pill container and checking with the TB clinic staff.

If paradoxical TB-IRIS is diagnosed (fulfilling INSHI case definition for paradoxical TB-IRIS), open label corticosteroids will be prescribed for IRIS treatment at physician discretion. Study drug will be stopped on the day the decision is made to start open label corticosteroids. The decision to commence corticosteroids to treat TB-IRIS and exact timing is an individualized one that is based on physician discretion and takes into account the severity of symptoms and the confidence with which alternative causes for clinical deterioration have been excluded.

The prednisone starting dose to treat TB-IRIS will be 1.5mg/kg/day and this will be reduced according to clinical response. In patients with central nervous system TB-IRIS the initial corticosteroid used may be dexamethasone intravenously.

13.3. Management of new opportunistic infections or malignancies

If patients are diagnosed with a new opportunistic infection or malignancy while on the trial they will be referred to the appropriate level of care and management will be according to national Department of Health guidelines for the treatment of opportunistic infections. For example, patients with cryptococcal meningitis will be treated with amphotericin B followed by fluconazole.

When a new WHO stage 4 opportunistic infection or malignancy is diagnosed while patients are on study medication this will be an indication for stopping study medication. For certain opportunistic infections it may be indicated to start corticosteroid therapy (eg. pneumocystis pneumonia with respiratory failure). This decision will be taken by the attending clinician and open label corticosteroid therapy will be documented.

13.4. Management of drug reactions

If TB drug-induced liver injury occurs during follow-up cases will be managed according to the local clinical guidelines (Western Cape Academic Hospitals Antimicrobial Recommendations). These guidelines are based on the American Thoracic Society guidelines for management of TB drug hepatotoxicity [46]:

Hepatotoxicity

TB drug-induced hepatitis is over-diagnosed: the case definition is transaminases more than 5-fold elevated or more than 3-fold elevated with symptoms/jaundice. Antituberculous therapy should be discontinued. The basis for the TB diagnosis should be reviewed. If the grounds for diagnosing TB were reasonable then commence three antituberculous drugs with low/no hepatotoxic potential (see background therapy below). Selected patients may then be rechallenged once symptoms of hepatitis have resolved, bilirubin levels return to normal and transaminases have decreased to <100. Rechallenge is **NOT** recommended for those who have had fulminant hepatitis (defined as hepatic encephalopathy with coagulopathy).

The rechallenge regimen of the American Thoracic Society (Am J Respir Crit Care Med 2006;174:935–52) have been followed as these are simple and quick. Rechallenge with PZA was previously not recommended, but a recent trial has shown that most patients tolerate it. PZA rechallenge should be considered in patients with severe TB (e.g. miliary, meningitis) or with drug resistance. Transaminase levels, especially ALT, should be monitored frequently (e.g. three times weekly) during rechallenge and every two weeks for a month following rechallenge.

If possible all patients with a drug induced liver injury should have their TB isolates sent for drug susceptibility testing. Do not rechallenge with an agent to which the isolate is resistant.

| | | |
|----------------------|--------------------|--|
| rechallenge regimen: | Background therapy | Ethambutol, streptomycin and moxifloxacin. |
| | day 1 | Rifampicin 450 or 600 mg daily depending on weight |
| | day 3 | Check ALT |
| | day 4 | Add INH 300mg daily |
| | day 7 | Check ALT |
| | day 8 | Consider PZA rechallenge (see text) |

NB: Duration of therapy should be individualised after rechallenge – consult ID for advice. The following are guidelines:

- Pyrazinamide not rechallenged/not tolerated: stop moxifloxacin and streptomycin, continue isoniazid, rifampicin and ethambutol for total duration 9 months
- Rifampicin not tolerated: continue streptomycin (for 2 months) and moxifloxacin, isoniazid, and ethambutol for total duration of 18 months
- Isoniazid not tolerated: stop moxifloxacin and streptomycin, add ethionamide (if tolerated – otherwise use moxifloxacin) to rifampicin and ethambutol for total duration 12 months

In addition, if significant liver impairment occurs or patients have symptoms of hepatitis with

liver function derangements then adjustments will be made to the ART regimen (omitting efavirenz and considering replacing it with an alternative ART agent, or stopping all ART if acute liver failure develops). In there is significant DILI co-trimoxazole and other potential hepatotoxic medication will also be stopped. Rechallenge of ART and other potential hepatotoxins will be considered and rechallenge will generally be done after rechallenge of TB medication. Management of each case will be individualized and discussed with the PI.

If patients develop drug rash on the trial this could be potentially related to co-trimoxazole (a frequent cause of drug rashes), efavirenz or one of the TB drugs. For very mild rashes they will be monitored with regular review and symptomatic therapy. In patients with more significant symptoms at presentation or who develop more severe clinical features (extensive rash, fever, systemic symptoms, blistering or desquamation, angio-oedema or mucosal involvement) all potential culprit drugs will be stopped immediately. Patients will be managed in consultation with the Groote Schuur Hospital Division of Dermatology either by sending digital photographs for an opinion or by referral of the patients for outpatient or inpatient management. Rechallenge of drugs after a drug rash will follow local guidelines.

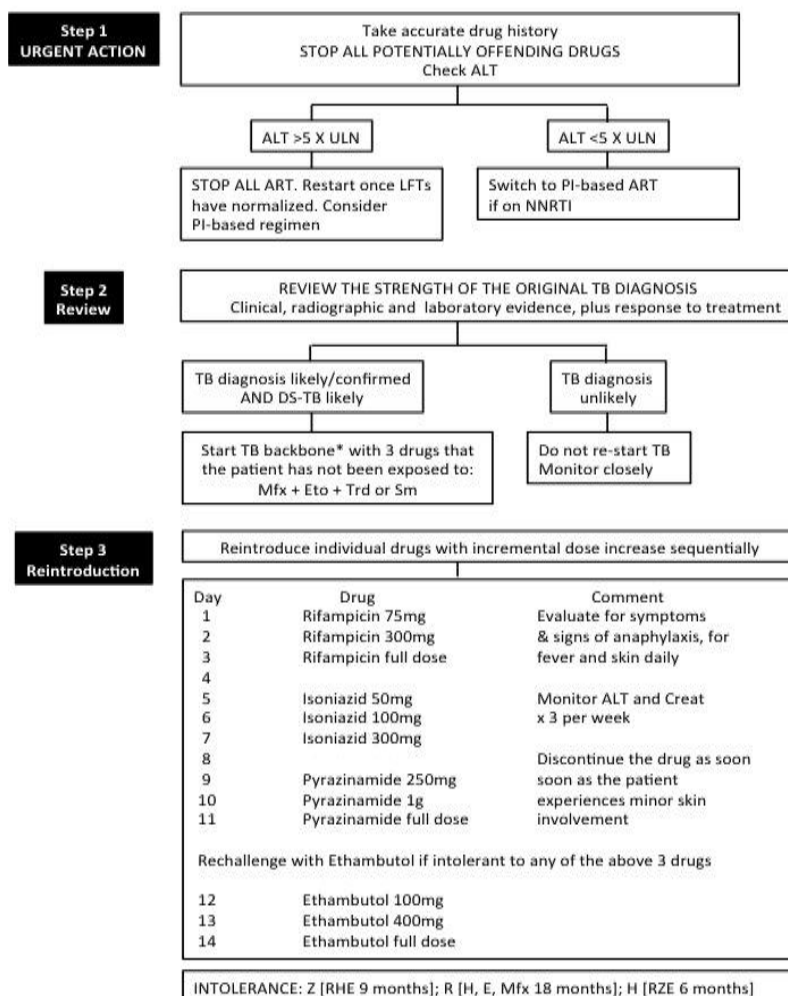
The Groote Schuur Hospital guidelines for the management of cutaneous drug reactions (CDRs) while on TB treatment will be followed:

Mild rash in isolation without systemic symptoms, mucosal involvement or abnormal LFTs can be treated with oral antihistamines and skin moisturizing agents, whilst continuing the drug under close observation.

Severe rashes involve mucosal membranes and are associated with systemic syndromes
Stevens-Johnson Syndrome - <10% skin detachment
Toxic Epidermal Necrolysis - >30% skin detachment
DRESS syndrome – Drug rash eosinophilia and systemic symptoms

The following algorithm will be used for management of severe CDR. If ART also needs to be stopped, then re-start after TB drug rechallenge is complete and consider a PI-based regimen should the patient have previously been on an NNRTI.

Management of severe CDR:



13.5. Management of drug-resistant TB

Any patient diagnosed with drug resistant TB (mono-resistance or multidrug resistant) will be managed according to South African National Drug-Resistant TB guidelines and referred for appropriate treatment and monitoring. Rifampicin-resistant TB is an exclusion criteria for the trial. If rifampicin-resistant TB is diagnosed only after a patient is enrolled on the trial then study medication will be stopped as soon as investigators become aware of the result, but follow-up will continue as planned to 12 weeks as per schedule of events.

13.6. Management of other medical co-morbidities

Participants who have medical co-morbidities or develop these while on the trial will be managed according to South African national Department of Health guidelines for these conditions.

13.7. Contraception and safe sex advice

Pregnancy is an exclusion criterion for this trial. Women of child-bearing potential will be encouraged to use two reliable methods of contraception (one being the use of condoms and the other a form of hormonal contraception) to avoid pregnancy for at least the duration of the trial because they have advanced HIV and are on treatment for active tuberculosis. Those who decline hormonal contraception will not be excluded from the trial. All patients will receive counseling regarding condoms to avoid HIV transmission.

If a female participant falls pregnant during the study, study medication will be stopped. Study follow-up will continue for the 12 weeks as planned and she will be immediately referred for appropriate antenatal care.

13.8. Interruptions in ART

When participants interrupt ART for drug toxicity, study drug will also be stopped on the same day. If participants interrupt ART due to non-adherence and this is reported then if the interruption is for five days or less then study drug will be continued. However, if ART interruption is more than 5 days then study drug will be stopped. Documentation of stops in study drug will be made in such instances.

13.9. Interruptions in study drug (by the participant)

If the participant interrupts the study drug then a discussion will occur regarding how long the interruption was for and the reasons the patient had for the interruption. If the patient interrupted because of side effects and does not want to restart then they will not be restarted and this will be documented. If the participant is agreeable to restarting study medication, then study medication will be restarted if the interruption was 5 days or less. If it was more than 5 days study medication will not be restarted. All interruptions will be fully documented.

In all cases participant follow-up will occur to week 12 unless consent for the trial is withdrawn by the participant.

13.10. Criteria for discontinuation of study medication (study follow-up will continue)

- Kaposi's sarcoma diagnosed
- Other new WHO stage 4 opportunistic condition diagnosed
- Rifampicin-resistant TB diagnosed
- Requirement for prohibited concomitant medication
- Development of TB-IRIS requiring open-label corticosteroid treatment (see section 13.2.)
- Pregnancy
- Request by participant to terminate
- Completion of 4 weeks of treatment as specified in protocol
- Clinical reasons believed life threatening by the clinical investigators, even if not addressed in the toxicity section of the protocol
- Patient interrupted ART for > 5 days
- Patient interrupts study medication > 5 days

The date of stopping study medication and reasons will be fully documented.

Unblinding of the randomization allocation of the participant would only occur under exceptional circumstances after a decision has been taken to stop study medication and when this information is deemed essential for ongoing clinical management by the attending clinician (see section 4).

13.11. Criteria for study discontinuation

- Request by participant to withdraw
- Completion of 12 weeks of trial as specified in the protocol
- At the discretion of the ethics committee or sponsor (in consultation with the investigators)

14. Statistical Methods

14.1. Sample size calculation

Assuming 35% cumulative incidence of TB-IRIS in the placebo arm and a 50% reduction in TB-IRIS in the corticosteroid arm (ie. to 17.5%) and requiring 80% power to test for the difference in TB-IRIS incidence at a two-sided significance level of 5%, the sample size required would be 110 in each arm. We will aim to recruit 240 patients assuming loss to follow-up of 10%. The estimate of 35% incidence of TB-IRIS in the placebo arm corresponds to the findings of Blanc et al (110/332 (33%) of participants who started ART in the early arm in this trial had TB-IRIS events (32)) and a study we conducted at Brooklyn Chest TB Hospital in Cape Town (42% IRIS incidence [47]) and reflects that these will be patients at high risk for TB-IRIS due to low CD4 counts and short interval between TB treatment and ART start. Lawn and colleagues demonstrated in a retrospective study conducted in Cape Town that low CD4 count and shorter interval between TB treatment and ART initiation were the strongest predictors of TB-IRIS. In their study 70% of patients with a CD4 count < 100/μl who started ART within 30 days of TB treatment developed TB-IRIS [48].

14.2. Statistical methods for analysis

The statistical analysis of the clinical trial will be performed according to a Statistical Analysis Plan which will be approved before database lock and study unblinding.

14.2.1 Primary Hypothesis

The primary hypothesis of the study is that the proportion of patients developing of paradoxical TB-IRIS within 12 weeks of starting ART, as determined by the INSHI case definition and confirmed by the endpoint review committee, will be lower in patients randomized to prednisone compared to patients randomized to placebo.

14.2.2. Analysis populations

The primary analysis and secondary efficacy analyses will be performed using an intention-to-treat approach. All patients randomized will be included under the treatment arm they were randomized to, irrespective of the fact that they actually received prednisone or started ART. In addition, an all-patients-treated approach will be performed excluding those participants who were randomized but never started study medication or ART. Safety analyses will be performed using the all-patients-treated approach.

14.2.3. Baseline characteristics

The number of patients screened and enrolled or excluded will be summarized by reason for exclusion. For the patients enrolled in the study, the number of patients discontinued or lost-to follow-up will be tabulated by reason and visit of study discontinuation. This information will be summarized in a CONSORT flow diagram.

Patients in each treatment group, overall and by site, will be described with respect to baseline characteristics. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics. The clinical importance of any imbalance will be noted but statistical tests of significance of imbalance in baseline characteristics will not be undertaken.

14.2.4. Primary analysis

The primary hypothesis will be tested comparing the proportion of patients with paradoxical IRIS among treatment groups using Fisher's exact test. In addition the relative risk of TB-IRIS will be estimated together with a 95% confidence interval.

A pre-specified subgroup analysis of those patients with a baseline CD4 count ≤ 50 cells/ μ L with respect to the primary and secondary outcomes will be performed.

14.2.5. Secondary and tertiary analyses

Secondary endpoints will be analyzed comparing study arms with the Fisher's exact test (for categorical data) or Wilcoxon rank sum test (for continuous data). Time to event analysis (constructing Kaplan Meier survival curves and comparing using the rank sum tests) will be used to compare time to IRIS event from start of ART by study arm.

As a tertiary analysis, we will assess possible risk factors for developing IRIS (such as baseline CD4 count, baseline HIV viral load, duration from TB treatment to ART initiation) in addition to treatment arm in a multiple logistic or Cox (time-to-event) regression model.

If we demonstrate that prednisone results in a reduction in the risk of developing paradoxical TB-IRIS we will perform a secondary cost-benefit analysis considering the cost of prednisone in relation to days of hospitalization, procedures and co-medication prescriptions averted.

14.2.6. Safety analyses

Adverse events will be coded following a standard dictionary (WHO-ART or Meddra) and the procedures will be clarified in a standard operating procedure. Safety endpoints, such as corticosteroid side effects, will be analyzed individually (eg. analysis comparing development of hyperglycaemia in each arm) and collectively (eg. analysis comparing all corticosteroid side effects that occurred in each arm).

15. Evaluation

15.1. Endpoint review committee

An endpoint review committee will be established to review the following endpoints

- Paradoxical TB-IRIS. The committee will adjudicate using the INSHI paradoxical TB-IRIS case definition as this will be used to define the primary endpoint.
- Severity of IRIS
- Drug toxicity (drug rashes and drug-induced liver injury)
- Cause of death
- New opportunistic infections

This committee will comprise investigators not active at the clinical site (Bob Colebunders, Lut Lynen) as well as William Burman an independent member of the TSC, and external reviewers. Two committees of three members will review endpoints. One committee will focus on IRIS endpoints and the other toxicity and deaths. The three reviewers in each committee will each independently adjudicate each event submitted to them. They will be supplied with the relevant CRFs and source document clinical notes relevant to the event and laboratory results. If they require further information this can be requested. Each committee member will decide independently regarding the classification of the event reported. If there is disagreement then consensus will be sought by email consultation. In the event that consensus cannot be reached then the committee members will vote and 2-1 vote will decide. This process will be recorded. Reviewers will be blinded to treatment arm allocation of the case they are adjudicating. The adjudication committee decision will be documented in the meeting minutes and each adjudication member will sign the final list of decisions. The data will be entered in a separate database using double-data entry.

16. Data handling and record keeping

16.1. Confidentiality

Information about trial participants will be kept confidential. All trial data will be de-identified and coded with a study number. The trial enrollment log and all signed ICFs and CRFs will be stored in a locked cabinet that is ICH-GCP compliant.

16.2. Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. For this trial, clinical data will be entered onto clinic visit sheets to provide a narrative record of the history and examination of the patient at each

visit. These sheets will constitute the clinical source document. Data from these sheets will be entered onto the CRFs at the time of that patients clinic visit and the clinician entering will check that the data on the source document and the CRF correspond before signing off and dating the CRF. For this trial, the laboratory result report will constitute the laboratory source document. When the laboratory result is received the study doctor will sign and date it and make a note of actions to be taken for any abnormal result. The laboratory results will be entered onto a corresponding laboratory CRF. The study team member entering this will check that the data on the source document (the laboratory result report form) and the CRF correspond before signing off and dating the CRF. Radiology reports will also constitute source documents and will be entered onto radiology CRFs and checked in the same way.

16.3. Case Report Forms (CRFs)

The case report forms (CRFs) are the primary data collection instruments for the trial. All data requested on the CRF will be recorded. If the data is missing this will be explained. All entries will be printed legibly in black ink. For corrections or to clarify illegible or uncertain entries, the original entry will be crossed out with a single line, signed and dated and the clarification or correction printed above the item. Clinical, radiological and laboratory data will be entered onto hard-copy case report forms (CRFs) from the source documents as described above. The clinical CRFs will be completed by the study doctor or nurse and the laboratory CRFs will be completed by the doctor, nurse or co-ordinator.

The following CRFs will be used

- Screening CRF
- Pre-enrollment CRF (optional)
- Informed consent CRF
- Enrollment CRF
- Week 0 visit clinical CRF
- Week 0 visit labs CRF
- Week 0 QOL assessment CRF
- Week 1 visit clinical CRF
- Week 1 visit labs CRF
- Week 2 visit clinical CRF
- Week 2 visit labs CRF
- Week 4 visit clinical CRF
- Week 4 visit labs CRF
- Week 4 QOL assessment CRF
- Week 8 visit clinical CRF
- Week 8 visit labs CRF
- Week 12 visit clinical CRF
- Week 12 visit labs CRF
- Week 12 QOL assessment CRF
- Unscheduled visit clinical CRF
- Unscheduled visit labs CRF
- IRIS assessment CRF
- IRIS management and outcome CRF
- Medications CRF

- Drug induced liver injury or rash CRF
- Adverse event CRF (will include documentation of opportunistic infections, ADR's and co-morbidities)
- Hospitalisation CRF
- Radiology CRF
- CRF for documentation of drug interruptions or stopping (ART, TB drugs or study drug), unblinding and open label corticosteroids

The CRFs will not contain the patient's name but will be identified using the study number and initials. There will be 2 study numbers: 1) a screening number for all patients screened and entered in the screening log; and 2) an enrollment number only for those enrolled in the trial and allocated a random assignment.

16.4. The electronic database (integerafrica)

For the purpose of monitoring and auditing the study, source documentation will consist of the laboratory report and the hard-copy CRFs developed and maintained by the investigator. Data recorded on source documents will be entered using electronic forms using an electronic data capture and management system provided by integerafrica. The hard-copy CRFs will be entered onto the electronic database by data-enterers at the clinic. integerafrica will design a tailor-made database with electronic forms that mirror the hard-copy CRFs. The electronic database will be a web-based research platform developed and maintained by integerafrica (www.integerafrica.org). The database will be ICH-GCP compliant. A subcontract will be awarded to integerafrica for the purpose of database design and maintenance. The portal is an easy-to-use web-enabled tool that assists medical researchers to track, analyze, and report on an individual case level. The platform allows researchers to enter data from multiple sites using one centralized database and provides tools for real-time data analysis. The architecture is developed according to clinical workflows and chronological procedures. Data are validated at the point of data entry. Multimedia data formats (chest radiographs, CT/MRI scans, ultrasound images or photographs) can be uploaded on the platform, allowing storage of complete clinical records. Training tools and support pathways are installed within the web-portal. The section Frequently Asked Questions (FAQ) serves as a guidance for the use of the platform. After login onto the TBIRIS website, the researchers have access to a variety of information regarding the study. In the download center, documents such as paper CRFs, informed consent forms, clinical guidelines, the study protocol, SOPs, study information sheets for patients, and patient education sheets can be made available. All data can be exported for further analysis in Excel format. The platform is available 24/7. The platform implements appropriate administrative, physical and technical safeguards to ensure the confidentiality, integrity and security of electronic health information. This database is being used in the EDCTP-funded eKhayaVac trial of the novel TB vaccine MVA85A being conducted in Khayelitsha (www.ekhayaovac.org), the Pan African Pulmonary Hypertension Cohort study (www.papuco.org) and the Registro Latinoamericano de Hipertensión Pulmonar (www.relahp.org).

integerafrica technology architecture

The main information technology design philosophy is an end-to-end solution approach, with specific focus on security, scalability, performance, and ease-of-use. The system is designed

to utilize the very best technology available to provide for a cost-effective and sustainable research environment in an African context. This is made possible by the extensive use of open-source technology. The system is built with hundreds of open-source components consisting of thousands of programs and millions of lines of code. The system architecture integrates five main components. 1) Devices: The system supports a wide variety of devices including mobile phones, tablet computers, laptops and desktop computers. Any computer with a browser and internet connection can use the system. 2) Browsers: The development of modern browsers have made it possible to develop and deliver much more powerful and secure web-based solutions for clients. The browser in many cases has replaced the reliance on propriety software and hardware. 3) Interfaces: The system supports any connection to the internet. It is designed for low bandwidth use without sacrificing a rich user experience. A connection speed as low as 512kbps is sufficient. The extensive use of caching technology throughout the system makes this possible. 4) User Experience: The user has access to a host of tools to manage research data with ease, securely. 5) Data Capture: The system allows for the secure capture of research data, geo-location data, imaging, videos, audio files and patient appointments. 6) Support: Training and support is provided in to study personnel. This is supplemented by online support in the form of training videos, frequently asked questions pages as well as a 24/7 online support request framework. 7) Security: Strict password policies, user access control logic, data encryption and SSL connection encryption are all in place to provide a secure user environment. 8) Data Storage: All data storage, as well as on- and off-site backup are provided. 9) Visualization: Real time project summary data is provided and well as basic analysis. All data can be visualized various formats including maps, charts and graphs. 10) Data Export: Data can be searched and selected from and exported in XLS or CSV format for further analysis, or sharing of results. 11) Reports: Custom reports can be generated and exported as PDF files or printed. 12) System Backend Firewall: As security is paramount, the first line of defense on web based systems is the use of various layers of firewall technology to guard against unauthorized intrusion. Both hardware and software firewalls are employed. 13) Server Security: Various industry standard, enterprise security policies are implemented and maintained across the various servers and server software components. 14) Cache and Proxy Servers: The advanced caching and proxy technology used for back-end optimization allows for fast, powerful and scalable solutions. Caching of various server components allows for the exponential increase in users, and fast responsive user experience managing research data online. 15) Web Server: The system utilizes Apache web-server technology that in 2009 was the first web-server to surpass the 100 million website milestone. 16) Application Server: On the application server side the system uses PHP and the Drupal framework to create fast and secure customized research solutions. The Drupal framework is a worldwide open source collaboration of more than 16000 developers in 228 countries. 17) Database Server: The system relies on the world's most popular database MySQL. 18) Backups: The backup policy extends to real-time RAID backups, On- and Off- Site data backups as well as database replication where needed.

The platform is accessible at www.TBIRIS.org and hosted at one of the world's most reliable hosting companies Hetzner online in Germany. Maintenance of the platform includes: daily server quick checks, daily security audits, and implementation of Drupal update releases, Drupal minor security updates, server security updates, server backups, annual SSL renewals. The main lines of our support services are: user and centre registration process guidance, user

personal data maintenance, online training for users on individual level using Skype, and online support requests for all the how-to items.

16.5. Data quality and completeness checking

Automated data checking mechanisms will be installed into the electronic database. Certain variables will be validated at the point of data entry, while data with reference values will be displayed in color codes according to reference range. Different field types (integer, decimal, range integer, range decimal, date, text field, text select list (drop down menu, multi-select, radio button for polar variables) will also be implemented to reduce errors at entry level.

The database is aware of the participant's position in the trial and will only allow certain procedures as per study protocol. Double electronic data entry will occur (hard-copy CRFs will be entered independently by two data enterers) and any discrepant entry will be flagged and/or the electronic record cannot be saved. Various list views allow for visual validation of data at entry and upon saving of the record. Every patient record is in a human readable format which allows the electronic record to be used as a electronic patient card. The database includes a complete check that gives a percentage of completeness of each record and the names of the missing fields. This information is available at the list view modules after login.

Additional data quality checking will be done (and documented) on every CRF by the study team. Any data errors or missing data detected will be corrected on both the hard-copy CRF and on the electronic database by the study doctor, who will sign and date changes on the hard-copy CRF and a system will be put in place to ensure all changes to the hard-copy CRFs are made onto the electronic database within one week of the change being made. Data entry and review will be co-ordinated by the Data Management Team at UCT with advice from the team at ITM (see section 20.1). Further details of the Data Management plan will be detailed in a specific standard operating procedure (SOP).

16.6. Records retention

The sponsor and the investigator will retain trial essential documents for at least 15 years after trial completion. The electronic database will be retained for an indefinite period.

17. Study monitoring, audit and inspection

17.1. Trial monitor

The trial will be monitored by a monitor that is subcontracted by the sponsor UCT to undertake monitoring. The monitor will be Jenny Henderson. She will perform a study initiation visit and thereafter monitoring visits every two months with an average of 3 hours per patient for source document verification as well as monitoring the regulatory binder, ethics committee communication, SAE reporting and informed consent documents.

17.2. Data and safety monitoring board (DSMB)

The trial will be monitored by an independent DSMB. This DSMB will be comprised of 3

independent internationally-recognized HIV-TB researchers and an independent statistician (subcontracted by UCT). The DSMB will be appointed by the sponsor before trial recruitment starts and will also have its first meeting with the investigators (via conference call) to make necessary modifications to the draft DSMB charter prepared by the investigators and approve the final charter before recruitment starts. The charter will specify the stopping rules for the trial that will be based on DSMB review of the blinded and/or unblinded data. It will also specify the specific data to be reviewed by the DSMB and whether or not these data needs to be presented to the DSMB in a blinded or unblinded manner or that the data summaries may be partly concealed.

Once the trial is underway, the DSMB will meet twice during the trial. The first meeting will occur after 80 participants (~33%) have been enrolled and completed follow-up and the second after 160 participants (~66%) have completed follow-up. The DSMB may also decide to convene an unscheduled DSMB review if warranted by safety or data quality concerns. The data for review will be prepared by the independent statistician.

Data including the following will be prepared for DSMB review:

- Study recruitment by month
- Eligibility violations
- Baseline characteristics
- Protocol violations
- Data completeness report
- Primary and certain secondary endpoint summaries
- Summary of adverse events (ACTG grade 3 and 4)
- Deaths on the trial

The task of the DSMB will thus be to review study recruitment, data quality and drug safety and advise the sponsor of major safety issues and data quality issues. The DSMB may advise that trial enrollment should be stopped based on significant safety concerns. However, although the DSMB will have access to the primary endpoint data (paradoxical TB-IRIS events) decisions regarding whether to stop the trial will not be based on this data. The DSMB may choose to review the efficacy data in a blinded fashion (following the DSMB charter). The DSMB meetings during the trial will take place via conference call and will include an open part when the investigators are present and update the DSMB members on unblinded aspects of the study. This will be followed by a closed part after the investigators have left the meeting when blinded data is reviewed and discussed and the DSMB recommendations are made.

The DSMB report and recommendations will be sent to the sponsor (the University of Cape Town represented by the Deputy Dean of the Faculty of Health Sciences at University of Cape Town). The sponsor will then make the decision based on the DSMB recommendations to continue the trial or to prematurely stop enrollment or to implement any alternative recommendation of the DSMB.

18. Adverse event grading, recording and reporting

18.1. Adverse event grading and recording

The ACTG adverse event grading system will be used (see addendum 2: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009).

All grade 1, 2, 3 and 4 clinical and laboratory adverse events will be recorded on the CRF and the adverse event log in each patients study folder.

18.2. Serious adverse event reporting

All adverse drug reactions, serious adverse events and deaths will be reported to the UCT Human Research Ethics Committee and the Medicines Control Council according to these bodies' guidelines on reporting as detailed below. Reporting to the sponsor's representative (UCT Faculty of Health Sciences Deputy Dean for Research) will be done at the same time that reporting is done to the Ethics Committee and to the DSMB. A cumulative report of all serious adverse events and deaths will be presented at each Trial Steering Committee meeting.

18.2.1. Reporting to UCT Faculty of Health Sciences Human Research Ethics Committee (UCT FHS HREC)

The study medical officer, study co-ordinator or principal investigator will report to the UCT FHS HREC using a cover letter and the UCT FHS HREC standard form (fhs008):

Definitions

- Unanticipated event: Must be all three of the following: a) unexpected, b) related to the research and c) harmful to participants or others
- Unexpected adverse event: Any of the following applies: a) Inconsistent with current information given to participants, b) inconsistent with current risk information in the protocol application, c) occurring more frequently than anticipated
- Serious Adverse Event: Results in any of the following: a) death, b) life-threatening incident, c) hospitalization, d) disability, e) congenital abnormality, f) requires medical or surgical intervention, g) inadvertent disclosure of confidential information.

Reporting

- Unanticipated problems will be reported within seven calendar days of being aware the occurrence.
- Adverse Drug Reactions (Fatal and life-threatening, Unexpected): As soon as possible, but no later than 7 calendar days after first learning about the event.
- Adverse Drug Reactions (Non-fatal, Serious, Unexpected): As soon as possible but no later than 15 calendar days.

- Adverse Drug Reactions (Expected): Only if occurring at significantly higher frequency or severity than expected and then within 15 calendar days.
- New Information that might impact the Conduct of a Clinical Trial: 3 Calendar days of first learning of its occurrence.

We will mail all Ethics Committee reports to:

The Chair, University of Cape Town Human Research Ethics Committee, Room 23, Floor E52, Old Main Building Groote Schuur Hospital, Anzio Road, Observatory, 7925.

We will at the same time mail these reports to the two ethics committees in Belgium, unless they request for a different reporting schedule:

Prof dr Anne Buvé, chairperson
 Institutional Review Board, ITM Antwerp
 Nationalestraat 155, 2000 Antwerp
 Tel: +32 3 247 65 33
 E-mail: abuve@itg.be

Prof dr Patrick Cras, chairperson
 Comité voor medische ethiek, University Hospital Antwerp
 Wilrijkstraat 10, 2650 Edegem
 Tel: +32 3 821 35 44
 E-mail: ethisch.comite@uza.be

18.2.2. Reporting to the Medicine Control Council of South Africa

Reporting of adverse events to the MCC will be done according to the document entitled Medicines Control Council: Reporting Adverse Drug Reactions in South Africa (May 2003). Section 5 refers to reporting of adverse events in clinical trials (Appendix C). The events will be reported using a cover letter and a copy of the form submitted to the ethics committee that provides a detailed narrative explaining the event. Expedited reporting is not appropriate if the event is not related to the study product. A named investigator will sign the report and submit a hard copy by mail as well as fax a copy to the MCC.

Key timelines for reporting:

a) Deaths:

All deaths will be reported within 7 days of becoming aware of the event, unless the death is unexpected and due to a serious adverse event, when it will be reported within 3 days as per the study protocol.

b) Adverse Drug Reactions: Fatal or life-threatening, unexpected

MCC requirement: Report as soon as possible but within 7 calendar days.

c) Adverse Drug Reactions: Not fatal or life-threatening, unexpected

Report as soon as possible, but within 15 calendar days.

d) Adverse Drug Reactions: Expected, but change in nature, severity or frequency, or new risk factor identified

Report within 15 calendar days.

e) Information which changes the risk-benefit assessment of trial:

Report within 3 calendar days.

f) Serious adverse events:

Report as part of the 6-monthly progress report in line listing format.

g) Non-serious adverse events:

Report as part of the 6-monthly progress report in line listing format.

We will mail all MCC reports to:

Medicines Control Council Clinical Trials Unit

41 Andries Street

Civitas Building

Pretoria

0001

19. Ethical considerations

Ethical and regulatory approval

Ethical permission for the clinical trial will be obtained from the University of Cape Town Human Research Ethical Committee, (<http://www.health.uct.ac.za/research/humanethics/adminteam/>) as well as from Institute of Tropical Medicine IRB and the Ethics Committee of Antwerp University Hospital, Belgium, before the trial is initiated. Written informed consent will be obtained from eligible patients prior to any study procedure being undertaken. The trial will adhere to ICH Good Clinical Practice guidelines, South African GCP guidelines of 2006 and the Declaration of Helsinki of 2008. All unexpected serious adverse events and deaths on the trial regardless of cause will be reported to the University of Cape Town Human Research Ethics Committee and the other 2 ethics committees in Belgium as per their guidelines. Regulatory approval for the trial will be obtained from the Medicines Control Council of South Africa (in Pretoria). All unexpected serious adverse events and all deaths on the trial regardless of cause will be reported to the MCC. Annual (UCT ethics committee and 2 ethics committees in Belgium) and six-monthly (MCC) reports will be submitted as per requirements.

Participant confidentiality

Documentation, data and all other information that relates to individual patients will be held in strict confidence during the trial and after trial completion. No information concerning the patient will be released to an unauthorized third party, without written approval of the participant except as necessary for trial monitoring or regulatory review.

The patient's name will be entered in a study log next to the trial number. Thereafter, all trial documentation will be with the study number and initials. This study log and all study documentation will be stored in a GCP compliant locked cabinet. The study database will be

fully anonymised. All data transported off the clinical site and all data sent to the sponsor and to ITM, Belgium, will be fully anonymised.

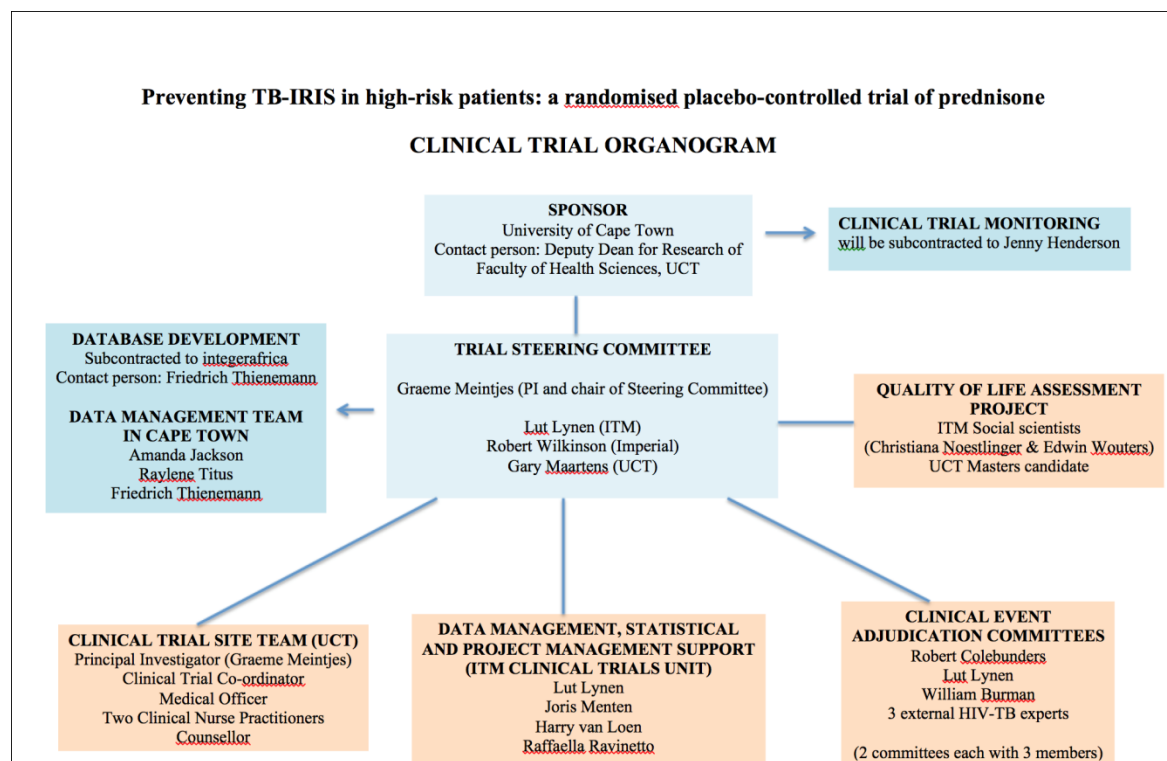
In this study, the database will be constructed, maintained and kept within South Africa, so no identifiable personal data will be transferred to ITM, Belgium. Only the final clinical and laboratory database will be shared with the Belgian partner, with no identifiable personal data. Any data that will be transmitted to Belgium (that is needed for statistical analysis) will be fully anonymized before transmission. This will involve removal of patient identifiers including initials, place of birth, place of residence, day and month of birth. The quality of life database will be shared with Belgian investigators prior to it being finalized as they will be involved in earlier phases of analysis of this database. However, all data that leaves the clinical site from the quality of life substudy and that is transmitted to Belgium will be de-identified in the same way described.

Standard of care

Neither corticosteroids nor any other medication are currently prescribed for preventing paradoxical TB-IRIS in the current standard of care with the implication that a placebo-controlled trial is ethically acceptable because placebo reflects current standard of care. Diagnosis and treatment of TB, antiretroviral therapy prescribed and the timing of antiretroviral therapy in this trial protocol all reflect standard of care in South Africa.

20. Administration, trial management and monitoring

20.1. Clinical Trial Organogram



*Additional members of TSC: Prof William Burman and representative from Ubuntu Clinic

20.2. Governance of the study

The governance and management plan for the trial is summarised in the organogram. The conduct of the clinical trial will be overseen by the Trial Steering Committee (TSC), which will include the PI and one representative from each participating institution (Graeme Meintjes (chair and PI), Lut Lynen, Robert Wilkinson and Gary Maartens). There will be one additional independent member of the TSC (Professor William Burman (University of Colorado, US)) and a member who represents the Ubuntu HIV-TB clinic in Khayelitsha. The TSC will have a meeting every 2 months with Skype conference call link-up with those members not in Cape Town. The TSC will review preparation for the trial, clinical enrolment and events on the study as well as all other aspects such as analysis and sub-studies. For each meeting of the TSC a progress report will be prepared that will include: participant accrual, study primary endpoint and deaths (blinded to study arm), all serious adverse events with details of each individual serious adverse event. This 2-monthly report will simultaneously be submitted to the sponsor's representative (Deputy Dean for Research, Faculty of Health Sciences, UCT).

Graeme Meintjes (PI) will be responsible for day-to-day management at the Clinical Trial site and for human resources issues. He will receive assistance in this regard from the HR Department at UCT who have very well established conditions of employment, contracts of employment and require clear job descriptions for staff (the PI will draft these job descriptions). The Core Office at the Institute of Infectious Disease and Molecular Medicine at UCT will manage the study finances and the financial report will be reviewed at the TSC meetings.

20.3. Sponsor and monitoring

The University of Cape Town is the Trial Sponsor. The official representative of the Sponsor is the Deputy Dean of Research, Faculty of Health Sciences, UCT. The sponsor will subcontract monitoring of the trial to a Clinical Trials Monitor with extensive experience of clinical trials monitoring in South Africa (Jenny Henderson, who previously monitored a study of adjunctive interferon- γ immunotherapy for cryptococcal meningitis that was conducted at our hospital [49]) (see section 17.1.). A copy of all monitoring reports and DSMB reports will be sent to the Deputy Dean for Research at the Faculty of Health Sciences at UCT. The Sponsor will also be informed of all unexpected serious adverse events and deaths occurring on the study at the same time as the ethics committees.

The trial will be conducted according to ICH GCP standards. GCP training and accreditation for all clinical research staff will take place prior to trial initiation. The laboratories used for this trial will be the National Health Laboratory Services (NHLS) in Green Point and at Groote Schuur Hospital. These laboratories are SANAS (South African National Accreditation System) accredited.

21. Quality of life substudy

21.1. Background

In our previous trial of prednisone for TB-IRIS treatment [3], we used the Medical Outcomes Study-HIV (MOS-HIV) Health Survey [50] and the Karnofsky performance score, in parallel. However, when using these tools we experienced practical problems with use in patients who are very ill as many of the questions were not relevant to their current state. Many of the validated HR-QOL measures, like the MOS-HIV, were developed prior to the ART era, and for a UK and US context. Many studies in developing countries have used these instruments often without proper validation and reliability testing [51, 52]. A review of HR-QOL measures for use in HIV/AIDS clinical trials found that the Functional Assessment of HIV-infection (FAHI) and MOS-HIV were the two most appropriate HIV-targeted measurement tools. Both can be self-administered and can be completed in 5-10 minutes. However, they would not be optimal in all HIV subgroups (e.g. adolescents versus adults, women versus men, ART naïve vs. treatment experienced) [53]. Recently a new HIV/AIDS-specific HR-QOL questionnaire, the Patient Reported Outcomes Quality of Life-HIV (PROQOL-HIV) was developed and has undergone psychometric validation in 8 countries. The instrument was developed simultaneously across several continents and accounts for ART and side effects of ART [54, 55]. It is a 38-item questionnaire covering 8 dimensions. Patients rate the different items over the past 2 weeks, on a 5-point scale ranging from 0="never" to 4="always". Although this questionnaire performed well in the 11 countries, it may not be the best questionnaire to assess HR-QOL in patients with an acute illness, as part of the questions deal with long-term side effects. Neither MOS-HIV nor PROQOL-HIV contain questions specific for TB patients.

Another more generic QOL tool, is the EuroQol generic health index, the EQ-5D-3L (<http://www.euroqol.org/home.html>). EQ-5D essentially consists of 2 pages - the EQ-5D-3L descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D-3L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, severe problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box corresponding to the most appropriate statement in each of the 5 dimensions. The EQ VAS records the respondent's self-rated health on a vertical, visual analogue scale where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state'. This information can be used as a quantitative measure of health outcome as judged by the individual respondents.

We aim to define a HR-QOL during this trial that is more suitable for the evaluation of a treatment directed at short-term impact on health outcome. If a novel HIV specific HR-QOL measure is demonstrated to work well in our setting, it can be used in future clinical trials. In order to explain the differences in HR-QOL measures between patient groups it is important to link it with a symptom list. The best known and validated list is the HIV symptom index developed by Justice [56]. This 20-item self-completed symptom index assesses whether symptoms are present or absent and their severity on a 4-point scale (0 = "does not bother me at all" to 4 = "bothers me a lot"). However, the 20-item list does not contain symptoms that are targeted to patients with possible side effects of steroids, or with TB-IRIS specific symptoms.

21.2. Methodology

Measures and their adaptation

1. Existing and validated HR-QOL measures will be used as originally designed: **PROQOL-HIV and EQ-5D-3L**, in combination with **Karnofsky score** and an adapted **HIV-symptom index**. As far as possible the tools will be self-administered by the patient. In case the patient is illiterate a trained study counsellor will read the questions to them. A brief review for completeness by the site investigators will be done. The PROQOL-HIV will be adapted by asking specifically for HIV and TB-related symptoms. The number of questions will not change, but for example the sentence "I have been satisfied with my HIV medicine" would become "I have been satisfied with my HIV and TB medicines".
2. The 20-item HIV symptom index will be complemented with symptoms targeted to patients with IRIS and side effects of steroids. This will be prepared by the investigators and presented and discussed with experts in TB-IRIS from the INSHI network before implementation.
3. The tools (PROQOL-HIV, EQ-5D and HIV symptom index) will be translated in Xhosa. This will include two forward translations and one reconciled version: one backward translation of the reconciled version. For the PROQOL-HIV a Zulu translation is already available, which will be used to facilitate the translation process, as these two language share similarities.
4. Initial validation of the comprehensiveness and the comprehensibility (cognitive interview) will be performed on a sample of 10 patients (including at least 5 with TB-IRIS). At the end of the surveys open-ended questions will be asked such as:
 - a. Did you have trouble understanding what we meant by any of the symptoms (items) listed? If so, please list them and explain why it was confusing to you.
 - b. Do you think that these symptoms are the most common and the most bothersome for patients with TB-HIV who start ART?
 - c. Are there any other symptoms that you have found particularly bothersome? If so, please describe the symptom and how much it has bothered you.
 - d. Are there any other symptoms that you hear about that bother people you know who have TB and HIV? Please describe the symptom and how much it seems to bother them.

These patients will be personally debriefed by trained interviewers, to make sure that the written responses as documented at the end of the survey accurately represent their impressions.

5. A social scientist from ITM, together with a master student from UCT and a local counsellor will select, based on Cape Town's patients' and providers' feedback, the final version of the two HR-QOL measures and the HIV/TB symptom index.
6. Cognitive interviews (see point 4 above) to pretest questionnaires (as described by for instance by DeMaio & Rothgeb [57] and Willis et al [58]) have some limitations, such as using only a very small number of purposively chosen respondents. Hence the results are not generalizable to a larger population.

Unlike the cognitive interview, respondent debriefing is often employed at a later stage in the questionnaire design. It offers the potential of larger and more representative data, and to assess whether findings from cognitive interview research can be re replicated in the actual survey setting ([58, 59]). Therefore, we suggest to employ respondent debriefing with a random sample of patients with and without IRIS enrolled in the trial, followed by in-depth interviews with purposively selected patients to make sure that the written responses adequately reflect the patients' impressions. Ideally, one should try to achieve data saturation

when conducting in-depth interviews, but we assume that 10-15 interviews should yield sufficient results. In case of discrepancies that cannot be solved and/or constantly new issues coming up the number of qualitative interviews would be increased.

Statistical methods

First, the statistical analyses aim to assess the validity and the reliability of the different scales in our population. Subsequently, the study aims to construct the optimal factor scores to measure the different latent concepts.

The Xhosa versions of the EQ-5D-3L and the Karnofsky Performance score have been applied and validated in HIV-positive populations on ART. The validity of the Xhosa translation of the recently developed PROQOL-HIV and the adapted version of the HIV Symptoms Index will be assessed in a series of tests. Firstly, we aim to test the *construct and concurrent validity* of the translated version of the PROQOL-HIV instrument in this population. In particular, we will explore whether the PROQOL-HIV score has the relationships with other variables (EQ-5D and EQ-VAS) in the expected direction. Similarly, we will test the *predictive validity* of the translated instrument by assessing the correlation with other QoL-measures at follow-up. *Convergent validity* of the QoL-scale will be assessed by employing the Karnofsky Performance score, which should be influenced by the same underlying concept (i.e. health). The *criterion validity* – the relationship with an objective measure – of the PROQOL-HIV will be assessed by calculating its correlation with objective clinical markers (eg. baseline CD4 cell count and viral load). The adapted HIV Symptoms Index will be validated in a similar manner testing its construct, convergent and criterion validity by exploring its association with the Karnofsky Performance score, EQ-5D and objective clinical markers.

The reliability of the different (sub)scales will be assessed by using a measure of internal consistency, Cronbach's alpha (> 0.7). In addition, we will also apply the test-retest method and the split-half method to further assess the external and internal reliability of the scales. The current study aims to optimally utilize the information gathered in the above-mentioned scales. For this reason the current study will employ confirmatory factor analysis (CFA) to test the a priori assumptions which are made in the often used sum scores and indices: we will explore (1) the weight of the different items, (2) the measurement errors, and (3) the number of dimensions measured by the scale. In practice and based on conceptual theory, the selected scales will be subjected to CFA using Mplus to assess whether the proposed factor structure fits with the gathered data and to compute factor scores which can be employed in future analyses containing the latent concepts. CFA provides two important analytic possibilities necessary to optimally address a number of challenges of our research. Firstly, our sample will contain a high number of respondents from vulnerable patient groups, which means that special attention will have to be devoted to the potentially disturbing influence of acquiescence (the tendency to agree) and method effects (negative and positive wording). If needed, the CFAs will include a method factor to control for these sources of response bias.

Secondly, it is important to note that the current study population contains several sub-groups (e.g. IRIS vs. non-IRIS patients, women vs men, patients at time 1 vs time 2, placebo vs prednisone) The majority of studies – including almost all previous QoL-studies on HIV/AIDS – have assumed equivalence of the structure of the measures they compare across

different respondent groups. However, legitimate comparison of means or structural relations across groups requires equivalence of the measurement structures underlying the indicators. The manifest means in a comparison does not only depend on the latent means but on the whole underlying measurement model (i.e., item intercepts and factor loadings). In other words, in order to make sure that the different patient groups (e.g. men vs women, placebo vs prednisone) identically interpret the different items and thus that the constructed factor scores of these different patient groups are measuring the same concept, multi-group confirmatory factor analysis – including measurement invariance testing – will have to be employed. At least partial measurement invariance (configural – metric – scalar invariance) is a prerequisite for cross-cultural or cross-group comparisons.

Using this procedure, we aim to produce refined factor scores, which represent the methodologically optimal measurement of the intended latent concepts and will thus be applicable in subsequent analyses incorporating the concepts measured by the selected scales. The qualitative data assessed will serve as important contextual information to interpret the quantitative results, for instance to explain a specific factor structure that could potentially be different compared to in the original instruments. All qualitative data (cognitive interviews) will be analyzed using an inductive approach. Data from the open-ended questions will first be transcribed verbatim and then translated into English, if conducted in another language (Xhosa). Systematic contextual data analysis methods will be applied using electronic software (NVivo8), by identifying recurrent patterns and themes [60]. A codebook will be developed by at least two independent coders and an iterative coding approach will be taken. Brief data analysis reports will summarize identified themes and rely on narrative text to illustrate and explain any newly emerging concepts in relation to how study participants perceive their health-related quality of life.

Thirdly, on the condition that multi-group scalar invariance is proven (see above), the QOL measures will be used as one of the outcome measures in the clinical trial comparing the use of prednisone with placebo in patients at high risk of TB-IRIS. Comparison of means (SD) and medians (IQR) of the scores (total and domain scores) obtained by the different tools in the placebo and the intervention group will be done using Wilcoxon rank sum, chi-square and Fisher's exact tests, as appropriate.

Table: Summary of Quality of Life assessments to be performed within the clinical trial

| Instrument | Method | Interviewees |
|---|--|---|
| PROQOL-HIV (including “TB” in the questions, next to “HIV”) | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| EQ-5D-3L | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| HIV-symptom index (complemented with specific symptoms related to IRIS and steroid side effects) | Self-administered questionnaire (or administered by counselor) | All participants at week 0, 4 and 12 visit |
| Karnofsky score | Score assigned by investigator in discussion with participant | All participants at week 0, 4 and 12 visit |
| Respondent debriefing interviews | In-depth interview conducted by an investigator specifically involved in QoL substudy with assistance of counselor for translation | 10-15 interviews with a random sample of participants |

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Addendum to protocol version 1.4, dated on 28 January 2015

Changes in follow up for current study participants:

1. All patients will attend an additional Week 28 study visit. Patient history will be taken and clinical examination will be performed specifically to assess for any history, symptoms or signs of HIV-associated cancer. Appropriate referrals will be done when needed.
2. We will call all study participants at 12 months on antiretroviral therapy and may in addition to this call their clinic and access clinical records from health care institutions to assess for any HIV-associated cancers.
3. The implications of the IMPI trial results will be discussed with each participant at their next scheduled follow up visit and we will document patients' continued consent to participation in the trial or withdrawal from the trial. We will use the document Additional Patient Information Regarding Prednisone dated 28 January 2015 information leaflet to inform patients about the results.
4. We will ask these participants to sign the following document: Pred-ART Trial Informed Consent Addendum, Version 1.0, 28 January 2015 if they are willing to have extended follow up on the Pred-ART trial.

Changes in follow up for participants who have completed Week 12 follow up visit:

1. All participants will be contacted and asked to attend a Week 28 study visit and this may occur at a later time point for patients who have progressed further than Week 28 since enrolment on the trial.
2. We will ask these participants to sign the following document: Pred-ART Trial Informed Consent Addendum, Version 1.0, 28 January 2015 if they are willing to have extended follow up on the Pred-ART trial.
3. We will discuss the implications of the IMPI trial with patients, take a history and do a clinical examination on all patients.

Changes in follow up for new patients:

1. Patients will sign the Pred-ART Trial Enrolment consent form (unchanged from previously) after the updated Pred ART Trial Patient Information Leaflet (Version 2.0, 28 January 2015) has been discussed with them. This updated patient information leaflet contains details of the amended follow up plans and results and implications of the IMPI trial.

Prepared by Charlotte Schutz
Reviewed by Graeme Meintjes

Summary of changes

Changes made when protocol version 1.3 was updated to protocol version 1.4:

- Expansion of participant recruitment sites to two additional sites
- Introduction of a window period to allow study drug initiation up to 48 hours after ART initiation in the case of patients who had initiated ART

Changes incorporated into the addendum to protocol version 1.4, made after IMPI trial findings [1] were released:

- An additional week 28 visit was added to assess specifically for (HIV-associated) cancers and vital status
- A phone call at one year was added to assess specifically for (HIV-associated) cancers and vital status

Informed consent forms and patient information sheets were updated accordingly.

References:

- 1) Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. N Engl J Med. 2014 Sep 18;371(12):1121-30.

Statistical analysis plan, dated 14 December 2016

Statistical Analysis Plan

Preventing tuberculosis-associated immune reconstitution inflammatory syndrome in high-risk patients: a randomized placebo-controlled trial of prednisone (PredART)

Date: 14 December 2016

Authors: Graeme Meintjes, Cari Stek and Lut Lynen

Approved by: Trial statistician
Jozefien Buyze, Institute of Tropical Medicine, Antwerp, Belgium



Date: 14 / 12 / 2016

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1. Executive Summary: The PredART trial

Tuberculosis (TB) is the most common opportunistic infection amongst HIV-infected patients starting antiretroviral therapy (ART) in developing countries and thus the most frequent form of immune reconstitution inflammatory syndrome (IRIS). Paradoxical TB-IRIS occurs in 4-54% of patients starting ART while on TB treatment and results in morbidity, hospitalisation, consumes health care resources and TB-IRIS may be fatal. We have previously demonstrated in a clinical trial that prednisone reduces morbidity when used for treatment of paradoxical TB-IRIS. This trial is a double-blind placebo-controlled trial of prophylactic prednisone (40mg/day for 2 weeks followed by 20mg/day for 2 weeks, started within 48 hours of initiating ART) in patients with TB who are identified as being at high risk for paradoxical TB-IRIS (starting ART within 30 days of initiating TB treatment and $CD4 \leq 100/\mu L$). The trial has enrolled 240 participants, randomised 1:1 (prednisone:placebo). The primary endpoint is development of paradoxical TB-IRIS, defined using an international consensus case definition. The trial is powered to determine a reduction in TB-IRIS events. The trial conduct is described in the Protocol.

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

2. Baseline characteristics

The number of patients screened and enrolled or excluded will be summarized by reason for exclusion. For the patients enrolled in the study, the number of patients discontinued or lost- to follow-up will be tabulated by reason and visit of study discontinuation. This information will be summarized in a CONSORT flow diagram.

Patients in each treatment group will be described with respect to baseline

characteristics. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics. The clinical importance of any imbalance will be noted but statistical tests of significance of imbalance in baseline characteristics will not be undertaken.

3. Analysis of the primary efficacy endpoint

The primary endpoint of the PredART trial is the development of paradoxical TB-IRIS within 12 weeks of study randomization that coincides with 12 weeks on ART (defined using the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition). The definition used will be that published by INSHI collaborators in 2008: *Meintjes G et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. Lancet Infect Dis. 2008 Aug;8(8):516-23 (see Panel 2, appended below).*

Panel 2: Case definition for paradoxical tuberculosis-associated IRIS

There are three components to this case definition:

(A) Antecedent requirements

Both of the two following requirements must be met:

- **Diagnosis of tuberculosis:** the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴
- **Initial response to tuberculosis treatment:** the patient's condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinitiation, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss
- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART—antiretroviral therapy. IRIS—immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as "probable paradoxical tuberculosis-associated IRIS". In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as "paradoxical tuberculosis-associated IRIS" cases.

For each participant who developed clinical deterioration during the first 12 weeks of ART and in whom paradoxical TB-IRIS was suspected as a possible cause for deterioration (even if the index of suspicion was low) a suspected TB-IRIS CRF and narrative were completed. In addition, a suspected TB-IRIS CRF and narrative were completed for all participants who died within the first 12 weeks of starting ART.

All these narratives were reviewed by an independent TB-IRIS review committee comprised of 3 expert clinicians not active at the clinical site. This review was performed in accordance with PREDART SOP 2016/19 ("PredART trial: Review of suspected TB-IRIS cases by independent endpoint review committee). Individual decisions regarding whether the case fulfilled the INSHI case definition were made, followed by consensus seeking according to the SOP to derive a final committee decision whether the case fulfilled the INSHI case definition for paradoxical TB-IRIS – this will be used as the primary endpoint event for the PredART trial.

The statistical analysis for the primary endpoint will compare the proportion of participants in the prednisone arm who fulfilled the INSHI paradoxical TB-IRIS definition to the proportion in the placebo arm, within 12 weeks from randomization. The analysis will be an intention to treat (ITT) analysis including all 240 participants randomized. A Chi-square test or Fisher exact test (as appropriate) will be used. A p-value of less than 0.05 will be regarded as significant for the primary endpoint analyses, and all other analyses performed. In addition, the relative risk of TB- IRIS will be estimated together with a 95% confidence interval. The primary hypothesis of the study is that the proportion of patients developing paradoxical TB-IRIS within 12 weeks of starting ART, as determined by the INSHI case definition and confirmed by the endpoint review committee, will be lower in patients randomized to prednisone compared to patients randomized to placebo.

In addition, as a secondary analysis, an all-patients-treated approach will be performed excluding those participants who were randomized but never started study medication and/or ART.

4. Analysis of secondary efficacy endpoints

A number of secondary efficacy analyses will be performed, all on the ITT population and the all-patients-treated population:

- a) Comparison of proportion of participants who fulfil at least one INSHI major criterion for diagnosis of paradoxical TB-IRIS in each arm. This will be done using Chi-square test or Fisher exact test (as appropriate).
- b) During review of suspected TB-IRIS cases by the independent endpoint review committee cases that the committee agreed had paradoxical TB-IRIS, but did not fulfil the INSHI case definition were identified. In a pre-specified secondary analysis, the proportion of all TB-IRIS cases (those fulfilling INSHI case definition plus additional cases not meeting this definition but considered by the committee to have had TB-IRIS) will be compared, using Chi-square test or Fisher exact test (as appropriate).
- c) In a pre-specified secondary analysis the proportion of participants who developed paradoxical TB-IRIS fulfilling INSHI criteria who had more sustained TB-IRIS symptoms will be compared. The criteria for this will be participants whose paradoxical TB-IRIS symptoms lasted more than 14 days plus any participant in whom symptoms lasted 14 days or less but who was prescribed open label corticosteroids to treat TB-IRIS symptoms. This will be done using Chi-square test or Fisher exact test (as appropriate).
- d) Comparison of the proportion of participants that fulfilled INSHI TB-IRIS criteria who were initiated on open label corticosteroids to treat paradoxical TB-IRIS, using Chi-square test or Fisher exact test (as appropriate).
- e) Comparison of time to paradoxical TB-IRIS event that fulfilled INSHI TB-IRIS criteria. This analysis will include all participants in the ITT population. The duration will be defined as the number of days from the initiation of ART to

onset of IRIS symptoms as reported by the participant to the study doctor. The two arms will be compared using cumulative incidence curves and the statistical comparison will be performed using Cox regression on cause-specific hazards.

- f) Comparison of duration of TB-IRIS for participants that fulfilled INSHI TB-IRIS criteria. Duration in days will be compared using cumulative incidence curves and Cox regression on cause-specific hazards. For those participants without a known date of IRIS remission the midpoint between the last visit date they were seen with TB-IRIS symptoms and the date they were seen with resolved TB-IRIS symptoms will be taken as resolution date. Patients who died or were lost to follow-up before TB-IRIS resolved will be censored at the date of death or loss to follow-up.
- g) Comparison of the duration of open label corticosteroid used to treat paradoxical TB-IRIS for participants that fulfilled INSHI TB-IRIS criteria. Duration in days will be compared using the Wilcoxon rank sum test.
- h) Comparison of the proportion of participants requiring hospitalization for paradoxical TB-IRIS event, within 12 weeks from randomization, using Chi-square test or Fisher exact test (as appropriate). This analysis will include only participants that fulfilled INSHI TB-IRIS criteria.
- i) Comparison of the proportion of participants requiring hospitalization for any cause (all-cause hospitalization), within 12 weeks from randomization, using Chi-square test or Fisher exact test (as appropriate). The total duration of hospitalization (in days) in each arm will also be compared using a zero-inflated Poisson model.
- j) Comparison of the proportion of participants who developed neurological manifestations of paradoxical TB-IRIS event (as decided by TB-IRIS review committee) using Chi-square test or Fisher exact test (as appropriate).

- k) Comparison of the C-reactive protein (CRP) concentrations in the two arms. CRP was measured at week 0, 2, 4 and 12. Values that fall below the detection limit will be replaced by half of the detection limit, i.e. 0.15. A mixed effects linear regression model on the log transformed CRP values with random intercept will be used to compare evolution of CRP over time between arms.
- l) Comparison of proportion of participants who die (all-cause mortality) within 12 weeks from randomisation between the two groups using Chi-square test or Fisher exact test (as appropriate).
- m) Comparison of proportion of participants who die and in whom death is attributed as due to paradoxical TB-IRIS within 12 weeks from randomisation between the two groups using Chi-square test or Fisher exact test (as appropriate). This will include only participants that fulfilled INSHI TB-IRIS criteria.
- n) Comparison of a composite endpoint of death, hospitalization, or hepatotoxicity (using the protocol-specified definition of Grade 3 or 4 increase in ALT or bilirubin) within 12 weeks of randomisation, using Chi-square test or Fisher exact test (as appropriate).
- o) Comparison of proportion of participants who develop other (non-TB) IRIS events within 12 weeks from randomisation, using Chi-square test or Fisher exact test (as appropriate).
- p) Comparison of quality of life assessment between the two arms at week 4 and at week 12. In the trial, the following measurements were performed: PROQOL-HIV, EQ-5D-3L, HIV symptom index and Karnofsky score. For EQ-5D-EL we will compare the proportion of patients in the different domain levels using the Chi-square test, and we will compare the means of the VAS score using the t-test or Wilcoxon rank sum test as appropriate. For the adapted ProQOL HIV the summary score per domain (adapted from [1]) will be compared using the t-test

or Wilcoxon rank sum test as appropriate. For the HIV-symptom index the frequency (proportion) and the severity of each symptom (score 1-2 vs score 3-4) will be compared using the Chi-square test. Because of multiplicity, Bonferroni correction will be applied in the analysis of HIV symptoms. Karnofsky score will be compared using the t-test or Wilcoxon rank sum test as appropriate.

- q) The cumulative proportion of participants who develop adverse events ascribed to TB treatment, ART or co-trimoxazole requiring interruption of one or more of these medications (for > 5 days) within 12 weeks of randomisation will be compared using Chi-square test or Fisher exact test (as appropriate).
- r) The cumulative proportion of participants who develop drug rash or drug-induced liver injury ascribed to TB treatment, ART or co-trimoxazole and requiring interruption of one or more of these medications (for > 5 days) within 12 weeks of randomisation will be compared using Chi-square test or Fisher exact test (as appropriate).

5. Analysis of secondary safety and tolerability endpoints

A number of secondary safety and tolerability analyses will be performed, all using the all-patients-treated approach (this excludes those participants who were randomized but never started study medication and/or ART).

- a) Comparison of proportion of participants in each arm who develop at least one of the pre-specified potential corticosteroid-associated adverse events within 4 weeks and within 12 weeks of randomization using Chi-square test or Fisher exact test (as appropriate). The proportion of participants in each group who develop individual potential corticosteroid-associated adverse events within 4 weeks and within 12 weeks of randomization will also be compared.

The pre-specified potential corticosteroid-associated adverse events are:

- New hypertension > 160/100
- New poor BP control on treatment > 160/100
- Hyperglycaemia (random glucose > 11.1 mmol/l)

- Hypomania/mania
- Depression
- Acne
- Epigastric pain
- Upper gastro- intestinal bleeding
- Cushingoid features
- New oedema
- Avascular bone necrosis

b) Comparison of laboratory safety bloods done at week 2, 4 and 12: sodium, potassium, creatinine, ALT, alkaline phosphatase, total bilirubin, glucose, haemoglobin, white cell count, neutrophil count, total lymphocyte count and platelets. Comparison of medians will be performed at each time point using the Wilcoxon rank sum test. The proportion of participants who develop an ACTG grade 3 or grade 4 abnormality in these blood tests will also be compared using Chi-square test or Fisher exact test (as appropriate).

c) Comparison of week 12 CD4 count and HIV viral load results. Comparison of medians will be performed using the Wilcoxon rank sum test. Proportion of participants with less than $2 \log_{10}$ drop in HIV viral load from screening to week 12 will be compared using the Chi-square test or Fisher exact test (as appropriate). Where screening viral load was too low to ascertain a $2 \log_{10}$ drop then an undetectable viral load at week 12 will be regarded as successful outcome.

d) Comparison of the proportion of participants who are diagnosed with other infections (AIDS-related, bacterial, fungal and viral) and malignancies (Kaposi's sarcoma) will be compared using Chi-square test or Fisher exact test (as appropriate). These will also be presented with individual listing. The proportion with severe infections (classified as any AIDS-defining (WHO stage 4) infection or invasive bacterial infection) will be compared in a separate analysis.

e) The proportion of participants with any clinical ACTG grade 1, 2, 3 and 4 adverse events (AEs) will be compared using Chi-square test or Fisher exact test (as appropriate). A similar comparison will be made for serious adverse events (SAEs) and adverse drug reactions (ADRs) attributed as definite, probably or possibly due to medication (the relationship between ADRs and medication has been determined by the study doctors and categorized as definitely not, unlikely, possibly, probably or definitely related to any medication).

In addition, a listing of AEs and SAEs categorized using MedDRA will also be presented. AEs and SAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be reported based on MedDRA preferred terms and body systems. All AEs and SAEs will be analyzed based on counts of patients with a specific category and not on counts of individual adverse events.

All safety and tolerability analyses will be performed for the 12 week trial period, as well as a separate analysis restricted to the first 4 weeks from randomization. The reason for performing these analyses at 4 and 12 weeks, is that certain participants may commence open label corticosteroid (generally from 2-3 weeks onwards) so the 4 week analysis will exclude most of the safety events related to open label corticosteroid and more directly compare study prednisone versus placebo.

6. Pre-specified subgroup analyses relating to the primary endpoint

A number of pre-specified subgroup analyses of the primary endpoint will be performed using an ITT population. These subgroups will be

- a) Participants with screening CD4 count \leq 50 cells/ μ L
- b) Participants with screening CD4 count $>$ 50 cells/ μ L
- c) Participants with screening HIV viral load $>$ 100,000 copies/ml
- d) Participants with screening HIV viral load \leq 100,000 copies/ml
- e) Participants with microbiologically proven TB (TB culture and/or Xpert)
- f) Participants not diagnosed with rifampicin-resistant TB during follow-up.

7. Analysis of risk factors for TB-IRIS

As a tertiary analysis, we will assess possible risk factors for developing IRIS (age, gender, extrapulmonary TB, lymph node TB, TB diagnosis method, baseline CD4 count, baseline HIV viral load, CD4 count increase at 12 weeks, log HIV viral load decrease at 12 weeks, duration from TB treatment to ART initiation, urine LAM status, baseline CRP, and baseline haemoglobin, neutrophil, white cell counts and Karnofsky score) in addition to treatment arm in multivariable logistic regression model and Cox regression on cause-specific hazards.

8. Missing data and sensitivity analyses

For those participants lost to follow-up before week 12, data was obtained on vital status from telephonic contact or health service databases. However, it was not possible to ascertain their outcome in terms of TB-IRIS after loss to follow-up and they were thus regarded as not having developed TB-IRIS for the primary analysis. A sensitivity analysis will be performed where all participants lost to follow-up before week 12 will be assumed to have developed TB-IRIS.

9. Protocol deviations

A list of all protocol deviations, major and minor, has been compiled. These were defined according to the relevant trial SOP, and in accordance with guidance of the UCT Human Research Ethics Committee. These will be presented as supplementary material in the trial publication.

10. Week 28 and month 12 safety assessments

In the original trial protocol follow-up was planned until week 12 in all participants. After the publication of the IMPI trial findings, the DSMB recommended additional follow-up to ascertain development of cancers. A week 28 visit and 12 month visit or phone call were added. Where this was/is not possible then the patient's clinic or hospital notes are checked. These safety assessments are only for the purposes of ascertaining vital status and whether a cancer diagnosis has been made at these time points. These data are entered on a separate database from the main trial database. Follow-up of the last participant at one year will be completed in February

2017. The DSMB agreed that we could lock and unblind the main trial database before this, given that ascertainment of death and cancer at these time points is at low risk of being biased by knowledge of trial allocation.

11. Calculation of durations

Some participants had two or more hospitalisation, or two or more courses of open label corticosteroid. In such cases the duration of hospitalisation or open label corticosteroid was calculated by summing the number of days of each hospitalisation or of each open label corticosteroid course.

12. Publication

This Statistical Analysis Plan will be published on-line (eg. clinicaltrials.gov or PredART website), before locking and unblinding of database.

References

[1] Duracinsky M, Lalanne C, Le Coeur S, Herrmann S, Berzins B, Armstrong AR, Lau JT, Fournier I, Chassany O. J Acquir Immune Defic Syndr. 2012 Apr 15;59(5):506-15. Psychometric validation of the PROQOL-HIV questionnaire, a new health-related quality of life instrument-specific to HIV disease.