# Supplementary Appendix

Title: Levofloxacin versus placebo for the treatment of latent tuberculosis among contacts of patients with multidrug-resistant tuberculosis (The VQUIN MDR Trial): a protocol for a randomized controlled trial

## Study objectives

### Primary objective

The primary objective of this study will be to determine the efficacy of a six-month regimen of levofloxacin in preventing the development of TB in infected household contacts of infectious MDR-TB patients.

### Secondary objectives

Secondary research objectives are to:

1. Compare the incidence ratio of all forms of TB (confirmed active TB plus clinically probable active TB ) in the intervention group, compared to the control group.
2. Compare the incidence ratio of confirmed active TB (confirmed active TB plus clinically probable active TB) in the intervention group, compared to the control group, among subjects who completed therapy.
3. Compare the incidence ratio of bacteriologically confirmed TB in the active levofloxacin group, compared to the placebo control group, among contacts of patients with MDR-TB bacilli that are susceptible to fluoroquinolones.
4. Determine the proportion of participants in each group discontinuing treatment owing to adverse events.
5. Determine the proportion of participants in each group completing at least 80% of doses of the trial therapy within 270 days after commencing therapy.



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6. Determine the incidence ratio of grade 3 or 4 adverse events in the active levofloxacin group, compared to the placebo control group.
7. Determine the ratio of death from any cause (except for violent or accidental causes) among the active levofloxacin group, compared to the placebo control group.
8. Evaluate the cost-effectiveness of 6 months of levofloxacin therapy, compared to placebo, in contacts of patients with MDR-TB, under programmatic conditions.
9. Determine the proportion of contacts with incident TB with acquired fluoroquinolone resistance in comparison to bacterial isolates of the index patient, in the levofloxacin group compared to the placebo group

Additional objectives for the biomarker sub-study are to:

10. Determine the difference in specific biomarkers (including microRNAs) between treatment initiation and treatment completion for compliant participants allocated to levofloxacin compared to the placebo group;
11. Determine the difference in specific biomarkers (including microRNAs) for patients with microbiologically proven TB, compared with enrolled contacts with infection, and enrolled contacts that are uninfected.

# Outcome definitions

## Bacteriologically confirmed tuberculosis

**Bacteriologically-confirmed TB** is defined as a positive identification of *Mycobacterium tuberculosis* by culture, GeneXpert MTB/RIF (Xpert) or another PCR-based diagnostic test in a contact with clinical or radiological evidence of disease.

A contact will be considered positive for bacteriologically-confirmed TB if: (a) at least one sample of sputum, or other body fluid or tissue is positive by culture, Xpert or another PCR-

based diagnostic test; or (b) at least two samples of sputum or other body fluid or tissue are positive by smear microscopy. Note that:

- AFB-positive TB alone on a single smear (that is not positive on culture, Xpert or another PCR-based diagnostic test) would not be considered "bacteriologically confirmed".
- If available cultures are contaminated or negative, then two samples that are both AFB-positive will satisfy the definition of "bacteriologically confirmed".
- Extrapulmonary TB will be considered "bacteriologically confirmed" if based upon either: additional investigations seeking microbiological confirmation (e.g. lymph node aspirate, CSF) with either a positive culture, Xpert or another PCR-based diagnostic test; OR two or more samples that are AFB positive on smear microscopy.

Bacteriologically confirmed TB should be classified as pulmonary, extra-pulmonary or both.

## Clinically probable tuberculosis

**Probable clinical TB:** Known exposure to TB plus "well-defined" clinical evidence AND supportive radiological or laboratory evidence of pulmonary or extra-pulmonary disease in a contact that is not bacteriologically-confirmed.

The diagnosis of "clinically probable TB" is equivalent to the secondary outcome of "clinical TB". The End Point Review Panel will reach a conclusion about the diagnosis of clinically probable TB on the basis of the following:

- Well-defined clinical evidence of clinically probable TB would include any TB-related symptoms of over 14 days' duration that was not improving (e.g. cough, fever,

night sweats, lethargy) or signs (documented weight loss, moderate or severe malnutrition, neck swelling or other clinical features of EPTB).

- In contacts with no clinical evidence, chest radiography is consistent with intrathoracic disease due to *M. tuberculosis*. In contacts with no clinical evidence, radiological evidence to support probable pulmonary TB would only include marked abnormality on CXR.
- AFB-positive alone on smear microscopy that is not culture or Xpert positive would be considered as supportive laboratory evidence.
- Probable EPTB will be based upon either additional investigations seeking microbiological confirmation (e.g. lymph node aspirate, CSF) or supportive clinical/radiological findings from laboratory investigation or imaging (e.g. pleural aspirate or ascitic tap, X-ray)
- Any form of EPTB with supportive evidence (see above) that is not also bacteriologically confirmed would be considered as Clinically Probable TB.

Clinical TB should be classified as pulmonary, extra-pulmonary or both.

## Possible clinical TB

Possible clinical TB is defined as known exposure to TB plus clinical or radiological evidence/abnormality that is not consistent with above definitions AND a decision was made to treat for TB. Possible clinical TB should be classified as pulmonary, extra-pulmonary or both.



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### Not TB

Known exposure to TB plus clinical symptoms or radiological features that resolve without treatment for TB.

### Latent tuberculosis infection (LTBI)

Known exposure to TB plus immunological evidence of infection with *M. tuberculosis* (TST/IGRA) plus no clinical or radiological evidence of disease

## Definitions of incident TB in child contacts aged <15 years

It is important to have consistency in reporting of cases within studies and as far as possible between studies for purposes of valid comparison or possible future meta-analysis.

While the clinical, non-bacteriologically-confirmed definitions have uncertainty, it should be emphasized that they are being used to strengthen consistency of reporting, not as definitions to inform treatment decisions. They are for the purpose of classification of study endpoints. Decisions to treat for TB (or not) will be made by a separate consensus panel on an individual patient basis. There are various factors that will influence treatment decisions that cannot be classified prospectively – for example, duration of symptoms, response of symptoms to other interventions, severity of illness (i.e. influencing urgency of referral and management), opportunity for follow-up and review prior to decision to treat, but that can be used retrospectively for classification purposes.

The previously suggested National Institutes of Health (NIH) case definitions were specifically designed for diagnostic assessment studies of intrathoracic TB in children and for



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where the entry point for the study would usually be children with symptoms suggestive of intrathoracic TB that presented to health services.

Note that in this MDR contact study, there are important differences to consider:

1. All children in this study will by definition already have "known exposure to TB".
2. Immunological evidence of infection with *M. tuberculosis* (i.e. positive TST or IGRA) adds to certainty of infection following known exposure but a negative result does not necessarily exclude infection. Definitions below do not include "immunological evidence of infection" as criteria but would be included in reporting of results.
3. Contact screening includes an active case-finding component that will identify children with TB at an earlier stage of disease than passive case-finding.
4. Some contact children may have extrapulmonary TB and so there is a need to include these in definitions.
5. There are no data to inform what might constitute an "adequate treatment response" as supportive evidence in this context and so this feature will not be included.

## Bacteriologically confirmed TB in children

**Bacteriologically-confirmed TB** is defined as a positive identification of *M. tuberculosis* by culture, Xpert or another PCR-based diagnostic test in a child with clinical or radiological evidence of disease.

A child will be considered positive for bacteriologically confirmed TB if: (a) at least one sample of sputum, or other body fluid or tissue is positive by culture, Xpert or another PCR-



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based diagnostic test; or (b) at least two samples of sputum or other body fluid or tissue are positive by smear.

- AFB-positive TB alone on a single smear (that is not positive on culture, Xpert or another PCR-based diagnostic test) would not be considered "bacteriologically confirmed".
- If available cultures are contaminated or negative, then two samples that are both AFB-positive will satisfy the definition of "bacteriologically confirmed".
- Extrapulmonary TB will be considered "bacteriologically confirmed" if based upon: either additional investigations seeking microbiological confirmation (e.g. lymph node aspirate, CSF) with either a positive culture Xpert or another PCR-based diagnostic test; OR two or more samples that are AFB positive on smear microscopy.

Bacteriologically confirmed TB should be classified as pulmonary, extra-pulmonary or both.

## Probable clinical TB in children

Probable clinical TB is defined as known exposure to TB plus "well-defined" clinical evidence AND supportive radiological or laboratory evidence of pulmonary or extra-pulmonary disease in a child who is not bacteriologically-confirmed.

The diagnosis of clinically probable TB is equivalent to the secondary outcome of "Clinical TB". The End Point Review Panel will reach a conclusion about the diagnosis of clinically probable TB on the basis of the following:

- Well-defined clinical evidence of clinically probable TB would include any TB-related symptoms of over 14 days' duration that was not improving (e.g. cough, fever, night sweats, lethargy) or signs (e.g. documented failure to thrive i.e. flattening of



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weight curve crossing centiles, documented weight loss, moderate or severe malnutrition [Weight-for-height Z score <-2], neck swelling or other clinical features of EPTB).

- In children with no clinical evidence, radiological evidence to support pulmonary TB would only include marked parenchymal abnormality on CXR
- AFB-positive alone on smear microscopy that is not culture or Xpert positive would be considered as supportive laboratory evidence.
- Probable EPTB will be based upon either additional investigations seeking microbiological confirmation (e.g. lymph node aspirate, CSF) or supportive clinical/radiological findings from laboratory investigation or imaging (e.g. pleural aspirate or ascitic tap, X-ray
- Any form of EPTB with supportive evidence (see above) that is not also bacteriologically confirmed would be considered as Clinically Probable TB.

Probable clinical TB should be classified as either pulmonary, extrapulmonary or both.

## Possible clinical TB in children

Possible TB is defined as known exposure to TB plus clinical or radiological evidence/abnormality that is not consistent with above definitions AND a decision is made to treat for TB. Possible clinical TB should be classified as pulmonary, extra-pulmonary or both.

## Not TB

This is defined as known exposure to TB plus clinical symptoms or radiological features that resolve without treatment for TB



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## Indeterminate TB status

A contact where documented results of the diagnostic evaluation of possible TB are insufficient for the End point Review Panel to reach determination of clinically probable TB.

## LTBI

This is defined as known exposure to TB plus immunological evidence of infection with *M. tuberculosis* (TST/IGRA) plus no clinical or radiological evidence of disease

## Drug toxicity

Drug toxicity will be evaluated during an interview with contacts once each month during treatment.

## Death

The secondary mortality outcome will be death from any cause except for violent (e.g. homicide) or accidental (e.g. motor vehicle accident) causes.

Details of the death will be obtained from health service medical records, family members or other official sources (including death certificates). Additional details of the circumstances surrounding the death will be collected to enable the IDMC to evaluate whether the death was likely attributable to the study medication.

A TB death is a death where TB is considered by the IDMC to be the most likely or major contributing cause of death.



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A non-TB death is a death where factors other than TB is considered the most likely or major contributing cause of death.

## Drug discontinuation owing to adverse drug reaction

Subjects will be deemed to have discontinued owing to an adverse drug reaction if the drug is ceased after recurring grade 3 or grade 4 toxicity.

For any recurring grade 3 or grade 4 toxicity, the study drug will be temporarily withheld, and may be permanently discontinued in the affected individual at the discretion of the Expert Clinical Panel.

## Drug discontinuation to causes other than adverse drug reactions

Causes of drug discontinuation not related to adverse drug reactions may include loss to follow-up, withdrawal from the study or not taking study therapy for at least 3 months. In such circumstances, the Principal Investigator may decide it is no longer advisable to continue the study drugs.

## Completion of six months of therapy

A contact will be defined to have completed the study therapy if they complete 80% or more of the total prescribed doses within a total period of 30 weeks after randomization.

## Adherence

Adherence will be assessed at the end of each month of treatment with pill counts. Standardized questionnaires will also be used to assess compliance. Contacts will be considered non-



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adherent if they fail to complete 80% of the total prescribed doses (i.e. ≥36 doses) within 270 days after commencing therapy.

### Acquired drug resistance

Acquired antibiotic resistance will be deemed to have occurred when (a) molecular testing of paired isolates (index patient and contact with incident TB) demonstrates transmission has occurred (i.e. the strain is the same) and, (b) the isolate of the contact demonstrates additional antibiotic resistance to that of the index patient.

### Proportion of contacts successfully treated

Contacts will be classified as successfully treated if they either achieve outcomes of (a) treatment success, or (b) cure according to WHO treatment definitions (1).

## Procedures for follow-up of contacts treated for TB disease

All contacts treated for co-prevalent TB, and randomized contacts treated for incident TB, will have the following follow-up performed.

Co-prevalent disease is defined as TB that is present at the time of enrolment, based upon diagnostic tests performed at the baseline. Contacts with co-prevalent TB will have samples collected and stored, for subsequent comparison with other contacts in their household who develop incident disease.

## Contacts in which tuberculosis is to be suspected

Symptoms that will be considered to indicate suspected TB, either at the time of baseline



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assessment or at any time during follow-up are listed in Table S1.

**Table S1: Symptoms consistent with suspected tuberculosis**

| **In contacts aged ≥ 10 years** | **In child contacts aged < 10 years** |
|---|---|
| Symptoms are defined as one or more of:<br>• Persistent cough (≥2 weeks)*<br>• Prolonged fever (≥2 weeks)*<br>• Night sweats<br>• Haemoptysis<br>• Documented weight loss<br>• Moderate or severe malnutrition | Symptoms are defined as one or more of:<br>• Persistent cough (≥2 weeks)**<br>• Prolonged fever (>1 week)**<br>• Lethargy//reduced playfulness<br>• Weight loss/Failure to thrive<br>• Documented weight loss<br>• Moderate or severe malnutrition |

* If participants report cough for < 2 weeks (and have no Xray abnormalities that lead them to be considered as suspects) then they will be reassessed again when 2 weeks has passed since symptom onset, to determine whether the cough is persistent. If the symptoms have resolved at the time of the second assessment, then no additional sputum testing will be required and the contact will not be considered a suspect on account of the cough. If the cough has persisted for a total of more than 2 weeks in total then they will be investigated as a suspect (including additional sputum testing as described below).

**Children with ANY symptom (cough or fever) defined in the above table should be followed until symptom resolution – with follow-up after 1-2 weeks if symptoms do not meet duration criteria specified above. Persistent cough or prolonged fever not responding to treatment for the most likely alternative cause requires TB investigation.



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## Procedures for suspected extrapulmonary TB

The appropriate tests for specific sites of suspected extra-pulmonary TB will be conducted according to the protocols of the National TB Program (NTP) and, where possible, in consultation with the Expert Clinical Panels. Investigations may include:

- Lymph node tuberculosis: Lymph node biopsy, with histological examination, culture (and if applicable drug susceptibility testing (DST))
- Pleural tuberculosis: culture, smear and biochemical analysis (lactate dehydrogenase (LDH), protein, glucose) for 2 separate pleural aspirates (and if appropriate pleural biopsy)
- Meningeal tuberculosis: smear, culture and biochemical analysis (LDH, protein, glucose) and cell count of cerebrospinal fluid
- Spinal tuberculosis: spinal radiography +/- biopsy if appropriate
- Abdominal tuberculosis: abdominal imaging, surgical biopsy if appropriate
- Other site: tissue biopsy and histology and/or culture and DST (if applicable)

## Procedures for TB meningitis diagnosis in adults and children

TB meningitis in adults and children are diagnosed and classified according to the following criteria:

**Tuberculous meningitis classification**

Standard diagnostic criteria will be used to classify TB meningitis into definite, probable, possible, and not tuberculosis meningitis (2)



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*Definite tuberculous meningitis*

Patients should fulfil criterion A or B:

A)

- Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; *M. tuberculosis* cultured from the cerebrospinal fluid (CSF); or a CSF positive commercial nucleic acid amplification test.

B)

- Acid-fast bacilli seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

*Probable tuberculous meningitis*

- Clinical entry criteria plus a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnoses. At least 2 points should either come from CSF or cerebral imaging criteria.

*Possible tuberculous meningitis*

- Clinical entry criteria plus a total diagnostic score of 6–9 points (when cerebral imaging is not available) or 6–11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses. Possible TB cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.



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*Not tuberculous meningitis*

- Alternative diagnosis established, without a definitive diagnosis of TB meningitis or other convincing signs of dual disease.

## Molecular testing of isolates

We will study isolates from contacts who develop culture-positive TB, at baseline or after treatment, to determine whether acquired drug resistance has developed, based upon concordant phenotypic DST and whether they have matching Mycobacterial Interspersed Repetitive Unit - 24 loci (MIRU-24) patterns. For greater resolution, we will also apply Whole Genome Sequencing to index-contact pairs, with samples to be tested in a facility with appropriate quality assurance, meeting international standards. With these methods, we will (a) determine the proportion of contacts who develop TB with isolates matching their index patients and (b) use genotypic testing to confirm known resistance mutations (especially in gyrA and gyrB, which are linked to quinolone resistance) and potentially identify new mutations that cause resistance.

## Data quality control

Monthly data checking and quality control will be performed at a central level. Study staff will be trained to use the study database. A manual of procedures for data management procedures will describe the steps for routine evaluation of data quality and checking of data.

## Interim safety analysis

The trial Independent Data Monitoring Committee (IDMC) will conduct an interim analysis after 1/3 of participants (600 contacts) have completed 6 months of therapy for grade 3 and 4 adverse events. If the incidence of severe adverse events (grade 3 and 4) are substantially



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higher in the intervention than control arms ($p < 0.025$), and the rate of severe adverse events is considered by the IDMC to be of major concern, then the IDMC may request the unblinding of the groups and may make a recommendation that the trial to be terminated early. In the event that the trial is to be terminated early, the final decision will be made by the Trial Steering Committee.



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## Supplementary appendix references

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