PROTOCOL TITLE

VALIDATION OF CORRELATES OF RISK OF TB DISEASE IN HIGH RISK POPULATIONS (CORTIS-HR)

A Companion Study of the CORTIS-01 Trial

PROTOCOL NUMBER: CORTIS-HR

PROTOCOL DATE: 26th August 2016

PROTOCOL VERSION: Version 1.0

SPONSORED BY: University of Cape Town

SITE PRINCIPAL INVESTIGATOR SIGNATURES OF AGREEMENT FOR PROTOCOL IMPLEMENTATION

SATVI Site PI	Date
Aurum Klerksdorp Site PI	Date
Aurum Rustenburg Site PI	Date
CAPRISA Site PI	Date
Stellenbosch University Site PI	Date

INVESTIGATOR APPROVAL STATEMENT

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make a reasonable effort to complete the study within the time designated.

I agree to personally supervise the study.

I will ensure that the requirements related to obtaining informed consent are in accordance with ICH Guidelines for Good Clinical Practice (GCP) section 4.8 and local requirements.

I agree to promptly report to the Ethics Committee (EC) all changes in the research activity and all unanticipated problems involving risk to the participants. I will not make any changes to the conduct of the study without the EC and Sponsor approval, except when necessary to eliminate apparent immediate harm to participants.

I agree to maintain adequate and accurate records and make those records available in accordance with ICH guidelines for Good Clinical Practices (GCP) section 4.11 and local requirements.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

I understand that the study may be terminated or enrolment suspended at any time by the Sponsor, with or without cause, or by me if it becomes necessary to protect the best interest of the participants.

_____ _____

National Principal Investigator Signature Date

RESPONSIBILITIES

Sponsor:	University of Cape Town
National Principal Investigator:	Mark Hatherill
Regulatory Functions:	Triclinium Clinical Development
Site Monitoring Functions:	Triclinium Clinical Development
Data Management Functions	Triclinium Clinical Development
Statistical Analysis:	The Statistical Center for HIV/AIDS Research & Prevention (SCHARP)
Clinical Laboratory:	BARC, Johannesburg
Analytical Laboratory:	South African Tuberculosis Vaccine Initiative (SATVI)

PROTOCOL SYNOPSIS

TITLE	**Validation of Correlates of Risk of TB Disease in High Risk Populations (CORTIS-HR)**
BACKGROUND	Effective tuberculosis (TB) control requires that people who progress from latent *Mycobacterium tuberculosis* (MTB) infection (LTBI) to TB disease are identified and treated before they become symptomatic and infect others. A prognostic correlate of risk (COR), based on mRNA expression signatures, which prospectively discriminates between TB cases and healthy controls, has been constructed and validated in HIV uninfected persons. Based on published microarray case-control datasets, this COR has 87% diagnostic sensitivity and 97% specificity for prevalent TB disease in HIV uninfected South African adults; and in two nested case-control studies, also among HIV uninfected persons, the COR has 70% prognostic sensitivity and 84% specificity for incident TB disease occurring within one year of sampling. Based on analysis of published microarray data, COR diagnostic performance for discriminating prevalent TB disease from latent TB infection in HIV infected persons appears to be reduced by approximately 10%, compared to HIV uninfected persons. ***Diagnostic and prognostic performance of the COR has not yet been tested in a prospective cohort of HIV infected persons***. The diagnostic and prognostic performance of the blood RNA signature of risk for TB will be validated in HIV infected persons, in a companion study of the CORTIS-01 trial. HIV infected persons bear a disproportionate TB disease burden, despite antiretroviral therapy (ART) and isoniazid preventive therapy (IPT). Modeling indicates that prevention of TB disease in HIV infected persons, using a COR 'screen and treat' strategy to target preventive therapy for those at highest risk, would have major impact on the TB epidemic in Sub-Saharan Africa, with considerable indirect benefit to the HIV uninfected population.
AIMS	**Primary Aim:** 1. Test whether COR status differentiates HIV infected persons with cumulative prevalent or incident TB disease from those without TB disease. **Secondary Aims:** **1.** Estimate whether COR status differentiates HIV infected persons with prevalent TB disease from those without prevalent TB disease 2. Estimate whether COR status differentiates HIV infected persons at high risk for incident TB disease from those at low risk for incident TB disease **3.** Compare prognostic performance of the COR for incident TB disease with Interferon-gamma release assay (IGRA) in HIV infected persons. **Exploratory Aims:** **1.** Assess and model the impact of a COR screen & treat strategy on reducing the rate of incident TB disease and TB mortality among HIV infected persons in South Africa. 2. Re-parameterize the COR assay for prevalent and incident TB disease in HIV infected persons. 3. Test the performance of additional validated COR signatures in distinguishing HIV infected persons with cumulative prevalent or incident TB disease from those without TB disease. 4. Compare prognostic performance of the COR for incident TB disease in HIV infected persons before and 3 months after starting IPT and/or ART.
STUDY SIZE	860 HIV infected adults

STUDY POPULATION	Adult volunteers living in TB hyperendemic communities of South Africa will be consented and screened. Persons aged 18 to less than 60 years, including those who are ineligible for the CORTIS-01 trial on the basis of HIV infection, will be approached to enroll in CORTIS-HR. *Inclusion criteria (at time of screening):* 1. Written informed consent 2. Aged ≥18 and <60 years 3. HIV infection 4. Likely to remain in follow-up and adhere to protocol requirements *Exclusion criteria (at time of screening):* 1. Pregnant or lactating 2. Diagnosed with TB disease within last 3 years 3. Household exposure to a TB patient with known multi-drug resistant (MDR-) TB disease within last 3 years 4. Any medical, surgical, or other condition, including but not limited to known diabetes mellitus (requiring oral or injectable therapy), liver disease, or alcoholism, that in the opinion of the Investigator is likely to interfere with COR performance; safety or efficacy of ART and/or IPT; or adherence to protocol requirements.
STUDY DESIGN	CORTIS-HR is an observational study of COR diagnostic and prognostic performance in HIV infected persons. Following sample collection for the COR assay at Visit 1 (Day 0), all participants will be screened for prevalent TB. All eligible participants without prevalent TB will be referred for at least 12 months (IGRA negative) or 36 months (IGRA indeterminate or positive) IPT. All HIV infected persons eligible for ART, as per SA national guidelines, will be referred in writing to the clinic for ART. Thereafter, all participants will be followed for 15 months for incident TB disease (3 telephone contacts and 4 site visits). Symptoms consistent with TB disease will be solicited at study visits and presence of one or more symptoms will trigger TB investigation. The performance of the COR will be evaluated by comparing the cumulative incidence of endpoint-defined TB disease over 15 months in COR+ versus COR- participants (RR_{COR}). We will perform an interim analysis of COR prevalence and diagnostic performance for prevalent TB cases. The results will inform a Stop/Go decision for continuation of study follow-up beyond 3 months in all subjects to evaluate COR prognostic performance. In subjects who continue follow-up for incident TB disease, we will perform a sensitivity analysis of month 3 COR prognostic performance to evaluate the impact of IPT and/or ART on COR score and TB risk.
INVESTIGATIONS	Blood will be sampled at Visit 1 (Day 0) for QuantiFERON-Plus (QFT) and CD4 cell count; blood will be collected for HIV viral load (VL) only in those subjects not yet established on ART; whole blood RNA will be collected in PAXgene tubes for the COR assay; and urine will be collected for lipoarabinomannan (LAM) assay. Aliquots of plasma, serum and urine will be stored for later molecular, proteomic and metabolomic studies. COR assay and serum and urine collection will be repeated in all subjects at month 3 to evaluate COR dynamics and the impact of IPT on the COR score. CD4 count and VL will be repeated at 3 months in those subjects starting ART. Risk of TB disease will be evaluated using the qualified BioMark HDFluidigm multiplex qRT-PCR COR assay at the SATVI Laboratory. All participants will undergo TB symptom screening and will provide two sputum samples for paired Xpert MTB/RIF and MGIT culture at Visit 1 (Day 0). Thereafter, symptoms consistent with TB disease will be solicited at all study visits; and presence of one or more symptoms will trigger TB investigation (paired sputum Xpert MTB/RIF and MGIT culture). All participants will undergo TB investigation again at end of study and submit two sputum samples for paired sputum Xpert MTB/RIF and MGIT culture. Throughout follow-up,

	additional tests for clinical suspicion of TB disease, including, but not limited to chest radiography and sputum induction, may be performed if deemed indicated by the investigator. Investigator decisions to start TB treatment on clinical grounds will be made independently of study-specific endpoint determination.
STUDY DURATION	All participants will be followed for 15 months for incident TB disease (3 telephone contacts and 4 site visits).
SITES	5 or more study sites in South Africa.
STUDY ENDPOINTS	The primary endpoint will be defined as Xpert MTB/RIF and/or MGIT culture positive TB disease, confirmed on two separate sputum samples; or on samples from any other site in the case of extrapulmonary TB disease.
STATISTICAL CONSIDERATIONS	The primary analysis will evaluate $RR_{COR}(15)$, relative-risk for TB disease over 15 months of follow-up. The primary outcome measure is Relative Risk (RR, 95% CI) for TB disease, as per the TB case endpoint definition. Based on our underlying assumptions for COR prevalence, ART and IPT usage, and TB prevalence and incidence, it is expected that of approximately 215 (25%) newly diagnosed HIV infected participants yet to start ART, we will identify 31 (95% CI 22, 42) prevalent TB cases at screening; and an additional 5 (95% CI 2, 10) participants who will develop active TB disease over 15 months of follow-up. It is expected that of the approximately 645 (75%) HIV infected participants already on chronic ART, we will identify 12 (95% CI 6, 20) prevalent TB cases at screening; and an additional 9 (95% CI 4, 15) incident TB cases over 15 months of follow-up. Thus, it is expected that we will measure the COR in a total of 58 (95% CI 44, 73) cumulative endpoint TB cases (43 prevalent and 14 incident TB cases) and 802 (95% CI 787, 816) controls. Based on these assumptions, it is estimated that the RR of a COR+ HIV infected participant for cumulative TB disease, relative to a COR- HIV infected participant, will be approximately 19 (95% CI 11-34). Corresponding estimates of precision for RR for prevalent cases only are 95% CI 10-37; and for incident cases only are 95% CI 6-65.
ETHICAL CONSIDERATIONS	*Risks:* HIV infected persons are at several-fold higher risk of TB disease compared to HIV uninfected persons, even if established on chronic ART, which reduces incident TB disease by between 45-65%. SA national guidelines now recommend initiation of ART for all HIV infected persons with CD4 count ≤500 cells/mm^3. IPT offers added protection, estimated to be in the range 30 – 50%, with isolated studies reporting up to 83% risk reduction. It is estimated that approximately 75% of CORTIS-HR participants will be receiving chronic ART at Visit 1 (Day 0), 10-20% of whom will be receiving chronic IPT. *Measures to Ensure Safety*: WHO guidelines currently recommend that all HIV infected persons in high TB transmission regions with unknown or positive tuberculin skin tests (TST) receive ART and at least 36 months of IPT. South African national guidelines additionally recommend that TST negative HIV infected persons receiving ART should also receive at least 12 months of IPT. Therefore, all HIV infected persons not receiving, but eligible for ART, will be referred in writing to the state health services to access the appropriate care. Additionally, all eligible participants will be referred for at least 12 months (QFT negative) or 36 months (QFT indeterminate or positive) of IPT. All participants diagnosed with prevalent TB disease at Visit 1 (Day 0) will discontinue study follow-up and will be referred for curative treatment. Thereafter, active symptom-based surveillance and investigation for incident TB disease will allow early diagnosis and effective treatment; all participants will be investigated for TB at end of study. Symptomatic participants who test sputum Xpert MTB/RIF negative on two samples, or who are sputum unproductive, may undergo additional investigations if deemed necessary by the investigator. Symptomatic, Xpert MTB/RIF negative participants may, at the discretion of the investigator, undergo a course of broad-spectrum antibiotics as trial of therapy prior to further investigation. Participants with a clinical or radiological suspicion of TB disease not meeting the study endpoint definition will be referred for curative treatment if indicated in the judgment of the investigator.

LIST OF ABBREVIATIONS

Abbreviation	Text
AFB	Acid-fast bacilli
BMI	Body Mass Index (BMI)
cDNA	Copy DNA
CI	Confidence interval
COR	Correlate of Risk
CRA	Clinical research associate
CRF	Case report form
CRO	Clinical Research Organization
DAIDS	NIH Division of AIDS
eCRF	electronic CRFs
FDA	Food and Drug Administration
GCP	Good Clinical Practice
H_0	Null hypothesis
HIV	Human Immunodeficiency Virus
IEC	Independent ethics committee
IGRA	Interferon gamma release assay
INH	Isoniazid
IPT	INH preventive therapy
LTBI	Latent tuberculosis infection
MDR-TB	multi-drug resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
mRNA	Messenger RNA
MTA	Material Transfer Agreement
MTB	*Mycobacterium tuberculosis*
NHP	Non-human primate
NTP	National TB Programme
PI	Principal Investigator
QFT	QuantiFERON
RR	Relative risk
$RR_{COR}(15)$	Relative risk for TB disease over 15 months
SA	South Africa
SATVI	South African Tuberculosis Vaccine Initiative
TB	Tuberculosis
TCD	Triclinium Clinical Development
TST	Tuberculin Skin Test
WHO	World Health Organization

SCHEDULE OF EVENTS

All participants (n=860)

Description	Screening & Enrolment	Follow-up						End of Study
Trial Visit	**Visit 1**	**Contact 2**	**Contact 3**	**Visit 4**	**Visit 5**	**Contact 6**	**Visit 7**	**Visit 8**
Day	**D0**	**M1**	**M2**	**M3**	**M6**	**M9**	**M12**	**M15**
Informed consent[1]	x							
Age verification	x							
Medical history	x							
Height	x							
Weight	x			x	x		x	x
Urine pregnancy test (females)	x							
HIV counselling & testing	x							
Vital signs	x			x	x		x	x
Targeted physical examination	x			x	x		x	x
Verification of eligibility	x							
Phlebotomy[2]	x			x				
COR (PAXgene RNA)	x			x				
CD4 count (cells/mm^3)	x			x[7]				
HIV Viral load (copies/mL)	x[6]			x[7]				
IGRA (IU/mL)	x							
Serum (proteomics)	x			x				
Plasma (molecular assays)	x							
Urine (LAM and metabolomics)	x			x				
TB symptom screen	x	x	x	x	x	x	x	x
TB Investigations	xx[3]	xx[4]	xx[4]	xx[4]	xx[4]	xx[4]	xx[4]	xx[5]
Concomitant Medications	x	x	x	x	x	x	x	x

[1] May be conducted at prior field visit
[2] Phlebotomy for further investigations only if eligible
[3] One sputum sample for Xpert MTB/RIF; one sputum sample for MGIT culture; store aliquot of unprocessed sputum from each (all participants)
[4] If indicated by positive TB symptom screen, one sputum sample for Xpert MTB/RIF; one sputum sample for MGIT culture
[5] One sputum sample for Xpert MTB/RIF; one sputum sample for MGIT culture (all participants)
[6] HIV viral load only in subjects not yet established on ART
[7] CD4 count and viral load repeated at 3 months only in participants starting ART

BACKGROUND

Two billion people worldwide, including the majority of adults in TB endemic countries, are *Mycobacterium tuberculosis* (MTB) infected. These latently infected individuals, identified by a positive tuberculin skin test (TST) or interferon-gamma release assay (IGRA), have higher risk of developing TB disease than uninfected people. Unfortunately, TST and IGRA have poor specificity for incident TB disease in endemic populations, including HIV infected people.

We have previously developed a highly specific prognostic correlate of risk (COR) to identify healthy, HIV uninfected, South African adults at high risk of active TB disease[1]. This validated COR, based on mRNA expression signatures in blood, prospectively discriminates between TB cases and healthy controls among HIV uninfected persons [1]. Based on published microarray case-control datasets, the COR has 87% diagnostic sensitivity and 97% specificity for prevalent TB disease in HIV uninfected South African adults [2-4]; and in two nested case-control studies, also among HIV uninfected persons, the COR has 70% prognostic sensitivity and 84% specificity for incident TB disease occurring within one year of sampling [1-4].

Based on analysis of published microarray data, COR diagnostic performance for discriminating prevalent TB disease from latent TB infection in HIV infected persons appears to be reduced by approximately 10%, compared to HIV uninfected persons [5]. Our preliminary data support this estimate. In a small pilot study of 100 participants, diagnostic performance (area under the Receiver Operating Characteristic curve) of the qRT-PCR-based COR decreased by 13% when applied to HIV infected participants, compared to HIV uninfected participants. Reduced COR sensitivity and specificity in HIV infected persons might be related to chronic stimulation of anti-viral type-I interferon responses and/or immune dysfunction. However, performance of the COR, which was discovered in HIV uninfected persons, might be further improved by re-parameterizing the gene expression signature to account for HIV status. Smaller, parsimonious COR models suitable for point-of-care testing are also being evaluated in ongoing studies.

The Dual Epidemics of TB and HIV

TB disease constitutes a major morbidity and mortality burden for HIV infected patients, and a massive logistic and economic burden for healthcare systems in resource-constrained countries like South Africa [6]. Prevalence of HIV infection in adult South Africans is approximately 17% (9.2 million people). HIV infected persons remain at several-fold higher risk of TB disease compared to HIV uninfected persons, even if established on chronic ART, which reduces rate of incident TB disease by between 45-65%[7-9]. Approximately 79% of HIV infected TB patients in South Africa receive chronic ART. However, the World Health Organization (WHO) estimates there were 450,000 TB cases in South Africa in 2014, of which 270,000 were HIV co-infected; with 72,000 deaths due to TB occurring in HIV infected persons (*WHO Global Tuberculosis Report 2015*) [6]. In that year alone, 1.1 million HIV infected South Africans (12%) were screened for TB disease and 500,000 patients were provided with IPT. National TB/HIV services are increasingly integrated and might provide a platform for targeted TB triage, treatment, and prevention, on a wider scale.

Effect of ART on Risk of TB Disease

ART alone is an effective TB preventive therapy for HIV infected persons [7-9]. A recent meta-analysis reported 65% average risk reduction for incident TB disease, with no difference in effect by CD4 cell count strata [8]. Overall, risk for incident TB disease in HIV infected persons on chronic ART is not affected by baseline CD4 cell count [8]. However, some data are conflicting [7,9,10]. The exceptions to the rule are at the upper and lower range of CD4 cell count; and during the period soon after the start of ART [7,9,10]. For example, in a study of 74,000 HIV infected South Africans, ART decreased the rate of incident TB by 45% among those patients with CD4 cell counts less than 350 cells/mm^3, but was not associated with statistically significant protection among those with CD4 cell counts above 350 cells/mm^3 [9]. By contrast, in a study of 65,000 HIV infected persons from high income countries, ART reduced the rate of incident TB by 44% overall, but was not associated with statistically significant protection among those with CD4 cell counts below 50 cells/mm^3 [7]. In a third

study, TB disease incidence among HIV infected South Africans with CD4 cell counts less than 200 cells/mm^3 were 1.7-fold higher in the first 4 months of ART than during chronic ART [10].

Effect of IPT on Risk of TB Disease
Provision of chronic ART is thought to exert greater impact on risk for incident TB disease than IPT alone, but IPT does offer added protection, estimated to be in the range 30 – 50%, with isolated studies reporting up to 83% risk reduction [11-14]. In a study among HIV infected South Africans, addition of IPT to an ART regimen was associated with average 37% risk reduction among both TST positive and TST negative persons, with the greatest benefit within the first year of starting therapy [11]. Adjustment for CD4 cell count did not significantly change the risk for incident TB disease [11]. A recent Cochrane review reported a similar 32% risk reduction overall, with the greatest effect seen among TST positive persons [12]. By contrast, the Brazilian THRio trial, conducted among 2,000 HIV infected TST positive persons, reported 83% reduction in risk for TB disease, with the greatest effect seen among those with CD4 cell counts below 200 cells/mm^3 [14]. It is possible that the effect of IPT may be more modest in high, compared to low, TB burden countries.

Durability of Protection Offered by IPT
We might expect that IPT would not offer durable protection after conclusion of therapy, due in part to reactivation of dormant *Mycobacterium tuberculosis* (MTB) bacilli; and in part to exogenous reinfection. Mathematical modeling suggests that provision of IPT to HIV infected persons receiving ART results in sterilizing cure of LTBI in only 35% of patients [15]. In a trial in Botswana, receipt of chronic IPT for 36 months was shown to reduce the annual incidence of TB disease in HIV infected persons to 0.7%, compared to 1.3% with 6 months of IPT [16], and with waning, but persistent protection after conclusion of IPT only in TST positive participants [17]. It is clear that HIV infected persons need additional protection against 'breakthrough' reactivation TB disease occurring during IPT, as well as TB disease occurring after IPT has ended.

Current Guidelines
SA national guidelines now recommend initiation of ART for all HIV infected persons with CD4 count ≤500 cells/mm^3. WHO guidelines currently recommend that all HIV infected persons in high TB transmission regions with unknown or positive tuberculin skin tests (TST) receive ART and at least 36 months of IPT. South African national guidelines additionally recommend that TST negative HIV infected persons receiving ART should also receive at least 12 months of IPT. It is estimated that at the study sites, approximately 75% of eligible HIV infected persons are receiving ART; and that 10-20% of those receiving ART are currently receiving chronic IPT.

Summary of Factors Affecting TB Risk
Risk of prevalent TB among HIV infected South Africans receiving chronic ART, across all CD4 cell count strata, is estimated at 2%, with historically higher rates in the range 4-6% [18,19]. Twelve-month risk of incident TB is also estimated at 2%, with historically higher rates in the range 4-10% [20]. Corresponding risk of prevalent TB in HIV infected persons not receiving, or newly established on ART, is estimated at 15% [21,22]; and 12-month risk of incident TB is estimated at 4% [21,22]. Addition of IPT to a chronic ART regimen might further reduce these estimates by 30 – 50%. Although some data are conflicting, it is unlikely that baseline CD4 cell count will have major impact on TB incidence in persons receiving chronic ART and IPT [7,9,10], but it is possible that any added benefit of IPT is maximal in TST positive persons [12,14].

RATIONALE
A conservative model of the South African epidemic indicates that a COR targeted 'screen & treat' strategy, independent of parallel improvements in TB therapeutics, could reduce overall TB incidence by 27% and TB mortality by 35% within 5 years. Key to population-level impact of the strategy is that COR targeted TB preventive therapy is effective for both HIV uninfected and HIV infected persons, in any community in which the strategy is rolled out. Additional modelling suggests that if the strategy were targeted only at HIV infected adults, this population would benefit directly by 15% reduction in TB incidence; and the HIV uninfected population would benefit indirectly by 16%

reduction over the same period. Thus, prospective validation of COR performance in HIV infected persons, and thereafter, demonstration of efficacy of TB preventive therapy in HIV infected COR+ persons, is crucial to success of the 'screen and treat' strategy in the countries of Sub-Saharan Africa affected by the dual epidemics of TB and HIV.

RISKS

Risk of TB Disease: HIV infected persons are at several-fold higher risk of TB disease compared to HIV uninfected persons, even if established on chronic ART, which reduces incident TB disease by between 45-65%. SA national guidelines now recommend initiation of ART for all HIV infected persons with CD4 count ≤500 cells/mm^3. IPT offers added protection, estimated to be in the range 30 – 50%, with isolated studies reporting up to 83% risk reduction. We estimate that approximately 75% of CORTIS-HR participants will be receiving chronic ART at baseline, 10-20% of whom will be receiving chronic IPT.

Measures to Minimize Risk of TB Disease: WHO guidelines currently recommend that all HIV infected persons in high TB transmission regions with unknown or positive tuberculin skin tests (TST) receive ART and at least 36 months of IPT. South African national guidelines additionally recommend that TST negative HIV infected persons receiving ART should also receive at least 12 months of IPT. Therefore, all HIV infected persons not receiving, but eligible for ART, will be referred in writing to the state health services to access the appropriate care. Additionally, all eligible participants will be referred for 12 months (QFT negative) or 36 months (QFT indeterminate or positive) of IPT.

All participants diagnosed with prevalent TB disease at Visit 1 (Day 0) will discontinue study follow-up and will be referred for curative treatment. Thereafter, active symptom-based surveillance and investigation for incident TB disease will allow early diagnosis and effective treatment. All participants will also be investigated for TB at end of study. Symptomatic participants who test sputum Xpert MTB/RIF negative on two samples, or who are sputum unproductive, may undergo additional investigations if deemed necessary by the investigator for diagnosis of suspected pulmonary or extra-pulmonary TB. Symptomatic, Xpert MTB/RIF negative participants may, at the discretion of the investigator, undergo a course of broad-spectrum antibiotics as trial of therapy prior to further investigation. Participants with a clinical or radiological suspicion of TB disease not meeting the primary endpoint definition will be referred for curative treatment if indicated in the judgment of the investigator.

Other risks: Other potential risks to participants include risk of breach of confidentiality and disclosure of HIV and/or TB status, which may be associated with social stigma; minor discomfort and bruising associated with phlebotomy; and the time and inconvenience of attending study visits.

Measures to Minimize Other Risks: Identifiable personal information and source documentation will be locked in secure cabinets accessible only to study staff to maintain confidentiality. All study procedures will be performed according to ICH-GCP. Study data will be coded using a personal identifier and stored in a password secured database. All the study sites are experienced research sites with study staff trained in phlebotomy. Discomfort and bruising associated with blood sampling is deemed a minor risk, as is the small volume of blood (<50mL) to be sampled at Visit 1 (Day 0) and 3 months.

BENEFITS

Benefits of Active Case-finding and Active Surveillance for TB Disease: All participants will benefit from active case finding for undiagnosed prevalent TB disease at screening, by symptom screening and collection of sputum for investigation. Similarly, all participants will benefit from TB education and active surveillance for incident TB disease; by active symptom screening and symptom-triggered TB investigation during follow-up; and by repeat sputum screening for undiagnosed TB disease in all participants at end of study. Earlier diagnosis of previously undiagnosed and pre-

symptomatic or incipient TB disease will allow earlier, effective treatment, reduced morbidity, and reduced MTB transmission to susceptible contacts.

Benefits of HIV Diagnosis and Referral for Care: Participants who are newly diagnosed with HIV infection during screening will benefit from post-test counseling and referral for early ART and IPT, which would be expected to reduce AIDS-related morbidity and mortality, including that due to TB co-infection. Participants with known HIV infection who are receiving chronic ART, but not receiving IPT, will benefit from referral and improved linkage to care for TB preventive therapy.

Benefits of Participation in Research: Persons with other previously undiagnosed medical, surgical, or other conditions identified at screening, will benefit from early diagnosis, referral and rapid access to treatment systems. Similarly, participants who develop new conditions during follow-up will also benefit from early diagnosis, referral and linkage to care.

PRIMARY AIM

1. Test whether COR status differentiates HIV infected persons with cumulative prevalent or incident TB disease from those without TB disease.

SECONDARY AIMS

1. Estimate whether COR status differentiates HIV infected persons with prevalent TB disease from those without prevalent TB disease
2. Estimate whether COR status differentiates HIV infected persons at high risk for incident TB disease from those at low risk for incident TB disease
3. Compare prognostic performance of the COR for incident TB disease with Interferon-gamma release assay (IGRA) in HIV infected persons.

EXPLORATORY AIMS

1. Assess and model the impact of a COR screen & treat strategy on reducing the rate of incident TB disease and TB mortality among HIV infected persons in South Africa.
2. Re-parameterize the COR assay for prevalent and incident TB disease in HIV infected persons.
3. Test the performance of additional validated COR signatures in distinguishing HIV infected persons with cumulative prevalent or incident TB disease from those without TB disease.
4. Compare prognostic performance of the COR for incident TB disease in HIV infected persons before and 3 months after starting IPT and/or ART.

STUDY DESIGN

CORTIS-HR is an observational study of COR diagnostic and prognostic performance in HIV infected persons. Following sample collection for the COR assay at Visit 1 (Day 0), all participants will be screened for prevalent TB. All eligible participants without prevalent TB will be referred for at least 12 months (IGRA negative) or 36 months (IGRA indeterminate or positive) IPT. All HIV infected persons eligible for ART, as per SA national guidelines, will be referred in writing to the clinic for ART. Thereafter, all participants will be followed for 15 months for incident TB disease (3 telephone contacts and 4 site visits). Symptoms consistent with TB disease will be solicited at study visits and presence of one or more symptoms will trigger TB investigation. The performance of the COR will be evaluated by comparing the cumulative incidence of endpoint-defined TB disease over 15 months in COR+ versus COR- participants (RR_{COR}).

We will perform an interim analysis of COR prevalence and diagnostic performance for prevalent TB cases. The results will inform a Stop/Go decision for continuation of study follow-up beyond 3 months in all subjects to evaluate COR prognostic performance. In subjects who continue follow-up for incident TB disease, we will perform a sensitivity analysis of month 3 COR prognostic performance to evaluate the impact of IPT and/or ART on COR score and TB risk.

STUDY POPULATION
The study population will include 860 HIV infected adults residing in TB hyperendemic communities at five or more study sites in South Africa.

RECRUITMENT
Primarily, persons screened for the CORTIS-01 trial who are ineligible on the grounds of HIV infection will be approached by study staff to participate in CORTIS-HR. In addition, persons with documented HIV infection may be approached through local ART clinics and Voluntary Counseling & Testing (VCT) services affiliated with or run directly by the study sites. Although recruitment efforts will be focused on persons with documented HIV infection status, in order to maintain confidentiality and avoid risk of stigmatization, community volunteers with unknown HIV status would also be eligible for screening. Any persons screened for CORTIS-HR who are found ineligible due to being HIV uninfected may be approached to participate in the CORTIS-01 trial.

ELIGIBILITY CRITERIA
A participant will be eligible for enrolment in the trial if all inclusion criteria are met. A participant will not be eligible for trial enrolment if any of the exclusion criteria are met.

Inclusion criteria (at time of screening):
5. Written informed consent
6. Aged ≥18 and <60 years
7. HIV infection*
8. Likely to remain in follow-up and adhere to protocol requirements

Exclusion criteria (at time of screening):
5. Pregnant or lactating
6. Diagnosed with TB disease within last 3 years
7. Household exposure to a TB patient with known multi-drug resistant (MDR-) TB disease within last 3 years
8. Any medical, surgical, or other condition, including but not limited to known diabetes mellitus (requiring oral or injectable therapy), liver disease, or alcoholism, that in the opinion of the Investigator is likely to interfere with COR performance; safety or efficacy of ART and/or IPT; or adherence to protocol requirements.

**A documented positive HIV test result obtained for the purpose of CORTIS-01 trial screening will not need to be repeated if the test was performed 28 days or less prior to CORTIS-HR screening.*

CONCOMITANT MEDICATIONS
ART and IPT data will be recorded in all participants throughout the study. Details to be recorded, if known, include the specific medication trade name, the dose and unit, frequency and route of administration, as well as the start and stop dates of the therapy, and prescribing clinic..

PARTICIPANT IDENTIFIER
All participants who are screened for eligibility to participate in CORTIS-HR will be allocated a unique participant identifier. The number will consist of a study-specific prefix, a 1-digit site identifier followed by a 4-digit participant identifier which will be allocated sequentially in accordance with the order in which participants present for screening i.e. the first, second and third participants presenting for screening at Site 1 will be 10001, 10002 and 10003 etc. This number will be used as the participant's primary identifier throughout the study and will be used for all labelling purposes.

VISIT SCHEDULE
After successful screening and enrolment, each participant will undergo a total of seven (7) study contacts or visits, including three (3) study contacts (telephonic or field visits) and four (4) study clinic visits, through 15 months of follow-up.

VISIT 1 (SCREENING & ENROLMENT): DAY 0

Potential participants must provide written informed consent for participation in the study prior to and within 28 days of performing any screening assessments or procedures. If necessary, the participant may take the study information document away with them and return at a later stage for screening examinations. All screening assessments must be completed prior to enrolment.

The following information will be obtained and procedures and assessments performed for the purpose of screening to determine eligibility:

- Written informed consent for study participation
- Verification of age
- Medical history
- Review of concomitant medications (ART and IPT)
- Measurement of height and weight
- Urine pregnancy test (women of child-bearing potential only)
- Blood will be collected for HIV rapid test (with pre- and post-test counselling)*
- Vital signs
- Targeted physical examination
- Verification of eligibility

**Not required if documented positive HIV test obtained during CORTIS-01 screening and within 28 days of CORTIS-HR screening assessment. Any participants newly diagnosed with HIV infection at CORTIS-HR screening may complete post-test counselling and return to complete enrolment within 3 calendar days of screening assessment.*

The following information will be obtained and procedures and assessments performed once eligibility has been determined:

- Blood will be collected for:
  - COR (PAXgene)
  - CD4 count
  - HIV viral load (only in participants not yet established on ART)
  - IGRA (QFT)
  - Serum for proteomics
  - Plasma for viral molecular assays
- Urine will be collected for LAM assay and metabolomics
- TB symptom screen
- Two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture; two aliquots of unprocessed sputum will be stored for additional MTB diagnostic tests at the end of study (potentially including, but not limited to Xpert MTB/RIF, MGIT culture, and line probe assay).

Review eligibility to start ART and/or IPT and refer to health services if indicated

CONTACT 2: DAY 28 (+/- 3)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- TB symptom screen
- If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture (field or study clinic visit)

CONTACT 3: DAY 56 (+/- 3)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- TB symptom screen
- If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture (field or study clinic visit)

All participants will be given an appointment and contact details confirmed for the next study visit (Month 3)

VISIT 4: DAY 84 (+/- 3)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- Measurement of weight
- Vital signs
- Targeted physical examination
- Blood will be collected for:
  - COR (Paxgene)
  - Serum for proteomics
  - And if recently started on ART
  - CD4 count (only in participants starting ART)
  - HIV viral load (only in participants starting ART)
- Urine will be collected for LAM assay and metabolomics
- Review of concomitant medications (including ART and IPT)
  - TB symptom screen
  - If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture

All participants will be given an appointment and contact details confirmed for the next study visit (Month 6)

VISIT 5: DAY 180 (+/- 7)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- Measurement of weight
- Vital signs
- Targeted physical examination
- TB symptom screen questionnaire
- If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture

CONTACT 6: DAY 270 (+/- 7)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- TB symptom screen
- If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture (field or study clinic visit)

All participants will be given an appointment and contact details confirmed for the next study visit (Month 12)

VISIT 7: DAY 365 (+/- 7)

All participants will undergo the following assessments and procedures:

- Review of concomitant medications (ART and IPT)
- Measurement of weight
- Vital signs
- Targeted physical examination
- TB symptom screen
- If indicated by one or more TB symptoms, two sputum samples will be collected, one for Xpert MTB/RIF and one for MGIT culture

All participants will be given an appointment and contact details confirmed for the next study visit (Month 15)

VISIT 9 (END-OF-STUDY VISIT): DAY 449 (+/- 7)
All participants will return to the study site for a final end-of-study evaluation. Every attempt will be made to ensure that participants are not lost to follow-up prior to this visit and the study team will attempt to trace participants who fail to present for this visit.

All participants will undergo the following assessments and procedures:
Review of concomitant medications (ART and IPT)
Measurement of weight
Vital signs
Targeted physical examination
TB symptom screen
Two sputum samples will be collected, regardless of presence or absence of symptoms, one for Xpert MTB/RIF and one for MGIT culture.
Contact details will be confirmed so that sputum results can be provided, with written TB clinic referral if necessary

EARLY WITHDRAWAL FROM THE STUDY
Participants will be advised that they are free to withdraw from the study at any time, for any reason, without prejudice. Every reasonable effort should be made by the study staff to keep participants in the study. Participants must, however, be withdrawn from the study for any of the following reasons

- At the request of the participant (withdrawal of informed consent), irrespective of the reason
- At the discretion of the investigator if he or she believes that continuation in the study would be detrimental to the participant's well-being

For participants who are lost to follow-up, study personnel should make at least three documented attempts to contact the participant. Determination of loss to follow-up will be made at end of study, if and when a participant does not return to follow-up after a missed visit/s. Unless lost to follow-up, withdrawn participants will attend an early discontinuation visit (procedures and assessments conducted as for End of Study Visit). Reason for withdrawal from the study will be documented to ascertain the cause of early termination.

Participants who are diagnosed with TB and referred for curative treatment should attend an End of Study Visit at the time of their next scheduled study contact/visit to confirm that the participant has accessed the appropriate care, after which an End of Study Visit form should be completed.

STUDY ASSESSMENTS

SCREENING DATA
Screening data will be collected prior to enrolment.

AGE VERIFICATION
Age of potential participants at date of screening will be verified by identification document, passport, or driver's license, copy of which to be kept in the participant file and participant identity checked at each visit.

SCREENING MEDICAL HISTORY
Potential participants will provide a targeted medical history, with a focus on socio-demographic data (gender, ethnicity, education level, & household economic indicators), risk factors for TB (including TB contact and smoking history), current and past medical and surgical conditions (including recent febrile episodes), and concomitant medications (including ART and IPT).

WEIGHT AND HEIGHT
Height in centimetres (cm) and body weight (to the nearest 0.1 kg in indoor clothing, but without shoes) will be measured at screening. Weight only will be repeated at each subsequent study visit. Body Mass Index (BMI) will be calculated using the formula:
BMI = weight (kg) / height $(m)^2$.

URINE PREGNANCY TEST
To confirm eligibility at screening, a urine β-hCG test will be performed for all females of child-bearing potential.

HIV RAPID TEST
Following appropriate pre-test counselling, evaluation for HIV seropositivity will be performed by rapid test, and, if positive, will be confirmed by a second rapid test as per site protocol. Discordant rapid test results will be confirmed by laboratory HIV ELISA. Appropriate post-test counselling will be made available by the investigator, and participants will be referred for ongoing HIV management in the event of a positive test. A documented positive HIV test that has been performed for CORTIS-01 screening will not need to be repeated if performed within 28 days of CORTIS-HR screening.

VITAL SIGNS & PHYSICAL EXAMINATION
A targeted physical examination will be performed at enrolment, including recording of vital signs (temperature, pulse rate, blood pressure); general examination including lymph nodes and skin; detailed examination of the respiratory system; and other systems as indicated on the basis of medical history or other physical findings. At all other visits, vital signs and an abbreviated physical examination will be performed if directed by presence of symptoms or occurrence of adverse events. Physical examination data will be recorded in the source documentation at the trial site.

VERIFICATION OF ELIGIBILITY
The investigator will review the screening medical history and tests and confirm eligibility.

COR ASSAY
Whole blood RNA will be collected in PAXgene tubes from all eligible persons at enrolment and repeated at Month 3 (1 tube of 2.5mL blood, stored at room temperature for 2-18 hours, then frozen at -20°C) and shipped frozen to the South African Tuberculosis Vaccine Initiative (SATVI) Cape Town Laboratory on a weekly basis. PAXgene tubes will be processed in batches of 94 (plus 2 internal assay controls), allowing for handling by standard assay plate format. cDNA synthesis and pre-amplification will be automated, and up to 6 Fluidigm chips will be run weekly. In order to extract high quality RNA and complete the first amplification steps of the COR assay using whole blood collected in PAXgene tubes in a high-throughput, standardized, reproducible and cost effective manner, a fully automated procedure using the TECAN EVO Freedom robotic platform will perform RNA extraction, cDNA-synthesis and pre-amplification steps on up to 465 samples per week. Participants will be evaluated for risk of TB disease using the PSVM.1 model on the BioMark HD Fluidigm multiplex qRT-PCR machine. COR analysis will be conducted by a locked-down R script, which includes Quality Control filters. COR data will not be used to guide clinical care.

IGRA
A 4mL whole blood sample will be collected for IGRA on Day 0 in all enrolled participants (4mL total, incubated at 37°C for 16-24 hours, then spun and supernatants stored at -80°C). The Day 0 IGRA result will be made available to the health services to guide clinical management.

SERUM PROTEOMICS
A 5mL clotted blood sample will be collected on Day 0 in all enrolled participants and repeated at Month 3. Serum will be spun and stored at -80°C for future proteomic analysis. Serum may be stored for up to 10 years for proteomic analysis before being destroyed

PLASMA MOLECULAR ASSAYS
A 6mL EDTA blood sample will be collected on Day 0 in all enrolled participants. Plasma will be stored at -80°C for viral assays, including molecular tests for HIV and other viral pathogens. Plasma may be stored for up to 10 years for molecular assays before being destroyed.

CD4 CELL COUNT
A 4mL EDTA blood sample will be collected for CD4 cell count on Day 0 in all enrolled participants and repeated at Month 3 only in those participants referred for ART. CD4 cell count results at Day 0 and Month 3 will be made available to health service providers to guide clinical care.

HIV VIRAL LOAD
A 6mL EDTA blood sample will be collected for HIV viral load on Day 0 only in those participants not yet established on ART and repeated at Month 3 after ART has been started. HIV viral load test results at Day 0 and Month 3 will be made available to health service providers to guide clinical care.

URINE LIPOARABINOMANNAN (LAM) AND METABOLOMICS
Urine (20mL) will be collected for urinary LAM assay and two aliquots of urine will be stored for specific protein and metabolomic studies. Urine may be stored for up to 10 years for analysis before being destroyed. Results of urinary LAM assays will not be used to guide clinical care in study participants, who will be non-hospitalized community volunteers.

TB EVALUATIONS
Investigation for TB disease will be conducted in all participants at Visit 1 (Day 0); in symptomatic participants during follow-up; and in all participants at the end of study visit.

TB SYMPTOM SCREENING
All participants will be asked about any new household or other close contact with a recently diagnosed TB patient, and about symptoms consistent with TB disease, at all study contacts and visits from Day 0 through end of study. Symptoms will include loss of weight or persistent unexplained cough, chest pain, fever, or night sweats for longer than two weeks; or any haemoptysis.

TB INVESTIGATIONS
Two sputum samples will be collected from all participants at Visit 1 (Day 0), regardless of presence or absence of symptoms, for Xpert MTB/RIF testing and MGIT culture. An aliquot of unprocessed sputum from each sample will be stored for additional MTB diagnostic tests at the end of the trial, potentially including, but not limited to Xpert MTB/RIF, MGIT culture, and line probe assay. Thereafter, TB investigation will be symptom-triggered throughout follow-up. A participant with any one or more symptoms consistent with TB disease will be asked to provide two sputum samples, one for Xpert MTB/RIF and one for MGIT culture. An additional MGIT culture will be requested if only one sample is positive for either test. If a participant cannot produce a sputum sample spontaneously, this will be recorded in the source notes and no sample will be sent to the laboratory. Participants who are unproductive of sputum will be deemed Xpert MTB/RIF and MGIT culture negative. However, if clinical suspicion of TB disease persists in any participant, additional samples may be obtained, including induced sputum, or samples from other sites in the case of suspected extra-pulmonary disease. All participants will provide two sputum samples for Xpert MTB/RIF testing and MGIT culture at the end of study visit.

STATISTICAL CONSIDERATIONS

We have estimated the expected number of prevalent and incident TB cases in HIV infected participants in CORTIS-HR, based on data from previous and ongoing studies in South Africa. In order to estimate the expected number of TB cases and a probable range, we used stochastic simulations of the CORTIS-HR study under a range of assumptions. The simulation assumed a 10% HIV prevalence rate, ranging between 5% - 25%, which would allow 860 HIV infected participants to be enrolled from approximately 10,000 persons screened for the CORTIS trial and the CORTIS-HR study.

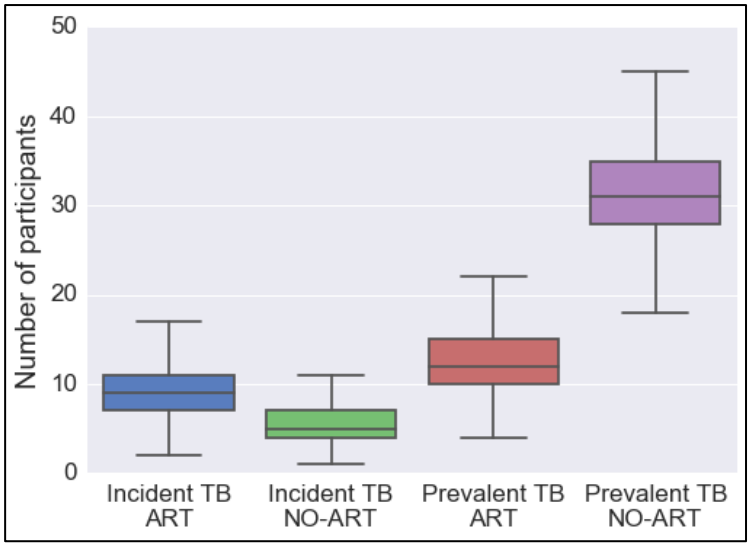

Figure 1: Expected number of incident and prevalent TB endpoints among HIV infected participants. Estimates are based on stochastic simulations.

We estimate that 75% of these participants will be receiving chronic ART and the remainder will begin ART. Based on studies conducted in South Africa, the rate of prevalent TB will be considerably higher among participants newly starting ART (15%) [21, 22], compared to those receiving chronic ART (2%) [18-22]. We also assume that 15% of participants on chronic ART will also be on IPT, thus further reducing the expected level of prevalent TB by 40%. Similarly, we expect the annual rate of incident TB will be higher among participants newly starting ART (4%) [21, 22], than among those already established on chronic ART (2%) [20]. In both these groups, we expect up to 70% to begin IPT upon enrollment, which will reduce expected levels of incident TB by 40%.

Based on these pre-specified parameter distributions, we expect that of the 215 newly diagnosed HIV infected participants yet to start ART, we will identify 31 prevalent TB cases (95% CI 22, 42) at screening; and an additional 5 (95% CI 2, 10) participants who will develop active TB disease over 15 months of follow-up. We expect that of the 645 HIV infected participants already on chronic ART, we will identify 12 prevalent TB cases (95% CI 6, 20) at screening; and an additional 9 (95% CI 4, 15) incident TB cases over 15 months of follow-up. Thus, we expect to measure the COR in a total of 58 (95% CI 44, 73) cumulative endpoint TB cases (43 prevalent and 14 incident TB cases) and 802 (95% CI 787, 816) controls.

We will evaluate the relative-risk of endpoint-defined TB over 15 months, $RR_{COR}(15)$, in COR+ versus COR- participants using a cumulative incidence based approach. The primary analysis will evaluate $RR_{COR}(15)$ on endpoints using the two-sample endpoint definition and according to the formula:

$$RR_{COR}(15) = \frac{H_A}{H_B}$$

where H_X is the cumulative incidence estimated for each group using the Product-Limit estimator of Nelson-Aalen (Aalen, O.O., “Non-parametric inference for a family of counting processes”, Annals of Statistics, 1978). A point-estimate for $RR_{COR}(15)$ will be presented with 90% confidence intervals and a p-value for the null-hypothesis H_0: $RR_{COR}(15) <= 2$. Descriptive plots will include Kaplan-Meier estimators with 95% confidence intervals for each group. In addition we will present time-dependent estimates of sensitivity, specificity, positive predictive value (PPV) and number needed to treat (NNT) using the methods of Heagerty et al. (2000, Biometrics, “Time-dependent ROC Curves for Censored Survival Data and a Diagnostic Marker”) as these will offer important insights into the performance and application of the COR in future strategies.

Our preliminary data suggests that the COR will perform similarly in HIV infected participants, with a possible 10% reduction in sensitivity to 77%, compared to HIV uninfected persons. Based on these characteristics, and the expected total number of cumulative TB disease endpoints (prevalent and incident), we estimate that the Relative Risk (RR) of a COR+ HIV infected participant for cumulative TB disease, relative to a COR- HIV infected participant, will be approximately 19 (95% CI 11-34). **See Figure 1.** Corresponding estimates of precision for RR for prevalent cases only are 95% CI 10-37; and for incident cases only are 95% CI 6-65.

We will perform an interim analysis of COR prevalence and diagnostic performance for prevalent TB cases only, in order to inform a Stop/Go decision for continuation of study follow-up beyond 3 months in all subjects to evaluate COR prognostic performance. In subjects who continue follow-up for incident TB disease, we will perform a sensitivity analysis of month 3 COR prognostic performance to evaluate the impact of IPT and/or ART on COR score and TB risk.

MODELLING

Data from the CORTIS-HR study on the performance of the COR in HIV-infected individuals will be used to refine our preliminary models and to predict the population-level impact of COR 'screen and treat' strategies in HIV infected populations. The model will be used to explore the impact under alternative scenarios of ART rollout and uptake of IPT; and alternative assumptions about the efficacy of COR-targeted 3HP regimens in HIV infected individuals. Models will make use of screening data (including age of trial participants, prevalence of undiagnosed TB), diagnostic performance of COR (for prevalent and incident TB), and effect of potential confounders including CD4 count, HIV viral load, ART and IPT on COR score, ideal threshold value, and performance.

DATA HANDLING AND QUALITY ASSURANCE

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for all participants under this protocol. This includes the maintenance of both source documentation and accurate electronic CRFs (eCRFs) for all participants who consent to participation in the study. Study data will be collected using a 21 CFR Part 11 compliant electronic system. Information recorded in the eCRFs will be supported by corresponding source documentation. All paper and electronic source documents pertaining to this study will be maintained by the investigators. eCRFs are considered confidential documents and will be handled and accessed accordingly. The Data Centre will provide the necessary training on the use of the specific eCRF system utilised during the study to ensure that data are captured accurately and appropriately. Each completed eCRF will be reviewed, signed, and dated by the investigator in a timely manner.

MONITORING THE STUDY

Clinical sites will be monitored by a clinical research associate (CRA) to ensure compliance with the protocol, GCP and applicable regulations and guidelines. The CRA(s) will conduct site visits and will be responsible for ensuring that the study protocol is adhered to. The assigned CRA(s) will visit the investigator and clinical site at periodic intervals and maintain periodic communication. The CRA(s) will maintain current personal knowledge of the study through observation, review of trial records and source documentation, and discussion of the conduct of the study with the investigator and staff. While on site, the CRA(s) will review all study, regulatory and ethics documents, compare entries in the CRF system with the source documents, and review endpoint laboratory sample storage. The CRA(s) will ask for clarification and/or correction of any noted inconsistencies. Any necessary corrections will be made in such a way that the original entry, the date of the correction and the identity of the person making the correction is accessible. By signing the protocol, the Investigator agrees to meet with the CRA(s) during clinical site visits, to ensure that site staff are available to the CRA(s) as needed, to provide the CRA(s) access to all study documentation and agrees to assist the monitors in their activities, if requested.

DATABASE MANAGEMENT AND QUALITY CONTROL

Data management, including the development and management of a database, will be performed in accordance with regulatory requirements by the Triclinium Clinical Development (TCD) Data Centre. Triclinium will review the eCRF data for completeness and accuracy. A formal querying process will be followed whereby the data management group will request the site personnel to clarify any apparent erroneous entries or inconsistencies and will request additional information from the site as required. Medical history/current medical conditions will be coded using the Medical dictionary for regulatory activities (MedDRA, version 18.1 or higher) terminology. Concomitant medications will be coded using the MIMS classification.

After all data have been captured and reviewed in the eCRF, all queries have been resolved with the site and any protocol non-compliances that were identified during the data management processes have been confirmed by the site, the database will be declared to be complete and accurate. It will then be locked and the COR status of participants in the Observation Arm will be unblinded and made available for data analysis. Any changes to the database after that time may only be made by the data manager, in consultation with the sponsor and in accordance with documented database unlock and relock procedures. Data management procedures will be described in detail in the Data Management Plan which will be documented and approved prior to study start.

INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit study-related monitoring, audits, IEC review, and regulatory inspection(s) by providing direct access to all study records. The confidentiality of records that can identify participants will be protected, respecting the privacy and confidentiality rules in accordance with regulatory requirements. The investigator must promptly

notify the sponsor of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the sponsor.

RETENTION OF RECORDS

Essential documents should be retained for not less than 10 years after study completion. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

ETHICAL CONSIDERATIONS

REGULATORY AND ETHICAL COMPLIANCE

This study will be conducted according to the ethical principles set forth in the Declaration of Helsinki (Fortaleza, Brazil 2013),[21] ICH-GCP,[22] European Directive 2001/20/EC,[23] US Code of Federal Regulations Title 21,[24] South African Good Clinical Practice Guidelines,[25] and other local regulatory requirements.

INFORMED CONSENT PROCEDURES

Eligible participants may only be included in the study after providing written, IEC-approved informed consent in the language of their choice. Illiterate participants require an impartial witness to be present for the informed consent process, and to sign and date that all information in the informed consent form was shared with participant, and the participant must sign by means of a thumbprint. Informed consent must be obtained before conducting any study-specific procedures (i.e. any of the procedures described in the protocol). The timing and process of obtaining informed consent must be documented in the participant source documents. The investigator must provide a copy of the signed informed consent document to the participant. The original form must be maintained together with the participant's source documents at the site.

RESPONSIBILITIES OF THE INVESTIGATOR AND IEC

The protocol and informed consent form must be reviewed and approved by a properly constituted IEC before commencement of the study at any site. By signing this study protocol, the principal investigator agrees to conduct this study in accordance with all laws, regulations and guidelines of the pertinent regulatory authorities, including the 2013 Version of the Declaration of Helsinki. While delegation of certain aspects of the study to sub-investigators and study coordinators is appropriate, the principal investigator will remain personally accountable for overseeing the study and for ensuring compliance with the protocol and all applicable regulations and guidelines.

The PI must ensure that all persons who have been delegated study-related responsibilities are adequately qualified and informed about the protocol and their specific duties within the context of the study. The principal investigator is responsible for providing the sponsor with documentation of the qualifications, GCP training, and research experience of site staff as required by the sponsor and the relevant governing authorities. In addition to this, the principal investigator is responsible for maintaining a list of all personnel who have been trained and to whom study-related responsibilities have been delegated, including the specific study-related duties concerned. Proof of training on the protocol and protocol-specific procedures must be kept on file to allow verification of training for delegated duties.

PROTOCOL ADHERENCE

A protocol non-conformance is an unintended and/or unanticipated departure from the procedures and/or processes approved by the sponsor, the IEC, and agreed to by the investigator. Investigators must agree to apply due diligence to avoid protocol non-conformances and must document and explain any such events. Protocol non-conformances will also be documented by the CRA throughout the course of monitoring visits. The investigator will be notified of non-conformances in writing by the CRA. The IEC must be notified of major protocol non-conformances in accordance with the IEC standard operating procedures.

AMENDMENTS TO THE PROTOCOL

Any change or addition to the protocol can only be made by means of a written protocol amendment that is approved by the sponsor and the IEC. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any participant included in this study, even if this action represents a deviation from the protocol. In such cases, the sponsor should be notified of this action immediately and the IEC at the study site should be informed within 10 working days of the action.

PARTICIPANT INJURY
The sponsor (University of Cape Town) will ensure that provisions are made for insurance or indemnity by a third party to cover the liability of the investigator and sponsor in relation to the study. In the event of any injury, suffering, deterioration in health or well-being resulting from a participant's participation in the study, the participant will receive appropriate compensation irrespective of their ability to prove fault on the part of the sponsor or anyone else connected with the study.

SAMPLE RETENTION
Biological samples may only be used for purposes related to this research. The samples will be stored until the study team has determined that specimens are no longer needed and the decision has been made that there are no samples to be re-assayed, up to a maximum period of 10 years. In addition, identifiable samples can be destroyed at any time at the request of the participant.

STUDY TERMINATION BY SPONSOR
The study may be terminated at the sponsor's discretion at any time for any reason. If the sponsor discovers conditions that warrant early termination of the study, the investigator will be notified by the sponsor or by its designee.

CLINICAL SITE CLOSURE
On termination of the study, all screening and ongoing study-related procedures conducted at the study site will be closed. The sponsor may terminate participation of the clinical site at any time. Examples of conditions that may warrant premature termination of a clinical site include, but are not limited to non-compliance with the protocol and/or applicable regulations and guidelines, or inadequate participant enrolment.

PUBLICATION OF THE STUDY PROTOCOL AND RESULTS
All information concerning the sponsor's operations, patent applications, formulae, and scientific data supplied by the sponsor to the investigator and not previously published, is considered confidential and remains the sole property of the sponsor. The complete participant CRFs also remain the property of the sponsor. The investigator agrees to use this information for purposes of the study execution only. The sponsor will post the key design elements of this protocol in a publicly accessible database such as clinicaltrials.gov. In addition, upon study completion the results of this study will be submitted for publication and/or posted in a publicly accessible database. Publications or other public presentations of the data resulting from this study will be planned and prepared by a Writing Committee chaired by the PI and including the study investigators.

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