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Optimisation strategies for the first line treatment of tuberculosis

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DOCTORAL THESIS

Optimisation strategies for the first line treatment of **tuberculosis**

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Berta, coherence with one's decisions, once you have made them. Persistance. Bravery to look within. Making everyone around you a better version of themselves.You helped me get here in one piece.

List of abbreviations

Abbreviation	Definition
CI	Confidence Interval
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CrI	Credibility Interval
CRO	Clinical Research Organisation
CTA	Clinical Trial Agreement
DINAVISA	Dirección Nacional de Vigilancia Sanitatria
DM	Diabetes Mellitus
DS-TB	Drug Susceptible tuberculosis
EBA	Early Bactericidal Activity
EMB	Ethambutol
EUPEARL	EU Patient Centric Clinical Trial Platforms
EUSAT-RCS	European-Latin American TB Clinical Research Network
HIV	Human Immunodefficiency Virus
HR-TB	Isoniazid Resistant tuberculosis
IGRA	Interferon Gamma Release Assay
INERAM	Instituto Nacional de Enfermedades Respiratorias y del Ambiente
INH	Isoniazid
LMIC	Low-Middle Income Countries
MAFLD	Metabolic dysfunction Associated Liver Disease
MDR-TB	Multi-Drug Resistant tuberculosis
NMA	Network meta-analysis
OR	Odds Ratio
PLWHIV	People Living With HIV
pre-XDR-TB	pre-XDR-tuberculosis
PZD	Pyrazinamide
RIF	Rifampicin
RR	Risk Ratio
RR-TB	Rifampicin Resistant tuberculosis
SCC	Sputum Culture Conversion
SUCRA	Surface Under the Cumulative Ranking
TB	Tuberculosis
TNF	Tumor Necrosis Factor
TST	Tuberculin Skin Test
UMCG	University Medical Center Groninguen
VHIR	Vall d'Hebrón Institut of Research
WHO	World Health Organisation
XDR-TB	Extensively Drug-Resistant TB

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Summary

Tuberculosis (TB) is one of the leading causes of disease and death due to a single infectious agent worldwide. The objectives of this thesis were 1) assessing the status of fist-line TB treatment, with special focus on the optimal rifampicin dose, and 2) use these results to inform the design of a phase 2b/c clinical trial evaluating optimised doses of rifampicin.

This thesis summarizes and discusses the results from two articles published in peer-reviewed journals. First, although the first-line treatment for TB has not changed substantially for more than 40 years, there is enough evidence showing that the current standard dose of rifampicin leads to underexposure in a significant subgroup or people, but it is still unclear what the optimal dose of rifampicin should be. Therefore, in the first article we report a systematic review and Bayesian Network Meta-Analysis (NMA) evaluating all doses of rifampicin evaluated in clinical trials in adults with pulmonary and extrapulmonary TB in the last two decades. The review included data from 3654 participants that were young adult males, and with a low comorbidity burden. The NMA showed an increased risk of overall and hepatic adverse events (AE) for 40 mg/kg/day but no other doses (including 50 mg/kg/day). Increasing doses improved sputum culture conversion at week 8 (RR 1.3, 95% CrI: 1.1, 1.7 for SCC with 35 mg/kg/day).

The second article describes the design of the RIAlta trial, a phase 2b/c non-randomized clinical trial with matched historical controls comparing the standard dosing of rifampicin with 35mg/kg/day (R^{35} group). The inclusion criteria focused on vulnerable groups typically excluded from previous trials (age > 60, diabetes, malnutrition, HIV, hepatitis B or hepatitis C coinfection), and extrapulmonary TB. The primary outcome was safety, with early sputum conversion as co-primary endpoint.

Finally, we present a brief report on the status of the RIAlta trial as of February 2025. The historical cohort included 117 participants, and recruitment is ongoing in Paraguay, the Netherlands, and Spain, with 28 participants enrolled in the R^{35} group. There were more rifampicin-related grade 3 or higher AE in the R^{35} group compared to the historical controls (4/23, 17.4% vs 3/63, 4.8%). However, we did not find significant differences in the adverse events leading to rifampicin dose modification between the two groups, and the proportion of grade 3 or higher AE is in line with that published in clinical trials with non-vulnerable population. Finally, interim data showed a significant effect on the sputum culture status at week 4 (Odds-Ratio, OR, for a positive culture 0.22, 95% CI 0.056-0.861), and non-significant at week 8 (OR 0.393, 95% CI 0.041-3.759).

Optimal doses of rifampicin may be between 25 and 35 mg/kg/day, but should be tailored at the individual or, at least, at the population level. Safety seems similar in vulnerable populations as compared to low-risk populations in previous trials, whilst the benefits in terms of early sputum clearance and, possibly, in treatment shortening, may be larger.

Resum

La tuberculosi (TB) és una de les principals causes de malaltia i mort per un sol agent infecciós a tot el món. Els objectius d'aquesta tesi eren 1) avaluar l'estat actual del tractament de la TB de primera línia, amb especial atenció a la dosi òptima de rifampicina, i 2) utilitzar aquests resultats per informar el disseny d'un assaig clínic de fase 2b/c que avalués les dosis optimitzades de rifampicina.

Aquesta tesi resumeix i discuteix el resultats d'articles publicats a revistes amb revisió per pars. Primer, tot i que el tractament de la TB no ha canviat gaire als darrers 40 anys, n'hi ha prou evidència que demostra que la dosi estàndard de rifampicina condueix a una subexposició en un subgrup important de persones, però encara no està clar quina hauria de ser la dosi òptima de rifampicina.

Al primer article resumim les troballes d'una revisió sistemàtica amb metaanàlisi bayesià en xarxa (NMA) que avalua totes les dosis de rifampicina avaluats en assajos clínics en adults amb tuberculosi pulmonar i extrapulmonar als darrers 20 anys. La revisió va incloure dades de 3.654 participants que eren joves, homes i amb una baixa càrrega de comorbiditat. La NMA va mostrar un risc més gran d'esdeveniments adversos (AE) i AE hepàtics per a 40 mg/kg/dia, però pas per a altres dosis (incloent-hi 50 mg/kg/dia). L'augment de dosis va millorar la conversió del cultiu d'esput a la setmana 8 amb 35 mg/kg/dia.

El segon article descriu el disseny de l'assaig clínic RIAlta, un estudi no aleatori de fase 2b/c amb controls històrics que compara la dosi estàndard de rifampicina amb 35 mg/kg/dia (grup R^{35}). Els criteris d'inclusió es centren en els col·lectius vulnerables exclosos dels assaigs clínics previs (edat > 60 anys, diabetis, desnutrició, VIH, coinfecció per hepatitis B o hepatitis C), i TB extrapulmonar. El resultat primari va ser la seguretat, amb la conversió precoç de l'esput com a variable co-primària.

Finalment, presentem un breu informe sobre l'estat de l'assaig RIAlta a febrer de 2025. La cohort històrica va incloure 117 participants, i el reclutament està en curs al Paraguai, els Països Baixos i Espanya, amb 28 participants inscrits al grup R^{35}. Hi va haver més AE de grau 3 o superior relacionat amb la rifampicina al grup R^{35} en comparació amb els controls històrics (4/23, 17,4% vs. 3/63, 4,8%). Tanmateix, no vam trobar diferències significatives en els esdeveniments adversos que van provocar una modificació de la dosi de rifampicina entre els dos grups, i la proporció d'AE de grau 3 o superior està en línia amb la publicada en assaigs clínics amb població no vulnerable. Finalment, les dades provisionals van mostrar un efecte favorable sobre l'estat del cultiu d'esput, significatiu a la setmana 4 (OR, per a un cultiu positiu 0.22, 95% CI 0.056-0.861) i no significatiu a la setmana 8 (OR 0.393, 95% CI 0.041-3.759).

Les dosis òptimes de rifampicina poden estar entre 25 i 35 mg/kg/dia, però s'han d'adaptar a l'individu o, almenys, a nivell de població. La seguretat sembla similar a les poblacions vulnerables en comparació amb les poblacions de baix risc en assaigs anteriors, mentre que els beneficis en termes d'eliminació precoç de l'esput i, possiblement, en l'escurçament del tractament, poden ser més grans.

1: Introduction

-What's wrong? I mean, what appear to be the symptoms?
-You heard them. I am coughing...
-Is there any blood?
-Sometimes...
-Fair enough. Now here, breathe... again... Let me see your tongue...
-What is it?
-It is no good news. You got tuberculosis. I'm really sorry for you, son. It's a hell of a thing.
-What do you mean?
-You're really sick. It's a progressive disease, you'll be... The best thing is rest, getting somewhere warm and dry, take it easy.
Red dead redemption 2. Rockstar Games, 2010

1.1. An overview of TB epidemiology: we are still far from ending TB

The global TB epidemic in numbers

In 1935, Prof. Edward Mellanby, secretary of the British Medical Research Council, stated that tuberculosis (TB) is a disease that was going away by itself. His data showed that, before any antibacillary treatments were available, tuberculosis-related mortality decreased from 34 per 1000 to 7 per 1000 between 1850 and 1934 (1).

But ninety years later, despite giant advances in diagnosis and treatment, TB is still the world's leading cause of death from a single infectious agent, returning to this position after the meteoric appearance of the SARS-CoV2 pandemic. The WHO estimated that more than 10 million people continue to fall ill with TB, and that 1.25 million deaths were directly related to TB in 2023 (figure 1) (2). The challenges

that limit the control of TB affect the diagnosis, surveillance, treatment adherence, adverse events, and treatment success. Regarding diagnosis, only 62% of the 6.9 million cases of pulmonary TB were bacteriologically confirmed in 2023. In terms of surveillance, there is a 2.7 million case gap between the estimated number of people developing TB each year, and the number of oficially reported cases. Although this gap is shrinking each year, it reflects that there is a considerable number of cases that escape epidemiological vigilance.

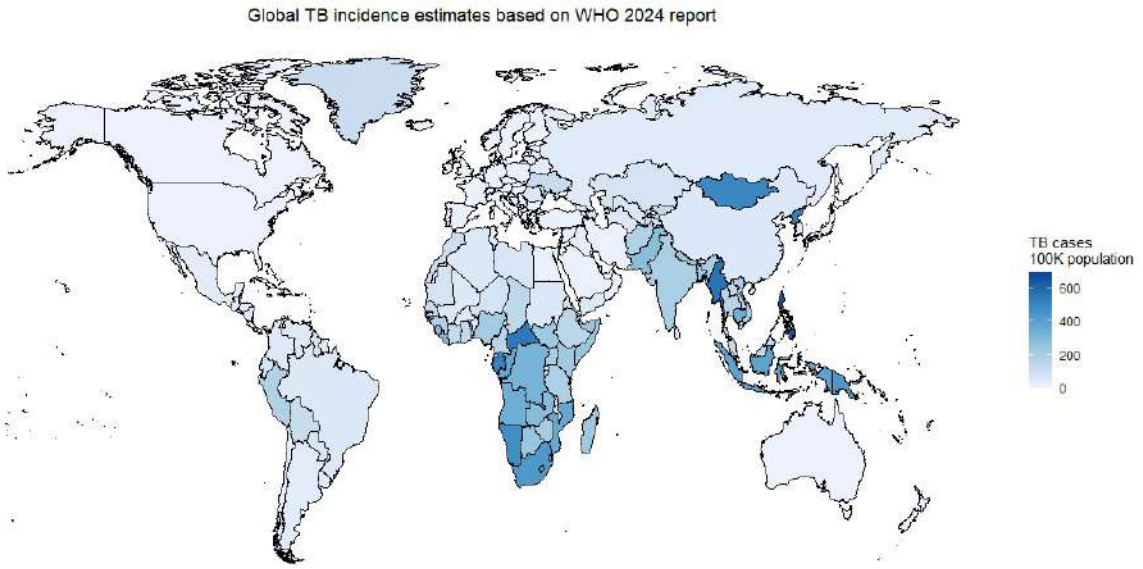

Figure 1. Global TB incidence in cases per 100000 population. Reconstructed from the public 2023 data made available by the WHO. Note that, despite a moderate incidence, countries with large populations such as India and China contribute to approximately 1 in 4 cases of TB.

Despite a partial recess related to the COVID-19 pandemic, the percentage of new cases that are started on treatment is improving by the year, but was estimated to be 75% in 2023, which would leave 1 out of each 4 TB cases without treatment. One must note, however, that these figures are based on the number of cases as reported by official institutions. There is probably underdiagnosis and underreporting in many resource-limited settings, where data are collected in a few sites that could not be fully representative of the local epidemiology.

Treatment success for rifampicin-susceptible TB (DS-TB) has remained in high levels in recent years (88% in 2022). However, microbiological confirmation of cure is not available for all cases. Figure 2 shows the differences between microbiologically confirmed cure and success defined as treatment completion in the different WHO regions using the last available data that differentiated both outcomes, in 2011. Post-treatment follow-up data is scarce, so there is little information about relapses, and even less about long-term disability or recurrence of the same infection compared to reinfections.

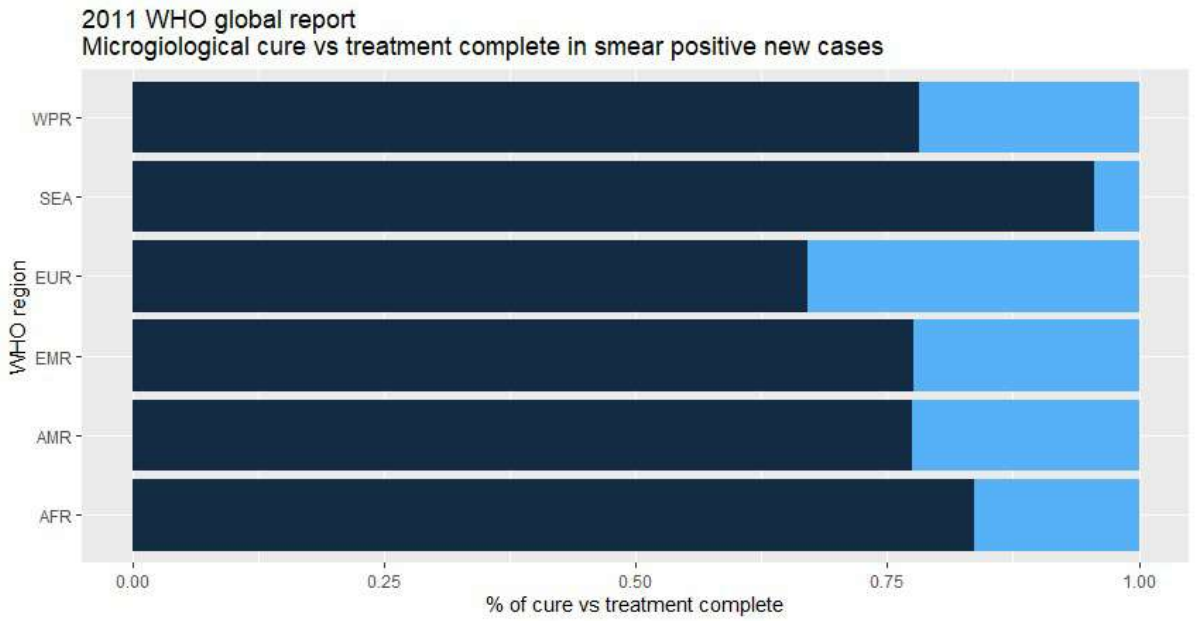

Figure 2. Microbiological cure vs. treatment complete in all smear-positive cases reported to WHO in 2011. That year was the last one in which microbiological cure and treatment complete are available as separate variables in the public datasets for all WHO regions. Microbiological cure was defined as bacteriologically confirmed TB where at least two consecutive cultures or smears taken on different occasions at least 7 days apart, are negative with no positive test afterwards. On average, up to 1 in 4 TB cases do not have microbiological evidence of cure. However, there were wide variations in the different regions that were not discussed in the original report but may be related to differences in follow-up and reporting at the programmatic level. WPR, West Pacific Region; SEA, Southeast Asia; EUR, European region, including East Europe; EMR, East Mediterranean Region; AMR, Region of the Americas; AFR, African Region.

Following the advances in the treatment for drug-resistant TB, the WHO categories of drug resistance were updated in 2020 (3):

- Isoniazid-resistant TB (HR TB): TB that is resistant to isoniazid, but susceptible to rifampicin.
- Rifampicin-resistant TB (RR TB): TB that is resistant to rifampicin, but susceptible to isoniazid.
- Multi-drug-resistant TB (MDR-TB): TB that is resistant to rifampicin and isoniazid.
- Pre-extensively drug-resistant TB (pre-XDR-TB): TB that is resistant to rifampicin and any fluoroquinolone.
- Extensively drug-resistant TB (XDR-TB): TB that is resistant to rifampicin, any fluoroquinolones and at least one of either bedaquiline or linezolid (any group A drug).

Out of the 10 million estimated cases, 4.3 were bacteriologically confirmed and 3.4 million (79% of those bacteriologically confirmed) were tested for rifampicin resistance (2). There were 188666 cases of confirmed rifampicin-resistant TB (combining RR-TB, MDR-TB and XDR-TB), and 93% started second-line treatment according to WHO's guidelines. However, it is estimated that a total of 400000 people developed MDR-TB in 2023, so less than half of them were properly diagnosed, and only 44%

of the total estimate started adequate treatment. With the new short, all-oral MDR-TB regimens, treatment success has reached 68% of the MDR/RR-TB cases at the programmatic level, and >80% in clinical trials (4,5).

TB burden is unevenly distributed between and within countries

Most people who developed TB in 2023 were in South-East Asia (45%), Africa (24%), and Western Pacific (17%) WHO regions (2). Eight countries account for two-thirds of the global burden of the disease (namely, India, Indonesia, China, Philippines, Pakistan, Nigeria, Bangladesh, and Democratic Republic of Congo). Furthermore, the WHO keeps an updated list of TB high-burden countries according to three categories: overall TB burden, TB/HIV burden, and MDR/RR-TB burden, with the top 30 countries in each category. There are 10 countries that appear on all 3 categories: China, Democratic Republic of Congo, India, Indonesia, Mozambique, Myanmar, Nigeria, Philippines, South Africa and Zambia. This is probably related to socioeconomic determinants of certain populations within these countries, and poor implementation of public health control measures at the programmatic level. For instance, India, Indonesia, Pakistan, China and Myanmar account for more than 50% of the gap between estimated incidence and reported cases.

There are several determinants that have a significant impact on the global TB burden. In 2020, an estimated 1.9 million incident cases of TB were attributable to undernutrition, 0.74 million to HIV infection. 0.74 million to alcohol use, 0.73 million to smoking, and 0.37 million to diabetes (6). At the individual level, factors such as advanced HIV infection and other immune suppression situations such as treatment with tumour-necrosis factor (TNF) inhibitors have the highest risk for developing TB. However, due to their prevalence, undernutrition and diabetes combined contribute with a greater absolute number of cases.

However, poverty is the TB determinant that rules over the previous ones, since it is a risk factor for them. Crowded households and working places, undernutrition, suboptimal control of chronic health problems (diabetes, HIV) and poor life habits (alcohol and tobacco consumption) aggregate in poor socioeconomic strata. Even in high-income countries, TB incidence is distributed unequally. For example, in 2022 in the city of Barcelona, overall TB incidence was 16.9/10000 population, but it varied from <10 in autochthonous population from high-income neighbourhoods to 60.3/100000 population in migrant population from the Ciutat Vella district (7). Migrant population is affected by the incidence in their countries of origin, but poor living conditions in the hosting country add to their overall risk for developing TB. People experiencing homelessness may have an incidence 30 times higher compared to the general population in Barcelona, although an accurate measure of both the absolute number of TB cases and the denominator population is difficult to obtain.

The EndTB strategy

One of the millennium development goals was to "halt and begin to reverse the incidence of tuberculosis by 2015". To guide the actions towards this vague goal, the WHO developed a set of ambitious targets, summarised in the EndTB strategy (8). The key performance indicators between 2015 and 2035 and are summarized in table 1.

Indicators with baseline values for 2015	Milestones			Target	WHO report
	2020	2025	2030	2035	2024
Percentage reduction in deaths due to tuberculosis (estimated 2015 baseline: 1.3 million deaths)	35%	75%	90%	95%	23%
Percentage and absolute reduction in tuberculosis incidence rate (estimated 2015 baseline 110/100000)	20% (<85/100000)	50% (<55/100000)	80% (<20/100000)	90% (<10/100000)	8.3%
Percentage of affected families facing catastrophic costs due to tuberculosis (no baseline estimate)	Zero	Zero	Zero	Zero	49% (DS-TB) 80% (MDR-TB)

Table 1. Key global indicators for the EndTB strategy. Adapted from the WHO (8).

Although the decline in TB incidence is very ambitious, the WHO deemed it feasible based on an even faster decline documented at national level in the context of universal access to health care and rapid socioeconomic development in Western Europe and North America during the second half of the past century. For example, in the Bajo Deva Region, an area with a strong mining tradition in the Basque Country in northern Spain, the incidence of TB went down from 84 in 2000 to 27/100000 population in 2007 after the implementation of a TB control program by the regional government (9). However, as of 2024 we are still far from these goals (table 1, rightmost column)(2).

Partial implementation of existing tools to diagnose, prevent, and treat TB, disruption of TB control programmes during the COVID-19 pandemic, and poor socioeconomic development (i.e., universal health coverage and social protection) explain most of the difference between the 2025 goals and the actual achievements in WHO reports. New tools such as point-of-care diagnostics, new TB vaccines, and improved treatments for TB infection and active disease are also needed to reduce the gap between the milestones and the estimated data from WHO's, and this can be achieved only by increasing the investment in TB research and development.

Limited access and incomplete adherence to TB treatment jeopardize its contribution to the objectives of the EndTB strategy. The 2022 success rates were 88% for DS-TB (2). Considering this and the global gap of estimated cases and treatment initiation data, there are at least 3.5 million people (35% out of the estimated 10 million new cases per year) with active TB that escape the control strategies for TB. The situation is even more alarming for MDR/RR-TB: if the treatment success is

68% and the coverage is 44% out of an estimated of 400000 cases, about 70% of the MDR/RR-TB are either not treated or suffer treatment failure each year.

1.2. A brief review of tuberculosis pathophysiology

TB is caused by *Mycobacterium tuberculosis* complex, with most cases caused by *M. tuberculosis* sensu stricto. Other species such as *M. africanum*, *M. bovis*, *M. caprae*, etc., have a smaller weight in the global TB burden. Humans are the only reservoir for *M. tuberculosis*, making it theoretically possible to eradicate TB (10). The host-pathogen interaction is complex, and studies trying to identify a single virulence factor that explains a certain clinical pattern have shown inconsistent results. Evidence suggests that *M. tuberculosis* 4 main lineages reflect co-evolution of pathogenicity factors in specific human populations, and their distribution reflects the migration patterns of such groups (11,12). Hence, specific clinical patterns are probably the result of the combination of host and pathogen genetic backgrounds, and the interactions between them, underlying specific factors such as virulence factors or comorbidities, respectively (10).

Figure 3 depicts the pathophysiology of *M. tuberculosis* infection. If the innate immune system, mainly through alveolar macrophages, fails to control the bacilli arriving at the alveoli, TB infection is established. In about 90% of the people with an established TB infection, the granuloma, a coordinated effort that requires an adaptive immune response, contains the infection without inducing substantial tissue pathology. We say then that the infection becomes latent, but we know now that this is a dynamic process with a spectrum of activity that may advance to active disease or recede to infection elimination and depends on a host-pathogen balance (13). Either if the granuloma fails to contain the initial infection or its control becomes deficient several years later, the host can develop active TB disease. There seems to be an ideal set point for the immune response against *M. tuberculosis*. For example, an excessive activation of TNF or the leukotriene pathway can be deleterious, and insufficient activation leads to lack of immune containment of the infection (14).

If left untreated, TB results in death or spontaneous recovery. There are interesting models of the natural history of TB using data from the pre-treatment era (15). The posterior estimates for mortality were 38.9% and 2.5% per year for smear positive and smear negative, respectively. For spontaneous recovery these were 23.1% and 13.0% per year for smear positive and smear negative TB, respectively. Smear positive TB had a disease duration of somewhat less than 2 years. In contrast, smear negative TB could last for 4-6 years on average, depending on background overall mortality, which highlights the importance of this form of disease with less bacillary burden but more time to maintain the transmission in the community.

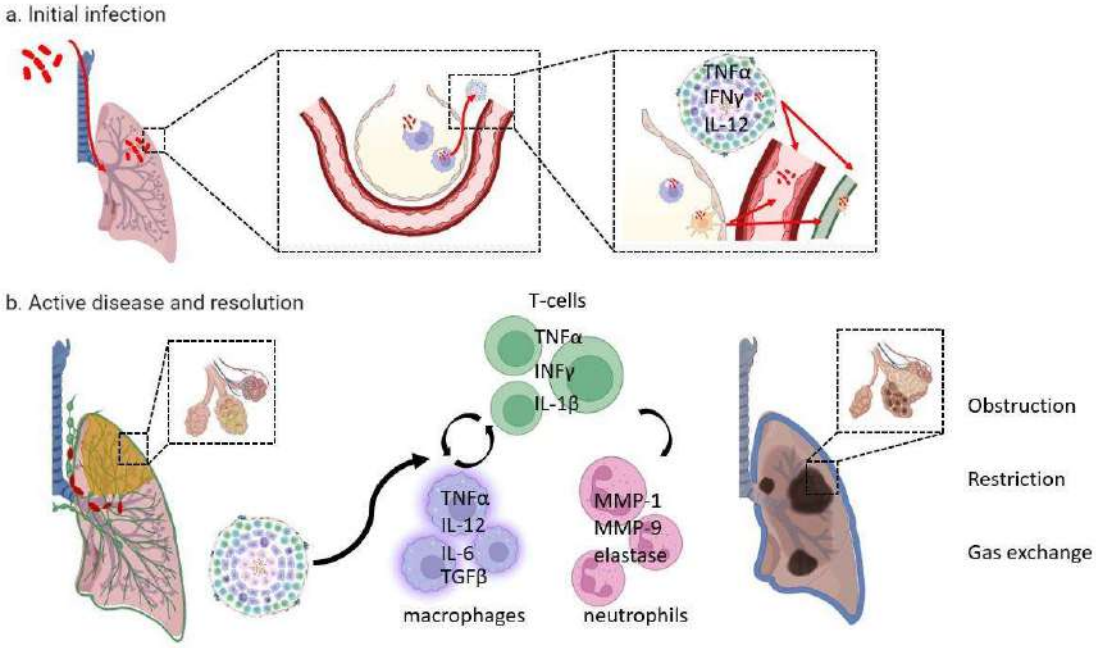

Figure 3. Pulmonary tuberculosis pathophysiology from exposure to long-term sequelae. a) After entry through inhaled aerosols, alveolar macrophages (purple) are often enough to eliminate all bacilli before infection is established. This people will not develop an adaptive immune response and therefore the tests like tuberculin skin test (TST) or Interferon Gamma Release Assays (IGRA) remain negative. If *M. tuberculosis* succeeds in inhibiting the fusion of lysosomes and phagosomes, it can persist in the cytosol and modify the macrophages' behaviour via virulence factors. Then, *M. tuberculosis* invades the interstitial space, either from free bacilli from the alveolar space, or from macrophages with persistent bacilli. As the primary infection is established, dendritic cells and monocytes (orange) carry *M. tuberculosis* to local lymph nodes to establish an adaptive immune response based on T cells. The host tries to isolate the bacteria by building granulomas. The formation of a granuloma is based on TNFα, IFNγ and IL-12 signalling. It implies the gathering of new phagocytic cells that *M. tuberculosis* can infect and the creation of a lymphoid crown around the nucleus. The bacilli can spread further through the bronchial tree and the blood, leading to local and systemic dissemination, respectively. b) Either if the primary infection progresses immediately or reactivates after something tilts the immune balance keeping *M. tuberculosis* replication at check, tissue inflammation with further development of granulomas and caseification ensues. The ongoing granuloma milieu depends on the cytokine intertalk between several cell types. We have highlighted the three most relevant to sustain the inflammatory response (T cells and activated macrophages) and drive tissue destruction and remodelling (neutrophils). In the long term, these tissue changes lead to destruction and fibrosis. Pulmonary TB lesions lead to functional impairment: obstruction (peribronchial fibrosis, cavitation, bronchiectasis), restriction (pleural thickening, broncovascular distortion, fibrotic bands), and impaired gas exchange (reduction in alveolar area cavitation and fibrotic scars).

TB diagnosis is based on symptoms, radiologic imaging of the organs affected, and microbiological confirmation. The host-pathogen interaction is so complex that the combination of these diagnostic tools can be misleading. In a recent meta-analysis, people with a chest x-ray suggestive of active disease had an estimated annual progression to open TB disease (positive sputum microbiology) of 10%, regardless of symptoms. In contrast, for those with lesions suggesting inactive disease or no lesions at all, the progression rates were 1% and 0.1%, respectively (15). Most cohorts included in this review were studied before the widespread availability of bronchoscopy and computed tomography scans, which could increase the sensitivity of microbiological confirmation. The opposite situation may also happen, with *M. tuberculosis* shedding into the sputum up to one year before clinical presentation (16). Factors such as HIV infection, diabetes, and anti-TNF therapy modify the dynamics

of this natural history, with faster progression to open disease and different radiographic and clinical presentation (17).

An important aspect of complex infections such as TB is the resolution of symptoms and normalization of acute-phase response biomarkers following the elimination of planktonic bacteria, but persistence of sessile populations that can cause a relapse of the disease in the future. The persistent bacilli form a relapsing reservoir in some treated patients (18). They can survive inside the host cells that should eliminate them, in places where drug penetration is suboptimal, setting the biological basis for the emergence of drug resistance. Optimal treatments must address persistent bacilli, which may be achieved by optimizing drug regimens, enhancing host's response, or modifying the metabolic and physiological status of the pathogen.

During all its stages, TB infection triggers tissue destruction and remodelling, even some time after successful treatment. This leads to long-term sequelae and organ malfunction. The role of antibacillary and immune modulatory drugs is unclear in preventing these sequelae once the disease is clinically advanced. Although this happens in all the affected tissues (e.g., spine, meninges, skin), most studies have focused on lung sequelae because of its prevalence, ease of measurement, and long-term consequences. For example, a history of treated TB had an OR of 3.05 (95% CI 2.42-3.85) for chronic airway obstruction independent from smoking history (19). The variability in host response to *M. tuberculosis* and subsequent superinfections can explain the heterogeneity in pulmonary deficits seen in TB survivors (figure 3) (20).

1.3. Overview of the first-line treatment of tuberculosis

The WHO recommends the use of rifampicin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by rifampicin and isoniazid for 4 months as the first-line treatment for TB (21). This treatment was established in the 1980s and had no major changes ever since, except that intermittent dosing is no longer recommended since 2010 (22).

According to the last update of the WHO's guidelines, the treatment can be shortened children and adolescents between 3 months and 16 years of age and with uncomplicated disease, with a continuation phase of 2 months for a total of 4 months. This recommendation is based on the results of the SHINE trial, in which uncomplicated TB was defined as lymph node involvement without airway obstruction, pleural effusion and smear-negative, non-cavitary single lobe and non-miliary TB (23). Data from the first rifampicin clinical trials in the late 70s and early 80s suggests that adults with paucibacillary disease (sputum smear negative, no immunosuppression and no lung cavitation on chest X-ray) could be also treated 4 months (24).

In contrast, some forms of TB often require longer treatments to obtain relapse-free cure. For TB affecting the Central Nervous System (CNS), bones and joints, the continuation phase is normally extended for a total duration of 12 months. In addition, some people have a high individual risk of treatment failure or relapse. Thus, in clinical practice treatment duration is often tailored according to baseline characteristics (i.e., age, immune suppression, diabetes, malnutrition, cavitary disease) and treatment response (i.e., smear and culture conversion to negative, radiological improvement) that are associated to a higher risk of relapse (see table 2 in appendix I). Since the 2010 WHO's guidelines, it is not recommended to extend the intensive, but the continuation phase of the treatment (25).

Therefore, further optimisation of the treatment for DS-TB is needed to reduce treatment duration and reduce the risk of treatment failure for high-risk subgroups. There are two main strategies to achieve this goal: 1) review the posology of the current drugs to find the best balance between safety and efficacy, and 2) substituting current drugs with new or repurposed drugs with increasing the overall bactericidal and sterilizing power of the regimen.

A recent example combining these two strategies is the high dose rifapentine and moxifloxacin regimen proposed in Study 31; the first that could cure most people, including high-risk populations (caverns and HIV coinfection), after *only* 4 months (26). The treatment based on optimized doses of rifapentine has not been widely implemented due to the limited availability of rifapentine, the pill burden, and the cost. Despite this important limitation, the last ATS/ERS guidelines recommend this regimen as the first option for DS-TB (27).

Regarding rifampicin, the current standard of 10mg/kg/day was selected in the late 1970's because it provided serum concentrations above the minimum inhibitory concentration of rifampicin against *M. tuberculosis*, adverse events seemed dose-dependent, and it was an expensive drug at that time (28). Recent research showed that the standard dose of rifampicin is at the lower end of the dose-response curve, leading to suboptimal serum concentrations in an important subset of people with TB, and nowadays, rifampicin is a cheap antibiotic. Higher doses of rifampicin could improve exposure for most people but potentially increasing adverse events. There is no consensus on which dose has the best risk-benefit balance.

2: Hypothesis

"The disease of the lady Madeline had long baffled the skill of her physicians. Her frame had been gradually and silently wasting away, and her character, if not her appearance, had undergone a radical alteration."

The Fall of the House of Usher, Edgar Allan Poe

The optimal dose of rifampicin for the treatment of tuberculosis is unknown

As discussed in Chapter I, the current standard dose was established decades ago based on early efficacy and safety studies, and high production costs. Since these first studies, rifampicin has become a cheap and widely available antibiotic, used not only for TB, but also for other mycobacterial infections, brucellosis, or bacterial infections where biofilm is likely involved (osteosynthesis material, prosthetic valve infections).

Several studies in recent years suggest that rifampicin used at doses higher than the standard is safe and may improve treatment efficacy. However, many studies used different doses, ranging from 13 to 50mg/kg/day, with no clear consensus on the optimal range balancing safety and efficacy of rifampicin. Vulnerable populations have been excluded from previous trials fearing an increased rate of adverse events because a more severe TB disease or a high comorbidity burden. However, these vulnerable subgroups have an increased risk of a poor outcome.

Therefore, there is a need to summarise previous evidence focusing not only on safety and efficacy endpoints, but also on a thorough description of the population included in TB clinical trials so far, to inform the design of future, more inclusive clinical trials.

Hypothesis: rifampicin dose optimization does not jeopardize safety while reducing the risk of poor treatment outcomes, especially in vulnerable populations.

The two main hypothesis of this work are:

1- If rifampicin is used at doses higher than the standard 10mg/kg/day, the rate of severe adverse events will not increase significantly. If the former assumption is accepted, the efficacy of the treatment for DS-TB could be enhanced using optimised rifampicin dosing.

2- Despite being excluded from previous clinical trials, if vulnerable people with a higher risk of poor treatment outcomes have similar safety results as in previous clinical trials, they could have a larger benefit in terms of bacterial clearance and clinical outcomes.

3: Objectives

-"You may have tuberculosis or whatever, but if you don't do your job, I'm going to tell him, and then, he won't show you any dreams".
Chapter 58, Mugen Train Arc, Kimetsu no Yaiba

The main objective of this work is to optimize the first line treatment for tuberculosis focusing on rifampicin as part of the first line treatment for tuberculosis with isoniazid, ethambutol, and pyrazinamide.

The secondary objectives were as follows:

1- Review current literature and summarize the current landscape and areas of uncertainty for the treatment of tuberculosis, from rifampicin-susceptible to MDR-TB.
2- Systematically analyse the available and most relevant literature regarding the use of high doses of rifampicin, with a primary focus on safety and describe the populations included in recent rifampicin dosing clinical trials.
3- Design and register a phase II clinical trial using the results of the systematic review from objectives 1 to 3.Create the capacity to conduct a phase II clinical trial within the EUSAT consortium, using high rifampicin dose to treat drug susceptible TB in Latin America and Europe in subpopulations known to have worse outcomes.
4- Evaluate the safety and efficacy of optimizing the dose of rifampicin plus the standard treatment in people with DS-TB from sites in Paraguay, the Netherlands, and Spain, focusing on people from vulnerable groups: people living with HIV, elderly people, patients with co-morbidities such as diabetes, immunosuppression and malnutrition.

4: Compendium of articles

"She suffers from pulmonary tuberculosis. Eradicating the germ that causes this disease is very difficult and slow. Mrs. Yasuda has been ill for three years and, to be honest, there is little chance of her ever recovering completely. Her husband already knows this. The only thing we can do is to control the progression of the disease by injecting her with a new medicine."

Tokyo express. Seicho Matsumoto.

4.1. Article 1: Safety, efficacy and pharmacokinetics of daily optimised doses of rifampicin for the treatment of tuberculosis: a systematic review and bayesian network meta-analysis

Espinosa-Pereiro J, Aguiar A, Nara E, Medina A, Molinas G, Tavares M, Tortola T, Ghimire S, Alfenaar JC, Sturkenboom MGG, Magis-Escurra C, Sánchez-Montalva A, Barros H, Duarte R. Safety, Efficacy and Pharmacokinetics of daily optimised doses of Rifampicin for the Treatment of Tuberculosis: A Systematic Review and Bayesian Network Meta-Analysis. Clin Infect Dis. 2025 Jan 10:ciaf003. doi: 10.1093/cid/ciaf003. Epub ahead of print.

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https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciaf003/7951397?redirectedFrom=fulltext&login=false

4.2. Article 2: Safety of rifampicin at high dose for difficult-to-treat tuberculosis: protocol for RIAlta phase 2b/c trial

Article 2: Espinosa-Pereiro J, Ghimire S, Sturkenboom MGG, Alffenaar JC, Tavares M, Aguirre S, Battaglia A, Molinas G, Tórtola T, Akkerman OW, Sanchez-Montalva A, Magis-Escurra C. Safety of Rifampicin at High Dose for Difficult-to-Treat Tuberculosis: Protocol for RIAlta Phase 2b/c Trial. Pharmaceutics. 2022 Dec 20;15(1):9. doi: 10.3390/pharmaceutics15010009.

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Communication

Safety of Rifampicin at High Dose for Difficult-to-Treat Tuberculosis: Protocol for RIAlta Phase 2b/c Trial

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Abstract: Previous clinical trials for drug-susceptible tuberculosis (DS-TB) have shown that first-line treatment with doses of rifampicin up to 40 mg/kg are safe and increase the early treatment response for young adults with pulmonary tuberculosis. This may lead to a shorter treatment duration for those persons with TB and a good baseline prognosis, or increased treatment success for vulnerable subgroups (age > 60, diabetes, malnutrition, HIV, hepatitis B or hepatitis C coinfection, TB meningitis, stable chronic liver diseases). Here, we describe the design of a phase 2b/c clinical study under the hypothesis that rifampicin at 35 mg/kg is as safe for these vulnerable groups as for the participants included in previous clinical trials. RIAlta is an interventional, open-label, multicenter, prospective clinical study with matched historical controls comparing the standard DS-TB treatment (isoniazid, pyrazinamide, and ethambutol) with rifampicin at 35 mg/kg ($HR^{35}ZE$ group) vs. rifampicin at 10 mg/kg (historical $HR^{10}ZE$ group). The primary outcome is the incidence of grade $\geq$ 3 Adverse Events or Severe Adverse Events. A total of 134 participants will be prospectively included, and compared with historical matched controls with at least a 1:1 proportion. This will provide a power of 80% to detect non-inferiority with a margin of 8%. This study will provide important information for subgroups of patients that are more vulnerable to TB bad outcomes and/or treatment toxicity. Despite limitations such as non-randomized design and the use of historical controls, the results of this trial may inform the design of future more inclusive clinical trials, and improve the management of tuberculosis in subgroups of patients for whom scientific evidence is still scarce. Trial registration: EudraCT 2020-003146-36, NCT04768231.

Keywords: tuberculosis; rifampicin; clinical trial; vulnerable population

1. Introduction

Current standard treatment for drug susceptible tuberculosis (DS-TB) was designed 40 years ago and has remained unchanged except for a 4-month regimen using high-dose rifapentine and moxifloxacin following the results of a recent clinical trial [1]. Rifampicin is still the backbone drug in the treatment of DS-TB because of its potent bactericidal and sterilizing activity. The currently used once daily 10 mg/kg dosing of rifampicin was selected in the 1970s and was based on pharmacokinetics, toxicity, and cost concerns [2]. Under clinical trial conditions and using per protocol analysis, the current standard regimen achieves a 95% efficacy [3]. However, several pharmacological, bacterial, and host factors may negatively affect treatment outcomes in daily practice.

Some groups of people are more vulnerable to TB because of risk factors (e.g., advanced age, immunosuppression, diabetes, or liver disease) that increase their risk of active disease and poor treatment outcomes [4]. For example, treatment success rate was 86% globally, but only 77% for people living with HIV [5]. Drug-induced liver injury is the best-known adverse reaction associated with rifampicin, isoniazid, and pyrazinamide. In addition, rifampicin typically causes gastrointestinal symptoms and hypersensitivity reactions (pruritus, rash, flu-like syndrome, hemolytic anemia). Although for clinicians these groups are also considered more vulnerable to adverse reactions, different studies show contradictory results. For example, in a retrospective study including 1149 patients, age > 60, diabetes, and hepatitis B virus (HBV) infection were not associated with an increased risk of adverse reactions [6].

Optimal drug regimen, dose, and treatment duration are still not well established, as these vulnerable groups were underrepresented in previous clinical trials as these were conducted in mid-to-high TB burden countries. However, the population in these studies included young participants with pulmonary TB and no comorbidities except 20% of participants with HIV coinfection (data from a systematic review by the European South American TB Research Collaborative Network (EUSAT-RCS, manuscript in preparation). A rifampicin dose ranging trial in DS-TB patients by Boeree and colleagues reported that doses (up to 50 mg/kg) were safe and tolerated, and further improved the extended early bactericidal activity in TB patients [7,8]. The optimized doses achieved up to 10-fold higher exposure in plasma (AUC0-24) and a higher early bactericidal activity measured by a decline in the colony-forming units count in sputum culture [7,9,10]. A meta-analysis of 13 studies from 1979 to 2021, including adults with TB meningitis and pulmonary TB receiving doses up to 35 mg/kg, did not show an increase in the incidence of Severe Adverse Events (SAE), with a pooled Incidence Risk Ratio of 1.00 (95% confidence interval 0.82–1.23) [11]. Therefore, increased doses might have the potential to shorten DS-TB treatment. Pharmacokinetic studies show that a low proportion of patients reach target exposures with the current standard doses of rifampicin. For example, in a clinical trial in Indonesia, only 48% of the participants receiving standard dose rifampicin reached the 8 mg/L threshold for maximum plasma concentrations (Cmax) deemed to be effective [12]. In addition, adults with TB and other comorbidities such as HIV and diabetes may have lower exposure to rifampicin [13]. In the absence of actual AUCs, clinicians often use the concentration 2 h after drug intake as a predictor of rifampicin exposure [14].

The RIAlta study aims to evaluate the safety and efficacy of rifampicin at 35 mg/kg daily for the intensive phase of the first line TB treatment in vulnerable adults with TB, usually excluded from clinical trials due to age and/or comorbidities. This is a single arm prospective study using matched historical controls. This article summarizes the trial protocol and the rationale behind key aspects of its design.

2. Methods

2.1. Study Design

This is an interventional, open-label, multi-center, historically matched controlled prospective clinical study of high dose rifampicin (35 mg/kg, $HR^{35}ZE$ group) versus standard-dose (10 mg/kg/day, historical $HR^{10}ZE$ group) in a vulnerable population. Clini-

cal trials with rifampicin are typically open label as the drug causes orange discoloration of the urine and other body fluids that makes it difficult to maintain blinding for participants and investigators [15–17]. Table 1 shows a comparative of key design aspects in different phase 2 studies using high-dose rifampicin in the last 15 years.

2.2. Objectives and Endpoints

The study primary objective is to evaluate the safety of high-dose rifampicin (35 mg/kg/d) as part of a standard first line regimen using standard doses of isoniazid (5 mg/kg), pyrazinamide (25 mg/kg), and ethambutol (15–25 mg/kg) for 8 weeks in adult subjects with pulmonary or extrapulmonary DS-TB belonging to difficult to treat subgroups. The secondary objectives are to evaluate the tolerability, efficacy, bactericidal activity, pharmacokinetic/pharmacodynamics, and cost effectiveness of high dose rifampicin, among others.

The primary safety endpoint is the proportion of participants and historical controls with one or more grade 3 or higher adverse events (AE) according to the CTCAE v5 or SAE at 8-weeks after treatment with high-dose rifampicin onset. Table 2 below describes in detail the analysis population and primary safety outcomes. The secondary tolerability endpoint includes the proportion of participants with any AE. The secondary efficacy endpoint includes the proportion of participants and historical controls who have sputum culture conversion at 8 weeks after treatment onset or a proportion of participants with clinical improvement according to the treating physician (if follow-up images are available for extra-pulmonary TB) but without a follow up sample at 8 weeks after treatment onset.

Most TB phase 2 trials complete their intervention at week 8 or earlier and then participant care is transferred to a public institution within the National TB programs, so the information about end of treatment and post-treatment outcomes is scarce [10,17,18]. In the RIAlta trial, after the intervention and the primary safety objective evaluation, an extended follow-up period will be offered to participants, in line with the phase 2b/c design suggested by Phillips et al. [19]. This will provide information about end-of-treatment outcomes and relapse incidence in the prospective group.

2.3. Study Setting

The European South American TB Research Collaborative Network (EUSAT-RCS) is a consortium supported by the European Commission (Marie Sklodowska-Curie grant number 823890). The study will be carried out at four different TB treatment centers across Europe and one in South America. Vall d'Hebron Institute of Research (VHIR, Barcelona, Spain) is the sponsor of the study, and Hospital Universitario Vall d'Hebron (Barcelona, Spain) is the coordinating center and serves as the primary contact point for this trial. Other participating centers include: Radboud University Medical Center, Nijmegen, the Netherlands; University Medical Center Groningen, Groningen, the Netherlands; Centro Hospitalario Universitário de São João, Porto, Portugal; and Instituto Nacional de Enfermedades Respiratorias y del Ambiente (INERAM), Asuncion, Paraguay.

According to WHO 2020 data, TB incidence per 100,000 population is low in The Netherlands, Spain, and Portugal, whereas it is still high in Paraguay [5]. Diabetes prevalence is estimated to be 15% among people with TB, in contrast with 9.3% globally in 2019 [20]. Median age is between 42 and 46 years in Europe, whereas in high burden countries, the population is some 20 years younger on average (26 years in Paraguay, 27 in South Africa). Both South America and the European region have relatively low incidence rates of HIV-TB coinfection (3 in 100,000) [5]. In a UK prospective study, 18.1% of the patients with active TB had markers for hepatitis B or C infection were significantly higher than the 0.3% prevalence for the general population in the same period [21]. However, despite the need to study the possible increase in incidence, the potential drug interactions, and the risk of drug-induced liver injury with TB treatment, these coinfections are exclusion criteria for many TB trials (Table 1).

Table 1. Comparison of recent phase 2 trials using high-dose rifampicin for tuberculosis.

Study	Trial ID	Design	Population	Key Exclusion Criteria	Arms	Max. Rifampicin (mg/kg/day)	Follow-Up (Weeks)
Ruslami 2013	NCT01158755	Phase 2b, open label, randomized	≥15 years old with clinically suspected TB meningitis	Body weight < 30 kg, liver disease	2	13	26
HR1	NCT01392911	Phase 2a, open label, sequential allocation	18–65 years old with confirmed pulmonary DS-TB	High alcohol consumption, diabetes	7	50	2
RIFATOX	ISRCTN55670677	Phase 2b, open label, randomized	18–65 years old with confirmed pulmonary DS-TB	High alcohol consumption, diabetes, HIV	3	20	16
HIGH RIF-2	NCT00760149	Phase 2b, double blind, randomized	18–65 years old with confirmed pulmonary DS-TB	Body weight < 50 kg, clinical hepatitis/cholestasis	3	20	12
MAMS-TB	NCT01785186	Phase 2b,c, open-label, randomized	≥18 years old with confirmed pulmonary DS-TB	High alcohol consumption, HIV infection with <200 CD4	5	35	48
HIRIF	NCT01408914	Phase 2b, double blind, randomized	18–60 years old with confirmed pulmonary DS-TB	Body weight < 30 kg, viral hepatitis	2	20	26
RifT	ISRCTN42218549	Phase 2, open label, randomized	≥18 years old with clinically suspected TB meningitis	Cirrhosis or clinical jaundice	3	35	24
RIFAVIRENZ	NCT01986543	Phase 2, open label, randomized	≥15 years old with confirmed pulmonary DS-TB	AIDS-defining infection, pharmacological immunosuppression	2	20	28
RIAlta	NCT04768231	phase 2b,c, open label, historical controls	≥60 or ≥18 years old (with HIV, HBV, HCV, DM), pulmonary and extrapulmonary TB	Decompensated chronic liver disease, oral anticoagulation	1	35	72

Table 2. Analysis populations and endpoints.

Assessment	Primary Safety	Efficacy
1. Per protocol population	Includes participants who completed assigned follow-up AND were at least 80% adherent to study treatment.	Includes those participants who complete assigned follow-up AND were at least 80% adherent to study treatment.
2. Intention to treat population	Includes all the participants that received at least one dose of anti-TB drugs.	Includes all the participants enrolled in the study.
3. Modified intention to treat population	Includes a subset of intention-to-treat population with exclusion of some subjects in a justified way (subjects having non-tuberculous mycobacteria, early withdrawal of consent, etc.).	Includes subset of intention-to-treat population with exclusion of some subjects in a justified way (participants having non-tuberculous mycobacteria, early withdrawal of consent, etc.).
4. Microbiological intention to treat population	Includes all the participants that received at least one dose of anti-TB drugs AND have culture confirmation of DS-TB.	Includes all the participants enrolled in the study AND have culture confirmation of DS-TB.
5. Microbiological per-protocol population	Includes those participants who complete assigned follow-up AND were at least 80% adherent to study treatment AND have culture confirmation of DS-TB.	Includes those participants who complete assigned follow-up AND were at least 80% adherent to study treatment AND have culture confirmation of DS-TB.

2.4. Study Population and Eligibility

Adult participants ≥18 years with confirmed or probable pulmonary or extrapulmonary TB will be eligible to participate in the study. Additionally, trial candidates must be older than 60 years or have one of the following: diabetes, HIV, HBV or HCV coinfection, malnutrition (body mass index below 18.5 kg/m^2), or any other chronic but stable liver disease.

Adult participants with *M. tuberculosis* that is rifampicin-resistant by Xpert MTB/RIF or by drug susceptibility testing; with a Barthel index < 40 or life expectancy of less than 2 months regardless of anti-TB treatment; with signs of hepatotoxicity characterized by AST or ALT > 5x upper limit of normal, total bilirubin > 3x upper limit of normal, Child–Pugh grade C cirrhosis or acute decompensation at enrollment, or any grade 3–4 hepatobiliary alteration; who were previously treated with first-line anti-TB drugs or quinolones for at least 14 days or current treatment for more than 7 days; with solid organ or bone marrow transplantation; with active neoplasm requiring chemotherapy or immunotherapy treatment; or with previous severe pulmonary disease other than TB according to the investigator's judgment will be excluded from the study. Participants with aischemic heart disease OR severe arrhythmia within 6 months OR subjects with atrial fibrillation and indication of oral anticoagulant therapy when transitioning to low-molecular weight heparin is not feasible will also be excluded from the study. Lastly, pregnant or breastfeeding women and participants not suitable to be included according to the investigator will not be enrolled for the study.

This is one the key differential aspects of RIAlta's design. Previous trials have included people living with HIV, but few participants with diabetes, age over 60, or chronic liver disease were included [11,22].

2.5. Recruitment

Participants will be prospectively included and compared with historical controls from the same sites. Participants meeting all the inclusion and not any of the exclusion criteria will be offered to start on the HR^{35}ZE arm. After 8 weeks, participants will be managed according to the national TB guidelines.

The selected retrospective participants will provide a comparison for the primary safety outcome, and to the secondary efficacy outcome of culture conversion at 8 weeks using MGIT media.

We adhered to all Pockock's principles for historical controls except for the use of information from participants that were previously randomized in clinical trials [23]. To avoid the selection bias that may arise because of this limitation, we aim to include all adult patients fulfilling the inclusion criteria treated with $HR^{10}ZE$ in the participating sites between January 2017 and December 2019 (to avoid the influence of the 2020–2021 SARS-CoV-2 pandemic in TB diagnoses and follow-up). Thus, the records of all patients from that period with pulmonary and extrapulmonary rifampicin susceptible tuberculosis will be reviewed. We will capture the severe or grade 3 adverse events and week 8 microbiological status from those who are ≥60 years, or ≥18 years and have a diagnosis of diabetes, malnutrition, HIV, HBV or HCV coinfection, other stable chronic liver diseases, or TB meningitis, and have no pharmacological immunosuppression. We intend to control the retrospective cohort to match the prospective one for age, sex, cavitation in lung TB, and severity in TB meningitis. In case not enough matched controls are not found, we will extend the search with patients until the calculated sample size in the control arm is achieved. The historical cohort will include participants from only Spain, Portugal, and Paraguay and not likely from the Netherlands, as optimized doses of rifampicin have been used in both Dutch sites for more than a decade in the same participant subgroups as included in our study.

2.6. Intervention

The intervention in this study is a once daily high-dose of rifampicin at 35 mg/kg. A dose-finding study conducted in South Africa found that 40 mg/kg/day was the maximum tolerated dose in young participants without significant comorbidities [8]. Participants should start anti-TB treatment as soon as possible after signing written informed consent. This protocol allows a window of up to 7 days to complete screening tests (whether TB treatment was initiated or not; see study design diagram: Figure 1). Pyridoxine (vitamin B6) supplementation will be allowed as per local guidelines recommendations. The investigation team will carefully assess other concomitant drugs. When interactions are detected, dose adjustments or substitutions by an alternative drug will be at the discretion of the investigators.

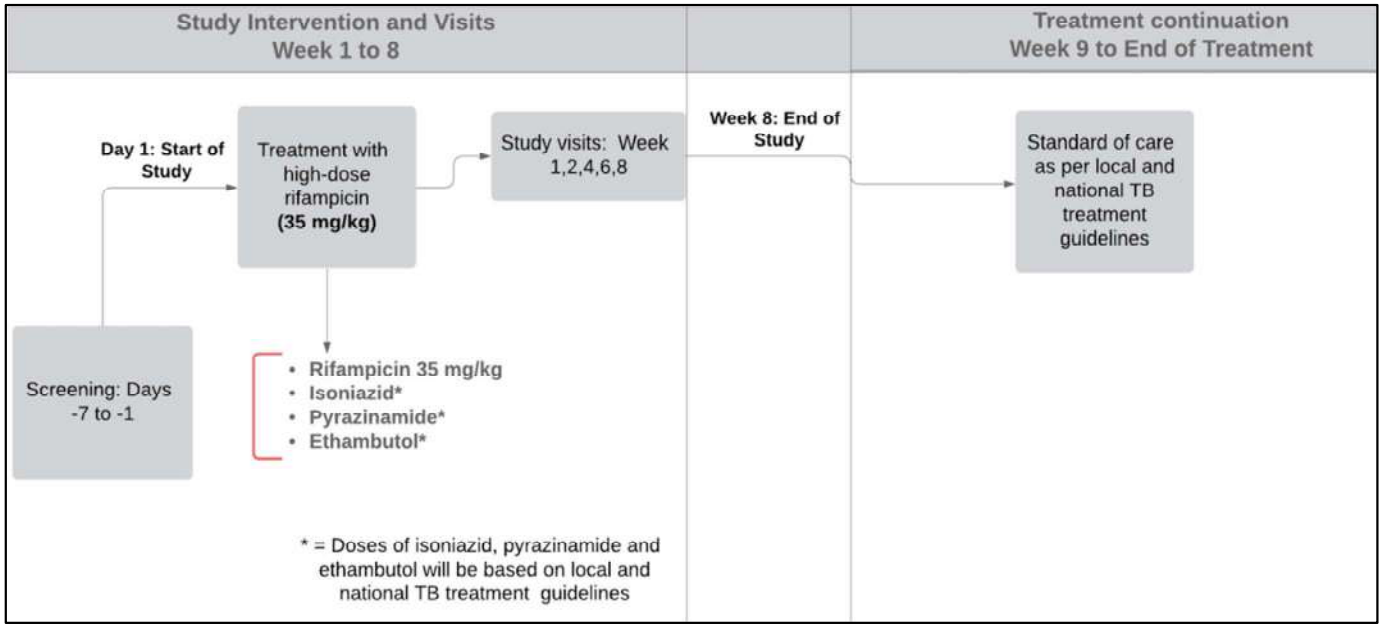

Figure 1. Study flow diagram.

2.7. Rifampicin Dosing Strategies

Study participants will receive high dose rifampicin 35 mg/kg for 8 weeks (maximum once daily dose of 3150 mg) by combining fixed-dose combination (FDC) tablets with rifampicin loose capsules (Table 3). We have adapted the WHO guidelines weight band dosing scheme for a more accurate rifampicin dosing. The dose for each band was calculated for the medium weight in each band (45 mg × 35 mg = 1575 mg, rounding 1500 mg), and for those with >80 kg, the dose is calculated for 90 kg (90 mg × 35 mg = 3150 mg).

Table 3. Weight-band dosing of rifampicin.

High Dose Rifampicin (35 mg/kg)	**Weight-Band Dosing**					
	35–40 kg	**41–50 kg**	**51–60 kg**	**61–70 kg**	**71–80 kg**	**≥80 kg**
Rifampicin tablets (300 mg)	900 mg 3 tablets	900 mg 3 tablets	1500 mg 5 tablets	1500 mg 5 tablets	1800 mg 6 tablets	2400 mg 8 tablets
Rifampicin tablets (150 mg)	-	150 mg 1 tablet	-	150 mg 1 tablet	150 mg 1 tablet	-
Fixed dose combination (*150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol*)	450 mg 3 tablets	450 mg 3 tablets	450 mg 3 tablets	600 mg 4 tablets	750 mg 5 tablets	750 mg 5 tablets
Total rifampicin dose	1350 mg	1500 mg	1950 mg	2250 mg	2700 mg	3150 mg

One limitation is the scarcity of the 600 mg presentation for rifampicin, which would reduce the pill burden for the participants in this trial. For instance, as optimized doses of rifampicin come closer to daily practice, public health decision makers should advocate to increase the availability of rifampicin in high dose presentations to reduce the number of pills taken by patients.

To achieve a similar drug exposure to oral rifampicin at 35 mg/kg/day, the dose for intravenous administration should be 26 mg/kg/day [13].

2.8. Assessment of Study Outcomes, Analysis Groups, and Duration of Follow-Up

The primary safety hypothesis is that the incidence of adverse events in the high dose rifampicin (35 mg/kg) supplemented with isoniazid, pyrazinamide, and ethambutol [$HR^{35}ZE$] during the first 8 weeks of treatment will not exceed that of controls on a standard HRZE regimen (rifampicin 10 mg/kg) [$HR^{10}ZE$]. The $HR^{35}ZE$ regimen will be considered unsafe if in a non-inferiority analysis, the upper limit of the 95% confidence interval of the difference of proportions in grade ≥ 3 AE (according to CTCAE scale version 5) between the experimental and the historical control arm exceeds 8%. The most common adverse events related to therapy with higher rifampicin doses were vomiting, headache, hyperuricemia, pain in the extremities, and pruritus. No significant differences according to rifampicin dose (from 10 to 35 mg/kg) were found in a recent systematic review and in a retrospective study collecting the real-life experience from a Dutch center [24,25].

To assess the AE and ensure a homogeneous evaluation of their grade, severity, expectedness, and treatment relatedness, we will administer a standardized training for the investigators from all different sites. In addition, both for the prospective and the retrospective cohorts, grade 3 or higher adverse events and SAE will be reviewed by the trial's Steering Committee for relatedness classification. When no consensus is reached, the Data and Safety Monitoring Board formed by experts independent from the trial will make the final decision.

Tolerability will be measured as any adverse event (grade 1 to 4), treatment dropout rate, and any dose reduction. For efficacy outcome, the hypothesis is that in DS-TB participants (pulmonary disease), the proportion of participants experiencing successful treatment outcomes (culture and smear negative) at the end of the intensive phase of treatment on $HR^{35}ZE$ will be non-inferior to that of controls on $HR^{10}ZE$. The $HR^{35}ZE$ regimen will be

considered ineffective if the lower limit of the 95% CI of the proportion of participants with a negative sputum culture (sputum sterilization), or unable to produce sputum if they have a previous negative culture at week 8, is more than 10% lower than that of historical controls treated with $HR^{10}ZE$. Other exploratory outcomes will be reported descriptively.

After the trial intervention ends at week 8, participants will be transferred to a standard of care as per national guidelines. We will extend the follow-up duration up to one year after the completion of the treatment to capture relapse-free cures.

We will use different subsets of the study population for the primary safety, tolerability, and efficacy objectives. Table 2 shows a detailed analysis plan as described in the protocol.

2.9. Sample Size Assumptions and Justification

For the sample size calculation, a non-inferiority design is assumed and the upper limit of the 95% confidence interval (CI) of the difference of proportions in grade 3 or higher adverse events at 8 percentage points has been set. We assume that 5% of patients will not be assessable. Alpha risk is set in 0.1 and power in 0.8. Based on the previous literature and our previous experience, expected standard treatment grade $\geq$ 3 AE are 5–10% (mean 8%) among experimental arms and standard treatment arms. Considering this, the number of participants per arm has been calculated to be 134 participants. Because of the limited recruitment capacity for this trial, we could not target specific numbers of participants for each sub-group of risk factors.

Based on a feasibility survey performed at the planning stage, the target enrollment rates per country were: Paraguay 6.25 participants per month; Spain, Portugal, and the Netherlands 1.6 participants per month.

2.10. Main Outcomes Analysis

The primary safety outcome will be analyzed as the proportion of participants in the $HR^{35}ZE$ and historical $HR^{10}ZE$ groups that received at least one dose of treatment with an unfavorable outcome after 8 weeks of experimental treatment start. According to the clinical and laboratory information, participants will be classified as having an unfavorable outcome if they suffered one or more grade $\geq$ 3 AE or SAE that are possibly, probably, or definitely related to rifampicin OR they did not complete their treatment because of other reasons OR were lost-to follow-up. High doses of rifampicin will be considered non-inferior to standard doses if the upper boundary of the 95% CI of the proportion of participants with an unfavorable outcome is less than 8% that of the point estimate in the historical standard dose group.

Efficacy will be assessed as the proportion of participants with pulmonary TB in the $HR^{35}ZE$ and the historical $HR^{10}ZE$ groups that have sputum culture conversion in liquid media at week 8. The unfavorable efficacy outcome will be defined as having a positive sputum culture at week 8.

2.11. Secondary Objectives

Secondary objectives can be grouped into 4 categories. The analyses will be descriptive as these data will not be available from most of the historical $HR^{10}ZE$ controls.

2.11.1. Microbiological Evaluation

We will collect sputum samples from the experimental treatment pulmonary TB participants at week 1, 2, 4, 6, and 8, and compare the baseline bacterial load with the bacterial load at each of these time points as estimated by the time to positivity in days, as compared to that at baseline [9]. Time to positivity (TTP) is a good correlate of bacterial load and this information is readily available from the MGIT system. In addition, we should be able to estimate CFU count using a formula previously published [26]. In addition, we will compare the changes in TTP with a baseline and follow-up breath signal of the AeoNose™ device, an electronic nose that analyzes volatile organic compounds (VOC) in exhaled air.

Pilot studies show that the VOC signal decreases during treatment; hence, it has potential as a biomarker to estimate bacterial load [27].

2.11.2. Pharmacokinetics

Drug exposure is a better predictor of treatment success than the total dose. There is extensive pharmacokinetic data on rifampicin at doses up to 50 mg/kg/day, but the target population in RIAlta was not represented. To capture rifampicin exposure in steady state, we will collect PK samples at week 4 after experimental treatment starts following a simplified sampling strategy (2, 4, and 6 h) to predict exposure (AUC0-24) [28].

2.11.3. Pharmacogenetics

The genes involved in rifampicin metabolism and hepatotoxicity, whose expression is modified by rifampicin itself, are thought to explain the wide inter-individual variations observed in rifampicin exposure and development of toxicities among certain individuals. The RIF gene is mutated at three sites: CYP2C9*2, CYP2C9*13, and CYP2C19*2, which might have significance in drug-induced liver injury. The study by Su et al. found that the CYP2C9*2 genotype was significantly associated with drug-induced liver injury [29]. In Europeans and admixed Americans, CYP3A4*22 is the most common allele (minor allele frequency, MAF 5% and 2.6%, respectively) with *3 and *2 contributing to the genetic variability in the former study. In contrast, CYP3A4*15 (MAF 2.5%) and CYP3A4*18 (MAF 1.9%) constitute the only common CYP3A4 alleles in Africans and East Asians [30]. A subset of participants will be offered to participate in a genetic analysis searching for variants in the genes *SLCO1B1*, *ABCB1*, *UGT1A*, or *PXR*. We will correlate these results with the exposure obtained from the PK sub-study as well as with the clinical and microbiological outcomes [31].

2.11.4. Health Economics and Quality of Life

We will assess the costs (direct and indirect), the incidence of catastrophic costs (those that are $\geq$20% of the household's annual income), and the changes in quality of life associated with the use of high dose rifampicin in this trial population. The EUSAT-RCS consortium has developed a survey tool to capture the costs for TB patients and their caregivers based on that described by the WHO [32,33].

2.12. Ethical Approvals

The trial protocol has been registered in EudraCT and Clinicaltrials (EudraCT 2020-003146-36, NCT04768231 respectively), and has already been approved by the Medical Ethics Committees from Vall d'Hebrón Hospital in Barcelona, Spain, and Radboudumc in Nijmegen for The Netherlands (approval number: NL75346.091.20). Additional ethics approval will be acquired by each participant country.

2.13. Sharing of Trial Findings

Trial results regarding its main safety and efficacy outcomes, and all secondary outcomes will be summarized in the final trial report and submitted to regulatory authorities and ethics committees as required in each participating country. In addition, the results will be made public through peer-reviewed journals, and the generated datasets will be made available according to the European Commission requirements and data protection regulations.

2.14. Participant and Public Engagement

No structured community engagement was followed during the design of this trial. We will engage with participants, TB patient associations, and the community to discuss the results of this study and contextualize them and ensure a relevant impact. Furthermore, these results will inform the design of future projects, in which a continuous dialogue with the TB research community will be of utmost importance.

2.15. Strengths and Weaknesses of the Design

The population included in the RIAlta trial includes people from groups typically excluded from clinical trials, and will thus outline the applicability of optimized doses of rifampicin, with exploratory data on late clinical efficacy endpoints (relapse-free survival). The simultaneous collection of pharmacokinetic, microbiologic, and a breath signature will help provide a complete perspective on the intervention.

However, the non-randomized design and the use of historical controls make the RIAlta trial especially prone to bias. Although preventive measures have been taken, the results should be considered as informative to future trial designs rather than general practice changing.

3. Discussion

RIAlta is a multinational, open label, non-randomized phase 2b/c trial that aims to bridge an important knowledge gap on the safety of optimized rifampicin doses in vulnerable TB patients that are more prone to suboptimal treatment outcomes and drug toxicities because of age and comorbidities. The trial will provide useful information on the efficacy and pharmacokinetics of 35 mg/kg rifampicin dosing and explore possible correlations between occurring adverse events and the rifampicin genetic polymorphisms involved in metabolism and hepatotoxicity. It will evaluate the usefulness of exhaled VOCs as biomarkers of early treatment response. Finally, the study will evaluate the social and economic circumstances of the participants.

There are no validated surrogates for early treatment response for extra-pulmonary TB. Efficacy studies at 8 weeks based on sputum culture conversion does not include this patient population. The cure at the end of treatment is also difficult to confirm in this group because of the need of invasive samples that are not indicated as the procedure might pose extra/unnecessary risks to patients' lives. As a result, most of the trials exclude persons with extrapulmonary TB and the safety and efficacy information are extrapolated from pulmonary TB studies. It is well known that the pharmacokinetics of antimicrobials may vary according to the site of infection [13]. To address this, our study will obtain information about pulmonary and extrapulmonary TB in parallel. The correlation between intermediate data about sputum culture conversion and change in sputum bacterial load as estimated by the TTP for pulmonary TB participants and change in VOC in all participants will enable us to explore if the breath signature changes could be used as surrogate markers for extra-pulmonary TB. This could open the path for non-invasive biomarker validation and future inclusion of people with extrapulmonary TB in regulatory trials.

Therefore, the population included in this study belong to the other risk factors groups for failure and relapse that have been under-represented in previous clinical trials [4,22]. As a phase 2 study, the main aim of this clinical trial is to evaluate safety of rifampicin defined as the rate of participants suffering grade 3 or higher adverse events or severely adverse events. This information will be likely available from historical controls although it was not systematically collected. Grade 1–2 adverse events, which are considered tolerability problems, are not typically reported in clinical records, or the information about them is not complete, thus making it difficult to make comparisons. To address this, we have developed adverse events monitoring and management guidelines, which will be harmonized at all centers for the prospective part. Extensive training will be provided to the investigators at each site.

It is important to acknowledge the main limitations related to this trial design. Because of the limited recruitment capacity within our consortium and budgetary constraints, we decided to use historical controls (rifampicin 10 mg/kg along with the same TB regimen) and assign all prospectively recruited participants to the 35 mg/kg dose. This results in a non-randomized design as all participants will be assigned to the experimental arm and compared with historical controls. Hence, another limitation is that information about tolerability is often incomplete or not reported in clinical records, and the endpoints in the different sub-studies are not performed in daily clinical practice. For similar reasons, the

sample size is too small for a formal subgroup analysis. The results will inform a larger phase 3 trial with power calculations for relevant subgroups.

Having one PK sampling at week 4 will allow us to show the percentage of participants reaching the target exposure (AUC/MIC) when in a steady state after rifampicin induction of its own metabolism. In contrast, this approach will not provide information about exposure during the first week of treatment, when most of the bacterial killing occurs. Nevertheless, this knowledge will aid our understanding if optimized rifampicin dosing is more likely to meet target rifampicin exposure.

Any attempt to improve TB treatment outcomes must also consider its impact on the socio-economic background of the people who suffer because of TB. This includes patients with active TB infection and extends to their families and communities. A treatment that is safe and effective, but not well tolerated or takes longer to treat has consequences not only on individuals and families but also on a society. For example, frail TB patients might be absent from the workforce for a longer time, threatening financial security of the whole family in informal economies. Furthermore, caregiving activities of a family member might be over-stretched to meet the needs of patients that affects both direct and indirect costs of TB. Hence, the global impact of a new treatment scaled to a programmatic level could be unforeseen inconveniences for the patients and their families beyond the biological aspects of health.

In the past few decades, very few clinical trials on TB have been conducted in the European region because of low TB incidence (and hence low recruitment speed) and increased costs as compared to other regions. For those conducted, none of the trials have focused on the use of an optimized rifampicin dose in the targeted patient population [34]. European sites are still important for external validation and, importantly, to offer innovative treatment opportunities for people with TB. In addition, whereas implementing clinical trials in low-and middle-income countries has specific logistic challenges, working closely in both realities through collaborative consortiums such as ours, EUSAT-RCS, creates a unique milieu for capacity building. Via mutual exchange of personnel and expertise, researchers can develop the skills, knowledge, and competencies needed to plan, design, and execute clinical trials adapted to the local setting while adhering to the international ICH-GCP guidelines. In the long term, the implementation of this trial will help the establishment of a sustainable research network integrating EU and non-EU sites.

4. Conclusions

This paper provides a roadmap for using historical controls and setting up multi-center, multi-national pragmatic trials in real-life settings for diseases such as TB that take a long time to treat and follow up, and for which research resources are rather scarce. We believe that the results from the RIAlta study will inform future, larger trials and thus contribute to the generalization of the use of optimized doses of rifampicin in the real-world setting.

5. Trial Status

The trial is registered with the EudraCT number 2020-003146-36 and the NCT04768231. Site initiation visits and the recruitment of the first participants are expected to start in the last quarter of 2022 or first quarter of 2023.

Author Contributions: Conceptualization, J.E.-P., S.G., A.S.-M. and C.M.-E.; methodology, A.S.-M., S.G., C.M.-E., M.G.G.S. and J.-W.C.A.; writing—original draft preparation, J.E.-P. and S.G.; writing—review and editing M.T., S.A., A.B., G.M., T.T., O.W.A., A.S.-M. and C.M.-E.; project administration, C.M.-E. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study protocol was designed in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board from Vall d'Hebrón University Hospital (ref. nº 538, 20220715) and from Radboudumc (ref. NL75346.091.20).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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4.3. Clarifications on the methodology

The EUSAT-RCS and the EU-PEARL consortia

Most of the work presented in this thesis was performed in the ecosystem of two international, multidisciplinary projects funded by the European Union. The European Latin American TB Clinical Research Network (EUSAT-RCS) was started in 2019, funded by the Marie Sklodowska-Curie Actions (MSCA) Research and Innovation Staff Exchange (RISE) program (grant number 823890). The consortium fostered a collaborative network with public and private stakeholders from Europe, Paraguay, and South Africa. Its main objective was to build TB clinical research capacity through multidisciplinary studies and impactful initiatives focusing on the most vulnerable, high-risk groups affected by TB.

The main achievements of the EUSAT-RCS consortium have been summarized in a recent editorial(29). The consortium focused on innovative approaches for mass screening in Paraguayan prisons with the PriNose study (NCT04407325), the evaluation of optimized doses of rifampicin with the RIAlta trial (EudraCT 2020-003146-36), and the description of the costs associated to TB for Paraguayan families with the "Estudio Longitudinal de Costes Asociados a la TB", ELC-TB study (manuscript under preparation).

The EU Patient-cEntric clinical tRial pLatforms (EU-PEARL) was an Innovative Medicines Initiative 2 Joint Undertaking project running between 2019 and 2023 (grant number 853966-2). This was a strategic public and private sector alliance aiming to support the transformation of the classical trial approach into a cross-company collaborative platform centred around patients rather than around diseases. The project delivered a common enabling framework for platform trials in any disease area, using as examples 4 different diseases, namely Metabolic-Disfunction Associated Fatty Liver Diasease (MAFLD), Neurofibromatosis, Major Depressive Disorder, and TB. Annexes 3 and 4 are the result of our work as part of the TB working package within the EU-PEARL project (30).

Network meta-analysis as means to handle multi-arm trials

When reviewing the status of DS-TB treatment we found that most trials studying rifampicin doses in recent years had 3 or more arms including the control (articles 1 and appendix 1), which add complexity in a meta-analysis. For example, comparing three active arms to placebo would mean using the data from the placebo groups thrice. This situation is known as unit-of-analysis error and is important because using correlated data underestimates heterogeneity and therefore the variance is artificially low.

There are several methods to deal with multi-arm trials in meta-analysis and control bias (31,32). One can combine all groups that represent different modalities of a treatment to a single large group, split the control group in as many subgroups as experimental arms, or use specific statistical tools to account for correlation. The first two methods avoid the unit-of-analysis error but no other bias, as the data are still correlated. Depending on the method used, the variance estimates can over or underestimate trial heterogeneity leading to a bias in study weights within random effects meta-analysis. A proposal that is becoming more common in recent years is to conduct a multiple treatment meta-analysis, also called a network meta-analysis (NMA), which generalizes pairwise meta-analysis to more than two treatments.

NMA are based on graph mathematics, which were developed to describe the interrelation of electrical networks (33). When this model is applied to a meta-analysis, the nodes correspond to interventions, and the edges are the existing comparisons between treatments, variance corresponds to resistance and precision corresponds to conductance. In Ohm's law, I is the electrical current, U the potential difference (voltage), and R the resistance:

$$U = I \cdot R, or\ \frac{U}{R} = I$$

This shows that the voltage is proportional to the current, with resistance as a proportionality factor. In meta-analysis, variance corresponds to resistance, ; if we interpret that the effect estimates as voltages and the weighted treatment effects as current, the outcome of the treatment can be interpreted as the potential at that given node (differences between the outcomes of a pair of treatments). As in electrical networks, the variance (resistance) can be estimated indirectly for a pair of nodes with known effect size (voltages). This allows to successfully estimate the treatment effects directly and indirectly (estimated from the parameters of the surrounding network). Therefore, an additional advantage of the NMA is that it can estimate comparisons that were not performed in the source trials (figure 4).

Including multi-arm studies has the added complication that the control arms are correlated, so in the frequentist approach this correlation must be considered when calculating the variance of the pooled effect sizes, both in a pairwise or in a network meta-analysis. In the Bayesian approach, this issue is solved by assuming that the effects of the i treatments contained in the i + 1 treatment arms of a multi-arm study k stem from a multivariate normal distribution.

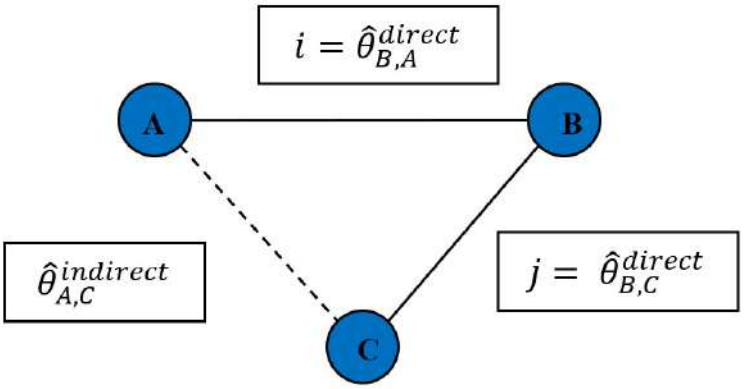

Figure 4. Network meta-analysis concept scheme. A network analysis is the mathematical representation of the figure above. It represents two trials, *i* and *j*, comparing treatments A to B and B to C, respectively. Each trial provides a real parameter for the effect estimate, that can be a mean difference, Odds Ratio, Risk Ratio, etc., as appropriate. This data provides an indirect pathway that allows us to compare treatments A and C, although these were never tested head-to-head in real life. Thus, we will obtain an indirect effect size between A and C.

Therefore, considering that several articles evaluating rifampicin doses in recent years had 3 or more arms, we decided to conduct a NMA. This technique has, however, some limitations that should be considered when interpreting its results. First, it does not avoid the influence of zero cells. No special precautions are needed in general to account for zero cells, but in datasets with a low number of events they may have a greater contribution to imprecision in the estimation of the variance for each outcome. If Bayesian statistics are used, this can be balanced using informative priors for the between-trial variance, although this may not suffice, or previous data be lacking (34). In addition, a NMA assumes that both direct and indirect comparisons are consistent with each other. Unnoticed lack of consistency in the network may lead to biased estimates, especially when indirect comparisons have more weight. There are different methods to measure inconsistency, being node-splitting one of the most accurate ones. This consists in 'splitting' each comparison calculating a direct and indirect estimate with its variance and testing the null hypothesis that there are no differences between direct and indirect estimates (35). Considering these limitations, the rankings produced by NMA need careful interpretation.

Designing and implementing a clinical trial

Clinical trial design and implementation are multidisciplinary tasks that begin with the outline of the trial protocol (article 2) and should involve all relevant stakeholders as soon as possible (appendix IV). The protocol delineates the interventions, objectives, how to measure these objectives (outcomes and endpoints), how to summarise them with statistics, trial population and essential procedures. All

related documents and procedures should align with the International Conference for Harmonization Good Clinical Practice guidelines (ICH GCP E6). Tuberculosis research faces specific challenges that need to be tackled from the design phase to avoid startup delays and inefficiencies. One of the most important ones is that research conducted in low-middle income countries (LMIC), where resource constraints and socioeconomic barriers complicate trial implementation.

Beyond the protocol, there are many other documents and procedures that need to be developed to ensure data quality and participant safety in clinical trials. We designed the case report forms selecting the minimal variables needed to answer the research questions for the trial while maintaining data consistency and completeness, while enabling for central monitoring. A key element to ensure data quality and adherence to good clinical practice is monitoring. Trial monitoring often combining central data analysis to find inconsistencies and missing data, and on-site trial monitoring to check adherence to the trial procedures, verifies participant safety and data integrity. This is normally performed by Clinical Research Organisations (CRO) who have their own protocols. For RIAlta trial, given the restricted budget, we had to develop our own protocols and procedures for monitoring (see the Capacity Building Section in chapter IV).

Other trial documents that needed to be developed for this trial, but that are not included in this thesis to keep the focus on the core scientific content, include the informed consent forms that were tailored to different languages and cultural settings, including one of the first informed consents for clinical trials in guaraní, investigator's brochure, standard operating procedures (SOP), insurance policies, clinical trial agreements, regulatory, and ethics review files.

5: Overall summary of the results

"The air is so stagnant, and the heat so oppressive, that the tortured little frame of the child lies withering in its bed, and hardly ventilates its languid and feeble soul. [...] Here, in a room at the top of a crazy house, barricaded with the rest of the world to prevent the disease from creeping forth [...]; and those who are born in it, live in it, and die in it, [...] have no home until the coffin comes, and they are laid in the ground by strangers who knew nothing about them."

Bleak House, Charles Dickens.

Our systematic review included 3962 participants from 19 studies spanning from early phase 2 to phase 3 trials. One study had a high risk of bias due to its non-randomized design, so it was excluded from the safety meta-analysis (but included for the Early Bactericidal Activity, EBA analysis). Most participants were young males with a mean age of 32.8 years old (95% Confidence Interval, CI 30.5-35.3). Comorbidity burden was low, with 15.8% with HIV coinfection, and less than 1% for diabetes and viral hepatitis. More than 25% of the participants had CNS TB, but there were no other forms of extrapulmonary TB. More than half of the participants were from 5 countries (Vietnam, India, Bangladesh, and Uganda). In these studies, there were 29 different regimens testing 11 different doses of rifampicin, including intravenous administration.

Safety outcomes were reported for 3265 participants from 15 studies. There was a significant increase in overall and hepatic grade ≥3 AE with 40mg/kg/day (RR 4.8, 95% Credibility Interval, CrI 1.1-25, and 15.00, 95% CrI 1.1; 58.0 respectively), but not for 50mg/kg/day (RR 0.83, 95% CrI 0.03-7.1, and 0.001, 95% CrI 0.0001-2.7). Note that there were only 15 and 17 participants for each of these dose groups leading to wide credibility intervals. In contrast, the probability rank and the Surface Under the Cumulative Ranking analysis did not order the probability of AE and hepatic AE according to increasing doses. No

significant increase in the risk of overall and hepatic grade ≥3 AE was found for doses between 13 and 35mg/kg/day (figure 4 in article 1). Of note, the only study exploring doses above 35mg/kg/day reported that 11 out of 17 participants in the 50mg/kg arm had to stop rifampicin due to tolerability issues.

There was a positive effect of higher rifampicin doses on early microbiological outcomes, which was significant for doses of ≥30mg/kg/day for EBA (416 participants at day 14, MD -1.4 logCfU, 95% CrI -2.5; -0.26 for 30mg/kg/day) and sputum culture conversion at week 8 (987 participants in liquid media, RR for a negative culture 1.1, 95% CrI 1.1–1.7 for 35 mg/kg/day). Interestingly, doses between 15 and 25 did not show a significant improvement in both EBA and sputum culture conversion (figure 5 in article 1). There were no significant differences regarding relapse in pulmonary TB or mortality in TB meningitis, although the number of events were relatively low for the regimens using ≥20mg/kg/day. This efficacy benefit is probably related to the more than proportional increase in exposure seen in the Cmax and AUC0–24 NMA, whereas Tmax and t1/2 were slightly affected by dose or administration route of rifampicin.

We used these results to inform the design of a clinical trial. RIAlta is a multinational, open label, non-randomized phase 2b/c trial that aims to bridge an important knowledge gap on the safety of optimised rifampicin doses in vulnerable people with TB that are more prone to suboptimal treatment outcomes and drug toxicities because of age and comorbidities. Table 1 in article 2 lists similar phase 2 trials with their key exclusion criteria. Half of these trials had an upper limit of age for enrolment, and excluded people with low weight, diabetes, HIV coinfection, and high alcohol consumption. Because of the limited recruitment capacity within our consortium and budgetary constraints, we decided to use historical controls (rifampicin 10mg/kg along with the same TB regimen) and assign all prospectively recruited participants to the 35mg/kg dose. This results in a non-randomized design as all participants will be assigned to the experimental arm and compared with historical controls. The use of historical controls in clinical trials offers the advantage of reduced costs and faster recruitment when the standard of care has not changed. However, historical controls introduce biases due to differences in participant populations, changes in the settings where the trial takes place, and memory bias if the information was imperfectly recorded. We planned to setup 5 sites to enrol participants, two in the Netherlands, one in Spain, one in Portugal, and one in Paraguay. At the time of writing this thesis, only 3 sites had completed setup and were actively recruiting participants: Radboud, Vall d'Hebrón Hospital, and INERAM. The protocol version 6.2 was approved by this committee on July 17th, 2022, and subsequently authorized by the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) on November 3rd, 2022. The protocol was approved in the Netherlands on September 7th, 2022, for Radboud and UMCG. In Paraguay, the evaluation process was longer due to changes in the regulatory framework and the limited experience of ethics committees

and regulators in evaluating academic clinical trials. The protocol was first submitted on December 2022, and ethics approval by the ethics committee in the Instituto Nacional del Cancer (INCAN) on October 27th, 2023. Regulatory authorisation was obtained April 9th, 2024, 16 months after the first submission.

Little was needed beyond the site initiation visit for trial setup in Radboud and Vall d'Hebrón. In INERAM, all procedures needed a thorough revision and adaptation, as it is the first clinical trial in that institution. An intense training plan on clinical trials was developed by the members of the consortium with special focus on young researchers. In INERAM, the capacities of the site's pharmacy and microbiology laboratory were limited. To cope with this, we provided specific training and secondments in the facilities from Radboud and Vall d'Hebrón. We established standard operating procedures and circuits with the local teams at the pharmacy of the National TB program and INERAM to ensure an adequate supply and monitoring of the trial medication, and with the Laboratorio Central de Salud Pública, the national microbiology reference laboratory, for the cultures, drug-susceptibility testing, and sample storage.

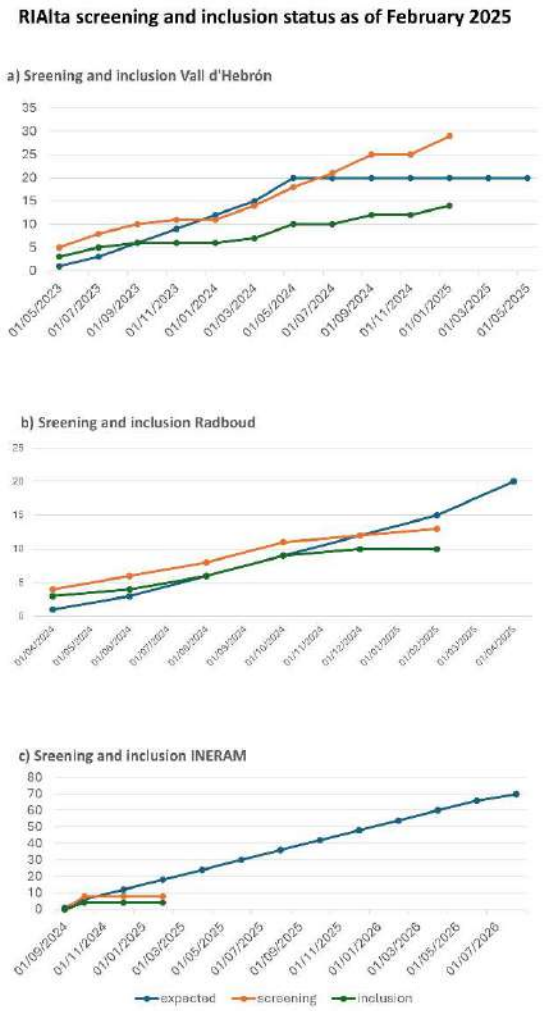

Figure 5. RIAlta screening and inclusion status as of February 2025. The graphics show screening and recruitment status, as compared to the original recruitment targets, in all 3 active sites. The original estimation was a recruitment period of 1 year, but in that time, sites reached half of the target sample size.

The Clinical Trial Agreements (CTA) needed to start recruitment took longer than anticipated: the CTA with RUMC was signed on September 4th, 2023, 12 months after local ethics and regulatory approval. The adaptation of the CTA for INERAM was relatively faster and was signed on September 6th, 2024. The first participant of the RIAlta trial was included on June 9th, 2023, in Vall d'Hebrón. The historical controls data were collected in Vall d'Hebrón and INERAM.

Due to the limited budget for the trial, a Clinical Research Organization (CRO) could not be appointed. Thus, we designed two strategies of monitoring that are complementary. First, the CRF was designed

to allow central monitoring of critical procedures such as informed consent and outcomes relevant to the primary objective. In addition, crossed monitoring with investigators from one site visiting other sites was planned. The first monitoring visit in was performed by EUSAT partners from Task, South Africa, in Vall d'Hebrón in November 2023.

We performed an interim analysis of the trial as of February 14th 2025, as part of the central monitoring activities of the trial with about 25% of the recruitment for the R^{35} group completed (figure 5). We present a summary of the data for the primary safety outcome and the coprimary efficacy outcome. Note that further actions are needed to ensure data quality of both the historical and prospective groups.

There were 117 participants in the historical controls, 63 from Spain and 54 from Paraguay, and 28 participants were included in the prospective group (14 from Vall d'Hebrón, 10 from Radboud, 4 from INERAM) (figure 6).

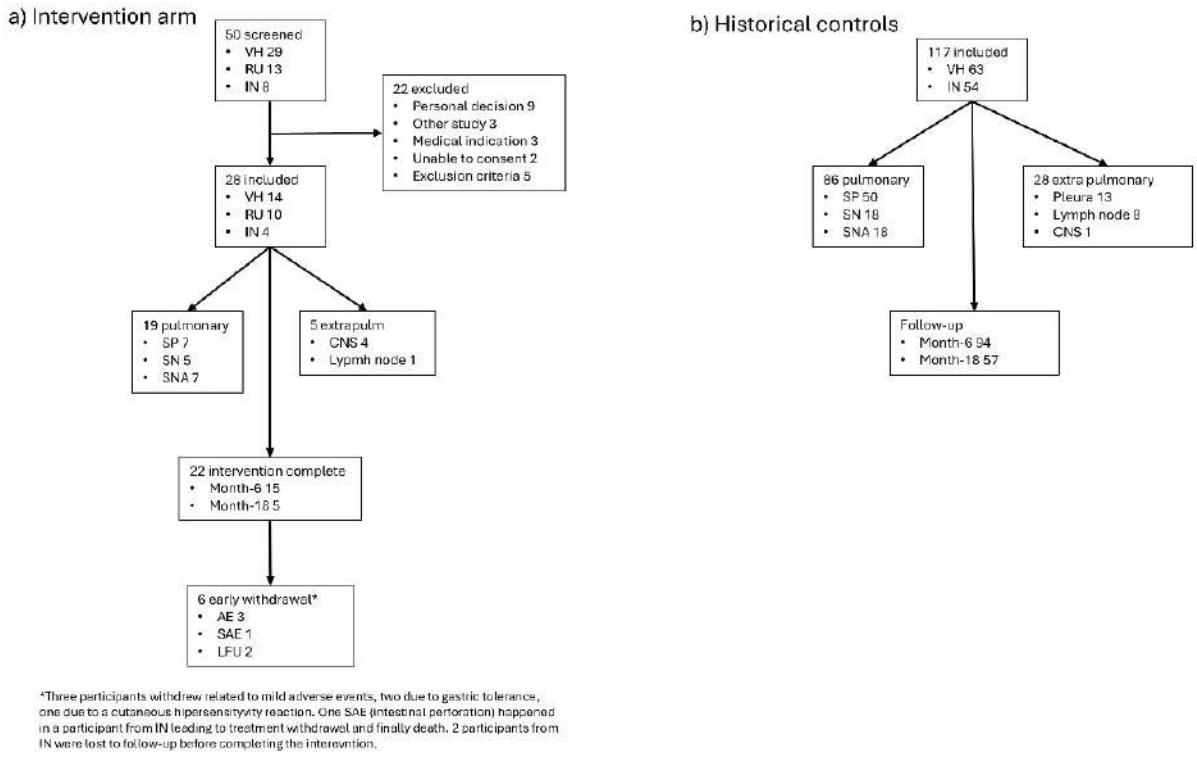

Figure 6. RIAlta flowchart as of February 2025**.** Inclusion in a) the R^{35} group and b) the historical cohort as of February 14th, 2025. VH, Vall d'hebrón; RU, Radboud; IN, INERAM; SP, sputum smear positive; SN, sputum smear negative; SNA, sputum smear not available; CNS, Central Nervous System; AE, Adverse Events; SAE, Serious Adverse Events; LFU, Lost to Follow-up.

The two groups were balanced in most demographic variables, but not all (table 2). Median age was 47.3 (IQR 28.8-63.0) and 66.1 (IQR 39.9-74.8) years in the historical and R^{35} groups. South American ethnicity was underrepresented in the R^{35} group (7.69% vs 42.6%). Regarding medical history, there

were only 7 (6.09%) participants with HIV coinfection in the historical group, and a higher proportion of diabetics in the R35 group (9/27, 33.3% vs. 16/117, 13.9%). A larger proportion of participants with pulmonary involvement TB had a positive smear at baseline in the historical cohort (50/86 73.5% vs. 8/16 50%), but the difference was not significant (p = 0.079), but cavitary disease was similar in both groups for all participants with available baseline chest x-rays (21.6% and 27.3% in the historical and R^{35} groups, respectively).

	Historical cohort *N=117*	R^{35} group *N=28*	p-value
female	37 (32.2%)	8 (29.6%)	0.979
Age (median, IQR)	47.3 [28.8;63.0]	66.1 [39.9;74.8]	0.026
Ethnic group			<0.001
Asian	10 (8.70%)	0 (0.00%)	
Caucasian	42 (36.5%)	13 (50.0%)	
Other	9 (7.83%)	5 (19.2%)	
Polynesian	3 (2.61%)	0 (0.00%)	
South American	49 (42.6%)	2 (7.69%)	
Sub Saharan African	2 (1.74%)	6 (23.1%)	
Smoking			0.008
current smoker	43 (44.8%)	7 (26.9%)	
no smoker	39 (40.6%)	8 (30.8%)	
previous smoker	14 (14.6%)	11 (42.3%)	
Alcohol			0.386
current use	37 (41.6%)	6 (26.1%)	
no consumption	46 (51.7%)	15 (65.2%)	
previous oh	6 (6.74%)	2 (8.70%)	
Previous TB	11 (9.57%)	3 (11.5%)	0.723
PLWHIV	7 (6.09%)	0 (0.00%)	0.347
Solid organ neoplasm	6 (5.22%)	2 (7.41%)	0.647
Hematological neoplasm:	1 (0.87%)	0 (0.00%)	1.000
Autoimmune disease	2 (1.74%)	0 (0.00%)	1.000
Diabetes	16 (13.9%)	9 (33.3%)	0.025
Chronic liver disease	13 (11.3%)	2 (7.41%)	0.736
HBV	1 (9.09%)	2 (7.41%)	1.000
HCV	10 (83.3%)	0 (0.00%)	<0.001
TB type			0.265
pulmonary	86 (74.8%)	13 (59.1%)	
extrapulmonary	28 (24.3%)	9 (40.9%)	
miliary	1 (0.87%)	0 (0.00%)	
Positive baseline smear (pulmonary TB)	50 (73.5%)	8 (50.0%)	0.079
Organ affected (extrapulmonary TB)			0.001
bone or joint	1 (3.57%)	1 (14.3%)	
cerebrospinal fluid	0 (0.00%)	3 (42.9%)	
lymph node	8 (28.6%)	1 (14.3%)	
other	3 (10.7%)	1 (14.3%)	
pericardium	0 (0.00%)	1 (14.3%)	
pleura	13 (46.4%)	0 (0.00%)	
skin	3 (10.7%)	0 (0.00%)	
Cavities at baseline (pulmonary TB)	19 (21.6%)	6 (27.3%)	0.776
Isoniazid monorresistance	3 (6.25%)	0 (0.00%)	1.000

Table 2. Baseline characteristics of RIAlta participants at the time of the interim report.

Table 3 lists all adverse events from both groups. There were 70 adverse events registered for the 63 participants from Spain in the historical cohort (no AE data was registered for the controls from Paraguay), which contrasts with 124 AE for 28 participants in the R^{35} group.

	Historical cohort *N=70*	R^{35} group *N=124*	p-value
Site			<0.001
VH	70 (100%)	36 (29.0%)	
RUMC	0 (0.00%)	87 (70.2%)	
IN*	0 (0.00%)	1 (0.81%)	
AE grade			0.001
1	53 (75.7%)	56 (46.7%)	
2	14 (20.0%)	47 (39.2%)	
3	3 (4.29%)	15 (12.5%)	
4	0 (0.00%)	2 (1.67%)	
Relation to rifampicin			<0.001
Definitely related	20 (28.6%)	0 (0.00%)	
Possibly related	11 (15.7%)	30 (25.0%)	
Probably related	24 (34.3%)	23 (19.2%)	
Unlikely related	1 (1.43%)	18 (15.0%)	
Unrelated	14 (20.0%)	49 (40.8%)	
Action on rifampicin			0.059
Interrupted	3 (5.08%)	1 (0.84%)	
Stopped	5 (8.47%)	4 (3.36%)	
Unchanged	51 (86.4%)	114 (95.8%)	
AE outcome			<0.001
Improved	0 (0.00%)	17 (16.5%)	
Resolved	70 (100%)	79 (76.7%)	
Unknown	0 (0.00%)	1 (0.97%)	
Worse	0 (0.00%)	6 (5.83%)	
Time (weeks) to the first AE	2.65 (2.59)	2.58 (2.26)	0.847

Table 3. All adverse events of RIAlta participants at the time of the interim report. *At the time of this interim analysis INERAM had not included the adverse events of the historical cohort. Radboud did not contribute cases to the historical cohort as this site has been using optimized doses of rifampicin in difficult-to-treat cases for more than a decade. Therefore, only Vall d'Hebron historical patients are included in the safety analysis. VH, Vall d'Hebrón; RUMC, Radboud University Medical Centre; IN, INERAM.

In line with this, there were 4/23 (17.4%) participants experiencing at least one grade ≥3 AE possibly to definitely related to rifampicin in the R^{35} group, as compared to 3/63 (4.8%) in the historical cohort (OR of 4.21 95% CI 0.86–20.51). However, if we look at the AE that led to an interruption or withdrawal of rifampicin, the difference was smaller (4/23 and 6/63 participants in the R35 and the historical groups, respectively (OR 2.00, 95% CI 0.51 – 7.85) (table 4). Interestingly, the median time from treatment onset to AE was around 2.6 weeks in both groups (table 3).

	Historical cohort N = 63	R^{35} group N = 23	OR	95% CI
≥g3 AE	3 (4.8%)	4 (17.4%)	4.21	0.86-20.51
≥g3 AE OR treatment interruption	6 (9.5%)	8 (34.8%)	5.07	1.52-16.85
Treatment interruption	6 (9.5%)	4 (17.4%)	2.00	0.51-7.85

Table 4. Incidence of grade 3 adverse events and treatment interruptions at the time of the interim analysis if RIAlta.

There was a positive effect of optimized rifampicin dose on the early microbiological outcomes. At week 4, 4/12 (33.3%) and 39/54 (72.2%) participants (OR 0.22, 95% CI 0.05 – 0.86), and at week 8 1/12 (8.3%) and 21/54 (38.9%) participants (OR 0.393, 95% CI 0.04 – 3.76) in the R35 and the historical group, respectively, had a positive sputum culture (table 5).

	Historical cohort N = 54	R^{35} group N = 12	OR	95% CI
Positive culture w4	39 (72.2%)	4 (33.3%)	0.220	0.056-0.861
Positive culture w8	21 (38.9%)	1 (8.3%)	0.393	0.041-3.759
Positive culture w8*	21 (38.9%)	1 (8.3%)	0.143	0.017-1.189

Table 5. Co-primary efficacy endpoint: sputum culture status at week 4 and 8. *We performed a weighted imputation of the missing values at week 8. Participants assigned negative results by the imputation were reviewed keeping the negative assignment when there was evidence of clinical improvement and of their impossibility to produce sputum samples.

Finally, there were only a reduced number of participants in the R^{35} group with a complete follow-up and end of treatment outcomes. Therefore, we only summarized the clinical outcomes of the historical cohort (table 6).

	IN *N=54*	VH *N=63*
Treatment outcome (WHO)		
Death	3 (6.38%)	1 (1.59%)
lost to follow-up	13 (27.7%)	1 (1.59%)
microbiological cure	2 (4.26%)	27 (42.9%)
treatment complete	29 (61.7%)	34 (54.0%)
Treatment outcome (composite)		
favourable outcome	31 (66.0%)	61 (96.8%)
unfavourable outcome	16 (34.0%)	2 (3.17%)
Treatment duration (months, median [IQR])	6.96 [6.53;9.29]	7.11 [6.59;9.80]
Post-treatment outcome		
Death	3 (6.00%)	2 (3.17%)
Relapse	4 (8.00%)	0 (0.00%)
relapse-free cure	9 (18.0%)	53 (84.1%)
Unknown	34 (68.0%)	8 (12.7%)
Post-treatment follow-up duration (months, median [IQR])	13.0 [0.00;12765]	12.8 [6.14;15.8]

Table 6. Clinical outcomes for the historical cohort for the RIAlta trial control arm. This corresponds to the extended follow-up outcomes in the prospective cohort, but there was very little data from the R^{35} group at the time of this interim analysis. Note the extreme values in the INERAM group for the post-treatment follow-up. Only 26 cases had a post-treatment follow-up of at least one month, and from these, 17 had very extreme values that we are currently reviewing.

We found 117 patient records with full available data and fulfilling inclusion criteria between 2016 and 2019. As explained in article 1, Radboud has been using optimized doses of rifampicin for the profile of patients included in this study for more than a decade and thus were considered not adequate as standard dose controls (36).

The primary objective of the trial is to measure the safety of rifampicin in a vulnerable population. Note that the observations here are adverse events, not individuals, so there can be more observations than participants in each group.

The primary endpoint of the RIAlta trial is the incidence of grade 3 or superior adverse events possibly, probably, or definitely related to rifampicin. As the use of historical controls introduces an important

memory bias, we updated the endpoint combining grade 3 or higher adverse events and all adverse events leading to a temporary or permanent interruption of rifampicin, which is more likely to be recorded in historical controls, as many interruptions were expected because of adverse events, regardless of their grade. As a sensitivity analysis, we also analysed only the treatment interruptions without grade 3 or higher adverse events

6: Overall summary of the discussion

The left lung had been for eighteen months in semiosseous or cartilaginous state, and was, of course, entirely useless for all purposes of vitality. The right, in its upper proportion, was also partially, if not thoroughly, ossified, while the lower region weas merely a mass of purulent tubercles, running one into another. Several extensive perforations existed; and, at one point, permanent adhesion to the ribs had taken place. [...]. It was the opinion of both physicians that M. Valdemar would die about midnight on the morrow.

The facts in the case of M. Valdemar. Edgar Allan Poe.

6.1. It is time to optimize the use of rifampicin in clinical practice

The current 6-month treatment for DS-TB using rifampicin at 10mg/kg/day achieves relapse-free cure rates of >95% under clinical trial conditions (37). In real life-however, success rates are far lower. For example, in the historical cohort used as controls for RIAlta, success rate was only 66% (57% if we consider the missing data as incomplete treatments), close to the 68% reported by the 2023 report from the National TB Program from Paraguay (38). And as shown in the historical cohort clinical outcomes, in vulnerable, difficult-to-treat populations such as those included in the RIAlta trial, treatments are longer in practice even with the best care standards (IQR 6.5-9 months). Shorter treatments are easier to adhere, but clinical trials should include these groups who have a higher risk of poor treatment outcomes in order to provide external validity and gather information which is as close as possible to the real clinical practice.

The results from our NMA suggest that the optimal dose of rifampicin is likely between 2 and 3 times the current standard dose. A new meta-analysis, updating a previous review commissioned by the WHO, was published almost at the same time as article 1 (39). This review included focused on long-term clinical outcomes, and inclided 3 studies that were not included in our systematic review. One was published in 1979, and the highest dose used was 750mg of rifampicin (40). Another is not available as a peer reviewed publication (PACTR201105000291300). The third trial showed an increase in grade

3 hepatic adverse events with 35 but not 25mg/kg/day (41). When added to our NMA as a sensitivity analysis, the results for this trial lowered the dose for a significant increase of hepatic AE from 40 to 35 mg/kg/day. The authors concluded that there is not enough evidence supporting the use of optimized doses of rifampicin in clinical practice.

Yet another review was published in the same number as article 1 (42). This new review included also children, and rifampicin doses were pooled in three groups to deal with multi-arm trials (10-14, 15-29, and ≥30mg/kg/day), and then performing two separate meta-analyses with the low dose group as control of the two high dose groups. The meta-analysis showed an increased risk of overall and hepatic AE with the high-dose group (incidence RR 1.48, 95% CI 1.12-1.96, and 1.96, 95% CI 1.21-3.18). This strategy decreases variance by increasing the available data for each comparison but lacks granularity to evaluate each dose against other doses. Note that this second review included one trial using both high-dose rifampicin and isoniazid which, like in article 1, may have influenced the results for the 35mg/kg/day arm (43). Finally, another NMA on rifamycins evaluated full regimens, found that rifapentine regimens had the best efficacy-safety balance (44). Only 4 out of its 15 trials overlap with our review and the highest rifampicin dose in the efficacy network was 20 mg/kg/day. No matter the design and units of analysis of all these reviews, estimating the effect on long-term outcomes is challenging because of the low numbers of treatment failure and relapse events in the arms using ≥20mg/kg/day rifampicin (i.e., only 12 relapses in article 1).

Despite these conflicting results and limitations, the *Clinical Infectious Diseases* published an editorial following the publication of article 1 the review by Arbiv *et. al* supporting that current evidence is enough to support the doubling the starting dose of rifampicin in routine care to improve early microbiological outcomes (42,45). Doses between 25 and 35mg/kg/day probably offer the best balance between safety and enhanced bactericidal activity. Within this range, the optimal dose may be different for some populations: people from India may have an efficacy benefit with 25 mg/kg/day, and 35mg/kg/day may cause unacceptable toxicity (41) , whereas people from Sub Saharan Africa may have an efficacy benefit only with 35mg/kg/day without toxicity concerns (46).

PK data support the proposal of at least doubling the rifampicin dose in standard treatment. Previous studies correlating PK parameters and treatment outcomes suggest a lower risk of treatment failure for people with a C_{max} of at least 8μg/mL for pulmonary TB (47) and at least 22μg/mL for CNS TB (48). In our NMA, doses of ≥20mg/kg/day had a lower CrI above this threshold, suggesting that these doses could ensure an adequate exposure for most people with pulmonary TB. For CNS-TB, only doses of 35mg/kg/day ensure an adequate exposure for most people (figure 9 in article 1).

Furthermore, the efficacy benefit may be more evident in vulnerable populations with risk factors for poor treatment outcomes (see table 2 appendix I). These subgroups have been underrepresented in clinical trials (article 1) as were exclusion criteria in many of them (table 1 article 2). This justified the inclusion criteria for the RIAlta trial. The decision of using historical controls and enrolling all prospective participants to the R^{35} group was made considering the safety data from article 1, the retrospective cohort from Radboud(36), and the limited recruitment capacity and funding in our consortium. The rationale was to collect as much data as possible from this population, and inform a larger, better designed, more inclusive trials.

The interim results presented after article 2, although far from the size required for a minimal statistical power are encouraging. Imbalances in the two groups were expected due to the lack of randomisation, but despite this, the demographic characteristics are reasonably balanced. We expect that, as recruitment advances in Paraguay, the unbalance in South American participants and smear positive pulmonary cases will be solved.

Safety data showed a great difference in the number of all AE and grade ≥3 AE between historical controls and the R^{35} group, although this result is probably affected by reporting bias to an extent greater than that anticipated during the trial design. The misbalance in AE between the two groups exemplifies the limitations for the use of historical controls in interventional trials, and we can expect it to grow larger as recruitment advances. However, the incidence of grade 3 adverse events in the R^{35} group from RIAlta is in the same range as that recorded in the rifampicin trials with the same dose included in article 1 (46,49,50). Finally, most AE did not require the modification of rifampicin doses, and there were no significant differences between the AE leading to treatment modification in the R^{35} group as compared to the historical controls. When data are complete, we plan a more balanced analysis using statistical methods such as a propensity score. We also plan to analyse the association with treatment modifications and safety confounders for age and comorbidity, such as medication burden and previous use of gastrolesive drugs such as non-steroidal anti-inflammatory drugs or corticosteroids.

In contrast, the efficacy benefit seemed higher in this population with a higher baseline risk of delayed sputum culture conversion and treatment success. This preliminary analysis showed that participants with optimized doses of rifampicin are less likely to have a positive sputum culture after 1 month of treatment. For a vulnerable population this means a faster withdrawal of isolation measures, which may in turn hasten the functional, psychological, and social recovery of those affected by TB. From the public health perspective this also means a shorter transmission time.

Optimizing rifamycin dose is only one step towards shorter treatments. The optimization of the treatment for DS-TB probably needs a combination of replacement of the drugs with lower potency by new or repurposed ones (ethambutol by moxifloxacin or linezolid, for example), and the revision of the dosing of key drugs (i.e., finding the optimal dose of rifamycins). A previous NMA showed the benefit of adding fluoroquinolones to the first-line treatment (51). But as with rifampicin dose optimization, this modification alone may be beneficial in people with TB and a low bacillary burden, but not all the cases (52). The results from Study 31 and the TRUNCATE trials suggest that the combination of two strategies, optimizing rifamycins and adding other highly bactericidal drugs is the path towards shorter treatments (26,53). As discussed in appendix I, the optimization of rifampicin dose could solve part of the limitations for the implementation of a 4-month regimen combining high-dose rifapentin and fluoroquinolones, as rifampicin is cheaper and more widely available than rifapentine. In line with this, we look forward to completing the RML-TB trial (EUCT 2023-509075-17-00), using 30mg/kg/day of rifampicin together with moxifloxacin and linezolid, sponsored by our group and running completely on Spanish public institutions.

6.2. Perspectives on academic clinical trials in low-middle income countries

We have the most promising drug pipeline for TB in decades, and several consortia working to facilitate the evaluation of several possible combinations to find the shortened regimens (PANTB, UNITE4TB, FAST-TB, etc.) (54,55). Prioritising all these drugs and their combinations for testing in phase 3 clinical trials is a huge challenge. Adaptive platform trials and multi-comparison techniques such as the NMA performed in article 1 are helpful tools to rank new regimens. Biomarker development is essential to optimize the use of clinical data for such ranking, but efficacy biomarker development in TB has been an uncoordinated effort characterized by high attrition rates and very low numbers of biomarkers fully validated for trial-level comparisons, and even less making it to regulatory approval and market registration (see appendix III).

Whatever the innovations are, these cannot substitute the need of large studies that are representative of the global TB burden. As shown in the figure S14 in the appendix II, clinical trials in TB are being conducted in a reduced number of expert sites in a relatively low number of countries. Although some consortia have invested in capacity building to add new sites to their networks (53), the global clinical trial capacity is still far from sufficient to produce evidence in a reasonable amount of time and produce effects on patient care in upcoming years (56).

But establishing new clinical trial networks has many challenges. We did not have specific funding for RIAlta allocated within the EUSAT-RCS budget, where the focus was supporting the staff exchange.

This allowed us to train young researchers from EU and Paraguay in clinical trials. Other expenses such as trial drugs, participant insurance, ethics and regulatory taxes, and laboratory materials were scrapped from the general budget of the consortium and local resources. The challenges of the regulatory process and staff changes made the local team to withdraw before submitting the protocol for ethical and regulatory review in the Portuguese site, and UMCG setup ran aground while discussing the clinical trial agreement. Similarly, due to changes in our consortium, the electronic nose evaluation as a treatment response biomarker was cancelled, and it is still unclear whether we will be able to conduct a pharmacogenomics analysis.

We started preparing the RIAlta protocol in July 2019. Excluding the first year of the COVID pandemic, it took us 2.5 years to include the first participant at the sponsor's site, and somewhat more than 4 years to recruit the first participant in Paraguay. There, RIAlta is the 6th academic trial (4th not considering COVID-19 trials) authorised by the regulatory agency Dirección Nacional de Vigilancia Sanitaria (DINAVISA). Having almost all the factors identified as relevant for startup delays in clinical trials in a recent review, these times are quite reasonable (57).

As happened in our case, unduly long approval processes, and in international trials, the multitude of applications with different processes may wane enthusiasm and commitment to enrol participants and lead to a loss of motivation to engage in clinical research (58). This complexity also increases the dependence on CROs, limiting the genuine independence of the clinical research. Furthermore, these times are too long for the periods established by competitive grants. The 4-to-5-year periods of public funding are too short for a sustainable capacity building. A mid-term economic plan for projects such as our consortium, and stepwise grants that allow groups to apply for subsequent stages of funding given the completion of pre-specified milestones would be needed to ensure the sustainability of capacity building and complex research projects such as EUSAT-RCS, which was closed in May 2024.

There is an increasing interest in improving the efficiency of the current TB treatment research and development pipeline. An important aspect is making the clinical trials more efficient so that evidence can be produced at a reasonable cost in time and resources (58). But importantly, this can only be achieved by expanding the global clinical trial capacity. So far, there is little generic guidance, and almost no TB-specific tools for site assessment and monitoring of capacity building interventions. During the EU Patient Centric Clinical Trial Platforms (EUPEARL) we tried to contribute to filling this gap with the Site Assessment and Capacity Building Handbook for TB platform trials (59).

Improving clinical trial capacity does not only benefit evidence generation but can contribute improving the health care quality to international standards. The secondments fostered knowledge exchange and exposed young researchers to different healthcare environments. The setup for RIAlta

made us review the clinical paths that patients underwent in each site, identifying gaps that are not only related to material resources and that could be solved with organisational interventions. Simple things such as creating an agenda for TB outpatients and a reminder system for patients to attend their visits were put in place at INERAM. History provides overwhelming examples of the positive consequences of improving local capacities to fight tuberculosis as well as what happens when these capacities are not sustained over time together with changes in the epidemiology of the disease. For example, after the closure of the British Medical Council TB units in Zambia in 1986 and boosted by the spread of HIV during the 80s, the case notification rates of TB had a steep increase (60).

Engaging civil society organisations, advocacy groups, patients and their families is critical not only to ensure that the research results are meaningful to and uptaken by the affected populations, but to the development and acceptance of the research ecosystem by that community. This was not formally embedded in the RIAlta trial, but during the EUPEARL project our group has worked with community representatives to establish a conceptual framework for the involvement of TB community representatives in complex clinical trials, from design to communication of the results (annex IV).

Despite all this, the challenges, the RIAlta trial is still recruiting participants. We hope to have informative results in the upcoming years and draw attention to the design of more inclusive trials with wide inclusion criteria and less stringent exclusion criteria.

7. Conclusions

1. The optimal dose of rifampicin is probably between 25 and 35mg/kg/day, depending on host factors. Current evidence is enough to support the use of personalised doses of rifampicin cases with an increased risk of poor treatment outcomes.
2. Safety of optimized doses of rifampicin seem similar among people with advanced age, undernutrition, diabetes, and stable chronic liver disease, compared to the young, healthy, people with low risk of poor treatment outcomes who were enrolled in previous trials.
3. Similarly, rifampicin-related AE seem not to lead to a higher percentage of modifications of rifampicin as compared to standard-dose rifampicin in historical controls from vulnerable populations.
4. Having a similar safety profile to low-risk groups, the efficacy benefit of optimized doses of rifampicin may be higher for the vulnerable populations with a high risk of poor treatment outcomes.
5. Clinical trials in LMIC are challenging but offer a unique opportunity for capacity building that has repercussions not only on research but also in the quality of clinical practice.

8. Future research perspectives

Many things are to be completed even after 5 years since we started working in this project. There is a high risk of not completing the RIAlta trial with power enough to provide meaningful conclusions. However, our main objective was the setting of a clinical research network and this we can say was a success.

The collaboration of each colleague, their expertise, willingness to learn, and energies, allowed to move the RIAlta trial to the current point. After the end of the EUSAT project, the sustainability of several ongoing works is quite uncertain. However, we are making our best efforts to maintain the collaboration network emerged from the project wile transferring leadership to our Paraguayan colleagues through local competitive funds.

As our network matures, we will succeed in steering up more complex enterprises in the future. It is important to keep the work within the group to avoid "cold" periods driven by project-based research and intermittent funding, which result in inefficiencies and may lead to loss of the skills and capacities, even of human resources, of the local teams.

The low success rates seen in Paraguay's historical cohort may be related to poor treatment adherence due to economic needs or limited access to health care. Symptoms disappear after some weeks of treatment so the people affected by TB, who may be stigmatised and in a difficult situation from the economic perspective, prioritise resuming their normal activities to cope with economic losses during the limiting part of the disease. The report of a health economics study analysing the incidence of catastrophic costs in those affected by TB in Paraguay, and a tool to evaluate site readiness for TB clinical trials are still ongoing. Most reviews TB highlight the challenge of navigating the different trial designs and endpoint definitions. Following the work done within the EUSAT-RCS and the EUPEARL projects, the author was invited to participate as a young researcher in the FAST-TB network, an

inter-consortium platform that has the objective of accelerating TB treatment development by acting as a forum where different research groups can coordinate their efforts. Some of the ongoing work within the FAST-TB network is aimed at a better understanding of design priorities and homogenization of definitions and procedures. We have planned further analysis to test the trial-level prediction of biomarkers in the special population of the RIAlta trial and an update of the review from appendix III is currently ongoing.

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10. Appendices

10.1. Appendix I: Update on the treatment of tuberculosis

Martínez-Campreciós J, Espinosa-Pereiro J, Sánchez-Montalvá A. Update on the treatment of tuberculosis. Med Clin (Barc). 2024 Sep 13;163(5):245-252. English, Spanish. doi: 10.1016/j.medcli.2024.02.030. Epub 2024 May 4.

This article is a narrative review of the current status of the treatment of TB, from DS-TB to MDR-TB trying to provide a hands-on document that can be used as a reference for clinicians. It complements the background information provided in chapter I. Here we showed that we are about to break the 6-month barrier of treatment duration for most cases of DS and MDR-TB. One of the knowledge gaps to reach this goal is the optimal dose of rifampicin.

doi: 10.1016/j.medcli.2024.02.030

http://elsevier.es/es-revista-medicina-clinica-english-edition--462-articulo-update-on-treatment-tuberculosis-S2387020624003632

doi: 10.1016/j.medcli.2024.02.030

http://elsevier.es/es-revista-medicina-clinica-english-edition--462-articulo-update-on-treatment-tuberculosis-S2387020624003632

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10.2. Appendix II: Supplementary material for article 1

1- Search term (accessed June 15th, 2023)

((Rifampin[Mesh] OR rifampicin OR rifampin OR antitubercul* OR antimycobacterial OR antimycobacterial activit* OR Antitubercular Agents[Mesh]) AND (Tuberculosis[Mesh] OR Tubercul*) AND (Clinical Trial[ptyp]) AND (Humans[Mesh]))

2- PRISMA NMA checklist

Section/Topic	Item #	Checklist Item	Reported on Page #
TITLE			
Title	1	Identify the report as a systematic review *incorporating a network meta-analysis (or related form of meta-analysis).*	*1*
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: **Background:** main objectives **Methods:** data sources; study eligibility criteria, participants, and interventions; study appraisal; and *synthesis methods, such as network meta-analysis.* **Results:** number of studies and participants identified; summary estimates with corresponding confidence/credible intervals; *treatment rankings may also be discussed. Authors may choose to summarize pairwise comparisons against a chosen treatment included in their analyses for brevity.* **Discussion/Conclusions:** limitations; conclusions and implications of findings. **Other:** primary source of funding; systematic review registration number with registry name.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known*, including mention of why a network meta-analysis has been conducted.*	4
Objectives	4	Provide an explicit statement of questions being addressed, with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists and if and where it can be accessed (e.g., Web address); and, if available, provide registration information, including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility,	*5*

		giving rationale. *Clearly describe eligible treatments included in the treatment network, and note whether any have been clustered or merged into the same node (with justification).*	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Suppl. Section 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
Geometry of the network	**S1**	Describe methods used to explore the geometry of the treatment network under study and potential biases related to it. This should include how the evidence base has been graphically summarized for presentation, and what characteristics were compiled and used to describe the evidence base to readers.	6, 8
Risk of bias within individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). *Also describe the use of additional summary measures assessed, such as treatment rankings and surface under the cumulative ranking curve (SUCRA) values, as well as modified approaches used to present summary findings from meta-analyses.*	6
Planned methods of analysis	14	Describe the methods of handling data and combining results of studies for each network meta-analysis. This should include, but not be limited to: ● *Handling of multi-arm trials;* ● *Selection of variance structure;* ● *Selection of prior distributions in Bayesian analyses; and* ● *Assessment of model fit.*	6
Assessment of Inconsistency	**S2**	Describe the statistical methods used to evaluate the agreement of direct and indirect evidence in the treatment network(s) studied. Describe efforts taken to address its presence when found.	6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	**8**
Additional analyses	16	Describe methods of additional analyses if done, indicating which were pre-specified. This may include, but not be limited to, the following: ● Sensitivity or subgroup analyses; ● Meta-regression analyses; ● *Alternative formulations of the treatment network; and*	6

		• *Use of alternative prior distributions for Bayesian analyses (if applicable).*	
RESULTS†			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Presentation of network structure	**S3**	Provide a network graph of the included studies to enable visualization of the geometry of the treatment network.	***8 (fig 3)***
Summary of network geometry	**S4**	Provide a brief overview of characteristics of the treatment network. This may include commentary on the abundance of trials and randomized patients for the different interventions and pairwise comparisons in the network, gaps of evidence in the treatment network, and potential biases reflected by the network structure.	7-10 (for each outcome)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7- 8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment.	7 (fig 2)
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: 1) simple summary data for each intervention group, and 2) effect estimates and confidence intervals. *Modified approaches may be needed to deal with information from larger networks.*	***Table 1***
Synthesis of results	21	Present results of each meta-analysis done, including confidence/credible intervals. *In larger networks, authors may focus on comparisons versus a particular comparator (e.g. placebo or standard care), with full findings presented in an appendix. League tables and forest plots may be considered to summarize pairwise comparisons.* If additional summary measures were explored (such as treatment rankings), these should also be presented.	***8-10***
Exploration for inconsistency	**S5**	Describe results from investigations of inconsistency. This may include such information as measures of model fit to compare consistency and inconsistency models, *P* values from statistical tests, or summary of inconsistency estimates from different parts of the treatment network.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies for the evidence base being studied.	7 (fig S1)
Results of additional analyses	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression analyses*, alternative network geometries studied, alternative choice of prior distributions for Bayesian analyses,* and so forth).	***Suppl sect 4:7***
DISCUSSION			
Summary of evidence	24	Summarize the main findings, including the strength of evidence for each main outcome; consider their relevance to	12

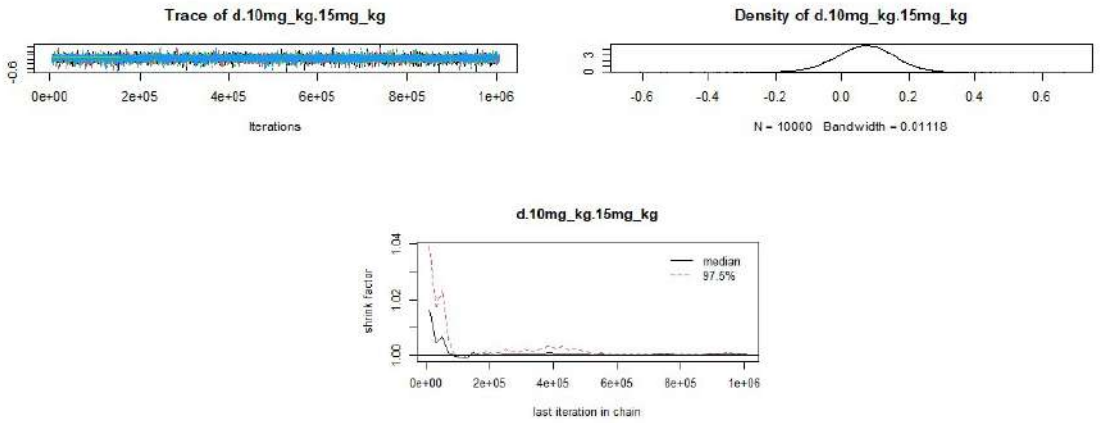
		key groups (e.g., healthcare providers, users, and policy-makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias). *Comment on the validity of the assumptions, such as transitivity and consistency. Comment on any concerns regarding network geometry (e.g., avoidance of certain comparisons).*	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
			17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. This should also include information regarding whether funding has been received from manufacturers of treatments in the network and/or whether some of the authors are content experts with professional conflicts of interest that could affect use of treatments in the network.	

3- Additional information on the statistical analysis

Previous studies show that the effects of rifampicin on the safety and efficacy outcomes vary between countries, TB clinical form, and severity. Furthermore, rifampicin PK is known to have a wide interindividual variability even in similar populations. Therefore, we considered that the fixed effect model could underestimate this variability and thus we favored the random effects model.

Given that most trials were multi-arm ones and that there were 11 regimens, with some trials using different control arms, there was a high risk of statistical errors such as finding false associations or inadvertent increase of the weight of the control arm, leading to unit-of-analysis error. This error happens when the information from a treatment arm is used more than once or with disproportionate weight. In conventional meta-analysis the unit-of-analysis error appears when the experimental arms in multi-arm trials are compared individually with the standard of care (i.e., if there are k experimental arms, the information from the control will be used k times). Previous review on optimized doses of rifampicin have overlooked this error (see for example Zhang Frontiers in Medicine 2022) or pooled all experimental arms and perform a conventional comparison of “standard” vs “high” dose rifampicin (for example Onorato CMI 2021).

Model adjustment was assessed using trace, density, and Gelman-Rubin plots, with a threshold Potential Scale Reduction Factor (PSRF) of less than 1.05. Below we include as an example these plots for the 10 vs 15mg/kg comparison in the sputum culture conversion at week 8 in liquid media outcome.



We tested different combinations of chains and iterations until an appropriate fit was observed for the outcomes included in the NMA. As number of iterations increased, we tested different parameters for thinning, as these can reduce the average costs of iterations making it possible to un thinned Markov chains longer, keeping the balance to avoid a decay of the correlations in the estimation. The final model used four chains, and 10^5 iterations and a thin of 10.

There was a need to check for inconsistencies in the network as not all studies included the same control arm. To contrast the estimates of the direct and the indirect evidence we used the node splitting method with a significance threshold of <0.05, using an automated loop that selects which comparisons to split as published by van Valkenhoef et al.

References

- Rücker G, Cates CJ, Schwarzer G. Methods for including information from multi-arm trials in pairwise meta-analysis. Res Synth Methods. 2017 Dec;8(4):392-403. doi: 10.1002/jrsm.1259. Epub 2017 Aug 25. PMID: 28759708.
- Rücker G. Network meta-analysis, electrical networks and graph theory. Res Synth Methods. 2012 Dec;3(4):312-24. doi: 10.1002/jrsm.1058. Epub 2012 Sep 25. PMID: 26053424.
- Zhang M, Wang M, He JQ. Intensified Antituberculosis Therapy Regimen Containing Higher Dose Rifampin for Tuberculous Meningitis: A Systematic Review and Meta-Analysis. Front Med (Lausanne). 2022 Feb 25;9:822201. doi: 10.3389/fmed.2022.822201. PMID: 35280900; PMCID: PMC8916538.
- Onorato L, Gentile V, Russo A, Di Caprio G, Alessio L, Chiodini P, Coppola N. Standard versus high dose of rifampicin in the treatment of pulmonary tuberculosis: a systematic review and meta-analysis. Clin Microbiol Infect. 2021 Jun;27(6):830-837. doi: 10.1016/j.cmi.2021.03.031. Epub 2021 Apr 1. PMID: 33813119.

- Art B. Owen. Statistically efficient thinning of a Markov chain sampler. University of Stanford. April 2017
- van Valkenhoef G, Dias S, Ades AE, Welton NJ. Automated generation of node-splitting models for assessment of inconsistency in network meta-analysis. Res Synth Methods. 2016 Mar;7(1):80-93. doi: 10.1002/jrsm.1167. Epub 2015 Oct 13. PMID: 26461181

4- Supplementary tables

Table S1. Participant characteristics.

	Overall	Ruslami 2007	Diacon 2007	Ruslami 2013	Boeree 2015	Heemskerk 2016
N (total)	3962 (3654 with R)	50	13	60	100	817
Geographic origin (%)	Vietnam 817 (20.6) Bangladesh 698 (17.6) Indonesia 494 (12.5) Uganda 467 (11.8) South Africa 373 (9.4) Tanzania 306 (7.7) Philippines 237 (6) Peru 180 (4.5) Bolivia 150 (3.8) Niger 127 (3.2) Nepal 50 (1.2) Thailand 49 (1.2) India 14 (0.3)	Indonesia	South Africa	Indonesia	South Africa	Vietnam
Males (%)	2718 (68.6)	26 (52)	8 (61)	33 (55)	74 (74)	560 (68.5)
Median age (r, IQR)	32.8 (30.5-35.3)	28 (r 18-55)	27 (mean, SD 9)	28 (r 16-64)	31 (r 18-60)	35 (IQR 29-46)
Median weight (r, IQR)	51.6 (49.5-53.7)	46 (r 35-71)	55 (mean, SD 16)	48 (r 34-75)	53.6 (r 40.2-84.2)	48 (IQR 44-55)
Median BMI (r, IQR)	19.2 (18.11-20.2)	-	-	18.4 (r 15.1-26)	19.4 (r 14.7-30.9)	-
Comorbidities						
HIV positive (%)	628 (15.8)	1 (2)	3 (23)	7 (12)	5 (5)	349 (42.7)
Diabetes mellitus (%)	37 (0.9)	4 (8)	-	-	Excl.	-
HBV	24 (0.6)	Excl.	Excl.	-	Excl.	-
HCV	14 (0.3)	Excl.	Excl.	-	Excl.	-
Disease characterist.						
Confirmed TB (%)	2742 (69.2)	50 (100)	13 (100)	31 (52)	100 (100)	407 (49.8)
Probable TB (%)	289 (7.2)	Excl.	Excl.	20 (33)	Excl.	214 (26.2)
Possible TB (%)	231 (5.8)	Excl.	Excl.	9 (15)	Excl.	174 (21.3)
Other or unlikely (%)	22 (0.5)	Excl.	Excl.	Excl.	Excl.	22
Lung involvement (%)	3274 (82.6)	50 (100)	13 (100)	28 (47)	100 (100)	509 (62)
Cavitary disease (%)	485 (12.2)	-	13 (57)	1 (1)	-	-
Milliary TB (%)	119 (0.3)	-	-	6 (10)	Excl.	109 (13.3)
Menigitis	1079 (27.2)			60		817
G1 (%)	378 (35)	NA	NA	4 (7)	NA	318 (38.9)
G2 (%)	535 (49.5)	NA	NA	49 (82)	NA	357 (43.7)
G3 (%)	181 (16.7)	NA	NA	7 (12)	NA	142 (17.4)
Bacterial load						
Sputum						
Negative/missing (%)		-	-	-	-	-
1 + (%)		-	Excl.	-	-	-
2 + (%)		-	13**	-	-	-
≥3 + (%)		-	-	-	-	-
Culture						
Negative/missing (%)						
1 + (%)		-	-	-	-	-

2 + (%)		-	-	-	-	-
≥3 + (%)		-	-	-	-	-
Median Log_{10}CFU/mL (r, IQR)	5.8 (4.9-6.7)	-	7.0 (mean, 0.4 SD)	-	6.1 (1.0-8.5)	-
Median TTP MGIT (days; r, IQR)		-	-	-	4.0 (2.2-19.3)	-
Median Xpert MTB/RIF cycle threshold (r, IQR)		-	-	-	-	-
*3 patients had HBV and HCV coinfection; **Diacon: sputum smear with a load of at least 2+. Do not report individual bacterial load; Excl. this was an exclusion criterion accorting to the protocol; IQR Inter-Quartilic Range; r Range. Note: median age and weight are presented excluding Dian, 2018.						

TableS1. Participant characteristics. (cont.)

	Yunivita 2016	Jindani 2016	Aarnoutse 2017	Boeree 2017	Velásquez 2018	Dian 2018
N (total)	30	300	150	365	180	60
Geographic origin	Indonesia	Bolivia 150 Uganda 100 Nepal 50	Tanzania	Tanzania 156 South Africa 209	Peru	Indonesia
Males (%)	18 (60)	205 (68.3)	135 (90)	258 (71)	114 (63)	32 (53%)
Median age (r, IQR)	33 (r 17-81)	28.5 (r 18-67)	33.5 (IQR 27-40)	33 (r 26-40)	25 (IQR 21-37)	29.5
Median weight (r, IQR)	45 (r 40-60)	51 (r 38-81)	55.5 (IQR 52-59)	53 (r 49-58)	54.1 (IQR 50.5-60.8)	45
Median BMI (r, IQR)	18.4 (r 14.7-25.0)	-	-	-	21.2 (IQR 19.6-23.7)	18
Comorbidities						
HIV positive	6 (20)	Excl.	15 (10)	24 (7)	5 (2.8)	6 (10)
Diabetes	-	Excl.	-	-	1 (0.5)	-
HBV	-	7*	-	-	Excl.	-
HCV	-	10*	-	-	Excl.	-
Disease characteristics						
Confirmed TB (%)	4 (13)	300 (100)	150 (100)	365 (100)	180 (100)	43 (71)
Probable TB (%)	11 (37)	Excl.	Excl.	Excl.	Excl.	17 (28)
Possible TB (%)	15 (50)	Excl.	Excl.	Excl.	Excl.	Excl.
Other or unlikely (%)	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.
Lung involvement (%)	13 (43)	300 (100)	150 (100)	365 (100)	180 (100)	35 (58)
Cavitary disease (%)	0	-	-	-	66/178 (37.1)	-
Milliary TB (%)	4 (13)	-	-	Excl.	-	-
Meningitis						
G1 (%)	4 (13)	NA	NA	NA	NA	1 (12)
G2 (%)	24 (80)	NA	NA	NA	NA	54 (90)
G3 (%)	2 (7)	NA	NA	NA	NA	5 (10)
Bacterial load						
Sputum						
Negative/missing (%)	-	-	5 (3)	-	-	-
1 + (%)	-	-	15 (10)	-	-	-
2 + (%)	-	-	51 (34)	-	87 (48.3)	-
≥3 + (%)	-	-	79 (52.6)	-	93 (51.7)	-
Culture						
Negative/missing (%)	-	-	31 (20.6)	-	-	-
1 + (%)	-	-	40 (27)	-	-	-
2 + (%)	-	-	49 (33)	-	-	-

≥3 + (%)	-	-	30 (20)	-	-	-
Median Log_{10}CFU/mL (IQR)	-	-	5.25 (4.04-6.79)	-	5.0 (4.5-5.9)	-
Median TTP MGIT (days, IQR)	-	-	5.13 (3.96-6.71)	-	4.3 (0.1-5.0)	-
Median Xpert MTB/RIF cycle threshold (IQR)	-	-	-	16 (14-19)	-	-
*3 patients had HBV and HCV coinfection; **Diacon: sputum smear with a load of at least 2+. Do not report individual bacterial load; Excl. this was an exclusion criterion accorting to the protocol; IQR Inter-Quartilic Range; r Range. Note: median age and weight are presented excluding Dian, 2018.						
BMRC British Medical Research Council TBM scale: 1) no neurological symptoms other than meningeal signs, GCS 15; 2) meningeal signs, cranial nerve or focal signs or GCS 11-14; 3) GCS <10, seizures, severe focal signs (hemiplegia, paraplegia); CFU Colony Forming Units;						
Age tau^2 = 10.0 (SE 5.7); I^2 93.79% | Weight tau^2 = 13.8 (SE 7.4); I^2 98.03% | BMI tau^2 = 1.5 (SE 1.5); I^2 78.03%						

Table S1. Participant characteristics. (cont.)						
	Maug 2020	Atwine 2020	Wasserman 2021	Cresswell 2021		
N (total)	701†	97	51	61		
Geographic origin	Bangladesh	Uganda	South Africa	Uganda		
Males (%)	511 (73.6)	71 (72.4)	26 (56.5)	34 (55.7)		
Median age (r, IQR)	43.5 (40.7-46.4)	33.6 (31.8-35.4)	38.5 (35.6-41.3)	33.5 (30.9-36)		
Median weight (r, IQR)	-	53.1 (51.5-54.7)	59.7 (56.2-63.2)	51.4 (48.9-53.8)		
Median BMI (r, IQR)	16.3 (16.1-16.5)	19.5 (18.4-20.6)	22.2 (21.1-23.3)	-		
Comorbidities						
HIV positive	Excl.	97 (99)	46 (100)	56 (92)		
Diabetes	32 (4.6)	-	-	-		
HBV	Excl.	4 (4)	-	-		
HCV	Excl.	1 (1)	-	-		
Disease characteristics						
Confirmed TB (%)	698 (100)	98 (100)	15 (24)	31 (50.8)		
Probable TB (%)	Excl.	Excl.	13 (28)	14 (23)		
Possible TB (%)	Excl.	Excl.	18 (39)	12 (20)		
Other or unlikely (%)	Excl.	Excl.	Excl.	-		
Meningitis	-	-	46 (100)	61 (100)		
Lung involvement (%)	698 (100)	98 (100)	-	-		
Cavitary disease (%)	-	42 (43)	-	-		
Milliary TB (%)	-	-	-	-		
Meiningitis						
G1 (%)	-	-	28 (60.9)	21 (34)		
G2 (%)	-	-	18 (39.1)	31 (51)		
G3 (%)	-	-	0	24 (39)		
Bacterial load						
Sputum						
Negative/missing (%)	Excl.		-	-		
1 + (%)	-		-	-		
2 + (%)	-		-	-		
≥3 + (%)	-		-	-		
Culture						
Negative/missing (%)			-	-		
1 + (%)	-		-	-		

2 + (%)	-		-	-		
≥3 + (%)	-		-	-		
Median Log_{10}CFU/mL (IQR)	-		-	-		
Median TTP MGIT (days, IQR)	-		-	-		
Median Xpert MTB/RIF cycle threshold (IQR)	-		-	-		
*3 patients had HBV and HCV coinfection; **Diacon: sputum smear with a load of at least 2+. Do not report individual bacterial load; Excl. this was an exclusion criterion accorting to the protocol; IQR Inter-Quartilic Range; r Range. Note: median age and weight are presented excluding Dian, 2018. †293 participants in the 31-44kg weight band underdosed by mistake.						
BMRC British Medical Research Council TBM scale: 1) no neurological symptoms other than meningeal signs, GCS 15; 2) meningeal signs, cranial nerve or focal signs or GCS 11-14; 3) GCS <10, seizures, severe focal signs (hemiplegia, paraplegia); CFU Colony Forming Units;						

Table S1. Participant characteristics. (cont.)

	Souleymane 2023	Sekaggya 2023	Paton 2023		
N (total)	127	128	674 (365 with R)		
Geographic origin	Niger	Uganda	Indonesia 294 Philippines 237 Uganda 80 Thailand 49 India 14		
Males (%)	111 (87.4)	81 (63.3)	421 (62.5)		
Median age (r, IQR)	37 (30-48)	36 (30-43)			
Median weight (r, IQR)	-	-	50 (30-97)		
Median BMI (r, IQR)	18.1 (IQR 16.3-19.7)	19.3 (IQR 17.7-21.7)	19 (12-33)		
Comorbidities					
HIV positive	3 (2.4)	-	-		
Diabetes	-	-	-		
HBV	13 (10.2)	-	-		
HCV	3 (2.4)	-	-		
Disease characteristics					
Confirmed TB (%)	127 (100)	128 (100)	674 (100)		
Probable TB (%)	Excl.	Excl.	Excl.		
Possible TB (%)	Excl.	Excl.	Excl.		
Other or unlikely (%)	Excl.	Excl.	Excl.		
Meningitis	-	-	46 (100)		
Lung involvement (%)	127 (100)	128 (100)	674 (100)		
Cavitary disease (%)	-	-	363 (53.8)		
Milliary TB (%)	-	-	-		
Meiningitis					
G1 (%)	-	-	28 (60.9)		
G2 (%)	-	-	18 (39.1)		
G3 (%)	-	-	0		
Bacterial load					
Sputum					
Negative/missing (%)	-		191 (28)		
1 + (%)	-		98 (15)		
2 + (%)	-		177 (26)		
≥3 + (%)	-		206 (30.6)		
Culture					

Negative/missing (%)			-		
1 + (%)	-		-		
2 + (%)	-		-		
≥3 + (%)	-		-		
Median Log_{10}CFU/mL (IQR)	-		-		
Median TTP MGIT (days, IQR)	-		-		
Median Xpert MTB/RIF cycle threshold (IQR)	-		-		
BMRC British Medical Research Council TBM scale: 1) no neurological symptoms other than meningeal signs, GCS 15; 2) meningeal signs, cranial nerve or focal signs or GCS 11-14; 3) GCS <10, seizures, severe focal signs (hemiplegia, paraplegia); CFU Colony Forming Units;					

Table S2. Background treatments.

Study	Background			
	Intensive	Weeks	Continuation	Weeks
Ruslami 2007	HZE daily	8	H 3 a week	16
Diacon, 2007	Standard treatment start upon discharge			
Ruslami, 2013	HZ for 2 weeks, then HRZE	8	HR 3 a week	16
Boeree, 2015	HZE week 2	8	HR	16
Heemskerk, 2016	HZE	12	HR	24
Yunivita, 2016	HZ	8	HR 3 a week	16
Jindani, 2016	HZE	8	HR	16
Aarnoutse, 2017	HZE	8	HR	16
Boeree, 2017	HZE	8	HR	16
	HZ	12	HR	14
Velásquez, 2018	HZE	8	HR 3 a week	16
Dian, 2018	HZE	8	HR	16
Maug, 2020	HZE	8	HR	16
Atwine, 2020	HZE	8	HR	24
Cresswell, 2021	HZE	8	HR	24
Wasserman, 2021	HZE	8	HR	24
Souleymane, 2023	H*ZE	24		
Sekaggya, 2023	HZE	8	HR	16
Paton, 2023	HZE+Cz	8-12		
	HZE+Lzd	8-12		
R, rifampicin (10mg/kg/day); H; isoniazid (H* triple dose isoniazid); Z, pyrazinamide; E, ethambutol; L levofloxacin; M, moxifloxacin; Cz, clofazimine; Lzd, Linezolid				

Table S3. All 3 or higher AE model results

Log risk ratio (95% CrI) relative effect table

	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg
10mg_kg	0.4217 (-0.8989, 1.785)	0.1052 (-0.425, 0.6323)	0.2279 (-0.1513, 0.7739)	-0.1363 (-1.031, 0.8037)	-22.54 (-78.5, -1.003)	0.4797 (-0.3205, 1.343)	0.2236 (-0.2123, 0.8218)	1.579 (0.05393, 3.2)	-0.1922 (-3.557, 1.966)

13mg_kg_iv		-0.3084 (-1.773, 1.1)	-0.1927 (-1.566, 1.292)	-0.5664 (-2.159, 1.057)	-22.99 (-78.78, -1.351)	0.05467 (-1.512, 1.647)	-0.1921 (-1.6, 1.284)	1.151 (-0.8521, 3.264)	-0.65 (-4.227, 1.922)
15mg_kg			0.1047 (-0.3823, 0.8467)	-0.2469 (-1.267, 0.8411)	-22.66 (-78.67, -1.112)	0.3668 (-0.5387, 1.376)	0.108 (-0.5035, 0.9242)	1.464 (-0.1106, 3.176)	-0.3009 (-3.676, 1.899)
20mg_kg				-0.3602 (-1.431, 0.5305)	-22.78 (-78.8, -1.247)	0.2494 (-0.6461, 1.072)	0.001387 (-0.6046, 0.5592)	1.343 (-0.2314, 2.918)	-0.4307 (-3.818, 1.703)
20mg_kg_iv					-22.42 (-78.35, -0.8556)	0.6188 (-0.5898, 1.844)	0.3591 (-0.4787, 1.337)	1.713 (-0.03356, 3.547)	-0.07432 (-3.536, 2.257)
25mg_kg						23.01 (1.467, 79.13)	22.8 (1.249, 78.75)	24.14 (2.593, 80.13)	22.21 (0.517, 78.33)
30mg_kg							-0.2499 (-1.165, 0.7003)	1.095 (-0.4884, 2.743)	-0.6811 (-4.081, 1.511)
35mg_kg								1.34 (-0.2246, 2.951)	-0.4317 (-3.81, 1.706)
40mg_kg									-1.788 (-5.157, 0.3068)
SUCRA									
25mg_kg	0.99132778								
20mg_kg_iv	0.67399722								
10mg_kg	0.65058056								
50mg_kg	0.59134444								
15mg_kg	0.52576667								
35mg_kg	0.42591111								
20mg_kg	0.42531944								
13mg_kg_iv	0.36803889								
30mg_kg	0.300225								
40mg_kg	0.04748889								
Model									
Gelman PSRF	1.010385								
Node splitting									
15mg_kg vs 20mg_kg	0.61875								
30mg_kg vs 20mg_kg	0.00835								
35mg_kg vs 20mg_kg	0.6912								
35mg_kg vs 30mg_kg	0.8283								

Table S4. Hepatic grade 3 or higher AE results										
Log risk ratio (95% CrI) relative effect table										
	13mg_kg	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg

10mg_kg	-1.339 (-4.861, 0.9587)	-0.2082 (-1.266, 0.7721)	-0.3266 (-0.9689, 0.2806)	-0.01193 (-0.5305, 0.5552)	-0.9974 (-4.365, 1.074)	-20.29 (-77.07, 1.216)	0.7028 (-0.3182, 1.99)	0.6658 (-0.09501, 1.453)	2.718 (0.05595, 6.356)	-20.9 (-78.14, 0.9987)
13mg_kg		1.145 (-1.419, 4.739)	1.01 (-1.392, 4.545)	1.334 (-1.02, 4.904)	0.3149 (-3.694, 4.364)	-18.86 (-75.69, 3.076)	2.089 (-0.4532, 5.833)	2.018 (-0.4194, 5.561)	4.176 (0.5349, 9.047)	-19.39 (-76.61, 2.834)
13mg_kg_iv			-0.121 (-1.194, 0.9937)	0.1935 (-0.8121, 1.348)	-0.8017 (-4.259, 1.521)	-20.07 (-76.83, 1.441)	0.9147 (-0.4658, 2.586)	0.875 (-0.3328, 2.18)	2.936 (0.1093, 6.727)	-20.7 (-78.04, 1.234)
15mg_kg				0.315 (-0.3318, 1.053)	-0.6744 (-4.095, 1.506)	-19.98 (-76.66, 1.574)	1.031 (-0.1046, 2.465)	0.9911 (0.05682, 1.99)	3.052 (0.3188, 6.725)	-20.59 (-77.83, 1.316)
20mg_kg					-0.9931 (-4.397, 1.102)	-20.29 (-77.03, 1.225)	0.7098 (-0.3459, 1.991)	0.6747 (-0.1923, 1.538)	2.727 (0.04104, 6.371)	-20.9 (-78.14, 1.015)
20mg_kg_iv						-19.07 (-76.21, 2.615)	1.747 (-0.6017, 5.286)	1.66 (-0.3483, 5.022)	3.859 (0.3729, 8.613)	-19.74 (-76.92, 2.522)
25mg_kg							21.02 (-0.5104, 77.88)	20.95 (-0.5345, 77.79)	23.13 (1.76, 80.15)	0.2706 (-64.03, 61.6)
30mg_kg								-0.04016 (-1.468, 1.201)	1.992 (-0.7074, 5.602)	-21.64 (-78.83, 0.2724)
35mg_kg									2.055 (-0.6323, 5.69)	-21.59 (-78.87, 0.3664)
40mg_kg										23.69 (2.014, 80.92)
SUCRA										
50mg_kg	0.9103675									
25mg_kg	0.909035									
13mg_kg	0.69252									
20mg_kg_iv	0.648215									
15mg_kg	0.584425									
13mg_kg_iv	0.50984									
20mg_kg	0.4271375									
10mg_kg	0.419225									
30mg_kg	0.190775									
35mg_kg	0.1829975									
40mg_kg	0.0254625									
MODEL										

Gelman PSRF	1.055592									
Node splitting										
10mg_kg vs 13mg_kg_iv	0.25080									
10mg_kg vs 15mg_kg	0.89360									
10mg_kg vs 20mg_kg	0.43505									
13mg_kg_iv vs 15mg_kg	0.39745									
13mg_kg_iv vs 20mg_kg	0.24105									
15mg_kg vs 20mg_kg	0.98585									
20mg_kg vs 30mg_kg	0.00495									
20mg_kg vs 35mg_kg	0.19910									
30mg_kg vs 35mg_kg	0.03525									

Table S5. EBA at 5 days results							
Log risk ratio (95% CrI) relative effect table							
	15mg_kg	20mg_kg	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg
10mg_kg	-0.1843 (-0.6584, 0.2925)	-0.1778 (-0.5345, 0.2029)	-0.5256 (-1.088, 0.1652)	-1.419 (-1.984, -0.7177)	-0.3663 (-0.9325, 0.3308)	-2.157 (-3.319, -0.9695)	-3.708 (-4.974, -2.396)
15mg_kg		0.006129 (-0.4554, 0.4921)	-0.341 (-1.012, 0.4564)	-1.234 (-1.904, -0.4275)	-0.1819 (-0.8561, 0.6222)	-1.973 (-3.186, -0.7282)	-3.524 (-4.84, -2.158)
20mg_kg			-0.3487 (-0.9005, 0.3127)	-1.242 (-1.793, -0.5739)	-0.1898 (-0.7395, 0.478)	-1.981 (-3.13, -0.8123)	-3.53 (-4.792, -2.235)
25mg_kg				-0.8925 (-1.55, -0.2294)	0.1595 (-0.4993, 0.8182)	-1.644 (-2.83, -0.4676)	-3.191 (-4.478, -1.891)
30mg_kg					1.052 (0.3903, 1.71)	-0.751 (-1.94, 0.4255)	-2.3 (-3.592, -0.9968)
35mg_kg						-1.803 (-2.989, -0.6241)	-3.351 (-4.644, -2.05)
40mg_kg							-1.544 (-3.179, 0.09657)
SUCRA							
50mg_kg	0.99499286						
40mg_kg	0.84546929						
30mg_kg	0.72310679						
25mg_kg	0.5043						
35mg_kg	0.38486071						
15mg_kg	0.24851857						
20mg_kg	0.23543286						
10mg_kg	0.06331893						
Model							
Gelman PSRF	1.000158						

No inconsistencies to check							

Table S6. EBA at 14 days results							
Log risk ratio (95% CrI) relative effect table							
	15mg_kg	20mg_kg	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg
10mg_kg	-0.1238 (-0.929, 0.7409)	-0.1009 (-0.8051, 0.6469)	-0.02499 (-1.061, 1.109)	-1.399 (-2.454, -0.2582)	-1.343 (-2.417, -0.1782)	-1.085 (-2.394,0.2761)	-2.297 (-3.667, -0.8707)
15mg_kg		0.0223 (-0.8094, 0.843)	0.09984 (-1.141, 1.373)	-1.275 (-2.523, 0.003226)	-1.22 (-2.48, 0.07675)	-0.9638 (-2.419,0.5156)	-2.174 (-3.689, -0.6397)
20mg_kg			0.07752 (-0.9536, 1.155)	-1.297 (-2.339, -0.2108)	-1.242 (-2.3, -0.1396)	-0.9864 (-2.268,0.3248)	-2.196 (-3.543, -0.8138)
25mg_kg				-1.375 (-2.508, -0.2503)	-1.32 (-2.467, -0.1803)	-1.065 (-2.404,0.2679)	-2.276 (-3.672, -0.8778)
30mg_kg					0.05528 (-1.098, 1.203)	0.3094 (-1.041,1.651)	-0.9011 (-2.304, 0.5039)
35mg_kg						0.2539 (-1.105,1.618)	-0.957 (-2.376, 0.4737)
40mg_kg							-1.209 (-2.829, 0.4092)
SUCRA							
50mg_kg	0.9655786						
30mg_kg	0.7548154						
35mg_kg	0.7278293						
40mg_kg	0.6366450						
15mg_kg	0.2747425						
20mg_kg	0.2587518						
25mg_kg	0.2017796						
10mg_kg	0.1798579						
Model							
Gelman PSRF	1.000061						
No inconsistencies to check							

Table S7. SCC at 8 weeks in MGIT media			
Log risk ratio (95% CrI) relative effect table			
	15mg_kg	20mg_kg	35mg_kg
10mg_kg	0.0697 (-0.1309, 0.2519)	0.1087 (-0.02867, 0.2563)	0.2998 (0.05679, 0.5385)
15mg_kg		0.03908 (-0.1337, 0.2382)	0.2305 (-0.06342, 0.5287)
20mg_kg			0.1911 (-0.07249, 0.4444)
SUCRA			

35mg_kg	0.9541750		
20mg_kg	0.5664583		
15mg_kg	0.3867417		
10mg_kg	0.0926250		
Model			
Gelman PSRF	1.000385		
No inconsistencies to check			

Table S8. SCC at 8 weeks in LJ media			
Log risk ratio (95% CrI) relative effect table			
	15mg_kg	20mg_kg	35mg_kg
10mg_kg	0.005044 (-0.1387, 0.1356)	0.03348 (-0.05476, 0.1275)	0.08194 (-0.0658, 0.2214)
15mg_kg		0.02888 (-0.09757, 0.1715)	0.07598 (-0.1066, 0.2647)
20mg_kg			0.04859 (-0.1029, 0.1873)
SUCRA			
35mg_kg	0.8273917		
20mg_kg	0.5616417		
15mg_kg	0.3465917		
10mg_kg	0.2643750		
Model			
Gelman PSRF	1.000301		
No inconsistencies to check			

Table S9. Recurrence rate at 12 months post randomization in pulmonary TB			
Log risk ratio (95% CrI) relative effect table			
	15mg_kg	20mg_kg	35mg_kg
10mg_kg	-1.002 (-4.68, 1.762)	0.3035 (-1.152, 1.865)	-0.1708 (-2.402, 1.695)
15mg_kg		1.301 (-1.409, 5.06)	0.8459 (-2.691, 4.848)
20mg_kg			-0.4731 (-3.058, 1.642)
SUCRA			
15mg_kg	0.7585750		
35mg_kg	0.5199833		
10mg_kg	0.4460083		
20mg_kg	0.2754333		
Model			
Gelman PSRF	1.00028		
No inconsistencies to check			

Table S10. Mortality in TB meningitis
Log risk ratio (95% CrI) relative effect table

	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	30mg_kg	35mg_kg
10mg_kg	-0.6352 (-2.509, 1.246)	-0.009509 (-1.844, 1.813)	0.2802 (-1.73, 2.271)	-0.01348 (-1.821, 1.809)	-1.983 (-5.563, 0.5262)	0.267 (-1.096, 1.633)
13mg_kg_iv		0.6277 (-2, 3.261)	0.9183 (-1.823, 3.667)	0.6206 (-1.973, 3.256)	-1.369 (-5.402, 1.855)	0.9099 (-1.425, 3.243)
15mg_kg			0.2836 (-2.4, 3.002)	-0.004193 (-2.572, 2.627)	-1.981 (-5.958, 1.114)	0.278 (-1.966, 2.547)
20mg_kg				-0.2918 (-2.967, 2.411)	-2.249 (-5.847, 0.2573)	-0.004975 (-2.437, 2.407)
20mg_kg_iv					-1.984 (-5.959, 1.112)	0.2822 (-1.497, 2.067)
30mg_kg						2.263 (-0.603, 6.103)
SUCRA						
30mg_kg	0.9151292					
13mg_kg_iv	0.6967542					
20mg_kg_iv	0.4390833					
15mg_kg	0.4328167					
10mg_kg	0.4268167					
20mg_kg	0.3100042					
35mg_kg	0.2793958					
Model						
Gelman PSRF	1.000376					
No inconsistencies to check						

Table S11. Pharmacokinetics: Cmax										
Mean difference (95% CrI) relative effect table										
	13mg_kg	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg
10mg_kg	5.094 (-1.11, 11.28)	15.36 (10.63, 19.92)	6.29 (3.359, 9.487)	10.08 (7.849, 12.48)	30.8 (26.77, 34.74)	15.21 (9.528, 20.61)	19.83 (14.53, 25.07)	24.91 (21.92, 27.9)	31.47 (24.78, 38.09)	43.36 (32.8, 53.82)
13mg_kg		10.24 (2.499, 17.93)	1.182 (-5.535, 8.248)	4.981 (-1.568, 11.6)	25.7 (18.32, 33.07)	10.11 (1.751, 18.24)	14.72 (6.662, 22.78)	19.82 (12.96, 26.65)	26.4 (17.2, 35.25)	38.28 (25.99, 50.15)
13mg_kg_iv			-9.061 (-14.14, -3.706)	-5.274 (-9.987, -0.2448)	15.46 (9.327, 21.58)	-0.1218 (-7.361, 6.93)	4.452 (-2.412, 11.49)	9.563 (4.16, 15.08)	16.13 (8.096, 24.19)	27.98 (16.53, 39.34)
15mg_kg				3.793 (0.5157, 6.958)	24.5 (19.31, 29.37)	8.922 (2.502, 14.92)	13.5 (7.476, 19.35)	18.63 (14.33, 22.65)	25.18 (17.84, 32.25)	37.01 (26.01, 47.8)
20mg_kg					20.73 (16.03, 25.1)	5.12 (-0.6839, 10.6)	9.716 (4.328, 15.07)	14.84 (11.36, 18.16)	21.38 (14.52, 28.05)	33.24 (22.52, 43.72)
20mg_kg_iv						-15.59 (-22.32, -9.086)	-10.97 (-17.45, -4.42)	-5.874 (-10.02, -1.664)	0.6878 (-6.89, 8.153)	12.56 (1.379, 23.5)
25mg_kg							4.62 (-2.389, 11.78)	9.703 (4.1, 15.61)	16.27 (8.495, 24.01)	28.15 (16.89, 39.3)

30mg_kg								5.103 (-0.6764, 10.92)	11.64 (3.66, 19.56)	23.54 (12.03, 34.76)
35mg_kg									6.566 (-0.4162, 13.4)	18.44 (7.705, 29.03)
40mg_kg										11.83 (0.1295, 23.68)

SUCRA										
50mg_kg	0.9961275									
40mg_kg	0.8561650									
20mg_kg_iv	0.8434375									
35mg_kg	0.6994675									
30mg_kg	0.5855875									
13mg_kg_iv	0.4583350									
25mg_kg	0.4523475									
20mg_kg	0.2986925									
15mg_kg	0.1661675									
13mg_kg	0.1389475									
10mg_kg	0.0047250									
Model										
Gelman PSRF	1.000437									
Node splitting										
10mg_kg. 13mg_kg_iv	0.75185									
10mg_kg. 15mg_kg	0.22335									
10mg_kg. 20mg_kg	0.77530									
13mg_kg_iv.15mg_kg	0.47470									
13mg_kg_iv.20mg_kg	0.92965									
15mg_kg. 20mg_kg	0.90230									
20mg_kg. 35mg_kg	0.82120									
30mg_kg. 35mg_kg	0.25570									

Table S12. Pharmacokinetics: AUC0-24

Mean difference (95% CrI) relative effect table

	13mg_kg	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	25mg_kg	30mg_kg	35mg_kg	40mg_kg	50mg_kg
10mg_kg	31.05 (-45.09, 107.6)	50.76 (-3.451, 105.2)	41.2 (4.393, 79.7)	68.49 (37.49	163.7 (110.7, 214)	102.4 (35.41	145.6 (87.84	215.6 (178.3	224.7 (155, 294.3)	337.6 (227.9

				, 100.7)		, 169.2)	, 203.2)	, 253.5)		, 447.6)
13mg_kg		19.6 (-74.44, 114.1)	10.21 (-75.08, 96.26)	37.37 (-45.12, 121)	133 (39.02, 223.4)	71.58 (-29.74, 173.1)	114.5 (18.68, 210.2)	184.7 (99.23, 270.2)	193.8 (89.8, 297.1)	306.3 (174.1, 438.9)
13mg_kg_iv			-9.348 (-67.58, 49.65)	18.1 (-38.75, 75.28)	113.2 (37.51, 186.5)	51.77 (-32.54, 136.1)	95 (17.34, 171.6)	164.9 (100.2, 230.8)	174 (86.98, 260.5)	286.9 (165.4, 409)
15mg_kg				27.32 (-13.3, 67.14)	122.5 (57.06, 182.9)	61.1 (-13.69, 135.8)	104.1 (37.32, 169.9)	174.4 (122.4, 225.3)	183.7 (105.8, 260.1)	296.4 (181.3, 411.3)
20mg_kg					95.25 (35.56, 151.8)	33.74 (-33.85, 101.2)	76.93 (17.87, 135.5)	147 (103.7, 190.3)	156.3 (85.02, 226)	269.1 (158.5, 378.8)
20mg_kg_iv						-61.54 (-142.3, 21.71)	-18.34 (-92.78, 59.27)	51.73 (0.4424, 107.6)	60.85 (-22.13, 145.9)	174.2 (57.15, 293.3)
25mg_kg							43.08 (-36.25, 122.8)	113.2 (43.48, 183.9)	122.1 (39.33, 204.9)	235.3 (117.1, 353.3)
30mg_kg								70.2 (5.653, 134.6)	79.08 (-3.198, 160.5)	192.5 (74.14, 310.3)
35mg_kg									8.99 (-63.37, 81.06)	122.1 (10.48, 233.1)
40mg_kg										113.3 (-5.89, 232.7)
SUCRA										
50mg_kg	0.9948725									
40mg_kg	0.8532850									
35mg_kg	0.8370875									
20mg_kg_iv	0.6706225									
30mg_kg	0.6191925									
25mg_kg	0.4823250									
20mg_kg	0.3656575									
13mg_kg_iv	0.2640325									
15mg_kg	0.2091675									
13mg_kg	0.1791600									
10mg_kg	0.0245975									
Model										

Gelman PSRF	1.00058									
Node splitting										
10mg_kg.13mg_kg_iv	0.92915									
10mg_kg.15mg_kg	0.29635									
10mg_kg.20mg_kg	0.73805									
13mg_kg_iv.15mg_kg	0.72015									
13mg_kg_iv.20mg_kg	0.83215									
15mg_kg.20mg_kg	0.41445									
20mg_kg.35mg_kg	0.00765									
30mg_kg.35mg_kg	0.49580									

Table S13. Pharmacokinetics: Tmax							
Mean difference (95% CrI) relative effects table							
	13mg_kg	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	30mg_kg	35mg_kg
10mg_kg	-0.4664 (-1.38, 0.4406)	-0.1085 (-0.7882, 0.5649)	0.003122 (-0.4582, 0.5369)	0.3365 (0.00427, 0.9051)	-1.52 (-2.167, -0.873)	1.747 (0.2259, 3.26)	0.6365 (0.07594, 1.236)
13mg_kg		0.3541 (-0.7724, 1.498)	0.4724 (-0.5268, 1.544)	0.8112 (-0.09972, 1.936)	-1.053 (-2.169, 0.05816)	2.217 (0.4721, 3.979)	1.102 (0.05105, 2.208)
13mg_kg_iv			0.1175 (-0.5852, 0.8864)	0.4547 (-0.2189, 1.333)	-1.412 (-2.335, -0.4675)	1.857 (0.1895, 3.513)	0.7447 (-0.112, 1.655)
15mg_kg				0.3238 (-0.1266, 0.9779)	-1.53 (-2.354, -0.7476)	1.736 (0.1484, 3.307)	0.6241 (-0.1116, 1.368)
20mg_kg					-1.868 (-2.715, -1.203)	1.383 (-0.2099, 2.928)	0.2865 (-0.4365, 0.8789)
20mg_kg_iv						3.269 (1.619, 4.93)	2.158 (1.522, 2.831)
30mg_kg							-1.105 (-2.718, 0.5096)
SUCRA							
20mg_kg_iv	0.99462500						
13mg_kg	0.77828929						
13mg_kg_iv	0.62809286						
10mg_kg	0.56395357						
15mg_kg	0.55314643						
20mg_kg	0.28310714						

35mg_kg	0.17233214						
30mg_kg	0.02645357						
Model							
Gelman PSRF	1.000737						
Node splitting							
10mg_kg.13mg_kg_iv	0.58460						
10mg_kg.15mg_kg	0.44540						
10mg_kg.20mg_kg	0.88975						
13mg_kg_iv.15mg_kg	0.48515						
13mg_kg_iv.20mg_kg	0.92595						
20mg_kg.35mg_kg	0.55080						

Table S14. Pharmacokinetics: T1/2							
Mean difference (95% CrI) relative effect table							
	13mg_kg	13mg_kg_iv	15mg_kg	20mg_kg	20mg_kg_iv	30mg_kg	35mg_kg
10mg_kg	0.302 (-0.6706, 1.271)	-0.447 (-1.298, 0.5927)	0.2645 (-0.2167, 0.9593)	0.1688 (-0.1583, 0.7167)	-2.078 (-3.596, -0.5655)	0.5013 (-0.5601, 1.658)	0.7944 (0.2097, 1.66)
13mg_kg		-0.7475 (-2.004, 0.6944)	-0.0325 (-1.068, 1.204)	-0.1165 (-1.097, 1.04)	-2.376 (-4.175, -0.5687)	0.1994 (-1.226, 1.726)	0.503 (-0.5932, 1.828)
13mg_kg_iv			0.7164 (-0.1514, 1.593)	0.631 (-0.2665, 1.51)	-1.641 (-3.478, 0.08848)	0.9397 (-0.4666, 2.318)	1.252 (0.1475, 2.382)
15mg_kg				-0.08186 (-0.6437, 0.4471)	-2.359 (-4.041, -0.797)	0.2227 (-0.9865, 1.4)	0.5361 (-0.3012, 1.4)
20mg_kg					-2.276 (-3.881, -0.7504)	0.3094 (-0.7876, 1.399)	0.6164 (-0.0698, 1.37)
20mg_kg_iv						2.588 (0.7473, 4.485)	2.896 (1.334, 4.56)
30mg_kg							0.311 (-0.9417, 1.597)
SUCRA							
20mg_kg_iv	0.99240000						
13mg_kg_iv	0.79204643						
10mg_kg	0.64561429						
20mg_kg	0.44328214						
13mg_kg	0.38627143						
15mg_kg	0.37601071						
30mg_kg	0.28046786						
35mg_kg	0.08390714						
Model							
Gelman PSRF	1.000381						
Node splitting							
10mg_kg.15mg_kg	0.8698						
20mg_kg.35mg_kg	0.3618						

5- Influence of the administration route

In individual study data the peak exposure of subjects who received intravenous rifampicin at a dose of 13 mg/kg/day was similar to that of oral 20 mg/kg/day, as was the overall exposure. The intravenous 20mg/kg/day provided lower exposure than the oral 35 mg/kg/day dose (Table S17).

The Cmax for intravenous 13 mg/kg/day dose was similar to oral 25 mg/kg/day dose when compared to that of 10 mg/kg/day: Cmax MD (mg/dL) 15 (95% CrI 11;20) and 15 (95% CrI 9.6; 21). Intravenous 20 mg/kg/day and oral 35 mg/kg/day showed different exposures: Cmax MD (mg/dL) 31 (95% CrI 27; 35) and 25 (95% CrI 22; 28). A similar relationship can be seen for the AUC0-24 between intravenous and oral doses: AUC0-24 MD (mg·h/L) 51 (95% CrI -3; 110) and 69(95% CrI 37; 100) for intravenous 13mg/kg and oral 25mg/kg; AUC0-24 MD (mg·h/L) 160 (95% CrI 110; 210) and 220 (95% CrI 180; 250) for intravenous 20mg/kg and oral 35mg/kg.

Table S15. Comparison between intravenous and oral exposure in individual trials.

	13 mg/kg/day iv	20 mg/kg/day po	20 mg/kg/day iv	35 mg/kg/day po
C_{max}	23.9 (range 14.9-36.4)*	19.9 (range 12.8-29.6)*		
AUC_{0-6}	78.7 (range 71-87.3)∫	55.5 (IQR 35.7-73.2) †		
AUC_{0-24}	94.9 (range 59.7-161.2)*	101.2 (range 41.7-167.8) *	206.5 (95% CI 154.6-275.8) ‡	295 (189.9-458.8) ‡

* Yunivita 2016

∫ Ruslami 2013

† Peloquin 2018

‡ Wassermann 2021

$C_{max,}$ maximum concentration (mg/L); AUC_{0-6} Area Under the Curve for rifampicin concentration 6h after single dose administration (mg·h/L); AUC_{0-24} Area Under the Curve for rifampicin concentration 24h after single dose administration.

6- Supplementary figures

Figure S1. Traffic plot for the risk of bias assessment

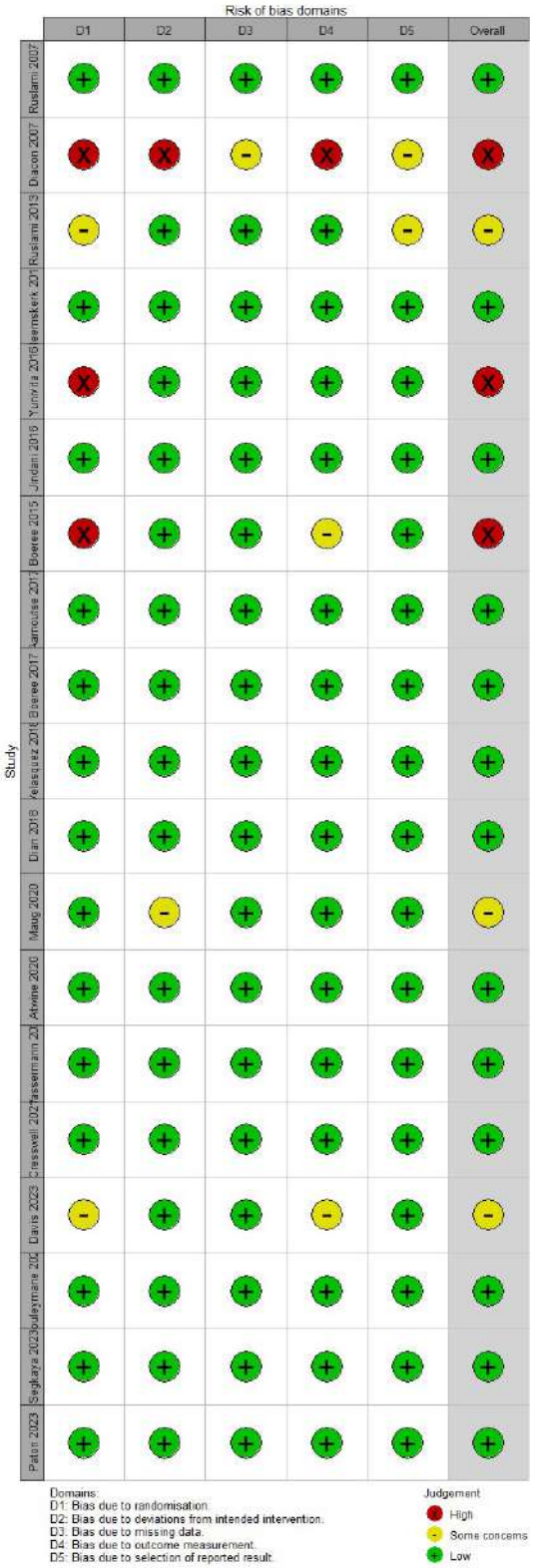


Figure S2. All grade ≥ 3 AE

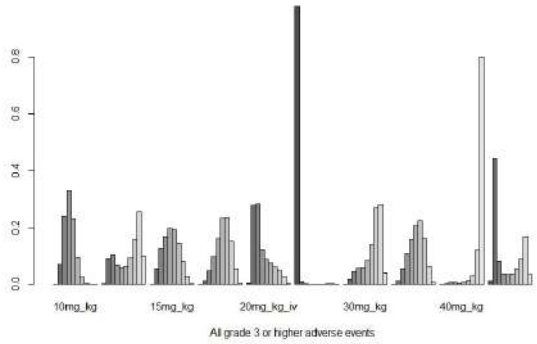


Figure S3. Hepatic grade ≥ 3 AE

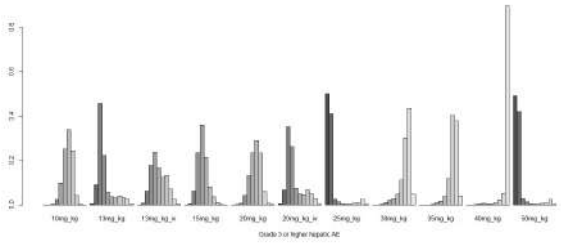


Figure S4. Early bactericidal activity at 5 days

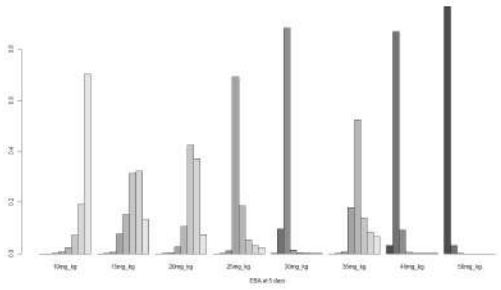


Figure S5. Early bactericidal activity at 14 days

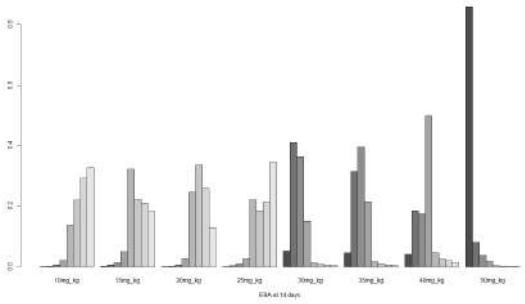


Figure S6. Sputum conversion at w8, MGIT

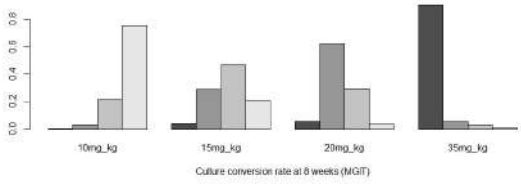


Figure S7. Sputum conversion ay w8, LJ

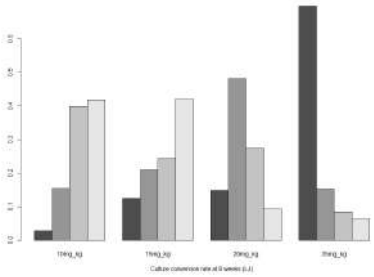


Figure S8. Recurrence

Figure S9. Mortality (TB meningitis)

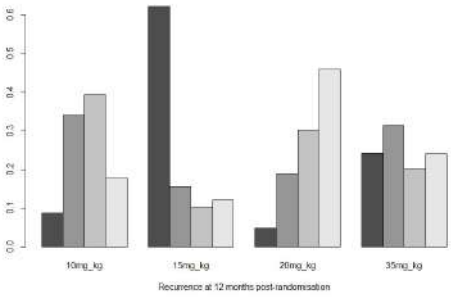


Figure S10. Cmax

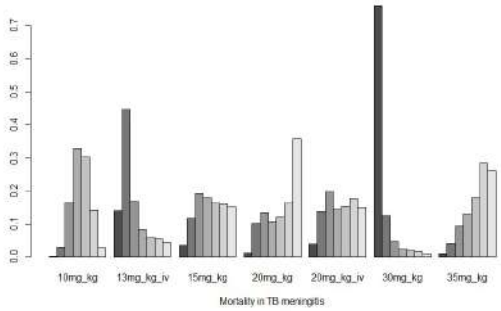


Figure S11. AUC_{0-24}

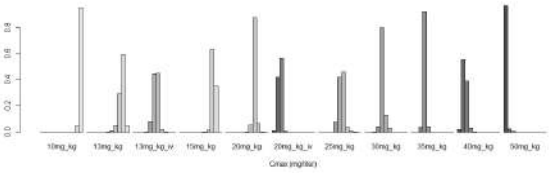


Figure S12. Tmax

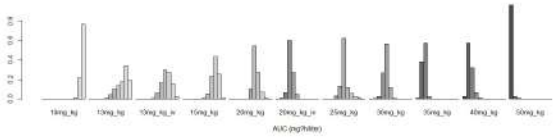
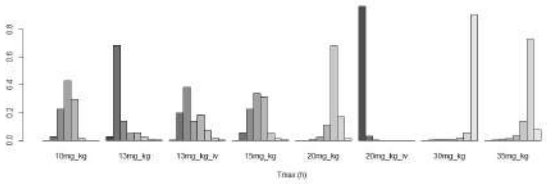


Figure S13. $T_{1/2}$



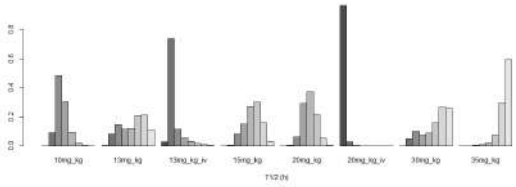
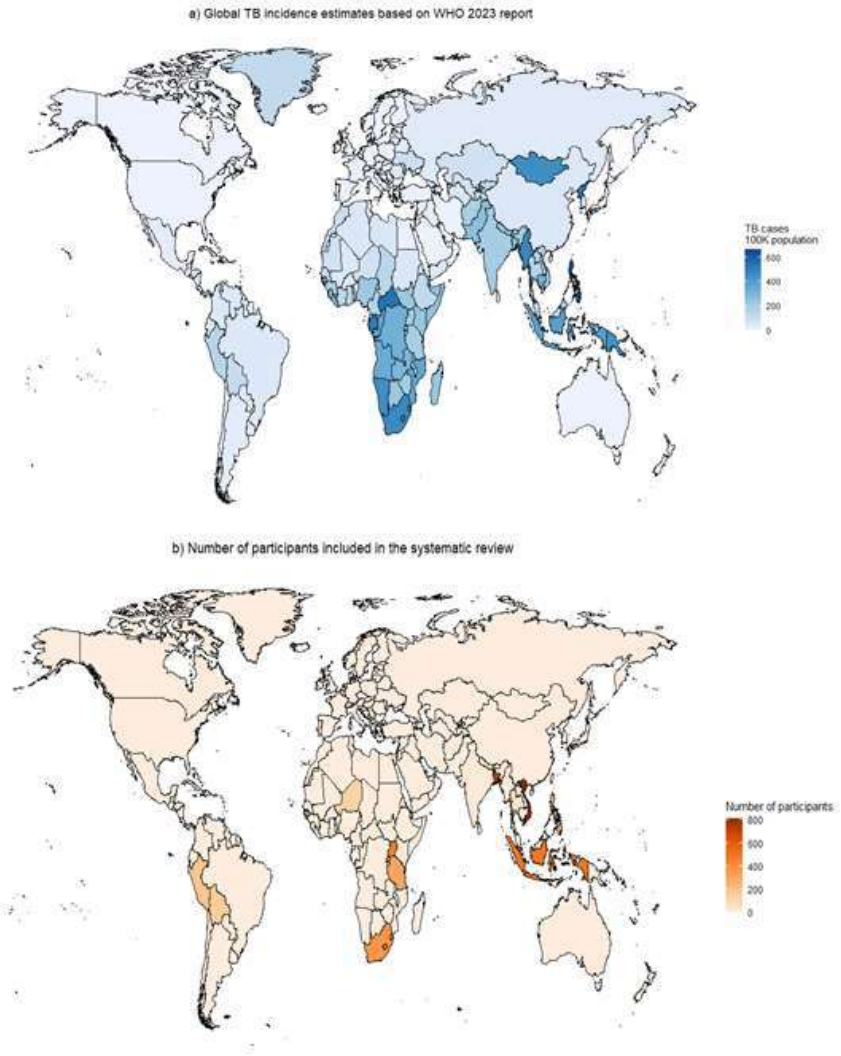


Figure S14. Global TB incidence (adapted from WHO global TB report 2023) compared to the distribution of participants included in the meta-analysis per country.



7- Sensitivity analysis

7.1- Excluding PK sampling during the first week of treatment

MD (95% credibility intervals) of each pharmacokinetic parameter from each rifampicin dose as compared to oral 10mg/kg				
Dose	C_{max} (mg/L)	AUC_{0-24} (mg·h/L)	T_{max} (h)	$T_{1/2}$ (h)
13mg/kg	5.1 (-2.2; 12.0)	31.0 (-27.0; 90.0)	0.3 (-0.6; 1.2)	0.3 (0.6; 1.2)
13mg/kg iv	14.0 (5.1; 23.0)	41.0 (-22.0; 110.0)	-0.4 (-1.3; 0.5)	-0.5 (-1.3; 0.5)
15mg/kg	6.3 (2.6; 10.0)	36.0 (6.1; 67.0)	0.3 (-0.2; 0.9)	0.3 (-0.2; 0.9)
20mg/kg	10.0 (7.1; 13.0)	60.0 (34.0; 86.0)	0.2 (-0.2; 0.7)	0.2 (-0.2; 0.7)
25mg/kg	15.0 (8.2; 21.0)	89.0 (35.0; 140.0)		
30mg/kg	20.0 (14.0; 26.0)	140.0 (87.0; 190.0)	0.5 (-0.6; 1.6)	0.5 (-0.6; 1.6)

35mg/kg	25.0 (20.0; 29.0)	180.0 (140.0; 220.0)	0.8 (-0.1; 1.5)	0.8 (0.1; 1.5)
C_{max}, maximum concentration (mg/L); AUC_{0-24} Area Under the Curve for rifampicin concentration 24h after single dose administration; T_{max} time to C_{max}; $t_{1/2}$ elimination half-life; MD mean difference				

7.2- *Post hoc*: Safety and Sputum Culture Conversion at week 8 in liquid media including a recently published trial from India showing an increased risk of toxicity with 35 mg/kg/dose.

Figure S15. Sensitivity analysis

Figure S15a. All grade ≥3 AE

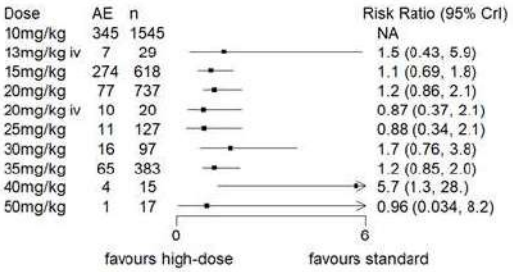


Figure S15b. Hepatic grade ≥3 AE

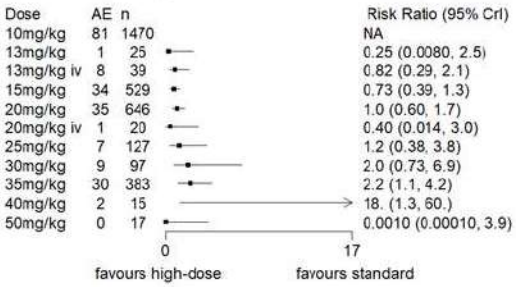
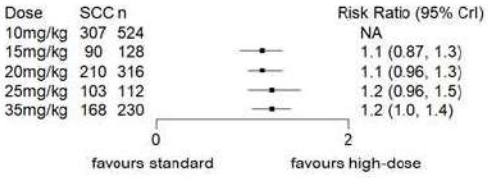


Figure S15c. SCC in liquid media at week 8



Ref 51 in main text: Kannabiran B. Safety and Efficacy of 25 mg/kg and 35 mg/kg vs 10 mg/kg Rifampicin in Pulmonary TB: A Phase IIb Randomized Controlled Trial. Open Forum Infect Dis. 2024 Feb 2;11(3):ofae034. doi: 10.1093/ofid/ofae034.

10.3. Appendix III: A systematic review of potential biomarkers for bacterial burden and treatment efficacy assessment in tuberuclosis platform-based clinical trials

Espinosa-Pereiro J, Alagna R, Saluzzo F, González-Moreno J, Heinrich N, Sánchez-Montalvá A, Cirillo DM. A Systematic Review of Potential Biomarkers for Bacterial Burden and Treatment Efficacy Assessment in Tuberculosis Platform-Based Clinical Trials. J Infect Dis. 2024 May 15;229(5):1584-1595. doi: 10.1093/infdis/jiad482.

This is a systematic review performed during the EU-PEARL project in which we analyse the landscape of biomarkers for treatment monitoring in TB clinical trials, and their development stage. It adds relevant information for the chapter VI, as the development of new biomarkers that can be used as efficacy surrogates is one of the critical steps to make clinical trials more efficient and, if these biomarkers are validated in extrapulmonary TB, more inclusive.

We found that, from 1356 biomarker-outcome pairs, only 41 were good predictors, and only sputum culture status was fully validated as a surrogate marker (with a limited performance as such). We concluded that, for multi-stage trials, a combination of biomarkers from different categories could help in designing more efficient platform trials.

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REVIEW

A Systematic Review of Potential Biomarkers for Bacterial Burden and Treatment Efficacy Assessment in Tuberculosis Platform-Based Clinical Trials

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Adaptive platform trials can be more efficient than classic trials for developing new treatments. Moving from culture-based to simpler- or faster-to-measure biomarkers as efficacy surrogates may enhance this advantage. We performed a systematic review of treatment efficacy biomarkers in adults with tuberculosis. Platform trials can span different development phases. We grouped biomarkers as: α, bacterial load estimates used in phase 2a trials; β, early and end-of treatment end points, phase 2b-c trials; γ, posttreatment or trial-level estimates, phase 2c-3 trials. We considered as analysis unit (biomarker entry) each combination of biomarker, predicted outcome, and their respective measurement times or intervals. Performance metrics included: sensitivity, specificity, area under the receiver-operator curve (AUC), and correlation measures, and classified as poor, promising, or good. Eighty-six studies included 22 864 participants. From 1356 biomarker entries, 318 were reported with the performance metrics of interest, with 103 promising and 41 good predictors. Group results were: α, mycobacterial RNA and lipoarabinomannan (LAM) in sputum, and host metabolites in urine; β, mycobacterial RNA and host transcriptomic or cytokine signatures for early treatment response; and γ, host transcriptomics for recurrence. A combination of biomarkers from different categories could help in designing more efficient platform trials. Efforts to develop efficacy surrogates should be better coordinated.

Keywords. tuberculosis; biomarkers; platform trials; surrogate end points; surrogacy.

Tuberculosis affects 10 million people worldwide each year, being one of the infectious diseases with higher mortality [1]. Current treatments are long and can cause important side effects, which makes it difficult to complete the treatment for those affected by tuberculosis. In the past decades, the development of new antituberculosis drugs, and optimization of drug combinations, has been slow and severely underfunded.

The EU Patient-Centric Clinical Trial Platforms (EU-PEARL) has brought many stakeholders together, with patients as central actors. Its aim is to optimize the entire process for developing new treatments, including the tools needed to design platform trials. The EU-PEARL project used 4 different diseases to test the flexibility of this framework, including tuberculosis.

Conventional clinical trials are designed to answer whether a small set [1–3] of interventions are better than the current standard of care in a specific disease population. The drug development process is rather inefficient with only 25% of new compounds reaching large preregulatory clinical trials, and about 10% finally authorized [2]. In platform trials, a single overarching protocol, termed the master protocol, is used to evaluate multiple treatments or treatment combinations (ie, interventions) in one disease [3]. Interventions can enter, be promoted to the next phase of development, or leave the trial under prespecified criteria spanning several years [4]. Platform trials can include adaptive design elements in which interim analyses inform operational modifications such as the allocation ratio or intervention drop-out [5]. Surrogate end points are often used for accelerating this decision making process [6]. In tuberculosis, for instance, different end points are used to progress new drugs or regimens through the different phases of clinical development: in phase 2a, early bactericidal activity trials are based on changes in bacterial load over time; in phase 2b, early clinical response trials are based on culture status (eg, time to negative culture, culture conversion at 2 months

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postrandomization, etc.); and in phase 2c-3, late clinical response trials are based on clinical and microbiological cure at end of treatment, or relapse-free cure after treatment completion. Platform trials can use these end points for adaptations and seamless progress of novel drugs or regimens. But end points based on mycobacterial cultures are technically demanding, have accuracy limitations, and have relatively long readout times [7, 8].

However, regulators, researchers, and the community should be acquainted with the limitations intrinsic to the use of surrogate end points [9]. Surrogacy is statistically defined as "a response variable for which a test of the null hypothesis of no relationship to the treatment groups under comparison is also a valid test of the corresponding null hypothesis based on the true end point" [10]. In practice, this means that the effect of the treatment on the final clinical end point is completely explained through its effect on the surrogate end point [7, 8].

We performed a systematic review of the literature to assess host and bacterial biomarkers able to predict the clinical outcomes used in tuberculosis clinical trials from phase 2a to 3, including individual-level and trial-level surrogacy.

METHODS

We performed a systematic review of biomarkers able to estimate bacterial burden as well as predict treatment outcomes in accordance with Cochrane guidance [11]. We grounded this report on the PRISMA standards [12]. The review protocol was registered at the international prospective register of systematic reviews PROSPERO (CRD42021225507) [13]. Search terms were designed and adapted to PubMed and Embase engines for articles published in English, Spanish, French, Portuguese, or Italian between January 2010 and September 2020. The search term structure can be found in the PROSPERO protocol and in the Supplementary Material.

Population Inclusion/Exclusion Criteria

We included studies reporting the results from subjects with bacteriologically confirmed pulmonary and confirmed or clinically diagnosed extrapulmonary tuberculosis who were 15 years of age or older, regardless of their ethnicity, geographical origin, comorbidities including diabetes and HIV infection, or clinical setting. We included drug-susceptible and drug-resistant, pulmonary and extrapulmonary tuberculosis cases and previously treated participants.

Study Type

Cohort, cross-sectional, case-control, and clinical trials (randomized or not) were included. Post hoc analysis of cohorts or clinical trials were also included. There were no restrictions for conventional or experimental tuberculosis drugs or regimens. Conference reports, abstracts, and non–peer-reviewed articles were excluded.

Biomarker Types, Index Tests, and Sample Type

We considered studies reporting biomarkers from mycobacterial and/or host origin, both individually and as part of different biosignatures. We included all modalities of ancillary tests, microbiology laboratory procedures, and radiology imaging. We included all studies that reported a statistical association between a biomarker and the clinical outcome, not restricted to sensitivity or specificity. Studies that did not clearly report any statistical measurement were excluded.

Outcomes and Reference Standard

The aim of this review was to provide tools to inform the design of tuberculosis adaptive platforms, able to progress novel drugs or combinations seamless, through different phases of the development. We adapted the classification published by McLeod, which groups biomarkers according to the reference standard and whether performance information is provided [14]. We defined 3 groups using as reference standard the classical outcomes for phase 2a, 2b, and 2c-3 tuberculosis trials (groups α, β, and γ, respectively), which are the outcomes of interest for this review:

1. Group α, biomarkers estimating the bacterial load and early bactericidal activity (EBA) during the first weeks of treatment. The reference standards are therefore the commonly used mycobacteriology tests: sputum smear bacterial load, and culture-based bacterial load.
2. Group β, biomarkers estimating early treatment outcomes such as culture conversion rate or time-to-culture conversion at interim time points and/or end-of-treatment clinical outcomes at a patient level.
3. Group γ, biomarkers estimating end-of-treatment outcomes at trial level, or posttreatment outcomes such as 12- or 18-month postrandomization relapse-free survival.

We excluded studies that reported only diagnostic performance of the biomarkers. We also excluded studies reporting biomarker changes during treatment without providing a clinical correlation.

Please note that these are the groups as defined in the protocol. After data extraction, we noted that several studies used composite end points such as treatment outcome (success or failure) and death, thus potentially falling in categories β and γ. To avoid double counting their biomarker entries, we classified these as a fourth, β/γ group.

Data Screening and Extraction

The search was run in October 2020. All publications captured by the search were uploaded in Rayyan Qcri [15]. The tool helps

to detect duplicates with artificial intelligence, and allows 2 reviewers to work in real-time for reference screening. After eliminating the duplicates, 2 independent reviewers (J. E. P. and R. A.) reviewed the titles and abstracts of all the citations for eligibility criteria. In instances of discrepancies or when a field was unclear, the reviewers conferred to reach consensus through a third independent reviewer (A. S. M.). Data extraction was performed independently by J. E. P. and R. A. Additional studies that were identified through reverse citation of the selected ones were also added.

Training was organized before data extraction. The data extraction sheet was prepared in Microsoft Excel and was piloted with the first 10 studies for further adjustment. We included basic manuscript information (main author, year of publication, and journal of publication), data about study design, the biomarkers included, the outcomes predicted, and their measurement time, test characteristics (including turnaround time and commercial availability), performance parameters as reported in the text, figures, or supplementary material, and overall characteristics of the study cohort. All time intervals were transformed into weeks as time measure to allow for comparison.

Data Quality and Risk of Bias Assessment

We adapted a panel of 6 questions for the 4 domains in the QUADAS-2 tool (available in the Supplementary Material). It assesses the risk of bias, the applicability of the results, and the quality of the report [16]. However, during the development of the article a decision was made to exclude from the summary the last domain, flow and timing. The rationale behind the decision is that this domain is not really relevant for our review question because timing between index and reference test, and prospective recruitment were inclusion criteria during the selection phase, hence all studies appeared with a low risk of bias.

Data Synthesis

We considered a biomarker entry (either a lone parameter or a biosignature) as an individual measurement from the host or the pathogen, at a specific time point or time interval, correlated to a specific outcome and time. Biosignatures can combine measurements from the same or different categories. For example, a biosignature can include different combinations of several host genes, or different combinations of host genes with clinical features or mycobacterial markers. We classified the performance of each entry as poor, promising, or good. To the best of our knowledge, when we designed the systematic review and until the preparation of this article, there were no target product profiles (TPP) specific for tuberculosis treatment monitoring or for the development of surrogate markers for clinical trials. Therefore, for sensitivity, specificity, and area under the receiver-operator curve (AUC), the thresholds were 75% for promising and 90% for good biomarkers, borrowing the thresholds from the World Health Organization TTP for markers of progression from latent to active tuberculosis [17]. For correlation metrics (ie, ρ or R^2), the thresholds were 0.8 for promising and 0.95 for good biomarkers following previous publications [7, 18]. Some publications use a lower threshold for defining a strong correlation (ie, $\rho = 0.6$). In adaptive trials, insufficient correlation will lead to inadequate adaptations of trial operations, and affect trial power and type I error [19, 20]. We did not consider those biomarkers reported as associated with an outcome by means of odds ratio or hazard ratio, as these statistics are not considered suitable to guide clinical decision making [21]. Finally, we indicate which of these biomarkers are based on commercially available tests and therefore ready to be implemented in regulatory clinical trials. The data was synthesized and analyzed using R software version 4.0.1.

RESULTS

Results of the Literature Search

After deduplication, 1961 publications were identified. Figure 1 illustrates the selection process. A total of 245 publications underwent full-text review, from which 16 articles were identified by reverse citation. Most excluded studies did not correlate the results of the biomarkers with clinical outcomes or did not correlate these results with microbiological information at any time point during follow-up.

Figure 2 shows the global evaluation of the 86 studies included in the review. Supplementary Table 1 shows the evaluation per QUADAS-2 domain (including flow and timing results) for each study, and Supplementary Figure 1 summarizes the studies reporting at least 1 promising biomarker entry as a traffic light plot. Overall, only 15% had a high risk of bias, mainly due to concerns about index test and participant selection. Most studies did not report blinding for results interpretation. Most of the data originated from cohort studies enrolling successive participants and randomized clinical trials. Almost all the studies used cultures for the assessment of early and final clinical outcomes. Finally, the summary plots do not show the flow and timing domain for the reasons stated in the "Methods" section: as part of the selection criteria, all studies making comparisons between 2 groups used contemporaneous participants, and the timing between measurements and treatment effect were indeed the objectives of the review.

Summary of the Study Characteristics and Biomarker Entries

Study characteristics and biomarker entries are summarized in Table 1. The 86 studies reported data from 22 864 adult participants, from cohort studies (59, 68.60%) and clinical trials (15, 17.44%). Most were in a single country (72, 85.73%). Only 5 studies included extrapulmonary tuberculosis alone or combined with pulmonary tuberculosis, 8 multidrug resistant

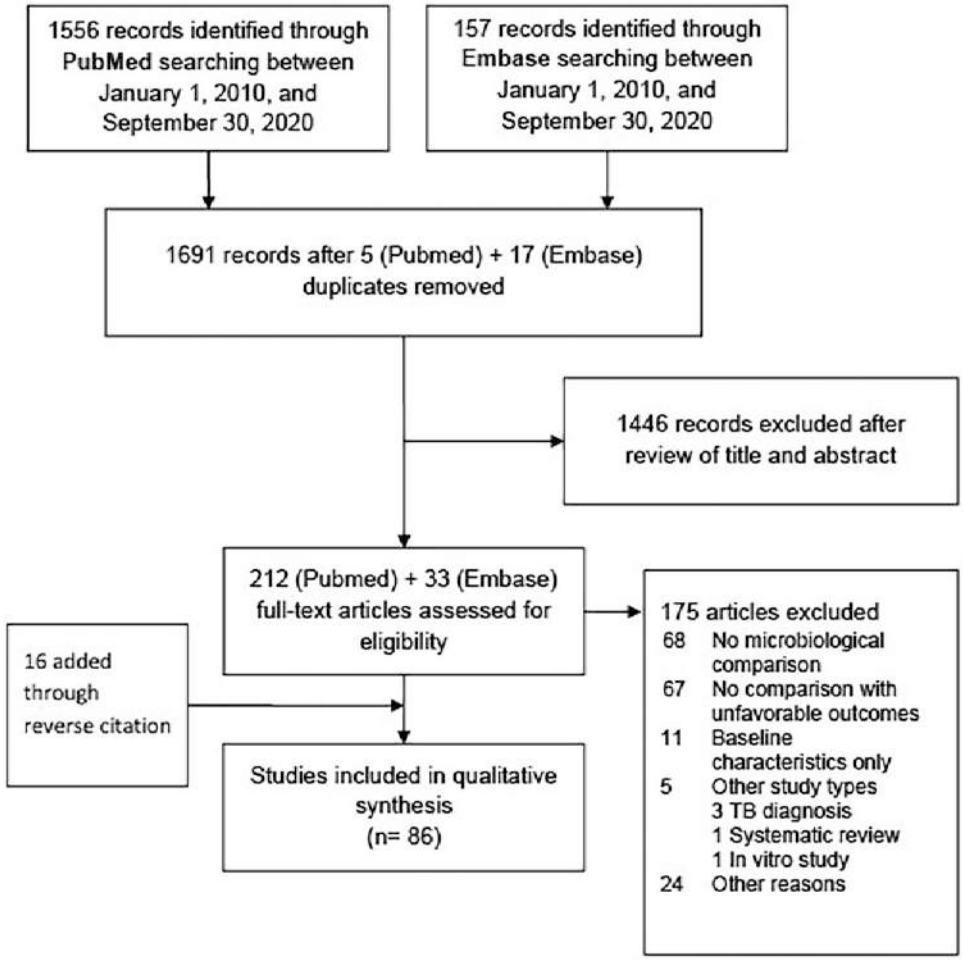

Figure 1. Identification, screening, and eligibility of publications.

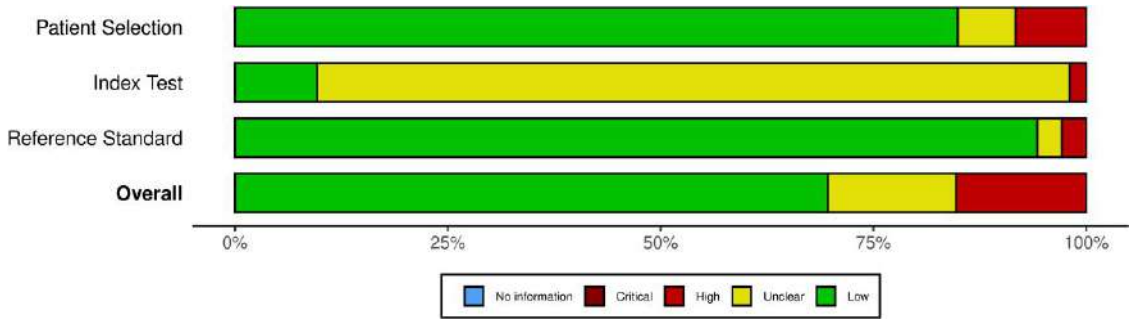

Figure 2. Risk of bias assessment using the QUADAS-2 tool.

(MDR) tuberculosis, and 12 a combination of MDR and drug-susceptible tuberculosis.

Overall, 1356 single-biomarker entries were identified. Among all biomarker entries, 633 were reported by authors as having some statistical association with the clinical outcome. However, only 318 of 1356 biomarker entries (23.45%) [22] were reported with the performance parameters of interest. Among the remaining, 130 entries (29.10% of 448) were reported with odds ratios, and the rest did not provide an association statistic. Supplementary Tables 2 and 3 display the 66 host and 37 pathogen biomarker entries (103 of 1356, 7.56%) that fulfilled the criteria for promising performance. Among the

Table 1. Summary of Studies Included

#	Study	Design	No.	α	β	γ	β/γ	Total BM
1.	Meyvisch (2018)	Clinical trial	132	0	4	0	0	4
2.	Thompson (2017)	Cohort	128	1	0	1	16	18
3.	Choi (2016)	Cohort	75	0	4	0	0	4
4.	Honeyborne (2015)	Cohort	38	0	0	0	1	1
5.	Agrawal (2018a)	Cohort	12	0	1	0	0	1
6.	Chen (2014)	Clinical trial	28	0	0	0	17	17
7.	Riou (2012)	Cohort	42	0	33	0	0	33
8.	Lima de Moraes (2014)	Cohort	55	0	12	0	0	12
9.	Kurbatova (2015)	Meta-analysis	2043	0	0	0	2	2
10.	Hoel (2019)	Cohort	14	0	6	0	0	6
11.	Riou (2014)	Cohort	18	0	11	0	0	11
12.	Jiang (2018)	Cohort	73	0	3	0	1	4
13.	Lee (2015)	Cohort	167	12	12	0	0	24
14.	Sabiiti (2020)	Mixed	178	1	4	0	0	5
15.	Waitt (2015)	Cohort	22	0	22	0	0	22
16.	Imperial (2018)	Meta-analysis	2854	0	0	0	2	2
17.	Ahmed (2018)	Clinical trial	39	0	10	0	0	10
18.	Denkinger (2013)	Cohort	150	0	4	0	0	4
19.	Trauner (2017)	Cohort	12	0	2	0	0	2
20.	Martínez (2012)	Cohort	20	0	1	0	0	1
21.	Cliff (2016)	Cohort	58	0	4	14	0	18
22.	Chen (2010)	Cohort	64	0	28	1	0	29
23.	Nyendak (2013)	Cohort	50	0	8	0	0	8
24.	Singh (2012)	Cohort	50	1	6	6	0	13
25.	Sivro (2017)	Clinical trial	195	0	0	53	0	53
26.	Hesseling (2010)	Cohort	231	0	7	8	0	15
27.	Wells (2015)	Clinical trial	464	0	0	0	1	1
28.	Montenegro (2014)	Cohort	56	0	3	0	0	3
29.	Svensson (2019)	Mixed	105	3	0	0	0	3
30.	Chen (2011)	Cohort	51	3	12	0	12	27
31.	Chung (2015)	Cohort	19	0	3	0	0	3
32.	Mukamolova (2009)	Cohort	8	1	0	0	0	1
33.	Ugarte-Gil (2013)	Cohort	68	0	17	0	0	17
34.	Kaneko (2015)	Cohort	49	0	0	3	0	3
35.	Chung (2016)	Cohort	83	0	13	0	5	18
36.	Sloan (2015)	Cohort	133	2	0	0	8	10
37.	Lu (2017)	Cohort	139	0	0	0	5	5
38.	Dupnik (2018)	Case-control	51	21	0	0	0	21
39.	Kumar (2017)	Cohort	88	6	12	0	0	18
40.	Goodridge (2012)	Clinical trial	38	0	5	0	5	10
41.	Kriel (2015)	Cohort	295	3	3	0	2	8
42.	Mthiyane (2015)	Cohort	65	1	4	0	1	6
43.	Alene (2018)	Cohort	429	0	0	0	10	10
44.	Ferrian (2017)	Cohort	41	1	29	0	0	30
45.	Baumann (2013)	Case-control	21	0	17	0	0	17
46.	Drain (2015)	Cohort	90	0	3	5	0	8
47.	Miranda (2017)	Cohort	139	2	4	0	0	6
48.	Phillips (2013)	Meta-analysis	6974	0	0	0	3	3
49.	Ronacher (2019)	Cohort	105	0	0	26	24	50
50.	Hatsuda (2015)	Cohort	626	0	4	2	0	6
51.	Kedia (2018)	Clinical trial	289	0	51	0	0	51
52.	Friedrich (2013)	Clinical trial	221	24	29	0	3	56
53.	Shenai (2016)	NA	96	0	23	0	19	42
54.	Phillips (2016)	Clinical trial	1540	0	0	4	14	18
55.	Li (2010)	Clinical trial	40	8	10	6	0	24
56.	Honeyborne (2016)	Cohort	15	6	3	0	0	9

Table 1. Continued

#	Study	Design	No.	α	β	γ	β/γ	Total BM
57.	Agrawal (2018b)	Cohort	12	6	14	0	0	20
58.	Honeyborne (2011)	Cohort	112	1	0	1	0	2
59.	Honeyborne (2014)	Cohort	94	4	1	0	0	5
60.	Penn-Nicholson (2020)	Cohort	85	0	2	0	4	6
61.	Qian (2016)	Retrospective	13	0	2	2	0	4
62.	Sigal (2017)	Clinical trial	319	7	39	0	0	46
63.	Nikolayevskyy (2020)	Cohort	89	0	122	0	131	253
64.	Xia (2020)	Clinical trial	34	22	0	0	0	22
65.	Kawasaki (2019)	Cohort	265	5	2	0	0	7
66.	Luies (2017)	Cohort	41	0	0	0	4	4
67.	Sariko (2017)	Case-control	70	0	0	0	1	1
68.	Adekambi (2015)	Case-control	10	0	12	0	0	12
69.	Lima de Moraes (2015)	Cohort	34	0	8	0	0	8
70.	Malherbe (2016)	Cohort	96	0	0	1	9	10
71.	Andrade (2013)	Cohort	38	2	1	0	0	3
72.	Chedid (2020)	Cohort	144	0	0	0	8	8
73.	Darboe (2019)	Clinical trial	129	2	5	3	0	10
74.	García-Basteiro (2017)	Cohort	115	0	0	0	1	1
75.	Hai (2019)	Cohort	56	12	0	0	1	13
76.	Jayakumar (2015)	Clinical trial	39	0	22	0	0	22
77.	Matsushita (2015)	Cohort	370	0	0	0	2	2
78.	Mesquita (2016)	Cohort	66	8	10	0	0	18
79.	Osawa (2020)	Cohort	252	4	4	0	7	15
80.	Rabna (2012)	Cohort	278	0	2	0	17	19
81.	Ralph (2013)	Clinical trial	123	0	3	0	0	3
82.	Rockwood (2017)	Cohort	130	0	0	0	6	6
83.	Sivakumaran (2020)	Cohort	90	0	0	6	15	21
84.	Warsinske (2018)	Case-control	98	0	0	0	3	3
85.	Wood (2012)	Cohort	199	1	0	0	2	3
86.	Yan (2018)	Cohort	377	0	4	0	2	6
	Overall		22 864	170	680	142	364	1356

Biomarker groups according to the predicted outcome: α, bacterial load estimates at baseline or during treatment; β, early and late clinical end points; γ, end or posttreatment and trial-level end points; and β/γ, combines characteristics of the 2 categories (ie, combines death and late clinical end points). A list of complete references is available in the supplementary material.

Abbreviations: BM, biomarker entries; No., number of participants.

promising biomarkers, 4 of 29 studies used data from randomized clinical trials [7, 23–25]. In addition, 40 of 66 (54.22%) of the host and 34 of 37 (91.89%) of the pathogen promising biomarkers were based on available commercial tests.

For the estimation of the bacterial load (group α), only 2 host biomarker entries, both based on *N*1, *N*12-diacetylspermine levels in urine, showed a promising performance (AUC > 80%), but a poor correlation ($R^2 < 40$) [24]. Among pathogen biomarkers, 1 was based on the measurement of resuscitation factors in culture supernatant [26], 2 on lipoarabinomannan (LAM) levels in sputum [27], and 2 on bacterial mRNA in sputum [25].

For early clinical response or treatment success (group β), there were 20 promising host biomarker entries, most of them measuring either cytokines or human RNA expression, all in blood or plasma samples. For pathogen biomarkers, the Xpert cycling threshold (Ct) and minimum cycling threshold (Ctmin) in sputum showed a varying performance for the prediction of concurrent sputum culture conversion (weeks 1 to 24) [28, 29]. Finally, rRNA detection in sputum paralleled the sputum liquid culture conversion from baseline to end of treatment in 1 study [30].

In the posttreatment or trial-level prediction (group γ) there were 4 host entries. Interestingly, the changes in 2 RNA signatures during the first 4–8 weeks of treatment were correlated with posttreatment risk of failure [31].

Forty host and 12 pathogen entries were biomarkers for end-of-treatment failure or death. These were classified as belonging to a fourth group, β/γ [32]. Host promising predictors of treatment failure after a few weeks of treatment and upon treatment completion included lung imaging scores using computed tomography (CT) and positron-emission tomography with CT (PET-CT) [23], and blood RNA signatures [31–35]. Regarding the pathogen biomarkers, 2 entries from a patient-level meta-analysis corresponded to the sputum culture in solid media at weeks 8 or 12 as predictors of treatment failure or

Table 2. List of Promising Host Biomarkers With at Least 1 Good Performance Parameter

Biomaker			Group				Performance						
Category	Biomarker	Predicted Outcome	α	β	γ	No.	Se	Sp	AUC	R^2	ρ	Commercial	Ref
Antibody	Tpx IgG + ESAT-6 IgA, week 0	Culture (liquid) conversion 2 mo	0	1	0	21	84.6	100	0	0	0	No	Baumann (2013)
Cytokine	CXCL9, week 0–8	Early treatment response	0	1	0	83	83	96	0	0	0	Yes	Chung (2016)
	CXCL9/CXCL10, week 0–8	Early treatment response	0	1	0	83	88	96	0	0	0	Yes	Chung (2016)
	CXCL9/CXCL11, week 0–8	Early treatment response	0	1	0	83	88	92	0	0	0	Yes	Chung (2016)
	CXCL10/CXCL11, week 0–8	Early treatment response	0	1	0	83	83	92	0	0	0	Yes	Chung (2016)
	CXCL9/CXCL10/CXCL11(any), week 0–8	Early treatment response	0	1	0	83	93	92	0	0	0	Yes	Chung (2016)
Image	CT manual-2, week 8	Treatment failure	0	1	1	75	0	0	92	0	0	Yes	Chen (2014)
	CT manual-2, week 24	Treatment failure	0	1	1	75	0	0	93	0	0	Yes	Chen (2014)
	CT automated, week 24	Treatment failure	0	1	1	75	0	0	97	0	0	Yes	Chen (2014)
	CT automated, week 8 (harder)	Treatment failure	0	1	1	75	79	75	91	0	0	Yes	Chen (2014)
	CT automated, week 24 (harder)	Treatment failure	0	1	1	75	96	75	98	0	0	Yes	Chen (2014)
	CT automated, week 24 (harder)	Treatment failure	0	1	1	75	0	0	99	0	0	Yes	Chen (2014)
	PET/CT manual, week 8	Treatment failure	0	1	1	75	96	75	86	0	0	No	Chen (2014)
Protein	HO-1, week 20	Treatment failure	0	1	1	130	90	67.4	81	0	0	Yes	Rockwood (2017)
RNA	RESPONSE5 + Xpert, week 0	Treatment failure	0	1	1	128	83	99	0	0	0	No	Thompson (2017)
	RESPONSE5 + Xpert, week 4	Treatment failure	0	1	1	128	83	97	0	0	0	No	Thompson (2017)
	RESPONSE5 + Xpert, week 24	Treatment failure	0	1	1	128	100	100	0	0	0	No	Thompson (2017)
	Clinical 5 + Transcriptional 3, week 0	Culture (liquid) conversion, week 8	0	1	0	90	0	0	97	0	0	No	Sivakumaran (2020)
	Clinical 3 + transcriptional 2, week 8	Treatment failure	0	1	1	67	0	0	97	0	0	No	Sivakumaran (2020)
	Clinical 1 + transcriptional 1, week 0	Treatment failure	0	1	1	84	0	0	92	0	0	No	Sivakumaran (2020)
	ACS COR, week 24	Treatment failure	0	1	1	128	0	0	95	0	0	No	Thompson (2017)
	DISEASE, week 24	Treatment failure	0	1	1	128	0	0	99	0	0	No	Thompson (2017)
	8-gene relapse signature, week 0–8	Relapse at 2 y	0	0	1	20	0	0	93.6	0	0	No	Cliff (2016)
	10-gene nonrelapse signature, week 0–4	Relapse at 2 y	0	0	1	20	0	0	93.1	0	0	No	Cliff (2016)
	RISK-6, week 24	Treatment failure	0	1	1	85	0	0	95.2	0	0	No	Penn-Nicholson (2020)
	3-gene tuberculosis score 1	Treatment failure	0	1	1	98	0	0	93	0	0	No	Warsinske (2018)

Promising performance: sensitivity and specificity >75%, or AUC >75%, or correlation statistics >0.8. Biomarker groups: α, bacterial load estimates; β, early treatment outcomes and/or end-of-treatment clinical outcomes at a patient level; γ, end-of-treatment outcomes at trial level and posttreatment outcomes such as relapse-free survival. A list of complete references is available in the supplementary material.

Abbreviations: AUC, area under the receiver-operator curve; CT, computed tomography; IgA, immunoglobulin A; PET, positron-emission tomography; Se, sensitivity; Sp, specificity.

relapse [7]. The other 10 entries were Xpert Ct and RNA signatures in sputum for assessing end of treatment cure [28, 29].

Among the 103 promising biomarker entries, 41 (26 host, 15 pathogen) fulfilled at least 1 criterion of good performance (Table 2 and Table 3). The changes, rather than static measurements of the interferon-γ (IFN-γ) pathway chemokines (CXCL9, CXCL10, or CXCL11) between baseline and week 8 were good predictors of early treatment response defined as a combination of negative sputum smear and culture with clinical and radiological improvement by week 8 of treatment [36]. Both manual and automated CT scan scores at the end of the intensive phase or at end of treatment were good predictors of treatment failure at end of treatment [23]. Ten different blood RNA signatures, 3 combined with clinical characteristics and 1 with Xpert results at end of treatment, were good predictors of treatment failure [29, 32].

None fulfilled the criterion for promising or good biomarkers based on correlation statistics (Table 2 and Table 3). The closest was a signature composed of 50 metabolites in tuberculosis patient urine analyzed by mass spectrometry [37]. When measured at baseline or at week 4 after treatment onset (R^2, 0.93 and 0.91, respectively) the metabolomic signature could differentiate between successful and unsuccessful treatment.

In our search, only 2 studies used clinical trial data to explore treatment-level surrogacy. In the meta-analysis by Phillips et al, a positive sputum culture at week 8 or 12 was strongly associated with treatment failure or relapse (R^2, 0.86 and 0.81, respectively) at trial level [7]. In the study by Li et al, the

Table 3. List of Promising Pathogen Biomarkers With at Least 1 Good Performance Parameter

Biomaker			Group				Performance						
Subcategory of Biomaker	Biomarker	Predicted Outcome	α	β	γ	No.	Se	Sp	AUC	R^2	ρ	Commercial	Study
DNA	Ctmin, week 2	Culture (mixed) and sputum conversion, week 2	0	1	0	40	98.8	100	0	0	0	Yes	Friedrich (2013)
	Ctmin, week 26	Culture (mixed) and sputum conversion, week 26	0	1	0	40	100	76.6	0	0	0	Yes	Friedrich (2013)
	Ctmin	Smear conversion	0	1	0	40	100	75.3	0	0	0	Yes	Friedrich (2013)
	Ctmin, week 26	Culture (solid) conversion, week 26	0	1	1	40	100	77.1	0	0	0	Yes	Friedrich (2013)
	Ctmin, week 26	Culture (liquid) conversion, week 26	0	1	1	40	100	76.8	0	0	0	Yes	Friedrich (2013)
	Ct, week 1	Culture (liquid) conversion, week 1	0	1	0	96	0	0	96.7	0	0	Yes	Shenai (2016)
	Ct, week 4	Culture (liquid) conversion, week 4	0	1	0	96	94.1	79.7	91.2	0	0	Yes	Shenai (2016)
	Ct, week 24	Culture (liquid) conversion, week 24	0	1	0	96	87.9	75	90.2	0	0	Yes	Shenai (2016)
	Ct, week 0–1	Culture (liquid) conversion, week 1	0	1	0	96	0	0	97.5	0	0	Yes	Shenai (2016)
	Ctmin, week 1	Culture (liquid) conversion, week 1	0	1	0	96	0	0	96.6	0	0	Yes	Shenai (2016)
	Ctmin, week 4	Culture (liquid) conversion, week 4	0	1	0	96	0	0	91.6	0	0	Yes	Shenai (2016)
	Ct, week 24	Treatment failure, week 24	0	1	1	96	75	88.9	90.2	0	0	Yes	Shenai (2016)
	Ct week 0+ Ct, week 0–4	Culture (liquid) conversion, week 4	0	1	0	96	94.1	79.7	0	0	0	Yes	Shenai (2016)
	Ct week 0 + Ctmin, week 4	Culture (liquid) conversion, week 4	0	1	0	96	94.1	81.1	0	0	0	Yes	Shenai (2016)
RNA	SAT-TB, week 0–24	Culture (liquid) status, week 0–24	0	1	1	377	86.92	100	0	0	0	Yes	Yan (2018)

Promising performance: sensitivity and specificity >75%, or AUC >75%, or correlation statistics >0.8. Biomarker groups: α, bacterial load estimates; β, early treatment outcomes and/or end-of-treatment clinical outcomes at a patient level; and γ, end-of-treatment outcomes at trial level and posttreatment outcomes such as relapse-free survival. A list of complete references is available in the supplementary material.

Abbreviations: AUC, area under the receiver-operator curve; Ct, cycle threshold; Se, sensitivity; Sp, specificity.

Mycobacterium tuberculosis icl gene RNA was a good correlate with sputum culture bacterial load, but it did not detect differences in bactericidal activity at the trial level (between isoniazid and fluoroquinolones) [25].

DISCUSSION

Only 167 (12.42%) of the biomarker entries reached a promising performance, and among them, only 25 25 (1.86%) reached a good performance, of all 1356 biomarker entries. No single biomarker entry fulfilled the criteria for good performance considering correlation metrics. These results show the considerable extent of research in tuberculosis biomarker discovery, but also highlight design shortcomings in the clinical evaluation of tuberculosis biomarkers. We found a common misleading tendency to interpret a significant change in a biomarker during treatment as a potential surrogate marker of a good clinical outcome at the individual level, and a lack of evaluation of trial-level surrogacy.

Despite an overall fair quality of the studies, about two-thirds of the entries included were not reported with performance metrics. Among those that did, one-third relied on statistical association measures such as the odds ratio, which are inadequate to reflect prediction of an outcome. Some studies reported both association and prediction metrics and allow us to grasp the difference between them. For example, in Lee et al, the cytokine RANTES measured at baseline and MMP-8 measured at week 8 had odds ratios of 8.29 (95% confidence interval [CI], 3.303–22.6] and 3.36 [95% CI, 1.25–10.5) regarding sputum culture status in liquid media at week 8, but the AUC for the biomarkers were 72.5% and 63.2%, respectively [38]. Similarly, PET activity in the lungs, and bacterial DNA by Xpert and mRNA in bronchoalveolar lavage, or serum biomarkers, were not able to predict sputum culture conversion at month 2, cure, or mortality [39, 40].

Surrogate validation needs data from several randomized trials testing different treatments. In conventional trials, this is limited by different clinical definitions of safety and efficacy, and the use of different assays in different populations. A platform trial can overcome these problems [41], providing the basis for a ranking of biomarkers using network analysis [42–44]. It is noteworthy that in the group α (bacterial load estimates) and group β (time to or rate of sputum culture conversion) no biomarkers based on molecular tests in sputum were selected with at least a promising performance, except for the estimation of baseline bacterial load. This includes mycobacterial DNA such as Xpert Ct or Ct min, and ribosomal RNA such as the MBLA assays [25, 28, 45–49]. Therefore, these tests could be used to estimate isolated bacterial loads but fail in capturing the effect of treatments on it, thus making suboptimal surrogates. As this is a matter of some debate in the design of contemporaneous clinical trials, we included a more in-depth discussion in the Supplementary Material. To the best of our knowledge, this is the first systematic review of biomarkers for tuberculosis clinical trials. A recent review by Zimmer focused on biomarkers for tuberculosis treatment monitoring; this describes commercial biomarkers that show significant changes during tuberculosis treatment but does not inquire into the predictive correlation between biomarkers and the different clinical outcomes [50]. However, as discussed above, mere statistical significance of changes during treatment does not make a promising surrogate.

Tuberculosis biomarker development is a blooming research field in constant change. As new evidence becomes available, the results of this review can become outdated. To illustrate this, we repeated the search limiting it to the period between 1 January 2020 and 28 February 2023, finding 535 new references. From these, 24 fulfilled our inclusion criteria, of which 6 were already included in the systematic review. We could not perform a full update of the systematic review but summarized some relevant findings from this period in Supplementary Tables 4 and 5. However, the studies showed similar results to those included in the systematic review. Notable additions to the pipeline of biomarkers included sputum LAM to estimate bacterial burden at baseline ($\rho = 0.77$), although its performance started to decline when measuring dynamic changes over a 2-week period ($\rho = 0.63$) [51]. Similar to the systematic review results, mRNA seemed a better predictor than rRNA of culture status and bacterial burden a few weeks into antitubercular treatment [52, 53]. At the time of writing of this article, there is no single test that can be used as biomarker for EBA, early clinical efficacy, and late treatment outcomes. Our aim was to provide a comprehensive overview of the end points used in tuberculosis clinical trials from phase 2 to 3. Therefore, our review cannot provide an in-depth analysis as if we were focusing on a single outcome. Another important limitation is that we collected only published data, and we did not attempt contacting authors for additional data, nor extracted raw result to calculate missing correlation statistics. However, the intention behind our work was to bring attention upon the importance of how biomarkers are reported. Trial design teams and regulators should read biomarker reports critically. In addition, standardized reporting could help researchers developing biomarkers and ensure that their work does not go unnoticed.

CONCLUSION

New molecular tests based on *M. tuberculosis* RNA are promising surrogates for bacterial load and early clinical end points, and blood cytokine and host RNA signatures are promising host biomarkers for early and late clinical end points, allowing for the inclusion of extrapulmonary tuberculosis in clinical trials. A combination of categories, rather than a single

biomarker, may be needed to obtain the maximum efficiency of adaptive platform trials in tuberculosis. However, the conjunction between discovery, clinical utility demonstration, and regulatory compliance remains a major challenge for biomarker development in tuberculosis. Better coordinated efforts, for example with a biomarker-reporting consensus statement, are needed.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. This article reflects only the author's view. The Joint Undertaking is not responsible for any use that may be made of the information it contains.

Data availability. The dataset used to generate the results is available upon request to the corresponding author and approval from the post-EUPEARL management team.

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Potential conflicts of interest. R. A. is employed by Quiagen. J. G. M. is employed by Janssen Pharmaceuticals. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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10.4. Appendix IV: Community engagement in tuberculosis research: the EU-Patient-Centric Clinical Trial Platforms (EU-PEARL) experience

Saluzzo F, Espinosa-Pereiro J, Dressler S, Tàvora Dos Santos Filho E, Seidel S, Gonzalez Moreno J, Heinrich N, Sanchez-Montalva A, Cirillo DM. Community engagement in tuberculosis research: the EU-Patient-cEntric clinicAl tRial pLatforms (EU-PEARL) experience. Int J Infect Dis. 2023 May;130 Suppl 1:S20-S24. doi: 10.1016/j.ijid.2023.03.008. Epub 2023 Mar 9.

This article summarises the experience of our group in Community Engagement activities within the EU-PEARL project. It adds to chapters IV and VI as it describes a useful approach for capacity building and integration of community representatives in clinical trials since their conception and design. Community representatives are key to ensuring that tuberculosis (TB) research is relevant, culturally sensitive, and appropriate. For all trials (new drugs or treatment regimens, diagnostics, or vaccines) this can result in improvement of recruitment, retention, and adherence to the trial schedule. Developing strategies to address these needs can contribute to preventing tokenism and in- crease the acceptability and appropriateness of TB research.

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Community engagement in tuberculosis research: the EU-Patient-cEntric clinicAl tRial pLatforms (EU-PEARL) experience

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ABSTRACT

Objectives: Community representatives are key to ensuring that tuberculosis (TB) research is relevant, culturally sensitive, and appropriate. For all trials (new drugs or treatment regimens, diagnostics, or vaccines) this can result in improvement of recruitment, retention, and adherence to the trial schedule. The early engagement of the community will, later in time, support the process of implementation of new policies designed for successful products. We aim at developing a structured protocol for the early engagement of TB community representatives developed in the context of the EU-Patient-cEntric clinicAl tRial pLatforms (EU-PEARL) project.

Design: The EU-PEARL Innovative Medicine Initiative 2 (IMI2) project TB work package has developed a community engagement (CE) framework to ensure fair and efficient participation of the community in the design and implementation of TB clinical platform trials.

Results: We showed that early engagement of the EU-PEARL community advisory board highly contributes to the process of development of a community-acceptable Master Protocol Trial and Intervention-Specific Appendixes. We identified capacity building and training as major gaps in advancing CE in the TB field.

Conclusion: Developing strategies to address these needs can contribute to preventing tokenism and increase the acceptability and appropriateness of TB research.

Introduction

It is an exciting moment to conduct research in the tuberculosis (TB) field. More than 15 new compounds are currently in different phases of development [1] and new strategies, such as adaptive trial designs and trustworthy longitudinal biomarkers, are now needed to prioritize the different possible regimens [2].

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Even if we have witnessed incredible advances in TB clinical trial design and implementation in the last couple of years, the need remains to provide people-centered interventions, that are not only effective but also acceptable and endorsed by the affected population and appropriate for the local context [3]. The World Health Organization has defined community engagement (CE) as "a cost-effective intervention to improve health service coverage and deliver accessible and people-centered integrated care" [4].

Community representatives can be strong advocates for TB research, contributing to ensuring that research procedures are relevant, culturally sensitive, and appropriate. This can result in improvement of recruitment, retention, and adherence to the trial schedule, finally affecting trial quality and helping to build a supportive environment for research. Moreover, the in-depth understanding of local cultures, priorities, and languages provided by community representatives can be pivotal in helping to close the gaps in the TB care cascade [3].

According to several sources and studies [4,5], CE promotes local ownership of the study(ies) and provides valuable input throughout study planning and implementation finally favoring more effective and transparent dissemination of the main results. Moreover, CE creates a trustworthy environment in which the researchers can communicate with affected communities, finally creating a path of critical information exchange between community members, civil society organizations, and academic and governmental institutions. Ultimately, CE can strongly contribute to the implementation of new technologies and/or treatment strategies.

If tokenism is to be avoided and true partnership between communities and researchers is sought, a structured approach to CE is needed. Here, we report the steps taken and lessons learned in the engagement and active participation of community representatives during all activities of EU-Patient-cEntric clinicAl tRial pLatforms (EU-PEARL) TB work package, including the elaboration of the TB Master Protocol (MP) for platform trials.

Material and methods

The EU-PEARL project

The EU-PEARL (EU Patient-cEntric clinicAl tRial pLatforms) innovative medicine initiative 2 (IMI2) project aims to develop new methods, tools, and frameworks to create a novel enabling infrastructure for conducting people-centered platform trials through an integrated system where pharmaceutical companies, non-profit product developers, academic institutions, and healthcare providers work together, in the context of an integrated research platform (IRP). The intent is to shape future clinical trials that will be more people-friendly by design and people-focused by outcomes. The project is designing platform studies for four different diseases: major depressive disorder (MDD), TB, non-alcoholic steatohepatitis (NASH), and neurofibromatosis (NF). Moreover, general frameworks and tools for designing and executing IRPs in any other disease area are under development.

In this context, the EU-PEARL IMI2 project TB work package has developed a CE framework to ensure fair and efficient participation of the community in the design and implementation of TB clinical platform trials.

Platform trials allow for testing simultaneously and seamlessly several interventions under an overarching MP. The development of MP templates (MPTs) and intervention-specific appendixes (ISA) templates can allow trialists and consortia who are developing their own MPs to have a base to start from with guidance on how to populate the different sections and subsections of the protocol.

The EU-PEARL TB work package has developed MPT and ISA templates in collaboration with the established EU-PEARL community advisory board (CAB). The CE process has been carefully planned and structured throughout this process to allow the community representatives full participation in the different stages of the process.

EU-PEARL community advisory board

At the beginning of the project, a general EU-PEARL patient and community advisory group has been created including 1-2 representatives from each disease area (MDD, NASH, NF, and TB), in accordance with the available resources. To select the two persons who would participate in the EU-PEARL patient and community advisory group and become the main members of the EU-PEARL TB CAB, TB work package members have performed a search of relevant community advisers with personal experience of TB contacting different CABs and organizations. Thus, candidates from Latin America, Africa, and Asia, as well as some candidates from Europe have been identified. Because of the nature of the project, extended previous experience in TB and the research environment as well as good knowledge of the English language have been pivotal parameters in the selection of the candidates. Moreover, as EU-PEARL is an EU-focused project, but most TB burden lies outside the EU, one person from an EU country and one from a TB high-burden country have finally been selected. Compensation has been foreseen for the participants to warrant significant participation.

The specific training that the TB CAB has received to support the TB work package activities is described in detail in the results section. General training on the main aims and methods of EU-PEARL has been provided at the initiation of the activities.

Results

CAB participation algorithm

In Figure 1 we summarized the established process of CE for a TB IRP.

Immediately after the project began the EU-PEARL CAB has been established and a training program has been developed by the ad hoc created Expert Advisory Group (EAG) including academic and industry representatives with experience in CE in TB clinical trials. The EAG had the role of providing training and guidance to the community representative regarding the main characteristics of platform trials in general and the EU-PEARL TB design, in particular. The training consisted of a first introductory online meeting to present the aim and challenges of TB platform trials and to introduce the CAB to the trial design. Then the CAB has been introduced to the MP task force and included first in the review activities of the EU-PEARL General MP and the TB MPT.

CAB and tuberculosis master protocol template

Clearly structured CAB roles and objectives for MP review were agreed upon using the relevant MP sections as guidance to facilitate community inputs in trial design and procedures (Table 1).

CAB structure and role description have been included in the relevant MPT section and defined as follows:

CAB: An independent group composed of community representatives (from community-based organizations, non-profit civil society organizations, representatives of networks of people affected by the study diseases, or similar entities). The group members should be interested in providing input in the overall clinical trial process and in reviewing scientific works. This can happen by means of capacity building and empowering community representatives in treatment and research literacy activities. The CAB is expected to include people affected by TB or by vulnerable groups such

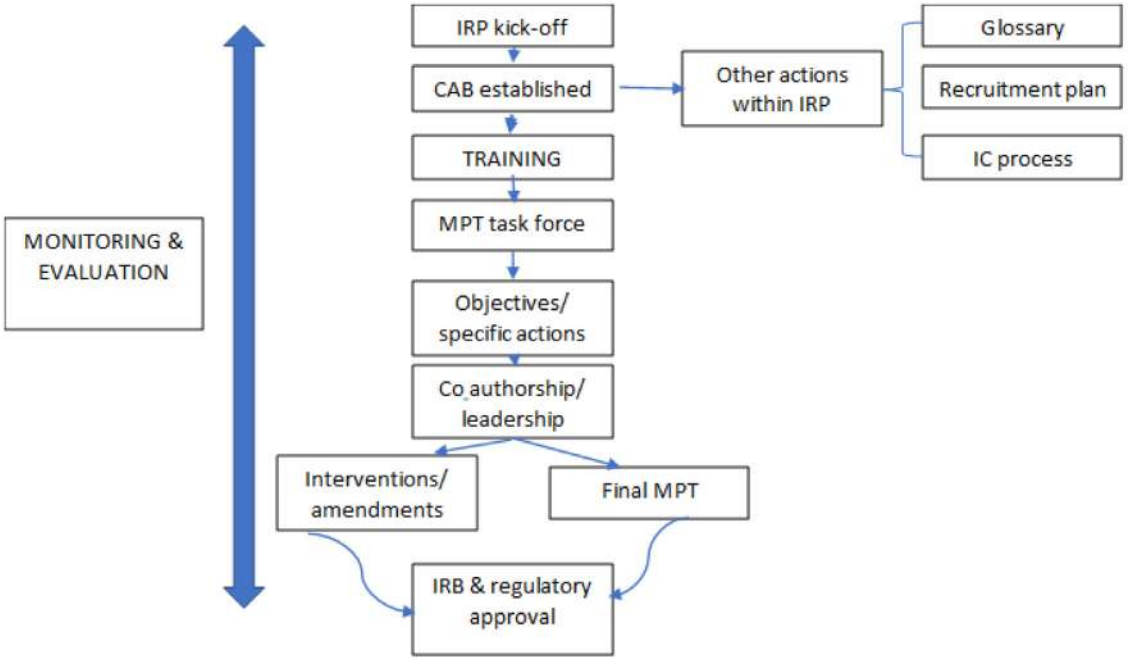

Figure 1. CAB participation algorithm. CAB, community advisory board; IC, informed consent; IRB, institutional review board; IRP, integrated research platform; MPT, master protocol template.

Table 1
CAB Role in MP drafting.

Section	CAB Role and Contribution
Introduction	Provide evaluation of overall risk/benefit balance.
Endpoints & outcomes	Review and select relevant endpoints, including the identification of relevant participant reported outcome measures and participant-reported experience measures.
Design	To be informed of the rationale for the design and ensure the adequacy, consistency, and proportionality of the study procedure to the objective of the study and participants' expectations.
Population	To be involved in the evaluation of inclusion/exclusion criteria and in the development of outreach strategies. Provide feedback on other Challenges and Barriers to Eligibility and Enrollment Outside of Inclusion and Exclusion Criteria. Assessment of the feasibility and capability of the inclusion of vulnerable population.
Study intervention(s) and concomitant therapy:	To contribute to the subsections "Continued access to intervention", "Concomitant therapies/ disallowed medications" and "Rescue strategies after treatment failure or arm is stopped for futility", providing feedback on the adequacy and proportionality of the intervention to the condition to treat.
Study assessments and procedures	To help select and adapt the tools used to assess personal outcomes such as quality of life and Health Economics. To appraise study procedures and adequacy with the objective of the study. To adapt procedures to the cultural background of the community. To help define possible compensation of study participants.
Statistical considerations	To provide feedback regarding the relevant analysis sets of population and subgroups analysis.
Supporting documentation and operational considerations	To be actively involved in the definition of the informed consent process and to provide feedback on amendments and re-consent. Ensure that all documents are available in plain language and accessible format. Assess complements to the written information for participants (e.g., video, graphics) to favor inclusion of illiterate population.

as people living with HIV/AIDS (PLWHA), and represent the main regions included in the study. The CAB should be appointed before the approval of the protocol, and be involved in the concept and development, as well as in the review of its instruments. The Sponsor must provide CAB members training in platform trials in general, and this study in particular. The MPT should support the CAB's regular follow-up of the study, by means of meetings and training and encourage strong and regular interaction with the study team.

Moreover, besides discussing the entire concept of the protocol, consultation with CAB has been planned for each of the areas where the community input is more relevant, providing input on the overall risk/benefit balance and support in the review and selecting relevant endpoints, including participant reported outcomes measures and participant reported experience measures. Furthermore, important feedback has been gathered regarding the study design as the CAB ensured the adequacy and proportionality of the study procedure to the objective of the study and participants' expectations.

The CAB also supported the finalization of the inclusion/exclusion criteria and the development of outreach strategies. Community representatives have underlined that beyond eligibility criteria, other several challenges and barriers that may prevent people from enrolling in clinical trials should be taken into consideration and that CE may provide strategies to overcome these challenges/barriers.

Among those,

1. Geographical: Isolated or nomad communities with a high TB burden are often difficult to reach and retain in treatment. Innovative solutions like technology-assisted follow-up applications may be of use to include this population in clinical trials.
2. Financial: Trials can require a substantial time commitment for treatment or travel. Absence from work and transportation expenses can represent barriers to enrollment. According to the United States Food and Drug Administration (FDA) guidance, reimbursement for travel expenses to and from the clinical trial site and associated costs such as airfare, parking, food, and

lodging do not raise issues of undue influence and are generally considered acceptable practice.
3. Transportation: In some areas, even with a budget dedicated to coping with participants' expenses, travel may be a relevant challenge because of a lack of infrastructure or public transport both for participants traveling to trial sites and the logistics for implementing the trial.
4. Lack of available caregivers for those who are dependent on the trial candidate. In most settings, women are more affected than men in this sense. For example, the lack of childcare facilities may discourage young women from enrolling in a trial or adhering to the trial schedule of visits.
5. Cultural: Some communities pay a historical mistrust toward 'western' medicine in general, and clinical trials in particular.
6. Literacy: TB disproportionally affects communities with significant levels of illiterate individuals. As trials grow in complexity, the informed consent process becomes harder to be truly informed for participants.

To overcome these barriers and challenges mitigation strategies and trial dimensions should be discussed with CAB in order to allow efficient participant enrollment and finally improve the trial recruitment rate.

Finally, the CAB provided input on the informed consent process and was included in the revision of the instruments (e.g., questionnaires and other data collection instruments) to be used in the trial to establish a clear and understandable language for any participant.

CAB and language

The active participation of community experts in MP drafting and in the other transversal activities of the consortium led to identifying language as a transversal element in which community advice is of utmost importance.

In particular, several documents refer to 'patient representative', 'patient engagement,' or 'patient representation'. The precise definition of 'patient' remains unclear, and there exists no generally accepted definition of 'patient'.

In the context of TB, the description of someone as 'patient' may be misleading: is a patient someone with an undiagnosed TB infection? Is this person a patient when latent TB infection is diagnosed? Is a person with active TB but not in medical care a patient? Is a person who denies treatment a patient? Is a person who had a successful TB therapy a patient because there might be a risk of relapse? Is a person cured a patient?

What do all these people have in common? They have a health issue in common: the risk of being harmed by TB if not even the risk of being killed by TB.

In this understanding, these people belong to an informal group or 'community'. TB organizations and programs acknowledge this fact by referring to "TB people", "People with TB" or "TB survivors" (used by several organizations, including TB Alliance, World Health Organization, and others). This terminology is now widely accepted by the TB research community even if the term 'patient' can be still found, mostly in medical publications.

Clinical trials and studies in the TB field are often multifaceted: while testing a new (experimental) combination treatment, surrogate markers for treatment success or diagnostic interventions may be evaluated in the same study. The term "patient" is therefore not only rather unclear but does not even adequately describe study participants.

Another term that sounds rather inadequate is "subject". There is a strong trend to avoid any term that may objectify the individual participating in a trial and "Trial participant" represents the most correct term to replace it. Consequently, the TB CAB had proposed to replace the term "patient representative" with the term "community representative" throughout all EU-PEARL documents.

The EU-PEARL CAB aimed to ensure that the research conducted is relevant to the diverse community needs and advocated for the well-being of study participants. This resulted in the elaboration of a glossary of terms to use in trial documents that take the community's views into consideration.

The EU-PEARL glossary for tuberculosis platform trials

Community: No generally accepted definition exists; here used as a description of formal associations, groups, and networks or informal groups of people with common (health) issues, or most affected or vulnerable to a disease (TB in this case).

Community engagement: Involvement of communities in research activities with the aim to improve their health; CE happens through a process of consultation and feedback with community representatives.

Community representatives: Persons who represent the interest and/or needs of groups of people with common issues. They have to ensure that the research is relevant to community needs and does not answer scientific questions only. Community representatives should advocate for the well-being of study participants, ensure appropriate informed consent, and secure access to research benefits.

Patient engagement: Used if a patient participates in a CE process; it does not confound with recruitment.

Patient representatives: (i) Sometimes used for community representative; (ii) in the legal context used to describe someone empowered to make or communicate health care decisions on behalf of an incompetent patient; (iii) someone who will assist patients in obtaining services.

Study participant: Any person who is participating in a study. The use of this term should not be limited to participation in clinical trials which investigate new treatments but should also be used in the context of studies that investigate diagnostic or other (e.g., preventive) interventions.

Study subject: The technical term for study participant. The term is currently not endorsed by the TB research community.

Discussion

Structured CE and participation have proved pivotal in the activities performed by our project. Nonetheless, guidance is lacking on this topic in the TB field and the most recent guideline available on Good Participatory Practice in TB drug trials have been published more than 10 years ago [3].

In our experience, with the EU-PEARL CAB, capacity building and training were identified as crucial steps to favor CE. In other studies, the gap in knowledge between the researchers and the CAB members has been identified as a major issue in forming and implementing CABs, leading to miscommunication [6]. Therefore, when planning new TB drugs clinical trials, it emerges the need to include the community from the very first stages of the process. This ensures that the communication pathway between researchers and CAB is well established before the design and implementation of the trial start and the objectives of the CAB participation are clearly stated. Moreover, during this time it is possible to thoroughly train the CAB regarding the characteristics of TB trials. Nevertheless, we are currently not aware of any available CAB training packages on TB clinical trials. The development and continuous update of such documents may facilitate CAB creation and implementation in this renewed TB research scenario and should not be limited to new drug trials. Furthermore, considering the possible different levels of literacy and experience in the TB research field of the CAB members, it is important to evaluate the

development of individualized or ad hoc training, according to the participants' needs.

The inclusion of the community in vaccine studies and new diagnostic trials is pivotal to ensure that innovative, acceptable, and reliable tests and preventive tools are developed, as too often we forget that TB care starts from diagnosis and prevention. In the context of other diseases, the inclusion of the community in preclinical laboratory research has been proved to be an occasion of mutual learning, finally leading to establishing new collaborations and improving the research efficiency [7,8]

Language is also a transversal topic that has been widely discussed in the last years in TB research, prevention, and care. The first language guide for TB communications "Every word counts" [9] has represented an important landmark and now a new updated document "Words Matter" has recently been published [10]. The EU-PEARL CAB in this context advocated once again to replace the word "patient" with "trial participant" and to use a more appropriate and empowering language, producing a positive change, and contributing to ending stigmatization. A small TB trials glossary has been then developed in the context of the project to promote this language shift and once again sensitize trialists on this topic. The glossary has been limited to the main terms used in trial documents and does not include other terms, such as TB survivors, that are accepted and used by the TB research community.

The main limitation of our study is represented by the inadequate geographical, gender, and cultural representativeness of our CAB. The need to ensure that all these elements are properly represented in the study CAB can contribute to improving the obtained outcomes, finding strategies, and defining research tools and frameworks that are acceptable and appropriate for all the trial participants. This can finally ensure that the trials performed are really fair and accessible to the entire TB community.

Conclusion

Engaging the community in TB research, especially in TB clinical trials, from the very beginning is crucial to favor the trial-related process and to design and implement people-centered studies, finally ensuring that the trial design and implementation are relevant to the community needs and promote the well-being of the participants.

The developed EU-PEARL TB CE algorithm and the plan for CE in the MP drafting represent an example of how to proactively involve the community in the conception of TB platform trials from design to implementation.

Nonetheless, these tools represent only a first step, and a long unexplored road is still ahead of us. Considering the rapidly evolving of this renovated TB research scenario, guidance on how to perform effective and meaningful CE is deeply needed not only in clinical trials for new drugs but also to study rapid, reliable, and user-friendly diagnostic tests and innovative and effective TB vaccines.

Declaration of competing interest

The authors have no competing interests to declare.

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Authors' Contributions

The authors confirm contribution to the paper as follows: study conception and design: Francesca Saluzzo, Juan Espinosa Pereiro, Stephanie Seidel, Daniela Maria Cirillo; contributed data: Stephan Dressler, Ezio Tavora dos Santos Filho; analysis and interpretation of results: Juan Espinosa Pereiro, Adrian Sanchez Montalva, Stephan Dressler, Francesca Saluzzo; draft manuscript preparation: Francesca Saluzzo, Juan Espinosa Pereiro; critical revision of the draft: Daniela Maria Cirillo, Adrian Sanchez Montalva, Norbert Heinrich, Jesus Gonzalez Moreno. All authors reviewed the results and approved the final version of the manuscript.

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10.5. Appendix V: Funding

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