Study Protocol
(Epi & non-clinical studies)

"Linking the within-host and between-host evolution in multidrug-resistant *Mycobacterium tuberculosis*"

Type of Research Project	1) Research projects (HRO) - Research projects – involving measures or sampling of biological material or collection of health-related data from persons. 2) Research projects (HRO) - involving further use of biological material and health-related personal data

Study acronym/ID	ECOEVODRTB		
Protocol Version Nr	1.0	**Date**	13.07.2020
Funding organisation	**ERC-European Research Council** B-1049 Brussels W https://erc.europa.eu/		
Project Leader / Responsible	Prof. Sebastien GAGNEUX, Swiss Tropical and Public Health Insitute (Swiss TPH) Socinstrasse 57, 4002 Basel, Switzerland +41 61 284 8369 sebastien.gagneux@swisstph.ch		
Funding Agency	European Research Council (ERC), Brussels		

Protocol Template Sources:
- Swissethics HRO_Research_Plan_Template_e2.0, 19.07.17
-WHORecommended format for a Research Project (https://www.who.int/rpc/research_ethics/format_rp/en/index1.html)

1 GENERAL INFORMATION

I. List of Project Leaders and other key persons involved in the study

Title	Names	Institution	Position	Function in study
Prof.	Sebastien Gagneux	Swiss TPH	Head of Department	Project Leader
Dr.	Nestani Tukvadze	NCTLD	Head of Research	Local Project Leader
Dr.	Levan Jugheli	Swiss TPH	Senior Research Scientist	Study Coordinator

The project leaders are qualified individuals by education and training, and responsible for the whole project. All further key persons are also qualified by education and training to perform their assigned tasks and responsibilities.

II. Signatures

Study Title: Linking the within-host and between-host evolution in multidrug-resistant *Mycobacterium tuberculosis*

The following project leaders have approved the protocol version ***[883582 – ECOEVODRTB Version_1.0_13.07.2020)]*** and confirm hereby to conduct the project according to the current version of the World Medical Association Declaration of Helsinki /*, and Essentials of Good Epidemiological Practice issued by Public Health Switzerland (EGEP) / ISO EN 14155/ CIOMS International Ethical Guidelines for epidemiological studies 2009* as well as all national legal requirements and guidelines as applicable.

Project Leader

- I have read this protocol (*Version_1.0_13.07.2020*) and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.
- I will ensure that all individuals and parties contributing to this study are qualified and I will implement procedures to ensure integrity of study tasks and data.
- I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed and trained regarding their activities within the study conduct.
- I will use only approved informed consent formsand will fulfil all responsibilities for submitting pertinent information to the Independent Ethics Committees responsible for this study.
- It is understood that this protocol will not be disclosed to others without prior written authorisation from the Project Leader or Sponsor, except where required by applicable local laws.

Project Leader (lead center/site)

Signature		
Name	Sebastien Gagneux	Date of Signature
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Local Project Leader at local center/site

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Main Study Coordinator

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III. Abbreviations / Glossary of terms

ERC	European Research Council
BSL2	Biosafety Level 2 Laboratory
BSL3	Biosafety level 3 Laboratory
CDC	Center for Disease Control
CRF	Case Report Form
DST	Drug susceptability Test
ECOEVODRTB	Ecology and Evolution of Drug Resistance Tuberculosis
ERCEA	European Research Council Executive Agency
GDPR	General Data Protection Regulation
HRA	Human Research Act
HRO	Ordinance of Human Research with the Exception of Clinical Trial
ICF	Informed Consent Form
IEC	Independent Ethics Committee
KOFAM	KoordinationsstelleForschung am Menschen (Federal Office of Public Health's (FOPH) portal for human research in Switzerland)
LEC	Local Ethics comitee
MDR-TB	Multidrug Resistant Tuberculosis
MIRU-VNTR	Mycobacteria Interspread Repetitive Unit- Variable Number Tandem Repeat
Mtb	*Mycobacterium tuberculosis*
NCTLD	National Center for Tuberculosis and Lung Disease
NTP/MoH	Georgian National TB Control Program/ Ministry of Health
PBMCs	Peripheral Blood Mononuclear Cells
sciCORE	Center for Scientific Computing
SNP	Single Nucleotide Polimorphism
SOP	Standard Operating Procedure
SWISS TPH	Swiss Tropical and Public Health Institute
TB	Tuberculosis
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR-TB	Extensive Drug Resistant Tuberculosis

IV. Synopsis

Sponsor	ERC
Project Leader	Sebastien Gagneux
Study Title	**Linking the within-host and between-host evolution in multidrug-resistant *Mycobacterium tuberculosis***
Short Title/Study ID	ECOEVODRTB
Protocol Version and Date	Version_1.0_13.07.2020
Study Category with Rationale	Research projects (HRO) - Research projects – involving measures or sampling of biological material or collection of health-related data from persons. Research projects (HRO) - involving further use of biological material and health-related personal data
Background and Rationale	*Mycobacterium tuberculosis*(*Mtb*) is the main cause of human tuberculosis (TB) and kills 1.5 million people each year. In particular, multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) represent major threats to global health.Recently, three new TB drugs have been approved for the treatment of MDR/XDR-TB: bedaquiline, delamanid and pretomanid. These new drugs are currently being rolled out, particularly in regions with a high burden of MDR/XDR-TB such as the country of Georgia. There is a real danger that resistance to these new drugs will arise rapidly. However, little is known on how Mtb develops resistance inside TB participants undergoing treatment. Such knowledge could help reduce the development of resistance to these and future new TB drugs. Hence, the goal of this project is to explore the causes and consequences of within-host evolution in multidrug-resistant *Mtb* over time and across different body compartments, to look for potential trade-offs with between-host evolution during participant-to-participant transmission, and to define the role of suboptimal participant treatment and phenotypic drug tolerance on drug resistance evolution inside patients and across patient populations.
Objective(s)	1) Study the genomic characteristics and evolutionary forces shaping multidrug-resistant *Mtb* populations in individual TB patients over time and across different body compartments. 2) Compare the genomic and phenotypic properties of multidrug-resistant *Mtb* populations in individual patients to those circulating within the corresponding patient population. 3) Determine the effect of suboptimal patient treatment and phenotypic drug tolerance on drug resistance evolution in *Mtb* inside participants. 4) Capacity building:Georgian scientists, students and institutions will benefit from the collaboration with Swiss TPH.
Primary Endpoint Secondary Endpoints	To understand the transmission dynamics of drug-susceptible and drug-resistant *Mtb* in Georgia over time. To understand how these dynamics are influenced by the genetic and phenotypic diversity of *Mtb* within participants during treatement.
Study Design	This study comprises four Modules. For Study **Modules 1 and 2**, the design of is population-based and nationwide, and thus includes all culture-positive TB cases presenting at any of the health facilities of the Georgian National TB Control Program/ Ministry of Health (NTP/MoH) during the study period. The study will be conducted retrospectively and a prospectively. The retrospective arm will use the data collected during our previous study conducted from 2013 to 2019 (Module 1). The prospective arm will use the same design and run from 2020 to 2025 (Module 2). Combining the data from the retrospective

	and prospective arms allows for a detailed analysis of the TB transmission dynamics in Georgia. Moreover, the prospective arm is particularly important for inferring the effect of the within-patient dynamics of *Mtb* evolution on TB transmission, which is the main research focus of this project.Molecular analyses of bacterial Mtb isolates will be performed using established techniques, which include SNP-typing, spoligotyping, MIRU-VNTR, targeted gene sequencing, and whole-genome sequencing.All bacterial Mtb isolates will be genetically characterized by whole genome sequencing. **Module 3**: this module will focus on MDR/XDR-TB patients who will be recruited prospectively, following informed consent, and for whome more detailed demographic and epidemiological data as well as additional sputum samples will be obtained. Moreover, for a subset of study participants, for whom at least two sequential Mtb isolates were recovered during their TB episode 50ml of human blood will be obtained upon successful treatment completion and cure. patients, This blood will be used for the patient-matched macrophage-based virulence assays. **Module 4**: we will take advantage of the fact that in Georgia, the NTP/MoH occasionally carries out lung surgical resection in difficult-to-treat TB patient as an adjunctive treatment procedure. Through these procedures, we can have access to the resected lung tissue, from which we will characterize the Mtb bacteria by culture and/or direct whole genome sequencing of bacterial DNA. Hence, this module will recruit prospectively any TB patient undergoing lung surgery and who agrees to participate in the study. Like Module 3, additional demographic and epidemiological data as well as additional sputum samples will be obtained following informed consent.
Inclusion/Exclusion Criteria	For Study **Modules 1 and 2**, study participants will be all culture-positive TB patients presenting at any of the health facilities in Georgia and no inclusion or exclusion criteria will apply. For Study **Modules 3 and 4**, the study participants are required to meet all the following inclusion criteria: 1.Signed written consent prior to undertaking any study-related procedures by participants or legally authorized representative. 2.Willingness and ability to attend scheduled follow-up visits and undergo study assessments. 3.Male or female, aged 18 years or over. 4. Patients diagnosed with MDR XDR-TB or/and undergoing lung surgery for adjunctive TB treatment For Study **Modules 3 and 4**, potential participants will be excluded if they meet any of the following criteria: 1. Individuals who are unable to provide informed consent 2.Children (< 18 years) 3.Any condition in the investigators' opinion, where participation in the study would compromise the well-being of the individual or prevent, limit or confound protocol specified assessments. 4.Prisoners
Measurements and Procedures	**Module 1 and 2**: The study participants will be all culture-positive TB patients, who will be included in the study through the routine diagnostic procedures established at NTP/MoH and by NTP/MoH staff. These routine procedures include a questionnaire, sputum smear-microscopy, primary (i.e. initial) culture of *Mtb* isolates from the sputum of these patients, phenotypic drug susceptibility testing (DST), and molecular DST. Participant questioning and the smear-microscopy are being performed routinely in one of 62 TB clinics in the country by NTP/MoH staff, and the primary bacterial cultures are carried out in the two culture facilities in Georgia by NTP/MoH staff. All of the above is part of the routine work performed in the frame of the routine NTP/MoH activities in Georgia, and is independent of this ERC project. After DST has been performed at NCTLD, the bacterial *Mtb* isolates will be subcultured by NTP/MoH staff

	and stored at NCTLD for further analysis in the frame of the ERC project as described above. The NTP/MoH staff will pseudonymize the samples before forwarding the sub-cultured bacterial isolates to ERC staff specially recruited for this project at NCTLD, who will perform the additional work. **Module 3**: Study participants will be MDR/XDR patients,from whom additional sputum samples will be collected at baseline (in addition to the one collected for the routine diagnosis) and at every monthly visit (in addition to the one collected for routine treatment monitoring) for i) direct sequencing of *Mtb* bacteria (without previous culturing step), and ii) sequencing of individual *Mtb* colonies after bacterial culturing on solid growth media, which allows the isolation of individual bacterial colonies.Upon successful treatment completion and cure, 50ml of human blood will be obtained from a subset of the Module 3 studyparticipants, for whom at least two sequential *Mtb* isolates were recovered during their TB episode For **Module 4**,we will take advantage of the fact that in Georgia, the NTP/MoH occasionally carries out lung surgical resection in difficult-to-treat TB participants as an adjunctive treatment procedure. Through these procedures, we can have access to the resected lung tissue, from which we will characterize the *Mtb* bacteria by culture and/or direct sequencing of bacterial DNA
Number of Participants with Rationale (if no Power Analysis conducted)	**Module 1 and 2**: Our nationwide epidemiological study of TB transmission in Georgia comprises a retrospective and prospective arm. The retrospective arm will include all the culture-positive TB patients (including children) and their bacterial *Mtb* isolates collected from 2013 to 2019, and which have been stored at NCTLD (N=11,419 participants, N=18,223 *Mtb* isolates). The prospective arm will include all culture-positive TB patients in the country recruited from 2020 to 2025 (approx. N=2,000 per year). **Module 3**:Based on the reported cases in recent years in Georgia, we expect to recruit 100-200 MDR/XDR-TB patients per year as study participants for this arm of the project. **Module 4**: According to the available information, 70-80 lung surgeries are carried out on TB patients by the Georgian NTB/MoH at NCTLD each year. Isolating bacterial Mtb isolates from the resected lung is not very efficient. Hence, to maximize the number of *Mtb* isolates recovered from such lesions, we will include all TB patients undergoing lung surgery as our study participants.
Study Duration	The study will be performed during the 5 years funding period of the corresponding ERC Advanced Grant
Study Schedule	September 2020 of First-Participant-In (planned) September 2025 of Last-Participant-Out (planned)
Investigator(s)	Prof. Sebastien Gagneux, Swiss TPH, Head of Department,Principal Investigator Dr. NestaniTukvadze,NCTLD, Head of Research, Local Project Leader Dr. LevanJugheli, Swiss TPH, Senior Research Scientist, Study Coodinator
Study Centre(s)	Swiss TPH, Basel,Switzerland NCTLD, Tbilisi,Georgia
Statistical Analysis incl. Power Analysis	The main purpose of the genomic epidemiological arm of this study is to study the transmission dynamics of drug-susceptible and drug-resistant *Mtb* as a function of the different participant and bacterial variables, and how these dynamics are influenced by the evolution of *Mtb* within individual study participant. For this, we will use the genomic data of the *Mtb* bacterial strains to define chains of ongoing transmission and to calculate secondary case rates and compare the ratios of these rates between the different *Mtb* strains as described previously. In addition, we will explore possible associations between different *Mtb* strain genotypes

	and participant characteristics based on the pseudonymised project-relevant participant data collected through the standard diagnostic activities of NTP/MoH. Statistical analyses will be performed using R and univariate and multivariate analyses will be performed. We will use the Kruskal-Wallis, Chi2, or Fishers' exact tests as appropriate.
Ethical consideration	This project will be carried out in accordance with the research plan outlined in this protocol and with principles enunciated in the current version of the Declaration of Helsinki / Essentials of Good Epidemiological Practice issued by Public Health Switzerland (EGEP) / ISO EN 14155 / CIOMS International Ethical Guidelines for epidemiological studies 2009 as well as all national legal requirements: Order of Health Minister # 233/o (04.08.2010), about Implementation of "ICH: E6 Good Clinical Practice: Consolidated Guidance" (1996) including WMA: Declaration of Helsinki (2010) (Georgian): http://rama.moh.gov.ge/res/docs/9539N233.pdf and guidelinesas applicable. This protocol will be reviewed by the EthikkommissionNordwest- und Zentralschweiz (EKNZ, Ethics Committee of Northern and Central Switzerland) and also be reviewed and approved by the Local Ethics Committee (LEC) of the National Center for Tuberculosis and Lung Diseases of Georgia before starting the study.

2 BACKGROUND INFORMATION

2.1 Introduction

Mycobacterium tuberculosis (Mtb), the etiological agent of human tuberculosis (TB), kills 1.5 million people each year[1], despite of TB being a curable disease. Of the estimated 10 million new TB cases that occurred globally in 2018, an estimated 0.5 million (i.e. ~5% of all TB cases) were multidrug-resistant (MDR). MDR strains of *Mtb*are resistant to at least isoniazid and rifampicin, the two most important first-line TB drugs. MDR strains are often resistant to additional drugs, leading to extensively drug-resistant (XDR) TB. MDR/XDR-TB represents a major threat to global TB control, as the treatment of MDR/XDR-TB participants requires the use of second-line drugs that are more expensive, more toxic and less effective than first-line drugs[2]. As a result, treatment of MDR/XDR-TB requires lengthy regimens of up to 24 months or more, compared to the standard 6-months therapy for drug-susceptible TB. Recently, three new TB drugs have been approved for the treatment of MDR/XDR-TB: bedaquiline, delamanid and pretomanid. These new drugs are currently being rolled out, particularly in regions with a high burden of MDR/XDR-TB such as the country of Georgia. There is a real danger that resistance to these new drugs will arise rapidly. However, little is known on how *Mtb* develops resistance inside TB patients undergoing treatment. Such knowledge could help reduce the development of resistance to these and future new TB drugs. Hence, the main purpose of this project is to enhance our understanding of the evolution of drug resistance inside individual TB patients over time, and to determine how this influences the spread of drug-resistant TB from one patient to the next.

This work builds on our previous project, in which we studied the details of the patient-to-patient transmission of TB in Georgia between 2013 and 2019 using a genomic epidemiological approach (ERC project 309540-EVODRTB). In this new project, we will take advantage of, and extent upon, the existing data and bacterial *Mtb* isolates collected in the previous study period by adding a new "within-patient" component. This new work will include several experimental approaches carried out in the laboratory, which are described in detail in the **Description of Action** of the ERC Project. In addition, for the detailed epidemiological characterization of clinical strains of *Mtb*, we will continue our longitudinal population-based study of TB transmission in the country of Georgia. For the new "within-host" component, we will put an extra focus on MDR/XDR-TB patients by i) collecting detailed clinical information and serial *Mtb* isolates during the course of treatment, and ii) obtaining blood following successful treatment to be used for the detailed phenotypic characterization of the *Mtb* bacteria isolated from these patients previously. Finally, for the subset of TB patients undergoing lung surgery because of programmatic reasons (Georgia routinely performs lung surgery as an adjunctive treatment against severe TB), we will take advantage of the lung lesions dissected to isolate the *Mtb*bacteria for detailed genomic and phenotypic characterization.

3 OBJECTIVES AND PURPOSE

3.1 Study rationale and objectives

3.1.1 Study rationale

The goal of this project is to explore the causes and consequences of within-host evolution in multidrug-resistant *Mtb* over time and across different body compartments, to look for potential trade-offs with between-host evolution during patient-to-patient transmission, and to define the role of suboptimal patient treatment and phenotypic drug tolerance on drug resistance evolution inside patient and across patient populations.

We hypothesize that:

i) The genetic heterogeneity of *Mtb* within TB patient over time and across body compartments varies as a function of treatment efficacy;

ii) The genetic and phenotypic variation of *Mtb* within patient differs from the corresponding between-patient variation due to the effects of short-sighted evolution;
iii) The within-patient genetic variation further translates into phenotypic heterogeneity affecting bacterial fitness and drug tolerance, which together contribute to the development and spread of clinically relevant drug resistance.

3.1.2 Primary objective

1) Study the genomic characteristics and evolutionary forces shaping multidrug-resistant *Mtb* populations in individual TB patient over time and across different body compartments
a.Perform population genomic analyses of sequential *Mtb* isolates from patient with/without relapse and with/without amplification of drug resistance;
b. Compare the population genomic characteristics of *Mtb* from sputum to *Mtb* populations isolated from different lung lesions of the same patient.

2) Compare the genomic and phenotypic properties of multidrug-resistant Mtb populations in individual patient to those circulating within the corresponding patient population
a. Compare the population genomic characteristics of multidrug-resistant *Mtb* populations within participants to the *Mtb* population circulating in the country of Georgia;
b. Use a patient-matched ex vivo model of infection as a proxy to compare the virulence of 'early' versus 'late' versus 'transmitted' *Mtb* isolates.

3) Determine the effect of suboptimal patient **treatment and phenotypic drug tolerance on drug resistance evolution in *Mtb* inside** patient
a. Simulate different within-host environments by experimentally evolving *Mtb* ex vivo in the presence of different concentrations and combinations of TB drugs;
b. Compare the genomic and phenotypic characteristics of *Mtb* populations generated experimentally to those from 'early versus 'late' versus 'transmitted' patient isolates with respect to drug tolerance and amplification of resistance.

3.1.3 Secondary objectives

The proposed longitudinal study will allow several PhD thesis for Georgian students who will be enrolled at the University of Basel. There will be further opportunities for fellowships and Master student candidates to conduct a thesis on a topic within this study. Basic mycobacteriology such as *Mtb* cultures, phenotypic drug resistance testing and DNA extraction will be performed at the BSL3 TB laboratory in Tbilisi. This will ensure continued training of the local laboratory staff. The genotyping and sequencing of bacterial genomes and analyses of genomic data require sophisticated equipment and extensive experience with the bioinformatics analyses of large-scale data, and hence will be primarily conducted in Switzerland. However, local collaborators will always be involved in this work (including during PhD trainees), and an exchange of collaborators is planned. Finally, the key staff of NCTLD in Tbilisi, Georgia, will also be involved in this project, and benefit from the collaboration and exchanges with Swiss TPH staff.

3.2 Scientific justification of study population

Three new TB drugs have been approved for the treatment of MDR/XDR-TB. These new drugs are currently being rolled out, particularly in regions with a high burden of MDR/XDR-TB such as the country of Georgia. There is a real danger that resistance to these new drugs will arise rapidly. However, little is known on how *Mtb* develops resistance inside TB patients undergoing treatment. Such knowledge could help reduce the development of resistance to these and future new TB drugs. The goal of this project is to explore the causes and consequences of within-host evolution in multidrug-resistant *Mtb* over time and across different body compartments. The focus will be on

MDR/XDR-TB patients as these are the most likely to require an extended period of treatment, which also requires sequential culturing of *Mtb* isolates for routine evaluation of the treatment response. This work builds on our previous project, in which we studied the details of the patient-to-patient transmission of TB in Georgia between 2013 and 2019 using a genomic epidemiological approach (ERC project 309540-EVODRTB). In this new project, we will take advantage of, and extent upon, the existing data and bacterial *Mtb* isolates collected in the previous study period by adding a new "within-patient" component.

4 STUDY DESIGN

4.1 Overview of Study Design and Methods

This project includes experimental studies carried out in the laboratory, as well as a series of genomic epidemiological studies based on clinical data and samples obtained from TB patients in the country of Georgia. The majority of the experimental research work as well as the detailed characterization of the patient samples collected in Georgia will be carried out at Swiss TPH in Basel, Switzerland. All the baseline activities related to the genomic epidemiological investigations, such as participant recruitment, collection of clinical data and patientsamples, will be conducted at theNCTLD in Tbilisi, Georgia, in collaboration with Swiss TPH. NCTLD is an integral part for the NTP/MoH. NCTLD also houses the Georgian National TB Reference Laboratory, which carries out most of the routine TB diagnosis work in the country. The overall coordination and oversight of the whole project will be by Prof.Gagneux at Swiss TPH.

An overview of the different experimental and epidemiological study modules, their interconnections, as well as the type of patient data and samples collected are shown in Figure 1. For the detailed lists of patient variables and patient samples collected during this project, see **Annexes 1, 2, and 3** attached. This Study Protocol focusses on the activities related to the genomic epidemiological arm of the project. With respect to the study design of the genomic epidemiological arm of the project, the following study modules are comprised (**Figure 1**):

Module 1: A retrospective nationwide and population-based genomic epidemiological study of TB transmission in Georgia, which relies on a subset of the patient data collected through routine diagnostics activities at NCTLD (see **Annex 1**) and the corresponding bacterial *Mtb* isolates collected during our previous study from 2013-2019 (ERC project 309540-EVODRTB; see **Annex 2**). This retrospective study includes all culture-positive TB patients in Georgia, including children. Exemption for individual informed consent was obtained from the Georgian Ministry of Health under the condition that the corresponding clinical data and bacterial *Mtb* isolates were appropriately pseudonymised (this is further discussed below).

Module 2: A prospective nationwide and population-based genomic epidemiological study of TB transmission in Georgia, which relies on a subset of the patient data collected through routine diagnostics activities at NCTLD (see **Annex 1**) and the corresponding bacterial *Mtb* isolates collected prospectively from 2020-2025 (see **Annex 2**). This prospective study is a continuation of Module 1 and includes all culture positive TB patients in Georgia, including children, and will be combined with the data from the retrospective study mentioned above. Exemption for individual informed consent will be obtained from the Georgian Ministry of Health, and all the corresponding clinical data and bacterial *Mtb* isolates will be appropriately pseudonymised (this is further discussed below).

Module 3: Detailed genomic and phenotypic analyses of the *Mtb* bacteria collected sequentially from MDR/XDR-TB patients during routine treatment. Individual informed consent will be obtained from all MDR/XDR-TB patients before inclusion into the study, but children below 18 years of age and prisoners will be excluded. Informed consent will be sought for the collection of additional study module-specific participant data (see **Annex 3**), the collection and storage of additional sputum specimens, detailed analysis of all bacterial *Mtb* isolates recovered from the routinely NCTLD-collected and additional sputum samples, drawing of 50ml blood following successful

treatment termination and cure for use in ex vivo patient-matched macrophage-based virulence assays as described in the **Description of Action** of the ERC Project (see **Annex 2**).

Module 4: Detailed genomic and phenotypic analyses of the *Mtb* bacteria isolated from lung resections of TB patients undergoing lung surgery for routine adjunctive treatment purposes. Individual informed consent will be obtained before inclusion into the study from all TB patients who have been selected by the NTP/MoH for programmatic reasons to undergo lung surgery. Children below 18 years of age and prisoners will be excluded from this project module. No participant will be asked/encouraged to undergo surgery specifically for the purpose of this project. Informed consent will be sought for the collection of additional project module-specific patient data (see **Annex 3**), the use of the resected lung tissues for microbiological analyses of the *Mtb* bacteria included in these tissue samples (see **Annex 2**), obtaining an additional sputum samples before surgery, and the use of all bacterial *Mtb* isolates recovered from the routinely NCTLD-collected and the newly collected sputum sample, as well as those recovered from the resected lung tissue samples (see **Annex 2**).

In summary, all of the research activities of this project are focused on the *Mtb* bacteria isolated from TB patients in Georgia (as opposed to the patients themselves). Importantly, this project includes no patient intervention.

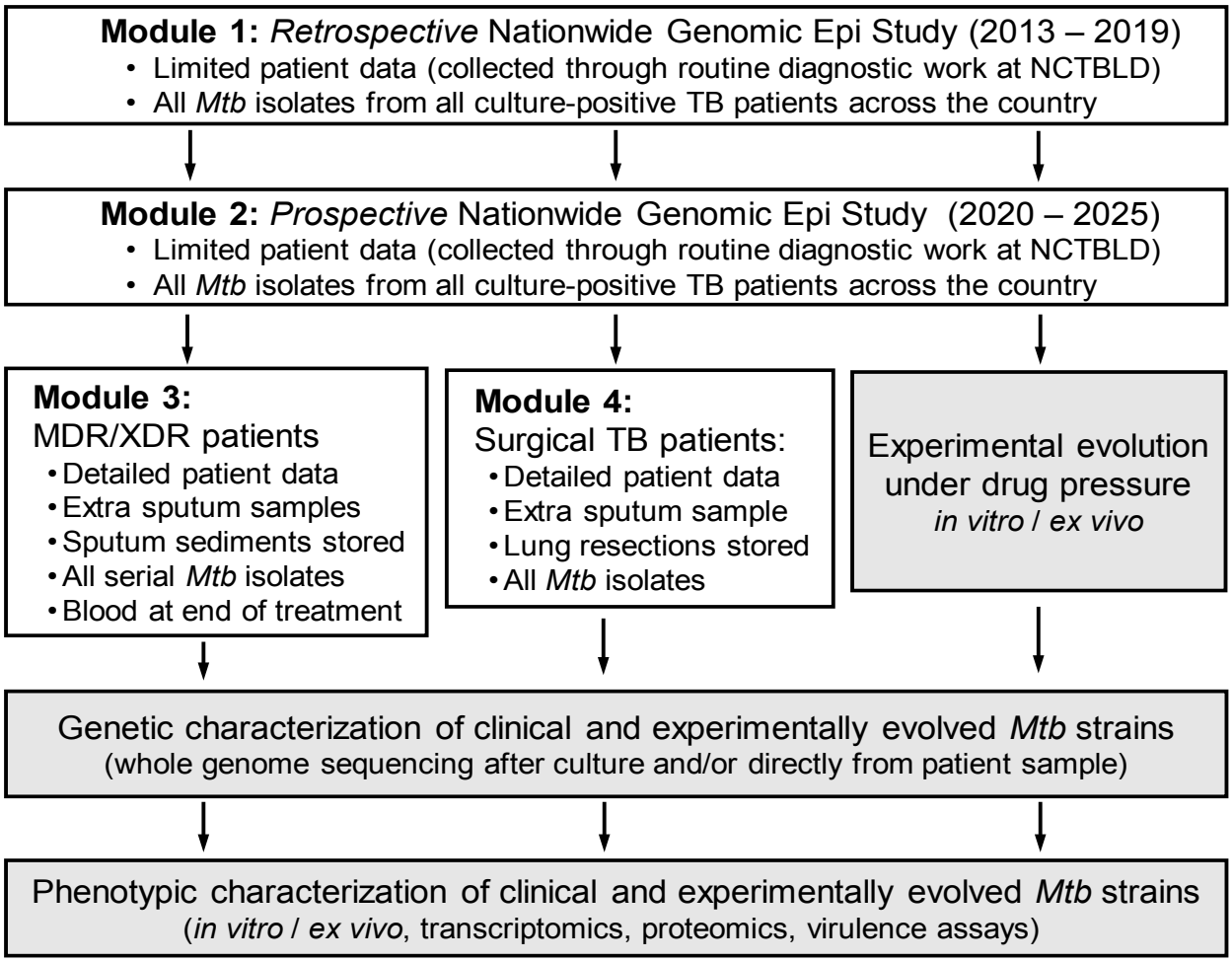

Figure 1. *Research approach and working modules. Epidemiological and experimental modules are indicated in white and grey, respectively. Arrows indicate the flow of data and/or bacterial Mtb samples. For detailed lists of the* patient *variables and* patient *samples collected in these different modules, see* ***Annexes 1, 2, and 3****.*

Module 1 and 2: Nationwide genomic epidemiological study of TB transmission in the country of Georgia (carried out at NCTLD in collaboration with Swiss TPH)

The main purpose of these two modules is to study the transmission dynamics of drug-susceptible and drug-resistant TB in Georgia over time, and to explore how these dynamics are influenced by the evolution of drug resistance within individual TB patients during routine treatment (Modules 3 and 4).

The design of this study is population-based and nationwide, and thus includes all incident culture-positive TB cases presenting at any of the facilities of the Georgian NTP/MoH during the study period. This study will be conducted retrospectively and a prospectively. The retrospective arm will use the data collected during our previous study conducted from 2013 to 2019 (Module 1). The prospective arm will use the same design and run from 2020 to 2025 (Module 2). Combining the data from the retrospective and prospective arms allows for a detailed analysis of the TB transmission dynamics in Georgia. Because TB is a chronic disease with a long period of latency, studies aimed at determining the transmission dynamics of *Mtb* increase in precision the longer they last[3]. Moreover, the prospective arm is particularly important for inferring the effect of the within-patient dynamics of *Mtb* evolution on TB transmission, which is the main research focus of this project.

Molecular characterization of bacterial Mtb isolates

Molecular characterization of all bacterial *Mtb* isolates obtained during the study period is necessary to define the number of secondary TB cases in a patient population and to measure the different transmission efficiencies of the various *Mtb* strains circulating in Georgia. For this, **pseudonymisedbacterial *Mtb* cultures obtained from TB patients recruited through routine diagnostic activities by the NTP/MoH** will be used to extract bacterial DNA according to standard procedures. The TB diagnostic procedures currently performed routinely on all patients suspected of TB by NTP/MoH at NCTLD are outlined below (**Section 4**). Molecular analyses of bacterial *Mtb* isolates will be performed using established techniques, which include SNP-typing, spoligotyping, MIRU-VNTR, targeted gene sequencing, and whole-genome sequencing.

The bacterial subculture, DNA extraction and some of the standard genotyping will be performed at NCTLD by dedicated ERC staff. For other molecular analyses (e.g. whole-genome sequencing), DNA samples extracted from these *Mtb* bacterial strains will be sent to Swiss TPH and analysed there. No ERC staff will have access to any participant identifying information at any time (see **Section 4**).

Inclusion Criteria for Module 1 and 2:

- All culture-positive TB patients diagnosed in the country of Georgia

Exclusion Criteria for Module 1 and 2:

- None

Module 3: Detailed genomic and phenotypic analyses of the Mtb bacteria collected sequentially from MDR/XDR-TB* patients *during routine treatment (carried out at NCTLD in collaboration with Swiss TPH)

The goal of this study arm is to explore the evolution of *Mtb* virulence and drug resistance over time within individual patients . The focus will be on MDR/XDR-TB patients as these are the most likely to require an extended period of treatment, which also requires sequential culturing of *Mtb* isolates for routine evaluation of the treatment response. Hence, following informed consent, detailed patient data will be collected with a project specific questionnaire and through additional CRFs with clinical and laboratory information (see **Annex 3**), and all sequential *Mtb* bacteria isolates will be recovered and stored from the routine diagnostic work (see **Annex 2**). For a subset of study participants in this Module, additional sputum samples will be collected at baseline (in

addition to the one collected for the routine diagnosis) and at every monthly visit (in addition to the one collected for routine treatment monitoring) for i) direct sequencing of *Mtb* bacteria (without previous culturing step), and ii) sequencing of individual *Mtb* colonies after bacterial culturing on solid growth media, which allows the isolation of individual bacterial colonies. These additional sputum samples are necessary for the additional analyses such as not to interfere in any way with the routine diagnostic and treatment monitoring procedures. Future technological advances might allow analysing stored (as opposed to fresh) sputum. Hence, we will also take advantage of the sputum samples collected for routine purposes, which otherwise would be discarded. Specifically, following routine processing of the sputum collected for routine diagnosis and treatment monitoring, the left-overs of the sputum sediments will be stored at -80 degrees for future analyses using direct sequencing and/or single colony sequencing of the *Mtb* bacteria contained in these samples. All bacterial *Mtb* isolates will be genetically characterized by whole genome sequencing. Upon successful treatment completion and cure, 50ml of human blood will be obtained from a subset of studyparticipants, for whom at least two sequential *Mtb* isolates were recovered during their TB episode. This blood will be used for the patient-matched macrophage-based virulence assays described in the **Description of Action of the ERC Project** (see also below).

Inclusion Criteria for Module 3:

Participants are required to meet all the following inclusion criteria:

1. Signed written consent prior to undertaking any study-related procedures.
2. Willingness and ability to attend scheduled follow-up visits and undergo study assessments.
3. Male or female, aged 18 years or over.
4. Patient diagnosed with MDR/XDR-TB

Exclusion Criteria for Module 3:

Participants will be excluded from participation if they meet any of the following criteria:

1. Individuals who are unable to provide informed consent
2. Children (< 18 years)
3. Any condition in the investigators' opinion, where participation in the study would compromise the well-being of the individual or prevent, limit or confound protocol specified assessments.
4. Prisoners

Module 4: Detailed genomic and phenotypic analyses of the Mtb bacteria isolated from lung resections of TB* patients *undergoing lung surgery for routine adjunctive treatment purposes (carried out at NCTLD in collaboration with Swiss TPH)

The goal of this study arm is to explore the evolution of *Mtb* virulence and drug resistance within individual patients from a spacial point of view. Specifically, we will take advantage of the fact that in Georgia, the NTP/MoH occasionally carries out lung surgical resection in difficult-to-treat TB patients as an adjunctive treatment procedure. Through these procedures, we can have access to the resected lung tissue, from which we will isolate the *Mtb* bacteria by culture and/or direct sequencing of bacterial DNA. Hence, following informed consent, detailed patient data will be collected with a project module-specific questionnaire and through additional CRFs with clinical and laboratory information (see **Annex 3**), and the lung tissue resection will be processed to isolate *Mtb* bacteria as described in the Description of Action (see also **Annex 2**). **No additional analyses (human genetic or otherwise) will be conducted on these lung tissue resections.** An additional sputum sample will be collected right before the surgery for i) direct sequencing of *Mtb* bacteria (without previous culturing step), and ii) sequencing of individual *Mtb* colonies after bacterial culturing on solid growth media, which allows the isolation of individual bacterial colonies. All bacterial *Mtb* isolates genetically characterized by whole genome sequencing.

Inclusion Criteria for Module 4:

Participants are required to meet all the following inclusion criteria:

1. Signed written consent prior to undertaking any study-related procedures
2. Willingness and ability to attend scheduled follow-up visits and undergo study assessments
3. Male or female, aged 18 years or over
4. TB patients undergoing lung surgery for adjunctive TB treatment

Exclusion Criteria for Module 4:

Participants will be excluded from participation if they meet any of the following criteria:

1. Individuals who are unable to provide informed consent
2. Children (< 18 years)
3. Any condition in the Investigator's opinion, where participation in the trial would compromise the well-being of the individual or prevent, limit or confound protocol specified assessments
4. Prisoners

Experimental studies performed at Swiss TPH

In addition to the genetic characterization of bacterial isolates described above, a subset of live pseudonymised*Mtb* strains will be sent to Swiss TPH in Basel for various experimental studies, with the aim of defining phenotypic differences across *Mtb* strains, particularly with respect to their virulence and tolerance and/or resistance to TB drugs. These will involve competitive fitness and virulence assays, experimental evolution, and detailed molecular characterization, including comparative genomics, transcriptomics and proteomics (**Figure 1**). All experimental work involving live *Mtb* bacteriawill be performed in a biosafety-level 3 (BSL-3) laboratory available at Swiss TPH following strict and thoroughly validated protocols, and according to international standards. Only thoroughly trained personnel will be allowed to work in the BSL-3 laboratory. All *Mtb* strains will be stored within the BSL-3 laboratory, to which only authorised personnel has access (i.e. an electronic batch system is implemented throughout Swiss TPH). For all the experimental evolution, virulence and drug tolerance assays, we will use the standardized THP-1 human cell-line, which is commercially available like any other laboratory reagant.

For the **patient-*matched macrophage-based virulence assay***, we will obtain informed consent from study participants to draw 50ml of blood following successful treatment completion and cure. PBMCs will be isolated from this blood, frozen in liquid nitrogen, and shipped to Swiss TPH for further use. Briefly, the PBMCs will be differentiated into macrophages and infected with different *Mtb* isolates obtained from the corresponding participant during his/her treatment and the virulence of these bacterial strains inside the macrophages will be compared.

4.1 Primary and secondary endpoint

4.1.1 Primary endpoint

To understand the transmission dynamics of drug-susceptible and drug-resistant *Mtb* in Georgia over time.

4.1.2 Secondary endpoints

To understand how these dynamics are influenced by the genetic and phenotypic diversity of *Mtb* within patients during treatement.

4.2 Measures to minimize bias

Study Modules 1 and 2 are population-based and nation-wide studies that include all culture-positive TB patients in the country of Georgia. Hence, we do not anticipate any bias. For Study Module 3, all MDR/XDR-TB patients who consents to participate will be included. Similarly, for Study Module 4, all TB patients undergoing lung surgery and who consent to participate will be included. Hence, we do not anticipate any major bias there either.

4.3 Study duration

The study will be performed during the 5-year funding period of the corresponding ERC Advanced Grant. Study participants will have 24h to decide whether they agree to participate in the study, and the sputum/ blood donation appointment at the NCTLD will take no longer than 1 hour.

4.3.1 Schedule of events

Period	Treatment						Follow up
Visits	Baseline[a]	Visit1[a]	Visit2[a]	Visit3[a]	Visit4[a]	Visit5[a]	FUP_Visit
Informed Consent	X						
Inclusion/Exclusion	X						
Demography	X						
Med/Trtmnt/Smoking History	X						
HIV Status[b]	X						
TB Symptoms Profile	X						
Participant Reported Health Status	X						
Vital Signs	X						
Sputum Collection	X	X	X	X	X	X	
Blood Collection[c]							X
Lung Tissue	X						

a) The visits depend on the Georgian NTP treatment regimen and timing is defined according to the Georgian national guidelines.
b) HIV status will be determined from test records performed according to the Georgian national guidelines. If no tests are done by NTP/MoH,no additional tests will be performed and the status will be recorded as unknown.
c) Total of 50ml of blood will be collected from selected participants, who have been declared cured. The follow-up visit should be done within 1 month from the end of treatment.

4.4 Amendments

Substantial changes to the project set-up; the protocol and relevant project documents will be submitted to the Ethics Committee for approval according to the Georgian and Swiss guidelines.

4.5 Discontinuation of the study

The Project Leader may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns
- insufficient participant recruitment

5 SELECTION OF STUDYPARTICIPANTS

5.1 Recruitment

Module 1 and 2: Our nationwide epidemiological study of TB transmission in Georgia comprises a retrospective and prospective arm. The retrospective arm will include all the culture-positive TB patients (including children) and their bacterial *Mtb* isolates collected from 2013 to 2019, and which have been stored at NCTLD (N=11,419 participants, N=18,223 *Mtb* isolates). The prospective arm will include all culture-positive TB cases in the country recruited from 2020 to 2025 (approx. N=2,000 per year).

All culture-positive TB patients will be included in the study through the routine diagnostic procedures established at NTP/MoH and by NTP/MoH staff. These routine procedures include a study questionnaire, sputum smear-microscopy, primary (i.e. initial) culture of *Mtb* isolates from the sputum of these participants, phenotypic drug susceptibility testing (DST), and molecular DST. We will not collect any additional patient data or perform any additional analyses on the participants themselves (or human cells collected from these participants) for the purpose of this arm of the project (Modules 1 and 2). All additional laboratory work performed on this arm of the project will focus on the bacterial *Mtb* isolates obtained from the studyparticipants through routine diagnostic procedures performed by NTP/MoH. Collection of basic patient data and the smear-microscopy are being performed routinely in one of the 67 TB clinics in the country by NTP/MoH staff, and the primary bacterial cultures are carried out in the two culture facilities in Georgia by NTP/MoH staff (i.e. NCTLD in Tbilisi and the TB Hospital in Kutaisi). All primary cultures from Kutaisi are sent to NCTLD for routine DST by NTP/MoH staff. All of the above is part of the routine work performed in the frame of the routine NTP/MoH activities in Georgia, and is independent of the ERC project. After DST has been performed at NCTLD, the bacterial *Mtb* isolates will be subcultured by NTP/MoH staff and stored at NCTLD for further analysis in the frame of the ERC project as described above. The NTP/MoH staff will pseudonymize the samples before forwarding the sub-cultured bacterial isolates to ERC staff specially recruited for this project at NCTLD, who will perform the additional work as outlined above. Similarly, a project relevant subset of patient data (see Annex 1) collected through the routine TB questionnaire used by NTP/MoH will be pseudonymized by the NTP/MoH staff before forwarding to the ERC staff. Hence, the ERC personnel will not have access to any participant identifying information.

Incidental Findings Policy

The link between the bacterial isolate of the study participant, the clinical data and the identification of the participant will be maintained by the NTP/MoH in case some of the information generated during the ERC project becomes beneficial to individual participants or to NTP/MoH more generally. NTP/MoH will be in charge of informing the “Central Drug-Resistant TB committee (DRC)” composed from NCTLD medical doctors, clinical microbiologists, clinical pharmacists and social workers. DRC is a countrywide entity to assign the appropriate MDR/XDR-TB treatment regimen and/or take clinical decisions on regimen modifications for the whole country of Georgia. DRC members are regional and WHO experts having a vast experience in clinical management of TB patients and amongst the first in the region to start compassionate use of new anti-TB drugs such as bedaquiline and delamanide and also first to roll-out new drugs for country-wide use per most recent WHO guidelines. Any incidental findings provided by the project staff, on additional or acquired resistance missed by standard phenotypic tests, will be discussed within the DRC committee and appropriate modification of treatment algorithm will be ensured for the patient.

Module 3: Based on the reported cases in recent years in Georgia, we expect to recruit 100-200 MDR/XDR-TB patients per year for this arm of the project. As at the beginning of treatment, it will be unclear how many study participants will have multiple sequential *Mtb* isolates available for study, we will recruit all MDR/XDR-TB participants that agree to participate in the study. However, children below of 18 years of age and prisoners will be excluded. Individual informed consent will include providing additional sputum samples at baseline and during the monthly follow-up visits. These additional sputum samples will be collected from a random subset of 50 participants per year. The informed consent will also include donating 50ml of blood after successful treatment completion and cure. However, we will restrict the total number of blood draws to 100 over the whole 5-year study period; 50 from participants who developed additional resistance to TB drugs

during treatment and 50 who did not. All collected participant data and samples (bacterial *Mtb* isolates and blood) will be pseudonymised before shipment to Swiss TPH in Basel and further processing.

Module 4: According to the available information, 70-80 lung surgeries are routinely carried out on TB patients by the Georgian NTB/MoH at NCTLD each year. Isolating bacterial *Mtb* isolates from the resected lung is not very efficient. Hence, to maximize the number of *Mtb* isolates recovered from such lesions, we will recruit all TB patients undergoing lung surgery. No lung surgery will be performed for the sole purpose of this study. Instead, we will only take advantage of the availability of resected lung tissue samples that were generated because of routine TB treatment processes and that would be discarded otherwise. Children below the age of 18 years and prisoners will be excluded from this study module. Individual informed consent will include giving one additional sputum sample before lung surgery and the permission to use the resected lung tissue for microbiological research work on the *Mtb* bacteria as outlined in the **Description of Action**. All collected participant data and samples (bacterial *Mtb* isolates) will be pseudonymised before shipment to Swiss TPH in Basel and further processing.

5.2 Inclusion criteria

Described in 4.1

5.3 Exclusion criteria

Described in 4.1

5.4 Criteria for discontinuation of study

5.4.1. Discontinuation of individual participants

A participant will be withdrawn from the study due to the following:

- Participant withdraws consent
- Investigator considers it for safety reasons in the best interest of the participant that he/shebe discontinued

6 STATISTICS

6.1 Hypothesis

We hypothesis that a) the genetic heterogeneity of *Mtb* within TB patients over time and across body compartments varies as a function of treatment efficacy; b) the genetic and phenotypic variation of *Mtb* within patients differs from the corresponding between-patient variation due to the effects of short-sighted evolution, and c) the within-patient genetic variation further translates into phenotypic heterogeneity affecting bacterial fitness and drug tolerance, which together contribute to the development and spread of clinically relevant drug resistance.These hypotheses will be tested by characterizing genomically and phenotypically MDR/XDR TB strains in Georgia.

6.2 Determination of sample size

For Study Modules 1 and 2, the design is population-based and nationwide, and thus includes all culture-positive TB cases presenting at any of the 67 facilities of the Georgian NTP/MoH from 2013 to 2025. For Study Module 3, based on the reported cases in recent years in Georgia, we expect to recruit 100-200 MDR/XDR-TB patients per year for this arm of the project. As at the beginning of treatment, it will be unclear how many studyparticipants will have multiple sequential *Mtb* isolates available for study, we will recruit all MDR/XDR-TB patients that agree to participate in the study. For Study Module 4,70-80 lung surgeries are routinely carried out on TB participants by the Georgian NTB/MoH at NCTLD each year. Isolating bacterial *Mtb*strains from the resected

lung is not very efficient. Hence, to maximize the number of *Mtb* isolates recovered from such lesions, we will recruit all TB patients undergoing lung surgery during the study period.

6.3 Description of statistical methods

Statistical analyses will be performed using R and univariate and multivariate analyses will be performed. We will use the Kruskal-Wallis, Chi2, or Fishers' Exact Test as appropriate.

Datasets to be analysed, analysis populations: The datasets to be analysed will comprise of WGseq data, phenotypical DST data, clinical data, colony forming unit data and cytokine data obtained from *Mtb* infected macrophages of the study participants.

Primary Analysis: The main purpose of the genomic epidemiological arm of this study is to study the transmission dynamics of drug-susceptible and drug-resistant *Mtb* as a function of the different patient and bacterial variables, and how these dynamics are influenced by the evolution of *Mtb* within individual patients. For this, we will use the genomic data of the *Mtb* bacterial strains to define chains of ongoing transmission and to calculate secondary case rates and compare the ratios of these rates between the different *Mtb* strains as described previously.

Secondary Analyses: We will explore possible associations between different *Mtb* strain genotypes and patient characteristics based on the pseudonymised project-relevant patient data collected through the standard diagnostic activities of NTP/MoH.

Interim analyses: The genomic epidemiological data will also give the opportunity to evaluate in deep the percentage of MDR/XDR TB recently transmitted over the years, providing extremely relevant data to the TB control programme in Georgia.

6.4 Handling of missing data

Great care will be taken to collect all relevant study data. If, nevertheless, there will be cases where data is missing for some participants, it will be attempted to restore/obtain information from source documents (e.g. patent treatment file, lab records, etc). If it will not be possible to retrieve data, the participant will not be included in the final data analysis.

7 DESCRIPTION OF DATA MANAGEMENT

7.1 Specification of source documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's study participants (source data). The investigator may need to request previous medical records or reports (if available), depending on the study. Source documents will be filed at the investigator's site. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary.

All source documents pertaining solely to this study (**Modules 3 and 4**) will be maintained by the study investigators. These are:

- Completed paper based participant informed consent forms will be stored in a locked cabinet at the NCTLD and will only be accessible by the local study PI in Georgia.
- Completed paper based contact sheet which will contain participant NTP/MoH Case ID and two phone numbers (primary and secondary), which will be attached and stored together with the ICFs. These phone numbers will not be entered into electronic ECOEVODRTB project database and will be used only to contact the participants for study follow-up visit.
- Electronic CRF (eCRF) for clinical data for Modules 3 and 4 (**Annex 3**).

- Molecular and experimental data generated will be securely stored at Swiss TPH
- Next Generation Sequencing data of the bacterial *Mtb* isolates will be stored on sciCORE in Basel (www.scicore.unibas.ch)

Source documents of the routine NTP/MoH data collection (DS1 and DS2) will be maintained by NCTLD according to local standards.

7.2 Data management system

Four sources of data will be used for this study (see **Figure 2**):

DS1. National TB case database (data used for Modules 1, 2, 3, 4)
DS2. TB Reference laboratory database (data used for Modules 1, 2, 3, 4)
DS3. Clinical Data collected specifically for this study (data used for Modules 3 and 4).
DS4. Experimental data collected during the course of this study

The relevant data (see **Annex 1**) will be stripped of all participant personal information (pseudonymised) and will be extracted from sources 1 and 2 into the ECOEVODRTB electronic database. The data extraction will be performed by the NCTLD staff and will be imported into the ECOEVODRTB database by the local Study Coordinator. Data collected specifically for the study (see **Annex 3**) will be electronically captured directly into the ECOEVODRTB project database without any participant personal information (**Figure 2**). Data capture will be done by the Study Doctor and the Clinical Research Coordinator (CRC).

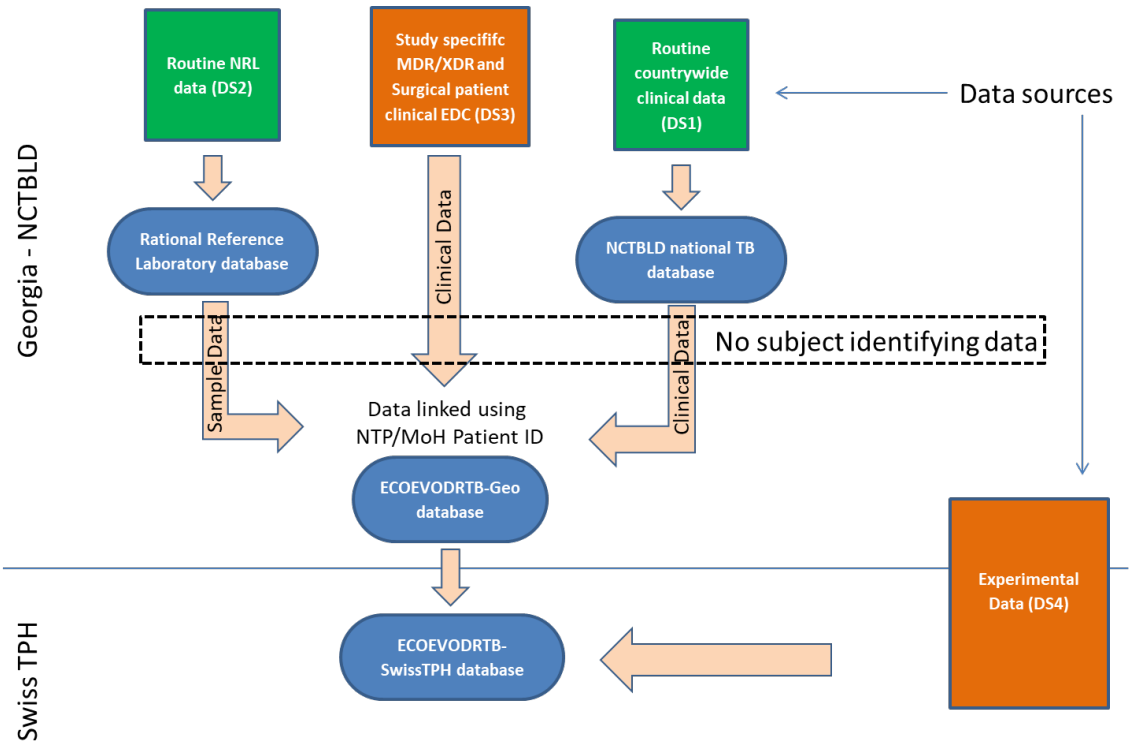

Figure 2.*The collection and flow of participant data and clinical samples in this project. Some participant data and clinical samples are collected through the routine diagnostic processes by the Georgian NTP/MoH via the Rationale Reference Laboratory Database and the NCTLD National TB Database. For* ***Module 3*** *and* ***4*** *additional data and participant samples will be collected for this project. All participant data, all clinical specimens and all bacterial Mtb isolates from* ***Module 1,2, 3 and 4*** *will be* ***pseudonymisedbefore entering the ERC project database in Georgia and only pseudonymiseddata and samples will be sent to Swiss TPH in Switzerland****.*

Experimental data generated during the course of the study will be electronically collected using various tools depending on used methods, and will be joined to the main database and analysed by the Swiss TPH project personel.

The data from all different sources will be linked using unique NTP/MoH Case ID. The ECOEVODRTB project data will be stored, managed and analyzed on separate and dedicated computers. This database will be password protected and access will be granted only to the ECOEVODRTB project staff.

7.3 Confidentiality and coding

All participant clinical and sample data will be "pseudonymised", as defined in General Data Protection Regulation (GDPR) of EU under article 4(5). Namely, participants will be assigned a unique case identifier ("Case ID") generated by the NTP/MoH (**Figure 3**). Any participant records or datasets that are transferred to the ECOEVODRTB personnel will contain this identifier only, **while participant names or any information which would make the participant identifiable will not be transferred.** The participant will be informed that his/her personal study-related data will be used by Swiss TPH in accordance with local and EU data protection law. The level of disclosure will also be explained to the participant. The participant will be informed that his/her medical records may be examined by quality assurance auditors or other authorized personnel appointed by the sponsor, by the appropriate ethics review board members, and by inspectors from regulatory authorities.

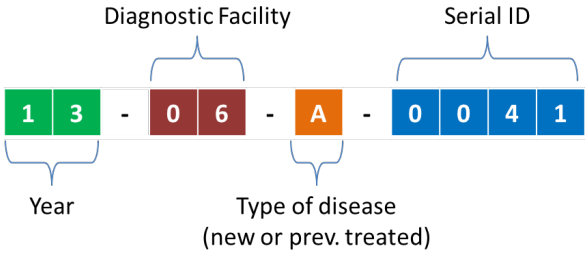

Figure 3. *Example of the unique NTP/MoH Case ID*

NTP/MoH will maintain the link between the Case ID and the study participants information on their system in case some of the information generated during the ERC project becomes beneficial to individual participants (e.g. additional drug resistance information for treatment adjustments) or to NTP/MoH more generally (e.g. information on TB transmission hot-spots).NTP/MoH will be in charge of informing the participants or treating physicians, and sharing this information within the NTP/MoH accordingly.

In addition to study related data, the participant will be requested to provide two phone numbers, which will be recorded on a separate paper based contact sheet. These phone numbers will not be entered into electronic ECOEVODRTB project database and will be used only to contact the participants for any potential follow-up visit (i.e. for blood drawings in Study Module 3). These separate paper sheets will be stored in a locked cabinet at the NCTLD and will only be accessible by the local study CRC who will contact those participants who will be selected for follow-up visit for blood collection (see paragraph 4).

7.4 Retention and destruction of study data and biological material

The bacterial samples and data collected during this study will not be destroyed at the end of the project as we are planning to pursue this study long-term in the frame of our on-going collaboration between NCTLD and Swiss TPH with future funding. Hence, these data will be extremely valuable for the following phases of the project.

Participant-related data will be stored indefinitely after publication of the research findings. Biological material will also be partially stored (backup sample) indefinitely after data collection. DNA will be stored at -80 °C until it will be entirely consumed to build libraries, required to generate genome sequencing data.

Participant contact sheets with contact phone numbers will be destroyed at the end of the project.

7.5 Data security, access, archiving and back up

The data will be securely stored at Swiss TPH. All sequencing data will be stored at the sciCORE high performance computing cluster of the University of Basel with access restricted to the study investigators.
All the data analysis will be stored or copied to Swiss TPH's file server. The file server is protected by a documented multi-level backup procedure which is audited on a regular basis. At the end of the project, data will be deposited in an appropriate data depository that complies with the FAIR principles (e.g. Zenodo). Where possible files will be stored in open archival formats e.g. word files converted to .pdf or .txt files and excel files converted to .csv. Where this is not possible information on software used and version number will be included.

8 QUALITY CONTROL AND QUALITY ASSURANCE

Process compliance will be regularly supervised by a senior scientist from the Swiss TPH. Data integrity will be assured by testing for consistency. The ERC project will only use a project-relevant subset of these data (after pseudonymisation) in addition to the pseudonymisedbacterial isolates, sputum and blood samples for further analyses.

The data generated through this project will be stored and managed in dedicated database, which will be accessible in case of audits. For example, ERCEA requested for us to appoint an independent ethics advisor who will need to file an ethics report together with each of our Periodic Reports to ERCEA.

The pseudonymisedbacterial samples and ERC project-relevant participant data will not be destroyed at the end of this 5-year project as we are planning to pursue this study long-term in the frame of our ongoing collaboration between NCTLD and Swiss TPH with future funding. Hence, the data from the first 5 years will be extremely valuable for the following phase of the project.

8.1 Supervision / Continuous Checks

The Investigator and study coordinator is responsible for the validity of all data collected at the site and mustaccept the various supervision procedures employed by the Swiss TPH. The purpose of supervisionis to verify that the rights and well-being of human participants are protected, that study data areaccurate, complete and verifiable with source data, and that the study is conducted in compliancewith the protocol.

The Study Coordinator will conduct regular site visits before, during and after the study for the purpose of supervising various aspects of the study, and to assure appropriate conduct ofthe project in accordance with the Declaration of Helsinki and the applicable regulatory requirements. The Investigator and site staff will allow the StudyCoordinator to inspect all CRFs, written informed consent documents and corresponding source documents (e.g., original medical records), participant records and laboratory raw data, site SOPs (where applicable), training records, facilities and other study related systems/processes. The Study Coordinator will query any missing, confusing, spurious, or otherwise ambiguous data with theInvestigator.

8.2 Confidentiality, data protection

Described in detail in 7.3 "Confidentiality and coding"

Only investigators involved in the study will have access to protocols, datasets, statistical codes, etc. during and after the study. In some cases, scientific journals may request access to raw data, which will be provided. All parties will be bound to strict confidentiality.

8.3 Translations - Reference language

All study documents will be available in English and some in Georgian (ICF and CRF).

8.4 Storage of biological material and related health data

Described in detail e.g. to “Data security, access, archiving and back up ”

9 ETHICAL CONSIDERATIONS

9.1 Independent Ethics Committee (IEC)

This Study Protocol and associated documents will first be sent to the relevant Georgian Ethical Review Board and the Georgian Ministry of Health, and then to the relevant Swiss Ethical Review Board (i.e. EthikkommissionNordwest- und Zentralschweiz, Switzerland) for approval. These documents and all the authorisation documents will then be forwarded to the Ethics Review Sector of the ERCEA.

9.2 Risk-benefit ratio

This project will offer local benefit to the NTP/MoH in Georgia, both in terms of medical and epidemiological terms. On the one hand, individual participant might benefit from some of the additional molecular analyses performed on their bacterial isolate (e.g. detection of additional drug resistance). On the other hand, the NTP/MoH will benefit from a better understanding of the *Mtb* strains circulating in Georgia, by the identification of transmission hot-spots and on the nation-wide situation and dynamics of drug resistance in the country over time. Moreover, the Georgian collaborators will continue to benefit in terms of capacity building (i.e. training of Georgian scientists, laboratory technicians, and other local staff in molecular techniques and data analysis), as has been the case during the many years of our joint collaborative partnership. Finally, the Georgian collaborators will be co-authors on all joint publications, conference abstracts and oral presentations based on data generated through this collaborative research project, as we have been doing until now.

9.3 Participant information and consent

The nationwide genomic epidemiological study of TB transmission (**Module 1 and 2**) has to be population-based in order to generate reliable and informative data on TB transmission in Georgia. This means that all culture-positive TB cases will be included nationwide, including children, as was done in the previous project phase. This makes obtaining written informed consent on all suspected TB patients unpractical because several thousand TB-suspectedpatients are seen at 67 different clinics in the country every year. **Hence, instead of obtaining individual written informed consent from each TB patient, we will apply for exemption at the Georgian Ministry of Health** and all the relevant Ethical Review Boards in Georgia and Switzerland, both for the retrospective as well as the prospective TB patients included in our study. We think that such an exemption is justified when considering the following: The study **Modules 1 and 2** build on routine TB diagnosis work performed by NTP/MoH at NITBLD in Tbilisi, Georgia. These routine activities are part of the national Standard of Care for TB patients in Georgia and include sputum collection, sputum smear-microscopy, culture of sputum samples, phenotypic and molecular drug susceptibility testing (DST) on isolated bacterial *Mtb* cultures. All the additional experimental work proposed in this project will be performed on pseudonymized bacterial *Mtb* isolates that are already being collected through routine diagnostic procedures performed by NTP/MoH at NITBLD, and no additional patient samples will be collected for the purpose of these study modules. This is true for both the retrospective and the prospective samples (Modules 1 and 2). Moreover, these additional analyses will be performed on

the bacteria and not on the participants themselves, their human cells, or on their blood. The only participant data used in these study modules are a subset of the pseudonymized demographic and epidemiological data that are also being (have been) collected through routine diagnosis procedures by NTP/MoH (**Annex 1**). As mentioned above, NTP/MoH staff will remove all participant identifying information from the bacterial cultures and the participant data before making them available to the ERC project crew (**Figure 3**). Hence, participant confidentiality will be guaranteed at all times during this project.

For the genomic epidemiological **Modules 3 and 4,** written individual informed consent will be obtained before any study-related procedures are performed. (see **ICF-1 and ICF-2** attached) Investigators may discuss the availability of the study and the opportunity for entry with a potential participant without first obtaining consent.

The Investigators have both ethical and legal responsibility to ensure that each participant being considered for inclusion in this study is given a full explanation of the protocol. Participants must be informed that their participation is voluntary. The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study. In addition the participant will be asked if he/she agrees to have GPS home data collected, to which the participant might opt out without being excluded from the rest of the studyThis will be documented on a written informed consent form that shall be approved by the same ethics review authorities responsible for approval of this protocol. Each informed consent form shall adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Once the appropriate essential information has been provided to the participant and fully explained by the Investigators (or qualified designees) and it is felt that the participant understands the implications of participating, the written informed consent form will be signed and dated by both the participant and the person obtaining consent (Investigators or designees). The original signed informed consent form will be kept with the study records and a copy of signed informed consent form will be provided to the participant or the participant's legally authorized representative.

Children below the age of 18 years and prisoners will be excluded from study Modules 3 and 4.

9.4 Participantconfidentiality

All site staff, the ERC project staff will preserve the confidentiality of all participants taking part in the study, in accordance with principles of the Declaration of Helsinki and applicable local legislation/regulations. Project data will be handled with uttermost discretion and will only be accessible to authorized personnel who require the data to fulfil their duties within the scope of the research project. On the CRFs and other project specific documents, participants will only be identified by the NTP/MoH Case ID.

Access to the sciCORE servers at Swiss TPH/University of Basel where the pseudonymized bacterial genome sequencing data will be stored will be restricted to the investigators of this project. Hence, data will be protected from alteration, deletion, copying, and theft. All backups will be performed on sciCORE.

Biological material (**Annex 2**) in this project will be identified by sample ID. Biological material will be appropriately stored in a restricted area only accessible to authorized personnel. Restricted access to BSL-2 and BSL-3 laboratories will prevent unauthorized or accidental disclosure of biological material.Hence, the samples will be protected from alteration, destruction, or theft. Biological material will be processed only by the study investigator to ensure traceability.

9.5 Participants requiring particular protection

For Study Modules 1 and 2, children will be included but only through the routine TB diagnostic activities as outlined in 4.1. The study Modules 3 and 4 will solely includeculture-positive TB patients who are adults

9.6 Participant compensation

Study participants of Module 3 and 4 will receive an economical compensation.

9.7 Other aspects

In Georgia, all work related to the genomic epidemiological modules of the project (as outlined above) will be carried out at NCTLD. All work involving live*Mtb* will be performed in the BSL-3 National TB Reference laboratory available at NCTLD according to the national standards of the Georgian NTP/MoH by specially trained personnel. As mentioned above, all the routine aspects of TB diagnosis, including the primary culturing and DST of *Mtb* will be conducted by NTP/MoH personnel. ERC personnel will then take over the pseudonymised anonymized cultures and perform some sub-culturing and DNA extractions, all of which will also be performed in the BSL-3 facility of NCTLD. Cultures will be stored in dedicated -80 freezers which is only accessible by authorized personnel.

All experimental work will be conducted at Swiss TPH. All the work performed with live *Mtb* will be carried out in the BSL-3 laboratory of Swiss TPH by specially trained personnel and following well defined Standard Operating Procedures (SOPs), which are based on international recommendations by the CDC and WHO and have been approved by the Swiss authorities for occupational health. The planned experimental evolution of *Mtb* in presence of drug will be limited in generating resistances to a maximum of two drugs. Hence, the resulting mutants will never reach the level of drug resistance observed in clinical strains (some of which are resistant to more than 4 drugs), and therefore will never be more dangerous than any *Mtb* strains naturally occurring in Georgia or other clinical settings.

All live *Mtb* strains, including clinical isolates obtained from Georgia will be stored in a -80 freezer within the BSL-3 laboratory of Swiss TPH. This BSL-3 laboratory is highly secure, and only a few authorized individuals have access to it. Transport of BSL-3 material from Georgia to Swiss TPH will be carried out according to standard international regulations using specialized commercial agencies (e.g. World Courier). Note that Georgia (as many countries with a high TB endemicity) regularly ships *Mtb* strains (including highly drug-resistant ones) out for the country to international WHO Supranational Reference Laboratories for quality control of their local diagnostic capacity.

Finally, potential concerns relating to military/bio-terror abuse or misuse (i.e. “dual use”) of the *Mtb* strains used in this project do not apply because i) as outlined above, none of the experimentally evolved *Mtb* strains in this project will be more dangerous than those occurring naturally (i.e. in clinical settings), and ii) because all *Mtb* strains will be stored in dedicated -80 freezers within laboratory facilities which are highly restricted and only accessible by authorized personnel.

10 FUNDING

This project is funded by the ERC with the grant number 883582-ECOEVODRTB. The research team attests that there is no conflict of interest and that this study will be conducted independently of outside influences in terms of specific intellectual, financial, and proprietary agendas.

Budget overview

Item	Value (EUR)
Personnel	1,521,200

Travelling and meetings	20,000
Supplies (labprocessing, culture, etc.)	421,300
Other (database, ethics fees, telecommunication)	37,500
Subtotal (direct costs)	2,000,000
Overhead costs (25%)	500,000
Total	**2,500,000**

11 DISSEMINATION OF RESULTS AND PUBLICATION POLICY

11.1 Dissemination to scientific community; incl. lead in publications

Data will not be transferred to any other parties. Raw data may only be shared upon request of the scientific journal for publication purposes, and all parties will be bound to strict confidentiality. The research team declares no conflict of interest for this study.
The exact timeline for dissemination of the project's results will depend on the progress of the work. Research outcomes will be presented on at least one international and one national conference, both to disseminate it and to receive guidance and feedback from the scientific community. In addition to presentation at conferences, the results of the project will be written up and submitted to high-ranking international journals. These traditional academic methods of dissemination will be combined with a comprehensive outreach program in order to communicate the most interesting and broadly relevant elements of the research to the general public.

11.2 Information of community and policy makers

In order to maximize impact, the outreach activities will be conducted with collaborations with established outreach organizations in Basel that will provide the support, training and opportunities to ensure the effectiveness of the proposed activities. The following outreach opportunities in Switzerland will be explored: (i) Open days at Swiss TPH and the University of Basel. These events will particularly target students and school children. (ii) The Basel Museums Night. This large annual event is particularly suitable for reaching the general public. (iii) The "Science on Stage" national festival 2021. It is suitable for people of all ages and abilities. (iv) Results will further be disseminated online through the collaborating platforms "SytemsXch" and "Institute Systems Biology" disseminated through blogposts and their respective "Youtube" channels. Importantly, opportunities for interviews with the local press arise on irregular intervals and they will be taken advantage of in disseminating the research outcome of this project.
Occasionally, parliamentarians visit Swiss TPH, where TBresearch and the BSL3 laboratory are being advertised, amongst others. The agenda will be expanded to also include parts of the human research aspect included in this project.

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13 APPENDICES

Annex 1: List of patient and laboratory variables collected during routine TB diagnosis by the Georgian NTP/MoH to be included in this project

The Georgian NTP/MoH collects routinely a set of demographic and other data from each TB patient during the standard diagnostic procedure at any of the NTP/MoH facilities. NTP/MoH staff will remove all participant personal data and only forward **project-relevant** data to the ERC staff for inclusion in the overall analysis. The list of variables to be forwarded is outlined below. The NTP/MoH will keep the link to the participant identifying information in case the ERC project generates data that might be useful for the individual participant or to NTP/MoH more generally. NTP/MoH will be in charge of sharing these data accordingly.

List of participant variables

Demographics

- Case code (unlinked to any participant identifying information)
- Participant age
- Participant sex
- Place of TB diagnosis
- Date of TB diagnosis

History and risk factors for TB

- Previous history of TB
- Outcome of previous treatment
- History of imprisonment
- Employment

Disease location

- Pulmonary versus extrapulmonary disease
- Organs involved

Case definition

- New *vs* retreatment case *vs* relapse

Outcome

- Treatment outcome (Cured *vs* died *vs* failure *vs* default)

List of laboratory variables

- Culture ID
- Diagnostic or Follow-up sample
- Sample type
- Date of sample collection
- Acid-fact bacilli result
- Date of inoculation
- Date of MGIT culture result
- MGIT culture result
- Date of LJ culture result
- LJ culture result
- Date of Drug Susceptibility Test start
- Date of Drug Susceptibility Test result
- Streptomycin resistance result
- Isoniazid resistance result

- Rifampicin resistance result
- Ethambutol resistance result
- Kanamicin resistance result
- Ofloxacin resistance result
- Etionamide resistance result
- Capreomicin resistance result
- PAS resistance result
- PZA resistance result

Annex 2: List of samples collected in this project

Module 1 and 2

- Bacterial *Mtb* isolates (recovered from the sputum collected through routine diagnostic procedures)

Module 3

- Additional sputum samples collected at baseline and during monthly follow-up visits
- All sputum sediments following processing for routine diagnostics
- All bacterial *Mtb* isolates recovered from all sputum samples
- 20ml of participant blood following successful treatment completion and cure

Module 4

- Additional sputum samples collected right before lung surgery
- Resected lung tissue following lung surgery
- All bacterial *Mtb* isolates recovered from all sputum samples and lung resections

Annex 3: List of variables collected from study participants specifically for this project (Study Modules 3 and 4)

Contact details

Primary and secondary phone numbers (only to be kept on a paper form stored separately and not in the electronic ECOEVODRTB project database. See details in paragraphs 7.1. and 7.3.)

Demographics

Date of interview
Participant Study ID and visit number
TB district number
Date of birth
Sex

Social Economic status

Education level
Occupation
Household monthly income
Household size
Household contribute

HIV information

HIV status
Date of HIV diagnosis

TB symptoms

Productive cough
Fever

Haemoptysis
Excessive night sweat
Chest pain
Weight loss
Night sweat
Weight loss
Abdominal pain

TB treatment history

Previous TB episodes
Date of episodes

TB diagnosis criteria

TB site (Pulmonary/Extrapulmonary)
TB participant category

Previous treatment TB medication

Previous treatment regimens
Date start of TB treatment
Other anti-TB medication given to the participant
IPT
Risk factors for TB (Smoking, Drug abuse, Imprisonment, BMI, HepC)

Nutritional assessment

Meals per day
Type of foods

Household member information

Year of birth
Sex
HIV Status

Measurements

Weight
Height
Body temperature
Respiratory rate
Mid upper arm circumference
Hip circumference
Waist circumference
Mid Upper Arm circumference (MUAC)
Skin fold thickness (TSF)
X-ray
Current episode treatment regimen
Current episode treatment outcome.

General condition

General condition
Pallor / anemia
Dehydration
Malnutrition
Cyanosis
Clubbing
General oedema
In pain / in discomfort

Systematic examination

Lymphatic system *(enlarged lymph nodes)*
Respiratory system
Abdominal system