The Regional Prospective Observational Research for Tuberculosis (RePORT)
India Phase II Common Protocol

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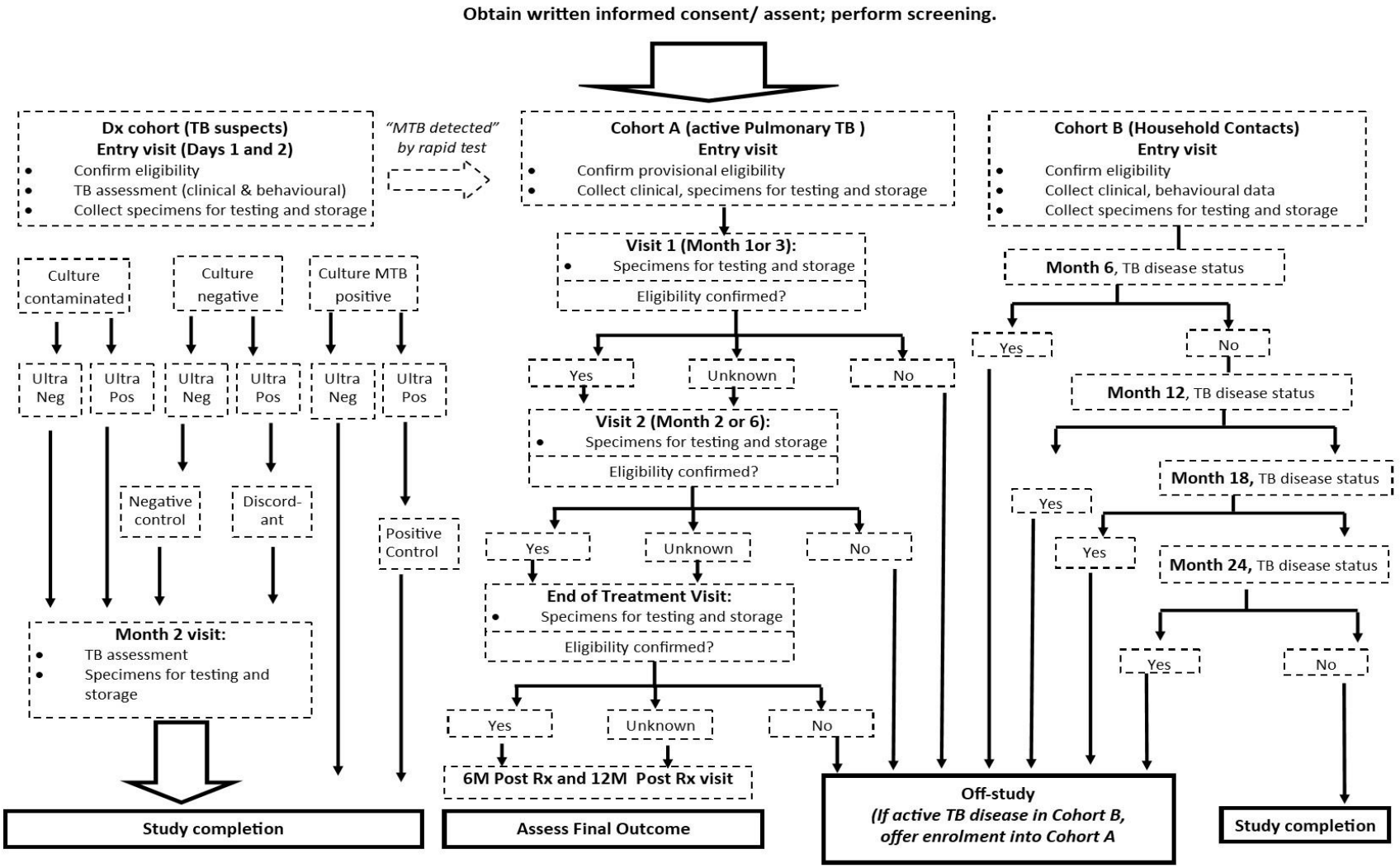
LIST OF ABBREVIATIONS AND ACRONYMS

AFB	Acid-Fast Bacilli
ADCC	Antibody-dependent cell-mediated cytotoxicity
AUDIT	Alcohol Use Disorder Identification Test
BJGMC	Byramjee Jeejeebhoy Government Medical College
BMMRC	Bhagwan Mahavir Medical Research Centre
BPHRC	Blue Peter Public Health & Research Centre
CAT	COPD Assessment Test
CBC	Complete Blood Count
CD	Cluster of Differentiation
CHRD-SAS	Centre for Health Research and Development-Society for Applied Studies
CMC	Christian Medical College
COVID-19	Coronavirus Disease of 2019
CP	Common Protocol
CSF	Cerebrospinal Fluid
CRF	Case Report Form
CRS	Clinical Research Site
CRU	Cohort Research Unit
CTB2	Consortium for Tuberculosis Biomarkers
CTU	Clinical Trials Unit
CXR	Chest X-Ray
DAIDS	(United States) Division of AIDS
DBT	(India) Department of Biotechnology
DLco	Diffusing capacity of the lungs for carbon monoxide
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
DR	Drug-Resistant
DS	Drug-Susceptible
DST	Drug Susceptibility Testing
DX	Diagnostic
EMB	Ethambutol
EPTB	Extra-pulmonary Tubcerculosis
GL	Gastric Lavage
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c (Glycated Hemoglobin)
HHC	Household Contact
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HR-CT	High-resolution computed tomography

ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMR	Indian Council of Medical Research
IEC	Independent Ethics Committee
IGRA	Interferon-Gamma Release Assay
IL	Interleukin
IFN	Interferon
INH	Isoniazid
IRB	Institutional Review Board
IS	Induced Sputum
IS	Infection Susceptible
IR	Infection Resistant
JIPMER	Jawaharlal Institute of Postgraduate Medical Education & Research
Kg	kilogram
LAM	Lipoarabinomannan
Lbs	pounds
LC	Large colonies
LFT	Liver Function Tests
LJ	Löwenstein–Jensen
LTBI	Latent Tuberculosis Infection
MDR	Multidrug-Resistant
MIC	minimum inhibitory concentration
MGIT	Mycobateria Growth Indicator Tube
MMRC	Modified Medical Research Council
MOP	Manual of Operating Procedures
mRNA	Messenger Ribonucleic Acid
MFX	Moxifloxacin
Mtb	Mycobacterium Tuberculosis
Prof. MVDRC	Professor M. Viswanathan Diabetes Research Centre
NACO	National AIDS Control Organization
NALC-NaOH	N-acetyl-l-cysteine-sodium hydroxide
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
NIRT	National Institute for Research in Tuberculosis
NPA	Nasopharyngeal aspirate
OAR	(United States) Office of AIDS Research
PBMC	Peripheral Blood Mononuclear Cell
PET-CT	Positron emission tomography–computed tomography
PI	Principal Investigator
PHQ	Patient Health Questionnaire
PID	Participant Identification Number

PGE2	Prostaglaindin –E2
POC	Point-of-Care
PPD	Purified Protein Derivative
PK/PD	Pharmacokinetic/pharmacodynamic
PTB	Pulmonary Tuberculosis
PY	Person-Year
PZA	Pyrazinamide
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
RePORT	Regional Prospective Observational Research for Tuberculosis
RIF	Rifampicin
RNA	Ribonucleic Acid
NTEP	National Tuberculosis Elimination Programme
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SGRQ	Saint Georges Respiratory Questionnaire
SOP	Standard Operating Procedure
ss-DNA	Single-stranded DNA
SCV	Small Colony Variant
TB	Tuberculosis
TBM	Tuberculous meningitis
TLC	Total Lung Capacity
TST	Tuberculin Skin Test
TX	Treatment
TX F/R/W	Treatment Failure/Recurrence/Withdrawal Evaluation
UPT	Urine Pregnancy Test
VAP	Vaccine Action Program
WHO	World Health Organization
XDR	Extensively Drug-Resistant

FIGURE 1: PROTOCOL SCHEMA DIAGRAM



A. Overview

A.1 Background:

The Regional Prospective Observational Research in Tuberculosis (RePORT) India was jointly established in 2013 by the Government of India's Department of Biotechnology (DBT) and the U.S. NIH, under the Indo- US Vaccine Action Program and is now the largest of 6 NIH-supported TB research consortia.[1] RePORT India's mission is to: 1) Advance regional TB science in India; 2) Strengthen TB research capacity and infrastructure; 3) Foster research collaboration within and with India focused on research that can lead to clinically important biomarkers, vaccines, drugs, and diagnostics.

Phase 1 (2013-18) commenced with 6 Clinical Research Sites (CRSs) in Western and Southern India that were partnered with 5 U.S. academic institutions. Hinduja was subsequently added as the 7th Indian site. Initially, each site its own "Parent Protocol", establishing prospective cohorts of TB disease cases (Cohort A) and household contacts (HHCs) (Cohort B). Each Parent Protocol had distinct research topics, with Cohort A studies focused on TB treatment outcomes and Cohort B on HHCs' TB infection risk and progression to disease after exposure. In 2017, RePORT India launched Phase I of the Common Protocol (CP) with standardized data elements and harmonized procedures for enrollment of TB disease cases (Cohort A) and HHCs (Cohort B) across 6 of the CRSs. The primary objective of the Phase I CP was to provide data and specimens to Indian biomarker researchers and collaborators to better understand: (1) prognosis of TB disease; and (2) pathogenesis of progression from TB exposure to disease. A RePORT India Central Biorepository was established at the National Institute of Research in Tuberculosis (NIRT) in Chennai.

A.2 Purpose:

To collect and utilize data and specimens for tuberculosis (TB) research, leveraging the existing infrastructure, processes, and scientific partnerships established under the Regional Prospective Observational Research for Tuberculosis (RePORT**)** India consortium. The RePORT India Phase II Common Protocol includes five specific scientific aims.

Scientific Aims:

1. **Diagnostics:** Evaluate novel diagnostics and biomarkers of diverse states of mycobacterium tuberculosis (Mtb) infection.
2. **Cohort A:** Identify markers of treatment response:
    - **2.A**: Identify TB Treatment Response Biomarkers
    - **2.B:** Investigate Host-Related Mechanisms of Treatment Failure
    - **2.C**: Investigate Pathogen-related Mechanisms & Predictors of Recurrence
3. **Cohort A: Lung Injury & Impairment:** Identify markers of lung injury associated with unfavorable TB treatment outcomes

4. **Cohort B: Resistance to Infection:** Examine mechanisms of protection against TB in exposed persons.

    4.A: Examine Host Antimicrobial Pathways in Inducing their infection resistant (IR) Phenotype in HHC

    4.B: Test if IR & Plasma Differ in Modulating Macrophage-Mediated Restriction of Mtb Growth & Evaluate AB Repertoires of Plasma from the IR and infection susceptible (IS) Cohorts

5. **Cohort B: Progression to disease:** Identify immunologic markers of persons at highest risk of progress of latent TB infection to TB.

    5.A: Stored Samples: Validation of PREDICT29 in Progressors & Nonprogressors from RePORT Sites

    5.B: Immune & Hormone Studies in Freshly Collected Samples

In addition to this, we will assess cross-cutting epidemiologic and COVID-19 related aims. See Section C for a detailed description of each scientific aim and the associated studies.

A.3. Design, Sample Size, and Population

To accomplish these five scientific aims, the RePORT India Phase II Common Protocol will carry out two types of research activities:

1. Establishing three new prospective, observational cohorts for collection of specimens and associated data:
    i. Diagnostic (Dx) Cohort: 1500 participants with suspected TB
    ii. Cohort A: 588 new adult (≥18years) pulmonary TB patients
    iii. Cohort B: 767 household contacts (HHCs) of adult (≥15years) active PTB patients
2. Analyzing stored specimens and associated data collected under this Phase II Common Protocol as well as existing IRB/IEC-approved protocols:
    iv. RePORT India Phase I Common Protocol[1] – Cohort A (active TB patients) and Cohort B (HHCs)
    v. RePORT India Site-Specific Parent Protocols -- Cohort A (active TB patients) and Cohort B (HHCs)

Table 01 outlines target sample size for the new cohorts and additional follow-up:

Table 01: Phase II - New Participant Enrollment Targets												
	New TB suspects (Dx Cohort)				New Adult Pulmonary TB cases (Cohort A)*						Household Contacts (Cohort B)	
	Aim 1 Diagnostics				Aim 2 Treatment response			Aim 3 Lung Health			Aim 4-resister, Aim 5-Progressor	Aim 4-Resisters, Aim 1-Diagnostics
Sites	DX Total	Adult PTB	Ped PTB	EPTB	Cohort A Total**	2A Biomarkers	2B Nutrition-PK	Core	HR-CT	PET-CT	DS-TB HHCs**	XDR-TB HHCs **
BJGMC	340	115	150	75	120	100	40	100	50	Pilot	0	0
BMMRC	0	0	0	0	0	0	0	0	0	0	500	0
CMC	300	200	0	100	120	100	40	100	50	50	0	0
JIPMER	260	260	0	0	78	65	40	0	0	0	0	0
MVDRC	200	200	0	0	60	50	30	0	0	0	0	0
NEIGRIHMS	0	0	0	0	30	25	25	0	0	0	0	0
NIRT	325	150	100	75	90	75	40	75	0	0	0	0
PGI	0	0	0	0	60	50	25	50	50	50	0	0
Hinduja	75	75	0	0	30	25	25	0	0	0	0	294
Total	1500	1000	250	250	588	490	240	325	150	100	500	294

Dx=Diagnostic; Adult PTB=Adult Pulmonary TB suspects; Ped PTB=Pediatric Pulmonary TB suspects; EPTB=adult or pediatric extrapulmonary TB suspects; FU=follow-up; HRCT= High-resolution computed tomography (HRCT) PET/CT= Positron emission tomography/computed HHC=household contacts of microbiologically confirmed adult pulmonary TB patients; *Data and specimens from newly enrolled adult pulmonary TB patients (Cohort A) will support analyses for multiple studies proposed in Aims 2 and 3. The required/proposed samples size required for each substudy is shown at the bottom of each column. To maximize efficient use of RePORT resources, most sites will co-enroll these Cohort A participants into multiple substudies. **Enrollment targets assume 20% loss to follow-up rate for Cohort A and 10% loss to follow-up rate for Cohort B (based on Phase I rates).

A.4. Study Duration:

- Dx Cohort: 2 months
- Cohort A: Approximately 18 months for Drug-sensitive TB cases and approximately 36 months for drug-resistance cohorts (duration of TB treatment and 12 months post-treatment).
- Cohort B: 24 months

A.5. Specimen Collection:

The following specimens will be collected and stored in the RePORT India Central Biorepository:

Dx Cohort: Mtb isolate subculture, whole blood (PAXgene, plasma for antibody testing, and genetic analyses), sputum and/or induced sputum or nasopharyngeal aspirate or gastric lavage, saliva, oral swabs, extra-pulmonary tuberculosis (EPTB) specimens, urine and stool.

Cohort A: Mtb isolate subculture, whole blood [PAXgene, plasma (antibody testing), peripheral blood mononuclear cells (PBMC), genetic analyses, pharmacokinetics, serum/ plasma for micronutrient testing], urine, sputum and/or induced sputum, stool, and saliva.

Cohort B: Whole blood [PAXgene, plasma for storage and antibody testing, PBMCs (only if sample remain post local site-testing at BMMRC), genetic analyses], Interferon-Gamma Release Assay (IGRA) supernatant, sputum and Mtb isolate subculture at both BMMRC and Hinduja sites. Urine for storage at Hinduja site only.

The following tests will be performed at the Clinical Research Site (CRS) local laboratories:

Dx cohort: Human immunodeficiency virus (HIV) test, cluster of differentiation 4 (CD4) count (if HIV-infected), complete blood count (CBC) and lymphocyte count, hemoglobin A1c (HbA1c) for adults (>18years) and Urine pregnancy test (UPT) if indicated. Samples may include any of the following: sputum or induced sputum or nasopharyngeal aspirate or gastric lavage or EPTB specimen for GeneXpert Ultra, smear/ XpertMTB/RIF, histopathology, culture, and drug susceptibility testing (DST).

Cohort A: UPT (if indicated), HIV test, CD4 counts (if HIV-infected), CBC and lymphocyte count, HbA1c for adults (>18years), liver function tests (LFT), albumin, creatinine, fasting lipid profile, sputum smear, culture and DST.

Cohort B: UPT (if indicated), HIV test, CD4 count (if HIV-infected), CBC and lymphocyte count, HbA1c for adults (>18years), Sputum or nasopharyngeal aspirate or gastric lavage for smear, Xpert testing (Ultra or MTB RIF), culture, and DST, IGRA (Quantiferon Gold In-Tube 4th generation) testing at both BMMRC and Hinduja. C-Tb skin test (TST replacement), Translational analysis of RNA from PAXgene tube using NanoString and Cephid 3-gene signature cartridges, stored mask for index aerosol testing and PET-CT testing at Hinduja only. IGRA (in-house assay), cell cultures and flow cytometry for immune markers using PBMCs and hormone studies using serum samples

A.6 Participating Clinical Research Sites: Participating CRSs are located in PGI, Chandigarh; NIRT and MVDRC at Chennai; BMMRC, Hyderabad; Hinduja, Mumbai; JIPMER, Puducherry; BJGMC, Pune; NEIGRIGHMS, Shillong; CMC, Vellore and may be expanded to other locations.

A.7 Coordination and Monitoring

The India-based Coordinating Hub will be housed at **Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) in Puducherry**. The U.S.-based Coordinating Hub will be housed at **Johns Hopkins University in Baltimore, Maryland**. The Indo-US Coordinating Hub will conduct research program management, data management, administrative coordination, protocol coordination/launch, and lab management activities for the consortium. Pharmaceutical Product Development, LLC (PPD) will conduct clinical and lab support activities for the consortium.

A.8 Central Biorepository

This protocol has been designed to provide a uniform schedule and methodology for collecting clinical data and specimens from participants in each of the three new cohorts so that they can be placed in biostorage for future studies. Samples collected under this

protocol will be curated, stored, and managed for life span of the biorepository after study completion at the RePORT India Central Biorepository. **The National Institute for Research in Tuberculosis (NIRT) in Chennai** where Phase I Common Protocol samples are currently stored will continue to serve as the Central Biorepository for the Phase II Common Protocol. NIRT will provide centralized training and laboratory support to the CRSs as needed.

To use biological samples for studies not described in this protocol, investigators must submit a concept sheet to the RePORT India Executive Committee for peer-review. See RePORT India Publication and Concept Sheet Process for details. Site-Specific Parent Protocol samples are stored at individual CRS biorepositories. To use biological samples for studies not described in this protocol, investigators must seek approval from the Site-Specific Parent Protocol PIs.

All participant samples will only be destroyed with written permission from the funding organizations and according to local regulatory guidelines. However, for participants who withdrew consent during the study for long-term storage and possible research testing of their biological samples, and have requested sample destruction at the time of consent withdrawal, samples will be destroyed with prior intimation to site study support team, lab and data management teams and will follow the sample destruction SOP. Samples from those who do not experience the outcome of interest will be available to serve as controls for those who do experience the outcomes.

A.7 Data Collection and Management:

A Data Management Center will be based in the Coordinating Hub at JIPMER in Puducherry, India and will provide centralized data management, analysis, and training to the CRSs.

The RePORT India EC Chair and RePORT India Program Manager (PM) based at JIPMER will oversee the RePORT Phase II Data Manager in coordinating activities across sites. Overall management of databases, quality management, generation of queries, and generation of data for interim data reports, Executive Committee (EC) meetings, study progress reports, and finalization reports will be done data coordinating hub. REDCap designer tool will be used to create customized Case Report Forms (CRFs) providing open-text, bubble, check-box, fill-in box, options for embedded data fields with the ability to configure logic and value range checks.

As a part of RePORT Phase I studies, humongous data from patients with active tuberculosis and their healthy household contacts were collected and stored. For active TB, data is available from baseline until end of treatment including TB recurrence and failure visits and for HHC, baseline until 12 months. Baseline and follow-up data of site protocols from about 3220 Cohort A participants and 3766 Cohort B participants of Phase I sites, have been checked for quality through internal and external query systems, harmonized and stored. Similarly, data collected through common protocol from all the participating sites (Cohort A= 724 and cohort B=898) were also harmonized.

The scientific priorities of Phase II are tailored based on the data and samples available from Phase I. Data hub will enable smooth transition of data from phase I to phase II and also from manual CRFs to REDCap format which will augment scientific publications and fruitful collaborations.
Refer the SOP for detailed information on data management and the Data Management Center's roles and responsibilities.

A.8 Ethical Conduct

All participating CRSs must be in compliance with Indian and U.S regulations applicable to research involving human subjects, and in accordance with the International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and ICMR guidelines. Should Indian and U.S regulations and guidelines differ, the more restrictive regulations and guidelines will apply.

This protocol, the informed consent forms (ICFs) and assent forms, and any subsequent modifications will be reviewed and approved by the IRB/IEC responsible for oversight of the protocol, including any national IRB/IEC, as required. Subsequent to the initial review and approval, the protocol will be reviewed in accordance with the IRB/IEC requirements. See the MOP for further details on the ethical conduct of the study.

A.8.1 Participant Information and Informed Consent

The study team will approach participants attending the public health, chest clinics, or other departments/facilities approved for study recruitment presenting with signs and symptoms of TB for both diagnostic (Dx) and active TB cohorts (Cohort A) screening. Household contacts of adult ($\geq$ 15years) pulmonary TB cases (Cohort B) will be approached through their index TB cases from their household, attending the approved recruitment centers or health care facilities. These household contacts will be asked to visit the nearest health care center of the household or the study recruitment center whichever is convenient for the participant. Each of the study participant in all three cohorts will be screened for eligibility at the initial visit, and eligible participants will be approached by the study counselor to provide a detailed overview of the study in the participant's preferred local language, a copy of the patient information sheet in the participant's local language will be offered in advance.

Participants who voluntarily agree to take part in any of the three cohorts (diagnostic, active TB and household contacts) in the study will be required to sign a Phase II Common Protocol informed consent form (ICF) before they can be screened for eligibility. Assent forms will be signed by children, as required by the local IRB/IEC, accompanied by an ICF signed by their parents/legal guardians (see Appendices A, C and D for sample ICFs). A copy of the ICF and assent will be offered for all participants. If a participant is illiterate or cannot read any of the languages that are used in the sites' consent forms the study counselor/nurse will read

the consent aloud in the presence of a witness. The participant will be given the option of choosing their witness. The witness will be given a copy of the consent to follow the text as it is read. The witness will be asked to sign the consent form to affirm that the written consent has been completely read to the participant. Participants will be asked to place their left thumbprint on the signature line of the consent form in the presence of the witness.

A.8.3 Confidentiality

All records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. The data will be entered into a secured database at each of the recruitment sites and will be stored in a central data storage centre administered by the RePORT India coordination hub at JIPMER in India. The RedCap database for RePORT India Phase II will be hosted on a server at the consortium's Data Management Centre at JIPMER in Pondicherry, India and will be responsible for managing the database for the consortium. RePORT India PIs and specific collaborators will have access to these data. Data may be reviewed by representatives of the IRB/IEC, funding organizations or their representatives, and by others tasked with duties of monitoring and quality assurance. Research and clinical information relating to participants may be shared with other researchers through lectures or publications, but the participants will not be identified by name. All specimens will be labelled with a PID with no identifying information. All ICFs, assent forms, and any other documents with participants' names or addresses will be stored separately and in secure facilities.

Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study. Specimens at the Central Biorepository will be labelled with a coded, unique identifier that will not contain identifying information. See the MOP for further details on participant confidentiality.

B. Description of Phase II Cohorts

B.1 Diagnostic (Dx) cohort

B.1.1 Aims

Aim 1: Evaluate Novel Diagnostics & Biomarkers of Diverse States of Mtb Infection.

B.1.2 Methodology

Two types of studies will be conducted:

1) **Exploratory Studies:** As shown in Table 02, we will utilize archived specimens for the initial evaluation of TB diagnostics platforms. 25-100 blinded specimens will be provided from culture-confirmed TB patients and 25-100 from suspected TB patients that are culture negative from Cohort A participants and/or uninfected HHC controls from Cohort B.

2) **Suspected TB Feasibility Studies:** We propose establishment of a new prospective cohort study of suspected cases of TB. We propose to conduct initial Exploratory Studies for 3 of the 4 prioritized novel diagnostic platforms (RNA gene expression-NanoString, plasma cytokines, and ultrasensitive urine LAM).

B.1.3 Anticipated Results & Impact

The diagnostic aim is likely to have direct impact on TB control in India through the demonstration that new tools are accurate and cost-effective. We will provide an unbiased comprehensive assessment of a promising diagnostic developed in India, M6 aptamer, with the potential to be applied at POC. It is highly likely that the RePORT India Consortium will demonstrate that host-directed diagnostics are of value for pediatric and extrapulmonary TB. We also will determine the feasibility of triage approaches and the adjunctive value of urine diagnostics. Finally, we may find that geographic differences in the host and prevalent organisms will require that host-directed diagnostics be site-specific or that comorbidities impact diagnostic accuracy. This will be particularly relevant to decisions to implement new diagnostics in India.

B.1.4. Table 02. Aim 1 studies

		RePORT India Phase II Studies				Other RePORT-related Studies	
Short Title		M6 Aptamer for TB Dx	TB NanoString for Dx	Plasma Cytokines for TB Dx	Urine LAM for TB Dx	RICC Pediatric Transcriptomic	Urinary Biomarker Assay and TB
RePORT Study ID		IND_AIM1_8	IND_AIM1_9	IND_AIM1_10	IND_AIM1_11	MULTI_RR_32	IND_RR_33
Laboratory		JIPMER, AIIMS (Delhi)	theraCUES	Prof.MVDRC, NIRT-ICER	Rutgers	BJGMC, NIRT, UCT, Imperial College (UK)	BJGMC, George Mason
Sample Types		Sputum, EPTB	PAXgene	Plasma	Urine	PAXgene	Urine
Gold Standard Assay		Culture Xpert UTRA	same	same	same	MGIT or LJ Culture	MGIT or LJ Culture
TB Infection State	Adult PTB	X	X	X	X		X (incl HIV)
	Adult EPTB		X	X			
	Pediatric TB		X	X		X	X
	LTBI vs Active TB	X	X	X	X		X
	Subclinical TB		X	X			
	DR-TB		X	X			
	Incident TBI		X	X			
	Uninfected controls		X	X	X	X	X
Archived Samples	Cohort A Participants	0	0	0	0	248	180
	Cohort B Participants	100	100	100	25		80
New Diagnostic Cohort	Adult PTB Suspects	1000	1000	1000	100		
	Adult EPTB Suspects	250	250	250	0		
	XDR-TB contacts		267				
	Pediatric PTB Suspects	250	250	250	0		
	Pediatric EPTB Suspects						

B.1.5.1 Schedule of Events for Dx Cohort:

Table 03a: Schedule of Events for Dx Cohort

Activities / **Visit**	**Baseline – Day 1**	**Baseline– Day 2**	**Month 2 (discordants, negative controls or status unknown)**
Informed consent	X		
Eligibility assessment	X		
Demographics	X		
Medical history, clinical questionnaires (symptoms, medications, MMRC, Karnofsky)	X		X
COVID-19 Questionnaire (screening, impact on TB and testing history)	X		X
Psychosocial questionnaire[a] (tobacco, AUDIT, PHQ-9, BAI)	X		
Impact of TB symptoms on income	X		X
Anthropometrics	X		X
Pregnancy Test[b]	X		
CXR[c]	X		
HIV Test (Rapid)[d]	X		
CD4 count if HIV-infected[d]		X	
CBC and lymphocyte count	X		
HbA1c[a]	X		
Sputum smear [e]	X (S1)[j]		
Sputum smear, Xpert Ultra and culture (MGIT and LJ)[f]	X (S2)[j]	X (S3)[j]	X
Sputum DST	X		X
EPTB sample for smear, Xpert Ultra, culture, pathology and storage[g]	X		X[k]
Mtb isolate subculture for storage	X		X
Whole blood (PAXgene) for storage (x 2)	X		X
Whole blood (plasma) for testing and storage[h]	X		X
Whole blood for storage (genetic analyses)	X		
Saliva for storage[e] (diagnosis or genetic analyses)	X		
Urine for storage	X		X
Sputum for storage (half in TRIzol, half untreated)		X (S4,5)[j]	X[l]
Stool for storage[i] (diagnosis, microbiome)	X		

AUDIT=alcohol use disorders identification test; CXR=chest radiograph; CBC=complete blood count; COVID-19=Coronavirus disease of 2019; LFT=liver function test; MGIT=mycobacteria growth indicator tube; LJ= Lowenstein Jensen; culture; DST=drug-susceptibility testing; EPTB=extrapulmonary TB (lymph node pleural, and TBM only); PHQ-9=Patient Health Questionnare-9, BAI=Beck's Anxiety Inventory

[a] Applicable for adults (≥18years) only

[b] Urine pregnancy test to be done before a performing a CXR in women of reproductive age (usually 10 to 50years)

[c] CXR at baseline, unless a good quality digital CXR was done within 4 weeks prior to the Baseline as part of standard of care evaluations or through the Parent Protocol and is available for the study team. Pregnant

women can have a CXR done if adequate shield can be provided (at discretion of study clinician). Do not repeat if enrolled for cohort A.

[d]HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV positive mother; abstract the data from the participant's medical chart or research record; CD4 count will only be performed on participants who are HIV-infected and who have not had a CD4 count performed in the preceding 6 months.

[e] For children (<15years) enrolled in the Dx cohort refer to Table 04b for micro and specimen collections.

[f]MGIT extension for discordant and negative controls

[g] EPTB specimens from lymph node pleural, and TBM cases will undergo smear/Xpert MTB/RIF, histopathology, Xpert Ultra and cultures by MGIT and LJ. Anything leftover will go for storage.

[h] Plasma samples will be used for Aim 1 (cytokine) and cross-cutting Aims (SARS-CoV-2 antibody) testing.

[i] Stool collection only for Paediatric (<15years) study participants.

[j] For sputum samples:

- Day 1- Sputum 1 (S1): Sputum 1 (S1): Spot sputum (preferably early morning specimen).
- Day 1- Sputum 2 (S2): Spot sputum collection.
- Direct florescence smear microscopy will be performed on both S1 and S2 samples. Both S1 and S2 will be mixed and homogenized using 10% sputasol or 0.1% DTT. The sample will be split, one half used for Direct Xpert ULTRA (version 2) and the other half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen cultures and MGIT liquid culture (with 4 weeks extended incubation for smear/ Xpert positive negative cultures)
- Day 2- Sputum 3 (S3), Spot sputum collection (preferably early morning).
- Day 2-Sputum 4 (S4). Spot sputum collection.
- Direct florescence smear microscopy will be performed on both S3 and S4 samples. Both S3 and S4 will be mixed and homogenized using 10% sputasol or 0.1% DTT. The sample will be split, one half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen culture and MGIT liquid culture (with 4 weeks extended incubation for smear negative cultures). The other half will be equally split and one half treated with Trizol and frozen for storage, and the other half directly frozen for storage. S5 spot sputum sample may be collected in a TRIzol prefilled sputum collection tube for sputum RNA stablity.
- For induced sputum or nasopharyngeal aspirates or gastric lavage, 2 specimens will be collected on day 1, four hours apart. Direct fluorescence smear microscopy or Xpert MTB RIF; Lowenstein Jensen culture and MGIT liquid culture (with 4 weeks extended incubation for smear negative cultures) will be performed on both samples. Detailed procedures for included sputum or nasopharyngeal aspirate or gastric lavage specimen processing and storage are mentioned in Lab MOP.

[k] Only if specimen was collected as standard of care for TB diagnosis

[l] If symptomatic

Note:

If blood volume in combination with other clinical or protocol blood collection requirements exceeds the allowable volume by the ICMR or IRB/IEC guidelines, the participant will be requested to return at the earliest possible time point to collect the baseline specimen, as long as the minimum specimen collection criterion is met before more than 1 week of anti-TB therapy was received.

All TB cases including EPTB will be offered sputum/ respiratory specimen collection.

B.1.5.2 Table 04b: Microbiologic SOE for Pediatric Dx Cohort

Activities / Visit	Baseline – Day 1	Baseline - Day 2	Month 2 (discordants, negative controls and status unknown)
2 induced sputum or nasopharyngeal aspirates (pooled): smear/Xpert MTB/RIF, Xpert Ultra, and cultures (MGIT and LJ)[a,]	X (x 2)[b]		
Induced sputum or nasopharyngeal aspirate: smear, direct Xpert Ultra, cultures (MGIT and LJ)[c]		X	X[d]
Sputum smear/ Xpert MTB/RIF	X (S1)[e]		X
Sputum smear/ Xpert MTB/RIF - Xpert Ultra, and culture (MGIT and LJ)[c]	X (S2)[e]	X (S3)[e]	X
DST [f]	X		X
EPTB sample for smear, Xpert Ultra, culture (MGIT and LJ), pathology and storage[h]	X[g]		
Mtb isolate subculture for storage[f]	X		X
Whole blood (PAXgene) for storage (x 2)	X		X
Whole blood (plasma) for storage	X		X
Whole blood for storage (genetic analyses)	X		
Saliva for storage (diagnosis or genetic analyses)	X		
Oral swab for storage[i]	X		
Sputum/ induced sputum or nasopharyngeal aspirate for storage (half in TRIzol, half untreated)		X (S4,5)	X[d]
Urine for storage	X		X
Stool for storage (diagnosis, microbiome)	X		

MGIT=mycobacteria growth indicator tube; LJ= Lowenstein Jensen; culture; DST=drug-susceptibility testing; EPTB=extra pulmonary TB (lymph node pleural, and TBM only)

Applies to induced sputum or nasopharyngeal aspirate or gastric aspirate samples collected among very young children who cannot produce adequate sputum:

[a]Perform prior to any respiratory sampling

[b]Two induced sputum or nasopharyngeal aspirates or gastric lavage can be collected at least four hours apart. Samples collected on the same day will be pooled and will be treated as 1 sample. If 2 samples were collected on two different days, both Day 1 and Day 2 sample can undergo testing for Direct fluorescence smear microscopy/ Xpert MTB RIF, Xpert Ultra, MGIT and LJ culture testing and any remaining samples from both days can be used for storage.

Pooled respiratory specimen (i.e. induced sputum or nasopharyngeal aspirates or gastric lavage collected on the same day will undergo the following:

- Both respiratory specimens (collected on the same day) will be mixed and homogenized using 10% sputasol or 0.1% DTT. If not pooled, each sample will be split and one half used for testing and other half for storage.
- The sample will be split, one half used for Direct Xpert ULTRA (version 2) and the other half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be

performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen culture; MGIT liquid culture (with 4 weeks extended incubation for smear/ Xpert positive negative cultures)

[c] MGIT extension for discordant and negative controls

[d] Induced sputum/ nasopharyngeal aspirate/ gastric lavage performed at month 2 for culture-negative untreated children with (1) unresolved symptoms or (2) positive Ultra result at baseline.

[e]Children <15years who are able to produce sputum adequately will follow the same sputum testing and storage procedures outlined in Table 04a for adults and adolescents (≥15years)

[f]Anytime culture is positive

[g] EPTB samples from lymph node pleural, and TBM cases only. EPTB specimen will be undergo smear, histopathology, Xpert Ultra and cultures by MGIT and LJ. Any leftover sample will go for storage.

[h] Only if specimen was collected as standard of care for TB diagnosis

[i]Oral swab applicable for children <6 years of age only

Note:

- If blood volume in combination with other clinical or protocol blood collection requirements exceeds the allowable volume by the ICMR or IRB/IEC guidelines, the participant will be requested to return at the earliest possible time point to collect the baseline specimen, as long as the minimum specimen collection criterion is met before more than 1 week of anti-TB therapy was received.
- When respiratory samples are required from children 14 years of age or younger, nasopharyngeal (NP) aspirates or gastric lavage (GL) may be collected instead of sputum. Children diagnosed clinically (PTB or EPTB) and are unable to produce any specimen will not be included in the study.
- All TB cases will be offered sputum / respiratory specimen collection and detailed storage instructions for sputum/ respiratory secretions, EPTB sample and blood are provided in lab manual.

B.2 Cohort A (Active pulmonary TB)

B.2.1. Aim 2:

Identify markers of treatment response

Aim 2 investigates host (2A and 2B) and microbial (2C) factors related to adverse treatment response. The **overall goal** is to produce new knowledge with the potential transform the approach to TB treatment by identifying patients and Mtb isolates that may be cured with shorter regimens using approved antibiotics.

Aim: 2.A: Identify TB Treatment Response Biomarkers

2.B: Investigate Host-Related Mechanisms of Treatment Failure

Objectives

1) To compare typical values and variability in concentrations of 1st line TB drugs among TB patients with or without undernutrition and DM and assess for interactive effects

2) To determine the degree to which changes in body composition during TB treatment alter drug PK;

3) To obtain preliminary data on the relationship between TB drug concentrations and clinical outcomes among those with undernutrition and DM

4) To evaluate for toxicity associated with increased drug levels.

2.C: Investigate Pathogen-related Mechanisms & Predictors of Recurrence

Objectives

1) Confirm the association between sub-breakpoint MICs and cure vs. recurrence in an Indian population

2) Define useful breakpoints for assigning TB treatment length

3) Define the contribution of small colony variants (SCVs) in pretreatment patient sputum to the measured MICs.

B.2.1.1 Table 04: Aim 2A Studies Using Archived Samples

Short Title		qRT-PCR TB signatures for Tx	TB NanoString for Tx	DR-TB signatures for Tx	Plasma Cytokine for Tx	Plasma Protemic Discovery for TB Recurrence/Death
RePORT Study ID		IND_AIM2A_12	IND_AIM2A_13	IND_AIM2A_164	IND_AIM2A_15	IND_AIM2A_16
Laboratory		SATVI, THSTI	theraCUES	theraCUES, Medgenome	Prof.MVDRC, NIRT-ICER	IIT-Bombay
Sample Types		PAXgene	PAXgene	PAXgene	Plasma	Plasma
TB Infection State	Adult PTB	X	X		X	X
	Adult EPTB					
	Pediatric TB					
	LTBI vs Active TB					
	Subclinical TB					
	DR-TB	X		X		
	Incident TBI					
	Uninfected controls					
Archived Samples	Cohort A Participants	243 (DS-TB)	243 (DS-TB)	87 (DR-TB)	243 (DS-TB)	120 (DS-TB)
	Cohort B Participants	0	0		0	0

B.2.1.2 Anticipated Results & Impact:

Aim 2A: Our goal is to produce new knowledge about treatment response biomarkers sufficiently robust to develop as clinical tests feasible for rollout into the Indian TB control program. This would most likely take the form of a chip or cartridge-based qPCR platform for automated analysis. In this regard, POC devices suitable for concise RNA signature measurement based on qRT-PCR and NanoString methods are already in development. Treatment response prediction based on clinical variables and biomarkers could be immediately translated to the clinic if results from this study demonstrate efficacy. If parsimonious RNA signatures underperform in the Indian population, then our focus would shift to the exploratory objective of Indian population-specific signatures discovery using RNAseq.

Aim 2B: Our goal is to identify the impact of under-nutrition and DM on TB drug PK and treatment outcomes. If our study identifies significant alterations, we anticipate the need for enhanced therapeutic drug monitoring and/or dose adjustments for these populations to improve outcomes. India has adopted a new daily ATT and our study will help inform whether TB drug concentrations, especially rifampin, which is critical for cure, remains suboptimal. In addition, the stool sample analysis on enteropathogen screening in the study can assess the burden of enteropathogens among persons living with TB, impact of enteropathogens on the pharmacokinetics of first-line antimycobacterials in adults, impact of enteropathogens on TB treatment outcomes and the effect of enteropathogens on the baseline nutritional status of TB patients. In addition, microbiome analysis can assess the impact of the gut microbiome on the pharmacokinetics of first-line antimycobacterials in adults TB patients.

B.2.2 Aim 3: Lung Injury & Impairment

Identify markers of lung injury associated with unfavorable TB treatment outcomes

Objectives

1. To measure the association between clinical, functional, and imaging markers of lung injury, and unfavorable treatment outcomes in adults with newly diagnosed drug-sensitive PTB cases in India

2. To identify plasma and sputum inflammatory markers associated with lung injury in PTB cases in India.

3. To characterize lung injury phenotypes and their relative burden in successfully treated PTB cases in India.

B. 2.2.1 Table 05 : Proposed Aim 3 Lung Health Assessments - Summary Table

Aim-3 Schedule of Evaluations		*Pulmonary Function and Exercise Testing*				*Lung Imaging*			*Respiratory Questionnaires*			*Inflammatory Markers*		*Storage*
		Spiro-metry	TLC	DLco	6MWT	CXR	HR-CT	PET-CT	SGRQ	CAT	MMRC	Plasma	Sputum	Sputum
Sample Size	325	325	100*	100*	325	325	150*	100*	325	325	325	100*	100*	100*
Schedule of Evaluations	Month 0	X			X	X		X	X	X	X	X	X	
	Month 2	X	X**	X**	X	X		X	X	X	X	X	X	
	End of TX	X	X	X	X	X		X	X	X	X	X	X	X
	6M-Post TX	X	X	X	X		X		X	X	X			X
	12M-Post TX													X
Participating CRSs	CMC	X	X	X	X	X	X	X	X	X	X	X	X	X
	PGIMER	X	X	X	X	X	X	X	X	X	X	X	X	X
	BJMC	X			X	X	X	X***	X	X	X			
	NIRT	X			X	X			X	X	X			

CRU – clinical research unit, TLC – total lung capacity, DLco – diffusing capacity of carbon monoxide, 6MWT – 6 minute walk test, CXR – chest radiograph, HR-CT – high resolution volumetric computerized tomography, PET-CT – positron emission tomography with computerized tomography, SGRQ – Saint George's Respiratory Questionnaire, CAT – COPD assessment test, MMRC – Modified Medical Research Council dyspnea scale, * - random subset, ** - only if smear negative ; *** pilot study with sample size subject to funding availability.

B.2.2.2 Aim 3: Overall Anticipated Results & Impact.

Results from our study, if significant, will provide the strongest evidence to date for the association between lung injury and unfavorable TB treatment outcomes, including emerging drug resistance and recurrent TB. In doing so, we will identify novel clinical, immunological, and imaging markers of early treatment response and identify TB patients at greatest risk of unfavorable treatment outcomes. A key strength and novelty of our study is the use of multiple clinically relevant modalities for evaluating lung injury, including PET-CT, in a relatively large and well-characterized cohort of never-smoking PTB patients with and without key comorbidities such as diabetes and HIV. **Whereas our primary focus for this project is on microbiological outcomes in TB, we are also well positioned to characterize the burden, phenotype, and risk-factors of chronic pulmonary sequelae in treated TB—a major contributor to respiratory morbidity, mortality, and burden of COPD globally.** Finally, by focusing primarily on circulating and sputum inflammatory markers identified a-priori, our study is well positioned to identify potentially modifiable immune pathways associated with microbiological and respiratory response to TB therapy; thereby identifying therapeutic targets for adjunctive HDTs in TB to improve treatment efficacy and prevent chronic pulmonary sequelae.

B.2.3 Table 06: Schedule of Events for Cohort A

Activities / **Visit**	TREATMENT PHASE					POST-TREATMENT PHASE		
	Screening	Baseline	Visit 1 (Month 1/ Month 3)	Visit 2 (Month 2/ Month 6)	End of TX (-4 wks/+6 wks)	6-M Post-TX (-4 weeks/+6wks)[m]	12-M Post TX (-4 weeks/+6wks)[m]	TX F/R/W
Informed consent	X							
Eligibility assessment	X	X						
Eligibility confirmation[a]			X	X	X			X
Demographics		X						
Medical history, clinical questionnaires (symptoms, medications, Karnofsky and COVID-19 screening)		X	X	X	X	X	X	X
Psychosocial questionnaire (tobacco, AUDIT-C, PHQ-9, BAI)		X			X	X		
Anthropometrics (detailed)		X	X	X	X	X	X	X
Dietary Assessment (MDD-W and HFIAS)		X	X	X	X			X
Medication Adherence and treatment side-effects			X	X	X			
TB economic costs		X		X		X		
Multidimensional Poverty Index Questionnaire		X				X		
Participant status[b]			X	X	X	X	X	X
CXR[c]		X			X			X
Urine pregnancy test (if indicated)		X			X			X
HIV Test (Rapid)[d]		X						X
CD4 count if HIV-infected[d]		X						X
CBC including Hb and lymphocyte count		X		X	X			
Haemoglobin A1c		X			X		X	
LFT and albumin		X	X	X	X			

Activities	TREATMENT PHASE					POST-TREATMENT PHASE		
	) e	e _	t h	t h	4 w	- 4 w	- 4	F /
Creatinine		X						
Fasting Lipid profile		X						
Sputum smear & culture		X	X	X	X			X
Sputum DST		X						X
Mtb isolate subculture for storage[p]		X			X			X
Whole blood (plasma and PBMC[o]) for testing and storage[e]		X[n]	X	X[n]	X[n]			X[n]
Whole blood (PAXgene) for testing and storage[e]		X	X	X	X			X
Whole blood for storage - sparse (0 and 2 hrs) PK[e, f]			X					
Whole blood for storage (genetic analyses)		X[g]			X			X
Whole blood for NAT genotyping and Isoniazid Acetylator status		X[g]			X			X
Saliva for storage (diagnosis or genetic analyses)		X			X			X
Urine for storage and adherence measurement		X	X	X	X			X
Sputum for storage		X	X	X				X
Aim 2B (Nutrition and PK) *(BMI<18.5+DM, BMI≥18.5+DM, BMI<18.5+No DM, BMI≥18.5+No DM)*								
Whole blood for storage - intensive (0, 2, 4, 6 and 8 hrs) PK[f]			X					
Whole blood for storage - micronutrient assessments			X					X
Stool for storage (diagnosis, microbiome and enteropathogen)			X					
Aim 3 (Lung Health)								
Spirometry, 6MWT and Respiratory Questionnaires[h]		X		X	X	X		
CXR (lung health subset only)		X		X	X			
DLco and TLC (subset)[i]				X[l]	X	X		
PET-CT (subset)[i,j]		X		X	X			
HR-CT without contrast (subset)[i]						X		
Sputum (expectorated or induced) for storage (subset)[k,l]					X	X	X	
Plasma and sputum for cytokine testing (subset)[i]		X		X	X			

AUDIT=alcohol use disorders identification test; BAI=Beck's anxiety inventory; BMI=Body Mass Index; CAT=COPD assessment test; CBC=complete blood count; COVID-19= Coronavirus disease of 2019; CXR=chest radiograph; DLco=diffusing capacity of carbon monoxide; DM=Diabetes Mellitus; DST=drug-susceptibility testing; HFIAS=household food insecurity access scale; hrs= hours; HR-CT=high resolution volumetric computerized tomography; LFT=liver function test; MDD-W= minimum dietary diversity for women of reproductive Age; MMRC=modified medical research council dyspnoea scale; 6MWT= 6 minute walk test; PET-CT=positron emission tomography with computerized tomography; PHQ-9=patient health questionnare-9; PK=Pharmacokinetic testing; SGRQ=Saint George's respiratory questionnaire; TLC=total lung capacity.

[a] Eligibility confirmation when possible, if not done previously.

[b] Clinical assessment, as needed

[c] If good quality digital CXR was done within 4 weeks prior to the visit as part of standard of care or through the Parent Protocol, the study team will not repeat the CXR testing. Pregnant women are not required to have a CXR but can have a CXR done if adequate shield can be provided (at the discretion of the clinician).

[d] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; abstract the data from the participant's medical chart or research record; CD4 count will only be performed on participants who are HIV-infected and who have not had a CD4 count performed in the preceding 6 months.

[e] If blood volume exceeds allowable volume by ICMR or IRB/ IEC guidelines in combination with other clinical or protocol blood collection requirements, the participant will be requested to return at the earliest possible time point to collect the baseline specimen, as long as the minimum specimen collection criterion is met before more than 1 week of anti-TB therapy was received.

[f] PK Sampling to be done at 0 and 2 hours for all participants and at 4, 6, and 8 hours additionally for intensive PK participants post-

Activities	TREATMENT PHASE					POST-TREATMENT PHASE		
	) e	e —	t h	t h	4 w	- 4 w	- 4	F /

dosing. Dose to be administered in the clinic. For further details on DS-TB and DR-TB participant PK collections refer to MOP.

[g] If samples remain post local-site testing (i.e. CBC or HbA1c), leftover blood may be stored.

[h] Respiratory questionnaire includes SGRQ, CAT, and MMRC

[i] Random subset, selected sites to pilot study with sample size subject to funding availability.

[j] Standard recommended tests or procedures for safety screening such as renal function tests will be performed before doing a PET-CT for individuals in the PET-CT sub-set.

[k] Sputum induction only if participant could not expectorate spontaneously.

[l] Only if smear negative

[m] If TB recurrence is suspected at post-treatment visits, complete evaluations required for the TX F/R/W Visit.

[n] Plasma samples collected at Baseline, Visit 2, End of Treatment and TX F/R/W visits will be used for Aim 2A (markers of treatment response), Aim 3 (markers of lung inflammation) and cross-cutting Aims (SARS-CoV-2 antibody testing); Additional 3 mL collection for plasma storage will be done at these time-points.

[o] PBMC sample processing and shipment to central biorepository storage from three RePORT India site participants (PGI Chandigarh, NEIGRIHMS and Hinduja) may not begin until necessary PBMC approvals are obtained as per the central biorepository requirements and will not be considered as off-study/ protocol deviation.

[p] Any culture positive after Visit 2 (i.e., EOT, 6M-Post TX, 12M-Post TX, TB F/R/W visits) MTB isolate storage is to be done

B.3 Cohort B (Household Contacts)

B.3.1 Aim 4. (Resistance & acquisition of infection)

Mechanisms of Protection Against TB in Exposed Persons

i. Aim 4A: Examine Host Antimicrobial Pathways in Inducing Their IR Phenotype in HHC
ii. Aim 4B: Test if IR & plasma Differ in Modulating Macrophage-Mediated Restriction of Mtb Growth & Evaluate AB Repertoires of Plasma from the IR and IS Cohorts

B.3.1.1 Table 07. Proposed Aim 4 studies summary table: RESISTANCE & ACQUISITION OF INFECTION

Assay	IL-36d, IL-1b, PGE2	Mtb killing	Th17	B cells	miRNA	QGIT-sup	MAIT & NK cells	MAIT, NK, $^{v\delta}$ cells, CyTOF panel
RePORT India Phase II Studies						Other RePORT-related Studies		
Laboratory	JIPMER	JIPMER	JIPMER	Rutgers	NIRT	ICER	ICER	BJGMC & U Colorado
Sample types	PBMC	PBMC	PBMC	PBMC	Plasma	QGIT-sup	PBMC	PBMC
Infection state	IR	IR	IR	IR	IR	IR	IR	IR
	IS	IS	IS	IS	IS	IS	IS	IS
Archived samples (CP)	20 20	20 20	20 20	20 20	20 20	20 20	20 20	20 40
New recruitment (CP follow-up of 877)	20 20	20 20	20 20	20 20	20 20	20 20	20 20	

Note:
IR – infection resistant.
HHC who are IGRA negative at the time of diagnosis of the index case and IGRA and TST negative on retesting at 6 months; for those studied previously and re-consented, remain IGRA and TST negative at least 12 months after initial ascertainment. At least one HHC is IGRA positive indicating that transmission has occurred.
IS – infection susceptible.
HHC who are IGRA positive at baseline visit and IGRA/TST positive at 3 months or IGRA negative at the time of diagnosis of the index case, IGRA and TST positive on retesting at 3 months.
For this aim, we will use existing samples from Cohort B (HHC) and add one collection time point post 12 months as needed to capture additional resisters in Parent and Common Protocol participants to determine if the HHC have remained infection free or have converted.

B.3.1.2 Aim 4: Overall Anticipated Results & Impact.

Overall, the identified innate pathways in IR that mediate protection from infection can be harnessed for host-directed therapy to induce activation of these pathways in the innate compartment. These innate pathways of protection from infection will be highly relevant in the HIV-infected. Clearly the identified innate pathways in IR that mediate protection from infection can be harnessed to design vaccines that can induce prolonged activation of these pathways in the innate compartmen

B.3.2 Aim 5: Progression to disease

Identify immunologic markers of persons at highest risk of progression of latent Tb infection to Tb. In addition to studies in Aim 5 for Stored Samples, we also propose detailed follow-up study and understanding immune mechanisms that regulate the progression to TB disease

i. **Aim 5A. (Stored Samples):** Validation of PREDICT29 in progressors & non-progressors from report sites
ii. **Aim 5B.** Immune & hormone studies in freshly collected samples

Objectives:

i. Study the Phenotype & Function of Immune Cells
ii. Determine the signaling pathways and microRNAs that regulate the immune response of HHCs those develop active TB
iii. Determine the Cellular & Molecular Mechanisms Involved in T4 Hormone Mediated Regulation of Mtb Growth in Macrophages.
iv. Developing an Elisa-Based Kit to Identify HHCs Likely to Develop Active TB Disease

B.3.2.1 Aim 5: Overall Anticipated Results & Impact

We anticipate that at the successful completion of Aim 5 studies, we will have identified biomarkers that predict risk of progression to TB disease. The precise mechanisms underlying protective immunity against TB remain unknown. The proposed studies will discover adaptive immune pathways that protect from Mtb infection and progression to disease. This understanding and the associated correlates of protection will contribute to innovative vaccine development

B.3.2.2 Table 08: Aim 5: PROGRESSION TO DISEASE: Identify Immunologic Markers of Persons at Highest Risk of Progress of Latent TB Infection to TB

ASSAY	Validation PREDICT29	NK cells	Macrophage function	Tregs and T-cell memory	miRNA	T4 hormone studies	FLOW cytometry-T cell response to for DosR-specific antigens
	RePORT India Phase II Studies						Other RePORT-related Studies
Laboratory	theraCUES	Mahavir	Mahavir	Mahavir	Mahavir/ UT Southwestern	Mahavir	IISc, Bangalore
Sample types	PAXgene	PBMC	PBMC	Plasma	Plasma and PBMC	PBMC and plasma	PBMC
Disease state	Progressor	Progressor	Progressor	Progressor	Progressor	Progressor	Progressor
	Non-Progressor	Non-Progressor	Non-Progressor	Non-Progressor	Non-Progressor	Non-Progressor	Non-Progressor
Archived samples	43 Progressors 80 Non-Progressors	Frozen Samples collected from various site during phase 1	Frozen Samples collected from various site during phase 1	Frozen Samples collected from various site during phase 1	Frozen Samples collected from various site during phase 1	Frozen Samples collected from various site during phase 1	Two time points 20 progressors 20 non-progressors
New recruitment	10 Progressors 20 Non-Progressors	14 to 16 progressors (2.2% of 500 HHCs)	14 to 16 progressors (2.2% of 500 HHCs)	14 to 16 progressors (2.2% of 500 HHCs)	14 to 16 progressors (2.2% of 500 HHCs)	14 to 16 progressors (2.2% of 500 HHCs)	None

B.3.3 Table 07: Schedule of Events for Cohort B

Activities / Visit	SCREENING	BASELINE	MONTH 6 (+1/-2 MONTHS)	MONTH 12 (+1/-2 MONTHS)	MONTH 18 (+1/-2 MONTHS)	MONTH 24 (+1/-2 MONTHS)	INCIDENT INFECTION[l]	TB ACTIVATION EVALUATION	PREM DC
Informed consent	X								
Eligibility assessment	X								
Demographics and TB exposure with Index case		X							
Medical history, clinical data (symptoms, medications, Karnofsky and MMRC), detailed anthropometrics and targeted exam		X	X	X	X	X		X	X
COVID-19 Questionnaire (screening, impact on TB and testing history)		X	X	X	X	X		X	X
Psychosocial Interview		X	X	X	X	X		X	
Urine Pregnancy test (If indicated)	X						X	X	
HIV test if status is unknown[a]		X						X	
CD4 count if HIV-infected[b]		X						X	
CBC and lymphocyte count[c]		X						X	
Haemoglobin A1c		X						X	
CXR[d]	X						X	X	
IGRA (QuantiFERON)[e]		X	X	X	X	X		X	
Xpert[f]		X					X	X	
Smear and culture from TB activation site[g, l]		X					X	X	
Mtb isolate subculture for storage[g]		X					X	X	
Sputum DST[g]		X					X	X	
Sputum/NPA/GL for storage[g]		X					X	X	
Whole blood (PAXgene) for storage[h]		X[i]	X	X	X	X		X	
Whole blood (Plasma) for storage and testing[j]		X[i]	X	X	X	X		X	
Whole blood (IGRA supernatant) for storage		X	X	X	X	X		X	
Whole blood (genetic analyses) for storage		X[i]							
BMMRC only:									
Whole blood (Plasma and PBMC) for testing and storage[j]		X[i]	X	X	X	X		X	
Whole Blood (Serum)		X	X	X	X	X		X	
IGRA (in-house assay)[e]		X	X	X	X	X		X	
CBC and Lymphocyte Count[d]			X	X	X	X			
Hinduja only:									
C-TB[e]		X							
Whole blood (PAXgene x 2) for Transcriptional analysis of RNA using NanoString and Cepheid 3-gene signature cartridges[h]		X						X	
Urine for storage		X	X	X	X	X		X	
Saliva (genetic analyses) for storage		X							

Stored mask for Index Aerosol Testing	X							X	
PET-CT[k]		X	X						

IGRA=Interferon Gamma Release Assay; CXR=chest radiograph; CBC=complete blood count; DST=drug-susceptibility testing; C-TB=Staten Serum Institute skin test for TB infection (replaces TST); PET-CT=positron emission tomography with computerized tomography; NPA=Nasopharyngeal aspirate; GL=Gastric Lavage.

[a] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV-positive mother; abstract the data from the participant's medical chart or research record.

[b] CD4 count will only be performed on participants who are HIV-infected and who have not had a CD4 count performed in the preceding 6 months as a part of standard of care or the Parent Protocol.

[c] CBC, lymphocyte count, and HbA1c are not required at baseline if collected within 4 weeks prior to the TB Activation Evaluation Visit as part of standard of care or as part of the Parent Protocol. Only BMMRC will collect additional samples for CBC and lymphocyte counts at every study visit. Both sites will repeat CBC and lymphocyte count at TB activation visits, if relevant.

[d] CXR data will be collected if a good quality digital CXR was conducted as part of standard of care or as part of the Parent Protocol. If not done already, a digital CXR will be done. Pregnant women are not required to have a CXR, but can have a CXR if adequate shield can be provided (at the discretion of the study clinician).

[e] C-TB to be performed at baseline. IGRA to be performed at each visit using commercially available assays (QuantiFERON) at both sites. BMMRC will also perform in-house IGRA for validation purposes.

[f] Rapid molecular testing will be performed for all participants at enrolment and evaluation of active TB. Xpert testing will be performed from all samples from BMMRC using Xpert Ultra (NAT). Xpert testing will be performed on all samples from Hinduja using Xpert Ultra.

[g] Smear and culture to determine participant's bacteriologic status. Gastric Lavage will be done for children who have symptoms highly suggestive of TB disease at any site or who have a CXR indicative of intrathoracic TB if they cannot produce an adequate sputum sample. Induced sputum as applicable for children and adults. Speciation and drug sensitivity to be performed if TB is suspected. All those determined to have active TB will have all specimens saved in the biorepository and will be requested to roll over to Cohort A.

[h] PAXgene tubes will be stored for central biorepository from BMMRC. PAXgene tubes from Hinduja will complete RNA isolation for NanoString and Cepheid 3-gene signature cartridges, with remaining RNA stored in central biorepository.

[i] If blood volume in combination with other clinical or protocol blood collection requirements exceeds the allowable volume by the ICMR or IRB/IEC guidelines, request the participant to return at the earliest possible time point to collect the baseline specimen.

[j] At each collection time point, plasma volume will include a 10mL collection at Hinduja and at least 20mL collection at BMMRC. Plasma will be harvested at both sites and PBMC isolation will happen at BMMRC site only from the heparinized tubes until Hinduja receives approval for PBMC processing and storage from the central biorepository. BMMRC will prioritize testing PBMCs for cell cultures, characterization of T cells, Monocytes and NK cells as markers of TB infection and disease spectrum and local storage for TRIzol storage for RNA extraction. Any leftover PBMCs will be shipped to Central Biorepository. Similarly, BMMRC will prioritize plasma samples for Cytokine, Chemokine and hormone testing locally and at least 1 mL plasma aliquots post testing will be shipped to Central Biorepository for storage. Hinduja will ship plasma samples to CR for storage and both sites will contribute plasma for SARS-CoV-2 infection antibody testing.

[k] PET-CT will be performed at baseline and 6 months for all NanoString-positive contacts (likely N=20), as well as matched NanoString-negative contacts (N=10). Standard recommended tests or procedures for safety screening before doing a PET-CT will be done for individuals in the PET-CT sub-set.

[l] If Xpert Ultra positive (trace) or PET CT positive or NanoString positive culture reading to be extended up to 10 weeks before calling them negative instead of stopping at 6weeks. Incident TB infection visit will happen only at Hinduja site

C. Cross-cutting aims

C.1 Epidemiologic aims

In addition to the scientific objectives proposed above, we will undertake key epidemiological analysis using data already collected through RePORT Phase I and Phase II. We are not proposing additional procedures, beyond those previously described, for these analyses.

Using data collected from RePORT Phase 1 Cohort A and RePORT Phase 2 Cohort A and diagnostic cohorts we will:

1. Describe index cases by RePORT study site to identify geographic differences in TB disease presentation and severity.
2. Evaluate population attributable fractions of relevant comorbidities (e.g. diabetes, undernutrition, alcohol use, and smoking) for TB disease.
3. Measure clinical and socio-demographic characteristics associated with TB disease severity (radiographic and microbiologic).
4. Identify predictors of poor TB treatment outcomes (culture conversion, failure, death, recurrence), particularly focused on the impact of comorbidities (e.g., undernutrition and diabetes) and where patients seek care (public, private clinics, etc.).
5. Measure population attributable risk of different comorbidities on TB treatment outcomes.
6. Study gender-related differences in TB disease presentation and outcomes.
7. Describe the causes of death and associated risk-factors in TB patients during and after therapy.
8. Validate and, if needed, recalibrate the Timika CXR score for predicting response to TB therapy.
9. Compare CXR reports with automated CXR reading programs to evaluate deep learning/machine learning techniques for TB diagnosis and monitoring therapy.
10. Develop and validate an epidemiological risk-score for predicting TB treatment outcomes using a combination of clinical, socio-demographic and microbiological parameters.
11. Describe the treatment seeking behavior and patients' pathways to TB diagnosis and treatment.
12. Validate the diagnostic algorithm for pediatric TB suspects.

Using data collected from Cohort B of RePORT Phase 1 and Phase 2, and in conjunction with relevant Cohort A data, we will:

1. Assess the burden of TB disease and infection at the household level to identify clustering of TB within communities.
2. Assess community and household exposure data from the Aim 1 cohort (e.g., whether suspects visit arrack shops, other community gatherings) would be used in conjunction with strain sequencing to generate models of where exposure is occurring.
3. Identify index case and household characteristics associated with prevalent and incident TB disease.
4. Identify index case and household characteristics associated with prevalent and incident TB infection.
5. Identify index case and household characteristics associated with TST/QFT discordance.
6. Measure population attributable risk of different comorbidities on incident TB infection and disease.
7. Develop and validate an epidemiological risk-score for predicting TB disease using a combination of clinical, socio-demographic and microbiological parameters from the index case and household members.
8. Detailed nutritional assessments to determine the impact of baseline nutritional status (and changes in nutritional status over time) on treatment outcomes and on drug PK, controlling for known confounders of TB treatment outcomes and PK values.
9. Clinical and epidemiologic data will be used in conjunction with basic science research to identify factors associated with resistance to infection, progression to TB disease, and how such clinical parameters correlate with biomarkers.
10. To study the differences in type and duration of symptoms, proportions having LTBI, subclinical TB and clinical types of TB between the gender, socio-economic groups and groups with various behavioral and clinical characteristics.
11. Assess the proportion of household contacts of drug-resistant TB patients with prevalent and incident LTBI during follow-up and assess if it is different from the proportions identified among household contacts of drug-susceptible TB patient

C.2 SARS-CoV-2 (COVID-19) AIMS:

We aim to assess the Impact of severe acute respiratory syndrome coronovirus-2 (SARS-CoV-2) on Indian TB suspects, patients with pulmonary TB and their TB-exposed household contacts. Specifically, we aim:

1. To compare the risk of active TB disease among new TB suspects (Diagnostic Cohort) with and without confirmed SARS-CoV-2 infection.
2. To study the prevalence and incidence of confirmed SARS-CoV-2 infection (symptomatic and asymptomatic), among TB suspects from diagnostic cohort (Dx Cohort), patients with pulmonary tuberculosis (Cohort A) and their household contacts (Cohort B).
3. To compare TB treatment outcomes between PTB patients (Cohort A) with and without confirmed SARS-CoV-2 infection (NAAT, antigen, antibody positive result).
4. To compare the incidence of TB disease between HHCs (Cohort B), with and without confirmed SARS-CoV-2 infection.
5. To compare the risk of pulmonary dysfunction between PTB patients enrolled in Lung Health sub-study (n=325), with and without SARS-CoV-2 infection.