Towards utilizing the full potential of old – *but not outdated* – drugs for tuberculosis and nontuberculous mycobacterial disease

A clinical pharmacological perspective

Ralf Stemkens

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Towards utilizing the full potential of old – *but not outdated* – drugs for tuberculosis and nontuberculous mycobacterial disease

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Promotor:
Prof. dr. R.E Aarnoutse

Copromotoren:
Dr. L.H.M. te Brake
Dr. J. van Ingen

Manuscriptcommissie:
Prof. dr. J.T. Bousema
Prof. dr. K.E. Dooley (Vanderbilt University Medical Center, Verenigde Staten)
Dr. C. Magis-Escurra

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Chapter 1

General introduction

Mycobacterial infectious diseases

Mycobacterial infectious diseases are caused by a group of bacteria called mycobacteria. The genus *Mycobacterium* (*M.*) consists of nearly 200 species (1). These include *M. tuberculosis* complex and *M. leprae*, which are the causative species of tuberculosis (TB) and leprosy, respectively. All other species are collectively labeled nontuberculous mycobacteria (NTM). Several of these NTM species are opportunistic pathogens, with *M. avium* complex (MAC) and *M. abscessus* as the most frequent causative agents of NTM disease worldwide (2). This thesis focuses on TB and NTM disease.

Tuberculosis

TB is an ancient disease that dates back thousands of years (3), but it remains a major global health problem to this day. In 2022, there were an estimated 10.6 million new TB cases and 1.3 million deaths worldwide (4). Furthermore, an estimated 410,000 people developed multidrug-resistant TB (MDR-TB; i.e. resistance against key first-line drugs isoniazid and rifampicin) or rifampicin-resistant TB (RR-TB). TB mostly affects developing countries. South-East Asia and sub-Saharan Africa have the highest TB incidence rates (figure 1). TB also still occurs in the Netherlands, but its incidence is low (710 cases in 2023) (5). The disease is spread by human-to-human transmission of *M. tuberculosis* through coughing by patients with active TB disease. The majority of individuals who are exposed to *M. tuberculosis* develop so-called latent TB infection without disease symptoms. Approximately one-fourth of the world's population is estimated to have latent TB infection. This serves as a vast 'seedbed' of potential new (active) TB cases, as about 5-15% of people with latent TB infection develop active TB over the course of their lives (6-8). Active TB predominantly manifests as pulmonary disease. However, TB can occur throughout most of the body including the spine, intestines, lymph nodes and the meninges (TB meningitis).

Treatment and outcomes of tuberculosis

The first effective antibiotic against TB (anti-TB drug), streptomycin, was discovered in 1944 (9). This was a huge leap forward in the fight against TB considering that treatment options before that time consisted mainly of rest, a healthy diet and open-air treatment in sanatoria. Although effective against TB, monotherapy with streptomycin led to resistance. Subsequently it was discovered that combination therapy with para-aminosalicylic acid could prevent the development of resistance. In the 1950s and 1960s several more effective anti-TB drugs were developed and the era of multidrug regimens was born. The addition of rifampicin to a regimen of streptomycin, isoniazid, and ethambutol proved pivotal as it enabled treatment shortening from 18 to nine months. In the 1980s, streptomycin was replaced by

pyrazinamide and this allowed for further treatment shortening to six months (9). This treatment regimen, consisting of isoniazid, rifampicin, pyrazinamide and ethambutol, has remained unchanged for nearly half a century and still is the first-line treatment for drug-susceptible TB (10, 11). These four drugs are combined in the first two months of treatment (intensive phase), followed by four months of treatment with isoniazid and rifampicin (continuation phase). The global success rates for people treated for drug-susceptible TB was 88% in 2021 (4).

TB meningitis represents only 1-4% of all TB cases, but is the most severe manifestation of TB with mortality rates of 30-50% (12-15). The antibiotic treatment regimen for TB meningitis is based on pulmonary TB, except with a longer treatment duration of 9-12 months (16). The same drugs are used in the same doses as for pulmonary TB. This is problematic considering that first-line drugs rifampicin and ethambutol have poor penetration in cerebrospinal fluid (CSF) across the blood–brain barrier (BBB) and blood–CSF barrier (BCSFB), leading to suboptimal concentrations at the site of infection (12, 17).

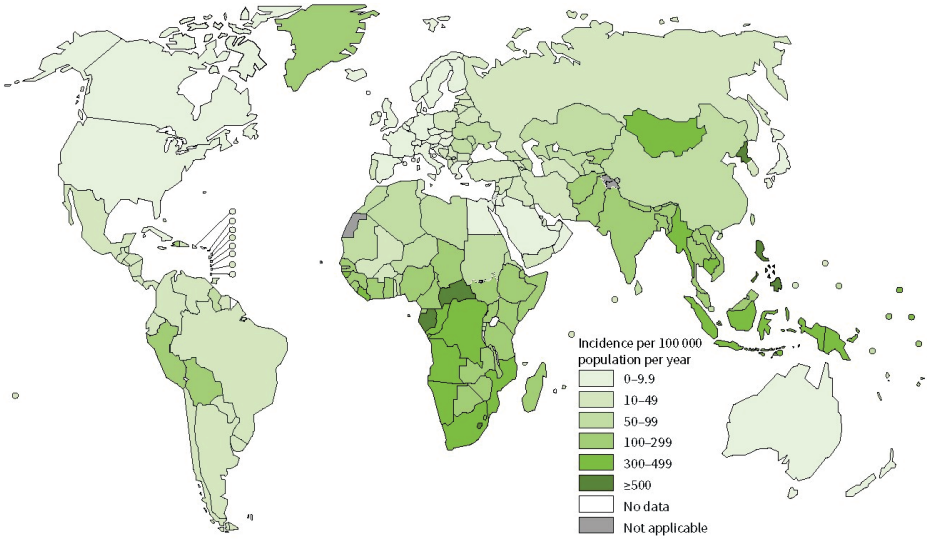

Figure 1. Global TB incidence rates in 2022
This figure is derived from the Global Tuberculosis Report 2023 from the World Health Organization (4)

In the treatment of MDR-TB significant advances have been made in recent years. First, shorter regimens (9-11 months) were found to be noninferior to longer treatment regimens (20 months) (18). Second, since the arrival of bedaquiline, treatment with (toxic) injectables (e.g. amikacin, kanamycin) is no longer necessary (19, 20). Currently,

a shorter all-oral regimen (9(-11) months) is favored over longer regimens for patients in whom resistance to fluoroquinolones has been excluded (21). This 9-month regimen includes bedaquiline (for 6 months), in combination with levofloxacin/moxifloxacin, ethionamide, ethambutol, isoniazid (high-dose), pyrazinamide and clofazimine (for 4(-6) months), followed by treatment with levofloxacin/moxifloxacin, clofazimine, ethambutol and pyrazinamide for 5 months. Thirdly, in the latest guideline from the World Health Organization (WHO) an even shorter 6-month treatment regimen is recommended consisting of bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) (22). Global MDR-TB treatment success rates have also increased over the years: from 50-63% between 2012 and 2020 (4) to 77-93%, 81% and 89% in trials for the new BPaL, BPaLC (BPaL + clofazimine) and BPaLM regimens, respectively (23-25). Despite these advances, a worrisome development is that resistance against pivotal drug bedaquiline is increasing (26, 27). Furthermore, data on the performance of these new regimens in clinical practice are currently emerging, unfortunately showing a high incidence of peripheral neuropathy and bone marrow suppression. These adverse effects were attributed to the use of linezolid, although higher initial linezolid doses (1200 mg) were used in these reports than the currently recommended daily dose of 600 mg (28, 29).

Nontuberculous mycobacterial disease

NTM are environmental bacteria, residing in soil and water, that are generally not transferred from one human to another. They are opportunistic pathogens, which means that they are capable of causing disease in patients with one or more predisposing host conditions, such as underlying lung disease or impaired immunity. NTM pulmonary disease (NTM-PD) is the most common NTM disease manifestation. It usually presents as nodular-bronchiectatic or fibro-cavitary disease, and is most frequently caused by MAC (MAC-PD). Other common causative pathogens of NTM-PD include *M. abscessus, M. kansasii* and *M. xenopi*. The less common extrapulmonary NTM disease can manifest as disseminated disease, skin and soft tissue infections, bone infections, and lymphadenitis (30, 31). Globally, NTM disease occurs less frequent than TB and the prevalence varies between geographical regions. The estimated annual prevalence rates for NTM-PD are 2.3-4.5/100,000 in the Netherlands, 6.2/100,000 in Europe (five countries), and 12.6/100,0000 in the USA (32-34). The highest estimated annual prevalence rates are reported in Asia (Japan, 24.9/100,000; South Korea, 39.6/100,000) (32, 35). Furthermore, the prevalence of NTM-PD appears to be increasing worldwide, although trend data for many parts of the world are lacking (figure 2) (36).

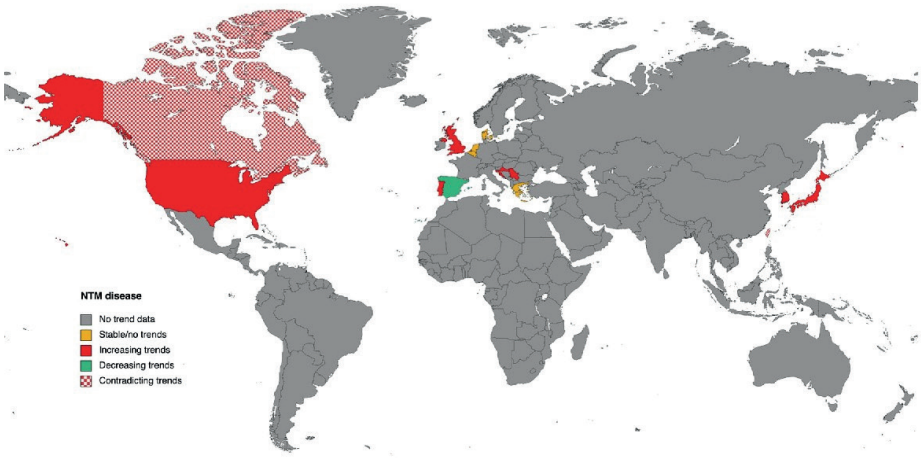

Figure 2. Global trend data on NTM disease. This figure is derived from Dahl et al. (36)

Treatment and outcomes of nontuberculous mycobacterial disease

Contrary to TB, NTM disease does not always require antibiotic treatment. Monitoring for progression of disease ('watchful waiting') may be considered for patients with NTM-PD, e.g. in case of mild symptoms, higher potential for intolerance to medication and causative pathogens that are less responsive to treatment. Initiation of treatment is recommended for patients with NTM-PD in case of cavitary disease and/or positive sputum smears, as well as the presence of symptoms with a significant decrease in quality of life (e.g. severe fatigue) (37).

Antibiotic treatment of NTM-PD requires multidrug regimens (typically ≥ three drugs) and treatment should be continued for at least 12 months after culture conversion. The recommended treatment regimen for MAC-PD consists of a macrolide antibiotic (preferably azithromycin), ethambutol and rifampicin (37). However, accumulating evidence suggests that clofazimine-based regimens have similar efficacy to rifampicin-based regimens, while avoiding the significant drug interaction potential of rifampicin (38-41). In case of fibro-cavitary MAC-PD, additional treatment with intravenous amikacin is recommended for the first two months of treatment (37). The treatment of *M. abscessus*-PD consists of an initial phase (2-3 months) and continuation phase (until at least 12 months after culture conversion). During the initial phase two oral drugs (a macrolide (preferably azithromycin), clofazimine or linezolid) are combined with 1-2 intravenous drugs (amikacin, imipenem/cilastatin or tigecycline) to a total of at least 3 *in vitro* active drugs. In the continuation phase two oral drugs are recommended in combination

with amikacin (inhaled). Despite the long and intensive treatment regimens, the success rates (defined by microbiological and/or clinical outcomes) are poor for MAC-PD (50-80%) and even worse for *M. abscessus*-PD (34-54%) (42-46). Several factors are associated with poor outcomes including cavitary disease, inadequate regimens, and macrolide resistance (47, 48). Furthermore, high recurrence rates of ~30% after successful treatment have been reported (49, 50).

Common challenges with antimycobacterial treatment of TB and NTM disease

Although TB and NTM disease are in some ways distinctly different (e.g. different causative species, patient populations, disease manifestations) there are many similarities with regard to antimycobacterial treatment and its challenges:

- Antibiotic treatment is complex, consisting of combination therapy with several antimycobacterial drugs (of which many are used for both TB and NTM disease), and requires long treatment durations
- Substantiation of drug doses is often limited
- Treatment outcomes are poor, especially in TB meningitis and NTM disease
- Drug resistance is a major problem
- Adverse effects occur frequently
- Antimycobacterial drugs (especially rifampicin) cause drug-drug interactions and are affected by them

The clinical pharmacological perspective: adequate drug exposure (concentrations) is essential

Looking at these challenges from a clinical pharmacological perspective, it is evident that they are interrelated. This interrelatedness is partly explained by suboptimal exposure to (concentrations of) antimycobacterial drugs, either systemically (plasma or serum) or at the site of action, and lack of target exposures associated with optimal outcomes. More specifically, the complex and long treatment contributes to non-adherence, resulting in suboptimal exposure of antimycobacterial drugs. Low exposures also occur due to large inter-individual variability in exposure of these drugs, contributing to treatment failure, relapse and the emergence of resistance (51-53). Insufficient penetration of drugs across the blood-brain and blood-CSF barriers and low CSF and cerebral drug exposure probably contribute to the high mortality in TB meningitis (12, 54, 55). Rifampicin causes a decrease in exposure of some antimycobacterial and many other drugs (56). On the other hand, unduly high drug exposure can contribute to the occurrence of adverse effects (57), which in turn may a negative effect on adherence to antimycobacterial drugs.

Clearly, there is a need to improve the treatment of TB and NTM disease and overcome the interrelated challenges. Although many antimycobacterial drugs have been used for decades, their optimal dose is often unknown (11). Therefore, an obvious strategy to improve treatment is to utilize the full potential of these old drugs through dose optimization. The underlying rationale is that optimized doses will in turn improve drug exposure in plasma and at the site of action (pharmacokinetics (PK)), and that this exposure could result in improved effects (i.e. more efficacy and less toxicity; pharmacodynamics (PD)) (11). According to this reasoning, the drug exposure is the intermediate link between the dose and effect (figure 3).

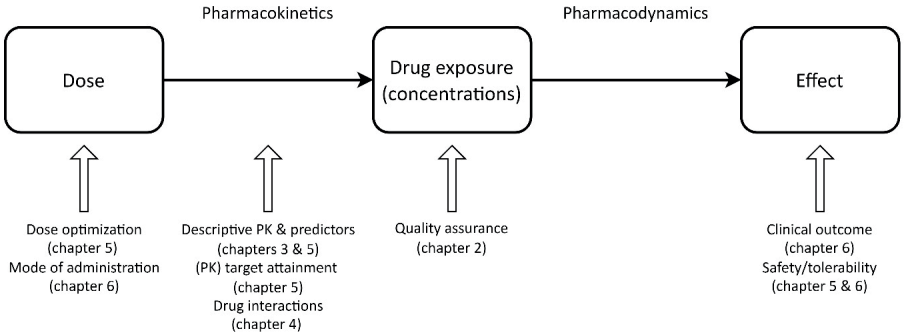

Figure 3. Relationship of pharmacokinetics and pharmacodynamics with the drug exposure (concentrations) being the intermediate link between dose and effect. The figure also shows the contents of this thesis.

Pharmacological research to optimize antimycobacterial drug doses and exposures

To optimize and substantiate antimycobacterial drug doses, clinical pharmacological researchers can use an array of methodological approaches.

In the first place, analytical methods need to be developed that can measure drug concentrations in plasma/serum, CSF or other relevant biological matrices. Intralaboratory (internal) method validation and internal quality control (QC) procedures are important steps to ensure sufficient accuracy and precision of these methods. In addition, participation in an external (interlaboratory) QC (or proficiency testing) program is an essential component of quality assurance. Validated analytical methods are key for research to optimize fixed doses for antimycobacterial drugs, but they are also applied to measure antimycobacterial drug concentrations in patient care to enable dose individualization (therapeutic drug monitoring, TDM).

When concentrations of antimycobacterial drugs can be measured, the PK of these drugs (absorption, distribution, metabolism, elimination) can be described in relevant patient populations. In such descriptive (phase I-II) PK studies, possible predictors of drug exposure (such as gender, weight, age, ethnicity, co-morbidity, drug-drug interactions and/or genetics) and attainment of PK targets are often evaluated as well. Other types of pharmacological studies aim to substantiate doses (dose-finding studies, phase I-II), identify drug-drug interactions (phase I-II), evaluate safety and efficacy and perform exposure-response analyses (phase II-III) and compare treatments (phase III). Drug-drug interaction studies are particularly relevant for the pivotal antimycobacterial drug rifampicin, which induces many metabolic enzymes and transporter proteins.

Aim and outline of this thesis

The overarching aim of this thesis is to contribute to dose and exposure optimization of antimycobacterial drugs that have been available for a long time ('old' drugs), to be able to utilize their full potential in the treatment of TB and/or NTM disease.

This thesis combines studies in patients with TB and those with NTM disease. Research questions focus on the measurement of concentrations of antimycobacterial drugs, PK and safety evaluations of available drugs with currently recommended and experimental doses, drug interactions, and the application and outcomes of alternative modes of drug administration (figure 3).

In **chapter 2** we evaluate the results, over a ten-year period, of an international interlaboratory (external) quality control program for measurement of anti-TB drug concentrations. Accurate measurement of drug concentrations is essential for dose and exposure optimization based on PK and PD principles.

In **chapter 3** we describe the PK of pyrazinamide in plasma and CSF during the initial phase of treatment in Indonesian patients with TB meningitis. We also identify predictors of exposure and assess the relationships between the dose of pyrazinamide and the resulting plasma and CSF exposures, all to help substantiate the dose of this drug in the most severe form of TB.

In **chapter 4** we assess the drug interaction potential of high-dose rifampicin in South-African patients with pulmonary TB by means of a phenotyping cocktail study. We compare the effect of high-dose rifampicin (40 mg/kg once daily) and standard-

dose rifampicin (10 mg/kg once daily) on the exposure to selective substrates (probe drugs) of five major cytochrome P450 enzymes and P-glycoprotein. Data on the drug interaction potential are relevant to the application of rifampicin in a higher dose.

In **chapter 5** we evaluate the PK and safety/tolerability of a clofazimine loading dose regimen in Dutch patients with NTM disease. The aim of our loading dose strategy is to achieve concentrations, similar to those at steady state, faster.

In **chapter 6** we describe the application and outcomes of topical antibiotic treatment, following surgery, in three patients with treatment-refractory NTM skin and soft tissue infections, aimed at reaching effective drug concentrations at the site of action.

Finally, In **chapter 7,** the findings of this thesis, and other strategies to improve treatment of TB and NTM disease, are discussed.

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Chapter 2

Ten-year results of an international external quality control program for measurement of anti-tuberculosis drug concentrations

Ralf Stemkens[1], Chaima Mouhdad[1], Eric J.F. Franssen[2,7], Daniel Touw[3], Jan-Willem Alffenaar[3,4,5,6], Lindsey te Brake[1], Marieke G.G. Sturkenboom[3,7], Rob E. Aarnoutse[1,7]

1. Department of Pharmacy, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands
2. Department of Clinical Pharmacy, OLVG Hospital, 1066 CX Amsterdam, The Netherlands
3. Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, Groningen, the Netherlands
4. Faculty of Medicine and Health, School of Pharmacy, The University of Sydney, NSW, Australia
5. The University of Sydney Institute for Infectious Diseases, Sydney, NSW, Australia
6. Westmead Hospital, Sydney, NSW, Australia
7. Drug Analysis and Toxicology section (KKGT), a section of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML), The Netherlands

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Synopsis

Objectives. Participation in an external (interlaboratory) quality control program is an essential part of quality assurance as it provides laboratories valuable insights in their analytical performance. We describe the ten-year results of an international QC program for the measurement of anti-tuberculosis (TB) drugs.

Methods. Each year, two rounds were organized in which serum (or plasma) samples, spiked with known concentrations of anti-TB drugs, were provided to participating laboratories for analysis. Reported measurements within 80-120% of weighed-in concentrations were considered accurate. Mixed model linear regression was performed to assess the effect of the measured drug, concentration level, analytical technique and performing laboratory on the absolute inaccuracy.

Results. By 2022, 31 laboratories had participated in the QC program and 13 anti-TB drugs and metabolites were included. In total 1407 measurements were reported. First-line TB drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) represented 58% of all measurements. Overall, 83.2% of 1407 measurements were accurate and the median absolute inaccuracy was 7.3% (interquartile range, 3.3-15.1%). The absolute inaccuracy was related to the measured anti-TB drug and to the performing laboratory, but not to the concentration level or to the analytical technique used. The median absolute inaccuracies of rifampicin and isoniazid were relatively high (10.2% and 10.9%, respectively).

Conclusion. The 10-year results of this external QC program illustrate the need for continuous external QC for the measurement of anti-TB drugs for research and patient care purposes, as one out of six measurements was inaccurate. Participation in the program alerts laboratories to previously undetected analytical problems.

Introduction

TB remains a major global health problem with 10.6 million new cases and 1.3 million deaths worldwide in 2022. (1) Adequate anti-TB drug concentrations in plasma or serum are crucial because they are the intermediary link between administered drug doses and the eventual drug effects of anti-TB drugs. Indeed, inadequate exposure to anti-TB drugs is a risk factor for suboptimal response and the development of resistance, whereas unduly high concentrations may cause adverse effects. (2)

For this reason studies evaluating the pharmacokinetics and concentration-effect relationships of anti-TB drugs, as well as drug interactions and/or food-drug interactions of these drugs, are essential during development of new anti-TB drugs and for dose optimization of existing drugs.

Achieving adequate anti-TB drug concentrations is also relevant in actual TB treatment. Therapeutic drug monitoring (TDM), i.e. dose individualization based on measurement and interpretation of drug concentrations, is performed in selected TB referral centres around the world. Studies describing the benefits of TDM in clinical practice have accumulated over the years, (3, 4) its availability across the world is spreading, (5) and the application of TDM has been incorporated in clinical standards for TB treatment and in guidelines. (6)

Clearly analytical methods to measure anti-TB drugs are required for both pharmacokinetic studies and TDM. Commercial (semi-)automatic immunoassays are not available for the measurement of anti-TB drugs. As a result, laboratories need to develop and internally validate their own methods. Participation in an external (interlaboratory) quality control (QC) or proficiency testing program is an essential part of quality assurance, as it provides laboratories valuable insights in their analytical performance. (7) It is also a requirement for medical laboratories. (8) In 2012, the first global external QC program for measurement of anti-TB drugs was initiated by the Drug Analysis and Toxicology section (KKGT), a part of the Dutch Foundation for Quality Assessment in Clinical Laboratories (SKML). (7)

The aim of this study was to describe the first 10 years of this program including an assessment of the performance of participating laboratories as well as an evaluation of factors that may contribute to the inaccuracy of anti-TB drug measurements.

Methods

Description of the quality control program

The design of the QC program was described previously. (7) Each year, two rounds were organized in which serum (or plasma) samples, spiked with known concentrations of anti-TB drugs, were provided to participating laboratories for analysis (only one round was organized in the first two years). Initially, the program included six anti-TB drugs: isoniazid, rifampicin, pyrazinamide, ethambutol, linezolid and moxifloxacin. Since the introduction of the program several drugs (levofloxacin, rifabutin, bedaquiline and clofazimine) and metabolites (acetylisoniazid, desacetylrifampicin and desacetylrifabutin) have been added.

All drug substances were of analytical quality with a high purity as specified in the certificate of analysis (>95%) for which was corrected during the preparation of stock solutions. Anti-TB drugs were weighed using calibrated analytical balances and dissolved in organic fluid/water using calibrated pipettes and volumetric flasks. Blank serum (first two rounds) or plasma from healthy volunteers was obtained from the Dutch Blood Bank (Sanquin, The Netherlands). QC samples were prepared by spiking blank serum or plasma with stock solutions of anti-TB drugs at concentrations ranging from the low to the high therapeutic or toxic range of the drug. Due to the instability of isoniazid in plasma, QC samples for this drug were prepared in water since 2019. Participants received instructions to mix this isoniazid-containing QC sample with blank plasma before analysis. All QC samples were dispensed in polypropylene tubes. After preparation, QC samples were freeze-dried (first two rounds only) or immediately frozen at -80°C until shipment. Stability of samples under these conditions had been assessed before (unpublished data; personal communication Rob Aarnoutse and Lindsey te Brake). Before shipment to the participants, the samples were analyzed with validated analytical methods and approved for release to the program if the deviation of the measured concentrations was ≤10% of the weighed-in concentrations. Apart from the first freeze-dried samples, all materials were shipped on dry ice to participating laboratories because of the instability of some of the drugs.

The samples of each round were accompanied by one or two clinical cases that served educational purposes. The mock cases reflected real-life TDM practice of patients with drug-susceptible or multi-drug resistant TB. Participants were asked to provide dosing advice via a multiple-choice question based on concentrations of anti-TB drugs measured in the received samples. Participants had to submit their analytical results and answers to the clinical cases before the annual deadlines.

Assessment of turnaround times was not part of the QC program. Participants received feedback within 6 weeks, which included an evaluation of the case by the program coordinator.

Statistical analyses

During the program, all laboratory measurements were standardized to percentages relative to the weighed-in concentrations, which were considered true values, by the following formula: (measured concentration/ weighed-in concentration) *100%. Measurements within 80-120% of the true concentrations were defined as accurate, based on guidelines for bioanalytical method validation and maximal allowable error specifications for the lowest level of quantification. (9, 10) This range is also commonly applied by external QC programs from the section KKGT of the SKML. The percentage inaccuracy from the true concentrations was calculated by subtracting 100% from these percentages.

Descriptive statistics were performed to assess the percentage of accurate measurements and the median absolute inaccuracy by (1) drug, (2) concentration level, (3) analytical technique and (4) performing laboratory. As to the concentration level, the weighed-in concentrations were divided in tertiles for each drug and classified as low, medium or high. This division was deemed suitable for drugs that were included in at least nine rounds (i.e. at least three weighed-in concentrations were available for each category). Mixed model linear regression was performed to test the effect of the four abovementioned factors on the absolute inaccuracy. The drug, concentration level and analytical technique were included as fixed factor and the performing laboratory as random factor. All statistical analyses were performed using SPSS version 27.0 (SPSS Inc., Chicago, IL, USA).

Results

Participating laboratories and overall results

In the 10-year period from 2012-2022 (in 2013 no rounds were organized) a total of 31 laboratories, representing 18 countries and five continents (Figure S1), participated in at least one round of the program, with a median of 13 participants per year (range 7-18). During this period 18 rounds were organized and a total of 1407 measurements were collected. Most measurements were collected for the first-line anti-TB drugs rifampicin, isoniazid, pyrazinamide and ethambutol (combined, n = 817; 58%), followed by linezolid (n = 155; 11%) and moxifloxacin (n = 149; 10.6%) (Table 1). Other compounds each represented less than 10% of all measurements. LC-MS was the most commonly used analytical technique (68.2%), followed by HPLC with other modes of detection (24.0%) and GC-MS (1.6%). The analytical technique was not reported for 87 measurements (6.2%, Table 1).

Overall, 1171 measurements (83.2%) were accurate according to the predefined range of 80-120%, whereas 105 measurements (7.5%) were below 80% and 130 measurements (9.2%) were above 120%. The median absolute inaccuracy was 7.3% (IQR, 3.3 - 15.1%). The median absolute inaccuracies per year of the program are depicted in Figure 1.

Results per drug or metabolite

Laboratories produced the highest percentage of accurate measurements for levofloxacin, moxifloxacin and acetyl-isoniazid (all above 90%) (Table 1). Isoniazid, rifampicin and desacetyl-rifampicin had the lowest percentages of accurate measurements (all below 80%), whereas 80-90% of measurements of the other drugs and metabolites yielded accurate results. In accordance with this, the order of compounds based on median absolute inaccuracies, from low to high, was: levofloxacin, linezolid, acetyl-isoniazid, desacetyl-rifabutin, ethambutol, pyrazinamide, moxifloxacin, rifabutin, clofazimine, bedaquiline, rifampicin, isoniazid and desacetyl-rifampicin (Table 1).

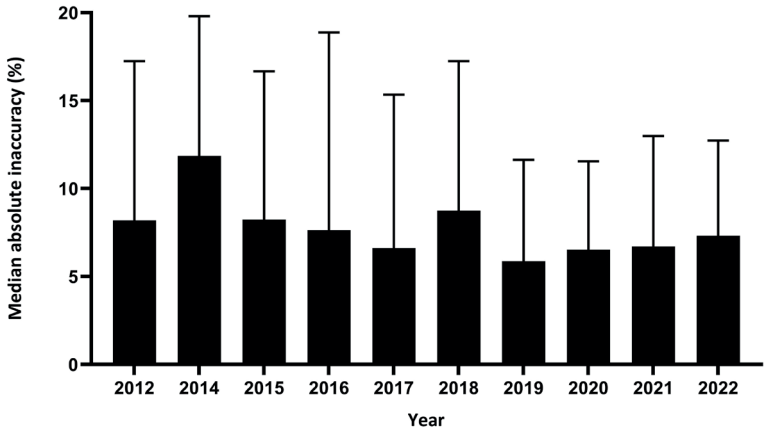

Figure 1. Median absolute inaccuracies (+ upper quartile limit) per year of the QC program. No rounds were organized in 2013.

Results per concentration level

The percentages of measurements with sufficient accuracy were 81.5%, 84.0% and 85.0% for the low, medium and high concentrations, respectively. Median absolute inaccuracies with IQR were 8.1% (3.5 - 16.2%), 6.9 (3.2 - 14.6%) and 6.7% (3.2 - 13.0%) for the low, medium and high concentrations, which means that results were comparable for the different concentration levels. The median absolute inaccuracies for each drug and concentration level are depicted in Figure 2. Bedaquiline, clofazimine and desacetyl-rifampicin were excluded from this analysis because less than nine (six, six and four, respectively) weighed-in concentrations were available.

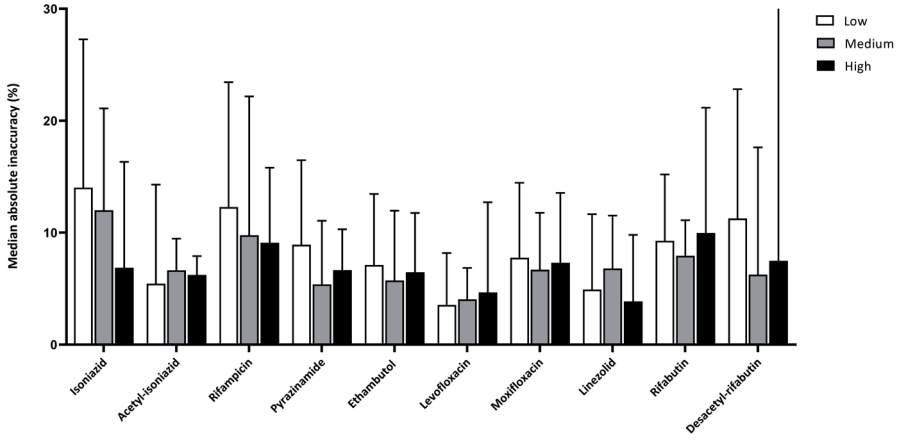

Figure 2. Median absolute inaccuracies (+ upper quartile limit) per drug and concentration level (low, medium, high). For desacetyl-rifabutin, the upper quartile limit for the high concentration level (56.0%) is not captured in the figure.

Table 1. Overall performance of the QC program for measurement of TB drugs

		Analytical method[a]		
Drug	**Analyses n (% of total)**	**LC-MS n (%)**	**HPLC n (%)**	**GC-MS n (%)**
First-line drugs and their metabolites				
Rifampicin	223 (15.8)	148 (66.4)	63 (28.3)	NA
Desacetyl-rifampicin	18 (1.3)	13 (72.2)	5 (27.8)	NA
Rifabutin	64 (4.5)	50 (78.1)	14 (21.9)	NA
Desacetyl-rifabutin	13 (0.9)	13 (100)	NA	NA
Isoniazid	209 (14.9)	134 (64.1)	61 (29.2)	NA
Acetyl-isoniazid	35 (2.5)	33 (94.3)	2 (5.7)	NA
Pyrazinamide	196 (13.9)	138 (70.4)	46 (23.5)	2 (1.0)
Ethambutol[b]	189 (13.4)	143 (75.7)	13 (6.9)	21 (11.1)
MDR-TB drugs				
Bedaquiline	33 (2.3)	9 (27.3)	10 (30.3)	NA
Levofloxacin	95 (6.8)	76 (80.0)	17 (17.9)	NA
Moxifloxacin	149 (10.6)	87 (58.4)	56 (37.6)	NA
Linezolid	155 (11.0)	110 (71.0)	41 (26.5)	NA
Clofazimine	28 (2.0)	6 (21.4)	9 (32.1)	NA
Total	1407 (100)	960 (68.2)	337 (24.0)	23 (1.6)

[a] The analytical method was not reported for 87 measurements (6.2%)

[b] One result for ethambutol could not be assessed. The weighed-in concentration was below the lower limit of quantification of the analytical method of the laboratory in question and consequently no quantitative result was reported

	Performance			
	Median absolute inaccuracy % (IQR %)	**Accurate n (%)**	**<80% n (%)**	**>120% n (%)**
	10.2 (4.2 – 20.1)	168 (75.3)	29 (13.0)	26 (11.7)
	20.8 (11.1 – 48.0)	11 (61.1)	3 (16.7)	4 (22.2)
	8.4 (4.4 – 14.7)	53 (82.8)	6 (9.4)	5 (7.8)
	6.3 (3.6 – 17.6)	11 (84.6)	1 (7.7)	1 (7.7)
	10.9 (4.9 – 21.5)	152 (72.7)	29 (13.9)	28 (13.4)
	6.2 (3.9 – 9.5)	32 (91.4)	2 (5.7)	1 (2.9)
	7.0 (3.2 – 12.3)	174 (88.8)	5 (2.6)	17 (8.7)
	6.4 (2.5 - 13.1)	164 (86.8)	7 (3.7)	17 (9.0)
	10.2 (4.6 – 15.9)	27 (81.8)	3 (9.1)	3 (9.1)
	3.8 (2.0 – 8.9)	90 (94.7)	3 (3.2)	2 (2.1)
	7.2 (3.3 – 13.2)	135 (90.6)	7 (4.7)	7 (4.7)
	5.4 (1.9 – 10.6)	130 (83.9)	8 (5.2)	17 (11.0)
	9.4 (5.2 – 17.3)	24 (85.7)	2 (7.1)	2 (7.1)
	7.3 (3.3 – 15.1)	1171 (83.2)	105 (7.5)	130 (9.2)

Results per analytical technique

The percentages of accurate measurements with LC-MS, HPLC and GC-MS were 84.8%, 78.0% and 100%, respectively. Median absolute inaccuracies were: 6.9% (3.2 - 14.2%) for LC-MS, 8.8% (3.7 – 17.6%) for HPLC and 3.9% (1.9 - 8.1%) for GC-MS.

Results per performing laboratory

There were large differences in the number of measurements per laboratory (median: 27, range: 2 - 180). The median percentage of accurate measurements per laboratory was 85.7% with a range of 0 to 100%. The laboratories with 0% (n= 1) or 100% (n= 3) accurate measurements only reported a small number of measurements (2 - 6). The median absolute inaccuracies ranged from 3.6% to 27% for the 31 participating laboratories.

Mixed model linear regression analysis

Eight outliers, with absolute inaccuracies of more than 200% (range 225% - 82567%), were excluded from this analysis because they could substantially affect the mean absolute inaccuracy. This cut-off was selected subjectively. Although the source of these inaccuracies was unknown, such large deviations were assumed to be the result of human errors rather than analytical errors. In agreement with the descriptive findings, there were significant differences in mean absolute inaccuracy between the anti-TB drugs ($p<0.001$). No significant differences in absolute inaccuracy were found between different concentration levels and analytical techniques ($p= 0.54$ and $p= 0.26$, respectively). The percentage of residual variance in the absolute inaccuracy, attributable to the performing laboratory, was 14.3%.

Clinical cases

A clinical case was included in the majority of the rounds. An overview of the topics is depicted in Table 2. These topics reflected typical indications for TDM of anti-TB drugs and highlighted specific drug characteristics relevant to each of the clinical scenarios, the limitations to TDM for anti-TB drugs, and specific items relevant to TDM. One case is depicted as an illustrative example (see Text S1).

Table 2. Overview of cases during 10 years of the program

Year plus round	Clinical scenario
2012	*No case*
2014 first case	Drug-susceptible TB: TB/HIV co-infection and slow response
2014 second case	MDR-TB: pyrazinamide and moxifloxacin, TDM of amikacin
2015.1	Drug-susceptible TB: diabetes mellitus and renal insufficiency
2015.2	Drug-susceptible TB: TB meningitis
2016.1	MDR-TB: moxifloxacin and linezolid
2016.2	Drug-susceptible TB: renal insufficiency
2017.1	Drug-susceptible TB: drug-induced liver injury
2017.2	Drug-susceptible TB: abdominal TB and malabsorption
2018.1	Drug-susceptible TB: TB meningitis
2018.2	MDR-TB: levofloxacin and linezolid
2019.1	Drug-susceptible TB: diabetes mellitus and QTc interval prolongation
2019.2	Drug-susceptible TB: osteo-articular TB
2020.1	Drug-susceptible TB: TB/HIV co-infection and drug interactions with rifabutin
2020.2	MDR-TB, moxifloxacin and linezolid and the relevance of MIC values
2021.1	Drug-susceptible TB: relapse TB and slow response
2021.2	*No case*
2022.1	Drug-susceptible TB: drug induced liver injury and renal insufficiency
2022.2	*No case*

Discussion

The number of participants in the international, external QC program for the measurement of anti-TB drugs has increased from seven laboratories in 2012 to a total of 31 laboratories that had participated (in at least one round) by 2022. (7) The program covers in total 13 different anti-TB drugs and metabolites. This expansion of the program and the overall results of ten years of the program illustrate the continuous need for a program that provides an external assessment of the performance of laboratories.

The majority of drug concentrations that were reported in the program (58%) related to the first-line anti-TB drugs isoniazid, rifampicin, pyrazinamide and ethambutol. This is not surprising, considering that the large part of new TB cases worldwide (96.1 % in 2022) falls ill with drug-susceptible TB. (1) The provision of external QC for measurement of the pivotal second-line anti-TB drugs bedaquiline, linezolid, the fluoroquinolones moxifloxacin and levofloxacin, and clofazimine is however equally important, as MDR-TB is a growing public health problem and its treatment warrants further optimization and individualization. (1) The program will likely continue to extend with other MDR-TB drugs.

Most analyses were performed with LC-MS, which reflects the increasing use of this technique in pharmacokinetic laboratories. This is probably related to the advantages of this technique, including the relative ease to develop analytical methods for simultaneous measurement of multiple compounds with different chemical structures, limited sample preparation, limited interference by endogenous compounds and other drugs, the achievement of lower limits of quantitation, and shorter run times, as compared to HPLC with other modes of detection. (11)

The actual 10-year results of this international QC program for anti-TB drugs show that 83.2% of all measurements were sufficiently accurate (within 80-120% of weighed-in concentrations). This is comparable with results from other QC programs, with 81.0%, 80.8% and 83.6% accurate measurements for programs focusing on antimicrobial, antifungal and anti-retroviral drugs, respectively. (12-14) Furthermore, the percentage of accurate measurements in the first round of our program was 82.7% and thus nearly identical to our 10-year results. (7) However, since both the participating laboratories and the included drugs have differed over the years, comparisons regarding analytical performance should be made with caution. Regardless, one out of six (16.8%) measurements is inaccurate which, although comparable with other programs, is clearly suboptimal. Accurate

measurements are essential to provide reliable results in pharmacokinetic studies that are meant to substantiate existing or new dosing regimens. In patient care, inaccurate measurements may lead to inadequate dose adjustments when applying TDM.

Our descriptive analysis and mixed model linear regression revealed that the absolute inaccuracy was related to the measured anti-TB drug and to the performing laboratory, but not to the concentration level of the drug or to the analytical technique used. We have questioned whether infrequently analyzed drugs were only measured by more experienced laboratories, possibly influencing the absolute inaccuracies of these compounds. However, follow-up explorative analysis did not reveal such an association (data not shown). Isoniazid and rifampicin had relatively high median absolute inaccuracies (10.9% and 10.2%, respectively). Isoniazid is known to be relatively unstable at room temperature. Bio-analysis of rifampicin may require an adjuvant (ascorbic acid) to prevent degradation as evidenced by many authors. (15-18) Adequate storage conditions (e.g. -80°C) and timely processing of plasma samples is recommended. (15) As to the performing laboratory, large variability between laboratories was observed in terms of percentage of accurate measurements and median absolute inaccuracies. This between-laboratory variability probably relates to differences in *intra*-laboratory (internal) QC, which includes the rigour of analytical method validation, validation of equipment, training and qualification of technicians, and other intra-laboratory QC procedures.

Our QC program has several strengths. First, it serves participating laboratories across the world and the portfolio of compounds has evolved to include many important anti-TB drugs. To our knowledge, there is only one other international QC program for the measurement of anti-TB drugs which so far includes only rifampicin and no results have yet been published. Secondly, by using human plasma that is no longer freeze-dried (requiring an additional dissolution step), the program aims to mimic clinical (or research) practice. One exception was made for isoniazid for which QC samples were prepared in water and subsequently needed to be mixed with blank plasma by participants. This was to ensure no degradation would occur during preparation, shipment, or handling of the samples. As a result of the similarities between QC samples and real samples, it can be inferred that the results of the QC program provide a measure of the intra-laboratory quality assurance in the participating laboratories. Still, it cannot be excluded that laboratories made additional efforts to achieve their results in this external QC program. This means that the results of the program could also reflect the best performance of the participants. Thirdly, the clinical cases served a unique educational purpose to this and several

other programs of the Drug Analysis and Toxicology section (KKGT) of SKML. Not only did these cases illustrate situations when TDM could be useful in patient care, they allowed participants to practice with the application of TDM.

A limitation of our QC program was that it did not include error evaluation questionnaires like certain other programs do. (13, 19) Including such forms would allow better assessment of the possible sources of measurement inaccuracies. This will be considered for future rounds.

To summarize, the 10-year results of our external QC program illustrate the need for continuous external QC for the measurement of anti-TB drugs. Participation of laboratories in the program either confirms their level of intra-laboratory quality assurance or alerts them to inaccuracies and underlying undetected problems, inciting them to optimize their methods or QC procedures. We continue to extend an open invitation to laboratories around the world to participate in this QC program.

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Supplementary data

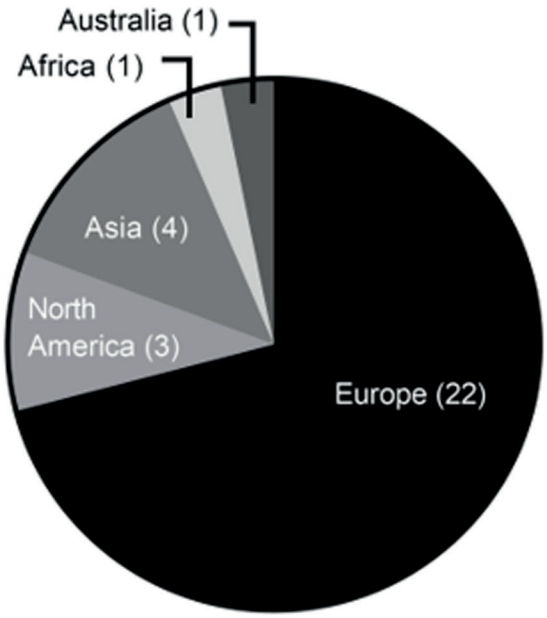

Figure S1. Global distribution of participating laboratories

Text S1

Example of a clinical case

The pharmacological laboratory was called by a concerned medical doctor from a municipal health center who treated a 24 year old male patient of 48 kg with drug-susceptible pulmonary TB and large pulmonary cavities. The patient had presented with relapse TB with a mycobacterium that was still susceptible to all first-line TB drugs. Unfortunately, the patient did not show sputum culture conversion after 3 months of his second course of directly observed treatment, he did not gain weight at all, kept on coughing and remained sick. The municipal health center had never considered TDM 'as it was not supported by randomized trials' and 'it was considered to be an expensive examination'. In case of this severely ill patient, the medical doctor was still advised to perform TDM. Samples were obtained at two and 6 hours after the dose to 'catch' peak plasma concentrations of isoniazid, rifampicin and pyrazinamide (ethambutol had been stopped). Peak concentrations were as follows: rifampicin: 5.1 mg/L, isoniazid: 2.0 mg/L, acetyl-isoniazid: 3.9 mg/L and pyrazinamide: 39.4 mg/L.

Your advice based on these results would be:

A: Keep the drug doses as they are
B: Increase the dose of isoniazid, monitor transaminases
C: Increase the dose of isoniazid and rifampicin, monitor transaminases
D: Increase the dose of isoniazid, rifampicin and pyrazinamide, monitor transaminases

Evaluation of the case

This is an example of a patient with relapse TB who does not show an adequate response to TB drugs. Based on this suboptimal response, the treatment regimen was empirically extended - the patient is still using pyrazinamide after 3 months of treatment.

There is an indication for Therapeutic Drug Monitoring (TDM) as both the relapse TB and the current suboptimal response may be due to low drug concentrations (1). Indeed, TDM proved to be of help in detecting low TB drug concentrations as a possible cause of the slow treatment response in this patient. The measured peak concentration of isoniazid (2.0 mg/L) is below the normal (reference) range for a once daily 300 mg (5 mg/kg) dose (3-6 mg/L (2)). This may be explained by the patient being a fast acetylator. The acetyl-isoniazid to isoniazid ratio is 2.0. At a sampling time of 3 h after the dose, a ratio above 1.5 is an indication for a fast acetylator (3). The peak concentration of rifampicin (5.1 mg/L) is also below the reference range of 8-24 mg/L (2). For this reason, it seems wise to increase the dose of isoniazid and rifampicin.

The pyrazinamide peak concentration (39.4 mg/L) is within the normal range (20-60 mg/L (2)) and also above a cut-off value of 35 mg/L that is used by some (4). It should be realized, however, that studies in the hollow fiber model and in animals as well as some clinical data (e.g. (5)) suggest that higher pyrazinamide exposures are more efficacious. In this case of non-response to TB drugs and a sick patient, an increase in the dose of pyrazinamide may be justified under monitoring of transaminases.

TDM was considered 'expensive' by the doctor, but it might prevent prolonged treatment in many similar patients and is cost-effective as shown by 'back of the envelope' calculations. Indeed randomized trials have not shown the value of TDM for TB drugs and many other drugs, but in clinical practice it has shown added value in selected cases.

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Chapter 3

Pharmacokinetics of pyrazinamide during the initial phase of tuberculous meningitis treatment

Stemkens R[a], Litjens CHC[a,b], Dian S[c,d], Ganiem AR[c,d], Yunivita V[d,e], van Crevel R[f], te Brake LHM[a], Ruslami R[d,e], Aarnoutse RE[a]

[a]Department of Pharmacy, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands

[b]Department of Pharmacology and Toxicology, Radboud Institute of Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands

[c]Department of Neurology, Faculty of Medicine, Universitas Padjadjaran & Hasan Sadikin Hospital, Bandung, Indonesia

[d]TB/HIV Research Centre, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

[e]Department of Biomedical Sciences, Division of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

[f]Department of Internal Medicine & Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands

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Abstract

Background. Tuberculous meningitis (TBM) is the most severe manifestation of tuberculosis (TB). Pyrazinamide (PZA) is a pivotal TB drug, but its dose has not been optimized for TBM.

Objectives. To describe the pharmacokinetics (PK) of PZA during TBM treatment; to identify predictors of PZA exposure; and to assess relationships between PZA doses, exposures in plasma and in CSF.

Methods. Plasma PZA PK data were assessed on days 2 and 10 of treatment in 52 adult TBM patients. A CSF-to-plasma concentration ratio was determined at day 2. Predictors of plasma PZA exposure, correlations between plasma and CSF exposures, and prediction of CSF concentrations based on dose, plasma $AUC_{0\text{-}24h,}$ C_{max} and plasma concentrations at the time of CSF sampling were evaluated.

Results. The geometric mean plasma PZA exposure ($AUC_{0\text{-}24h}$) and peak concentration (C_{max}) at day 2 were 709 h*mg/L and 59 mg/L, after a median dose of 33.3 mg/kg/day. The $AUC_{0\text{-}24h}$ on day 10 (523 h*mg/L) was lower than on day 2 ($p<0.001$). The dose in mg/kg and BMI correlated with $AUC_{0\text{-}24h}$ and C_{max}. The CSF concentration at 3-6 h was 42 mg/L and the CSF-to-plasma ratio was 90%. $AUC_{0\text{-}24h}$, C_{max} and CSF concentrations were highly correlated. CSF concentrations could be predicted based on dose, $AUC_{0\text{-}24h}$, C_{max} and plasma concentration at the time of CSF sampling with -4.4%, 1.5%, 1.0% and -2.7% bias and 20.6%, 12.0%, 16.9% and 9.9% imprecision.

Conclusions. Exposure to PZA decreases during the first days of TBM treatment, possibly due to the evolving inductive effect of rifampicin. PZA penetrates well in CSF. The association between PZA doses, exposures in plasma and CSF provides a rationale to study higher PZA doses for TBM.

Introduction

Tuberculosis (TB) is the leading infectious disease killer worldwide and Indonesia has the third highest TB case load in the world (1). TB meningitis (TBM) occurs in roughly 1% of TB cases but is disproportionally important as it is the most severe manifestation of TB, leading to death or permanent disability in more than 30% of those affected (2, 3).

The treatment of drug-susceptible TBM is based on the treatment regimen for pulmonary TB. An intensive phase of 2 months with rifampicin, isoniazid, pyrazinamide (PZA) and ethambutol is followed by a continuation phase of up to 10 months with rifampicin and isoniazid (2). However, rifampicin and ethambutol do not cross the blood-brain and blood-cerebrospinal fluid (CSF) barriers well (2, 4), and the dose of none of the first-line anti-TB drugs, including PZA, has been optimized for TBM. It is essential to find the optimal treatment regimen for this devastating disease.

PZA is a pivotal first-line anti-TB drug, showing little or no bactericidal activity during the first 2 days of treatment (5) and a strong sterilizing effect that enabled shortening of pulmonary TB treatment duration from 9 to 6 months (6). Most studies on TBM treatment have not compared treatment regimens with and without PZA (7), apart from one small nonrandomized study in children showing that a PZA containing regimen is more efficacious than longer treatment regimens without PZA (8). In any case, PZA is used worldwide as a standard first-line anti-TB drug in TBM (2).

In vitro, animal and clinical studies have shown that a higher dose of PZA could be more efficacious than a standard dose of 25-30 mg/kg in pulmonary TB (9-11). Using higher doses of PZA could be a potential strategy for optimizing TBM treatment as well. Considering that doses of PZA result in exposures that subsequently determine the effects of PZA, it is important to have a good understanding of the pharmacokinetics (PK) of PZA and the interrelationships of doses, exposures in plasma and in CSF in patients with TBM. Limited data is available, especially in the Indonesian population, on the PK of PZA during TBM treatment (4).

The aim of this study was to describe the pharmacokinetics of PZA during the critical initial phase of TBM treatment in Indonesian TBM patients, to identify predictors of PZA exposure in plasma and CSF, and to assess relationships between PZA doses, exposures in plasma and in CSF.

Material and methods

Study design and patient population

This was a descriptive pharmacokinetic study focusing on PZA, performed among patients with TBM who were included in a clinical trial. In this phase II clinical trial, 60 suspected TBM patients were randomized to three groups, receiving different daily doses of rifampicin (450, 900 or 1350 mg daily) in addition to standard doses of other anti-TB drugs, including 1500 mg PZA, according to Indonesian national guidelines. The study was approved by the ethical review board of the Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. Written informed consent to participate in the trial was obtained from all patients or from their relatives if the patient could not provide informed consent. In the latter case, patients who regained the capacity to consider participation were consulted, and the study was continued after obtaining informed consent. Detailed methods of the main study have been reported previously (clinicaltrials.gov NCT02169882) (3).

Pharmacokinetic sampling and bio-analysis

PK samples were taken twice, at day 2 +/-1 and at day 10 +/-1 of treatment. Blood samples were taken in fasting condition just before and at 1, 2, 4, 8 and 12 hours after administration of anti-TB medication. Additionally, one CSF sample was taken 3 to 9 hours after drug administration on day 2 +/-1. Total PZA concentrations in plasma and CSF were analysed using validated ultra performance liquid chromatography (UPLC) methods. Accuracy for pyrazinamide standard samples was between 99.8% and 104.8% for plasma and 85.6% and 95.5% for CSF depending on the concentration level. The intra- and inter-day coefficient of variation were less than 4.9% and 6.6% over the 0.2 - 60 mg/L concentration range for plasma and CSF, respectively.

Pharmacokinetic data analysis

PK parameters for PZA were assessed with non-compartmental pharmacokinetic methods using Phoenix WinNonlin version 7.0 (Certara USA Inc, Princeton, NJ), as described previously (3). CSF-to-plasma concentration ratios were determined based on PZA CSF concentrations and calculated corresponding plasma PZA concentrations at the time of CSF sampling. These PZA concentrations were calculated based on the nearest plasma concentrations before and after the time the CSF sample was taken, assuming first-order pharmacokinetics in the decay of PZA concentrations.

Statistical analysis

PK parameters were described using geometric mean and range, apart from time to peak concentration (T_{max}) and CSF-to-plasma ratio which were described as median and arithmetic mean (with range), respectively. PK parameters of PZA at days 2 and 10 were compared with a paired samples t-test on log transformed PK parameters; T_{max} values were compared using the Wilcoxon signed-ranks test. Univariate analyses were performed to assess the effect of gender, age, BMI, PZA dose (in mg/kg), HIV status, use of nasogastric tube, and rifampicin $AUC_{0\text{-}24h}$ on PZA plasma exposure measures on day 2. Predictors of PZA exposure ($p<0.1$) were to be included in a multiple linear regression analysis.

Plasma area under the concentration-versus-time curve ($AUC_{0\text{-}24h}$), peak concentration (C_{max}) and CSF concentration were correlated to each other using rank correlation. Linear regression formulas were used to predict CSF concentrations based on PZA dose in mg/kg, plasma $AUC_{0\text{-}24h,}$ plasma C_{max} and the plasma concentration calculated at the time of CSF sampling, using the jackknife method for resampling. Bias was assessed using the median percentage prediction error (MPPE) and imprecision by the median absolute percentage prediction error (MAPE), both should be <15-20% (12).

All statistical analysis were performed with SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). P values <0.05 were considered statistically significant in all analyses.

Results

Patients

PK samples for PZA on day 2 were available for 52 patients and a CSF sample was available for 51 patients. PK samples for PZA on day 10 were available for 36 patients. Half of the patients were male (51%) and the median age was 30 years. The vast majority of patients (91%) had British Medical Research Council (BMRC) disease grade 2, 7.5% was HIV positive. About 60% had a nasogastric tube on day 2 of treatment and the median PZA daily dose was 33.3 mg/kg (range: 19.2-44.5).

Pharmacokinetics of PZA

The geometric mean $AUC_{0\text{-}24h}$ and C_{max} values for PZA on day 2 were 709 h*mg/L and 59 mg/L, respectively (Table 1). The $AUC_{0\text{-}24h}$ on day 10 (523 h*mg/L) was significantly lower than the $AUC_{0\text{-}24h}$ on day 2 ($p<0.001$; Figure 1A, Table 1). CSF samples were taken on average 4.2 hours (range 3-6 hours) after administration. The mean CSF concentration at day 2 was 42 mg/L and the mean CSF-to-plasma ratio was 90% (range 55-115%). Individual CSF concentrations and calculated plasma concentrations at the time of CSF sampling are depicted in Figure 1B.

Univariate analysis showed that only PZA dose (r_s 0.530, $p<0.001$) and BMI (r_s -0.434, $p=0.001$) were significantly correlated with PZA $AUC_{0\text{-}24h}$ and C_{max} on day 2 and no multivariate analysis was performed. PZA $AUC_{0\text{-}24h}$, C_{max} and CSF concentrations were all highly correlated to each other (r_s at least 0.80, $p<0.001$). The PZA dose, plasma $AUC_{0\text{-}24h,}$ C_{max} and plasma concentration at the time of CSF sampling all predicted CSF concentrations with a MPPE of -4.4%, 1.5%, 1.0% and -2.7% and a MAPE of 20.6%, 12.0%, 16.9% and 9.9%, respectively.

Table 1. Pharmacokinetic parameters of pyrazinamide after a daily dose of 1500 mg in patients with tuberculous meningitis

Parameters	Day 2[a] (n = 52)	Day 10[a] (n = 36)	P value[b]
$AUC_{0\text{-}24h}$ (h·mg/L)	709 (355-1906)	523 (275-1047)	<0.001
C_{max} (mg/L)	59 (37-126)	57 (33-83)	0.532
T_{max} (hr)	1.2 (0.9-8.0)	1.4 (1.0-8.0)	0.725[c]
Cl/F (L/hr)	2.1 (1.3-4.3)	2.9 (1.4-5.5)	<0.001
Vd/F (L)	31 (16-54)	27 (20-36)	<0.001
$T_{1/2}$ (hr)	10.1 (5.9-24.6)	6.5 (4.2-17.8)	<0.001
CSF (mg/L) (n = 51)	42 (17-112)	n.a.	n.a.
CSF-to-plasma ratio (%) (n = 51)	90 (55-115)	n.a.	n.a.

Abbreviations: AUC_{0-24h}, area under the concentration-versus-time curve from 0 to 24 h; C_{max}, maximum plasma concentration; T_{max}, time to C_{max}; CL/F, apparent total clearance; V/F, apparent volume of distribution; $T_{1/2}$, elimination half-life; CSF, cerebrospinal fluid; n.a., not available.

[a] PK parameters are presented as geometric mean (minimum-maximum), except T_{max} and CSF-to-plasma ratio which are shown as median (minimum-maximum) and arithmetic mean (minimum-maximum), respectively.

[b] Paired t-test on log-transformed data of 36 patients for whom PK data were available on both day 2 and day 10.

[c] Wilcoxon signed-ranks test between day 2 and day 10.

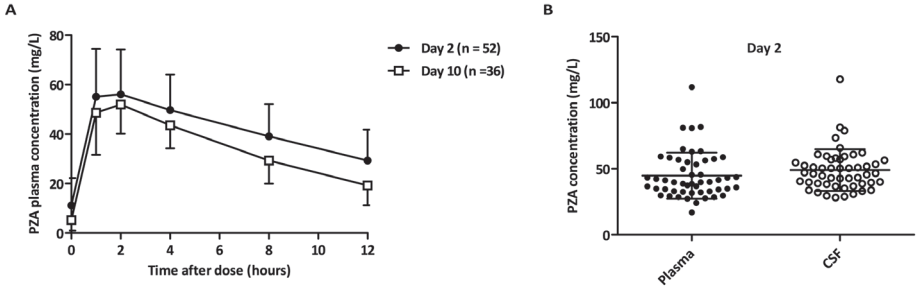

Figure 1. Pharmacokinetic profiles of pyrazinamide (PZA) after a daily dose of 1500 mg in patients with tuberculous meningitis. Figure A shows the plasma concentration versus time curves of PZA on day 2 (n=52) and day 10 (n=36) of treatment (mean ± standard deviation). Figure B depicts the individual plasma concentrations calculated at the time of CSF sampling and measured CSF concentrations (n=51) of PZA on day 2 of treatment. Bars represent the mean ± standard deviation.

Discussion

This study provides important data on the PK of PZA in adult Indonesian patients with TBM. The mean $AUC_{0\text{-}24h}$ (709 h*mg/L) and C_{max} (59 mg/L) during the first days of TBM treatment, after a median dose of 33.3 mg/kg, were relatively high compared to reference data ($AUC_{0\text{-}24h}$ 473 h*mg/L and C_{max} 44 mg/L) in an Indonesian population using the same dose, even though these reference data were recorded at steady-state (13). This is in agreement with our observation that the PZA $AUC_{0\text{-}24h}$ on day 2 was significantly higher than the $AUC_{0\text{-}24h}$ on day 10, while no significant difference in C_{max} was observed (Table 1). The higher exposure during the first days of treatment is remarkable since steady-state is not yet reached and thus an increase rather than a decrease in exposure would be expected. Something similar was observed previously in one study among pulmonary TB patients; PZA exposure was decreased after >2 weeks of treatment compared to <2 weeks (14). PZA is metabolized by both bacterial pyrazinamidase and host mediated metabolism and is also converted by xanthine oxidase (15). A likely explanation for the decrease in exposure to PZA is that rifampicin, a potent inducer of metabolic enzymes and transporters, influences the metabolism of PZA, considering that induction by rifampicin takes time and develops in 1-2 weeks time (16). The exact mechanism of this possible interaction is currently unknown.

An adequate exposure to PZA in the CSF and brain is essential for the treatment of TBM. The mean CSF concentration (42 mg/L) was within reference ranges for the C_{max} of PZA in plasma (20-60 mg/L) (17). The high mean CSF-to-plasma ratio of 90% also shows that PZA penetrates the CSF well, which is in agreement with previous studies (4).

We found a correlation between PZA doses administered and plasma exposures achieved, furthermore, we found high correlations between PZA plasma $AUC_{0\text{-}24h}$ and C_{max} on the one hand and PZA CSF concentrations on the other hand. We could predict CSF concentrations based on PZA dose and various measures of exposure to PZA in plasma. This implies that higher doses of PZA, leading to higher exposures in plasma, will in turn result in higher CSF exposures, and it should be investigated whether this results in improved outcome. It is important to take into account that this should be balanced against a possible increased risk of hepatotoxicity and neurotoxicity (18, 19).

A limitation of this study was that only one CSF sample was taken in a short time interval, whereas the CSF-to-plasma ratio could vary over the dosing interval. Ideally, a CSF-to-plasma ratio is determined for total exposure ($AUC_{0\text{-}24h}$), which requires multiple

CSF samples (20). We measured total PZA plasma concentrations, whereas unbound concentrations are ideally measured for determining CSF-to-plasma ratios (18), but this is not relevant for PZA which has only 1% protein binding in plasma (21).

In conclusion, exposure to PZA was high during the first days of TBM treatment and was significantly lower around day 10, possibly due to an interaction with rifampicin. PZA penetrates well in CSF and an association between doses, exposure in plasma and CSF concentrations was observed. This provides a rationale for follow-up research into the pharmacokinetics, efficacy and safety of higher doses of PZA for TBM and the interrelationships of these.

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Chapter 4

Drug interaction potential of high-dose rifampicin in patients with pulmonary tuberculosis

Ralf Stemkens[1], Veronique de Jager[2], Rodney Dawson[3], Andreas H. Diacon[2], Kim Narunsky[3], Sherman D. Padayachee[3], Martin J. Boeree[4], Stijn W. van Beek[1*], Angela Colbers[1], Marieke J.H. Coenen[5], Elin M. Svensson[1,6], Uwe Fuhr[7], Patrick P.J. Phillips[8], Lindsey H.M. te Brake[1], Rob E. Aarnoutse[1] on behalf of the PanACEA consortium

[1]Department of Pharmacy, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[2]TASK, Cape Town, South Africa

[3]Division of Pulmonology and Department of Medicine, University of Cape Town and University of Cape Town Lung Institute, Cape Town, South Africa

[4]Department of Pulmonary Diseases, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[5]Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, The Netherlands

[6]Department of Pharmacy, Uppsala University, Uppsala, Sweden

[7]Clinical Pharmacology, Department I of Pharmacology, Center for Pharmacology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

[8]UCSF Center for Tuberculosis, University of California San Francisco, San Francisco, CA, USA

*Present address: Stijn W. van Beek, Pharmetheus AB, Uppsala, Sweden

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Abstract

Accumulating evidence supports the use of higher doses of rifampicin for tuberculosis (TB) treatment. Rifampicin is a potent inducer of metabolic enzymes and drug transporters, resulting in clinically relevant drug interactions. To assess the drug interaction potential of higher doses of rifampicin, we compared the effect of high-dose rifampicin (40 mg/kg daily, RIF40) and standard dose rifampicin (10 mg/kg daily, RIF10) on the activities of major cytochrome P450 (CYP) enzymes and P-glycoprotein (P-gp). In this open label, single-arm, two-period, fixed-order phenotyping cocktail study, adult participants with pulmonary TB received RIF10 (days 1-15), followed by RIF40 (days 16-30). A single dose of selective substrates (probe drugs) was administered orally on days 15 and 30: caffeine (CYP1A2), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A) and digoxin (P-gp). Intensive pharmacokinetic blood sampling was performed over 24 hours after probe drug intake. Twenty-five participants completed the study. Geometric mean ratios (90% CI) of the total exposure (area under the concentration versus time curve, RIF40 versus RIF10) for each of the probe drugs were: caffeine, 105% (96-115%); tolbutamide, 80% (74-86%); omeprazole, 55% (47-65%); dextromethorphan, 77% (68-86%); midazolam, 62% (49-78%), and 117% (105-130%) for digoxin. In summary, high-dose rifampicin resulted in no additional effect on CYP1A2, mild additional induction of CYP2C9, CYP2C19, CYP2D6 and CYP3A, and marginal inhibition of P-gp. Existing recommendations on managing drug interactions with rifampicin can remain unchanged for the majority of co-administered drugs when using high-dose rifampicin.

Clinical Trials Registration. NCT04525235.

Introduction

Rifampicin is the cornerstone of treatment of drug sensitive tuberculosis (TB), a disease that remains a major global health problem with 10.6 million new cases and 1.6 million deaths worldwide in 2021 (1). The standard dose of rifampicin has been 10 mg/kg/day since its approval in 1971 by the US Food and Drug Administration (2). A growing body of evidence supports the use of higher doses of rifampicin. Doses up to 40 mg/kg in adults have yielded improved early bactericidal activity, may shorten TB treatment and are well tolerated (3-6). In addition, higher doses of rifampicin may reduce mortality in patients with TB meningitis (7, 8). Some TB referral centers already use higher doses of rifampicin in clinical practice (9). Many clinical trials in both adults and children are currently evaluating high-dose rifampicin in the treatment of active TB and latent TB infection.

Rifampicin is notorious for its capacity to cause drug interactions as it is a potent inducer of several metabolic enzymes and drug transporter proteins (10). It decreases the exposure to many co-administered drugs, including anti-retroviral, anti-diabetic and cardiovascular drugs (10). Little is known about the maximal inductive capacity of rifampicin. Some small studies suggest that maximal induction already occurs at lower rifampicin doses of 300-600 mg daily, but these studies only evaluated the effect of rifampicin on selected drugs and provided no data on higher rifampicin doses. (30-40 mg/kg) (11-13). High-dose rifampicin (35 mg/kg) does not affect the exposure to isoniazid, pyrazinamide and ethambutol, compared to standard dose rifampicin (5). In a recent study, patients receiving high-dose rifampicin (35 mg/kg) exhibited reduced plasma exposures to the antiretroviral drugs dolutegravir and efavirenz as compared to patients receiving standard dose rifampicin (14). However, the drug interaction potential of high rifampicin doses remains unknown for all other drugs.

Phenotyping for drug metabolizing enzymes or transporters, is defined as: measuring the actual *in vivo* activity in an individual. This is performed by single administration of a selective substrate for an enzyme or transporter (probe drug) and subsequent determination of a phenotyping metric, preferably the total exposure to the probe drug. Multiple probe drugs can be applied simultaneously as a 'cocktail' to assess the activity of metabolic enzymes and transporters at the same time (15, 16). We conducted a phenotyping cocktail study in participants with pulmonary TB to assess the effect of optimized, high-dose rifampicin (40 mg/kg/day; RIF40), as compared to a standard dose of 10 mg/kg/day (RIF10), on the activity of 5 major cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6

and CYP3A4/5 (CYP3A)) and P-glycoprotein (P-gp). Many commonly prescribed drugs are substrates of these enzymes (17).

Results

Study population

A total of 38 participants were screened, of whom 30 participants were enrolled in the study (see supplementary Figure S1). Five participants withdrew before study completion, four due to a positive COVID-19 test and one participant due to adverse events (headache, nausea and flushing after commencing RIF40). Twenty-five participants completed the study and were included in the demographic, safety and pharmacokinetic analyses. Patient characteristics are shown in Table 1.

Safety and adherence to treatment

A total of 35 AEs were reported for 15 participants: 24 grade 1 (n=14 participants), 5 grade 2 (n=4 participants) and 6 grade 3 (n=4 participants) AEs. Two of the ≥ grade 2 AEs were deemed definitely related to the study medication and 6 of the ≥ grade 2 AEs occurred in the same participant (see Table S7). In general, the study medication was well tolerated. Adherence to TB treatment was high and, according to pill count, ranged from 93% to 100%.

Table 1. Demographic and baseline characteristics

N	**25**
Age, years, median (range)	28 (21 – 47)
Body weight, kg, median (range)	56 (43 – 79)
Male sex, n (%)	14 (56)
Race, n (%)	
Black	12 (48)
Colored	13 (52)
Smoking status, n (%)	
Smoker	13 (52)
TB treatment duration before study, days, median (range)	84 (63 – 123)

Effect of RIF40 on the phenotyping metrics of the probe drugsThe GM $AUC_{0\text{-}24h}$ of rifampicin with RIF40 was 318.9 mg/L*h, ~8-fold higher than with RIF10. This greater than dose-proportional increase in exposure is consistent with the well-known non-linear pharmacokinetics of rifampicin (3, 5). The GM $AUC_{0\text{-}24h}$ of isoniazid was similar at both PK sampling days (Table 2). Rifampicin and isoniazid exposures corresponded well with reported data, but were somewhat higher in this study (4).

Table 2. Rifampicin and isoniazid pharmacokinetics during treatment with RIF10 (Day 15) and RIF40 (Day 30)

PK parameter	RIF10 (Day 15)	RIF40 (Day 30)
	n=25	n=25
Rifampicin $AUC_{0\text{-}24h}$ (mg/L*h)	39.4 (34.9-44.5)	318.9 (286.2-355.4)
Rifampicin C_{max} (mg/L)	7.6 (7.0-8.4)	43.2 (40.6-45.9)
Isoniazid $AUC_{0\text{-}24h}$ (mg/L*h)	10.8 (8.6-13.4)	10.9 (8.7-13.7)
Isoniazid C_{max} (mg/L)	2.5 (2.2-2.8)	2.5 (2.2-2.8)

PK parameters are depicted as geometric mean (95% CI)

The GMR estimates of all phenotyping metrics (RIF40 versus RIF10) are depicted in Figure 1 and Table 3. The GMR of the $AUC_{0\text{-}\infty}$ with 90%CI for caffeine (105%; 90%CI 96-115%) was within the standard bioequivalence range of 80-125%, whereas this range was exceeded for all other probe drugs (Table 3). The GMR (90%CI) for tolbutamide (CYP2C9), dextromethorphan (CYP2D6) and midazolam (CYP3A) were 80% (74-86%), 77% (68-86%) and 62% (49-78%), respectively. The GMR (90%CI) of the $AUC_{0\text{-}24h}$ and C_{max} for digoxin (P-gp) were 117% (105-130%) and 117% (102-135%). Due to atypical PK profiles of omeprazole, the $AUC_{0\text{-}\infty}$ could often not be calculated. The AUC until last measurable concentration ($AUC_{0\text{-}last}$) was used as an alternative. The GMR (90%CI) of this $AUC_{0\text{-}last}$ was 55% (47-65%). A detailed overview of the PK parameters of the probe drugs is depicted in Table S8. Based on these findings the additional interaction caused by high-dose rifampicin was classified as absent (CYP1A2) or mild (CYP2C9, CYP2C19, CYP2D6, CYP3A, P-gp) (18, 19).

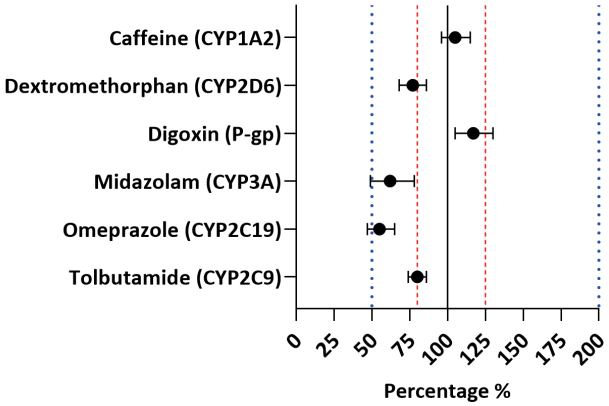

Figure 1. Geometric mean ratios (%) of AUC values (RIF40 versus RIF10) with 90%CI for all the probe drugs. The solid line is the unity line (i.e. no difference between RIF10 and RIF40). The dashed lines represent the standard bioequivalence range of 80-125% (20). GMR estimates with 90% CI entirely within this range were considered to indicate no significant additional interaction with RIF40. The dotted lines represent the range that indicates a mild additional interaction (≤ 2-fold decrease or increase) (18, 19).

Table 3. Primary phenotyping metrics of the probe drugs with RIF10 and RIF40

Primary phenotyping metrics	**RIF10 (Day 15)**	**RIF40 (Day 30)**	**GM ratio RIF40/RIF10 % (90% CI)**	**90% CI within 80 – 125%**
	Geometric mean h*ug/L (95% CI)			
	n=25			
Caffeine $AUC_{0-\infty}$ (CYP1A2)	20474 (16238-25814)	21574 (16548-28126)	**105 (96 – 115)**	Yes
Tolbutamide $AUC_{0-\infty}$ (CYP2C9)	88623 (75014-104701)	70722 (56742-88147)	**80 (74 – 86)**	No
Omeprazole AUC_{0-last} (CYP2C19)	55.7 (39.1-79.3)	30.8 (21.3-44.4)	**55 (47-65)**	No
Dextromethorphan $AUC_{0-\infty}$ (CYP2D6)	14.7[a] (9.2-23.5)	12.0[a] (7.3-19.8)	**77 (68 – 86)**	No
Midazolam $AUC_{0-\infty}$ (CYP3A)	7.1[b] (5.2-9.6)	4.4[b] (3.4-5.9)	**62 (49 – 78)**	No
Digoxin AUC_{0-24h} (P-gp)	8.6[c] (7.4-10.0)	10.1[c] (8.9-11.4)	**117 (105 – 130)**	No

[a] N=21 and N=19 with RIF10 and RIF40, respectively; AUC0-∞ of dextromethorphan could not be estimated reliably in all participants, because the percentage extrapolated was ≥ 20%. Of note, the AUC_{0-last} was calculated as an alternative metric for all participants and yielded similar results; GMR (90% CI) was 69% (61-79%).

[b] N=24; AUC0-∞ of midazolam could not be estimated in one participant due to insufficient data points for extrapolation

[c] N=24; One exclusion due to a decreased renal function (increase of ≥ 1.5 times the serum creatinine concentration between day 15 and day 30)

Subgroup analyses

Caffeine concentrations were 55-60% lower among smokers, regardless of rifampicin dose (Table S9). The GMR estimate of the $AUC_{0-\infty}$ for caffeine was similar for smokers (n=13) and nonsmokers (n=12), namely 100% (87-114%) and 112% (98-128%). Genetic evaluation (Table S10) revealed two participants with possibly reduced activity of CYP1A2 and one PM of CYP2C19. The GMR of caffeine and omeprazole without these participants were 106% (96-118%) and 56% (48-67%), which was similar to the results in the total study population.

Discussion

This study evaluated the drug interaction potential of high-dose rifampicin in a target population of TB patients, using metabolic phenotyping as an efficient instrument to screen for multiple drug interactions simultaneously. The results demonstrate that high-dose rifampicin (40 mg/kg daily) has no additional effect on the activity of CYP1A2, caused mild additional induction (≤50% reduction in exposure to probe drugs) of CYP2C9, CYP2C19, CYP2D6 and CYP3A, and slightly inhibited P-gp, compared to standard dose rifampicin (10 mg/kg daily). This infers that these effects will have no clinical implication for the majority of drugs that are co-administered with high-dose rifampicin in clinical practice and in clinical trials across the world.

The largest effects in this study were observed for midazolam (CYP3A) and omeprazole (CYP2C19). RIF40 reduced midazolam and omeprazole exposures by 38% and 45%. The additional induction of CYP3A and CYP2C19 as compared to RIF10, would formally be classified as mild, whilst the width of the 90%CIs details that a borderline moderate additional interaction (>50% reduction in exposure) cannot be excluded (18, 19). It is important to emphasize that these reductions are much smaller than the effect of standard dose rifampicin (versus no rifampicin) on exposures to midazolam and omeprazole. More specifically, it has been shown that rifampicin (600 mg daily) versus no rifampicin causes ~96% (~20-fold) and ~90% (~10-fold) reductions of midazolam and omeprazole exposures, respectively (21, 22), see Figure 2. Therefore, the observed additional induction is not expected to be clinically relevant for the majority of CYP3A and CYP2C19 substrates. It has no implications for CYP3A or CYP2C19 substrates that are already contra-indicated during treatment with RIF10 (e.g. direct oral anticoagulants). Additional induction is also not expected to have clinical implications for most co-administered drugs that require dose titration based on clinical effect, when administered with rifampicin (e.g. calcium channel blockers). For drugs with a narrow therapeutic

index, additional care may however be warranted when using RIF40. Additional induction may also be relevant for orally administered CYP3A substrates with fixed dosing recommendations (e.g. dexamethasone for TB meningitis; where examples for CYP2C19 are lacking) (23).

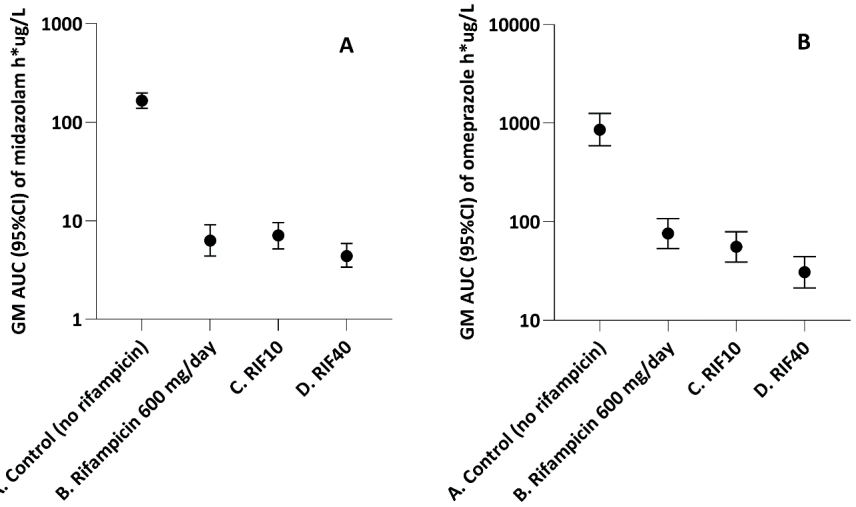

Figure 2. Comparison of midazolam and omeprazole PK with reference studies (with and without rifampicin). Figure **A** depicts GM $AUC_{0-\infty}$ values (95%CI) of midazolam after an oral dose of midazolam (15 mg) in healthy volunteers without and with rifampicin (A and B), as well as with RIF10 and RIF40 in our study (C and D). Figure **B** shows GM AUC values (95%CI) of omeprazole after an oral dose of omeprazole (20 mg) in healthy volunteers ($AUC_{0-\infty}$) without and with rifampicin (A and B), as well as with RIF10 and RIF40 in our study and our study ($AUC_{0\text{-last}}$, C and D). The AUC values from the reference studies were converted to h*ug/L for this figure (21, 22).

Of pharmacological interest, the decreased $AUC_{0-\infty}$ of midazolam with RIF40 in this study corresponded with a decreased peak plasma concentration (4.2 versus 2.6 µg/L), whereas the elimination half-life was similar with RIF10 and RIF40 (1.3h and 1.2h). This suggests that additional induction of CYP3A by RIF40 relates to first pass metabolism, corresponding with previous studies (24, 25), and is probably most relevant to orally administered drugs.

Atypical PK profiles were observed for omeprazole. One possible explanation for this observed PK behavior is its enteric-coated formulation, which may have led to irregular absorption of omeprazole (16). Consequently, results for omeprazole (CYP2C19) should be interpreted with caution. Furthermore, omeprazole is primarily metabolized by CYP2C19, but CYP3A4 also plays a role in its metabolism. The reduction in exposure with RIF40 may not be solely attributable to CYP2C19, but also to CYP3A4 (16).

RIF40 also caused a reduction of 23% in dextromethorphan $AUC_{0-\infty}$. Dextromethorphan is predominantly metabolized via CYP2D6, but CYP3A4 is also involved in its metabolism (26). Overall, the mild additional induction, whether (solely) attributable to CYP2D6 or not, is not expected to be clinically relevant.

A small decrease in tolbutamide $AUC_{0-\infty}$ (20%) was observed with RIF40. It has previously been shown that standard dose rifampicin reduces the $AUC_{0-\infty}$ of tolbutamide by 65% (27). The mild additional induction of CYP2C9 by RIF40 is not expected to be clinically relevant.

No differences in caffeine $AUC_{0-\infty}$ were observed between RIF40 and RIF10, indicating no additional induction of CYP1A2. These results were similar for smokers and non-smokers which suggests that smoking, known to cause induction of CYP1A2, did not affect the induction potential of RIF40 for this enzyme (28).

RIF40 was associated with a slightly increased digoxin AUC_{0-24h} and C_{max} (both 17%), which is not deemed clinically relevant. In contrast, previous work has shown that standard dose rifampicin decreased the digoxin AUC_{0-144h} by 30% as a result of P-gp induction (29). However, as rifampicin is also known to inhibit intestinal P-gp, this minimal increase in AUC_{0-24h} could be due to additional P-gp inhibition by RIF40, outweighing possible additional induction of P-gp (30). The extent of this inhibitory effect may be dependent on the interval between digoxin and rifampicin administration (30). The possibility of a stronger inhibitory effect during simultaneous administration cannot be excluded.

There are minimal data on the maximal inductive capacity of rifampicin. It has been long suspected that this induction capacity was maximal with standard rifampicin doses (11-13). A recent study has shown that a high dose of rifampicin (35 mg/kg daily) reduced the trough concentrations of dolutegravir (a substrate of UDP glucuronosyltransferase (UGT) 1A1 and CYP3A4) and mid-dose concentrations of efavirenz (a substrate of CYP2B6) by 43% and 37%, as compared to 10 mg/kg rifampicin (14). No loss of virological control of HIV was observed in participants with dolutegravir or efavirenz concentrations below target thresholds, but the study was not powered to assess virologic efficacy (14). Overall, these data correspond well with our results, showing some additional induction with higher doses of rifampicin.

This study had some limitations. First, the study did not include a control arm without rifampicin which would have provided a more complete picture of the effect

of increasing rifampicin doses versus no rifampicin. However, the drug interaction potential of standard dose rifampicin is well established and therefore our results can be evaluated in perspective (see Figure 2). Second, we assessed the effect of (high-dose) rifampicin in the presence of isoniazid, which is an inhibitor of CYP2C19 and CYP3A4 (31). However, we believe that this does not impact our results as the potent inductive effect of rifampicin outweighs the inhibitory effect of isoniazid and the same dose of isoniazid was used throughout the study. Finally, the effect of RIF40 on other metabolic enzymes (e.g. CYP2B6, UGT) and transporters was not evaluated in this study. It is however well-known that CYP3A4 is most susceptible to rifampicin induction and the additional effects on other enzymes are not expected to exceed those on CYP3A4 (10). Additionally, many commonly prescribed drugs are substrates of the investigated CYP enzymes and P-gp (10, 17, 32).

In conclusion, high-dose rifampicin results in no additional effect on the activity of CYP1A2, shows mild additional induction of CYP3A, CYP2C19 and, to a lesser extent, CYP2C9 and CYP2D6, whilst slightly inhibiting P-gp. These results indicate that clinicians can use existing recommendations on managing interactions with standard dose rifampicin for the majority of co-administered drugs when using high-dose rifampicin. Further interaction studies may be warranted, focussing on high-dose rifampicin and CYP3A or CYP2C19 substrates with a narrow therapeutic index.

Material and methods

Study population

Adults (18-65 years) with drug-sensitive pulmonary TB, in the continuation phase of standard TB treatment, using a standard dose of 10 mg/kg rifampicin and isoniazid, were included in this study (see Table S1 for in- and exclusion criteria). Written informed consent to participate in the trial was obtained from all participants.

Study design

This open label, single-arm, two-period, fixed-order phenotyping cocktail study was performed at two study sites, TASK Clinical Research Center and the University of Cape Town Lung Institute, in Cape Town, South Africa. The study protocol was approved by local ethical review boards and by the South African Health Products Regulatory Authority (SAHPRA) and was conducted according to Good Clinical Practice standards. This trial is registered with ClinicalTrials.gov as trial number NCT04525235.

All participants received RIF10 (i.e. continuation of the dose as per standard care) in a fixed combination with isoniazid (Rifinah®) for 15 days (period 1), followed by RIF40 (Rifinah® + additional loose rifampicin (Rifadin®) capsules) for 15 days (period 2, see Figure 3 for a schematic overview). The duration of treatment with RIF40 was selected to achieve maximal (additional) induction of CYP enzymes and P-gp after increasing the dose of rifampicin (10). During period 2, all TB drugs were administered with breakfast to prevent or alleviate possible adverse effects related to high-dose rifampicin. Dosing was weight-banded (Tables S2 and S3). Adherence to TB therapy was evaluated using treatment registration cards, self-assessment by participants and pill counts.

Participants were hospitalized during the last 3 days of each study period. At day 15 of each study period, a phenotyping cocktail consisting of 6 probe drugs was administered. Participants received a single oral dose of caffeine (150 mg), tolbutamide (125 mg), omeprazole (20 mg), dextromethorphan (30 mg), midazolam (15 mg) and digoxin (0.5 mg), to assess the activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A and P-gp, respectively. Table S4 shows details on the composition of the phenotyping cocktail. This cocktail was based on the well-established Cologne cocktail (15, 16, 33). The probe drugs were administered on an empty stomach following overnight fasting (of at least 8h duration) and participants remained fasted until 4 hours following probe drug administration. Rifampicin and isoniazid were administered 4 hours after administration of the probe drugs, with a standardized meal. This time interval was chosen to prevent a food effect on the PK of the probe drugs and to minimize any inhibitory effects of rifampicin on enzyme or transporter activities, which might mask additional inductive effects (30, 34).

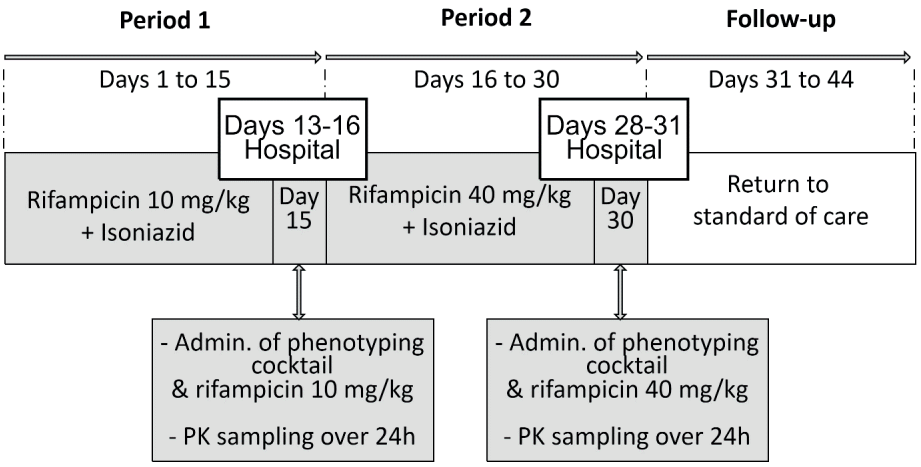

Figure 3. Schematic overview of the study design.

Monitoring of safety and concomitant medications

Participants were closely monitored throughout the study for evidence of clinical or laboratory-based adverse events (AEs). Grading and classification of AEs occurred according to the Common Terminology Criteria for Adverse Events (CTCAE, v 5.0) (35). All concomitant medication (and changes thereof) were reviewed for their inductive or inhibitory potential as well as to identify possible substrates of CYP enzymes that could potentially be affected by additional induction with RIF40.

Pharmacokinetic and pharmacogenetic blood sampling and bioanalysis

Blood samples for assessment of pharmacokinetic (PK) parameters of the phenotyping cocktail and the TB drugs were collected on day 15 and day 30. Samples for PK assessment of each of the probe drugs were drawn pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24h post-dose. Blood samples for PK assessment of the TB drugs were drawn pre-dose and at 2, 4 and 6h after rifampicin and isoniazid intake. Additionally, a blood sample was collected for pharmacogenetic testing to identify the genotypes of the CYP enzymes. Total (protein-bound plus unbound) concentrations of the probe drugs and rifampicin/isoniazid in plasma were measured with validated liquid chromatography–mass spectrometry (LC-MS/MS) methods at Nuvisan (Neu-Ulm, Germany) and Radboudumc (Nijmegen, the Netherlands), respectively. Details on the PK and pharmacogenetic assays are described in the supplementary texts S1, S2 and Tables S5 and S6.

Pharmacokinetic analysis

Noncompartmental pharmacokinetic analysis (NCA) was conducted using Phoenix WinNonlin v6.4 (Certara USA Inc., Princeton, NJ) to determine the phenotyping metrics (area under the curve until infinity ($AUC_{0-\infty}$) or until 24h (AUC_{0-24h}) for digoxin because of its long elimination half-life) and other PK parameters of each of the probe drugs when combined with RIF10 (day 15) and RIF40 (day 30). For digoxin the peak concentration (C_{max}) was assessed as a secondary phenotyping metric, specifically to assess intestinal P-gp activity. Individual PK parameters of rifampicin and isoniazid were estimated with established population PK models, based on relevant individual characteristics, dosing information and observed drug concentrations, using NONMEM software (36-38).

Statistical analyses

Based on reported data on intrasubject variability in pharmacokinetic data of probe drugs, a sample size of 25 participants was assessed (33, 39, 40). Participant demographics and study outcomes were reported for participants who completed the study. The effect of RIF40 in comparison to RIF10 on the phenotyping metrics of the different probe drugs was evaluated with a mixed-model bio-equivalence analysis, using Phoenix WinNonlin®. The main pharmacokinetic parameter under evaluation was the AUC, for which a geometric mean ratio (GMR) of all the probe drugs was calculated (RIF40 versus RIF10). GMR estimates with a 90% confidence interval (90%CI) entirely within the range of 80-125% were considered to indicate no significant additional interaction (20). The extent of additional induction was classified as mild, moderate or strong in case of ≤ 50%, > 50 to ≤ 80%, and > 80% reductions in AUC, respectively, based on guidelines for drug interactions (18, 19).

A subgroup bio-equivalence analysis was performed for caffeine to separate smokers from non-smokers, as smoking can cause CYP1A2 induction (28). In addition, a subgroup analysis excluding poor metabolizers (PMs; i.e. two nonfunctional alleles) or, in the case of CYP1A2, individuals with possibly reduced activity, was performed for all CYP enzymes.

Statistical analyses were performed using IBM SPSS Statistics for Windows (v.27.0 Armonk, NY: IBM Corp.)

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Supplementary material

Overview of content

Methods

Results

Methods

Table S1. In- and exclusion criteria

Inclusion criteria	
1.	The participant is able and willing to provide written, informed consent prior to all trial-related procedures.
2.	The participant is aged between 18 and 65 years, inclusive.
3.	The participant is a diagnosed pulmonary TB patient.
4.	The participant is currently being treated with a daily dose of 10 mg/kg rifampicin, i.e. 450 mg daily for patients with a body weight below 55 kg and 600 mg daily for participants with a body weight above 55 kg. This is in correspondence with the local South African TB treatment program. Furthermore, the participant has to be in the continuation phase of the treatment regimen (i.e. month 3 to 6), has demonstrated reasonable treatment compliance (≥80% of doses) and tolerates treatment well.
5.	The participant has a body weight (in light clothing and with no shoes) between 40 and 85 kg, inclusive.
6.	The participant is and stays non-pregnant (based on a negative serum pregnancy test,) and non-lactating (female participants of childbearing potential only).
Exclusion criteria	
1.	The patient is in poor general condition where any change in treatment cannot be accepted per discretion of the Investigator.
2.	The participant has active Hepatitis B.
3.	The participant has active Hepatitis C.
4.	The participant is receiving antiretroviral therapy (ART).
5.	There is evidence showing the participant has clinically significant metabolic, gastrointestinal, or other abnormalities than could possibly alter the PK of rifampicin and/or the probe drugs.
6.	The participant has a history of or current clinically relevant cardiovascular disorder such as: heart failure, atrioventricular (AV) block, arrhythmia, tachyarrhythmia or status after myocardial infarction.
7.	The participant has a family history of sudden death of unknown or cardiac-related cause, or of prolonged QTc interval.
8.	The participant has clinically relevant abnormalities in the ECG such as atrioventricular (AV) block, prolongation of the QRS complex over 100 milliseconds, or of a QTc interval over 450 milliseconds on the screening ECG.
9.	The participant has abnormal alanine aminotransferase (ALT) and/or aspartate transferase (AST) levels > 3 times the upper limit of the laboratory reference range at screening.
10.	The participant has a known or suspected, current drug or amphetamine abuse, that is, in the opinion of the Investigator, sufficient to compromise the safety or cooperation of the patient.

Table S1. Continued

11.	The participant used any drugs or substances known to be strong inhibitors or inducers of cytochrome P450 enzymes and/or P-glycoprotein (P-gp) within 2 weeks prior to day 1 (i.e. 1 month before administration of the phenotyping probes on day 15) of the study (including carbamazepine, barbiturates, St. John's Wort, clarithromycin, itraconazole, fluconazole, quinidine, ketoconazole, erythromycin). Exceptions may be made for participants who have received 3 days or less of one of these drugs or substances, if there has been a wash-out period equivalent to at least 5 half-lives of that drug or substance before day 1 of the study.
12.	The participant uses any of the phenotyping probe drugs (i.e. midazolam, caffeine, dextromethorphan, tolbutamide, omeprazole and digoxin) as part of standard medical treatment.
13.	The participant has as history of allergy to any of the phenotyping probe drugs (i.e. midazolam, caffeine, dextromethorphan, tolbutamide, omeprazole and digoxin)

Table S2. Weight banded dosing of standard dose rifampicin (10 mg/kg) and isoniazid

Bodyweight (kg)	**Rifinah® fixed combination 150/75 mg**	**Rifinah® fixed combination 300/150 mg**
	Number of tablets	
40-54	3	0
≥55	0	2

Table S3. Weight banded dosing of high-dose rifampicin (40 mg/kg) and isoniazid

Bodyweight (kg)	**Rifinah® fixed combination 150/75 mg**	**Rifinah® fixed combination 300/150 mg**	**Capsules rifampicin (Rifadin®) 150 mg**	**Capsules rifampicin 300 mg (Rifadin®)**
	Number of tablets and capsules			
40-54	3	0	1	4
55-70	0	2	0	6
>70	0	2	1	8

Table S4. Overview of the phenotyping cocktail

Probe drugs	Formulation	Dose[a]	Route of administration
Caffeine	Tablet 150mg (Regmakers®)	150 mg (= 1 tablet)	Oral
Tolbutamide	Tablet 500 mg (from Centrafarm BV)	125 mg (= ¼ tablet[b])	Oral
Omeprazole	Capsule 20 mg (Altosec®)	20 mg (= 1 capsule)	Oral
Dextromethorphan	Syrup 3 mg/ml (Benylin®)	30 mg (= 10 ml tablet)	Oral
Midazolam	Tablet 15 mg (Dormicum®)	15 mg (= 1 tablet)	Oral
Digoxin	Tablet 0.25 mg (Lanoxin®)	0.5 mg (= 2 tablets)	Oral

[a]The doses were based on studies performed with the Cologne cocktail [1, 2]. In our study we used a higher dose of midazolam, 15 mg instead of 2 mg as used in the Cologne cocktail. The reason was the strong induction of CYP3A4 by rifampicin which results in a (much) lower expected exposure to midazolam. A higher dose of midazolam was deemed justified and necessary in order to achieve plasma concentrations well above the lower limit of quantification to adequately describe the PK.

[b]The tolbutamide tablets do not contain score lines for exact splitting in quarter tablets. The administered quarter (and total) tablets were weighed. A paired samples t-test showed that the calculated administered doses at day 15 were not significantly different from day 30; the mean difference was -1.6 % with a 95% CI of [-4.1% to 0.9%] (p = 0.2). Therefore, no dose corrections were applied for the assessment of the phenotyping metrics.

Text S1. Bioanalysis of probe drugs and anti-TB drugs

The intraday and interday accuracy for the probe drugs and rifampicin/isoniazid were 96-107% and 94-104%, respectively. The intraday and interday coefficients of variation (CVs) for the probe drugs and rifampicin/isoniazid were ≤8.8% and ≤6.1%, respectively. More details are shown in tables 5 and 6.

Table S5. Validation parameters of the bioanalytical assays for the probe drugs

Compound	Calibration range	Intraday		Interday	
	Concentration (µg/L)	Accuracy %	Precision %	Accuracy %	Precision %
Caffeine	20.0 - 5000	100 – 107	3.1 – 8.8	98 – 106	3.6 – 6.4
Tolbutamide	50.0 – 25000	100 – 104	3.7 – 7.4	104 – 107	5.3 – 7.6
Omeprazole	1.00 – 500	98 – 102	1.8 – 3.3	98 – 101	2.7 – 5.2
Dextromethorphan	0.05 – 25.0	97 – 100	1.7 – 2.5	96 – 99	2.4 – 4.3
Midazolam	0.15 – 75.0	99 – 106	0.8 – 3.4	97 – 102	2.2 – 4.4
Digoxin	0.02 – 10.0	99 – 103	3.9 – 7.8	99 – 102	3.4 – 8.8

Table S6. Validation parameters of the bioanalytical assays for rifampicin and isoniazid

Compound	Calibration range	Intraday		Interday	
	Concentration (mg/L)	Accuracy %	Precision %	Accuracy %	Precision %
Rifampicin	0.09-60.0	96 – 103	2.6 – 4.9	96 – 101	0.0 – 1.9
Isoniazid	0.045-15.0	94 – 104	3.2 – 6.1	95 – 103	0.0 – 0.8

Text S2. Genotyping of cytochrome P450 (CYP) genes

Genomic DNA was extracted automatically using the Chemagic DNA isolation kit special (PerkinElmer, Waltham, MA, USA) according to the manufacturer's instructions.

Genotyping of the *cytochrome P450* (*CYP*) genes was performed using single-molecule molecular inversion probes as described previously at the Clinical Genetics department of the Maastricht University Medcial Center^{+} (MUMC^{+}) [3]. smMIPs for *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4* and *CYP3A5* were designed using the MIPgen pipeline [4]. smMIP library pools were prepared followed by sequencing on a NextSeq500 according to the manufacturer's instructions. The following variants were assessed *1A, *1C, *1F, *1K, *3, *4, *6 and *7 in *CYP1A2*, *6 and *18 for *CYP2B6*, *2-*6, *8 and *11-*13 for *CYP2C9*, *2-*10 and *17 for *CYP2C19*, *2-*12, *14, *15, *17-*21, *29, *31, *33, *35, *36, *38, *40-*42 and the duplication for *CYP2D6*, *1A, *1B, *1G, *6, *8, *11, *13, *16-*18, *20, *22 and *26 in *CYP3A4* and *2-*7 in *CYP3A5*. The copy number variant (deletion/duplication) in *CYP2D6* was assessed using the LightCycler 480 II (Roche; LightCycler 480 Software release 1.5.1.62 SP3) according to the protocol described by Langaee et al [5]. Two probes were used; one in exon 9 and one in intron 6 Genetic variants and according phenotypes were called automatically using an in-house designed calling algorithm. Phenotype assignment was done based on the guidelines of the Dutch Pharmacogenetics Working Group (DPWG) [6]. If variants were not assigned by the DPWG, the phenotypes on PharmVar were followed [7]. The *1 allele assignment was used when the analyzed variants were not present in a patient.

Results

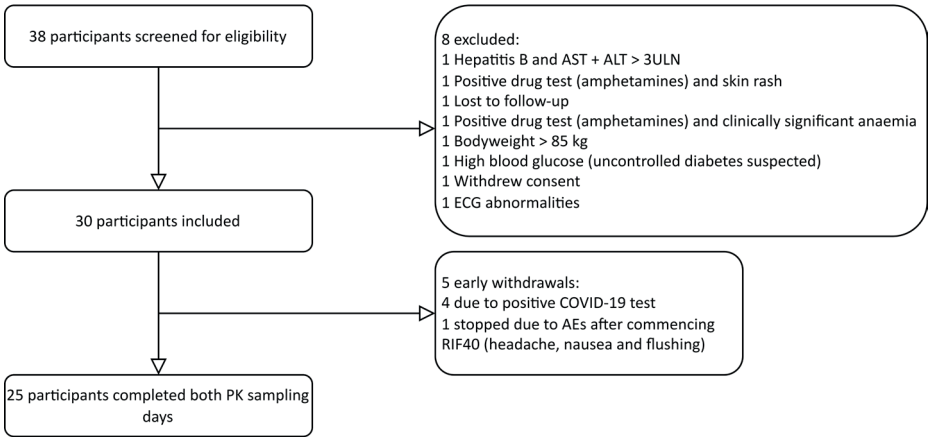

Figure S1. Overview of study population.

Table S7. Description of ≥ grade 2 adverse events (AEs)

Description AE	n	CTCAE severity grade	Relatedness to study medication
Participants with reported ≥ grade 2 AE, n (%)	**5 (20%)**		
≥ grade 2 AEs total	**11**		
AEs reported in period 1 (days 1-15)	**3**		
Hypoalbuminaemina with proteinuria	1[a]	3	Not related
Worsening of anemia	1	3	Not related
Tinea capitis	1[a]	2	Unlikely related
AEs reported in period 2 (days 16-30)	**8**		
Liver injury	1	3	Definitely related
Neutropenia	1	3	Possibly related
Worsening of anemia	1[a]	3	Possibly related
Nephrotic syndrome	1[ab]	3	Not related
Elevated ALT	1	2	Unlikely related
HIV diagnosis	1[a]	2	Not related
Impaired renal function	1[a]	2	Not related
Allergic reaction	1	2	Definitely related

[a] Six ≥ grade 2 AEs were reported for the same participant.
[b] The nephrotic syndrome required hospitalization during follow-up, which was reported as a serious adverse event

Table S8. PK parameters of the probe drugs

	Caffeine (CYP1A2) N=25		Dextromethorphan (CYP2D6) N=25		Digoxin (P-gp) N = 24[c]	
	RIF 10 mg/kg	RIF 40 mg/kg	RIF 10 mg/kg	RIF 40 mg/kg	RIF 10 mg/kg	RIF 40 mg/kg
C_{max} (ug/L)	3296 (1810- 5030)	3327 (2020 - 5360)	1.30 (0.20-10.3)	1.06 (0.12-15.9)	0.74 (0.25-1.41)	0.87 (0.46-1.42)
T_{max} (h)	1.5 (0.5-3.0)	1.6 (0.5-5.0)	2.0 (1.0-5.0)	2.0 (1.0-5.0)	1.5 (0.5-6.0)	4.0 (0.4-8.1)
AUC_{0-24h} (h*ug/L)	NA		NA		8.6[d] (3.1-16.4)	10.1[d] (4.6-15.9)
AUC_{0-last} (h*ug/L)	NA		10.3 (1.39-110)	7.1 (0.60-130)	NA	
$AUC_{0-\infty}$ (h*ug/L)	20474 (9989-75280)	21574 (7468-75144)	14.7[a] (3.61-124)	12.0[b] (3.43-154)	NA	
T½ (h)	3.3 (2.1-8.0)	3.5 (1.7-8.4)	6.3[a] (4.3-8.9)	6.2[b] (3.9-9.3)	NA	
CL/F (L/h)	0.007 (0.002-0.015)	0.007 (0.002-0.020)	2043[a] (241.9-8308)	2497[b] (194.8-8751)	NA	

PK parameters are depicted as geometric mean (range), except T_{max} which is depicted as median (range)

[a] N=21; $AUC_{0-\infty}$ and $T_{1/2}$ could not be reliably estimated for 6 participants because the percentage extrapolated was > 20%

[b] N=19; $AUC_{0-\infty}$ and $T_{1/2}$ could not be reliably estimated for 4 participants because the percentage extrapolated was > 20%

[c] N=24; One participant was excluded from the analysis due to the development of renal insufficiency between day 15 and day 30, with an increase of ≥ 1.5 times the serum creatinine concentration

[d] Due to the long $T_{1/2}$ of digoxin AUC_{0-24h} was estimated instead of $AUC_{0-\infty}$

[e] N=24 $AUC_{0-\infty}$ could not be estimated for 1 participant

[f] $AUC_{0-\infty}$ could not be estimated for many participants. AUC_{0-last} (0 to last measurable concentration) was calculated as an alternative. As a result, no $T_{1/2}$ could be calculated either

	Midazolam (CYP3A4) N=25		Omeprazole (CYP2C19) N=25		Tolbutamide (CYP2C9) N=25	
	RIF 10 mg/kg	RIF 40 mg/kg	RIF 10 mg/kg	RIF 40 mg/kg	RIF 10 mg/kg	RIF 40 mg/kg
	4.2 (1.1-20.9)	2.6 (0.5-14.7)	39.1 (6.1-194)	19.1 (3.6-59.6)	12983 (6900-20800)	11513 (2460-19100)
	1.0 (0.5-2.1)	0.5 (0.4-1.5)	2.0 (0.5-5.0)	2.0 (1.4-3.9)	3.0 (1.0-5.0)	2.1 (0.5-4.0)
	NA		NA		NA	
	NA		55.7[f] (8.1-427)	30.8[f] (3.4-142)	NA	
	7.1 (1.7-28.2)	4.4[e] (1.5-23.8)	NA[f]		88623 (28311-167684)	70722 (9261-134742)
	1.3 (0.7-5.8)	1.2[e] (0.6-3.5)	NA[f]		3.8 (2.1-6.6)	3.2 (1.1-6.0)
	2246 (570-8978)	3563 (669-9725)	NA[f]		1.45 (0.81-4.42)	1.79 (0.97-13.5)

Table S9. Subgroup analysis of the primary phenotyping metrics of caffeine for smokers and nonsmokers

Caffeine (CYP1A2)	RIF10 (Day 15)	RIF40 (Day 30)	GM RIF40/RIF10 ratio % (90% CI)	CI within 80 – 125%
	h*ug/L (range)			
$AUC_{0-\infty}$ total (n = 25)	20474 (9989-75280)	21574 (7468-75144)	**105 (96-116)**	Yes
$AUC_{0-\infty}$ smoking (n = 13)	14059 (9989-21195)	13990 (7468-31638)	**100 (87–114)**	Yes
$AUC_{0-\infty}$ non-smoking (n = 12)	30765 (14789-75280)	34491 (13343-75144)	**112 (98–128)**	No

Table S10. Overview of the CYP genotypes

CYP1A2	*CYP2C9*	*CYP2C19*	*CYP2D6*	*CYP3A4*	*CYP3A5*
			Genotype, n (%)[a]		
*1A/*1A 3 (12)	*1/*1 18 (72)	*1/*1 8 (32)	*1/*1 4 (16)	*1/*1 22 (88)	*1/*1 7 (28)
*1A/*1F 4 (16)	*1/*2 1 (4)	*1/*17 5 (20)	*1/*17 1 (4)	*1/*22 1 (4)	*1/*3 7 (28)
***1C/*1C[b] 2 (8)**	*1/*6 1 (4)	*1/*2 4 (16)	*1/*2 3 (12)		*1/*6 4 (16)
*1F/*1C 9 (36)	*1/*8 2 (8)	*1/*9 5 (20)	*1/*41 1 (4)		*1/*7 1 (4)
*1F/*1F 3 (12)		***2/*2 1 (4)[b]**	*1/*5 2 (8)		*3/*3 3 (12)
*1F/*1J 2 (8)			*17/*17 2 (8)		*6/*6 1 (4)
			*17/*41 1 (4)		
			*2/*10 1 (4)		
			*2/*17 1 (4)		
			*2/*29 1 (4)		
			*2/*4 2 (8)		
			*4/*29 1 (4)		
			*4/*35 (1 allele duplicated) 1 (4)		
			*5/*29 1 (4)		

[a] For 2 participants there was insufficient DNA material available to perform this analysis. In addition, the *CYP2D6* and *CYP2C9* genotype could not be determined for 1 participant

[b] Two participants were classified as possibly having a reduced activity of CYP1A2 (*1C/*1C) and 1 participant was classified as poor metabolizer of CYP2C19 (*2/*2), based on guidelines of the Dutch Pharmacogenetics Working Group (DPWG).

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Chapter 5

A loading dose of clofazimine to rapidly achieve steady-state-like concentrations in patients with nontuberculous mycobacterial disease

Ralf Stemkens[1*], Arthur Lemson[2*], Simon E. Koele[1], Elin M. Svensson[1,3], Lindsey H.M. te Brake[1], Reinout van Crevel[4], Martin J. Boeree[2], Wouter Hoefsloot[2], Jakko van Ingen[5], and Rob E. Aarnoutse[1]

[1]Department of Pharmacy, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[2]Department of Pulmonary Diseases, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[3]Department of Pharmacy, Uppsala University, Uppsala, Sweden

[4]Department of Internal Medicine and Infectious Diseases, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[5]Department of Medical Microbiology, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

*Ralf Stemkens and Arthur Lemson contributed equally.

Submitted for publication

Synopsis

Objectives. Clofazimine (CFZ) is a promising drug for the treatment of nontuberculous mycobacterial (NTM) diseases. Accumulation of CFZ to reach steady-state plasma concentrations takes months. A loading dose may reduce the time to steady-state-like concentrations. We evaluated the pharmacokinetics (PK), safety and tolerability of a loading dose regimen in patients with NTM disease.

Methods. Adult participants received a four-week loading dose regimen of 300 mg CFZ once daily (QD), followed by a maintenance dose of 100 mg QD (combined with other antimycobacterial drugs). Blood samples for PK analysis were collected on three occasions. A population PK model for CFZ was developed and simulations were performed to assess the time to reach steady-state-like (target) concentrations for different dosing regimens.

Results. Twelve participants were included. The geometric mean peak and trough CFZ concentrations after the four-week loading phase were 0.87 mg/L and 0.50 mg/L, respectively. Adverse events were common, but mostly mild and none led to discontinuation of CFZ. Our loading dose regimen reduced the predicted median time to target concentrations by 1.5 months compared to no loading dose (3.8 versus 5.3 months). Further time benefit was predicted with a six-week loading dose regimen (1.4 versus 5.3 months).

Conclusion. A four-week loading dose regimen of 300 mg QD reduced the time to target CFZ concentrations and was safe and well-tolerated. Extending the loading phase to six weeks could further decrease the time to target concentrations. Using a loading dose of CFZ is a feasible strategy to optimize treatment of NTM disease.

Clinical Trials Registration: NCT05294146

Introduction

Nontuberculous mycobacterial (NTM) diseases are increasingly reported worldwide and the most common clinical manifestation is NTM pulmonary disease (NTM-PD). (1, 2) Antibiotic treatment of NTM diseases requires multidrug regimens, characterized by long treatment durations, frequent occurrence of adverse effects, and poor cure rates. (3, 4)

Clofazimine (CFZ) is an old antibiotic that is primarily used in the treatment of leprosy (5). In more recent years, there has been an increased interest in the use of CFZ for the treatment of NTM disease and drug-resistant tuberculosis (DR-TB). (1, 6-8) CFZ is highly active in vitro against clinically important NTM species, i.e. *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MAB), and shows synergy with clarithromycin and amikacin. (9, 10) CFZ-based regimens have also shown comparable efficacy to first-line rifampicin-based regimens in patients with MAC-PD and it is considered a first-line oral antibiotic for the treatment of MAB-PD. (1, 11-13)

While CFZ has been increasingly recognized as an important drug, studies evaluating exposure-response relationships are scarce and the optimal dose of CFZ is unknown. (14) The dose that is commonly used for NTM disease (and DR-TB) is 100 mg once daily, although a range of 100-200 mg daily is recommended for NTM-PD. (1) In addition, there is experience with higher daily doses of 300 mg daily in the treatment of leprosy and DR-TB. (14-17) CFZ is characterized by complex pharmacokinetics (PK). It is highly protein-bound, very lipophilic, and accumulates particularly in adipose tissue and macrophage-rich organs. This results in a very long elimination half-life of ~30-70 days. As a result, it takes several months to reach steady-state ('stable') concentrations in plasma. (6, 18) This implies that the drug is not contributing fully to the NTM treatment regimen for months. We believe that treatment efficacy could be improved by using a higher dose at the start of treatment, i.e. a loading dose, to faster achieve concentrations similar to those at steady-state (steady-state-like concentrations). The use of loading doses is not uncommon for drugs that, similar to CFZ, have long elimination half-lives, such as the anti-TB drug bedaquiline. (19) To date, a loading dose strategy has not been evaluated for CFZ in the treatment of NTM disease.

We assessed the PK, safety and tolerability of CFZ in patients with NTM disease who received a loading dose regimen of 300 mg once daily (QD) for four weeks followed by a maintenance dose of 100 mg QD.

Methods

Study population

Adult patients with pulmonary or extrapulmonary NTM disease, eligible for treatment with CFZ, were included in this study (see supplementary table S1 for in- and exclusion criteria). Written informed consent was obtained from all participants.

Study design

This explorative, one-arm, open-label, PK study (acronym C-LOAD) was performed at Radboud university medical center (Radboudumc), Nijmegen, the Netherlands. Due to the explorative nature of the study, no sample size calculations were performed. We aimed to enroll ten participants but allowed for additional enrollments (a maximum of five), in case of early withdrawals. The study was approved by an ethical review board, METC Oost-Nederland, and was conducted under Good Clinical Practice standards. This study is registered with ClinicalTrials.gov under trial number NCT05294146.

All participants received a loading dose regimen of CFZ (Lamprene), 300 mg QD for four weeks (period 1), followed by a maintenance dose of 100 mg QD until a total study duration of four months (period 2; see figure 1 for a schematic overview). Other antimycobacterial drugs were used as per standard of care throughout the study. With the loading dose regimen, we aimed to reach steady-state-like concentrations of CFZ in a shorter time period. The loading dose regimen was selected based on explorative simulations with a population PK model for TB patients. (18) CFZ was administered with food, as recommended by the manufacturer and because this improves absorption. (6) Adherence to CFZ was evaluated using pill counts (only in period 1), a medication diary, and self-assessment by participants.

PK blood sampling and bioanalysis

Blood samples for assessment of PK parameters of CFZ were collected on three occasions (figure 1). Samples were collected after the last loading dose of 300 mg (at four weeks (+/- two days), with sampling time points at pre-dose and at 2, 4, 6, 8, and 24 hours post-dose. In addition, samples were collected after the first maintenance dose of 100 mg (at four weeks + one day), with sampling time points at 2 and 6 hours post-dose, and once more after four months (+/- one week). Total plasma concentrations of CFZ were measured using validated liquid chromatography–mass spectrometry methods (details are shown in Table S2).

Monitoring of safety and tolerability

Participants were monitored throughout the study for evidence of clinical or laboratory-based adverse events (AEs). Severity grading of AEs occurred according to the Common Terminology Criteria for Adverse Events (CTCAE, v 5.0). (20)

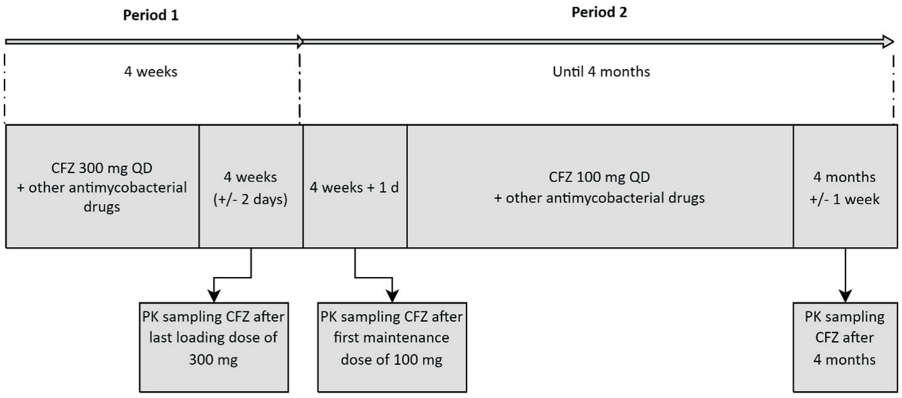

Figure 1. Schematic overview of the study periods and PK sampling occasions. Adherence to treatment and adverse effects were also monitored on PK sampling occasions as well as on additional study visits: day one, one week, two months and three months (phone call). ECGs were recorded 2-6 hours post-dose of CFZ on day one, at four weeks and four months and, without a specific time window, after one week and two months.

Noncompartmental PK analysis (NCA)

NCA was performed to determine the plasma PK parameters of CFZ, including the area under the time versus concentration curve (total exposure, $AUC_{0\text{-}24h}$), peak concentration (C_{max}), time to C_{max} (T_{max}) and the (pre-dose) trough concentration (C_{trough}), after the last loading dose of 300 mg.

Comparator study without a loading dose regimen

We used PK data from a completed randomized clinical trial (PERC trial), executed at the Radboudumc, that compared the efficacy of a rifampicin-based regimen and a CFZ-based regimen for MAC-PD. (21) Participants in the CFZ-arm of that study received a dose of 100 mg QD (without loading dose) and PK samples were collected, at 2 and 6h post dose, after one and four months of treatment, similar to PK sampling in our study. Plasma concentrations of CFZ in this PERC trial were measured with the same assay for CFZ. We used the PK data from the PERC trial to compare the highest measured CFZ concentrations with our study and to develop a population PK model.

PK model development

Three previously developed population PK models were evaluated to describe the CFZ PK. (18, 22, 23) The model evaluation is described in more detail in text S1. The model-fit was not satisfactory and therefore, non-linear mixed-effects modeling was used to develop a pharmacokinetic model for CFZ, based on the data from the current study and the PERC trial (21). Model development was described in text S2.

Simulations

The developed PK model was used to simulate alternative CFZ loading dose regimens to shorten the time to steady-state-like concentrations. A virtual patient population of 1,000 patients was generated for each simulated loading dose regimen. Body weight in the virtual patient population followed a log-normal distribution based on the combined demographics of our study and the PERC trial. (21) Loading doses of 200, 300, and 400 mg QD were assessed for periods of four weeks, six weeks, and eight weeks in total, followed by a maintenance dose of 100 mg QD. A regimen without a loading dose (100 mg QD) served as the reference. We evaluated the time required to reach target concentrations for each of the dosing regimens. The selected target was 80% of the individual PK-model predicted steady-state trough (pre-dose) concentrations, achieved with a dose of 100 mg QD. We assumed that at 80% of steady-state concentrations no clinically relevant difference in effect could be expected compared to true steady-state. Moreover, with this target we aimed to prevent reaching peak concentrations (associated with QTc prolongation (24)) during the loading phase that exceeded steady-state peak concentrations, achieved with a dose of 100 mg QD.

Software

NCA was performed using Phoenix WinNonlin v6.4 (Certara USA Inc., Princeton, NJ). Descriptive statistics were performed using IBM SPSS Statistics for Windows (v.29.0). PK model development was performed using NONMEM 7.5, and data management and post-processing of results were performed in R 4.1.3. (25, 26) Perl-speaks NONMEM was used for VPC generation, SCM analysis, and the SIR procedure.

Results

Study population

A total of 12 participants were screened and all were enrolled in the study. Their demographics and baseline characteristics are depicted in table 1. Four participants dropped out of the study. All dropouts occurred in period 2 of the study and none were deemed related to CFZ (details are presented in table S3). Adherence to CFZ was high and ranged from 95-100%.

Table 1. Demographic and baseline characteristics

N	**12**
Age, years, median (range)	69 (39 – 82)
Body weight, kg, median (range)	62 (51 – 105)
Fat-free mass, kg, median (range)[a]	50 (38 – 70)
Male sex, n (%)	6 (50)
Race, n (%)	
Caucasian	10 (83)
Middle Eastern	2 (17)
Pulmonary NTM disease, n (%)	9 (75)
Extrapulmonary NTM disease, n (%)	3 (25)
Causative species (n)	
M. avium complex	7
M. avium complex and M. abscessus	1
M. abscessus	1
M. chelonae	1
M. malmoense	1
M. xenopi	1
Other antimycobacterial drugs used (n)	
Amikacin (intravenous)	8
Amikacin liposomal (inhalation)	1
Azithromycin	11
Clarithromycin	1
Ethambutol	9
Imipenem/cilastatin	2
Moxifloxacin	1
Rifampicin	7

[a] Calculated according to Janmahasatian *et al.* 2005 (27).

NCA and descriptive PK

The geometric mean (GM) C_{max},, C_{trough} and $AUC_{0\text{-}24h}$ of CFZ after the last loading dose of 300 mg were 0.87 mg/L, 0.50 mg/L and 16.1 mg*h/L (table 2) respectively. The GM highest measured concentrations after approximately one (first dose of 100 mg) and four months of treatment were 0.75 mg/L and 0.98 mg/L. In the PERC trial,

GM highest measured concentrations after one and four months were 0.39 mg/L and 0.78 mg/L, respectively (table 2).

Table 2. Pharmacokinetic parameters of clofazimine in this study (C-LOAD) and the comparator trial (PERC) (21)

	Day 28 (+/- 2)	1 month		4 months	
	C-LOAD 300 mg	C-LOAD 100 mg	PERC 100 mg	C-LOAD 100 mg	PERC 100 mg
PK parameter[a]	**n = 12**	**n = 12**	**n = 19**	**n = 8**	**n = 16**
C_{max} (mg/L)	0.87 (0.69-1.11)				
T_{max} (h)	6.0 (2.0-8.1)				
$C_{highest}$ (mg/L)[b]	NA	0.75 (0.57-0.97)	0.39 (0.30-0.50)	0.96 (0.71-1.2)	0.78 (0.60-1.02)
C_{trough} (mg/L)	0.50 (0.39-0.64)				
AUC_{0-24h} (mg*h/L)	16.1 (12.8-20.4)				

[a] PK parameters are depicted as geometric mean (95% CI), except for T_{max} which is depicted as median (range).

[b] Due to the limited number of samples (2h and 6h post-dose), the C_{max} may not have been captured. The highest measured concentration ($C_{highest}$) was reported as an alternative PK parameter.

Adverse events

A total of 151 AEs were reported in 12 participants, including 109 grade 1, 25 grade 2 and 17 grade ≥ 3 events (see table 3). CFZ was well-tolerated and was not discontinued in any of the participants. The most common AEs were gastro-intestinal (GI; n= 28 in 11 participants), skin-related (n= 20 in 10 participants) and ototoxicity (n= 12 in eight participants). Seven serious AEs (SAEs) were reported in six participants. Five SAEs were unscheduled or prolonged hospitalizations and two SAEs were deaths. None of the SAEs were deemed related to the use of CFZ (details are described in table S4).

PK model development

In total, 112 CFZ concentrations from our study (12 patients) and 67 concentrations (19 patients) from PERC were available for the development of the population PK model. CFZ PK were best described by a two-compartment disposition model. Absorption was characterized by a depot and one transit compartment with first-order absorption into the central CFZ compartment. Estimation of a separate absorption-rate constant and mean-transit time did not significantly improve the model fit. A combined proportional and additive error model described the residual error in the data best. Allometric scaling was included based on total body weight. A schematic representation of the PK model is presented in figure S1.

Table 3. Overview of adverse events

	Period 1 (n)	Period 2 (n)
Total number of AEs	71	80
CTCAE severity grade[a]		
Grade 1	49	60
Grade 2	15	10
Grade ≥ 3	7	10
AEs contributing to >5% of total number of AEs		
Gastro-intestinal	15	13
Ototoxicity	4	8
Skin-related	9	11
Elektrolyte imbalance	1	9
QTc prolongation	6	3
Hypoalbuminemia	6	2
Other	12	19
Total number of SAEs	2	5
Unscheduled hospitalization	1	3
Prolonged hospitalization	1	
Death		2
Related to clofazimine	0	0

[a] In case the severity of an AE changed over time (e.g. from grade 2 to 3), this was described as one AE with the highest reported severity grade. The start date of an AE was the first reported date of this AE.

Covariate analysis revealed that the bioavailability of CFZ was ~26% lower (95% CI: 9.2-40%) for 300 mg doses compared to 100 mg doses ($p<0.01$). No other tested covariate significantly impacted the PK of CFZ. Moreover, no statistically significant differences in key CFZ PK parameters (absorption rate, volume of distribution, clearance, and bioavailability) were detected between our study and the PERC trial. Final PK model parameters are presented in table 4, and visual predictive checks are presented in figure 2. Goodness-of-fit plots and final model code are presented in figure S2 and text S3, respectively.

Table 4. Final pharmacokinetic model parameters for CFZ

Parameter	Typical value (95% CI)[a]
Fixed effects[b]	
Clearance (L/h)	4.17 (2.81-5.79)
Central volume of distribution (L)	460 (370-608)
Absorption rate constant (1/h)	0.683 (0.502-0.940)
Intercompartmental clearance (L/h)	24.6 (18.1-32.9)
Peripheral volume of distribution (L)	10100 (7670-13000)
Effect of high-dose on bioavailability (%)	73.6 (59.6-91.8)
Random effects[c]	
Interindividual variability in clearance (CV%)	85.6 (55.9-143)
Interindividual variability in absorption rate constant (CV%)	80.6 (47.8-139)
Interoccasion variability in bioavailability (CV%)	79.3 (59.7-122)
Residual error	
Proportional error (%)	8.00 (5.95-10.5)
Additive error (mg/L)	0.00174 (0.000701-0.00278)

[a] 95% confidence interval obtained using the SIR method.
[b] All disposition parameters are reported as apparent disposition parameters. Volumes and clearances are allometrically scaled using fixed exponents of 1 and 0.75, respectively, with 70kg as reference total body weight.
[c] Interindividual variability CV% calculated as SQRT(exp(OM^2)-1).

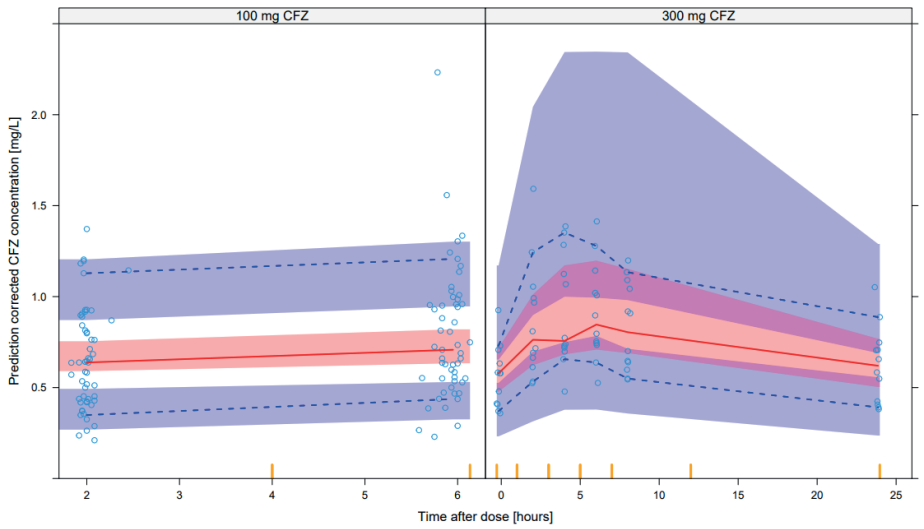

Figure 2. Visual Predictive Checks (VPCs) showing the observed 5th, 50th, and 95th percentiles (lines) and the 95% CI for the same percentiles (shaded areas) calculated from simulated data using the final PK model. Left: Observations and model predictions after 100 mg dosing. Right: Observations and model predictions after 300 mg dosing.

Simulations

In our virtual population of 1,000 patients, the median time to reach the target concentration (80% of individual steady-state concentration) for CFZ (100 mg QD) without a loading dose was 5.3 months. When using the loading dose regimen investigated in this study, 300 mg QD for four weeks, the median time to reach the target concentration was reduced to 3.8 months. Extending the loading phase to six weeks decreased the median time to target concentration to 1.4 months. No additional gain in time to target concentration was predicted with either a longer loading phase of eight weeks or a higher loading dose of 400 mg. Peak concentrations during the loading phase for loading regimens up to six weeks were similar compared to peak concentrations at steady-state with a dose of 100 mg QD. The results of the simulated dosing regimens are depicted in table 5. To illustrate the effects of different loading dose regimens, concentration-time profiles for a typical patient of 65 kg are presented in figure 3.

Table 5. Results for the simulated loading dose regimens

Dose regimen[a]	Time to target concentration[b] (months)	Highest C_{max} during loading dose period (mg/L)	C_{max} during steady-state (mg/L)
100 mg QD (reference)	5.3 (0.93 – 8.1)	0.44 (0.23 – 1.0)	1.1 (0.29 – 2.7)
200 mg QD 4 weeks	4.6 (0.53 – 7.9)	0.75 (0.42 – 1.6)	1.1 (0.32 – 2.9)
300 mg QD 4 weeks	3.8 (0.33 – 7.6)	0.96 (0.55 – 2.1)	1.1 (0.34 – 2.8)
400 mg QD 4 weeks	3.5 (0.20 – 7.6)	1.1 (0.55 – 2.3)	1.1 (0.28 – 2.8)
200 mg QD 6 weeks	3.9 (0.50 – 7.6)	0.87 (0.43 – 1.9)	1.1 (0.29 – 2.8)
300 mg QD 6 weeks	1.4 (0.30 – 7.3)	1.1 (0.58 – 2.3)	1.1 (0.32 – 2.7)
400 mg QD 6 weeks	1.2 (0.23 – 7.3)	1.2 (0.63 – 2.5)	1.1 (0.30 – 2.9)
200 mg QD 8 weeks	1.9 (0.53 – 7.4)	1.0 (0.47 – 2.0)	1.1 (0.30 – 2.8)
300 mg QD 8 weeks	1.4 (0.30 – 7.0)	1.3 (0.64 – 2.6)	1.1 (0.31 – 2.9)
400 mg QD 8 weeks	1.3 (0.23 – 7.0)	1.4 (0.70 – 2.9)	1.1 (0.29 – 3.1)

[a] All loading dose regimens were followed by a maintenance dose of 100 mg QD.
[b] Target concentration: 80% of the individual PK-model predicted steady-state trough (pre-dose) concentrations, achieved with a dose of 100 mg QD.
All results are depicted as median (2.5th – 97.5th percentiles).

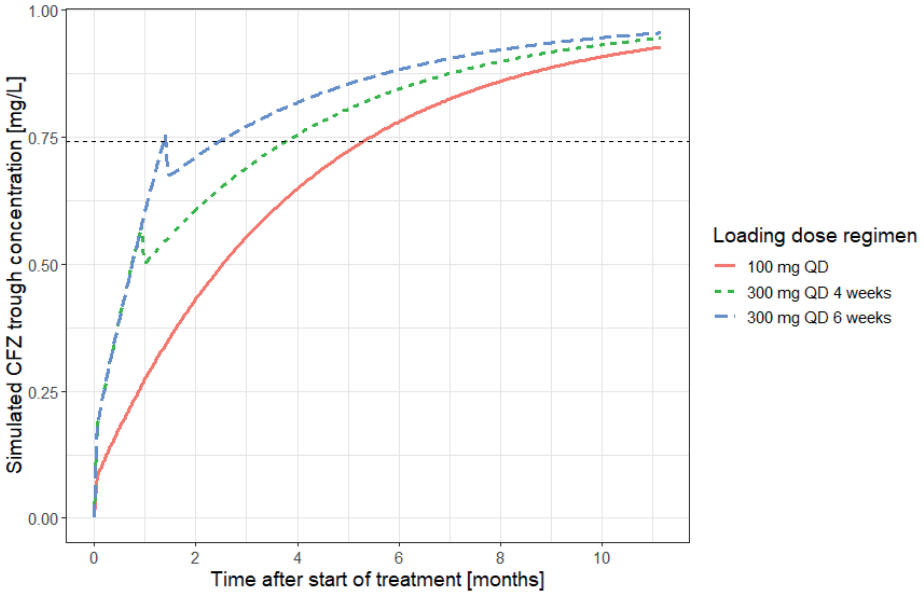

Figure 3. Simulated CFZ trough (pre-dose) concentration versus time curves for a typical patient (65 kg) following three different dosing regimens; 100 mg QD (reference), 300 mg QD for four weeks followed by 100 mg QD, and 300 mg QD for six weeks followed by 100 mg QD. The dashed line represents the target concentration, i.e. 80% of the predicted steady-state trough concentration.

Discussion

A dose of 300 mg CFZ QD for four weeks led to faster attainment of steady-state-like concentrations, as compared to no loading dose regimen, and was safe and well-tolerated. The PK model derived from our data revealed that a 300 mg QD dose for six weeks is expected to further decrease the time to steady-state-like concentrations. These results show the usefulness and feasibility of a loading dose of CFZ, which could be implemented in patient care and evaluated in follow-up studies.

After the loading phase, the highest measured CFZ concentration (with a dose of 100 mg QD) after one month of treatment (0.75 mg/L) approached the highest measured CFZ concentration after four months of treatment (0.96 mg/L) in this study. In contrast, a larger (two-fold) difference of the highest measured CFZ concentrations between one and four months of treatment (0.39 mg/L versus 0.78 mg/L) was observed in the PERC trial with a CFZ dose of 100 mg QD (without loading phase). (21) These results illustrate that a loading dose yields steady-state-like concentrations faster.

Adverse events were reported frequently throughout the study which is common among patients with NTM diseases undergoing treatment, (28) but none resulted in discontinuation of CFZ. Although attributing AEs to a specific antibiotic in multi-drug regimens is challenging, AEs associated with CFZ include QTc prolongation, gastro-intestinal (GI) and skin-related effects. (6) QTc prolongation was observed in nine participants, including two grade 3 events. All patients used two antimycobacterial drugs that can cause QTc prolongation (CFZ and a macrolide or moxifloxacin), and in some cases additional QTc-prolongating drugs. Our observations are in line with previous reports, especially when CFZ is combined with other QTc-prolongating drugs. (29) GI AEs frequently occurred, similar to previous reports on CFZ-based regimens. (30) They were mostly mild, with the exception of six grade 3 events (in three participants). Skin-related AEs characteristic for CFZ (discoloration, dryness and pruritis), were also common but generally mild. Overall, no unexpected events related to CFZ were reported in this study. Of course, the number of patients receiving a loading dose in our study was small for the purpose of a safety evaluation. However, in the treatment of leprosy and DR-TB there is experience with a daily CFZ dose of 300 mg (up to 600 mg for leprosy) for months and even years. (14-17) Furthermore, steady-state concentrations were not exceeded during the loading phase which further supports the safety of the loading dose regimen.

The population PK model for CFZ in NTM patients showed that CFZ PK were characterized by a large volume of distribution (~10.000L) and a long terminal half-life (~70 days), consistent with previous reports. (6, 18) An important finding was that the bioavailability of CFZ was 26% lower with a dose of 300 mg compared to a 100 mg dose. This could be explained by dose-dependent saturation of absorption, rather than a previously suggested time-dependent effect, (18) and is supported by previous observations in a study on fecal excretion in relation to different doses of CFZ. (31) This finding indicates there are limits to the dose that can be administered and further dose increases (above 300 mg QD) may not lead to substantially higher exposure in plasma.

Our loading dose simulations demonstrate that a large improvement can be achieved in the time required to reach steady-state-like concentrations when using a loading dose regimen. The loading dose investigated in this study reduced the time to target concentrations by 1.5 months, as compared to not using a loading dose (100 mg QD). Importantly, the peak concentration (associated with QTc prolongation (24)) during the loading phase did not surpass the peak concentrations at steady-state, reaffirming the safety of the loading dose regimen. The optimal loading dose regimen was determined to be a dose of 300 mg QD for six weeks. This

regimen reduces the time to target concentrations by 3.9 months compared to not using a loading dose, without exceeding steady-state peak concentrations. A higher loading dose of 400 mg QD did not further improve the time to target concentrations in our simulations due to a further decrease in bioavailability. It should be noted that this simulation is less certain as it builds on extrapolation outside of the investigated dose range. Furthermore, multiple daily dosing (e.g. 200 mg twice daily) may circumvent bioavailability limitations. We deemed once daily dosing preferable in the context of multidrug regimens and therefore we did not assess multiple daily dosing.

Previously, a different loading dose regimen of 200 mg QD for 2-4 weeks has been proposed for the treatment of TB based on model simulations. (18) This loading dose regimen was based on a target concentration of 0.25 mg/L. This target was derived from a murine TB model in which sustained antimicrobial activity after treatment cessation was associated with concentrations of ≥0.25 mg/L. (32) However, there is no evidence that this target is associated with maximum effect against *Mycobacterium tuberculosis*. In general, there are limited data on exposure-response relationships for CFZ in TB. In patients with MDR-TB an $AUC_{0\text{-}24h}$/MIC ratio of ≥50 was associated with faster culture conversion. (33) In another study, patients in whom culture conversion was achieved had higher $AUC_{0\text{-}24h}$/MIC ratios after two and six months of treatment ($AUC_{0\text{-}24h}$/MIC: 116 versus 64 and 101 versus 52, respectively). (34) For NTM disease, data on exposure-response relationships are even more scarce. In two studies in NTM-PD, lower MICs (≤0.25 mg/L) were associated with better outcomes. (35, 36) In the PERC trial, patients in whom culture conversion was achieved, had higher peak CFZ concentrations (0.95 versus 0.51 mg/L). (21) While these data suggest that higher CFZ exposures may be favorable, the optimal exposure-response relationships (and thus exposure targets) of CFZ remain to be established. As CFZ-based treatment regimens with a standard dose of 100 mg QD have shown promising outcomes in NTM disease, (11-13, 21, 30, 37) we deemed steady-state concentrations that are achieved with this dose as the most suitable target for our loading dose strategy. However, we recognize that the current standard dose of 100 mg QD is not well substantiated. (14) The evaluation of exposure-response relationships of CFZ should be a goal of future studies in order to define exposure targets and to further establish optimal dosing of CFZ for NTM disease.

Our study had limitations. The sample size was small and the study had no control arm without a loading dose. However, we believe that the PERC trial served very well as a comparator study because it was performed in the same setting, had a

similar patient population (except three patients with extrapulmonary NTM disease in our study), and CFZ plasma concentrations had been measured with the same analytical method. (21) The appropriate choice of the PERC trial as comparator was substantiated by the absence of differences in PK parameters between our study and PERC. A notable difference with the PERC trial is that seven patients in our study used rifampicin of whom five close to the PK sampling days. One study reported a limited reduction in bioavailability of CFZ (22%) when combined with rifampicin, (23) whereas other studies suggest that rifampicin has no effect on the exposure to CFZ. (38, 39) In our study rifampicin use was not identified as a significant predictor of CFZ exposure, but the sample size was small. There is significant variability in reported PK of CFZ between different studies and patient populations. (18, 23, 40) Moreover, existing population PK models did not describe our data well (text S1). As a result, it is unknown how our specific recommendations on the optimal loading dose regimen translate to other populations. However, we believe that our finding on the usefulness and feasibility of a CFZ loading dose strategy is universally applicable.

In conclusion, a four-week loading dose regimen of 300 mg QD significantly reduced the time to steady-state-like concentrations of CFZ and was safe and well-tolerated. Extending the loading phase to six weeks is expected to yield additional time benefit. These results show the feasibility of a loading dose of CFZ, which could be implemented in patient care and evaluated in follow-up studies.

Notes

Funding

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Supplementary material

Overview of content:

Methods:

Results:

Methods

Table S1. Overview of inclusion and exclusion criteria

Inclusion criteria:
The participant is diagnosed with pulmonary or extrapulmonary NTM disease and is eligible for treatment with CFZ
The participant is at least 18 years of age
The participant has a body weight (in light clothing and with no shoes) of at least 45 kg
The participant is able and willing to provide written, informed consent
Exclusion criteria:
The participant is in poor general condition where participation in the study cannot be accepted per discretion of the Investigator
There is evidence showing the participant has clinically significant metabolic, gastrointestinal, or other abnormalities that could possibly alter the PK of CFZ
The participant is diagnosed with cystic fibrosis
The participant has a prolongation of the QTc interval, > 450 milliseconds for males and > 460 milliseconds for females, on the screening ECG
The participant has abnormal alanine aminotransferase (ALT) and/or aspartate transferase (AST) levels of > 3 times the upper limit of the laboratory reference range at screening
The participant is pregnant or is using inadequate contraceptive measures (if applicable)
The participant is breastfeeding (if applicable)
The participant has a known or suspected, current drug or alcohol abuse, that is, in the opinion of the Investigator, sufficient to compromise the safety or cooperation of the patient
The participant has as history of allergy/hypersensitivity to CFZ
The participant has received clofazimine in the past 3 months before inclusion with the exception of short-term use of no more than 7 days in the period of 1 to 3 months before inclusion

Table S2. Validation parameters of the bioanalytical assay for clofazimine

Compound	**Calibration range**	**Intraday**		**Interday**	
	Concentration (mg/L)	Accuracy %	Imprecision %	Accuracy %	Imprecision %
Clofazimine	0.05 - 10.0	95.1-104.6	3.0-6.9	98.9-102.3	0.9-3.2

Text S1. Evaluation of previously published population PK models for clofazimine

Literature search identified three population pharmacokinetic models for clofazimine.

In summary, Abdelwahab *et al.* (1) pooled data from two studies (PROBeX and a phase IIa clinical trial (2, 3)) to study the CFZ PK in participants with pulmonary tuberculosis. In total, 1570 observations from 139 participants were included, from both sparse and intensive PK sampling. A three-compartmental PK model with an estimated number of transit compartments was reported. Allometric scaling was based on total body weight for clearances, and one peripheral volume, on fat mass for the second peripheral volume, and on fat-free mass for the central volume, standardized to their respective medians. Furthermore, an auto-inhibitory effect of clofazimine on P-glycoprotein was hypothesized, leading to a decreased bioavailability during the first days on treatment.

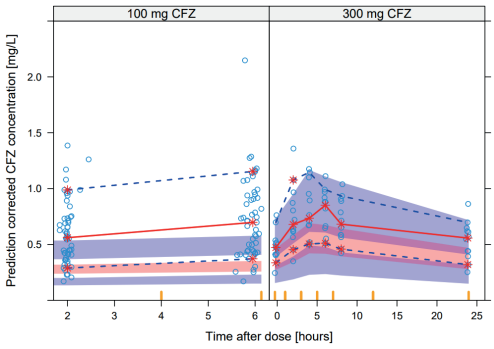
Faraj et al. (4) used data from a phase IIa clinical trial in participants with pulmonary tuberculosis. (3) In total, data 14 participants were included with intensive PK sampling. The authors reported a two-compartmental PK model with a lag time absorption model and allometric scaling of clearance and volume parameters using total body weight standardized to the population median, and fixed exponents of 0.75 and 1, respectively.

Watanabe *et al.* (5) used data form a trial in participants with a pulmonary NTM infection. In total, 144 PK observations from 45 participants were included for model development. All PK observations were pre-dose samples. The authors reported a one-compartmental PK model with allometric scaling of the clearance using an estimated exponent of 1.16 based on total bodyweight and of the volumes using an estimated exponent of 1.55 based on body mass index, both standardized to the population median. Furthermore, rifampicin co-administration was estimated to reduce the bioavailability of clofazimine by 22%.

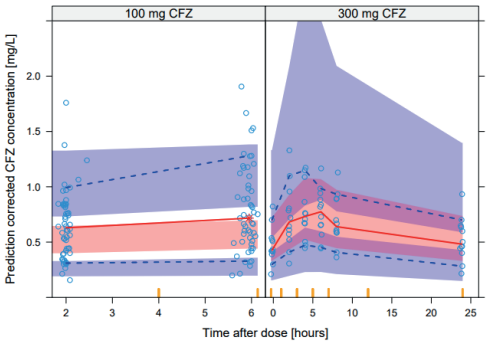
To evaluate the ability of the models to describe the data from this study and the PERC study, a two-step approach was used. First, an evaluation of the PK model was performed using the reported pharmacokinetic parameter estimates. Secondly, key pharmacokinetic parameters (clearance, volume of distribution, absorption, bioavailability, and their respective variability) were re-estimated for each model. For both steps, VPCs were created to assess the model fit. The VPCs for each model before and after the estimation of the key pharmacokinetic parameters are shown below. All VPC show the observed 5^{th}, 50^{th}, and 95^{th} percentiles (lines) and the 95% CI for the same percentiles (shaded areas) calculated from simulated data using the respective PK model.

Abdelwahab *et al.*

No estimation

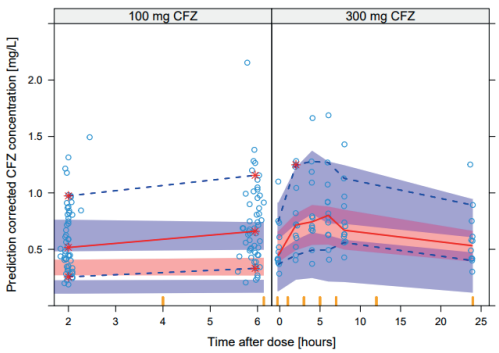


Key parameters re-estimated

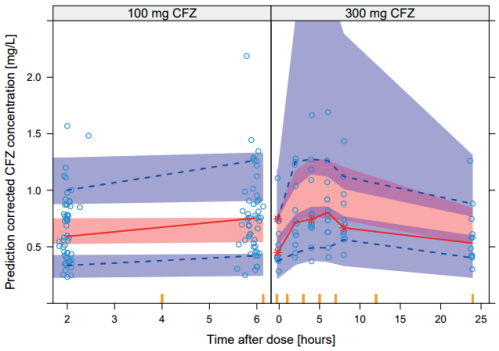


Faraj *et al.*

No estimation

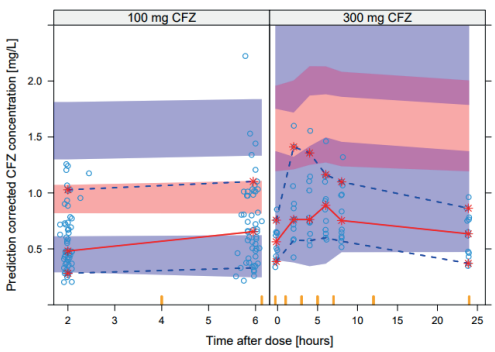


Key parameters re-estimated

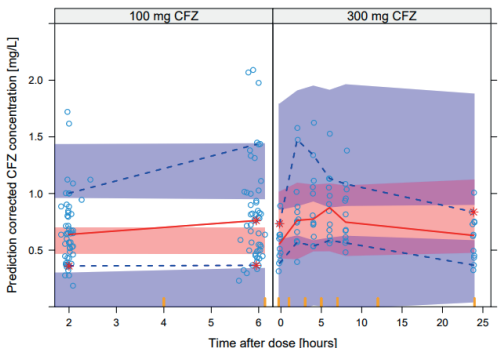


Watanabe *et al.*

No estimation



Key parameters re-estimated



The population PK models of Abdelwahab *et al.* and Faraj *et al.* both showed an underprediction of the CFZ concentration before re-estimation. Conversely, Watanabe *et al.* overpredicted the CFZ concentrations before re-estimation. Re-estimation of the PK modes showed that the models from Abdelwahab *et al.* and Faraj *et al.* were able to describe the shape of the concentration-time profile well, but were not able to describe both the 100 mg QD dose and the 300 mg QD dose. The model from Watanabe *et al.* did not follow the general shape of the CFZ concentration-time profile well. Concluding, none of the evaluated models were able to accurately describe the CFZ PK in this study combined with the PERC study.

Text S2. Development of pharmacokinetic model

One-, two-, and three-compartmental disposition models were investigated. First-order, zero-order, and transit compartment models were tested for their ability to describe the CFZ absorption. First-order clearance was assumed to take place from the central CFZ compartment. Interindividual variability was assumed to be log-normally distributed.

Allometric scaling was implemented using fixed standard exponentials of 1 for volumes and 0.75 for clearances. Total body weight, fat-free mass, (6) and fat mass were investigated as body size descriptors for use in allometric scaling. Analysis of other predictors of the PK of CFZ (covariates) were assessed using stepwise covariate modeling (SCM). A forward significance criterion of $p<0.05$ and a backward criterion of $p<0.01$ were used as significance cutoffs. Explorative covariates were: dose of CFZ, rifampicin co-administration, age, sex, and pulmonary versus extrapulmonary disease.

The 95% confidence interval for all model parameters was determined using the sampling importance resampling (SIR) procedure. (7) Model evaluation was performed using goodness-of-fit plots as well as visual predictive checks (VPCs). Additional VPCs of the accumulation of CFZ over time on treatment are depicted in figure S3. A decrease in objective function value of < -3.84 was considered significant at the $p=0.05$ level.

Results

Table S3. Description of dropouts

1	One participant died due to a massive pulmonary hemorrhage
2	One participant died due to respiratory insufficiency caused by an acute COPD exacerbation
3	One participant withdrew because of clinical deterioration leading to cessation of antimycobacterial treatment
4	One participant withdrew at their own request

Table S4. Description of SAEs

1	Hospital admission because of clinical deterioration (weight loss, dyspnea)
2	Death due to a massive pulmonary hemorrhage
3	Hospital admission because of clinical deterioration (weight loss, dyspnea and fever)
4	Hospital admission due to nausea (and vomiting), most likely related to the use of imipenem/cilastatin
5	Acute exacerbation of COPD resulting in prolonged hospitalization and shortly thereafter death
6	Hospital admission due to influenza A infection
7	Prolonged hospitalization due to tubulo-interstitial nephritis, related to the use of ethambutol

Table S5. Population PK model derived clofazimine PK parameters at month 1 and 4 of clofazimine treatment for this study (C-LOAD) and the comparator PERC study (8)

	Day 28 (+/- 2)	**1 month**		**4 months**	
	C-LOAD	**C-LOAD**	**PERC**	**C-LOAD**	**PERC**
	300mg	**100 mg**	**100 mg**	**100 mg**	**100 mg**
PK parameter [a]	**n=12**	**n = 12**	**n = 19**	**n = 8**	**n = 16**
$AUC_{0\text{-}24h}$ (mg*h/L)	16.0 (12.6-20.3)	14.6 (11.5-18.5)	7.75 (6.18-9.72)	19.8 (15.1-25.9)	15.8 (12.4-20.0)
C_{max} (mg/L)	0.832 (0.649-1.07)	0.734 (0.575-0.937)	0.399 (0.317-0.502)	0.930 (0.720-1.20)	0.767 (0.606-0.971)
T_{max} (h)	4.28 (3.37-5.44)	3.93 (3.22-4.80)	4.99 (4.26-5.83)	3.76 (2.83-5.01)	5.26 (4.68-5.92)
C_{trough} (mg/L)	0.507 (0.406-0.633)	0.517 (0.409-0.654)	0.233 (0.195-0.280)	0.716 (0.522-0.982)	0.499 (0.393 -0.635)

[a] PK parameters are depicted as geometric mean (95% CI).

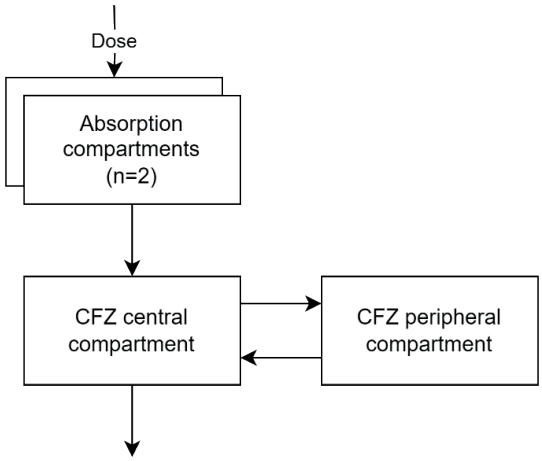


Figure S1. Schematic representation of the CFZ pharmacokinetic model

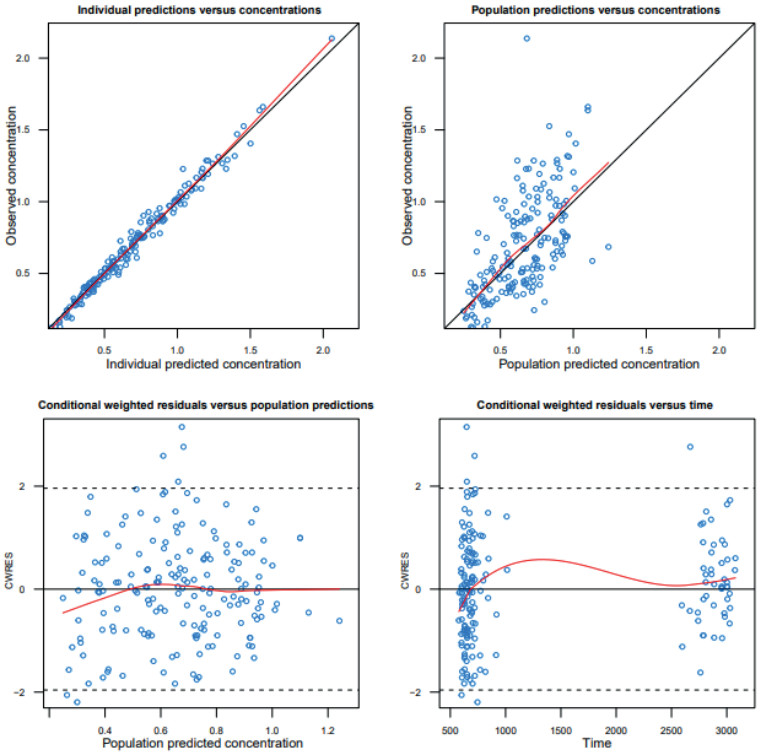


Figure S2. Goodness-of-fit plots for the final CFZ population PK model
Top left: Observed CFZ concentrations versus individual model predicted concentrations. Top right: Observed CFZ concentrations versus population model predicted concentrations. Bottom left: Conditional weighted residuals versus population model predicted concentrations. Bottom right: Conditional weighted residuals over time after the start of treatment.

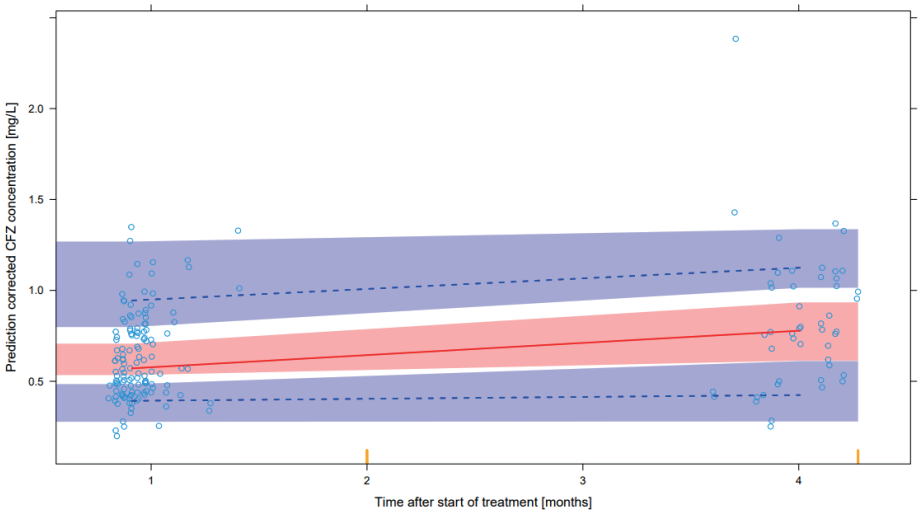


Figure S3. Visual Predictive Checks (VPCs) of CFZ accumulation over time on treatment showing the observed 5th, 50th, and 95th percentiles (lines) and the 95% CI for the same percentiles (shaded areas) calculated from simulated data using the final PK model.

Text S3: Model code

```
$PROBLEM  Clofazimine population pharmacokinetic model
$INPUT    ID TIME DV EVID MDV AMT OCC WT STUDY
$DATA     … .csv IGNORE=@

;Data dictionary
;ID                Subject ID number
;TIME              Time after start treatment (h)
;DV                Observed CFZ concentration (mg/L)
;EVID              Event ID
;MDV               Missing dependent variable
;AMT               Amount (mg)
;OCC               Occasion (0 for no PK sample, 1/2/3 for each PK day)
;WT                Weight (kg)
;STUDY             Study identifier for C-LOAD =0, for PERC =1

$SUBROUTINES ADVAN5
$MODEL
COMP=(DEPOT)
COMP=(TRANSIT)
COMP=(CENTRAL)
COMP=(PERIPHERAL)

$PK
;Allometric scaling parameters
AlloCL = (WT/70)**0.75
AlloV = (WT/70)**1

;PK parameters
TVCL              = THETA(1)
TVV               = THETA(2)
TVKTR             = THETA(3)
TVQ               = THETA(4)
TVVp1             = THETA(5)

highdoseEFF=1
IF(highdose.EQ.1) highdoseEFF = THETA(6)
```

```
;Variability
IIVCL                   = EXP(ETA(1))
IIVKTR                  = EXP(ETA(2))
IOVF = 1
IF(OCC.EQ.1)IOVF        = EXP(ETA(3))
IF(OCC.EQ.2)IOVF        = EXP(ETA(4))
IF(OCC.EQ.3)IOVF        = EXP(ETA(5))

;Parameters
CL                      = TVCL   * IIVCL    * AlloCL
V                       = TVV               * AlloV
KTR                     = TVKTR * IIVKTR
Q                       = TVQ               * AlloCL
Vp1                     = TVVp1             * AlloV

;Bioavailability
F1                      = 1* IOVF *highdoseEFF

;Rate constants
K12 =KTR
K23 =KTR
K30 =CL/V
K34 =Q/V
K43 =Q/Vp1

$ERROR

IPRED = A(3)/V
```

;Error model
PROPERR= EPS(1)
ADDERR= EPS(2)
Y = IPRED *(1+ PROPERR)+ADDERR

$THETA
(0, 4.17) ; 1 CL
(0, 460) ; 2 V
(0, 0.683) ; 3 KA
(0, 24.6) ; 4 Q
(0, 10100) ; 5 Vp1
(0, 0.736) ; 6 F highdose

$OMEGA
0.55 ; 1 IIV CL
0.501 ; 2 IIV KTR
$OMEGA BLOCK(1)
0.488 ; 3 IOV F
$OMEGA BLOCK(1) SAME
$OMEGA BLOCK(1) SAME

$SIGMA
0.00639 ; Proportional error
0.00174 ; Additive error

$ESTIMATION METHOD=1 INTER MAXEVAL=9999 NOABORT PRINT=1
$COVARIANCE

$TABLE …..

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4. Faraj A, Svensson RJ, Diacon AH, Simonsson USH. Drug Effect of Clofazimine on Persisters Explains an Unexpected Increase in Bacterial Load in Patients. Antimicrob Agents Chemother. 2020;64(5).
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Chapter 6

Successful addition of topical antibiotic treatment after surgery in treatment-refractory non-tuberculous mycobacterial skin and soft tissue infections

Ralf Stemkens[1]*, Maarten Cobussen[2]*, Erik de Laat[3], Wouter Hoefsloot[4], Reinout van Crevel[2], Rob E. Aarnoutse[1], Jakko van Ingen[5]

[1]Department of Pharmacy, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[2]Department of Internal Medicine and Infectious Diseases, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[3]Department of Plastic Surgery, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[4]Department of Pulmonary Diseases, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, The Netherlands

[5]Department of Medical Microbiology, Radboud university medical center, Nijmegen, The Netherlands

*Contributed equally.

Antimicrob Agents Chemother. 2023 Dec 14;67(12):e0078823

Abstract

Treatment of skin and soft-tissue infections with nontuberculous mycobacteria sometimes fails despite repeated debridements and long-term systemic antibiotic therapy. These treatment-refractory infections can cause significant morbidity and pose a treatment challenge. Following surgery, we treated three patients with negative pressure wound therapy with instillation and dwell time of topical antibiotics, in addition to systemic antibiotic treatment. Treatment was successful and well tolerated except for some local irritation.

Case Presentations

Case 1

A 44-year-old woman presented with a treatment-refractory tenosynovitis of metacarpophalangeal (MCP) joints 2-4 of the right hand due to *Mycobacterium chelonae,* despite multiple debridements and 10 months of systemic antimycobacterial treatment (Figure 1a).

Case 2

A 59-year-old male presented with a chronic and treatment-refractory soft tissue infection of the right shoulder with a fistula with *Mycobacterium fortuitum*. The patient had been treated with several combinations of anti-mycobacterial drugs and repeated debridements (Figure 1b).

Case 3

A 64-year-old male presented with a treatment-refractory tenosynovitis of the extensor tendon compartments 2-4 of the left wrist, due to *Mycobacterium intracellulare*. Despite long courses of antibiotic treatment and repeated surgical interventions, cultures remained positive (Figure 1c).

None of the patients had a known immune disorder or foreign material at the site of infection. All NTM isolates in these cases were identified using whole genome sequencing, using the Illumina-MISeq platform (Illumina, San Diego, CA, USA). Antimicrobial susceptibility testing was performed by broth microdilution according to CLSI guidelines applying the SensiTitre mycobacteria plates (Trek Diagnostics, Breda, NL) (Table 1). Outcome definitions were based on NTM-NET (1).

Challenge question

What is the optimal treatment for treatment-refractory skin and soft tissue infections caused by nontuberculous mycobacteria?

A. Systemic antibiotic treatment based on *in vitro* susceptibility
B. Surgical debridement in addition to systemic antibiotic treatment based on *in vitro* susceptibility
C. Option B, but with adjunctive topical antibiotics after surgery
D. There are no treatment options with proven efficacy

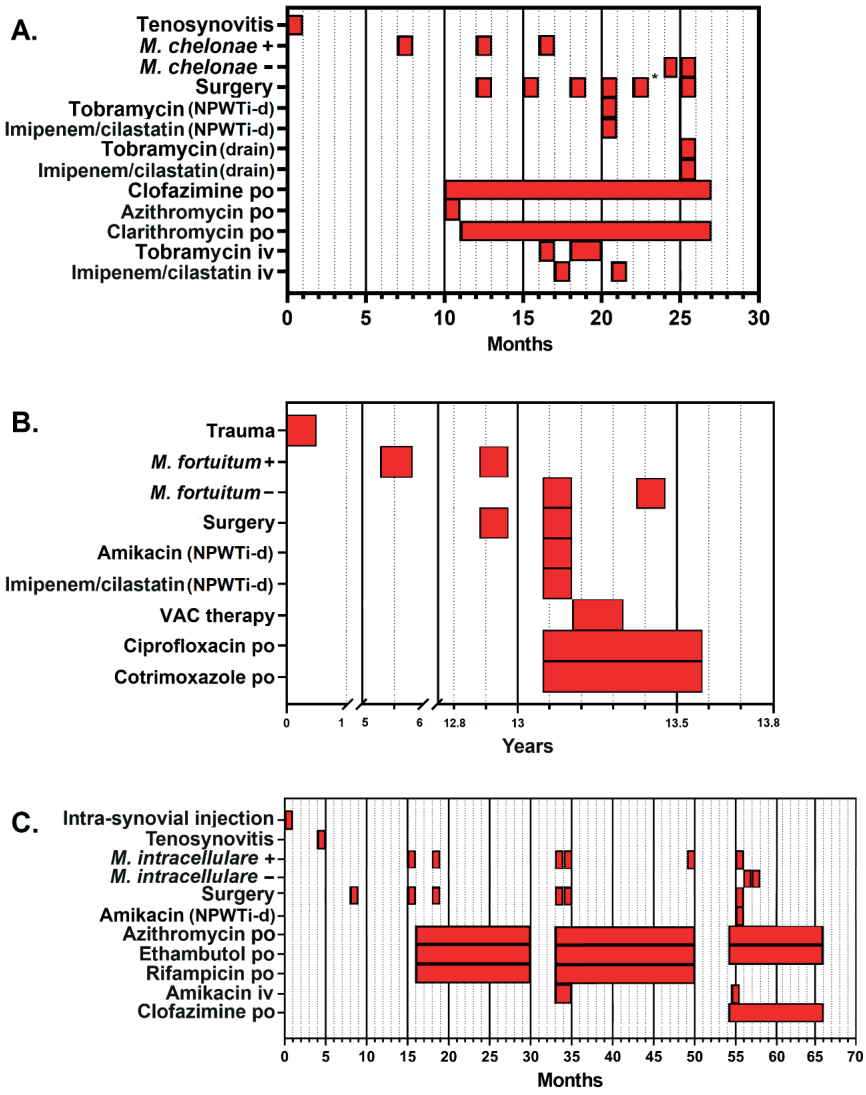

Figure 1. (A) Timeline of antibiotic treatment and surgery of case 1. *M. chelonae* +; culture positive. *M. chelonae* -; culture negative. T = 32 yielded negative results for mycobacterial cultures (data not shown in figure). NPWTi-d: negative pressure wound therapy with instillation and dwelling; po: per os; iv: intravenous. * Split skin graft used to close the wound. **(B)** Timeline of antibiotic treatment and surgery of case 2. Due to a very long treatment history (13 years between trauma and NPWTi-d), which largely took place in other hospitals, only the first isolation of *M. fortuitum* was captured for the first 12.5 years on the timeline. During the course of 13 years the patient had surgery at least 14 times with the development of a fistula in more recent years. Also, the patient was treated during at least 5 episodes with different combinations of clarithromycin, ciprofloxacin, cotrimoxazole and amikacin, without success. *M. fortuitum* +; culture positive. T = 5.7, T = 7.8, T = 8.4, and T = 9.2 all yielded positive results for *M. fortuitum* on culture (data not shown in figure). *M. fortuitum* -; culture negative. T = 14.1 years yielded negative results for mycobacterial cultures (data not shown in figure). **(C)** Timeline of antibiotic treatment and surgery of case 3. *M. intracellulare* +; culture positive. *M. intracellulare* -; culture negative.

Treatment and outcome

We have applied a novel treatment strategy for selected patients with treatment-refractory nontuberculous mycobacterial (NTM) skin and soft tissue infections (SSTI). We considered infections to be treatment-refractory if no cure was achieved despite guideline-compliant antibiotic treatment and surgical debridement. Our new strategy for these infections was to combine systemic antibiotic therapy and debridement (with synovectomy or fistulectomy) with adjunctive negative pressure wound therapy (NPWT) with instillation and dwell time of antibiotics (NPWTi-d), using a Vacuum Assisted Closure (VAC) system (V.A.C. VERAFLO Therapy, KCI USA Inc., San Antonio, Texas). NPWT uses subatmospheric pressure to remove excess exudate and infectious material from the wound and promotes blood flow, tissue granulation and wound healing (2). With NPWTi-d, NPWT cycles alternate with instillation of a topical solution on a sponge (inserted under aseptic conditions during surgery), to further facilitate wound cleansing and healing (2). NPWTi-d has been used to treat a variety of wound infections (2, 3), yet no published data are available on its use in NTM infections.

In all our cases, NPWTi-d was initiated immediately after debridement and performed in a cyclic manner; 6 cycles of 4 hours per day. Each cycle consisted of 30 minutes instillation and dwelling of an antibiotic in the wound, followed by 3.5 hours of NPWT. The instillation volume was set by the wound specialist. The sponge covering the wound was completely saturated with antibiotic fluids to maximize exposure of the wound surface to the antibiotics. NPWTi-d was performed for 1-2 weeks, although the optimal duration is unknown. The microbiological perspective, favouring longer treatment with topical antibiotics, had to be balanced against factors associated with the required prolonged hospitalization (patient's wishes, logistics, costs).

The selection of antibiotics for NPWTi-d therapy was NTM specific and based on current guidelines and in vitro susceptibility (Table 1) (4, 5). The concentrations of the antibiotics were derived from existing experience with other topical applications (eye and ear drops) (6-8). We assumed that these concentrations would be safe and effective for NPWTi-d as well. The solutions for topical application were made from commercially available drug preparations meant for intravenous application. During combination therapy, only one antibiotic was instilled during each cycle because of unknown compatibility in one solution. All patients received an aminoglycoside (tobramycin (10 mg/ml) or amikacin (50 mg/ml)), either as monotherapy or combined with imipenem/cilastatin (5/5 mg/ml). It should be noted that imipenem is relatively unstable and the abovementioned solutions should be replaced in a timely manner to prevent degradation (9).

Aminoglycosides exhibit concentration dependent killing and NPWTi-d enabled the administration of very high concentrations at the site of infection (up to a 1000-fold of peak plasma concentrations after IV administration) (10). This makes aminoglycosides attractive drugs for this treatment modality.

Imipenem/cilastatin was administered 4 times daily, considering that beta-lactam antibiotics exhibit time dependent killing. The administered concentration was ~1000-fold higher than reference trough plasma concentrations of imipenem (11). The exposure to imipenem/cilastatin was only 30 minutes per cycle, but we hypothesized that sufficient drug would diffuse into soft tissue to allow for adequate inhibition of mycobacteria.

Furthermore, we theorize that the abovementioned high concentrations may overcome antibiotic resistance, which is another potential benefit of NPWTi-d.

Table 1. Minimum inhibitory concentrations (in mg/L) of patient isolates and interpretation

	Case 1 M. chelonae	Case 2 M. fortuitum	Case 3 M. intracellulare
Amikacin	32 (I)	<1 (S)	8 (S)
Tobramycin	2 (S)	>16 (R)	n.a.
Clarithromycin	0.25 (S)	>16 (R, inducible)	2 (S)
Clofazimine	0.25	n.a.	0.12
Imipenem	16 (I)	4 (S)	n.a
Cotrimoxazole	>4 (R)	<0.25 (S)	n.a.
Ciprofloxacin	4 (R)	<0.12 (S)	n.a.
Moxifloxacin	2 (I)	<0.25 (S)	4 (R)
Linezolid	8 (S)	4 (S)	16 (I)

Note: interpreted according to CLSI M24-A3 guidelines at the time of initiating topical treatment, interpretation within brackets (S: susceptible, I: intermediate susceptibility. R: resistant); n.a. not applicable

Case 1

NPWTi-d was initiated after debridement and synovectomy and consisted of 4 consecutive cycles with instillation of imipenem/cilastatin (5/5 mg/ml) and 2 consecutive cycles with tobramycin (10 mg/ml) per day. In addition, IV imipenem/cilastatin 500/500mg (4 times daily) was started. Therapeutic drug monitoring (TDM) during NPWTi-d showed no detectable plasma concentration of tobramycin. Local irritation occurred during instillation of antibiotics and vacuum, but was manageable with analgesics (paracetamol, 1000 mg 4 times daily; piritramide via

PCA pump following surgery which was replaced after a few days by oxycodone slow release, 5 mg 2 times daily; oxycodone immediate release as needed). NPWTi-d was continued for 14 days. After discharge, the patient continued with imipenem/cilastatin (IV) for 2 months and clofazimine and clarithromycin (oral) for 6 months after debridement.

Four months after surgery the patient developed new symptoms of pain and swelling. Surgical debridement showed a granuloma, with negative mycobacterial cultures. Local treatment with imipenem/cilastatin and tobramycin was initiated via a local drain system, because the VAC system was not suitable for the small wound size, and was discontinued after 5 days upon the patient's request. Oral antibiotic treatment was completed 6 months after initial NPWTi-d. Cure was achieved and the patient regained function of the right hand. After 20 months of follow-up there were no signs of relapse.

Case 2

Surgical debridement and fistulectomy were performed and NPWTi-d was initiated consisting of 4 consecutive cycles with instillation of imipenem/cilastatin (5/5 mg/ml) and 2 consecutive cycles with amikacin (50 mg/ml) per day. TDM during NPWTi-d showed no detectable plasma concentration of amikacin. Local pain occurred during instillation and vacuum, which was manageable with analgesics (paracetamol, 1000 mg 4 times daily; oxycodone 5 mg as needed). NPWTi-d was continued for 14 days. Treatment with ciprofloxacin and cotrimoxazole was continued for 4 months after discharge. Microbiological cure was achieved, but after 19 months of follow-up a fistula remained.

Case 3

Following surgical debridement and synovectomy, NPWTi-d was initiated, consisting of 6 cycles with amikacin (50 mg/ml) per day. In addition, the patient was treated with azithromycin, ethambutol and clofazimine orally, and peri-operative amikacin IV (3 days). TDM during NPWTi-d showed a low amikacin plasma concentration (1 mg/L), which was most likely residual exposure after the last IV dose. NPTWi-d was continued for 1 week after which the wound was closed surgically. The systemic treatment has been ongoing for 10 months and will be continued until 12 months after debridement. A mild tenosynovitis remains, but without evidence of microbiological failure.

Treatment-refractory NTM SSTIs remain a challenge. The use of topical antibiotics as adjuvants to surgery and systemic antibiotic treatment was successful in these

3 patients. No relapses have occurred in the first two cases, although two stages of surgical debridement in combination with topical antibiotic treatment were required for case 1. The treatment of case 3 is ongoing, with conversion to negative cultures obtained during treatment. Local irritation occurred in two cases, but was manageable with analgesics. No systemic toxicity was observed and topical treatment with aminoglycosides did not lead to systemic exposure. Although these outcomes are promising, it should be noted that the precise relationship between using NPWTi-d and the observed clinical responses cannot be established based on this small case series. More data is needed to evaluate the efficacy and safety of using NPWTi-d for these difficult to treat infections. Also, there is literature that cautions against the use of local antibiotics via NPWTi-d due to concerns about resistance development (2). Considering the very high concentrations of antibiotics that we used we deem resistance development unlikely.

We propose that topical application of antibiotics using NPWTi-d, following surgery and combined with systemic antibiotic treatment, could be considered for patients with treatment-refractory NTM SSTIs. Follow-up studies are needed to evaluate the efficacy and safety of this treatment modality.

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Chapter 7

General discussion

The overarching aim of this thesis was to contribute to dose and exposure optimization of available (old – *but not outdated*) antimycobacterial drugs in the treatment of TB and NTM disease. We evaluated the ten-year results of an international QC program for measurement of TB drugs, since accurate measurement of drug concentrations is essential to perform PK studies (chapter 2). In the following chapters we evaluated the PK (and safety) of available drugs with currently recommended doses (pyrazinamide; chapter 3) and experimental doses (clofazimine; chapter 4), the effect of high-dose rifampicin on the exposure to other drugs (chapter 5), and the impact of an alternative mode of administration, at the site of action, on outcomes (topical antimycobacterial drugs; chapter 6). The research questions, main findings and implications of each of the chapters of this thesis are summarized in table 1.

In this final chapter the results are put in perspective. Five main topics will be discussed:

- Dose and exposure optimization of available antimycobacterial drugs, focusing on: pyrazinamide, clofazimine, rifampicin, and alternative modes of administration
- New treatment options for TB and NTM disease
- Dose individualization through therapeutic drug monitoring
- Clinical pharmacological methodologies and techniques
- Interplay between multidisciplinary patient care and research

Table 1. Research questions, main findings and implications of each of the chapters in this thesis

Chapter	Disease	Investigated drug(s)	Research question(s)
Chapter 2	N.A.	First-line and second-line anti-TB drugs[a]	What is the quality of measurement of anti-TB drugs concentrations in an external (interlaboratory) QC program over a 10-year period?
Chapter 3	TB	Pyrazinamide	1. What is the PK profile of pyrazinamide during the initial phase of treatment in patients with TB meningitis? 2. How well does pyrazinamide penetrate in the CSF
Chapter 4	TB	Rifampicin (PHENORIF study)	What is the drug interaction potential of high-dose rifampicin (40 mg/kg) compared to standard-dose rifampicin (10 mg/kg)?
Chapter 5	NTM disease	Clofazimine (C-LOAD study)	1. Does the application of a loading dose of clofazimine result in improved PK endpoints in patients with NTM disease? 2. Is a loading dose regimen of clofazimine safe and well-tolerated?
Chapter 6	NTM disease	Various antimycobacterial drugs (topical administration)[b]	Can topical administration of high concentrations of antimycobacterial drugs, following surgery, improve treatment outcomes in patients with treatment-refractory NTM skin- and soft tissue infections?

Abbreviations: N.A.: Not applicable, TB: tuberculosis, NTM: nontuberculous mycobacteria, QC: quality control, PK: pharmacokinetics, CSF: cerebrospinal fluid, CYP: cytochrome P450, SSTIs: skin- and soft tissue infections.

[a] The external QC program for measurement of anti-TB drugs covers 13 anti-TB drugs and metabolites

[b] Topical administration of an aminoglycoside (amikacin or tobramycin) with or without imipenem/cilastatin

	Main findings	Implications
	• The QC program expanded in both number of participants and number of anti-TB drugs and metabolites covered. • 83.2% of all measurements were accurate. • The absolute inaccuracy was related to the measured anti-TB drug and to the performing laboratory, but not to the concentration level or to the analytical technique used.	The 10-year results illustrate the need for continuous external QC for the measurement of anti-TB drugs. Participation of laboratories in the program either confirms their level of intra-laboratory quality assurance or alerts them to inaccuracies and underlying undetected problems.
	• Pyrazinamide exposure in plasma decreased between day 2 and day 10 of treatment, possibly due to a drug interaction with rifampicin (unknown mechanism). • Pyrazinamide penetrates well in the CSF (high CSF-to-plasma ratio). • The dose of pyrazinamide, exposure in plasma, and CSF concentration were highly correlated.	The results provide a rationale for follow-up research into the pharmacokinetics, efficacy and safety of higher doses of pyrazinamide for TB meningitis.
	High-dose rifampicin resulted in: • No additional effect on CYP1A2. • Mild additional induction of CYP2C9, CYP2C19, CYP2D6 and CYP3A. • Marginal additional inhibition of P-gp.	Existing recommendations on managing drug interactions with rifampicin can remain unchanged for the majority of co-administered drugs when using high-dose rifampicin.
	• A loading dose of 300 mg once daily for 4 weeks reduced the time to steady-state-like concentrations and was safe/well-tolerated. • Model-based simulations indicated that a six-week loading phase could further decrease the time to steady-state-like concentrations.	These results show the feasibility of a loading dose of clofazimine as a strategy to optimize treatment of NTM disease.
	The use of topical antibiotics as adjuvants to surgery and systemic antibiotic treatment was successful in 3 patients: • Microbiological cure was achieved in all patients. • Local irritation occurred in 2 patients, but was manageable with analgesics. • Topical administration of aminoglycosides did not lead to systemic exposure.	• Topical application of antibiotics, using negative pressure wound therapy with instillation and dwell time of topical antibiotics (NPWTi-d), following surgery and combined with systemic antibiotic treatment, could be considered for patients with treatment-refractory NTM SSTIs. • Follow-up studies are needed to evaluate the efficacy and safety of this treatment modality.

Dose and exposure optimization of available antimycobacterial drugs

The majority of available antimycobacterial drugs that are used to treat TB and/or NTM disease were developed decades ago. Despite (or perhaps because of) their long existence the currently used doses of these drugs are often not well substantiated. Optimizing the doses of (and exposures to) available drugs, based on their PK and PD properties, is an important strategy to utilize their full potential and to improve treatment of these mycobacterial infectious diseases. This part of the discussion builds further on the main research findings. The drugs that were studied in this thesis are discussed, our findings are put in perspective, and future directions are described.

Pyrazinamide

Pyrazinamide has been a key first-line anti-TB drug since its addition to a rifampicin-based regimen resulted in treatment shortening from nine to six months for pulmonary TB (1, 2). The optimal dose of pyrazinamide is unknown. The WHO currently recommends a daily dose of 20-30 mg/kg (3). This is lower than the doses used (30-40 mg/kg) in clinical trials that showed the treatment shortening potential of pyrazinamide for pulmonary TB in the 1970/80s (1, 2, 4).

The efficacy of pyrazinamide is exposure-dependent. A target $AUC_{0\text{-}24h}$/minimal inhibitory concentration (MIC) ratio of 209 in the lung was derived from a hollow fiber model (5). In clinical studies, lower exposures have been associated with worse outcomes in several studies. A C_{max} of < 35 mg/L was associated with worse outcomes in patients with pulmonary TB (69% with HIV/TB co-infection) in Botswana (6). In a South-African study among pulmonary TB patients, an $AUC_{0\text{-}24h}$ of ≤ 363 mg*h/L was among the most important predictors of poor long-term outcomes. In the same study, lower sputum culture conversion rates were observed in patients with a C_{max} of ≤ 58 mg/L (7). In addition, an $AUC_{0\text{-}24h}$/MIC ratio of > 11.3 in plasma is a proposed PK/PD target (8). Model-based simulations showed that these targets (C_{max}, $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}24h}$/MIC) are often not achieved with currently recommended doses (9). Further evidence for exposure-response relationships is provided in more recent studies. In a meta-analysis, including eight studies and a total of 389 patients, low pyrazinamide concentrations were found to increase the risk of poor outcome (10). In addition, PK-PD assessments of PK data from three phase 2 trials showed that higher pyrazinamide C_{max} values were associated with higher culture conversion rates. The greatest effect was observed when higher pyrazinamide exposures were accompanied with higher rifampicin exposures (11).

Based on these studies it appears that higher pyrazinamide exposures could be more efficacious. On the other hand, higher exposures may lead to increased toxicity of pyrazinamide (mainly hepatoxicity). Pyrazinamide doses of 40-50 mg/kg for longer periods of time caused high rates of hepatoxicity in earlier studies (11, 12). However, in a more recent meta-analysis high doses of up to 60 mg/kg were not associated with increased hepatotoxicity (13). The latter corresponds with another meta-analysis showing no increased risk of hepatotoxicity until an $AUC_{0\text{-}168h}$ (AUC over the course of a week) of > 5000 mg*h/L (14). Such exposures are high, as shown by median $AUC_{0\text{-}168h}$ values of 2100-2310 mg*h/L with median pyrazinamide doses of 23-26 mg/kg in a combined analysis of three studies (11).

The data above are mainly derived from studies in pulmonary TB, but optimizing exposures is also relevant to TB meningitis, the most lethal manifestation of TB (15-18). Pyrazinamide is also part of the first-line treatment regimen for TB meningitis. This regimen, including the doses of pyrazinamide and other anti-TB drugs, is based on pulmonary TB. In order to reach the site of infection, anti-TB drugs have to cross the blood–brain barrier or blood–cerebrospinal fluid (CSF) barrier. Rifampicin and ethambutol do not penetrate in the CSF well (17-19). In **chapter 3** we studied the PK of pyrazinamide during the initial phase of treatment in Indonesian patients with TB meningitis. We found that the pyrazinamide plasma exposure decreased by 26% from day 2 to 10, possibly as a result of a drug interaction with rifampicin, in line with a previous study (20). Furthermore, we found that pyrazinamide penetrates well in the CSF with a high mean CSF-to-plasma concentration ratio of 90% and a mean CSF concentration of 42 mg/L which corresponds with reference ranges for plasma C_{max} (21). Also, we found a strong correlation between the pyrazinamide dose, plasma exposure and CSF concentration. These findings support and further substantiate the important role of pyrazinamide in the treatment of TB meningitis and provide a rationale for studying higher dose of pyrazinamide for TB meningitis.

Future perspectives for pyrazinamide

There is a clear rationale for studying the efficacy and safety of higher pyrazinamide doses for TB. Currently, the efficacy of high-dose pyrazinamide (2000/2400 mg for below/above 50 kg) as part of a short 12-week regimen, in combination with high-dose rifampicin (2100 mg), isoniazid and moxifloxacin, is investigated in patients with pulmonary TB in a multi-arm phase 2B/C study (STEP2C, clinical trials.gov identifier: NCT05807399). In another ongoing study in pulmonary TB patients, a 4-month regimen with high-dose pyrazinamide (40 mg/kg) and rifampicin (35 mg/kg) combined with standard doses of isoniazid and ethambutol is being investigated (22). Although these developments are promising, there are currently

no studies investigating the efficacy of higher pyrazinamide doses for the treatment of TB meningitis. Our findings in **chapter 3** imply that, from a PK viewpoint, the same dosing strategy for pyrazinamide can be used in TB meningitis as for pulmonary TB. This also means that, in line with ongoing studies for pulmonary TB, studies investigating the efficacy and safety of higher doses of pyrazinamide (in combination with high-dose rifampicin) in TB meningitis are warranted.

Rifampicin

Rifampicin is a pivotal first-line anti-TB drug with strong sterilizing activities. The drug was approved in 1971 and several trials showed that rifampicin-containing regimens were very effective. The standard daily dose since its approval has been 10 mg/kg, but this this dose is not well substantiated. A review of historical literature revealed that the recommended dose was not based on optimal efficacy but on other reasons: serum concentrations were found to be above the MIC of *M. tuberculosis*, combined with a fear of toxicity, and associated high costs of rifampicin (23).

A growing body of evidence supports the use of higher doses of rifampicin in TB treatment. Several clinical studies have shown that increasing the rifampicin dose leads to a more than proportional increase in exposure (24-26). Doses of 35-40 mg/kg resulted in a 7-to-10-fold higher $AUC_{0\text{-}24h}$ than with a standard dose of 10 mg/kg (25-27), which corresponds well with our findings in **chapter 4**. Higher rifampicin doses, and in turn higher exposures, have shown to increase early bactericidal activity in patients with pulmonary TB (25, 27, 28). A higher dose of daily 35 mg/kg also shortened the time to culture conversion, which may suggest that it bears the potential for treatment shortening (26, 29). Doses up to 40 mg/kg administered for 2 weeks were safe and well-tolerated and did not result in more toxicity than the standard dose (25, 27). Three months of a dose of 35 mg/kg was also safe and tolerable (26). So called 'staggered' dosing of 20 mg/kg for 7 days followed by the higher 35-40 mg/kg dose is predicted to further improve tolerability while maintaining exposure levels associated with better efficacy (30). However, in one study exposure-related adverse events (including hyperbilirubinaemia) were observed with a dose 50 mg/kg and as a result 40 mg/kg was suggested to be the maximum tolerated dose (27).

A more recent development is the use of 'flat' doses of rifampicin rather than dosing on a mg/kg base, considering that the latter only yields a small and non-clinically relevant decrease in interpatient variability in exposure (31). Very recently, in a phase 3, randomized, controlled trial (RCT) two short 4-month regimens with higher 'flat' rifampicin doses of 1200 mg and 1800 mg were compared to a standard 6-month regimen in patients with pulmonary TB. The high-dose rifampicin regimens did not

meet the noninferiority criteria compared with the standard regimen. Nonetheless, all arms showed high favorable response rates (90% and 87% in the 1200 mg and 1800 mg groups, respectively, versus 93% in the control group) (32).

Optimization of the rifampicin dose is also particularly relevant for TB meningitis. Rifampicin does not penetrate well in the CSF, with a reported CSF-to-plasma partition coefficient of 5.5% (33), leading to suboptimal CSF concentrations with a standard dose (19). However, it should be noted that this coefficient is based on total plasma concentrations but the free (unbound) fraction of rifampicin in plasma is only ~10% (34). Several clinical studies have been performed in patients with TB meningitis, showing that higher doses lead to higher CSF concentrations (18, 35, 36). Studies with increased rifampicin doses have yielded varying results with regards to clinical outcome. In one study, the mortality rate was lower with an intravenous rifampicin dose of 13 mg/kg compared to a standard oral dose of 10 mg/kg (35% versus 65%) (35). In a phase 3 study, a slightly increased rifampicin dose of 15 mg/kg was not associated with higher survival rates than a standard dose of 10 mg/kg (37). In a phase 2 trial with higher rifampicin doses, there appeared a trend of reduced mortality with dose of 30 mg/kg (18), but the study was not powered for efficacy. Another phase 2 study found no association between higher rifampicin doses (35 mg/kg oral and 20 mg/kg intravenously) and reduced mortality (36), but also this study was not powered for efficacy. A meta-analysis of three phase 2 trials showed a clear survival benefit with higher doses of rifampicin (33). An increase in 6-month survival from 50% to 70% was predicted when increasing the oral rifampicin dose from 10 to 30 mg/kg. Furthermore, model-based simulations predicted that even higher doses (> 30 mg/kg) may further improve survival (33). Higher rifampicin doses (oral, 30-35 mg/kg; intravenous, 20 mg/kg)) were safe in three phase 2 trials on TB meningitis (18, 36, 38).

A challenge with the use of rifampicin is its well-known capacity to cause pharmacokinetic drug interactions (39). Rifampicin acts as an activator of nuclear pregnane X receptor (PXR) which in turn induces many metabolic enzymes and drug transporters, including various cytochrome P450 (CYP) iso-enzymes, glucuronosyltransferases (UGTs), and P-glycoprotein (P-gp). The result of the potent induction by rifampicin is often a decrease in exposure to other drugs, including (but not limited to) antiretroviral drugs, antibiotics, anti-diabetic drugs, and cardiovascular drugs including calcium channel blockers and anticoagulants (39, 40). In **chapter 4** we aimed to answer an important question related to the use of higher rifampicin doses for TB: do higher rifampicin doses cause more severe drug interactions than a standard dose? We found that high-dose rifampicin

(40 mg/kg) did not result in an additional effect on CYP1A2, mild additional induction of CYP2C9, CYP2C19, CYP2D6, and CYP3A, and marginal inhibition of P-gp, as compared to a standard dose of rifampicin (10 mg/kg). The largest effects in this study were observed for midazolam (CYP3A) and omeprazole (CYP2C19). RIF40 reduced midazolam and omeprazole exposures by 38% and 45%. However, the additional inductive effects of high-dose rifampicin are much smaller than the effects of a standard dose compared to no rifampicin (figure 2 of chapter 4). It has been shown that rifampicin (600 mg daily) versus no rifampicin causes ~96% (~20-fold) and ~90% (~10-fold) reductions in midazolam and omeprazole exposures, respectively (41, 42). Therefore, we suggest that existing recommendations on managing drug interactions with rifampicin can remain unchanged for the majority of co-administered drugs when using high-dose rifampicin. This important finding aids the (future) implementation of higher doses of rifampicin in clinical practice.

Future perspectives for rifampicin

Great strides have been made in the dose optimization of rifampicin for TB. Currently several studies with higher rifampicin doses are ongoing. This includes the (previously mentioned) STEP2C trial which evaluates the efficacy and safety of 3- and 4-month regimens with a daily rifampicin dose of 2100 mg, combined with isoniazid, moxifloxacin and standard-dose or high-dose pyrazinamide, in patients with pulmonary TB. This study is expected to provide important new data on the treatment shortening potential of high-dose rifampicin-based regimens for pulmonary TB. For patients with TB meningitis, important insights will likely come from the HARVEST study (43). In this phase 3 RCT the survival rates of a high-dose rifampicin (35 mg/kg) – based regimen will be compared to the standard of care. The results of these trials (and several others) will determine the role of higher (optimized) rifampicin doses in the treatment of TB. Finally, while the use of optimized rifampicin doses is a promising strategy to improve TB treatment, it currently causes a significant additional pill burden for patients. Therefore, ideally new drug formulations will be developed in the future.

Clofazimine

Clofazimine is probably most well-known for its role in the treatment of leprosy. However, initially it was introduced as a very promising anti-TB drug in the 1950s (44). Although clofazimine was very effective against *M. tuberculosis in vitro*, the excitement did not last long. Due to inconsistent results in early animal studies as well as the emergence of other potent anti-TB drugs (isoniazid and rifampicin), the drug was no longer prioritized (45). However, clofazimine proved to be a potent drug in the treatment of leprosy in the 1960s, and remains a part of treatment

regimens for leprosy to date (46). With the emergence of drug-resistant TB (DR-TB), there came renewed interest in clofazimine. Several clinical studies have demonstrated the efficacy of clofazimine in the treatment of MDR-TB (47-50). This is also reflected in WHO guidelines. In 2008 clofazimine was still considered a group 5 drug ('drugs of unclear role in DR-TB') (51). Currently, clofazimine is considered a Group B drug and is part of a recommended 9-month regimen that is preferred over longer (18-month) regimens in the WHO guideline for TB (52).

Clofazimine has also gained interest as a promising drug in the treatment of NTM disease. It is highly active, *in vitro*, against common pathogens MAC and *M. abscessus*. Also, clofazimine is synergistic with two key antibiotics, clarithromycin and amikacin, against MAC and *M. abscessus* (53, 54). In the current guideline for NTM-PD, clofazimine is considered an alternative for first-line drug rifampicin (combined with a macrolide and ethambutol), for the treatment of MAC-PD (55). Several retrospective studies reported comparable efficacy for clofazimine-based regimens and rifampicin-based regimens for MAC-PD (56-58). Randomized controlled trials (RCTs) are scarce in NTM disease. The results of a (recently published) RCT (PERC) were in line with previous studies, showing similar culture conversion rates for clofazimine-based and rifampicin-based regimens, although noninferiority could not be proven due to the limited sample size (59). Nonetheless, clofazimine should be considered as a treatment option for MAC-PD and individual patient characteristics (such as potential drug interactions with rifampicin or QTc-prolongation with clofazimine) should be taken into account when selecting a treatment regimen. For NTM-PD due to *M. abscessus,* clofazimine is already a first-line oral drug (55).

While clofazimine has been increasingly recognized as an important drug for NTM disease and TB, the optimal dose is unknown (60). The WHO currently recommends a daily dose of 100 mg for DR-TB disease (52). Interestingly, in 2008 the WHO still recommended a dose range of 100-300 mg, but the reason for this change was not stated. The common daily clofazimine dose for NTM disease is also 100 mg, although a range of 100-200 mg per day is recommended in the latest guideline on NTM-PD (55). In addition, there is experience with higher doses of up to 300 mg per day in the treatment of DR-TB, and even higher daily doses of 600 mg have been reported in old publications on leprosy (61-64). Currently, the highest recommended dose is 300 mg per day for a maximum of 12 weeks (for erythema nodosum leprosum) after which the dose is tapered (65, 66).

Studies evaluating dose-exposure relationships for clofazimine are scarce, both in TB and NTM disease. In a murine TB model clofazimine contributed to sustained antimycobacterial activity after discontinuation, which was associated with plasma concentrations above 0.25 mg/L (67). Dose-dependent efficacy of clofazimine (12.5-25 mg/kg, estimated to be equivalent with a human dose of 100-200 mg) was also observed in another murine TB model (68). However, in yet another murine TB model no dose-dependent bactericidal activity was observed during treatment (69). In a prospective cohort study, a clofazimine $AUC_{0\text{-}24h}$/MIC ratio of ≥50 was associated with faster culture conversion in patients with MDR/RR-TB. In another study on MDR-TB, patients in whom culture conversion was achieved had higher $AUC_{0\text{-}24h}$/MIC ratios after two and six months of treatment ($AUC_{0\text{-}24h}$/MIC: 116 versus 64 and 101 versus 52, respectively) (70, 71). The available data for NTM disease are even more limited. In a South Korean study with 58 patients treated with clofazimine for NTM-PD, lower MICs (≤ 0.25 mg/L) were associated with higher culture conversion rates in patients without amikacin or clarithromycin resistance, yet not in the overall population (72). In a recent RCT (PERC trial), there was a significant difference in the geometric mean of the highest measured clofazimine concentrations between patients with and without culture conversion (0.95 vs 0.51 mg/L; P = .018), but this was based on only 19 participants for whom clofazimine PK data were available (59). Data regarding dose-toxicity (or exposure-toxicity) relationships of clofazimine are also scarce. Clofazimine commonly causes gastro-intestinal (GI) and skin-related adverse effects (including dry skin, pruritus and skin discoloration), but is generally tolerated well (50, 73, 74). It can also cause concentration-dependent QTc prolongation, particularly when combined with other QTc-prolongating drugs (75-78).

Clofazimine is characterized by complex PK. It is highly protein bound, very lipophilic and thus accumulates particularly in fatty tissues and macrophage-rich organs. This results in a very long elimination half-life of ~30-70 days and it takes months to reach steady state concentrations in plasma (50, 60). This implies that clofazimine does not contribute fully to TB and NTM treatment regimens for months. Indeed studies in mice and humans have shown that clofazimine does not exhibit early bactericidal activity against *M. tuberculosis* during the first two weeks of treatment (69, 75). We believe that treatment efficacy could be improved by using a higher dose at the start of treatment, i.e. a loading dose, to faster achieve concentrations similar to those at steady-state (steady-state-like concentrations). In **chapter 5** we studied the pharmacokinetics and safety/tolerability of a loading dose regimen in patients with NTM disease at the Radboudumc. We found that a loading dose of 300 mg once

daily for four weeks, followed by a standard maintenance dose of 100 mg once daily, resulted in steady-state-like concentrations faster compared to no loading dose. Importantly, the loading dose was safe and well-tolerated in this study. Model-based simulations predicted that this 4-week loading dose regimen reduces the time to steady-state-like concentrations by 1.5 months compared to no loading dose (3.8 versus 5.3 months). The optimal loading dose regimen was predicted to be a dose of 300 mg QD for six weeks, achieving a time benefit of 3.9 months (1.4 versus 5.3 months), without exceeding steady-state peak concentrations achieved with a dose of 100 mg once daily. Furthermore, we found that the bioavailability of clofazimine was 26% lower with a dose of 300 mg compared to a 100 mg dose, which could be explained by dose-dependent saturation of absorption, and further dose increases (400 mg once daily) did not result in additional predicted time benefit. Overall, our results contribute to dose optimization of clofazimine in NTM disease. We did not study the effect of a loading dose on efficacy outcomes and the sample size was small. However, we believe that the clear pharmacological rationale for a loading dose, substantiated with attainment of PK endpoints (reduced time to steady-state-like concentrations) in our study, could be deemed sufficient to implement a loading dose of 300 mg once daily for six weeks in clinical practice. Moreover, this proposed loading dose regimen does not exceed the recommended (and approved) daily dose of 300 mg (100 mg three times a day) for up to 12 weeks, used for erythema nodosum leprosum (66). In addition, doses of up to 300 mg/day have also been used in the treatment of DR-TB and leprosy for long treatment periods of up to 24 months (61-63, 79, 80).

Future perspectives for clofazimine

It is important that the full potential of clofazimine is utilized in patient care and studies aiming to further establish its role in the treatment of NTM disease and TB. While we studied a loading dose in patients with NTM disease, we believe that our findings on the usefulness and feasibility of a clofazimine loading dose strategy could also apply to TB. However, due to significant variability in reported PK of clofazimine between different studies and patient populations the optimal loading dose regimen may vary between study populations (60, 78, 81). A loading dose regimen of 300 mg once daily for two weeks is being investigated in an ongoing phase 2 study in patients with drug-susceptible TB (CLO-FAST study; NCT04311502). In addition to the application of a loading dose of clofazimine, it is important to further study exposure-response (and toxicity) relationships, and define exposure targets of clofazimine in patients with TB and NTM disease, to establish the optimal maintenance dose. Furthermore, efforts have been made to develop less lipophilic clofazimine derivatives, which could lead to less tissue accumulation,

skin discoloration, as well as improved oral bioavailability. One novel compound, pyrifazimine (TBI-166), demonstrated equivalent anti-TB activity and less skin discoloration in mice, and synergistic activity with the same companion drugs as clofazimine (50). This is an interesting development, but clinical data are not yet available (NCT04670120). Another interesting development is a novel clofazimine inhaled suspension (CIS). Animal studies suggest that CIS could lead to higher concentrations in the lungs than oral administration and is well tolerated (82). No clinical data on CIS have yet been published.

Alternative modes of administration in NTM disease

Antimycobacterial drugs are predominantly administered systemically (oral or intravenously). Systemic administration leads to systemic drug exposure (e.g. in plasma) which in turn leads to drug exposure at the site of infection. Striving for optimal plasma exposures to combat infections has to be balanced against (exposure dependent) toxicity. In some cases, different modes of administration that directly target the site of action, may be beneficial. In **chapter 6** we described the use of topical antibiotics as adjuvants to surgery and systemic antibiotic treatment for three patients with treatment-refractory skin and soft tissue (SST) NTM disease. Following surgical debridement, negative pressure wound therapy with instillation and dwell time of topical antibiotics (NPWTi-d) was performed for 1-2 weeks, via a Vacuum Assisted Closure (VAC) system. The topically administered antibiotics included an aminoglycoside (amikacin or tobramycin) with or without imipenem/cilastatin. This system allowed for the administration of high concentrations at the site of infection, that were ~1000-fold higher than typical plasma concentrations that are achieved with systemic administration, without signs of systemic toxicity. This is particularly attractive for aminoglycosides, which exhibit concentration dependent efficacy, whereas intravenous administration commonly causes oto- and renal toxicity (83). Treatment was considered successful in these three patients. Microbiological cure was achieved in all patients. Local irritation occurred in two cases, but was manageable with analgesics. We propose that topical application of antibiotics using NPWTi-d, following surgery and combined with systemic antibiotic treatment, could be considered for patients with treatment-refractory NTM SSTIs.

There are other examples of local administration of antimycobacterial drugs. In a case series on *M. abscessus* otomastoiditis, topical administration of tigecycline and imipenem/cilastatin (ear drops), in combination with systemic antibiotics and adjunctive surgery, led to cure in four children (84). Local administration of antimycobacterial drugs (topical eye drops and intravitreal therapy) has also been described in several case series on NTM ocular and adnexal infections (85, 86). The

use of amikacin inhalation (amikacin liposomal inhaled suspension; ALIS) for NTM-PD is probably the most well substantiated example of local drug administration. Phase 2 and 3 studies have shown that the addition of ALIS to standard guideline-based therapy results in greater culture conversion rates in patients with treatment-refractory MAC-PD (87-89). ALIS is registered for this indication and recommended in the current guideline for NTM-PD (55). There is also some evidence for the use of ALIS in *M. abscessus*-PD treatment and it is currently recommended for the continuation phase of treatment (55, 87, 90). Published data on experiences with other inhaled antibiotics for NTM-PD include case series (imipenem/cilastatin) and animal studies (clofazimine, tigecycline) (82, 91-93)

Future perspectives for alternative modes of administration

With the exception of ALIS for treatment-refractory MAC-PD, the evidence for local administration of antimycobacterial drugs remains limited to case series. This is not surprising considering that NTM SST, ear and eye infections are relatively rare. These difficult to treat infections challenge treatment teams to develop novel treatment approaches such as described in chapter 6. Larger clinical studies are needed to further evaluate the safety and efficacy of local administration of antimycobacterial drugs. Also, treatment teams should be encouraged to report their experiences.

New treatment options for TB and NTM disease

Optimizing the dose of and exposure to existing antimycobacterial drugs is very important, but it is not the only strategy to improve treatment of TB and NTM disease. The development of new drugs, or drug regimens, is essential but has proven to be very challenging.

For TB, despite the high incidence and TB-related deaths, no new drugs were developed during several decades before the 2010s. TB mostly affects low income countries and TB drug development is perceived as a relatively unattractive market for pharmaceutical companies. Other challenges include the difficulty of identifying new drugs with activity against *M. tuberculosis*, and the necessity of lengthy and costly clinical trials, considering that no sufficiently validated surrogate marker for response is available (94, 95). Furthermore, while the development of one new drug already takes a long time, TB treatment consists of multidrug regimens and it could take 20-30 years to develop a new regimen (95, 96). Fortunately, the need to meet these significant challenges has led to several extensive, international collaborations since early 2000s. This includes large public-private partnerships such as the TB Alliance and UNITE4TB, and various other research consortia (e.g. PanACEA, TBTC, ACTG).

With a renewed interest in TB drug development the last decades, multiple new compounds have been developed. Since 2012 three anti-TB drugs (bedaquiline, delamanid, and pretomanid) have been approved by the EMA (European Medicines Agency) and FDA (U.S. Food and Drug Administration). Several new drugs are in various stages of preclinical and clinical development. Furthermore, many trials evaluating new regimens have been completed or are ongoing (97, 98). Some successful regimens so far include combinations of (optimized) existing drugs, new drugs, and repurposed drugs (available drugs for other infections; e.g. linezolid, moxifloxacin and clofazimine). A phase III trial showed that a regimen consisting of rifapentine, isoniazid, pyrazinamide and moxifloxacin can shorten the treatment duration of drug-susceptible TB to four months (99). The current WHO guideline states that patients with drug-susceptible TB, aged ≥12 years, may receive this regimen (100). Major advances have also been in the treatment of RR/MDR-TB. First, long treatment regimens (18-20 months), including oral drugs and injectables, have been replaced by a shorter, 9(-11)-months, all-oral regimen. Furthermore, the currently preferred option for RR/MDR-TB is an even shorter, 6-month regimen, including bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) (52).

Along with the development of new drugs and drug regimens, strategies for more individualized approaches may further improve TB treatment. In one study risk stratification tools for selecting optimal treatment durations were developed (101). In this study patients with drug-susceptible TB were stratified into low-, moderate-, and high-risk groups for TB-related outcomes based on various patient characteristics. The predicted optimal treatment durations for rifamycin-based regimens were four months for low-risk patients, six months for moderate-risk patients, and likely more than six months of treatment are required for high-risk patients. The authors conclude that their stratification approach may be useful for the design of clinical trials and patient care (101). However, potential obstacles regarding the applicability of this approach in resource-limited settings were identified by others (e.g. no routine performance of chest radiography) (102). Another study on a more individualized approach investigated a strategy of giving short 8-week treatment regimens to patients with pulmonary TB, with the possibility to extend treatment for persistent clinical disease and retreatment for relapse. An initial 8-week combination of bedaquiline, linezolid, isoniazid, pyrazinamide, and ethambutol was noninferior to the standard 6-month regimen, with a reduced mean total time on treatment (85 days versus 180 days), and without major safety concerns (103).

The available evidence for treatment of NTM disease is increasing, but remains limited. None of the currently used antimycobacterial drugs used for NTM disease

were developed for NTM disease. A promising development is that there are several drugs in pre-clinical and clinical development which hopefully will lead to more treatment options (104).

Treatment was initially based on anti-TB regimens. Since the 1990s there has been an important shift toward macrolide-based regimens for MAC-PD following several studies demonstrating their efficacy (105-108). Although MAC-PD has received the most attention, the available evidence is mainly comprised of data from relatively small, and often noncomparative or non-randomized, studies (55, 108). The evidence-base for the treatment of NTM-PD caused by other NTM species, or extrapulmonary NTM disease, is even more limited. A likely contributing factor is that NTM diseases are relatively rare diseases. This poses a significant challenge for performing large clinical trials. This was recently illustrated by the slow inclusion in a single-center RCT (PERC) comparing the efficacy of a clofazimine-based regimen with a rifampicin-based regimen for MAC-PD (59). The success of large international collaborations for TB may be an example for the field of NTM disease and could possibly spur the development of new drugs, and regimens, that are very much needed.

In addition to the development of new drugs, more individualized approaches to treatment may be beneficial for NTM disease. A scoring system, BACES (body mass index, age, cavity, erythrocyte sedimentation rate, and sex), was initially developed to predict the 5-year mortality risk in patients with NTM-PD, and may be a potential marker for other outcomes (e.g. disease progression, treatment response) as well (109-111). The BACES score might serve as a useful tool for informing treatment decisions for individual patients. Further research is needed to evaluate whether treatment strategies based on this score, or other risk-based strategies, (e.g. more aggressive treatment for higher scores or 'watchful waiting' for lower scores) could lead to better outcomes.

Another interesting approach to improve treatment of mycobacterial infections is so-called host-directed therapy (HDT). Rather than directly acting on pathogens, HDT may either stimulate host defense or control excessive immune responses. For most types of HDT, clinical trial evidence is currently lacking (97). However, there is evidence for the benefit of corticosteroids in selected TB cases. A clear example is the use of adjunctive dexamethasone in the treatment of patients with TB meningitis, which is associated with a 31% reduced risk of death (112). However, adjunctive dexamethasone did not result in a survival benefit in a recent trial among HIV-positive adults with TB meningitis (113). In NTM disease, HDT is sporadically applied in expert centers but there is no published evidence of its efficacy.

Dose individualization through therapeutic drug monitoring

The main focus of this thesis is dose and exposure optimization of antimycobacterial drugs on a population level. Although the rationale of this strategy is clear, aiming for doses that are effective and safe for as many patients as possible, it follows a 'one size (or dose) fits all' approach. However, there are large interindividual differences in pharmacokinetics of antimycobacterial drugs leading to variability in drug exposure. These differences can be caused by many factors including body composition, genetics, co-morbidities, co-medication, and concomitant food intake (21). The result of this variability is that some patients will have low drug exposures, increasing the risk of poor outcomes or development of drug resistance. On the other hand, high exposures may lead to toxicity (7, 114, 115). Therapeutic drug monitoring (TDM), i.e. individualized drug dosing based on measured plasma or serum concentrations, could contribute to improving treatment outcomes (21, 116). In addition to large interindividual variability in PK, another important prerequisite for the applicability of TDM is that there should be a clear relationship between drug exposure and response (i.e. exposure target). Although the knowledge about exposure-response relationships continues to expand, the recommended target exposure ranges (typically C_{max} or AUC) for TDM in TB or NTM disease often represent normal ranges that can be expected with standard doses (21, 116, 117). This approach follows the reasoning that standard doses are generally effective, especially in TB, and therefore standard drug exposures are also expected to be effective (21, 118). The same approach is typically recommended when applying TDM in NTM disease, because information on exposure-response relationships of antimycobacterial drugs used for NTM disease is scarce (119). TDM ideally also includes drug susceptibility data (MIC) in addition to drug concentrations. AUC/MIC, C_{max}/MIC and time/MIC ratios are the main so-called PK/PD indexes, and AUC/MIC and C_{max}/MIC ratios best explain the effect of first-line anti-TB drugs (116, 118). TDM is an important tool that can improve clinical outcomes and reduce the risk of development of resistance in individual patients. It can also be useful to resolve drug interactions and toxicity. Although several studies have reported on the benefits of TDM (mainly TB), larger randomized controlled trials evaluating the outcomes of TDM are lacking (21, 116). Furthermore, currently TDM of antimycobacterial drugs is predominantly used in referral centers (118, 120). It is rarely performed in endemic TB settings due to various reasons including cost constraints, lack of infrastructure (including analytical methods; **chapter 2**) and trained personnel (116, 121). Programmatic use of TDM for TB drugs in endemic countries is considered beyond the scope of this thesis, but probably warrants (1) a targeted approach, i.e. a focus on patients that may benefit most from TDM based on characteristics of the drug, the host and the pathogen, (2) development of immuno-assays that could replace

labor-intensive and costly HPLC-based assays, (3) a thought-out logistical system for transport of samples, and (4) reimbursement of the TDM service (120, 122).

Clinical pharmacological methodologies and techniques

In this thesis various methodological approaches and types of data analysis were used to substantiate and optimize doses of antimycobacterial drugs. As described in the introduction, a prerequisite for performing clinical pharmacological research is the availability of analytical methods that can accurately measure drug concentrations in relevant biological matrices (e.g. plasma/serum and CSF). Commercial (semi-)automatic immunoassays are not available for the measurement of antimycobacterial drugs. As a result, laboratories need to develop and internally validate their own methods. Participation in an external (interlaboratory) quality control (QC) or proficiency testing program is an essential part of quality assurance, as it provides laboratories valuable insights in their analytical performance. In **chapter 2** we evaluated the ten-year results of an international QC program for the measurement of antimycobacterial drugs. The program has expanded since its initiation in both number of participating laboratories and number of covered drugs (and metabolites). Overall, 83.2% of all measurements were accurate which is comparable with results from other programs (123-125). However, this means that one out of six measurements was inaccurate, which is suboptimal. Accurate measurements are essential to provide reliable results in pharmacokinetic studies and in TDM. Furthermore, the absolute inaccuracy was related to the measured anti-TB drug and to the performing laboratory, but not to the concentration level or to the analytical technique used. These results illustrate the need for continuous external QC for the measurement of antimycobacterial drugs. Participation of laboratories in the program either confirms their level of intra-laboratory quality assurance or alerts them to inaccuracies and underlying undetected problems, inciting them to optimize their methods or QC procedures.

Noncompartmental PK analysis (NCA) was performed to assess the PK of antimycobacterial drugs in plasma with standard doses (pyrazinamide; **chapter 3**) and experimental doses (clofazimine; **chapter 5**), as well as the PK of co-administered drugs (various probe drugs; **chapter 4**). PK data analysis in these chapters follows the so-called standard two-stage approach. First, PK parameters are estimated for individual study participants. Second, measures of central tendency (e.g. geometric mean) and spread are calculated. NCA is a useful technique to assess key PK parameters of a drug (e.g. AUC, C_{max}, clearance, distribution volume) without assumptions of a specific compartmental model. A prerequisite for NCA is intensive PK sampling, i.e. the recording of a full PK curve. In addition to NCA, **chapter 5**

included population PK modeling. First, NCA was performed to assess the PK of clofazimine with a loading dose in this dose optimization study. Then, a population PK model was developed based on PK data from C-LOAD and PERC (comparator study without a loading dose). Subsequently, this model was used to derive key PK parameters of clofazimine, assess predictors of exposure, and to evaluate PK target attainment with various simulated dosing regimens in a large virtual patient population. The latter provided a proposed optimal loading dose regimen of 300 mg once daily for six weeks. This study illustrates the benefits of population PK modeling, in addition to more conventional NCA. Population PK models are useful tools for determining the primary PK parameters such as clearance and bio-availability, are able to assess predictors of exposure, have more statistical power to assess PK-PD relationships than conventional statistics, and allow for simulations of different dosing strategies (126). Other benefits are that population PK can be used with sparse PK data, uneven sampling designs and missing data.

In chapter 6 we studied the drug interaction potential of high-dose rifampicin by means of metabolic phenotyping. Phenotyping for drug-metabolizing enzymes or transporters is defined as measuring their actual in vivo activity in an individual. This is performed by single administration of a selective substrate for an enzyme or transporter (probe drug) and subsequent determination of a phenotyping metric, preferably the total exposure to the probe drug. Multiple probe drugs can be applied simultaneously as a "cocktail" to assess the activity of metabolic enzymes and transporters at the same time. The great benefit of this approach is that it allows for screening of many potential drug interactions in one study, preventing the need for multiple drug interaction studies with individual drugs. Metabolic phenotyping is not a novel approach, but it is not widely used in TB. PHENORIF was the first study of the PanACEA consortium to employ this approach, but since then it has been used to evaluate the drug interaction potential of novel compounds.

The interplay between multidisciplinary patient care and research

The aim to provide the best possible treatment options to today's and future patients is a driving force to perform research. Research questions may arise from knowledge gaps in current multidisciplinary patient care. Reversely, research outcomes could lead to implementation of changes in patient care, which in turn may fuel further research. This 'cross-fertilization' between patient care and research was also crucial in this thesis. For example, the dose optimization strategy for rifampicin was born out of a need to improve TB treatment, and has led to extensive research. The use of higher rifampicin doses in clinical trials, and in patient care (127), led to new questions. One of these, regarding the drug interaction potential of

higher rifampicin doses, resulted in the PHENORIF study (**chapter 4**), the results of which are now applied in patient care.

The studies in this thesis also highlight the benefits and necessity of a multidisciplinary approach in performing research. Performing clinical studies in NTM disease is challenging. C-LOAD (**chapter 5**) was an academic study without external funding, and was a collaborative effort of various departments of the Radboudumc. Ultimately, it was made possible by a common cause, and dedication of our team, towards improving treatment of NTM disease. The developed loading dose strategy of clofazimine could lead to implementation of changes in patient care, and may be part of future studies. The novel treatment approach of applying high local concentrations for treatment-refractory SST NTM disease (**chapter 6**) was developed by our multidisciplinary team as a response to 'back against the wall situations' when established treatment options failed. We found it important to report our experiences with this approach, although limited to a few cases, so that others may benefit and to contribute knowledge to the field. Furthermore, chapters 5 and 6 may further increase attention to NTM disease which hopefully paves the way for future research.

Concluding remarks

I conclude this thesis on a more personal note. My first encounter with TB was several years ago when I was still a hospital pharmacist in training. While I had heard of TB, back then I was completely unaware of the major global health problem it represented and the millions of people who continue to suffer because of it. My knowledge of NTM disease was even worse, I simply had never heard of it. As I learned more through patient care and was confronted with the challenges involved with antibiotic treatment, I became increasingly intrigued and realized I wanted to pursue a PhD in this field. It took a few years, and required some persistence of all those involved, but thankfully an opportunity presented itself. In the research that followed we aimed to contribute to dose and exposure optimization of available antimycobacterial drugs. I hope that the findings in this thesis will bring the field closer towards utilizing the full potential of old – *but not outdated* – drugs for patients with TB and NTM disease.

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Appendices

Summary

Mycobacterial infectious diseases are caused by a group of bacteria called mycobacteria. The focus of this thesis was tuberculosis (TB) and nontuberculous mycobacterial (NTM) disease. TB is spread by human-to-human transmission of *M. tuberculosis* through coughing by patients with active TB disease. It is a major global health problem with an estimated 10.6 million new TB cases and 1.3 million deaths worldwide in 2022. NTM are environmental bacteria, residing in soil and water, that are generally not transferred from one human to another. There are many NTM species and several are opportunistic pathogens, with *M. avium* complex (MAC) and *M. abscessus* as the most frequent causative agents of NTM disease worldwide. Globally, NTM disease occurs less frequent than TB and its prevalence varies between geographical regions. There are many similarities with regard to antimycobacterial treatment of TB and NTM disease and its challenges:

- Antibiotic treatment is complex, consisting of combination therapy with several antimycobacterial drugs (of which many are used for both TB and NTM disease), and requires long treatment durations
- Substantiation of drug doses is often limited
- Treatment outcomes are poor, especially in TB-meningitis and NTM disease
- Drug resistance is a major problem
- Adverse effects occur frequently
- Antimycobacterial drugs (especially rifampicin) cause drug-drug interactions and are affected by them

From a clinical pharmacological perspective, it is evident that these challenges are interrelated. This is partly explained by suboptimal exposure to (concentrations of) antimycobacterial drugs, either systemically (plasma or serum) or at the site of action, and lack of target exposures associated with optimal outcomes. Although many antimycobacterial drugs have been used for decades, their optimal dose is often unknown. Therefore, an obvious strategy to improve treatment is to utilize the full potential of these old drugs through dose optimization. The underlying rationale is that optimized doses will in turn improve drug exposure in plasma and at the site of action (pharmacokinetics (PK)), and that this exposure could result in improved effects (i.e. more efficacy and less adverse effects; pharmacodynamics (PD)). This thesis combines studies in patients with TB and those with NTM disease. Research questions focused on the measurement of concentrations of antimycobacterial drugs, PK and safety evaluations of available drugs with currently recommended and experimental doses, drug interactions, and the application and outcomes of an alternative mode of drug administration.

In **chapter 2** we evaluated the ten-year results of an international, external quality control (QC) program for the measurement of antimycobacterial drug concentrations. Each year, two rounds were organized in which serum (or plasma) samples, spiked with known concentrations of anti-TB drugs, were provided to participating laboratories for analysis. Reported measurements within 80-120% of weighed-in concentrations were considered accurate. By 2022, 31 laboratories had participated and 13 anti-TB drugs and metabolites were included, showing an expansion of the program since its initiation. Overall, one out of six measurements (16.8%) of 1407 measurements was inaccurate, which is suboptimal. Accurate measurements are essential to provide reliable results in pharmacokinetic studies as well as in patient care when performing dose individualization based on drug concentrations (therapeutic drug monitoring). Furthermore, the absolute inaccuracy was related to the measured anti-TB drug and to the performing laboratory, but not to the concentration level or to the analytical technique used. These results illustrate the need for continuous external QC for the measurement of antimycobacterial drugs. Participation in the program alerts laboratories to previously undetected analytical problems.

In **chapter 3** we studied the PK of pyrazinamide in plasma and cerebrospinal fluid (CSF) during the initial phase of treatment in adult Indonesian patients with TB-meningitis, the most severe manifestation of TB. We found that the total exposure ($AUC_{0\text{-}24h}$) in plasma on day 10 of treatment was 26% lower compared to day 2. The higher initial exposure is surprising since steady-state is not yet reached and thus an increase rather than a decrease in exposure would be expected. We hypothesize that this could be the result of a drug interaction with rifampicin. We also found that pyrazinamide penetrates well in CSF as is illustrated by a high mean CSF-to-plasma concentration ratio of 90%. Furthermore, the dose of pyrazinamide, the exposure in plasma, and CSF concentrations were highly correlated. These findings support and further substantiate the important role of pyrazinamide in the treatment of TB-meningitis. Higher doses of pyrazinamide are currently being evaluated for pulmonary TB. Our findings imply that, from a PK viewpoint, the same dosing strategy for pyrazinamide can be used in TB-meningitis as for pulmonary TB. Studies evaluating the PK, efficacy, and safety of higher pyrazinamide doses for TB-meningitis are warranted.

In **chapter 4** we assessed the drug interaction potential of high-dose rifampicin (40 mg/kg), as compared to standard-dose rifampicin (10 mg/kg), in adult patients with pulmonary TB in South-Africa. Accumulating evidence supports the use of higher rifampicin doses as they have yielded improved early bactericidal activity,

may shorten TB treatment, may reduce mortality in patients with TB-meningitis, and are well tolerated. However, rifampicin is a potent inducer of metabolic enzymes and transports and causes many significant drug interactions. Our aim was to evaluate whether high-dose rifampicin causes more severe drug interactions than standard-dose rifampicin by means of a phenotyping cocktail study. All participants (25 completed the study) received standard-dose rifampicin (days 1-15), followed by high-dose rifampicin (days 16-30). A single dose of selective substrates (probe drugs) was administered orally on days 15 and 30: caffeine (CYP1A2), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A), and digoxin (P-gp). Following a standard within-subject bioequivalence approach with standard margins (80-125%), with total exposure of each the probe drugs as the main PK parameter, we found that high-dose rifampicin resulted in no additional effect on CYP1A2, mild additional induction of CYP2C9, CYP2C19, CYP2D6, and CYP3A, and marginal inhibition of P-gp. We believe that existing recommendations on managing drug interactions with rifampicin can remain unchanged for the majority of co-administered drugs when using high-dose rifampicin.

In **chapter 5** we evaluated the PK and safety/tolerability of a clofazimine loading dose regimen (i.e. higher starting dose) in adult patients with pulmonary or extrapulmonary NTM disease in the Netherlands. Clofazimine has a long elimination half-life and it takes months to reach steady-state ('stable') concentrations in plasma. This implies that clofazimine does not contribute fully to the treatment regimen for months. The rationale for this study was that a loading dose could reduce the time to concentrations similar to those at steady state (steady-state-like concentrations). Twelve patients received a loading dose of 300 mg once daily (QD) for four weeks, followed by a (standard) maintenance dose of 100 mg QD, in combination with other antimycobacterial drugs. We found that this regimen resulted in steady-state-like concentrations faster compared to no loading dose, and was safe and well-tolerated. A population PK model was developed and model-based simulations predicted the optimal loading dose regimen to be a dose of 300 mg QD for six weeks, followed by 100 mg QD. This regimen reduces the time to steady-state-like concentrations by 3.9 months compared to no loading dose (1.4 versus 5.3 months), without exceeding steady-state peak concentrations (achieved with a dose of 100 mg QD). These results show the feasibility of a loading dose of clofazimine as a strategy to optimize treatment of NTM disease.

In **chapter 6** we described the use of topical antibiotics as adjuvants to surgery and systemic antibiotic treatment for three patients with treatment-refractory skin and soft tissue (SST) NTM disease. Following surgical debridement, negative

pressure wound therapy with instillation and dwell time of topical antibiotics (NPWTi-d) was performed for 1-2 weeks, via a Vacuum Assisted Closure (VAC) system, in addition to systemic antibiotics. The topically administered antibiotics included an aminoglycoside (amikacin or tobramycin) with or without imipenem/cilastatin. This system allowed for the administration of high concentrations at the site of infection, that were ~1000-fold higher than typical plasma concentrations that are achieved with systemic administration, without signs of systemic toxicity. Treatment was successful and well tolerated, except for some local irritation. We propose that topical application of antibiotics using NPWTi-d, following surgery and combined with systemic antibiotic treatment, could be considered for patients with treatment-refractory NTM SSTIs. Follow-up studies are needed to evaluate the efficacy and safety of this treatment modality.

In **chapter 7** the main findings and implications of each the chapters of this thesis were summarized and put in perspective, and other strategies to improve treatment of TB and NTM disease were discussed. I hope that the results of the research in this thesis will bring the field closer towards utilizing the full potential of old – *but not outdated* – drugs for patients with TB and NTM disease.

Nederlandse samenvatting

Mycobacteriële infectieziekten worden veroorzaakt door een groep bacteriën die mycobacteriën worden genoemd. De focus van dit proefschrift lag op tuberculose (TB) en niet-tuberculeuze mycobacteriële (NTM) infecties. TB wordt verspreid door overdracht van mens op mens van *M. tuberculosis*, via hoesten door patiënten met actieve TB. Het is een groot wereldwijd gezondheidsprobleem met naar schatting 10,6 miljoen nieuwe TB gevallen en 1,3 miljoen doden wereldwijd in 2022. NTM komen in onze omgeving voor, o.a. in de bodem en het water, en worden over het algemeen niet van mens op mens overgedragen. Er zijn veel NTM species en diverse species zijn opportunistische pathogenen, met *M. avium complex* (MAC) en *M. abscessus* als de meest voorkomende veroorzakers van NTM infecties wereldwijd. Wereldwijd komen NTM infecties minder vaak voor dan TB en de prevalentie varieert per geografische regio. Er zijn veel overeenkomsten met betrekking tot de antimycobacteriële behandeling van TB en NTM infecties en de uitdagingen daarbij:

- Antibioticabehandeling is complex, bestaat uit combinatietherapie met verschillende antimycobacteriële geneesmiddelen (waarvan er veel voor zowel TB als NTM infecties worden gebruikt) en vereist een lange behandelduur
- De onderbouwing van doseringen is vaak beperkt
- De behandeluitkomsten zijn slecht, vooral bij TB-meningitis en NTM infecties
- Resistentie is een groot probleem
- Bijwerkingen komen vaak voor
- Antimycobacteriële geneesmiddelen (vooral rifampicine) veroorzaken geneesmiddelinteracties en worden hierdoor beïnvloed

Vanuit een klinisch farmacologisch perspectief is het duidelijk dat er een relatie bestaat tussen deze uitdagingen. Dit wordt gedeeltelijk verklaard door suboptimale blootstelling aan (concentraties van) antimycobacteriële geneesmiddelen, hetzij systemisch (plasma of serum) of op de plaats van werking, en het ontbreken van 'target' blootstellingen die geassocieerd zijn met optimale resultaten. Hoewel veel antimycobacteriële geneesmiddelen al tientallen jaren worden gebruikt, is hun optimale dosering vaak onbekend. Een voor de hand liggende strategie om de behandeling te verbeteren is dan ook om de volledige potentie van deze oude geneesmiddelen te benutten door middel van dosisoptimalisatie. De onderliggende gedachte is dat geoptimaliseerde doseringen op hun beurt de blootstelling aan het geneesmiddel in plasma en op de plaats van werking (farmacokinetiek (PK)) zullen verbeteren, en dat deze blootstelling vervolgens resulteert in verbeterde respons (d.w.z. betere effectiviteit en minder toxiciteit; farmacodynamiek (PD)).

Dit proefschrift combineert onderzoeken bij patiënten met TB en patiënten met NTM infecties. Onderzoeksvragen richtten zich op het meten van concentraties van antimycobacteriële geneesmiddelen, PK- en veiligheidsevaluaties van beschikbare geneesmiddelen met momenteel aanbevolen en experimentele doseringen, geneesmiddelinteracties en de toepassing en uitkomsten van een alternatieve toedieningswijze van geneesmiddelen.

In **hoofdstuk 2** evalueerden we de resultaten over een periode van 10 jaar van een internationaal, extern kwaliteitscontrole (QC) programma voor het meten van concentraties van antimycobacteriële geneesmiddelen. Elk jaar werden er twee rondes georganiseerd waarbij serum (of plasma) monsters, gespiked met bekende concentraties van anti-TB geneesmiddelen, voor analyse werden aangeboden aan deelnemende laboratoria. Gerapporteerde concentraties binnen 80-120% van de ingewogen concentraties werden als accuraat beschouwd. In 2022 hadden 31 laboratoria deelgenomen en waren 13 anti-TB-medicijnen en metabolieten opgenomen, waaruit blijkt dat het programma is gegroeid sinds de start ervan. Eén op de zes metingen (16,8%) van in totaal 1407 metingen was inaccuraat, hetgeen suboptimaal is. Accurate metingen zijn essentieel voor het verkrijgen van betrouwbare resultaten in farmacokinetische onderzoeken en in de patiëntenzorg bij het uitvoeren van dosisindividualisatie op basis van geneesmiddelconcentraties (therapeutic drug monitoring). De absolute onnauwkeurigheid was gerelateerd aan het gemeten anti-TB geneesmiddel en aan het uitvoerende laboratorium, maar niet aan het concentratieniveau of de gebruikte analysemethoden. Deze resultaten illustreren de noodzaak van externe QC voor het meten van antimycobacteriële geneesmiddelen. Deelname aan het programma attendeert laboratoria op eerder onopgemerkte analytische problemen.

In **hoofdstuk 3** bestudeerden we de PK van pyrazinamide in plasma en cerebrospinale vloeistof (CSF) tijdens de eerste fase van de behandeling bij volwassen Indonesische patiënten met TB-meningitis, de meest ernstige vorm van TB. We observeerden dat de totale blootstelling (AUC0-24u) in plasma op dag 10 van de behandeling 26% lager was dan op dag 2. De hogere initiële blootstelling is verrassend omdat steady-state nog niet is bereikt en dus eerder een toename dan een afname van de blootstelling zou worden verwacht. We veronderstellen dat dit het resultaat zou kunnen zijn van een interactie met rifampicine. We zagen ook dat pyrazinamide goed penetreert in CSF, hetgeen geïllustreerd wordt door een hoge gemiddelde CSF/plasma concentratieratio van 90%. Bovendien waren de dosis van pyrazinamide, de blootstelling in plasma en de CSF concentraties sterk aan elkaar gecorreleerd. Deze bevindingen ondersteunen en verder onderbouwen de

belangrijke rol van pyrazinamide bij de behandeling van TB-meningitis. Momenteel worden hogere pyrazinamide doseringen geëvalueerd voor pulmonale TB. Onze bevindingen impliceren dat, vanuit PK perspectief, dezelfde doseringsstrategie voor pyrazinamide kan worden toegepast bij TB-meningitis als bij pulmonale TB. Studies die de PK, effectiviteit en veiligheid van hogere pyrazinamide doseringen voor TB-meningitis evalueren, zijn nodig.

In **hoofdstuk 4** hebben we het geneesmiddelinteractie potentieel onderzocht van een hoge dosering rifampicine (high-dose rifampicin, 40 mg/kg) in vergelijking met een standaard dosering (10 mg/kg) bij volwassen patiënten met pulmonale TB in Zuid-Afrika. Er is steeds meer bewijs voor het gebruik van hogere doseringen van rifampicine, omdat deze een betere 'early bactericidal activity' opleveren, de behandeling van TB kunnen verkorten, de mortaliteit bij patiënten met TB-meningitis kunnen verlagen en goed worden verdragen. Rifampicine is echter een krachtige inducer van metabole enzymen en transporters en veroorzaakt veel relevante geneesmiddelinteracties. Ons doel was om te evalueren of high-dose rifampicine nog sterkere geneesmiddelinteracties veroorzaakt dan een standaard dosering, door middel van een 'phenotyping cocktail' onderzoek. Alle deelnemers (25 voltooiden de studie) kregen een standaarddosering van rifampicine (dag 1 t/m 15), gevolgd door high-dose rifampicine (dag 16 t/m 30). Op dag 15 en 30 werd een enkele orale dosis van selectieve substraten (probe drugs) toegediend: cafeïne (CYP1A2), tolbutamide (CYP2C9), omeprazol (CYP2C19), dextromethorfan (CYP2D6), midazolam (CYP3A) en digoxine (P-gp). Met een (within-subject) bio-equivalentie benadering met standaard grenzen (80-125%), met de totale blootstelling van elk van de probe drugs als belangrijkste PK parameter, vonden we dat high-dose rifampicine resulteerde in geen additioneel effect op CYP1A2, milde additionele inductie van CYP2C9, CYP2C19, CYP2D6 en CYP3A, en marginale remming van P-gp. Wij zijn van mening dat de huidige aanbevelingen voor het omgaan met geneesmiddelinteracties met rifampicine ongewijzigd kunnen blijven, voor de meerderheid van de gelijktijdig toegediende geneesmiddelen, bij gebruik van high-dose rifampicine.

In **hoofdstuk 5** evalueerden we de PK en veiligheid/verdraagbaarheid van een oplaaddosering van clofazimine (d.w.z. hogere startdosis) bij volwassen patiënten met pulmonale of extrapulmonale NTM infecties in Nederland. Clofazimine heeft een lange eliminatiehalfwaardetijd en het duurt maanden om steady-state ('stabiele') concentraties in plasma te bereiken. Dit impliceert dat clofazimine maandenlang niet volledig bijdraagt aan het behandelregime. De rationale voor dit onderzoek was dat een oplaaddosering de benodigde tijd om concentraties

te bereiken die vergelijkbaar zijn met steady-state concentraties (steady-state-like concentraties) zou kunnen verkorten. Twaalf patiënten kregen een oplaaddosering van 300 mg eenmaal daags (QD) gedurende vier weken, gevolgd door een (standaard) onderhoudsdosering van 100 mg QD, in combinatie met andere antimycobacteriële geneesmiddelen. Dit doseerregime resulteerde sneller in steady-state-like concentraties in vergelijking met geen oplaaddosering en was veilig en werd goed verdragen. Er werd een populatie PK model ontwikkeld en op basis van simulaties bleek het optimale doseerregime een dosis van 300 mg QD gedurende zes weken te zijn, gevolgd door 100 mg QD. Dit doseerregime verkort de tijd tot steady-state-like concentraties met 3,9 maanden in vergelijking met geen oplaaddosering (1,4 versus 5,3 maanden), zonder daarbij steady-state piekconcentraties te overschrijden (die worden bereikt met een dosis van 100 mg QD). Deze resultaten tonen de toegevoegde waarde aan van een oplaaddosering van clofazimine als strategie om de behandeling van NTM infecties te optimaliseren.

In **hoofdstuk 6** beschreven we het gebruik van topicale (lokaal toegediende) antibiotica als adjuvans voor chirurgie en systemische antibioticabehandeling bij drie patiënten met refractaire NTM infecties van huid en weke delen (SST). Na chirurgisch debridement werd gedurende 1-2 weken negatieve druk wondtherapie met instillatie en verblijftijd van lokale antibiotica (NPWTi-d) uitgevoerd via een Vacuum Assisted Closure (VAC) systeem, in aanvulling op systemische antibiotica. De lokaal toegediende antibiotica betroffen een aminoglycoside (amikacine of tobramycine) met of zonder imipenem/cilastatine. Dit systeem maakte de toediening van hoge concentraties van de antibiotica op de plaats van infectie mogelijk. Deze concentraties waren ~1000-maal hoger dan typische plasmaconcentraties die bereikt worden met systemische toediening, zonder tekenen van systemische toxiciteit. De behandeling was succesvol en werd goed verdragen, met uitzondering van lokale irritatie. We stellen voor dat lokale toepassing van antibiotica met NPWTi-d, na chirurgie en in combinatie met systemische antibiotica, kan worden overwogen voor patiënten met refractaire NTM SSTI's. Vervolgonderzoek is nodig om de effectiviteit en veiligheid van deze behandelmethode te evalueren.

In **hoofdstuk 7** werden de belangrijkste bevindingen en implicaties van alle hoofdstukken van dit proefschrift samengevat en in perspectief geplaatst. Tevens werden andere strategieën bediscussieerd om de behandeling van TB en NTM infecties te verbeteren. Ik hoop dat de resultaten van het onderzoek in dit proefschrift bijdragen aan het benutten van de volledige potentie van oude - *maar niet achterhaalde* - geneesmiddelen voor patiënten met TB en NTM infecties.

Data management

Ethics and privacy

This thesis is largely based on the results of medical-scientific research with human participants. The studies described in chapter 3, 4 and 5 encompassed human participants and were conducted in accordance with the ICH-GCP guidelines (Good Clinical Practice). The phase 2 clinical trial from which data for chapter 3 were derived, was approved by the ethical review board of the Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia. The phenotyping cocktail study in chapter 4 was approved by local ethical review boards and by the South African Health Products Regulatory Authority (SAHPRA). The medical ethical review committee 'METC Oost-Nederland' has given approval to conduct the C-LOAD study (chapter 5) (file number: NL77668.091.21). Informed consent was obtained from all research participants. Chapter 6 was a case series and all patients gave permission for the publication of their cases. Technical and organizational measures were followed to safeguard the availability, integrity and confidentiality of the data. These measures include the use of independent monitoring, pseudonymization, access authorization and secure data storage.

Data collection and storage

Chapter 3 was a pharmacokinetic study for which data were derived from a double-blinded, randomized, placebo-controlled phase II trial that was performed in Bandung, Indonesia. Universitas Padjadjaran, Bandung, Indonesia was Sponsor of this trial and data were shared with the Radboudumc for our study. Radboudumc was Sponsor of the phenotyping cocktail study described in chapter 4, which was performed at TASK Clinical Research Center and the University of Cape Town Lung Institute, in Cape Town, South Africa. Data was collected on paper based CRFs by the two study sites and was subsequently transferred to eCRFs using CASTOR EDC. Data for chapter 5 (C-LOAD study) was collected through electronic Case Report Forms (eCRFs) using CASTOR EDC. This pharmacokinetic study was performed at the Radboudumc. Pseudonymized data of chapters 4 and 5 were stored and analyzed on the Radboudumc department server and in Castor EDC and are only accessible by project members working at the Radboudumc. Paper (hardcopy) data are stored in secured cabinets on the department. Data for chapter 2 (non-human data), pseudonymized data for chapter 3, and data for chapter 6 (case series) were stored and analyzed on the department server and are only accessible by project members working at the Radboudumc.

Availability of data

All studies are published open access. The data will be archived for 15 years after termination of the study. Reusing the data for future research is only possible if this was agreed upon by study participants as outlined in the informed consent forms. The anonymous datasets that were used for analysis are available from the corresponding author upon reasonable request.

List of publications

A. Lemson, T.A. Koster, N. Carpaij, C. Magis-Escurra, M. Boeree, **R. Stemkens**, R.E. Aarnoutse, A. van Laarhoven, R. van Crevel, J. van Ingen, W. Hoefsloot. Evaluation of a national multidisciplinary meeting for non-tuberculous mycobacterial disease. *IJTLD OPEN 2024 1(6):279–281*

Ralf Stemkens, Chaima Mouhdad, Eric J F Franssen, Daniel Touw, Jan-Willem Alffenaar, Lindsey H M Te Brake, Marieke G G Sturkenboom, Rob E Aarnoutse. Ten-year results of an international external quality control programme for measurement of anti-tuberculosis drug concentrations. *J Antimicrob Chemother. 2024 Jun 3;79(6):1346-1352.*

Sanne M H Zweijpfenning, Rob Aarnoutse, Martin J Boeree, Cecile Magis-Escurra, **Ralf Stemkens**, Bram Geurts, Jakko van Ingen, Wouter Hoefsloot. Safety and Efficacy of Clofazimine as an Alternative for Rifampicin in Mycobacterium avium Complex Pulmonary Disease Treatment: Outcomes of a Randomized Trial. *Chest. 2024 May;165(5):1082-1092.*

Sandra Salillas, Jelmer Raaijmakers, Rob E Aarnoutse, Elin M Svensson, Khalid Asouit, Erik van den Hombergh, Lindsey Te Brake, **Ralf Stemkens**, Heiman FL Wertheim, Wouter Hoefsloot, Jakko van Ingen. Clofazimine as a substitute for rifampicin improves efficacy of Mycobacterium avium pulmonary disease treatment in the hollow-fiber model. *Antimicrob Agents Chemother. 2024 Mar 6;68(3):e0115723*

Ralf Stemkens*, Maarten Cobussen*, Erik de Laat, Wouter Hoefsloot, Reinout van Crevel, Rob E Aarnoutse, Jakko van Ingen. Successful addition of topical antibiotic treatment after surgery in treatment-refractory nontuberculous mycobacterial skin and soft tissue infections. *Antimicrob Agents Chemother. 2023 Dec 14;67(12):e0078823*
*Equal contribution

Ralf Stemkens, Veronique de Jager, Rodney Dawson, Andreas H. Diacon, Kim Narunsky, Sherman D Padayachee, Martin J Boeree, Stijn W van Beek, Angela Colbers, Marieke JH Coenen, Elin M. Svensson, Uwe Fuhr, Patrick PJ Phillips, Lindsey HM te Brake, Rob E Aarnoutse, on behalf of the PanACEA consortium. Drug interaction potential of high-dose rifampicin in patients with pulmonary tuberculosis. *Antimicrob Agents Chemother. 2023 Oct 18;67(10):e0068323*

Ralf Stemkens, Jeroen A Schouten, Sophie A M van Kessel, Reinier P Akkermans, Denise S C Telgt, Hanneke WHA Fleuren, Mark AA Claassen, Marlies EJL Hulscher, Jaap Ten Oever. How to use quality indicators for antimicrobial stewardship in your hospital: a practical example on outpatient parenteral antimicrobial therapy. *Clin Microbiol Infect. 2023 Feb;29(2):182-187*

R Stemkens, CHC Litjens, S Dian, AR Ganiem, V Yunivita, R van Crevel, LHM Te Brake, R Ruslami, RE Aarnoutse. Pharmacokinetics of pyrazinamide during the initial phase of tuberculous meningitis treatment. *Int J Antimicrob Agents. 2019 Sep;54(3):371-374*

Curriculum Vitae

Ralf Stemkens werd op 25 september 1989 geboren in Weert. In 2007 behaalde hij zijn VWO diploma aan het Pleincollege Sint-Joris te Eindhoven. Aansluitend begon hij met de studie Farmacie aan de Universiteit Utrecht waar hij in 2011 zijn bachelordiploma behaalde. Tijdens de daaropvolgende master heeft hij een onderzoeksstage uitgevoerd, gericht op de farmacokinetiek van mycofenolzuur, in het Cincinnati Children's Hospital Medical Center in Cincinnati (Ohio, Verenigde Staten). In 2014 behaalde Ralf zijn masterdiploma Farmacie. In 2015 begon Ralf met de opleiding tot ziekenhuisapotheker. De opleiding werd volbracht in het Canisius Wilhelmina Ziekenhuis en het Radboudumc in Nijmegen. Tijdens de verdiepingsfase van de opleiding heeft Ralf zich gericht op de klinische farmacologie, met in het bijzonder een focus op mycobacteriële infectieziekten. Het zogenaamde registratieonderzoek naar de farmacokinetiek van pyrazinamide bij patiënten met tuberculeuze meningitis maakt onderdeel uit van dit proefschrift.

Na afronding van de opleiding tot ziekenhuisapotheker heeft Ralf zijn loopbaan in 2019 vervolgd in het Radboudumc. Als ziekenhuisapotheker heeft hij in samenwerking met de afdeling Interne geneeskunde gewerkt aan de optimalisatie van OPAT (intraveneuze behandeling van antimicrobiële middelen in de thuissituatie). Dit heeft o.a. geleid tot een landelijke praktijkgids.

In 2020 is Ralf gestart met promotieonderzoek, hetgeen hij combineerde met klinische taken als ziekenhuisapotheker en projectmanagement voor geneesmiddelenonderzoeken van het Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA). Daarnaast heeft Ralf in 2022 zijn opleiding tot klinisch farmacoloog afgerond. Zijn promotieonderzoek richtte zich op het optimaliseren van het gebruik van antimycobacteriële middelen, middels klinisch farmacologisch onderzoek, voor patiënten met tuberculose en niet-tuberculeuze mycobacteriële infectieziekten. Het onderzoek werd begeleid door prof. dr. Rob Aarnoutse, dr. Lindsey te Brake en dr. Jakko van Ingen.

Ralf vervolgt zijn loopbaan als ziekenhuisapotheker in het Radboudumc. Hij woont samen met zijn vrouw Kelly en zoon Nathan in Malden.

PhD portfolio of Ralf Stemkens

Department: **Pharmacy**
PhD period: **01/06/2020 – 02/04/2024**
PhD Supervisor(s): **Prof. dr. R.E. Aarnoutse**
PhD Co-supervisor(s): **Dr. L.H.M. Te Brake, dr. J. van Ingen**

Training activities	Hours
Courses	
• RIHS - Introduction course for PhD candidates (2020)	15.00
• RIHS workshop - How to write a peer review (2021)	1.00
• Teach the Teacher in Farmacotherapie-onderwijs (2021)	8.00
• Radboudumc - eBROK course (for Radboudumc researchers working with human subjects) (2021)	26.00
• Introductory Biostatistics for Researchers (2021)	126.00
• Radboudumc - Scientific integrity (2022)	20.00
• RIHS workshop - boost your writing skills (2022)	1.00
• ESCMID course - Diagnosis and treatment of mycobacterial infections (2022)	16.00
Seminars	
SWAB Webinar during World Antimicrobial Awareness Week (2021)	2.00
RIHS PhD retreat (2022)	8.00
Conferences	
• NVZA ziekenhuisfarmacie Goes Digital: @WORK in the roaring 20's (2020)	8.00
• Oral presentation at SWAB A-team meeting: 'Introductie Praktijkgids OPAT' (2020)	7.00
• SWAB A-team meeting (2020)	4.00
• Wetenschappelijke Mededelingendag Nederlandse Vereniging voor Klinische Farmacologie & Biofarmacie (NVKFB) (2021)	8.00
• Nationale SWAB A-team meeting / SWAB symposium (2021)	8.00
• NVZA Ziekenhuisfarmaciedagen: Technologie & Innovatie - Back to the Future (2021)	8.00
• International Workshop on Clinical Pharmacology of Tuberculosis Drugs (2021)	16.00
• NVZA Ziekenhuisfarmaciedagen (2022)	8.00
• Symposium NVALT: 'Pulmonary Infectious Diseases' (2022)	8.00
• Presentation Symposium NVALT: 'Pulmonary Infectious Diseases' (2022)	7.00
• Wetenschappelijke Mededelingendag Nederlandse Vereniging voor Klinische Farmacologie & Biofarmacie (NVKFB) (2023)	8.00
• Poster presentation - European Congress of Clinical Microbiology & Infectious Diseases Copenhagen: 'Drug interaction potential of high-dose rifampicin: results from a phenotyping cocktail study in patients with pulmonary tuberculosis' (2023)	14.00
• European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) Copenhagen (2023)	23.00
• Oral presentation at the14th International Workshop on Clinical Pharmacology of Tuberculosis Drugs: 'Drug interaction potential of high-dose rifampicin in patients with pulmonary tuberculosis' (2023)	12.00
• 14th International Workshop on Clinical Pharmacology of Tuberculosis Drugs (2023)	8.00
• NVZA Ziekenhuisfarmaciedagen: 'Binnenste buiten' (2023)	12.00
• Oral presentation at European Congress of Clinical Microbiology & Infectious Diseases (ECCMID), Barcelona: 'A loading dose of clofazimine to optimize treatment in patients with nontuberculous mycobacterial disease' (2024)	12.00
• European Congress of Clinical Microbiology & Infectious Diseases (ECCMID), Barcelona, Spain (2024)	20.00

Other	
• Review scientific publication (2021)	7.00
• NOVA, Department of Pharmacy, Radboudumc, Nijmegen (2020-2024)	28.00
• Research meetings 'Broodje mycobacterie', Radboudumc (2020-2024)	14.00
Teaching activities	
Lecturing	
• Research presentations PanACEA consortium (2020-2021)	20.00
• Research presentations at NOVA - Pharmacy (2020-2024)	16.00
• Research presentations at 'Broodje Mycobacterie' - Radboudumc (2022-2024)	8.00
Supervision of internships / other	
• Co-supervision Biomedical Sciences research internship (2021)	56.00
• Co-supervision of research project ANIOS ziekenhuisfarmacie (2023)	36.00
Total	**599.00**

Dankwoord

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