Addressing critical knowledge gaps to improve and shorten multidrug-resistant tuberculosis treatment regimens in children

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Declaration

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This dissertation includes six original papers published in peer reviewed journals and two unpublished papers. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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Summary

Multidrug-resistant (MDR) tuberculosis (TB), defined as TB disease or infection caused by *Mycobacterium tuberculosis* with resistance to at least both isoniazid and rifampicin, threatens global TB control, with an estimated 490,000 incident cases of MDR-TB globally in 2016. The burden of paediatric MDR-TB has been poorly characterized to date. However, recent modeling studies estimate that there are approximately 26,000-32,000 incident MDR-TB cases in children (< 15 years of age) worldwide each year.

Traditionally, treatment regimens for adults and children were constructed using a minimum of four second-line antituberculosis drugs likely to be effective, including a second-line injectable medication, for up to 6 months, and a total duration of treatment of up to 18-24 months. In 2016, the World Health Organization (WHO) recommended a shortened (9-12 month) treatment regimen, which still includes an injectable drug for four months. In addition, the development and increasing use of the novel TB drugs bedaquiline and delamanid, are radically altering the MDR-TB treatment landscape, although children have lagged behind in accessing these important developments.

Treatment outcomes for adults with MDR-TB have been persistently poor, with 54% successfully treated in 2014 both overall globally, and in South Africa. In contrast, treatment outcomes among children with MDR-TB are generally good, with 78-90% successfully treated under routine clinical conditions. However, current paediatric MDR-TB treatment regimens have important limitations. These current regimens remain long (9-18 months or more), which is costly and burdensome. There are also frequent adverse effects, including from the second-line injectable medications (amikacin, kanamycin, capreomycin) that cause permanent sensorineural hearing loss in up to 24% of children treated long-term. Additionally, the injectables are mainly given by painful daily intramuscular injections, resulting in trauma and distress for patients, their caregivers and healthcare providers. Therefore, it is an urgent priority to develop more optimal treatment regimens for children with MDR-TB that retain their efficacy but are shorter, more child-friendly, are better tolerated, safer and which do not require the use of an injectable medication.

The purpose of this doctoral research was to address critical knowledge gaps in paediatric MDR-TB treatment, with the aim of informing more effective, safer, and more child-friendly MDR-TB treatment strategies in children. I identified critical knowledge

gaps related to the pharmacokinetics, including the effects of formulation, optimal dosing, safety, and tolerability of key second-line and novel antituberculosis drugs in children, and completed complementary studies on ofloxacin, levofloxacin, linezolid, amikacin and bedaquiline designed to address these knowledge gaps.

In an observational study of the pharmacokinetics and safety of ofloxacin in children routinely treated for MDR-TB disease or exposure, exposures after a daily 20mg/kg ofloxacin dose were well below target exposures from adults receiving the routine 800 mg dose. Ofloxacin was safe and well tolerated, with few musculoskeletal complaints or serious adverse events. This data adds to the evidence of the safety of fluoroquinolones in children even with long-term use, and identifies the need to revise ofloxacin paediatric doses.

Subsequently, in this large observational study, the population pharmacokinetics of levofloxacin among children with MDR-TB disease or exposure was characterized using non-linear mixed effects modeling. One hundred and nine children treated with the routinely available adult 250 mg tablet formulation of levofloxacin at daily doses of 15 mg/kg or 20 mg/kg were included. Levofloxacin's apparent oral clearance (CL/F) was higher than expected based on previously published data, possibly due to the formulation studied. Simulations using the final model targeting exposures in adults with TB receiving 750 mg of levofloxacin identified weight-banded doses that were much higher than previously in use (18 mg/kg to nearly 40 mg/kg daily). It was concluded that levofloxacin dosing in children should be reassessed, formulation effects explored further, and that safety should be carefully evaluated if higher levofloxacin doses are used.

Building on this data, I completed an evaluation of the safety of long-term levofloxacin in children treated for MDR-TB. Among 70 children, median age 2.1 years, treated for a median of 11.6 months, levofloxacin was generally safe and was well tolerated. There were no Grade 4 or serious adverse events, and few musculoskeletal events. There was no QT-interval prolongation and no association of QT interval with levofloxacin concentration. This study supported the safety of long-term fluoroquinolone treatment in children, and provided novel data on the QT prolonging effect of levofloxacin, which is needed, as increasingly levofloxacin is being combined with other QT prolonging medications.

The effects of drug formulation in pharmacokinetic studies are critically important. In a lead-in pharmacokinetics study to the TB-CHAMP trial (phase 3 cluster randomized trial comparing levofloxacin vs. placebo for prevention of TB in child contacts of MDR-TB cases), 24 children had pharmacokinetic sampling with a novel dispersible tablet formulation of levofloxacin. The levofloxacin exposures were much higher with this novel formulation compared to those seen in the previously reported study using the adult 250 mg levofloxacin tablet. Combining these two data sets using non-linear mixed effects modeling identified that reduced bioavailability of the adult 250 mg tablet formulation compared to the dispersible levofloxacin tablet was the explanation for the substantial differences in exposures. This study highlighted the importance of formulation considerations to paediatric pharmacokinetic studies and provided practical weight-banded dosing guidelines for use of this formulation now becoming available in the field.

Linezolid is a key drug with an increasingly important role in the treatment of MDR-TB strains with additional resistance and in central nervous system TB disease. I performed a structured review of the literature on linezolid to inform its use in children for MDR-TB treatment and identify knowledge gaps for future research. Few children treated with linezolid for MDR-TB were described in the literature. As in adults, linezolid appeared to be effective but was associated with frequent adverse events. There was no data on linezolid pharmacokinetics in children with TB. Practical interim guidance was provided for linezolid use in children. Priority research needs identified included studying linezolid pharmacokinetics in children with TB, characterization of its safety with long-term use, and its optimal dose for TB in MDR-TB regimens going forward.

Following on this review, an analysis of linezolid pharmacokinetics and safety from children with MDR-TB was performed with data from 48 children combined from two observational studies using non-linear mixed effects modeling. Seventeen children received long-term linezolid and were monitored longitudinally for safety; 31 children only contributed cross-sectional pharmacokinetic data after a single-dose of linezolid. After accounting for the effects of weight with allometric scaling, no other covariates significantly contributed to the model. Exposures were higher than expected, based on previously reported data. Ten of 17 participants had a linezolid related adverse event,

including five Grade 3 or 4 events; anaemia was the most common event. This first data on linezolid pharmacokinetics in children demonstrated higher than expected exposures and frequent, serious linezolid-related adverse events, and will inform the use and future dosing recommendations of this increasingly important antituberculosis medication in children.

While drug substitutions for injectable drugs are not yet available for many children, improving the tolerability of the continued use of second-line injectable medications is an important question to address in children. A randomized two-period crossover study was designed to characterize the effect of co-administration of lidocaine on the pain and pharmacokinetics of intramuscular amikacin. Children each received a dose of amikacin with and without additional lidocaine on separate days, and were randomized to the sequence of treatments; pain assessments and pharmacokinetic sampling were performed on each day. Twelve children were enrolled and completed the study. The addition of lidocaine reduced pain immediately after the injection, was safe, and did not affect the pharmacokinetics of amikacin in children, and should be considered as a routine policy in patients with MDR-TB receiving an injectable agent.

The novel drug bedaquiline is increasingly used globally and in South Africa for adults with MDR-TB, and ongoing paediatric trials will characterize the pharmacokinetics, safety and optimal dose in children. The paediatric formulation, which is being evaluated in at least one of the ongoing paediatric trials, may not be available for routine care for some time. In order to inform the rational use of the adult bedaquiline formulation in young children, a randomized two-period crossover study in healthy adult volunteers was designed. Adult bedaquiline tablets administered suspended in water were bioequivalent to adult tablets swallowed whole. The suspended tablets were also found to be acceptable and palatable to the majority of participants, an important finding considering that crushing or suspending some tablets, such as the fluoroquinolones, reduces their palatability and acceptability substantially. This data will accelerate access to bedaquiline for young children in research and routine care.

In conclusion, this doctoral research has addressed a number of important key knowledge gaps related to more optimal paediatric MDR-TB treatment. This research has raised a number of follow-up questions that have informed subsequent studies that

will continue to advance the field towards a goal of effective, safe, shorter MDR-TB treatment for all children.

Opsomming

Multimiddel-weerstandige (MMW) tuberkulose (TB), wat gedefinieër word as as TB siekte of infeksie wat veroorsaak word deur *Mycobacterium tuberculosis* met weerstandigheid teen ten minste isoniasied en rifampisien, bedreig wêreldwye TB beheer, met 'n geskatte 490,000 nuwe gevalle van MMW-TB wêreldwyd in 2016. Die lading van pediatriese MMW-TB is tot op hede swak omskryf. Onlangse modeleringstudies beraam egter dat daar elke jaar ongeveer 26,000-32,000 nuwe MMW-TB gevalle in kinders (<15 jaar oud) wêreldwyd voorkom.

Tradisioneel was die behandelingsregimens vir volwassenes en kinders saamgestel deur 'n minimum van vier waarskynlik effektiewe tweede-linie antituberkulosemiddels te gebruik, insluitend 'n tweede-linie inspuitbare middel vir tot 6 maande en totale behandelingsduur van tot 18-24 maande. In 2016 het die Wêreldgesondheids-organisasie (WGO) 'n verkorte (9-12 maande) behandelingsregimen, wat steeds 'n inspuitbare middel vir 4 maande ingesluit het, aanbeveel. Hierby het die ontwikkeling en toenemende gebruik van die nuwe TB middels, bedakwilien en delamanid, gelei tot radikale veranderinge in die MMW-TB omgewing, alhoewel kinders in die toegang tot hierdie belangrike ontwikkelinge agtergebly het.

Die uitkomste van behandeling van MMW-TB in volwassenes was aanhoudend swak met 54% suksesvolle behandeling in 2014 beide algeheel globaal, sowel as in Suid-Afrika. In teenstelling hiermee is die uitkomste van behandeling in kinders met MMW-TB oor die algemeen goed, met 78-90% suksesvolle behandeling onder roetine kliniese sorg. Huidige pediatriese MMW-TB behandeling het egter belangrike beperkings. Die huidige behandeling bly egter van lange duur (9-18 maande of langer) wat dit duur en moeilik maak. Daar is ook dikwels nadelige gevolge, soos byvoorbeeld permanente sensorineurale gehoorsverlies in tot 24% van kinders wat langtermyn behandeling ontvang en wat deur die tweede-linie inspuitbare middels (amikasien, kanamisien, kapreomisien) veroorsaak word. Daarby word die inspuitbare middels hoofsaaklik per pynlike binnespierse inspuiting toegedien wat trauma en angstigheid in die pasiënt, die versorger en die gesondheidsverskaffers veroorsaak. Daarom is dit 'n belangrike prioriteit om meer optimale behandelingsregimens vir kinders met MMW-TB te ontwikkel wat hulle effektiwiteit behou, van korter duur en meer kindervriendelik is, wat beter verduur word, veiliger is en wat nie enige inspuitbare middels bevat nie.

Die oogmerk van hierdie doktorale navorsing was om kritieke kennisleemtes in die behandeling van pediatriese MMW-TB aan te spreek, met die doel om meer effektiewe, veiliger en meer kindervriendelike MMW-TB behandelingstrategieë in kinders toe te lig. Ek het kritieke kennisleemtes verwant aan die farmakokinetika, insluitend die effek van formulerings, optimale dosering, veiligheid en verdraagsaamheid van sleutel tweede-linie en nuwe antituberkulose middels in kinders geïdentifiseer en het komplimentêre studies oor ofloksasien, levofloksasien, linezolied, amikasien en bedakwilien ontwerp om hierdie kennisleemtes aan te spreek.

In ‘n waarnemingstudie oor die farmakokinetika en veiligheid van ofloksasien in kinders wat normaalweg vir MMW-TB siekte of blootstelling behandel is, was die blootstellingsvlakke na ‘n daaglikse dosis van 20 mg/kg ofloksasien duidelik laer as die teikenblootstellingsvlakke wat bereik word deur volwassenes wat ‘n roetine dosis van 800 mg ontvang. Ofloksasien was veilig en is goed verduur met min muskuloskeletale klagtes of ernstige nadelige gevolge. Hierdie inligting dra by tot die bewyse dat die fluorokwinolone veilig is in kinders selfs met langtermyn gebruik en toon die behoefte aan om die pediatriese dosisse van ofloksasien te hersien.

Hierna, in hierdie groot waarnemingstudie, is die populasiefarmakokinetika van levofloksasien in kinders met MMW-TB siekte of blootstelling bepaal deur die nie-lineêre gemengde-effekte modeleringstegniek te gebruik. Eenhonderd-en-nege kinders wat met die normaalweg beskikbare volwasse-formulering 250mg levofloksasien tablette behandel is met ‘n daaglikse dosis van 15 mg/kg of 20 mg/kg, is ingesluit. Levofloksasien se oënskynlike mondelingse opruiming (CL/F) van levofloksasien was hoër as wat verwag was volgens vorige gepubliseerde inligting, moontlik as gevolg van die formulering wat bestudeer is. Simulasies wat die finale model gebruik het wat blootstellingsvlakke in volwassenes wat 750 mg levofloksasien ontvang, geteiken het, het gewigsgebaseerde dosisse geïdentifiseer wat baie hoër is as wat voorheen gebruik is (18 mg/kg tot byna 40 mg/kg daagliks). Daar is tot die slotsom gekom dat levofloksasiendoserings in kinders hersien moet word, formuleringseffekte verder ondersoek moet word en dat, as hoër doserings van levofloksasien gebruik sou word, veiligheid versigtig nagegaan moet word.

Op grond van hierdie inligting het ek die veiligheid van die langtermyngebruik van levofloksasien in kinders met MMW-TB bestudeer en voltooi. In 70 kinders, mediane

ouderdom 2.1 jaar, wat behandel is vir ‘n mediane duur van 11.6 maande, was levofloksasien oor die algemeen veilig en is dit goed verduur. Geen Graad 4 of ernstige nadelige effekte het voorgekom nie en net enkele muskuloskeletale effekte het voorgekom. Geen verlenging van QT-interval het voorgekom nie en daar was geen verband waargeneem tussen levofloksasienkonsentrasies en QT-intervalle nie. Hierdie studie het die veiligheid van die langtermyn-gebruik van fluorokwinoloon-behandeling in kinders ondersteun en het nuwe inligting oor die QT-verlengingseffek van levofloksasien verskaf wat baie nodig is, want levofloksasien word toenemend tesame met ander QT-verlengende middels aangewend.

Die effekte van middelformulerings in farmakokinetikastudies is van kritiese belang. Tydens ‘n inleidende farmakokinetikastudie vir die TB-CHAMP studie (fase-3 cluster ewekansige studie wat levofloksasien met plasebo vergelyk vir die voorkoming van TB in kinderkontakte van MMW-TB gevalle) het 24 kinders farmakokinetiese monsterneming gehad tydens die gebruik van ‘n nuwe oplosbare tablet van levofloksasien. Die levofloksasien blootstellingsvlakke was baie hoër met hierdie nuwe formulering in vergelyking met dié wat in die vorige studie met die gebruik van die 250 mg volwasse tablette gevind is. Deur hierdie twee inligtingstelle te kombineer deur gebruik te maak van die nie-lineêre gemengde-effekte modeleringstegniek, het dit geblyk dat die verminderde biobeskikbaarheid tussen die volwasse 250 mg tablette en die oplosbare levofloksasien tablette die rede is vir die aansienlike verskil in blootstellingsvlakke. Hierdie studie het die belangrikheid van middelformulerings oorwegings in pediatriese farmakokinetika beklemtoon en het praktiese gewigs-gebaseerde doseringsriglyne daargestel vir die gebruik van hierdie formulering wat nou in die veld beskikbaar raak.

Linezolied is ‘n sleutelmiddel met ‘n toenemend belangrike rol in die behandeling van MMW-TB stamme met bykomende weerstandigheid sowel as in sentraal senuweestelsel TB siekte. Ek het ‘n gestruktureerde oorsig van die literatuur oor linezolied onderneem om duidelikheid te kry oor die gebruik daarvan in kinders met MMW-TB en om kennisleemtes vir toekomstige navorsing te identifiseer. Slegs enkele kinders wat behandel is vir MMW-TB is in die literatuur opgeteken. Soos in die geval van volwassenes, het linezolied effektief blyk te wees, maar was met gereelde nadelige effekte geassosieer. Daar was geen inligting oor oor die farmakokinetika van linezolied

in kinders met TB beskikbaar nie. Praktiese tussentydse riglyne vir die gebruik van linezolied in kinders is voorsien. Navorsingsprioriteite wat geïdentifiseer is, het linezolied farmakokinetika in kinders met TB, kenmerke van linezolied se veiligheid met langtermyn gebruik en sy optimale dosering vir TB in MMW-regimens vorentoe, ingesluit.

Na hierdie oorsig is ‘n analise van linezolied-farmakokinetika en veiligheid in kinders met MMW-TB gedoen met inligting verkry van 48 kinders saamgevat uit twee waarnemingstudies deur gebruik te maak van nie-lineêre gemengde-effekte modelering. Sewentien kinders het langtermyn linezolied ontvang en is longitudinaal vir veiligheid opgevolg; 31 kinders het slegs deursnit farmakokinetiese inligting na ‘n enkeldosis linezolied verskaf. Na die effek van gewig met behulp van ‘n allometriese skaal in bereking gebring is, het geen ander medevariante betekenisvol tot die model bygedra nie. Blootstellingsvlakke was hoër as wat verwag was in vergelyking met vorige gerapporteerde inligting. Tien van 17 deelnemers het ‘n linezolied-verwante nadelige effek getoon, wat vyf Graad 3 of 4 effekte ingesluit het; anemie was die mees algemene effek. Hierdie eerste inligting oor linezolied-farmakokinetika in kinders het hoër as verwagte blootstellingsvlakke en gereelde, ernstige linezolied-verwante nadelige effekte aangetoon; dit sal die gebruik en toekomstige doseringsaanbevelings van hierdie toenemend-belangrike antituberkulose middel toelig.

Terwyl middelvervangings vir die inspuitbare middels vir baie kinders nog nie beskikbaar is nie, is dit belangrik om die verbeterde verduring van die voortgesette gebruik van die tweede-linie inspuitbare middels in kinders aan te spreek. ‘n Ewekansige twee-periode oorkruis-studie is ontwerp om die effek van mede-toediening van lidokaïen op die pyn en farmakokinetika van binnespierse amikasien te bepaal. Kinders het elk ‘n dosis van amikasien met en sonder addisionele lidokaïen op aparte dae ontvang en was ewekansig vir die volgorde van toediening ingedeel; pynevaluering en monsterneming vir farmakokinetika is op beide dae uitgevoer. Twaalf kinders is ingesluit en almal het die studie voltooi. Die toevoeging van lidokaïen het die pyn onmiddelik na die toediening van die inspuiting verlig, was veilig, het nie die farmakokinetika van amikasien beïnvloed nie en behoort as roetine beleid in pasiënte met MMW-TB wat inspuitbare middels ontvang, oorweeg te word.

Die nuwe middel bedakwilien word toenemend wêreldwyd en in Suid-Afrika vir die behandeling van volwassenes mt MMW-TB gebruik en voortgesette pediatriese studies sal die farmakokinetika, veiligheid en optimale dosering in kinders toelig. Die pediatriese formulering wat in ten minste een van die voortgesette studies evalueer word, sal moontlik nie binnekort vir roetinegebruik beskikbaar wees nie. Om die rasionele gebruik van die volwasse bedakwilienformulering in jong kinders toe te lig, is ‘n ewekansige twee-periode oorkruis-studie in gesonde volwasse vrywilligers ontwerp. Volwasse bedakwilien-tablette toegedien opgelos in water was bioekwivalent aan volwasse tablette wat heel ingesluk is. Die opgeloste tablette was ook aanvaarbaar en smaakaanvaarbaar vir die meerderheid van die deelnemers wat ‘n belangrike bevinding is as inaggeneem word dat die fyndruk en oplos van sommige tablette, soos byvoorbeeld die fluorokwinolone, die smaak en aanvaarbaarheid aansienlik verminder. Hierdie inligting sal toegang tot bedakwilien vir jong kinders in navorsing en in algemene sorg bespoedig.

Ten slotte het hierdie doktorale navorsing ‘n aantal belangrike kennisleemtes aangespreek in verband met die meer optimale behandeling van MMW-TB in kinders. Hierdie navorsing het gelei tot ‘n aantal opvolgvrae wat daaropvolgende studies toegelig het wat op hulle beurt die weg na ‘n doelwit van effektiewe, veilige, korter MMW-TB behandeling vir alle kinders baan.

Dedication

I dedicate this work to my wife Heather, for her continuous support. Working together has been a source of sincere enjoyment for me.

I also dedicate this to my daughters Mosa, Palesa, Amara and Celia who are a constant source of pride and happiness, and to my father Dr. Joseph Garcia-Prats, whose example in all aspects of his life I aspire to.

List of Abbreviations

ALT	Alanine aminotransferase
ART	Antiretroviral Therapy
ARV	Antiretroviral/s
AUC_{0-8}	Area under the concentration time curve from 0-8 hours
AUC_{0-24}	Area under the concentration time curve from 0-24 hours
C_{max}	Maximum plasma concentration
CI	Confidence interval
EBA	Early bactericidal activity
ECG	Electrocardiogram
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
HIV	Human immunodeficiency virus
IM	Intramuscular
IMPAACT	Infant Maternal Pediatric Adolescent AIDS Clincal Trial Network
IUATLD	International Union Against Tuberculosis and Lung Disease
IV	Intravenous
IQR	Interquartile range
LC MS/MS	Liquid chromatography tandem mass spectometry
MDR-TB	Multidrug-resistant tuberculosis
MIC	Minimum inhibitory concentration
NLME	Non-linear mixed-effects modelling
PAS	Para-aminosalicylic acid
PK	Pharmacokinetic/s
PMTCT	Prevention of mother to child transmission of HIV
QTcF	QT-interval corrected with the Fridericia correction
RMR-TB	Rifampicin-monoresistant tuberculosis
SA NTP	South Africa National Tuberculosis Programme
$t_{1/2}$	Half-life
T_{max}	Time to maximum plasma concentration
TB	Tuberculosis
XDR-TB	Extensively drug-resistant tuberculosis
WHO	World Health Organization

Chapter 1: Introduction, rationale and literature review

1.1 Epidemiology of multidrug-resistant tuberculosis, impact on children and public health relevance

Multidrug-resistant (MDR) tuberculosis (TB), defined as TB disease or infection caused by *Mycobacterium tuberculosis* with resistance to at least both isoniazid and rifampicin, is a growing health problem, threatening global TB control. Rifampicin monoresistant (RMR) TB (i.e.*; M. tuberculosis* with rifampicin resistance but isoniazid susceptibility), was previously unusual but is now being identified more often and is treated with the same drug regimens as MDR-TB. The World Health Organization (WHO) estimates that in 2016 there were 490,000 incident cases of MDR-TB globally, with an additional 110,000 TB cases with rifampicin resistance (without documentation of isoniazid susceptibility) (1). Of these 600,000 estimated incident cases, there were roughly 240,000 deaths and only 350,000 cases were notified (i.e. were registered and treated) (1). MDR-TB with additional resistance to either a second-line injectable drugs or a fluoroquinolone is often referred to as pre-extensively drug-resistant (pre-XDR)-TB, with extensively drug-resistant (XDR) TB defined as MDR-TB with additional resistance to both a fluoroquinolone and a second-line injectable TB drug. XDR-TB is also increasingly diagnosed worldwide, with 6.7% of MDR-TB cases having XDR-TB and 20% of MDR-TB isolates tested having fluoroquinolone resistance in 2016 (1).

There is an evolving understanding of how *M. tuberculosis* acquires drug resistance. Drug resistance may be acquired, primarily through true or functional monotherapy due to suboptimal regimen prescription or adherence to treatment, differential lesion penetration by different drugs, or pharmacokinetic variability that selects for resistant organisms (2, 3). Until recently it was believed that the majority of patients with MDR-TB had acquired their resistance during therapy for one of these reasons. However, patients with MDR-TB may also transmit their resistant strain on to others, including children. More recent analysis has demonstrated that globally, transmission of drug-resistant strains of *M. tuberculosis* likely accounts for 95.9% of MDR-TB in new TB cases and even 61.3% of MDR-TB in retreatment cases (4). This has important implications for children, for whom the risk of acquiring drug resistance during treatment is low

because they usually have a low burden of organisms (paucibacillary disease)(5), but who are susceptible to transmission of resistant strains (6).

The burden of paediatric MDR-TB has been underestimated and poorly characterized to date. Until recently it was largely believed that children were at low risk for MDR-TB, in part due to the inaccurate previous belief that MDR-TB was most commonly due to acquisition of resistance during inadequate treatment, rather than due to primary transmission of MDR-TB as described above. The underestimated risk of MDR-TB in children also persisted because of challenges in confirming the diagnosis in children, due to the paucibacillary nature of TB in the majority of paediatric cases, and challenges in obtaining respiratory samples, especially in young children (7, 8). However, recent data have shown that children have a similar setting-specific risk of MDR-TB as antituberculosis treatment-naïve adults (9). Modeling studies estimate that there are roughly 26,000-32,000 incident MDR-TB cases in children (defined by the WHO as < 15 years of age) worldwide each year (10, 11), and that there may be as many as two million children currently infected globally with MDR strains of *M. tuberculosis* (11). South Africa has one of the highest burdens of MDR-TB globally (12), including a substantial paediatric disease burden, with 13.2% of the overall national burden of TB in South Africa occurring in children <15 years of age in 2016 (13). In enhanced hospital-based surveillance at Tygerberg Hospital, Western Cape Province, South Africa from 2003-2013, the prevalence of MDR-TB among children with bacteriologically confirmed TB ranged from 5.4% to 8.9% (14). Due to improved implementation of prevention of mother to children transmission (PMTCT) of HIV, the HIV prevalence in children with bacteriologically confirmed TB has declined from a high of 29% during 2007-2009, to 15.3% between 2011-2013, and is expected to continue to decrease further with additional improvements to PMTCT programs (14).

MDR-TB will remain an important health problem in children in South Africa and globally for the foreseeable future.

1.2 Treatment of MDR-TB in children: evolving approaches, successes and limitations

1.2.1 A landscape of rapidly evolving approaches to MDR-TB treatment

Data contributing to this doctoral research was collected between 2012 and 2018, a time when new evidence and policy guidelines for MDR-TB treatment have been rapidly evolving, especially for adults.

Treatment regimens for MDR-TB in adults and children have traditionally been constructed using the "second-line antituberculosis drugs". Prior to 2016, the WHO categorized antituberculosis drugs in five groups (see Table 1.1) (15). MDR-TB treatment regimens during this period were recommended to include pyrazinamide, a second-line injectable agent from Group 2, a fluoroquinolone from Group 3, and then additional drugs from Groups 4 and 5 to create a treatment regimen with at least 4-5 effective drugs (15, 16). WHO guidelines recommended an intensive phase duration (i.e. injectable-agent duration) of at least 8 months, and a total treatment duration of at least 20 months (16). Prior to 2016, there was limited formal guidance on treatment regimens for children with MDR-TB. However, the same general approach to constructing a regimen was taken. One exception was that children, who frequently had paucibacillary TB, could often be successfully treated with shorter durations of injectables and overall treatment, as part of individualized treatment regimens (see 2.1.2) (17). Many of the studies contributing to this doctoral work were conceived and implemented during the time these approaches guided MDR-TB treatment.

WHO MDR-TB treatment guidelines were revised in 2016 and a number of important changes were made to global MDR-TB recommendations (18): antituberculosis drug groupings were altered to reflect emerging data (Table 1.2), with the fluoroquinolones recognized as the key drugs for MDR-TB treatment, stronger roles were identified for the repurposed drugs linezolid and clofazimine, and recommendations were made regarding the inclusion of the novel TB drugs bedaquiline and delamanid under specific conditions (18)

Table 1.1 World Health Organization antituberculosis drug groupings prior to 2016

Group	Group Name	Drugs
1	First-line oral agents	Isoniazid Rifampicin Ethambutol Pyrazinamide Rifabutin Rifapentine
2	Injectable agents	Kanamycin Amikacin Capreomycin Streptomycin
3	Fluoroquinolones	Moxifloxacin Levofloxacin Ofloxacin
4	Oral bacteriostatic second-line agents	Ethionamide Prothionamide Cycloserine Terizidone Para-aminosalicylic acid
5	Agents with unclear efficacy or concerns regarding usage	Clofazimine Linezolid Amoxicillin-clavulanic acid Thiacetazone Meropenem-clavulanic acid Imipenem/cilastatin High-dose isoniazid Clarithromycin

Table 1.2. Revised WHO groupings of medicines for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis (2016)

Group A. Fluoroquinolones		Levofloxacin Moxifloxacin Gatifloxacin
Group B. Second-line injectable agents		Amikacin Capreomycin Kanamycin (Streptomycin)
Group C. Other core second-line agents		Ethionamide/prothionamide Cycloserine/terizidone Linezolid Clofazimine
Group D. Add-on agents	**D1**	Pyrazinamide Ethambutol High-dose isoniazid
	D2	Bedaquiline Delamanid
	D3	Para-aminosalicylic acid (PAS) Imipenem-cilastin Meropenem Amoxicillin-clavulanate (Thioacetazone)

Most significantly, these guidelines have recommended a shortened (9-12 month) treatment regimen for patients, including for children, with pulmonary RMR-TB and MDR-TB who did not have evidence of fluoroquinolone or second-line injectable resistance and previous exposure to second-line drugs. The new shorter regimen included 4-6 months of kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol, followed by 5 months of moxifloxacin, clofazimine, pyrazinamide, and ethambutol (18). This recommendation was based on multiple high quality observational studies in adults with MDR-TB that consistently demonstrated cure in 80% (19-21), and is now further supported by preliminary results from a randomized controlled trial (STREAM 1, NCT02409290) of this regimen in adults (22). Children and adults not eligible to receive this 9-12 month shortened regimen were recommended to be treated with a more conventional approach for 20-24 months duration with at least five effective drugs, including pyrazinamide, a drug from Group A, a drug from Group B, and 2 drugs from Group C. If a regimen with five effective drugs cannot be constructed, then drugs from D2 and D3 should be added based on these guidelines.

The revised 2016 WHO MDR-TB treatment guidelines have for the first time included some specific paediatric recommendations. In addition to recommending the new 9-12 month shortened regimen and the more conventional approach described above, the new guidelines allow that children with clinically diagnosed MDR-TB (i.e. MDR-TB that is not microbiologically confirmed, and diagnosed based on signs, symptoms, radiography and exposure to MDR-TB, which usually reflects less severe clinical spectrum of disease), could be treated without an injectable drug in the standard (i.e. not shortened) regimen, usually for 15-18 months (see supportive data for this recommendation in 1.2.2) (18). These important changes reflect data from ongoing and emerging paediatric data, including individual studies and a global evidence synthesis (23), some generated during this doctoral research.

In addition to the new WHO MDR-TB treatment recommendations made in 2016, the development and increasing use of the novel TB drugs bedaquiline and delamanid is radically altering the MDR-TB treatment landscape. Bedaquiline is a diarylquinoline that inhibits mycobacterial ATP-synthase, resulting in potent activity against *M.*

tuberculosis in vitro (24) and in mice, with the potential to shorten treatment substantially (25-27). The U.S. Food and Drug Administration (FDA) granted accelerated approval for bedaquiline in 2012 for MDR-TB treatment in adults, and the WHO issued recommendations for its use (28), based on phase 2 studies in adults with MDR-TB showing significantly improved culture conversion (29) and treatment outcome at 120 weeks (30). Delamanid is a nitro-dihydro-imidazooxazole antibiotic that blocks *M. tuberculosis* mycolic acid synthesis (31), resulting in potent antimycobacterial activity *in vitro* and treatment shortening potential in mice (31). Delamanid was granted conditional registration from the European Medicine Authority (EMA) in 2013 for MDR-TB treatment (32), and WHO issued guidance for its use in adults with limited treatment options (33), based on a Phase 2 trial showing delamanid in combination with an optimized background regimen (OBR) in adults with MDR-TB, resulted in improved 2-month culture conversion (34) and 24-month treatment outcomes (35). Although both drugs are likely to be critical for future MDR-TB treatment regimens in adults and children (see Section 1.5.1), paediatric trials of both drugs have been delayed and few children have accessed these medications to date in routine care.

1.2.2 Successes of current MDR-TB treatment regimens in children: markedly better treatment outcomes compared to adults

Treatment outcomes for adults with MDR-TB have been persistently poor (12), with 54% reported successfully treated in 2014 both overall globally and in South Africa from routine programmatic data (1). This is also consistent with data from the metropolitan Cape Town area. Among adults with MDR-TB treated in the Khayelitsha sub-district from 2008-2012, only 359 of 757 (47.4%) were successfully treated (i.e. cured or treatment completed) (36). A systematic review and individual patient data meta-analysis of observational or experimental studies of adults treated for MDR-TB reported 61% of 12,030 patients had successful outcomes (37). In contrast, treatment outcomes among children with MDR-TB are generally good. In a 2012 systematic review including eight studies, 82% of 315 children treated for MDR-TB had a successful outcome (defined as a composite outcome of bacteriological cure or treatment completed) (38). In a recently completed large systematic review and individual patient data meta-analysis including 975 children from 18 countries treated

for probable or confirmed MDR-TB, 78% were successfully treated (23) according to WHO outcome definitions (39). Better outcomes, with >90% successfully treated (cured or treatment completed) have been reported in some observational studies in the context of routine care (17). All these data include children treated with traditional regimens prior to those recommended in 2016, including the use of older fluoroquinolones including ofloxacin in the majority of cases.

There is limited data on treatment outcomes among children treated with the WHO-shortened 9-12 month regimen recommended in 2016 several years after data collection for this doctoral thesis work was started. This policy recommendation was based on adult data (see Section 1.2.1), and only a small observational cohort from West Africa including 47 children <18 years of age with MDR-TB treated with the shortened regimen, which reported successful outcomes in 83% (40).

The good outcomes among children treated with more traditional and shortened regimens highlights the potential for children to benefit from effective but shorter and safer MDR-TB regimens, especially given the paucibacillary nature of the majority of TB and MDR-TB cases in children.

1.2.3 Limitations of current MDR-TB treatment regimens in children: regimens are long, toxic and poorly tolerated

Despite the good treatment outcomes described above, current paediatric MDR-TB treatment regimens have important limitations.

Even considering the newly recommended shortened regimen of 9-12 months, current regimens remain long, and some children will still require the more typical duration of 15-18 months or more. This is costly and burdensome for children, their caregivers and the health system, and is a challenge for adherence.

Secondly, the second-line antituberculosis medications are often poorly tolerated and associated with frequent and severe adverse effects. Although mostly mild, adverse events such as rash and vomiting are frequent, occurring in 24% and 18% of children treated for MDR-TB, respectively; other events such as pain, lethargy, headache, sleep disturbance, and reduced appetite are also common (17). Abnormal thyroid function tests have been documented in up to 54% of children treated with ethionamide or para-aminosalicylic acid, with 23% requiring levothyroxine supplementation (17, 41);

untreated, this hypothyroidism may have effects on neurodevelopment, particularly in young children. Additionally, the use of multiple hepatotoxic drugs raises the risk of liver injury during treatment, although this risk has not been well characterized. Additionally, the lack of child-friendly formulations of almost all second-line antituberculosis drugs means that adult tablets must often be crushed or split for administration in young children who cannot swallow these tablets whole. Although not well characterized, this formulation manipulation often reduces the palatability and acceptability of these medications further (42). Acceptability is the overall suitability of a dosage formulation, including factors such as palatability, dose volume or size, dosing frequency, dosing device for liquid medications, and directions for use (43). Palatability is defined as the overall acceptance of the taste, smell, volume or size, and texture of a medication to be taken orally (43), and is a particularly important determinant of medication acceptability in children.

The most troubling adverse effects are related to the second-line injectable medications (amikacin, kanamycin, capreomycin). These medications accumulate in cochlear hair cells, eventually resulting in cell death and permanent sensorineural hearing loss that progresses from higher to lower frequencies as the damage increases (44). Long-term treatment (i.e. for 4-6 months duration) with these injectable drugs has been reported to cause hearing loss in up to 24% of children (45). Although disastrous at any age, hearing loss is particularly devastating in young children, resulting in long-term developmental and functional challenges. Additionally, in most high MDR-TB burden settings, the injectables are mainly given by painful daily intramuscular injections, resulting in trauma and distress for patients, their caregivers and healthcare providers. This also contributes to the need for long-term hospitalization of children with MDR-TB in many settings, which may interrupt education, and peer and family bonds in older children, and may affect attachment in younger children.

Although the newly WHO recommended 9-12 month shortened regimen has the advantage over conventional regimens of a reduced treatment duration, safety and tolerability continue to be concerns, with limited available data in this regard. In the only cohort reported to date of children with MDR-TB receiving the 9-12 month shortened regimen, 75% experienced at least one adverse event, including 41% with some degree of hearing loss (40). These data highlight the persistent challenges

remaining for children; even with this newly recommended shortened regimen, an injectable drug continues to be required, with its associated pain and unacceptable risk of permanent hearing loss, adverse events remain frequent, and the duration (minimum of 9 months) is still long. This is ironic, given the excellent treatment outcomes and low bacillary burden in most children with MDR-TB, in whom less aggressive shorter regimens are likely to be highly successful.

Therefore, as treatment outcomes with previously and currently recommended MDR-TB regimens are good, and dramatically better than reported in adults, it is an urgent priority to develop more optimal treatment regimens for children with MDR-TB that retain their efficacy but are shorter, more child-friendly, better tolerated, safer and which do not require the use of an injectable medication.

1.3 Overall approach to improving MDR-TB treatment in children

The following three-pronged approach will inform the development of efficacious, but shorter, more child-friendly, better tolerated, safer and injectable-sparing treatment regimens for children with MDR-TB.

1) Optimize the use of existing second-line antituberculosis treatments: This aspect has formed the majority of this doctoral work, given the paucity of data in this regard, and is discussed in detail in Section 1.4.

2) Study currently available novel TB drugs in children to inform their effective, safe and acceptable paediatric use: Paediatric phase 1/2 for-registration trials to establish the dose and safety of both bedaquiline and delamanid have been in planning or implementation during the course of this doctoral work, but were beyond its scope. However some aspects of the paediatric use of these new medications, such as the impact of formulation manipulation (i.e. crushing or splitting tablets), have not traditionally been studied in such trials but could inform the practical use of these drugs in the field (see 1.4.3).

3) Combine existing and novel antituberculosis drugs into shorter, safer, injectable-sparing regimens: Once the optimal dosing and safety of existing second-line and novel antituberculosis drugs have been established, these drugs must be combined into short, all-oral regimens, and the efficacy, safety and acceptability of these regimens

established. Such research is beyond the scope of this doctoral work, but is being informed by data emerging from this doctoral research.

1.4 Approach to optimizing the use of existing second-line antituberculosis treatments

Because of differences in the pathophysiology of TB in children, especially regarding the disease spectrum and paucibacillary nature of the majority of paediatric TB, and the difficulties in obtaining sputum specimens in young children (7, 8), the evaluation of the efficacy of antituberculosis drugs or regimens, which has generally relied on microbiologic endpoints, is more challenging in children than in adults. Additionally, repeating efficacy studies of individual antituberculosis drugs in children is unnecessary. There is clear international consensus, including from regulatory authorities, that efficacy can be extrapolated to children from adult studies, as it is reasonable to assume that antituberculosis agents shown to be efficacious in adults will be at least as efficacious in children, as long as drug exposures are similar in children (46, 47). Key priorities therefore for evaluating and optimizing the use of antituberculosis drugs in children, are establishing the doses in representative ages that result in exposures similar to those in adults receiving doses shown to be efficacious, and rigorously evaluating safety and tolerability in children at those doses (48). Development of appropriate child-friendly formulations, especially relevant to younger children, is also a priority. Prior to this research, the pharmacokinetics, safety and tolerability of most of the second-line antituberculosis drugs in children had been poorly characterized and the evidence base for their optimal use in current or new treatment regimens was limited.

1.4.1 Pharmacokinetics and optimal doses of second-line antituberculosis drugs in children

Doses of the second-line antituberculosis medications in children that achieve adult target concentrations were not established (42), with most of these drugs being used off-label in children, with mg/kg doses extrapolated from adult data. This approach does not take into account the known effects of body size and age on pharmacokinetics (developmental pharmacology). Allometry, the study of how biological processes,

volumes and body parts scale with body size, can help describe the effect of body size on pharmacokinetics (49). Drug clearance, a key determinant of total drug exposure, scales with weight at a less than proportional rate, meaning that the same mg/kg dose will result in underexposure of drugs in children relative to adults, that is worse in small children (50). The relationship of clearance, and other key primary pharmacokinetic parameters, with body size can be accounted for mathematically with allometric scaling in population pharmacokinetic modeling that utilizes non-linear mixed effects (NLME) modeling techniques (50). The pharmacokinetics of drugs are also affected by the development and maturation of many physiologic processes, including changes in gut structure and function, metabolic capacity (enzyme function), renal function and body volumes (51). Maturation of these processes can affect drug absorption, distribution, metabolism and elimination. The development and maturation of these processes depend on post-gestational age and post-natal age, and vary from process to process in how rapidly they develop. There is often rapid development in the first days and weeks of life, and then more slow maturation thereafter. This has the greatest impact on pharmacokinetics during the first 6 months of life, and by 2-3 years of age, most relevant processes have matured to near adult levels (51, 52). The effect of age can be difficult to reliably predict and must be evaluated in young children, and for each relevant drug. It is therefore important to include a substantial number of young children, if possible of different racial and genetic origins, in studies of antituberculosis drug pharmacokinetics in order to characterize this maturation and inform dosing across the age spectrum.

Ensuring that paediatric doses of the existing second-line antituberculosis drugs are used that achieve target exposures will ensure that these agents are being utilized more optimally. This will help maximize their efficacy and thus potentially allow for treatment regimens in children that are shorter or that use fewer agents (i.e. could allow treatment without injectable drugs).

1.4.2 Safety and tolerability of existing second-line antituberculosis drugs in children

Knowledge of the safety profile of key second-line antituberculosis drugs at optimal doses, will be critical to inform the optimal use of existing drugs in current regimens and in combination with new and repurposed drugs in novel regimens.

The safety of medications in children can be informed by juvenile animal studies and adult trials. However this cannot be assumed and must be evaluated carefully in paediatric studies that include children across the age spectrum and of different racial and genetic origins. Children may have fewer or less severe adverse effects compared to animal studies or adult trials, which may result in potentially beneficial medications not being used in children (53, 54). An example is the fluoroquinolones in which juvenile animal studies showed a frequent severe arthropathy which has not been seen in children with short-term fluoroquinolone use (55). Children may also have more severe or unexpected adverse effects. Chloramphenicol, which was used widely in adults without serious concerns, caused "grey baby syndrome" in young infants due to immature metabolic pathways (56). The safety profile of current MDR-TB regimens in children has not been well characterized for key second-line drugs, such as the fluoroquinolones and linezolid (see Sections 1.5.2, 1.5.3). Safety evaluations of existing second-line antituberculosis drugs should also proactively consider overlapping toxicities with the novel medications bedaquiline and delamanid, as this will inform optimal regimen design. The key adverse effect of both bedaquiline and delamanid in adults is QT-interval prolongation (33, 57). Additional considerations are the study of these drugs in HIV-infected children in combination with appropriate antiretroviral therapy (ART) regimens.

Whereas the safety of a medication refers to the medical risk it poses to a patient, a medication's tolerability is the degree to which its adverse effects can be tolerated by a patient (58). The least well tolerated second-line antituberculosis drugs are the injectables. In addition to their serious safety concerns, the need for these medications to be given by daily, painful injections is a serious problem for their tolerability, and contributes to serious distress in MDR-TB patients. These injections are frequently cited as one of the worst aspects of current MDR-TB treatment and likely contribute to the high proportion of MDR-TB regimens not completed (59). Ulimately, the goal is to develop regimens which do not include the injectables, but in the meantime, strategies to improve the injectables' tolerability could substantially improve current regimens.

Understanding the appropriate, safe and tolerable use of the existing second-line antituberculosis drugs is a priority for improving MDR-TB treatment regimens in children.

1.4.3 Formulation considerations for existing second-line antituberculosis drugs in children

The impact of drug formulation on the pharmacokinetics and tolerability of medications in children is increasingly being recognized (60, 61). There have been few if any widely available child-friendly formulations of the second-line TB medications (62), and the routine practice of manipulating adult formulations by crushing or splitting tablets can affect drug exposures, with the magnitude and the direction of these effects difficult to predict (60). Formulation manipulation may result in reduced drug exposures, as has been seen with rifapentine in children (63), potentially increasing the risk of poor treatment response and theoretically the acquisition of additional resistance, or may increase drug exposures with a higher potential risk of adverse effects. Work is ongoing to develop child-friendly dispersible tablet formulations of the existing second-line and novel TB drugs. However there are many challenges with making these formulations available in the field, including financial, technical, market and regulatory barriers (62, 64). To improve MDR-TB treatment in children, a pragmatic approach to formulation development is needed, which in parallel seeks data on the optimal dosing of new child-friendly formulations in children and characterizes the effects of formulation manipulation where child-friendly formulations are unlikely to be readily available.

1.4.4 Summary

Addressing critical gaps in our current understanding of the pharmacokinetics, safety and tolerability of key second-line and novel TB drugs has the potential to substantially improve MDR-TB treatment strategies in children in the immediate future. Understanding the effects of formulation and formulation manipulation effects on drug pharmacokinetics in children is important for the effective and safe use of antituberculosis drugs and regimens in children. Improved knowledge in these areas will help optimize existing treatment regimens that utilize currently available medications. It will also inform future paediatric treatment of MDR-TB, by positioning children to be able to access novel, shorter and safer regimens currently being evaluated and even recommended in some adult populations in the rapidly evolving global MDR-TB treatment landscape.

1.5 Key existing and novel second-line antituberculosis drugs: the fluoroquinolones, linezolid, the second-line injectables and bedaquiline

1.5.1 Priority second-line and novel antituberculosis drugs

In order to address critical knowledge gaps for MDR-TB treatment with high potential impact on MDR-TB management, this PhD work has focused on the pharmacokinetics, dosing, safety and tolerability of key existing and novel antituberculosis medications. High priority medications were considered to be those that: 1) were critically important to current regimens from an efficacy, safety or tolerability perspective; 2) and/or were likely to be important in future treatment regimens (see Table 1.3 for list of ongoing or planned trials of MDR-TB treatment or prevention in adults or children); 3) and for which persistent important knowledge gaps had remained.

Of the existing second-line antituberculosis drugs, the fluoroquinolones, linezolid, the second-line injectables and bedaquiline were identified as priority agents for this doctoral work for which new data had high potential to improve the efficacy, safety or tolerability of MDR-TB treatment in children. Research on delamanid was also considered a high priority, but was collaboratively pursued beyond the scope of this body of doctoral research. Pretomanid, being very behind in its paediatric evaluation, was not considered an immediate priority.

Table 1.3. Planned or ongoing Phase 2 or 3 trials of MDR-TB treatment or preventive therapy

MDR-TB Treatment trials		*MDR-TB Preventive therapy trials*	
Trial	**Components of intervention arm**	**Trial**	**Components of intervention arm**
NC005	PZA, **BDQ,** PTA	VQUIN	**LFX**
Opti-Q	**LFX** + standard of care	TB-CHAMP	**LFX**
STREAM II	**BDQ**, CFZ, EMB, PZA, **LFX**, INH, PTO	PHOENIx	DLM
NIX-TB	**LZD**, **BDQ**, PTA		
STAND	PZA, MFX, PTA		
NEXT-TB	PZA, **LFX**, ETO/hdINH, **LZD, BDQ**		
C208	**BDQ** + standard of care		
Trial 213	DLM + standard of care		
endTB	Combinations including **LZD**, **BDQ**, CFZ		

PZA-pyrazinamide; BDQ-bedaquiline; PTA-pretomanid; LFX-levofloxacin; EMB-ethambutol; MFX-moxifloxacin; PTO-prothionamide; CFZ-clofazimine; hdINH-high dose isoniazid; LZD-linezolid; ETO-ethionamide; DLM-delamanid; **BOLD** indicates drugs included in this doctoral research.

1.5.2 The fluoroquinolones for treatment of MDR-TB in children

The fluoroquinolones inhibit the action of bacterial DNA gyrase, resulting in disruption of DNA synthesis and subsequent cell death (65). In addition to broad-spectrum activity against both Gram-negative and -positive organisms, the fluoroquinolones have potent anti-mycobacterial activity. The fluoroquinolones most commonly used for TB treatment to date have been ofloxacin, levofloxacin and moxifloxacin (66). Ofloxacin was one of the first quinolones used for treatment of MDR-TB and because of its low cost and widespread availability became the fluoroquinolone of choice for TB treatment until recently. Levofloxacin, the *l*-isomer and the active component of the ofloxacin racemate (67), has approximately twice the activity of ofloxacin, and since 2012-2013 has been recommended over ofloxacin for MDR-TB treatment globally. Moxifloxacin is the most potent of the three fluoroquinolones against *M. tuberculosis*, and has been the fluoroquinolone of choice for MDR-TB treatment. However there have been important barriers to its use in children, including limited paediatric pharmacokinetic data (68), lack of a child-friendly formulation (only 400 mg unscored tablets available in most settings), and very poor palatability especially when crushed (66). Therefore moxifloxacin has been used much less in children. Ofloxacin (200 mg and 400 mg tablets) and now levofloxacin formulations (250 mg tablets), although imperfect, are in strengths that allow reasonably accurate dosing in children and are less bitter than moxifloxacin, and have been the fluoroquinolones of choice for children (66), with good treatment outcomes documented in children (23).

The fluoroquinolones are the most important component of current MDR-TB regimens (16, 69, 70). The early bactericidal activity (EBA) of levofloxacin (1000 mg) and moxifloxacin (400 mg), defined as the fall in colony forming units per mL of sputum from days 0 to 2 of treatment (71), were 0.45 and 0.33 respectively, approaching that of 300 mg of isoniazid (0.67) (72). They are therefore critical for rapidly reducing the load of metabolically active mycobacteria early in treatment and for preventing acquisition of resistance to companion drugs. In addition to their role in current regimens, the fluoroquinolones are being evaluated as components of multiple novel regimens for MDR-TB treatment (Table 1.3) (73-75). Recent data has also shown that levofloxacin had comparable activity to isoniazid in a mouse model of latent infection with *M.*

tuberculosis (73), and appeared effective in high quality observational studies of MDR-exposed children and adults (76, 77). Two randomized controlled trials comparing levofloxacin versus placebo for the prevention of TB in in high-risk children (TB-CHAMP, South Africa, ISRCTN92634082) and adults (VQUIN, Vietnam, ACTRN12616000215426) exposed to MDR-TB were in development during the period during which this PhD research was undertaken, and both have now opened and are enroling (Table 1.3).

The pharmacokinetics and safety of ofloxacin and levofloxacin have been poorly described in children with TB. The fluoroquinolones are well absorbed with oral bioavailability of 85-95% for ofloxacin and >99% for levofloxacin; neither medication has significant food affects and both are primarily eliminated unchanged in the urine (66). Clinically significant drug-drug interactions between ofloxacin or levofloxacin and antiretrovirals or other antituberculosis drugs have not been described and are not expected (66) . The fluoroquinolones are concentration-dependent antibiotics with the area under the concentration time curve (AUC) to minimum inhibitory concentration (MIC) ratio most closely tied to efficacy (78); exposures in children should be targeted to approximate the AUC in adults receiving the recommended, efficacious dose (79-81). Prior to this PhD research, there was a single study of ofloxacin pharmacokinetics in children with typhoid fever (82), and limited published data on levofloxacin paediatric pharmacokinetics (83, 84). In previous work, we described a small cohort of 23 children receiving levofloxacin (15 mg/kg daily) or ofloxacin (20 mg/kg daily) for MDR-TB treatment or preventive therapy, and documented exposures of both drugs that were low relative to adult target values. However this study was small, had a limited ability to explore the impact of key covariates, such as age and HIV infection, on pharmacokinetics of the fluoroquinolones (85), and primarily used non-compartmental analysis (NCA). Data on levofloxacin pharmacokinetics in 50 children treated for MDR-TB exposure or disease in the Federated States of Micronesia and the Republic of the Marshall Islands suggested a dose of 15-20 mg/kg once daily would be appropriate, but few young children and no HIV-infected children were included (86).

Although the fluoroquinolones are generally well tolerated, they are known to cause a variety of adverse effects. Mild gastrointestinal effects such as nausea, vomiting, diarrhoea are the most common events described in clinical trials (66). A variety of

central nervous system effects have been described with the fluoroquinolones, including dizziness, headaches, confusion, psychosis, and seizures (66). Sleep disturbance, insomnia and hallucinations (with overdose), have been well documented in children receiving fluoroquinolones (77, 87, 88). QT prolongation is also a classwide effect of the fluoroquinolones, mediated by dose-dependent inhibition of cardiac potassium rectifier (HERG) channels (89). Moxifloxacin has the largest QT prolonging effect (89, 90), while even at doses as high as 1500 mg in adults, levofloxacin results in minimal clinically important QT prolongation (91). The QT prolonging effects of the fluoroquinolones in children have not been well described. However this information was urgently needed, as the fluoroquinolones will increasingly be combined in novel regimens with the new and repurposed drugs bedaquiline, delamanid and clofazimine, all of which cause QT prolongation (33, 57). This was also important for understanding the expected background QT interval in order to interpret the QT effects of bedaquiline and delamanid in paediatric trials, in which the drugs will be added to an optimized background regimen. The primary limiting factor for fluoroquinolone use in children to date has been a concern for arthropathy based on animal data (66). Damage to the weight-bearing joints induced by fluoroquinolones was demonstrated in all juvenile animal models tested, with juvenile dogs being the most sensitive (92). However, to date multiple large evaluations have not demonstrated a significant risk of serious arthropathy in children treated with fluoroquinolones for short durations (66). One non-blinded long-term follow-up study of levofloxacin-treated children did report a significant increase in musculoskeletal complaints, primarily subjective arthralgia; the lack of blinding may have biased the findings, especially given the subjective nature of the events (55). However other large studies, including a review of over 7000 children and another including 6000 children, did not identify patients with severe arthropathy or an increased risk of musculoskeletal disorders (92, 93). However there is limited high quality prospective data on fluoroquinolone safety for the long durations and at the doses in use for MDR-TB treatment and concerns about arthropathy have persisted.

Given the particular importance of the fluoroquinolones in current and future MDR-TB treatment and prevention, it was critically important to understand the pharmacokinetics, impact of key covariates (including the effect of formulation and formulation manipulation), optimal doses, safety and tolerability of ofloxacin (see Section 1.8, Chapter 2) and levofloxacin in children with MDR-TB (Section 1.8, Chapters

3, 4 and 5), in order to optimize current and future MDR-TB treatment regimens in children.

1.5.3 Linezolid for the treatment of MDR-TB in children

Linezolid is an oxazolidinone antibiotic, that binds the 50s ribosomal subunit, inhibiting protein synthesis (94). Linezolid is active against Gram-positive bacteria and is used in adults and children for the treatment of skin and soft-tissue infections and for resistant Gram-positive organisms (94). Relatively recently it has also been identified to have antimycobacterial activity, and has been used increasingly and very effectively in difficult to treat drug-resistant TB such as XDR-TB for which there are very few effective treatment options available (95-97). Its excellent cerebrospinal fluid (CSF) penetration makes it an important option for the treatment of MDR-TB meningitis (98, 99). Trials in adults to evaluate the best dose and duration of linezolid to optimize efficacy while minimizing adverse effects have been in planning or are underway, and linezolid is a component of a number of novel MDR-TB regimens under evaluation (Table 1.3). As in adults, linezolid has been used off-label in children with difficult to treat forms of DR-TB, however the evidence for this was limited to case reports and case series, and there was no formal guidance on its use or dose in paediatric MDR-TB. During the course of this PhD research, linezolid has become an even more important treatment option for MDR-TB. The Nix-TB study (NCT02333799), an open-label single arm phase 3 study that opened in 2015 is evaluating a regimen of bedaquiline, linezolid and pretomanid for 6 months among adults with XDR-TB or with MDR-TB failure or intolerance (100). This patient population has very poor treatment outcomes, with <25% successfully treated in some studies (101). However interim results of the trial were very encouraging, with death reported in only 4 of 61 and all surviving patients culture negative at 4 months and 74% culture negative at 8 weeks; there were substantial adverse effects mostly attributed to linezolid (100). Final results are pending and a follow-up study, ZeNix (NCT03086486) that aims to evaluate different linezolid dosing strategies is now underway.

Linezolid has nearly 100% oral bioavailability, and food does not significantly affect total exposures (94, 102). Linezolid has complex metabolism with formation of multiple metabolites and undergoes both renal and non-renal elimination (102). Linezolid pharmacokinetics has been studied in children treated short-term (<28 days)

for Gram-positive bacterial infections, and there are recommended paediatric doses that approximate exposures in adults receiving 600 mg twice daily (103). However there is no published data in children with TB with or without HIV-infection, and it is not clear what dose in children will result in exposures that approximate the 600 mg once daily dose of linezolid most commonly in use for adults with TB.

For the short durations that are recommended for treatment of Gram-positive infections (<28 days), linezolid has been shown to be safe and well tolerated in adults and children (104). However linezolid is associated with dose and time-dependent serious adverse effects, including peripheral neuropathy, optic neuropathy, myelosuppression (anaemia, thrombocytopaenia and leucopenia), and more rarely pancreatitis and lactic acidosis (105, 106). These have been described to occur frequently in adults treated with long durations of linezolid for MDR-TB (96, 107), however the frequency, severity, and risk factors for these serious adverse effects in children treated long-term with linezolid for MDR-TB have been poorly characterized.

Considering the importance of linezolid currently for treatment of children with XDR-TB or MDR-TB meningitis, its potential to substitute for the second-line injectable agents, and its likely key role in future MDR-TB treatment regimens, it was identified that there was insufficient evidence to inform its use in children with MDR-TB (see Section 1.8, Chapter 6). Data were urgently needed on linezolid pharmacokinetics, optimal dose and safety with long treatment duration in children with MDR-TB (Section 1.8, Chapter 7).

1.5.4 The second-line injectable TB medications for the treatment of MDR-TB in children

The aminoglycosides amikacin and kanamycin and the cyclic polypepetide capreomycin are often referred to together as the second-line injectable antituberculosis drugs. These medications have similar mechanisms of action, pharmacology, requirement for intravenous (IV) or intramuscular (IM) route of administration, and adverse effect profiles. All three bind to different bacterial ribosomal subunits, inhibiting protein synthesis (108, 109). Along with the fluoroquinolones, they have traditionally been considered a key component of existing MDR-TB treatment regimens. This has begun to be questioned, as these agents have a minimal EBA (110) and evidence from early clinical trials with streptomycin suggests that they have minimal if any sterilizing effect (111). However, for now they remain recommended components of routinely used

MDR-TB treatment regimens for most adults and children globally (18). The second-line injectables are rapidly degraded if given orally, so must be given either IV or IM, and are eliminated unchanged in the urine (108). The pharmacokinetics of amikacin, kanamycin and other aminoglycosides have been studied in children for non-TB indications but not in children with TB, and there is minimal data in general on capreomycin pharmacokinetics in children (112). The most important concern for using the second-line injectables in MDR-TB treatment regimens is the high risk of ototoxicity. These medications cause cochlear hair cell death resulting in permanent sensory neural hearing loss (44). The most important risk factor for this ototoxicity appears to be cumulative drug exposure (113, 114). The incidence of ototoxicity with long-term second-line injectable treatment varies widely, from 2.6-61% in adults, but is likely towards the higher end of this range (115). Paediatric studies have reported hearing loss in up to 24-41% of children receiving long-term injectables (17, 40, 45). MDR-TB treatment regimens that avoid the use of the injectables are thus desperately needed. But until other treatment options are available, improved characterization of the pharmacokinetics, optimal dose, and safety (including incidence of and risk factors for ototoxicity) of the second-line injectables in children are needed. This work was undertaken but was outside the scope of my PhD research thesis.

In addition to the serious safety concerns regarding ototoxicity, the daily intramuscular injections of the injectable agents are painful, poorly tolerated, and a significant source of distress for children, caregivers and healthcare workers. This has not been well documented in children, but in adults has been identified as one of the worst aspects of current MDR-TB treatment regimens (59). As the second-line injectables continued to be recommended as a component of existing MDR-TB treatment regimens in many countries, identifying approaches to improve the tolerability of the injectables was a priority. Strategies to reduce the pain of intramuscular injections could substantially improve their tolerability. The addition of local anaesthetic to other antibiotic injections, such as ceftriaxone or benzathine penicillin, has been shown to reduce injection pain without affecting pharmacokinetics (116, 117). A similar strategy had not been evaluated with the second-line injectables, but had the potential to substantially improve the tolerability of the second-line injectables when they are required to be given (see Section 1.8, Chapter 8). Such a risk-reduction strategy would be a

temporizing measure until other medications or injectable-sparing regimens could be developed, in part informed by work in this PhD.

1.5.5 Bedaquiline

As described in Section 1.2.1, the novel antituberculosis drug bedaquiline received accelerated approval in 2012 from the U.S. FDA for adults with MDR-TB, and the WHO provided recommendations for its use (28, 57). Since then it has been registered in many countries, and is becoming widely available globally (118). In South Africa, after registration in 2014, aggressive roll-out in routine care settings has provided access to bedaquiline for thousands of adults and some adolescents with good treatment outcomes including reduced mortality (119, 120). Bedaquiline is also being evaluated in adults as a component of many novel, shortened regimens for MDR-TB treatment (Table 1.3). Bedaquiline is clearly a critically important antituberculosis medication for current and future MDR-TB treatment. Paediatric bedaquiline trials have unfortunately been very delayed, and were in planning or ongoing during this PhD research. This has been a major barrier to paediatric access to bedaquiline. The Janssen-sponsored C211 phase 1/2 trial of bedaquiline pharmacokinetics and safety in HIV-uninfected children only (NCT02354014), only opened in 2016. A paediatric bedaquiline dispersible tablet formulation was developed for the trial and will be studied in children less than 6 years of age in the study. The IMPAACT P1108 phase 1/2 trial of bedaquiline pharmacokinetics and safety (NCT02906007) includes HIV-infected and –uninfected children with MDR-TB and opened at the end of 2017. These studies will characterize the pharmacokinetics, optimal dose and safety of bedaquiline in children, and should lead to a paediatric indication. However access to the paediatric formulation in routine care settings is likely to be very delayed, even after data from the paediatric trials on dosing and safety in young children is available. The adult formulation, already widely available, could be manipulated through crushing or suspending in water for administration to young children, but it is unclear if and how this would affect its bioavailability. This question, of significant practical importance, was not being studied in the paediatric trials. A trial characterizing the affect of suspending adult bedaquiline tablets in water on its bioavailability would inform the rational use of the adult formulation in young children, greatly improving access until the paediatric formulation is more widely available (Section 1.8, Chapter 9).

The novel TB drug delamanid received conditional approval from the EMA in 2013 and the WHO issued recommendations for its use in adults with MDR-TB in 2014 (see Section 1.2.1) (32, 33). Registration of delamanid in high TB burden settings has been very slow, however the paediatric phase 1/2 trials are further along than bedaquiline. Otsuka-sponsored phase 1 (242-12-232) (NCT01856634) and phase 2 (242-12-233) (NCT01859923) paediatric trials of delamanid opened in 2013, including in South Africa. These age de-escalation trials aimed to characterize delamanid pharmacokinetics and safety over 10 days (trial 232) and 6 months of dosing (trial 233). The older age cohorts (6 to <18 years of age) have completed the trials, and the WHO issued recommendations for delamanid use in children ages 6-17 years with MDR-TB (121). The younger cohorts have completed enrolment, with only long-term follow-up in the youngest group expected to be completed in early 2019. Two paediatric formulations (5 and 25 mg dispersible tablets) were developed for and studied in these trials. However, similar to bedaquiline, it is expected that access to these paediatric formulations in routine settings are likely to be very delayed. A similar pragmatic approach of studying the effect on bioavailability of manipulating the adult formulation through crushing or suspending in water would facilitate rational use of this formulation in young children until the paediatric formulation is available. Such a study would be very useful, but was not included in the scope of this PhD research, partly because delamanid was not registered in South Africa, so access to the adult formulation was limited.

1.6 Purpose and scope of proposed research

The purpose of this PhD research was to address critical knowledge gaps in paediatric MDR-TB treatment, with the aim of informing more effective, safer, and more child-friendly MDR-TB treatment strategies in children. This includes: characterizing the pharmacokinetics and safety of currently recommended doses of key second-line antituberculosis drugs including ofloxacin, levofloxacin, and linezolid in children with MDR-TB, investigating the impact on pharmacokinetics and pain of administration practices for intramuscular injections of amikacin in children with MDR-TB, and characterizing the effects of formulation and/or formulation manipulation on the pharmacokinetics of levofloxacin and bedaquiline. Identifying paediatric doses of these

second-line antituberculosis drugs that better approximate target exposures will ensure that the efficacy of these key medications is optimized and will allow for extrapolation of efficacy to children from adult MDR-TB regimens under evaluation. Optimal paediatric dosing may also facilitate the use of shorter, safer regimens in children for MDR-TB prevention and treatment compared to adults in future.

1.7 Overall objective

The overall objective of this doctoral research was to characterize the pharmacokinetics, including the effects of formulation, optimal dose, safety, and tolerability of key second-line and novel antituberculosis drugs in children in order to inform the safer and effective treatment of children with MDR-TB.

1.8 Hypotheses and proposed studies

The following complementary hypotheses and related studies were proposed to address the key knowledge gaps described above:

Hypothesis 1, Study 1 (Chapter 2): *The WHO-recommended dose of ofloxacin (20mg/kg once daily) is safe and achieves adequate target drug concentrations in children with MDR-TB exposure or disease.*

The study aim was to characterize the pharmacokinetics and safety of ofloxacin among HIV-infected and uninfected children aged 0 to <15 years routinely treated for MDR-TB disease or exposure. This was a prospective observational intensive pharmacokinetic study. Pharmacokinetic measures were estimated using non-compartmental analysis.

Hypothesis 2, Studies 2, 3, 4: *The paediatric dose of levofloxacin (15-20mg/kg once daily) in use for MDR-TB exposure or disease is safe and achieves target drug concentrations*

Study 2 (Chapter 3) aimed to characterize the pharmacokinetics of levofloxacin 15mg/kg and 20mg/kg oral doses, given routinely to children 0 to <15 years of age for prevention or treatment of MDR-TB. In this prospective observational pharmacokinetic study, children underwent intensive pharmacokinetic sampling. The analysis used

population pharmacokinetic modeling techniques and clinical trial simulations to characterize levofloxacin pharmacokinetics and optimal doses.

Study 3 (Chapter 4) characterized levofloxacin (15mg/kg and 20mg/kg) safety in children with MDR-TB disease in this same cohort. Clinical and laboratory adverse events were assessed for severity according to standard grading and for attribution to levofloxacin.

Study 4 (Chapter 5) characterized the pharmacokinetics and short-term safety of a novel, child-friendly 100 mg scored dispersible tablet. In an open-label pharmacokinetic study as a lead-in to the TB-CHAMP phase III trial (levofloxacin vs. placebo for MDR-TB prevention), children 0 to <5 years of age had intensive pharmacokinetic sampling following weight-banded doses of this novel levofloxacin formulation. NLME modeling was used to compare the primary pharmacokinetic parameters with the historical cohort evaluated in Study 2; simulations identified optimized weight-banded dosing for this formulation.

Hypothesis 3, Study 5, 6: *The currently used dose of linezolid for MDR-TB and XDR-TB in children is safe and achieves target concentrations.*

The aim of **Study 5 (Chapter 6)** was to review and synthesize the literature on the use of linezolid for the treatment of MDR-TB in children. This scoping review undertook a sensitive search of the literature for all evidence that could inform use of linezolid in children with MDR-TB, including pre-clinical and clinical data on its efficacy, its safety in adults and children with prolonged use, and its pharmacokinetics in adults and children. Reports of linezolid use in children with MDR-TB were combined to estimate its efficacy and safety in children, and other results were synthesized to create practical recommendations for linezolid in children with MDR-TB.

Study 6 (Chapter 7) combined data from two prospective observational intensive sampling pharmacokinetic studies of linezolid in children with MDR-TB. Linezolid pharmacokinetic parameters were estimated using NLME modeling and optimal doses were identified using clinical trial simulations, and safety described.

Hypothesis 4, Study 7 (Chapter 8): *Lidocaine co-administered with intramuscular injections of amikacin results in reduced pain and similar amikacin plasma concentrations in children with MDR-TB.*

This study assessed the effect of co-administering lidocaine with intramuscular amikacin injections on injection-associated pain and on the amikacin pharmacokinetics using intensive sampling. This was a randomized, double-blind, two-period crossover study in children aged 8 to <18 years with MDR-TB routinely receiving amikacin for MDR-TB. Children received a standard mg/kg dose of amikacin either with or without a weight-banded dose of lidocaine. Pain was assessed before, and then immediately, 30 minutes and 60 minutes after the injection. Pharmacokinetic measures were estimated using non-compartmental analysis.

Hypothesis 5, Study 8 (Chapter 9): *Bedaquiline 400 mg administered as tablets suspended in water is bioequivalent to bedaquiline 400 mg swallowed as whole tablets in healthy adult volunteers.*

This was a randomized, open label, crossover, bioequivalence study with two single treatment periods, separated by a 14 day washout period. Twenty-four healthy male and female volunteers with similar demographics were randomly assigned 1:1 to one of two treatment sequences in order to receive either first the crushed form of bedaquiline, as the experimental, and secondly the whole tablet as the approved dosing form, or vice versa. NLME modeling was used to estimate the potential difference in bioavailability for crushed compared to whole tablets. 95% confidence intervals of the estimate were compared to formal bioequivalence criteria.

Chapter 2: The pharmacokinetics and safety of ofloxacin in children treated for drug-resistant tuberculosis

Rationale

The fluoroquinolones are critical components of treatment regimens for MDR-TB. Until 2013-2014 ofloxacin was the most widely used fluoroquinolone for TB treatment in both adults and children globally. The WHO recommended a dose of 15-20 mg/kg once daily in children, however there was little data on ofloxacin use in children to inform this recommendation and it was unknown if this dose achieved the exposures achieved in adults after the standard recommended 800 mg dose (median $AUC_{0\text{-}24}$ 103 μg · h/ml) (81).

Study aims

The objective of this study was to characterize the pharmacokinetics and long-term safety of ofloxacin in HIV-infected and -uninfected children routinely receiving ofloxacin for MDR-TB disease or exposure.

Methods

The MDRPK1 study (*Pharmacokinetics and toxicity of second-line antituberculosis drugs in HIV-infected and –uninfected children*, PI Hesseling, Schaaf, HD 069169-01) was an NIH RO1 funded study targeting 318 HIV-infected and uninfected children receiving second-line antituberculosis drugs for pharmacokinetic sampling and longitudinal follow-up for safety and treatment outcomes. The primary objective of the study was to characterize the pharmacokinetics of the second-line antituberculosis drugs in children; other objectives were to characterize the safety of the second-line antituberculosis drugs and any potential drug-drug interactions with antiretrovirals (ARVs) in HIV-infected children. The study was open from 2011-2015, and recruited children with MDR-TB disease or exposure from Tygerberg Hospital and Brooklyn Hospital for Chest Diseases, Cape Town as well as Brewelskloof Hospital, Worcester. Children were eligible for the study if they were 0 to <15 years of age, routinely treated with second-line antituberculosis drugs for >2 weeks but <8 weeks for treatment of MDR-TB disease or as preventive therapy for MDR-TB exposure, HIV-infected or –uninfected, and on ART for >2 weeks if HIV-infected. Exclusion criteria included laboratory documented

anaemia (haemoglobin <8 g/dL) or weight <5 kg. Local guidelines in Cape Town used a three-drug MDR-TB preventive therapy regimen composed of ethambutol, high-dose isoniazid and either ofloxacin (prior to 2013) or levofloxacin (from 2013) to children <5 years of age or HIV-infected and <15 years of age with a recent documented exposure to MDR-TB but no evidence of TB disease at the time. Children enrolled in the study on MDR-TB preventive therapy had cross-sectional intensive pharmacokinetic sampling, and were followed in the routine care programme, but within the study were not followed for safety or long-term outcomes, as data on the safety of this regimen had already been documented (77). Children in the study who were receiving treatment for MDR-TB disease had intensive pharmacokinetic sampling and were followed longitudinally for safety and treatment outcome.

Children enrolled in MDRPK1 who were less than 15 years of age and routinely treated with ofloxacin for the prevention or treatment of MDR-TB were included, primarily during 2011-2013, prior to the introduction of levofloxacin. All children had pharmacokinetic sampling between 2 and 8 weeks of treatment with ofloxacin as a component of their routine treatment regimens. The routinely available formulation of ofloxacin was used (200 mg tablets, Sanofi Aventis, Midrand, South Africa). On the day of pharmacokinetic sampling, an exact 20 mg/kg dose of ofloxacin was administered by the study team, and samples taken pre-dose and at 1, 2, 4, 8 and either 6 or 11 hours post-dose. Ofloxacin was administered either as whole tablets, or as crushed tablets either orally or by nasogastric tube, depending on what the child was able to tolerate. Ofloxacin plasma concentrations were measured using a validated liquid chromatography tandem mass spectrometry (LC MS/MS) assay at the University of Cape Town Division of Clinical Pharmacology. Key pharmacokinetic measures were calculated using NCA, and univariable and multivariable linear regression used to assess associations between pharmacokinetic measures and clinical and other covariates of interest using Stata 12.1 SE software (StataCorp., College Station, Texas). Children receiving ofloxacin as a component of a treatment regimen for MDR-TB disease had 1-2 monthly safety monitoring until the completion of MDR-TB treatment (typically 12-18 months). All adverse events were assessed for both severity using standard Division of AIDS criteria (DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 1.0, December 2004, updated August 2009) (122) and attribution to

ofloxacin. Frequency and rates of adverse events were reported by grade and by relation to ofloxacin.

Results

Eighty-five children, median (IQR) age 3.4 years (1.9 to 5.2), were included in this study. Eleven (13%) were HIV-infected; 85% received ofloxacin as crushed tablets in water (as opposed to tablets swallowed whole, due to feasibility reasons). The mean ofloxacin (range) C_{max}, AUC_{0-8}, and $t_{1/2}$ were 8.97 µg/ml (2.47 to 14.4), 44.2 µg · h/ml (12.1 to 75.8), and 3.49 h (1.89 to 6.95), respectively. The mean AUC_{0-24}, estimated in 72 participants, was 66.7 µg · h/ml (range, 18.8 to 120.7). In multivariable analysis, AUC_{0-24} was increased by 1.46 µg · h/ml for each 1-kg increase in body weight (95% CI, 0.44 to 2.47; p=0.006). No other assessed variable contributed to the model. No grade 3 or 4 adverse events at least possibly attributed to ofloxacin were observed.

Conclusions and recommendations

In this largest study of ofloxacin in children to date, ofloxacin was safe and well tolerated in children routinely receiving an ofloxacin-containing regimen for MDR-TB treatment or prevention. However, ofloxacin exposures were well below the values typically achieved in adults. The long-term safety of ofloxacin demonstrated in this study is reassuring, as historical concerns about fluoroquinolone adverse effects in children have persisted in many settings despite emerging evidence to the contrary. Only weight was associated with AUC_{0-24} in multivariable analysis. Notably, neither HIV co-infection nor ofloxacin formulation manipulation (receiving crushed tablets vs. whole) was associated with ofloxacin exposure. The study identified that higher doses of ofloxacin than currently recommended will likely be needed in children to achieve the target exposures achieved in adults receiving an 800 mg daily dose. Although ofloxacin was no longer the fluoroquinolone of choice in the period following this publication, this work importantly demonstrated the safety of long-term fluoroquinolone treatment in young children and also identified that the recommended dose of this antituberculosis drug resulted in low exposures in children, raising the concern that there may be similar considerations for other fluoroquinolones.

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Pharmacokinetics and Safety of Ofloxacin in Children with Drug-Resistant Tuberculosis

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Ofloxacin is widely used for the treatment of multidrug-resistant tuberculosis (MDR-TB). Data on its pharmacokinetics and safety in children are limited. It is not known whether the current internationally recommended pediatric dosage of 15 to 20 mg/kg of body weight achieves exposures reached in adults with tuberculosis after a standard 800-mg dose (adult median area under the concentration-time curve from 0 to 24 h [AUC_{0-24}], 103 μg · h/ml). We assessed the pharmacokinetics and safety of ofloxacin in children <15 years old routinely receiving ofloxacin for MDR-TB treatment or preventive therapy. Plasma samples were collected predose and at 1, 2, 4, 8, and either 6 or 11 h after a 20-mg/kg dose. Pharmacokinetic parameters were calculated using noncompartmental analysis. Children with MDR-TB disease underwent long-term safety monitoring. Of 85 children (median age, 3.4 years), 11 (13%) were HIV infected, and of 79 children with evaluable data, 14 (18%) were underweight. The ofloxacin mean (range) maximum concentration (C_{max}), AUC_{0-8}, and half-life were 8.97 μg/ml (2.47 to 14.4), 44.2 μg · h/ml (12.1 to 75.8), and 3.49 h (1.89 to 6.95), respectively. The mean AUC_{0-24}, estimated in 72 participants, was 66.7 μg · h/ml (range, 18.8 to 120.7). In multivariable analysis, AUC_{0-24} was increased by 1.46 μg · h/ml for each 1-kg increase in body weight (95% confidence interval [CI], 0.44 to 2.47; $P = 0.006$); no other assessed variable contributed to the model. No grade 3 or 4 events at least possibly attributed to ofloxacin were observed. Ofloxacin was safe and well tolerated in children with MDR-TB, but exposures were well below reported adult values, suggesting that dosage modification may be required to optimize MDR-TB treatment regimens in children.

Globally, in 2013 there were an estimated 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB), defined as *Mycobacterium tuberculosis* resistant to isoniazid (INH) and rifampin (RIF) (1). Precise incidence data in children are unavailable, but modeling estimates suggest that there were 33,000 new pediatric MDR-TB cases in 2010 (2). In addition, assuming an average of two child contacts for each adult MDR-TB source case (3), there may be as many as 900,000 children newly exposed to MDR-TB globally each year. Fluoroquinolones are a key component of existing regimens for treatment (4) and prevention (5) of MDR-TB in adults and children.

Ofloxacin, a fluoroquinolone, has potent activity against *M. tuberculosis* (6, 7) and has been routinely used in MDR-TB treatment. The current World Health Organization (WHO) recommended adult dose of ofloxacin for MDR-TB is 800 mg daily. Ofloxacin is not metabolized; rather, it is excreted unchanged in the urine (8). It is well absorbed after oral administration, and food intake does not affect its pharmacokinetics appreciably (9–12).

There are limited data on ofloxacin pharmacokinetics in children, particularly in children <5 years of age, to guide appropriate dose selection (11, 13). The WHO recommends a pediatric ofloxacin dose for MDR-TB of 15 to 20 mg/kg of body weight daily (14); however, it is unknown if this dose achieves exposures in children approximating those in adults after the recommended 800-mg dose. Concerns regarding arthropathy (15, 16) had initially limited the use of fluoroquinolones in children. Although safe in short courses (16–18), there are limited data on fluoroquinolone safety in children with long-term use (5, 19).

The more potent fluoroquinolones levofloxacin and moxifloxacin (20, 21) are beginning to replace ofloxacin for MDR-TB treatment. However, because of its low cost and widespread availability, ofloxacin is still used for MDR-TB in many settings, and optimizing its use in children remains important.

The objective of this study was to evaluate the pharmacokinetics and safety of ofloxacin among a large cohort of HIV-infected and uninfected children of representative ages who were routinely receiving ofloxacin for the prevention or treatment of MDR-TB.

MATERIALS AND METHODS

Study design. This was a prospective observational pharmacokinetic study.

Study setting. The study took place in the Western Cape, South Africa, where in 2010 the TB notification rate was 954.1 cases per 100,000 population, and from 2009 to 2011 MDR-TB represented 7.1% of culture-

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confirmed cases in children <13 years old (22, 23). The diagnosis of MDR-TB was based on (i) culture of *M. tuberculosis* from sputum or other relevant specimens with drug susceptibility testing (DST) demonstrating resistance to INH and RIF, (ii) clinical and radiologic evidence of TB and contact with an MDR-TB source case, or (iii) failure of first-line TB treatment. Treatment for MDR-TB in children was provided independent of the study, according to local and international guidance, based on the DST of the child's isolate or the isolate of their most likely source case. Treatment included at least four drugs likely to be active given for at least 12 to 18 months (14, 24).

In the study setting, child contacts of adult MDR-TB cases are referred to a specialty clinic for preventive therapy. Children <5 years of age and those HIV infected without evidence of TB were prescribed 6 months of a three-drug preventive therapy regimen: ofloxacin, ethambutol, and high-dose INH (5).

Study population. Children were recruited from a large provincial referral hospital (Tygerberg Children's Hospital) and two provincial TB hospitals (Brooklyn Hospital for Chest Diseases and Brewelskloof Hospital). Children <15 years of age routinely started on ofloxacin for prevention or treatment of MDR-TB were eligible. Exclusion criteria were a weight of <5 kg or hemoglobin of <8.0 g/dl. Children treated for MDR-TB disease were followed longitudinally to assess safety and tolerability during treatment. The safety of this preventive therapy regimen has been previously documented; these children were followed independent of the study (5). Data from 23 children from a substudy of this cohort were previously published and are included in the present analysis (13).

Data collection. Children were categorized as receiving ofloxacin either for MDR-TB treatment or prevention. TB was categorized as confirmed, probable, or possible according to international consensus definitions (25) and as pulmonary, extrapulmonary, or both. HIV status was ascertained in all participants. Weight-for-age Z-score (WAZ) was calculated using the 1990 British growth reference (26).

All participants underwent intensive pharmacokinetic sampling 2 to 8 weeks after starting ofloxacin. Ofloxacin (200-mg tablets; Sanofi Aventis, Midrand, South Africa) at a dose of 15 to 20 mg/kg once daily was routinely prescribed. On pharmacokinetic sampling days, an exact 20 mg/kg dose of ofloxacin was weighed and administered by the study team after a minimum 4-h fast with a small amount of water. Medications were given either as whole tablets or were crushed and given by mouth or nasogastric tube, depending on what the child would tolerate. Crushed tablets were ground with a mortar and pestle, mixed with a small amount of water in a plastic cup along with any other crushed TB medications, and administered immediately. Any residue in the cup was rinsed 1 to 2 times with additional water and administered to the child. All other anti-TB medications in the regimen were given together with the ofloxacin. One hour after the TB medications, antiretrovirals were administered (if applicable) and a standard breakfast was offered. Samples for pharmacokinetic analysis were drawn predose and then at 1, 2, 4, 8, and either 6 or 11 h postdose. Whole-blood samples were collected in EDTA-containing tubes and immediately centrifuged, and plasma was separated and frozen at −80°C. Ofloxacin concentrations were determined by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) using a previously described method validated over a range of 0.0781 to 20.0 μg/ml (27).

Children receiving ofloxacin for MDR-TB treatment had clinical monitoring monthly for the first 6 months and then every 2 months thereafter and laboratory monitoring (potassium, creatinine, alanine aminotransferase [ALT], total bilirubin, thyroid functions) every 2 months. Adverse events were graded according to standardized Division of AIDS (National Institute of Allergy and Infectious Diseases) criteria (28) and were considered attributable to ofloxacin if they were (i) at least possibly drug related and (ii) thought by the investigator to be likely related to ofloxacin or if they were not otherwise attributed to another drug. The person-time of observation began at the initial study visit and ended at either the final study visit or the date of treatment completion; observation periods in which the child received an alternative fluoroquinolone for part of the time (treatment guidelines changed during the study period) were excluded from safety analyses.

Statistical analysis. Demographic and clinical characteristics were summarized using descriptive statistics. Pharmacokinetic measures were estimated using noncompartmental analysis (NCA). Observed maximum plasma drug concentration (C_{max}) and time to C_{max} (T_{max}) were recorded directly from the concentration-time data. The area under the concentration time curve from 0 to 8 h (AUC_{0-8}) was calculated using the linear trapezoidal rule. Oral clearance (CL/F), half-life ($t_{1/2}$), and AUC_{0-24} were estimated in patients with at least 3 concentration data points in the elimination phase, with the latter based on exponential extrapolation from the final three time-concentration data points. Fifteen predose drug concentrations below the limit of quantification (0.078 μg/ml) were set to zero in analyses.

The C_{max}, AUC_{0-8}, AUC_{0-24}, and $t_{1/2}$ were compared by age group (0 to <2 years, 2 to <5 years, and ≥5 years), HIV status, nutritional status (WAZ, <−2 versus ≥−2), and administration method (crushed versus whole tablets). Using simple linear regression, the AUC_{0-24} and C_{max} were analyzed separately for associations with age, weight, height, HIV status, nutritional status, gender, ethnicity, disease status (receiving preventive therapy versus treatment for MDR-TB), and administration method. Covariates with a P of <0.05 in univariable analysis, and factors known to affect drug disposition (age and weight) were included in multivariable models. We also assessed whether body surface area (BSA) (29) or lean body mass (LBM) (30) were better predictors than weight and height.

All analyses were performed using Stata 12.1 SE software (StataCorp, College Station, TX).

Ethical considerations. Written informed consent was obtained from the parents or legal guardian, and informed assent was collected from all children ≥7 years of age. Ethical approval was provided by the Health Research Ethics Committees of the Faculty of Medicine and Health Sciences of Stellenbosch University and the Faculty of Health Sciences of the University of Cape Town.

RESULTS

Baseline characteristics. Eighty-five children were included (Table 1). All age groups were well represented. The median age was 3.4 years (interquartile range [IQR], 1.9 to 5.2 years). Eleven (13%) participants were HIV infected. Fourteen of the 79 patients with evaluable data (18%) were underweight for age (WAZ, <−2) and 11 of these children were HIV infected (79%). Overall, 72 of 85 (85%) received crushed tablets on the day of pharmacokinetic sampling (97% of those <5 years old and 41% of those ≥5 years old).

Pharmacokinetics and determinants of drug exposures. With a dose of 20 mg/kg, the mean AUC_{0-8} ($n = 85$) was 44.2 μg · h/ml and AUC_{0-24} ($n = 72$) was 66.7 μg · h/ml; other summary measures with reported adult values for comparison are shown in Table 2. Pharmacokinetic values by age group, HIV status, WAZ category, and type of administration are presented in Table 3. Half-life was shorter in the youngest children, and there was a trend toward a higher C_{max} in children receiving crushed tablets. In simple linear regression, no variables assessed were significantly associated with C_{max}, and only weight was significantly associated with AUC_{0-24}. In multivariable analysis, C_{max} was reduced by 0.44 μg/ml for each 1-year increase in age (95% confidence interval [CI], −0.74 to −0.13; $P = 0.005$) and was increased by 0.13 μg/ml for each 1-kg increase in body weight (95% CI, 0.10 to 0.24; $P = 0.029$). In multivariable analysis, AUC_{0-24} was increased by 1.46 μg · h/ml for each 1-kg increase in body weight (95% CI, 0.44 to 2.47; $P = 0.006$). Controlling for age and weight, no other assessed

TABLE 1 Baseline demographic and clinical characteristics of children receiving ofloxacin for treatment or prevention of drug-resistant tuberculosis

Characteristic[a]	No. (%) with MDR-TB disease ($n = 55$)	No. (%) receiving MDR-TB preventive therapy ($n = 30$)
Age at enrollment		
0 to <2 yr	16 (29.1)	8 (26.7)
2 to <5 yr	17 (30.9)	22 (73.3)
5 to <15 yr	22 (40.0)	0 (0.00)
Male sex	32 (58.2)	15 (50.0)
Certainty of TB diagnosis		
Bacteriological confirmation	20 (36.4)	
Probable TB	32 (58.2)	
Suspected TB	3 (5.5)	
TB disease type ($n = 55$)		
PTB only	40 (72.7)	
EPTB only	5 (9.1)	
PTB and EPTB	10 (18.2)	
HIV infected	11 (20.0)	0 (0.0)
Weight-for-age Z-score <−2.0 ($n = 79$)[b]	11 (22.5)	3 (10.0)
Height-for-age Z-score <−2.0 ($n = 81$)[c]	19 (35.9)	4 (14.3)
Weight-for-length Z-score <−2.0 ($n = 60$)[c]	2 (6.3)	1 (3.6)

[a] PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.
[b] Weight-for-age Z-scores only available for patients aged <10 years.
[c] The sample size is <85 due to missing data.

TABLE 2 Summary statistics for ofloxacin pharmacokinetic measures in children receiving treatment or prevention for multidrug-resistant tuberculosis[a]

Parameter[b]	No. of children	Values for children in the present study	Values for adults with TB given an 800-mg ofloxacin dose[c]
C_{max} (μg/ml)	85	8.97 (2.47–14.4)	10.5 (8.0–14.3)
T_{max} (h)	85	2.0 (1.0–4.0)	1.03 (0.5–6)
$t_{1/2}$ (h)	72	3.49 (1.89–6.95)	7.34 (3.53–28.3)
CL/F (liter/h/kg)	72	0.31 (0.11–1.06)	0.12 (0.02–0.32)
V (liter/kg)	72	1.45 (0.86–6.49)	1.28 (0.78–2.83)
AUC_{0-8} (μg · h/ml)	85	44.2 (12.1–75.8)	
AUC_{0-24} (μg · h/ml)	72	66.7 (18.8–120.7)	103 (48–755)

[a] All values are presented as means (ranges), except for T_{max}, CL/F, and V, which are presented as medians (ranges); adult values are all reported as medians (ranges).
[b] C_{max}, maximum serum concentration; T_{max}, time to maximum serum concentration; $t_{1/2}$, half-life; CL, clearance; F, fraction absorbed; V, volume of distribution; AUC_{0-8}, area under the concentration time curve from 0–8 h; AUC_{0-24}, area under the concentration time curve from 0–24 h.
[c] $n = 11$ (10).

variables contributed to these models. Neither LBM nor BSA improved the model fit over weight.

Safety. Forty-six children contributed a total of 23.8 years of observation time on ofloxacin to the safety assessment, with a median time per child of 4.9 months (IQR, 1.2 to 10.2 months) (Table 4). Adverse events were mostly mild in severity; vomiting and pruritus were the most frequent. Most adverse events were not attributed to ofloxacin but represented known toxicities related to companion MDR-TB drugs. The only grade 3 or 4 events were two episodes of asymptomatic ALT elevation due to confirmed acute hepatitis A, which resolved without complication after brief interruptions of some TB medications while awaiting the hepatitis A results.

DISCUSSION

Ofloxacin given at the WHO-recommended dose of 20 mg/kg to children was safe and well-tolerated, but exposures in this substantial pediatric cohort were considerably lower than those achieved in adults taking the standard MDR-TB treatment dose of 800 mg daily.

Although ofloxacin has been widely used for treatment and prevention of MDR-TB in children, the appropriate dosage has not been established. Indeed, only one other study evaluating the pharmacokinetics of ofloxacin in children has been conducted to our knowledge. In a study in Vietnam, 17 children (aged 5 to 17 years) with typhoid fever received a single oral dose of 7.5 mg/kg of ofloxacin (11). A C_{max} of 5.73 μg/ml and an AUC_{0-12} of 26.5 μg/ml were achieved (11). The C_{max} (8.97 μg/ml) and AUC_{0-8} (44.1 μg · h/ml) in our study are lower than would be expected with a 2.5× higher dose given that ofloxacin exposures should be dose proportional in the dosing range tested (8, 10). It is unclear if this is because of differences in the study population or drug formulation used, but our findings underline the importance of not relying on a single study conducted in one geographic location to inform global dosing recommendations in children.

The differing AUC_{0-8} and AUC_{0-24} trends by age in univariable analysis may be due to the fact that the proportion of the total daily AUC that is captured in the first 8 h after dosing is greater in younger children (data not shown) due to more rapid absorption and clearance compared to older children. Children with slower absorption and elimination, and most likely a higher AUC, would be more likely to be excluded from our estimates of AUC_{0-24}. Indeed, AUC_{0-24} was not estimated in a higher proportion of older children (Table 3), suggesting we may have underestimated the AUC_{0-24} in children ≥5 years old. The differences in $t_{1/2}$ by age in univariable analysis and association of AUC_{0-24} with weight are consistent with the principle of allometric scaling, in which smaller body size is associated with more rapid clearance.

Our large sample allowed us to evaluate covariate effects on the pharmacokinetics of ofloxacin. In multivariable analysis, age and weight were associated with AUC_{0-8} and C_{max}, and weight was associated with AUC_{0-24}. HIV and undernutrition are frequent concomitant conditions among children with MDR-TB and have been associated with failure to culture convert at 2 months and death (31). HIV infection may affect concentrations of some TB medications (32); however, we did not observe any significant effect of HIV infection on ofloxacin pharmacokinetics. This is consistent with the available adult literature (9, 10). Undernutrition also did not have a clinically significant impact on ofloxacin pharmacokinetics. These data suggest that worse outcomes among children with HIV coinfection or undernutrition are not likely due to reduced concentrations of ofloxacin, the key bactericidal drug in the regimen.

The lack of child-friendly formulations of second-line TB medications is a major challenge for MDR-TB treatment in children, and the impact of formulation manipulation, such as the crushing or breaking of adult tablets, has not been evaluated fully.

TABLE 3 Pharmacokinetic measures for ofloxacin (20 mg/kg) in children receiving treatment or prevention for multidrug-resistant tuberculosis, by age, HIV status, nutritional status, and administration method[a]

Parameter	No. of children	C_{max} (μg/ml)	*P* value	AUC_{0-8} (μg · h/ml)	*P* value	No. of children	AUC_{0-24} (μg · h/ml)	*P* value	$t_{1/2}$ (h)	*P* value
Age group										
0 to <2 yr	24	10.43 (1.96)		45.9 (8.8)		23	63.9 (15.3)		3.01 (0.53)	
2 to <5 yr	39	8.52 (2.37)		43.8 (12.0)		35	66.5 (20.9)		3.52 (0.75)	
≥5 yr	22	8.18 (2.01)	<0.001	43.1 (8.9)	0.632	14	71.7 (17.8)	0.473	4.18 (1.22)	<0.001
HIV status										
HIV infected	11	8.42 (1.51)		42.5 (9.0)		9	63.4 (16.4)		3.35 (0.59)	
Not HIV infected	74	9.05 (2.44)	0.404	44.4 (10.6)	0.560	63	67.1 (19.0)	0.579	3.51 (0.93)	0.614
WAZ										
<−2.0	18	8.94 (2.35)		42.7 (11.4)		15	61.0 (20.1)		3.06 (0.49)	
≥−2.0	67	8.98 (2.35)	0.953	44.6 (10.1)	0.498	57	68.2 (18.1)	0.190	3.60 (0.94)	0.004
Administration										
Whole	11	7.87 (1.67)		42.2 (10.6)		8	72.4 (23.4)		4.32 (1.45)	
Crushed	72	9.16 (2.40)	0.089	44.6 (10.4)	0.481	62	66.1 (18.1)	0.375	3.39 (0.76)	0.114

[a] HIV status, nutritional status, and administration method comparisons were generated using *t* tests; age group comparisons were generated using one-way analyses of variance (ANOVAs); all values are presented as means (standard deviations). A total of 85 children participated in the study.

Many children in our study were unable to swallow whole ofloxacin tablets and took them crushed. In univariable analysis, there was a trend toward a higher C_{max} with crushed tablets; however, crushing did not contribute to the multivariable model, which included age and weight. The associations of C_{max} with age and weight described here are somewhat unexpected and should be interpreted cautiously, as crushing was highly associated with younger age and less so with weight, and it may have been difficult to separate these effects in the model. There was no association between crushing and AUC_{0-8} or AUC_{0-24}. Although this does not replace a formal assessment of relative bioavailability of crushed versus whole tablets, it suggests that crushing tablets does not negatively impact drug exposures and crushing may, in fact, increase the rate or magnitude of absorption.

When efficacy of a TB drug has been established in adults, efficacy studies may not be required in children, but studies characterizing a drug's pharmacokinetics and safety in children are essential. This allows the selection of dosages that achieve concentrations associated with treatment success in adults (9, 10). In a study of ofloxacin pharmacokinetics after an 800-mg dose (median dose, 14.5 mg/kg) in adults with MDR-TB at two sites in South Africa, estimated pharmacokinetic parameters were a $t_{1/2}$ of 7.8 h and a C_{max} of 8.8 to 10.4 mg/liter (9). In a U.S. study, 11 adults with TB (median age, 42 years [range, 22 to 57 years]; median weight, 64 kg [range, 50 to 86 kg]; 3 HIV infected) underwent intensive pharmacokinetic sampling on ofloxacin at steady state with a median dose of 800 mg (range, 600 to 1,200 mg). Assays were performed using high-performance liquid chromatography, and data were analyzed using population pharmacokinetic modeling (Table 2). Using simulations based on their population model generated from these data and from an additional group in this study having sparse pharmacokinetic sampling, estimated pharmacokinetic parameters after an 800-mg once-daily dose were an AUC of 100.7 μg · h/ml and a C_{max} of 9.35 μg/ml (10). The C_{max} in our children was only slightly below these reported adult values, although the children received a higher milligram per kilogram dose (20 mg/kg) compared to the adults (9). However, the estimated AUC_{0-24} in our children of 66.7 μg · h/ml was far below the adult value (103 μg · h/ml) (10). This is likely related to the more rapid clearance of ofloxacin in children; calculated $t_{1/2}$ in children in our study was 3.5 h compared to 7 to 8 h in the adult studies. That currently recommended dosages of ofloxacin result in AUCs in children well below those of adult targets has important implications for MDR-TB treatment and prevention, particularly given the fluoroquinolones' high bactericidal activity (33) and their key role in current treatment regimens (34). The AUC is believed to be the most important pharmacodynamic measure for the fluoroquinolones against *M. tuberculosis* (35). As our data were derived in an optimal setting with an exact 20-mg/kg dose, drug exposures with unsupervised dosages closer to the lower end of the recommended range (15 to 20 mg/kg) may be even lower. Although additional studies corroborating the findings in our study would be useful, it may not be prudent to wait on such studies before reevaluating pediatric dosing. Population pharmacokinetic modeling can be used to predict dosages most likely to achieve adult targets; this information is urgently needed, and such an analysis is planned from this cohort. Higher dosages should be introduced carefully, though, to assess their safety and tolerability, particularly given that the C_{max} may exceed the C_{max} in adults receiving 800 mg daily.

Ofloxacin was generally safe and well tolerated. The overall person-time of observation for adverse events was more limited than expected, as many children were switched from ofloxacin to levofloxacin or moxifloxacin during their treatment, following a national treatment guideline change mid-study. Adverse effects were of low grade, and there were no ofloxacin-related grade 3 or 4 events. There was no evidence of arthralgia or arthropathy in our cohort. Subtle arthralgia may not have been reported, but it is unlikely clinically significant arthralgia or arthritis would have been missed. There were two reports of insomnia attributable to ofloxacin, a well-described adverse effect of this medication (5, 36). Anecdotally, we have seen self-limited, mild insomnia and nightmares attributable to ofloxacin not infrequently; our data may underestimate the

incidence, as many children were admitted to hospital wards early in their treatment and sleep disturbance may be less noticeable by ward staff than by parents. Our safety assessment is limited by the lack of a control group and by the difficulty in attributing adverse effects to individual drugs in a multidrug regimen that typically includes ethambutol, pyrazinamide, amikacin, ethionamide, terizidone, and high-dose INH. Our approach was, however, conservative and more likely to have overestimated ofloxacin-related adverse effects. These data add to a growing body of evidence that the fluoroquinolones are safe in children, including in long-term use (37). The lack of adverse effects may be related to the relatively low exposures, and safety should continue to be monitored closely if dosages are increased.

Treatment outcomes in this cohort were generally good and will be reported elsewhere; however, one child had documented acquisition of ofloxacin resistance during treatment. This HIV-infected child had a complicated course with large recurrent tuberculous brain abscesses requiring the use of multiple immunosuppressant medications and was treated with multiple fluoroquinolones prior to resistance development, making it difficult to ascribe the resistance acquisition solely to ofloxacin concentrations. Additionally, this child's ofloxacin C_{max} and AUC were each above the median. Despite generally good outcomes, optimized dosing of the fluoroquinolones in children remains an important priority and may potentially improve outcomes further and facilitate the use of shorter, injectable sparing MDR-TB treatment regimens.

In conclusion, in this large cohort of children receiving ofloxacin, exposures were lower than those in adults. Although ofloxacin is being phased out of MDR-TB treatment regimens in favor of more potent fluoroquinolones, it is still used in many places and it is likely underdosed in children. That ofloxacin was safe and well tolerated is reassuring, particularly if higher dosages that will be needed to reach adult reference exposure targets are to be evaluated. A better understanding of the pharmacokinetics and safety profiles of all second-line anti-TB drugs is essential to ensure the provision of appropriate drugs at appropriate dosages to children with MDR-TB to optimize treatment outcomes.

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We declare no conflicts of interest.

TABLE 4 Adverse events in children treated for multidrug-resistant tuberculosis with ofloxacin[a]

Adverse event	Adverse event by grade							Adverse effects possibly, probably, or definitely attributed to ofloxacin by grade						
	No. of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	Total no. of events	Event rate (per person-yr)	No. of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	Total no. of events	Event rate (per person-yr)
Arthralgia	1	0	0	0	1	1	0.042	0	0	0	0	0	0	
Arthritis	0	0	0	0	0	0		0	0	0	0	0	0	
Pain other than traumatic injury	10	10	2	0	0	12	0.504	1	0	0	0	1	1	0.042
Headache	7	5	3	0	0	8	0.336	1	0	0	0	1	1	0.042
Neurosensory alteration	0	0	0	0	0	0		0	0	0	0	0	0	
Insomnia	2	0	2	0	0	2	0.084	2	2	0	0	0	2	0.084
Fatigue/malaise	3	3	0	0	0	3	0.126	1	0	0	0	0	1	0.042
Nausea	8	9	0	0	0	9	0.378	2	0	0	0	0	2	0.084
Vomiting	12	16	1	0	0	17	0.714	3	4	0	0	0	4	0.168
Anorexia	4	3	1	0	0	4	0.168	0	0	0	0	0	0	
Cutaneous reaction	4	5	0	0	0	5	0.210	1	0	0	0	0	1	0.042
Pruritus	12	13	0	0	0	13	0.546	4	4	0	0	0	4	0.168
Acute systemic allergic reaction	0	0	0	0	0	0		0	0	0	0	0	0	
ALT	8	5	1	1	1	8	0.336	5	4	1	0	0	5	0.210
Bilirubin	0	0	0	0	0	0		0	0	0	0	0	0	

[a] Fourty-six patients were followed for a median time of 149.5 days (IQR, 36 to 308 days); total number of person-years was equal to 23.80.

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Chapter 3: Levofloxacin population pharmacokinetics in children treated for multidrug-resistant tuberculosis

Rationale

Levofloxacin is the l-isomer and the active component of the ofloxacin racemate, conferring levofloxacin with nearly twice the antimycobacterial activity of ofloxacin. Therefore levofloxacin became favoured over ofloxacin for MDR-TB treatment and prevention. This was reflected in WHO guidance, which recommended phasing out ofloxacin and prioritizing levofloxacin or moxifloxacin for MDR-TB during 2012-2013, during the time field research contributing towards this doctoral dissertation was underway. The WHO recommended variable doses of levofloxacin for children, including: 7.5-10 mg/kg once daily (15, 123) and 15-20 mg/kg/day divided twice daily for ≤ 5 years of age, 10-15 mg/kg once daily for > 5 years of age (124). However the optimal levofloxacin doses in children that achieve target concentrations in adults after a 750 mg dose (96.8 μg · h/ml) (79) had not yet been characterized. This routine change in policy recommendation by WHO was followed by a South African National TB Programme (SA NTP) recommendation in 2012-2013 to use levofloxacin in children below 8 years of age, and moxifloxacin in older children and adults with MDR-TB during (125). The rationale for recommending different fluoroquinolones in children was primarily due to formulation considerations. Moxifloxacin was only available as an unscored 400 mg tablet, making accurate dosing in young children very difficult, and when crushed or dissolved/suspended in water it is very bitter and poorly palatable. In contrast, levofloxacin was available as a 250 mg tablet, which could be split to accurately dose most children, and although still bitter when crushed was more palatable than moxifloxacin.

Study aims

The aim of this prospective observational pharmacokinetic study was to characterize the pharmacokinetics and optimal dosing strategy of levofloxacin in children routinely treated for MDR-TB disease or exposure.

Methods

Children in this evaluation of levofloxacin were enrolled in the MDRPK1 study (see Chapter 2). Similar in design to the study of ofloxacin in Chapter 2, children <15 years routinely receiving levofloxacin for MDR-TB disease or exposure at one of the three study facilities in Cape Town and Worcester, were included in this study, primarily from 2013-2015. On pharmacokinetic sampling days, samples were taken pre-dose and at 1, 2, 4, 8 and 6 or 11 hours after an observed exact dose of 15 mg/kg or 20 mg/kg, taken as either whole tablets, or crushed tablets either swallowed or administered via nasogastric tube. The routinely available levofloxacin adult 250 mg tablets (Austell, Johannesburg, South Africa) were used throughout the study. Levofloxacin plasma concentrations were measured using a validated LC MS/MS assay at the University of Cape Town Division of Clinical Pharmacology. Pharmacokinetic parameters were estimated using NLME modeling, and the effect on the model fit of key covariates was explored, including HIV status, MDR-TB status (disease vs. exposure), undernutrition, ethnicity, and administration method (whole tablets vs. crushed tablets taken orally vs. crushed tablets administered via nasogastric tube). Simulations using the final model were used to estimate weight-banded doses achieving the adult target exposure. This optimized dosing regimen was targeted to achieve a median AUC in each weight band within 20% of the target exposure, with the simulated C_{max} not exceeding by more than 20% that observed to be safe in adult studies. Safety data for levofloxacin are reported in Chapter 4.

Results

One-hundred and nine children were included in the study, with a large proportion of young (median age 2.1 years, range 0.3 to 8.7) and small (median weight 12 kg, range 6 to 22 kg) children. Levofloxacin followed two-compartment kinetics with first-order elimination and absorption with a lag time. Inclusion of allometric scaling improved the model fit, and clearance in a typical child (weight 12 kg, age 2 years) was 4.7 liters/h; HIV infection reduced levofloxacin clearance by 16%. Crushing of tablets or use of a nasogastric tube did not significantly affect the overall bioavailability. Levofloxacin exposures in children with the 250 mg adult tablet were considerably lower than reported in adults receiving a similar dose on a mg/kg basis even after accounting for the effect of body size with allometric scaling. The reason for this is not clear, but possibilities include effects of the formulation used in the study or effects of formulation

manipulation (crushing tablets). To achieve exposures in children equivalent to adult target exposure values with the routine adult formulation studied, higher levofloxacin doses than currently recommended would be required, varying from 18 mg/kg/day for younger children (3-4 kg) up to 40 mg/kg/day for older children.

Conclusions and recommendations

Currently recommended doses of levofloxacin with the routine adult levofloxacin formulation studied resulted in exposures in children well below current routine target values in adults. Based on our data, the largest paediatric levofloxacin pharmacokinetic study to date, weight-banded doses in children that would be expected to achieve target values with the studied formulation were proposed, for careful prospective evaluation of pharmacokinetics and safety. Even after accounting for the effects of body size, exposures were lower in this study than has been previously reported. This unexpected difference could not be explained by our data, however the effects of formulation or formulation manipulation were considered possibilities. The effects of formulation and formulation manipulation on levofloxacin pharmacokinetics need further evaluation. As pharmacokinetic targets evolve in adults based on ongoing trials, paediatric studies would be required to evaluate the required doses to achieve these new exposures and safety at those doses.

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PHARMACOLOGY

Levofloxacin Population Pharmacokinetics in South African Children Treated for Multidrug-Resistant Tuberculosis

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ABSTRACT Levofloxacin is increasingly used in the treatment of multidrug-resistant tuberculosis (MDR-TB). There are limited pediatric pharmacokinetic data to inform dose selection for children. Children routinely receiving levofloxacin (250-mg adult tablets) for MDR-TB prophylaxis or disease in Cape Town, South Africa, underwent pharmacokinetic sampling following receipt of a dose of 15 or 20 mg/kg of body weight given as a whole or crushed tablet(s) orally or via a nasogastric tube. Pharmacokinetic parameters were estimated using nonlinear mixed-effects modeling. Model-based simulations were performed to estimate the doses across weight bands that would achieve adult exposures with 750-mg once-daily dosing. One hundred nine children were included. The median age was 2.1 years (range, 0.3 to 8.7 years), and the median weight was 12 kg (range, 6 to 22 kg). Levofloxacin followed 2-compartment kinetics with first-order elimination and absorption with a lag time. After inclusion of allometric scaling, the model characterized the age-driven maturation of clearance (CL), with the effect reaching 50% of that at maturity at about 2 months after birth and 100% of that at maturity by 2 years of age. CL in a typical child (weight, 12 kg; age, 2 years) was 4.7 liters/h. HIV infection reduced CL by 16%. By use of the adult 250-mg formulation, levofloxacin exposures were substantially lower than those reported in adults receiving a similar dose on a milligram-per-kilogram basis. To achieve adult-equivalent exposures at a 750-mg daily dose, higher levofloxacin pediatric doses of from 18 mg/kg/day for younger children with weights of 3 to 4 kg (due to immature clearance) to 40 mg/kg/day for older children may be required. The doses of levofloxacin currently recommended for the treatment of MDR-TB in children result in exposures considerably lower than those in adults. The effects of different formulations and formulation manipulation require further investigation. We recommend age- and weight-banded doses of 250-mg tablets of the adult formulation most likely to achieve target concentrations for prospective evaluation.

KEYWORDS NONMEM, allometric scaling, dosing recommendations, fluoroquinolones, maturation, pediatric, population PK modeling

The fluoroquinolones, with their potent antimycobacterial activity, are increasingly important medications for tuberculosis (TB) prevention and treatment, particularly for multidrug-resistant (MDR) TB (i.e., TB caused by *Mycobacterium tuberculosis* strains resistant to both isoniazid and rifampin), against which the fluoroquinolones are a key component of current regimens. Levofloxacin, the L-isomer and the more active component of the ofloxacin racemate (1, 2), is the fluoroquinolone most commonly used in

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young children with TB, partly due to the availability of smaller, child-friendly formulations. Levofloxacin also has the advantage of having a reduced QT-prolonging effect compared to that of other fluoroquinolones (like moxifloxacin) (3), making it more suitable for use in explorations of the effects of increased doses and its combined use with the novel and repurposed anti-TB drugs bedaquiline, delamanid, pretomanid, and clofazimine, all of which can cause QT interval prolongation (4). Levofloxacin is also increasingly used as a substitute for isoniazid in the treatment of isoniazid-resistant TB and is being evaluated for the treatment of tuberculous meningitis. Data from an animal model of latent TB infection and from observational studies in children suggest a potential role for the fluoroquinolones in MDR-TB-preventive therapy as well, and two phase III clinical trials (the TB-CHAMP trial in South Africa [International Standard Randomized Controlled Trial number ISRCTN92634082] and the V-QUIN trial in Vietnam [trial identifier, ACTRN12616000215426]) comparing levofloxacin and placebo are ongoing (5, 6). Given these broad indications for the use of levofloxacin against TB, it is likely to be more widely used in children affected by TB (7, 8).

There are currently limited data on levofloxacin pharmacokinetics (PK) in children with TB. The fluoroquinolones are concentration-dependent antibiotics, with the area under the concentration-time curve (AUC) and maximum plasma concentrations (C_{max}) being considered the pharmacokinetic parameters most closely correlated with efficacy (9). Levofloxacin is rapidly absorbed after oral administration with a bioavailability of >90% and is mainly renally eliminated (10). The doses of levofloxacin in children that result in exposures approximating those in adults receiving the current World Health Organization (WHO)-recommended dose of 750 mg once daily for the treatment of TB are not well defined (11). The levofloxacin dose currently recommended by WHO for MDR-TB treatment in children is 15 to 20 mg/kg of body weight/day divided into two doses daily in children aged ≤5 years and 10 to 15 mg/kg/day once daily in children aged >5 years; however, published data supporting this recommendation are limited (11). A small study of 22 South African children aged 0 to 8 years with MDR-TB disease or exposure who received levofloxacin at 15 mg/kg once daily showed lower exposures (median C_{max}, 6.79 mg/liter; median AUC, 32.9 mg · h/liter) than healthy adults after a standard 750-mg dose (mean C_{max}, 9.3 mg/liter; mean AUC, 101 mg · h/liter) (10, 12). On the basis of data from a study of 50 children receiving levofloxacin for MDR-TB treatment or preventive therapy in the Federated States of Micronesia and the Republic of the Marshall Islands, a 15- to 20-mg/kg once-daily dose was recently recommended (13). However, few young children were included in that study, with the study including only three children younger than 2 years of age, the age range during which renal function is rapidly maturing (13). Data for HIV-infected children also remain limited.

The objective of this study was to describe the population pharmacokinetics of levofloxacin among children receiving levofloxacin for MDR-TB and to use this information in simulations to predict the dosing that would achieve the exposures seen in adults receiving the currently recommended 750-mg dose.

RESULTS

Study population and pharmacokinetic samples. The study included 109 children. The median age was 2.1 years (range, 0.3 to 8.7 years), and the median weight was 12 kg (range, 6 to 22 kg); 16 were HIV infected and on antiretroviral therapy (ART) containing either lopinavir-ritonavir ($n = 13$) or efavirenz ($n = 3$). Patient characteristics are shown in Table 1. A total of 662 samples were available for analysis (3 participants contributed data from more than one sampling occasion), with the levofloxacin concentrations in 36 (5.4%) samples being below the limit of quantification of the assay, and all of these were observed in the predose sample.

Pharmacokinetic model. The pharmacokinetics of levofloxacin was well described using 2-compartment disposition kinetics (with respect to a 1-compartment model, the change in the objective function value [ΔOFV] was 43.8; 2 degrees of freedom [df]; $P <$ 0.001), first-order elimination and absorption, and inclusion of an absorption lag time

TABLE 1 Characteristics of children with MDR-TB disease or exposure treated with levofloxacin[a]

Characteristic	Value
No. (%) of children of the following ethnicity:	
Black	69 (63.3)
Mixed race	40 (36.7)
No. (%) of male children	56 (51.4)
Median (range) age (yr)	2.1 (0.32–8.65)
Median (range) wt (kg)	12.4 (5.88–21.8)
Median (range) wt-for-age Z-score (WHO)	−0.39 (−4.18–3.32)[b]
Median (range) ht-for-age Z-score (WHO)	−1.31 (−4.70–1.45)[c]
Median (range) wt-for-length Z-score (WHO)	0.60 (−4.88–4.33)[d]
No. (%) of children with the following MDR-TB disease status:	
MDR-TB disease (treatment)	71 (65.1)
MDR-TB exposure (preventive therapy)	38 (34.9)
Median (range) levofloxacin total dose (mg)	212 (88.5–435)
Median (range) levofloxacin dose (mg/kg)	15 (10–21.4)
Median (range) creatinine clearance (ml/min)[e]	119 (66.6–181)
No. (%) of children administered drug by the following procedure:	
Whole tablet, orally	7 (6.4)
Crushed tablet, orally	12 (11)
Crushed tablet, nasogastric tube	90 (82.6)
No. (%) of children HIV infected	16 (14.7)

[a]Data are for 109 children. WHO, World Health Organization.
[b]Twelve children had a Z-score of <-2.
[c]Thirty-three children had a Z-score of <-2.
[d]Two children had a Z-score of <-2.
[e]The revised formula of Schwartz et al. (47) was used; the serum creatinine concentration was measured only in the patients treated for TB disease ($n = 71$, 65.1%).

(ΔOFV, 68.5; 2 df; $P < 0.001$). The final parameter estimates of the model, along with their precisions, are shown in Table 2.

After the inclusion of allometric scaling with total body weight, which substantially improved the fit (ΔOFV, 118), the model could characterize the effect of age, as shown in Fig. 1 (ΔOFV, 18.5; 2 df; $P < 0.001$): the model estimated that levofloxacin clearance (CL) reaches 50% maturity at about 2 months after birth and is nearly fully mature around 2 years of age. The use of fat-free mass (14) for allometric scaling did not provide meaningful improvements to the fit.

The model estimated the typical value of CL in a 12-kg, 2-year-old child to be 4.7 liters/h. The use of a nasogastric tube (NGT) for drug administration was found to increase the speed of absorption by shortening the absorption lag time (ΔOFV, 11.4; 1 df; $P < 0.001$), but no significant effect on bioavailability was detected when either the tablet was crushed or an NGT was used. Additionally, HIV-infected children had a 16% lower CL (ΔOFV, 9.3; 1 df; $P < 0.01$) and, hence, higher exposure. This difference could not be ascribed to a particular ART regimen, due to the small sample size of this subgroup. Creatinine clearance was tested as an alternative to weight and age as a predictor of CL in the subset for which serum creatinine concentration measurements were available but did not provide a better model fit. No significant differences were found between children receiving treatment for MDR-TB and those receiving preventive treatment, and there was no effect of undernutrition or ethnicity.

After adjustment for all the predictors mentioned above, the model still identified a moderate random between-subject variability (BSV) in CL, a large between-occasion variability (BOV) in the absorption parameters, and a moderate BOV in bioavailability. The model significantly improved (ΔOFV, 51.4; 1 df; $P < 0.001$) when the estimate of the greater variability (~4.5-fold) in the bioavailability of the unobserved doses was allowed. This additional parameter was included to adjust for the greater uncertainty

TABLE 2 PK parameter values for levofloxacin in children with MDR-TB disease or exposure[d]

PK parameter	Typical value (95% CI)	Variability as CV% (95% CI) [eta shrinkage (%)]
CL (liters/h)[a]	4.70 (4.37, 5.00) for HIV$^-$	BSV: 15.2 (10.6, 19.0) [24]
V_c (liters)[a]	19.2 (10.9, 21.8)	
Q (liters/h)[a]	0.796 (0.332, 4.76)	
V_p (liters)[a]	3.40 (2.53, 38.7)	
T_{lag} (h)	0.242 (0.0385, 0.654) for oral dosing	BOV: 130 (30.9, 303) [76]
k_a (1/h)	1.61 (0.855, 2.78)	BOV: 64.8 (43.4, 80.9) [43]
F	1 (fixed)	BOV: 21.8 (13.7, 28.4) [10]
HIV$^+$ on CL (%)	−15.9 (−26.6, −5.93)	
NGT on T_{lag} (%)	−85.6 (−99.3, −34.6)	
Scaling of BOV in F for unobserved doses (fold)[b]	4.48 (3.31, 7.08)	
$PMAGE_{50}$ (mo)	10.6 (7.55, 12.9)	
γ	3.39 (1.42, 4.98)	
Additive error (mg/liter)[c]	0.0160, i.e., 20% of LLOQ (fixed)	
Proporational error (%) [epsilon shrinkage (%)]	11.6 (10.0, 12.7) [29]	

[a]All clearance and volume parameters have been scaled with allometric scaling. The typical values reported here refer to a 12-kg child aged 2 years. Age affects clearance, since maturation was used. At 2 years after birth, maturation is predicted to be 97.9% complete.
[b]This is a multiplicative factor increasing the BOV in bioavailability for all predose concentrations, which follow an unobserved dose.
[c]The estimate of the additive error hit the stipulated lower boundary (20% of LLOQ), so it was fixed to this value.
[d]Values in parentheses are empirical 95% confidence intervals obtained with a 500-sample nonparametric bootstrap. The PK parameter variability was included either as between-subject variability or between-occasion variability, assuming a lognormal distribution. It is reported here as the approximate coefficient of variation (in percent). CL, clearance; V_c, central volume of distribution; Q, intercompartmental clearance; V_p, peripheral volume of distribution; T_{lag}, absorption lag time; k_a, absorption rate constant; F, bioavailability; PK, pharmacokinetic; $PMAGE_{50}$, postmenstrual age at which 50% maturation is reached; γ, shape factor for the maturation function; BSV, between-subject variability; BOV, between-occasion variability; HIV$^+$ and HIV$^-$ HIV-infected and uninfected children, respectively; NGT, drug administration with a nasogastric tube.

expected to affect the information on the timing of doses and accurate dosing procedures on the days prior to the day of the visit when the dose was observed to have been given and samples were collected for pharmacokinetic analysis (Table 2).

The model fit the data adequately, as shown in the visual predictive check (VPC) in Fig. 2, which also highlights the differences between the pharmacokinetic profiles obtained using different administration procedures and the effect of HIV infection.

Simulated exposures. The final model was used to simulate the exposures achieved in children with different weights and ages, and these were compared with the values for adults. We first explored the expected AUC and C_{max} in children receiving levofloxacin at a 20-mg/kg dose (the high end of the currently used dosing range of 15 to 20 mg/kg). The resulting exposures (AUC and C_{max}) across different weights are shown in Fig. 3. These simulations show that the AUC achieved in children after dosing of a 20-mg/kg dose using the 250-mg adult formulation was considerably lower than

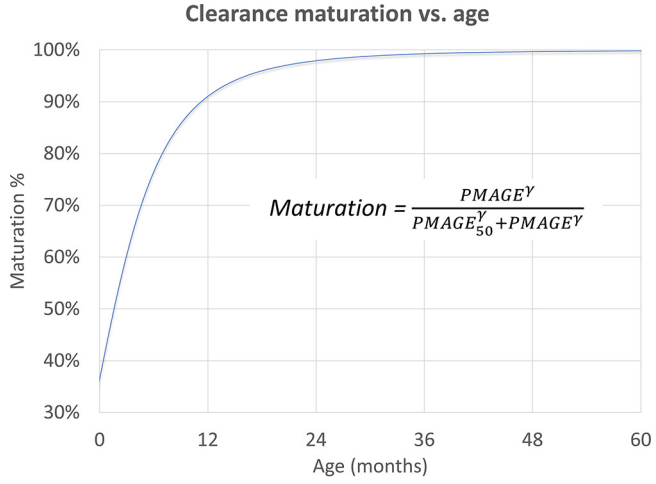

FIG 1 Maturation function of levofloxacin clearance. The percent maturation achieved versus postnatal age, assuming a standard duration of gestation (9 months), is shown.

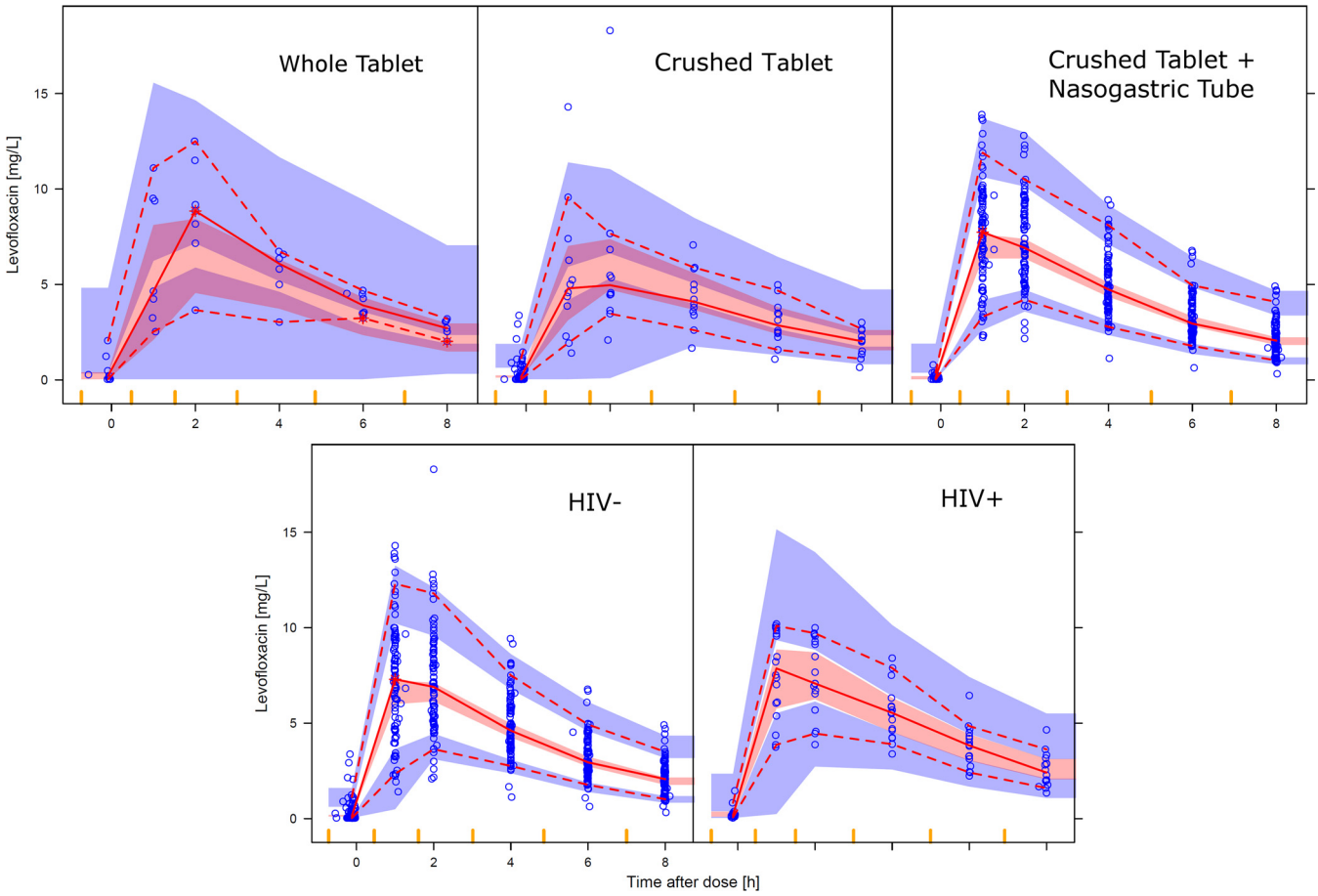

FIG 2 Visual predictive check of the levofloxacin concentration versus time after dose, stratified by either the administration method (top) or HIV infection status (bottom). The solid and dashed lines represent the 50th, 5th, and 95th percentiles of the observed data, while the shaded areas represent the model-predicted 95% confidence intervals for the same percentiles. The dots are the observed concentrations.

the chosen target for all age groups. Additionally, a fixed dose on a milligram-per-kilogram basis achieved a progressively lower AUC as age and weight decreased down to ~9 kg (for children <~1 year of age), at which point the effect of maturation reversed this trend. C_{max} was also lower than that previously reported in adults. We then evaluated an alternative dosing scheme, shown in Table 3, designed to more closely approximate the proposed target exposures in adults. To attain an AUC in children similar to that in adults, our model predicts that children in the weight band of between 8 and 11 kg may require considerably higher doses of this formulation of up to almost 40 mg/kg on a milligram-per-kilogram basis compared with the dose in adults. Children below 2 years of age, in whom the maturation of clearance is still incomplete, would require a slightly lower dose on a milligram-per-kilogram basis compared with the dose in adults, and the weight bands for very young children must be narrow to provide appropriate doses, given the substantial impact of body size and age on the pharmacokinetics of levofloxacin in younger children.

DISCUSSION

Among children who routinely receive the standard adult formulation of levofloxacin for the treatment or prevention of MDR-TB at currently recommended doses, exposures were substantially lower than those for adults receiving levofloxacin at the standard 750-mg dose for the same indication. This was true even in young children in whom renal function is still immature (15). As fluoroquinolones are considered the most important component of MDR-TB regimens, underdosing of these drugs is particularly concerning (16).

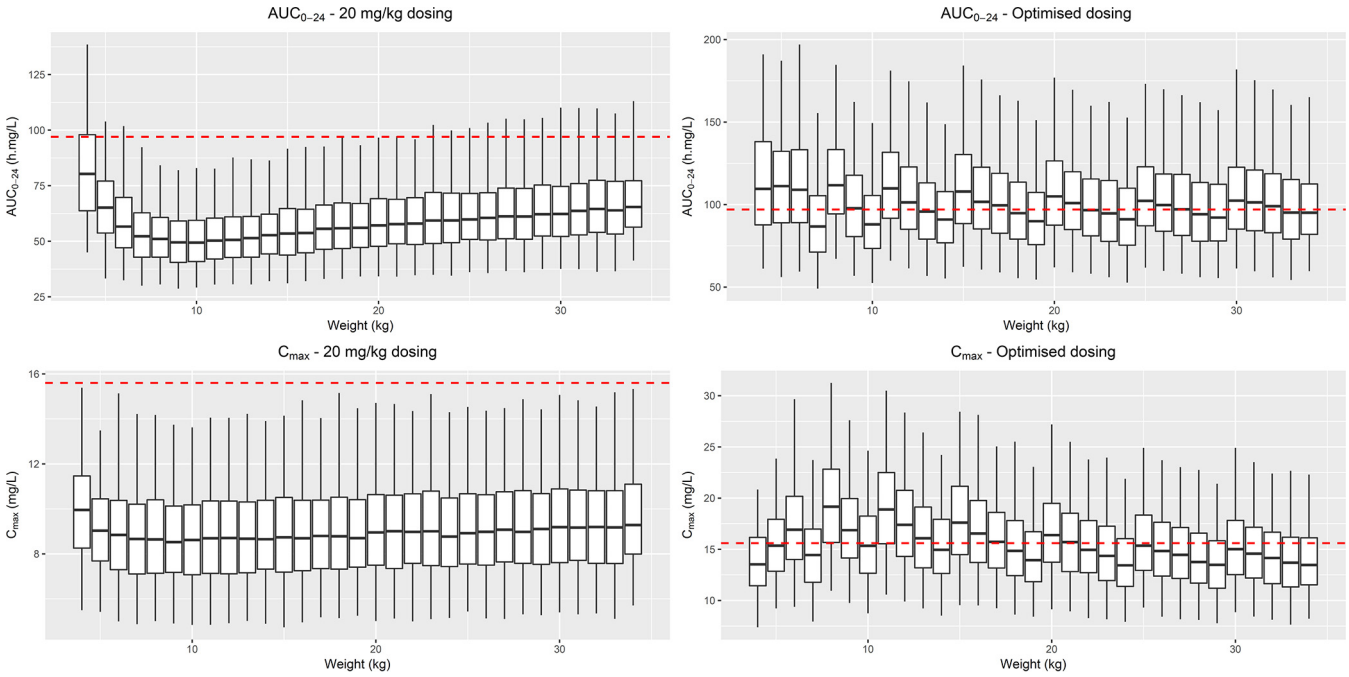

FIG 3 Simulated levofloxacin steady-state AUC from time zero to 24 h (AUC_{0-24}) (top) and C_{max} (bottom) versus body weight. (Left) The concentrations achieved with dosing at 20 mg/kg; (right) the suggested optimized dosing. The dashed lines for C_{max} (15.55 mg/liter) are the median values observed by Peloquin et al. (15) with dosing at 1,000 mg daily, while the dashed lines for AUC (96.8 mg · h/liter) represent the median exposure from the same study, after rescaling of the dose from 1,000 mg to 750 mg daily, the dose currently recommended for the treatment of tuberculosis in adults.

Our findings are generally consistent with those described in previous reports (13, 17) of the pharmacokinetics of levofloxacin in children (also determined using a standard 250-mg formulation), which showed lower exposures in children than in adults when both groups were dosed at the same milligram-per-kilogram level. Only part of the difference between the exposures in children and adults could be explained by the nonlinear effect of differences in body size described by allometric scaling. After standardizing the value of CL to a 70-kg individual with allometric scaling, our estimate is higher than the values previously reported for pediatric populations (13, 17) and, in keeping with the findings of other studies, is higher than the CL values reported in studies with adults (Table 4) (15, 18, 19). Why the levofloxacin concentrations in our study were lower than expected after adjustment for body size is unclear. Compared to other studies with pediatric populations, our CL values are greater than any of the values previously reported and are closest to those reported by Chien et al. (17) for the youngest cohorts in their study (<2 years old). As differences in body composition could play a role, we tested fat-free mass (FFM) as a descriptor of size for allometric

TABLE 3 Suggested optimized weight-banded dosing of once-daily levofloxacin in children to approximate an adult 750-mg once-daily dose[a]

Wt band (kg)	No. of 250-mg tablets	Daily dose (mg)	Median daily dose (mg/kg)
3-<4	0.25	62.5	18
4-<5	0.5	125	28
5-<6	0.75	187.5	34
6-<8	1	250	36
8-<11	1.5	375	39
11-<15	2	500	38
15-<20	2.5	625	36
20-<25	3	750	33
25-<30	3.5	875	32
30-<35	4	1,000	31

[a]The target was a steady-state AUC_{0-24} of 96.8 mg · h/liter (rescaled from 1,000 mg to 750 mg from Peloquin et al. [15]).

TABLE 4 Comparison of scaled AUC and CL of levofloxacin in South African children with MDR-TB treatment or exposure with previously reported values in adults and children

Author (reference or source)	No. of patients	Country	Median wt (kg)	Median and/or range age (yr)	Route of administration	Dose		AUC (mg · h/liter)	AUC scaled to 1,000 mg or 20 mg/kg	CL (liters/h) scaled to a 70-kg adult[a]
						mg	mg/kg			
Preston et al. (19)	272	USA	77.5	47	Oral	500	~6.5	72.53	145	8.35
Thwaites et al. (18)	15	Vietnam	48	33	Intravenous	1000	~20.8	155	155	8.56
Peloquin et al. (15)	10	Brazil	56	44	Oral	1000	~17.9	129	129	9.16
Mase et al. (13)	50	Pacific Islands		1–15	Oral gel		~14	~55	~80	11.6
Chien et al. (17)	8	USA	11	0.5–2	Oral		7	25.8		13.7
	8	USA	15.6	2–5	Oral		7	25.9		15.4
	8	USA	26.1	5–10	Oral		7	29.0		13.7
	8	USA	42.5	10–12	Oral		7	37.3		11.7
	8	USA	60.7	12–16	Oral		7	41.1		12.2
Denti et al. (this study)	109	South Africa	12	2.4 (0.6–8.6)	Oral		17.7	45	51	18.8

[a]Allometric scaling was applied with the exponent 3/4 to scale the value from the median weight in the original study to 70 kg.

scaling, but this did not significantly improve the model. Although we did not identify a significant effect of the use of crushed tablets versus whole tablets in the model, our study may have been underpowered to detect a difference, as only 7 of 109 children received whole tablets. Differences in bioavailability between formulations are increasingly recognized, and this is an important potential explanation for the variability in exposures between those found in previous work and those found in our study. This possibility should be evaluated in future work, as it has important practical implications. Other explanations could be differences in the ethnicities of the patients evaluated, differences between the drug formulations used our study and those used in previous studies, or the concomitant use of other antituberculosis drugs. Novel dispersible levofloxacin formulations (e.g., a 100-mg scored dispersible pediatric formulation [Macleods, India]) which are currently undergoing prequalification by WHO and which will be used in several trials for the treatment of MDR-TB in children should be evaluated, since bioavailability may vary substantially by formulation (20).

Our study was unique in that a large number of very young children were included, and we were able to characterize the developmental pharmacokinetics of levofloxacin. Specifically, levofloxacin CL was estimated to reach 50% of the value at maturity at about 2 months after birth. Seventeen children in our cohort were younger than 1 year of age. Since the youngest participants were about 4 months of age, the predicted exposure for infants under age 4 months is a model extrapolation and should be interpreted with caution. However, the parameter values for the maturation of levofloxacin clearance were estimated with reasonable precision and they are similar to those reported for the maturation of glomerular filtration rate (21). This is consistent with the fact that levofloxacin's main route of elimination is renal excretion. It is important to appropriately dose the youngest children, as these children are at the highest risk of progression to TB disease following *M. tuberculosis* infection; have the highest risk of disseminated TB in hard-to-reach compartments, such as the cerebrospinal fluid (CSF); and have the highest rates of TB-related mortality (22).

HIV infection was associated with a lower clearance of levofloxacin. The effect was small and is unlikely to be clinically significant. As all HIV-infected children were on ART, we could not separate the effects of HIV infection and ART. The lower levofloxacin clearance could be related to a drug-drug interaction, possibly with ritonavir, which has broad effects on metabolizing and transport enzymes. However, our study had an insufficient power to ascribe this finding to a single antiretroviral drug. Recently, high levels of immune activation have been associated with reduced isoniazid clearance in adults coinfected with *M. tuberculosis* and HIV (23). We did not routinely measure markers of inflammation in the children to explore this hypothesis, which may deserve further evaluation in the future. A previous study in adults failed to identify a significant

increase in levofloxacin concentrations when levofloxacin was given to HIV-infected patients treated with efavirenz or nelfinavir, but that study had no HIV-infected control group, and the comparison was instead made with historical data (24). The lack of an effect of the drug administration method on the overall bioavailability of levofloxacin is reassuring, given the limited availability of child-friendly levofloxacin formulations and the frequent need to crush levofloxacin tablets intended for use by adults for administration to young children. However, only 7 of the 109 children in this study received whole tablets, and those were the oldest children. This limits the power of our analysis to investigate this effect. Confirmation in other studies is necessary. Furthermore, the pharmacokinetics of a novel dispersible levofloxacin formulation should be evaluated in children, to inform the safe and effective dosing of levofloxacin for the treatment of MDR-TB.

The structure of our pharmacokinetic model, which has 2 compartments and delayed first-order absorption, differs from that of most previous models, which have 1 compartment and different models of absorption. These differences are likely due to different sampling schedules: the beta phase of the pharmacokinetic profile predicted by our model did not affect the concentration during first hours after the dose, but it improved our description of the minimum concentration at 24 h postdosing.

To achieve exposures similar to those in adults receiving the routine daily 750-mg dose used for the treatment of TB, the dose in children would need to be substantially increased from the currently recommended doses. In general, small children may need doses much higher on a milligram-per-kilogram basis (up to 40 mg/kg) than the 10- to 15-mg/kg dose used in adults. However, for infants (age, $<$1 year), who may have an immature clearance function, the dose increase needed to achieve the pharmacokinetic targets is more modest (18 to 28 mg/kg). Different formulations (e.g., dispersible tablets) may, however, have different dosing requirements.

The use of higher doses of levofloxacin in children may have implications for safety, particularly if similar exposures produce more serious adverse effects in children than in adults. Our most immediate concern would be for central nervous system (CNS) toxicity. Hallucinations, serious sleep disturbances, severe headaches, and other clinically significant CNS events, such as intracranial hypertension, have been described in children and adults receiving fluoroquinolones. These have particularly been observed with doses higher than those that were previously recommended but that were still within the range of the doses required to achieve the target concentrations in adults after the administration of 750-mg doses (25–28).

As can be seen from the simulations, if children are dosed once daily to target AUC values similar to adults on the current 750-mg dose, this will also result in C_{max} values in children substantially higher than those in adults; the C_{max} values in some children will exceed those observed in adults on a 1,000-mg dose. The relationship between C_{max} and some adverse effects of concern (such as CNS side effects or muscle or joint toxicity) is poorly characterized. As the C_{max} values expected with the once-daily simulated doses exceed what has been shown to be safe in adults to date, these doses would need to be carefully assessed in children before they are recommended for routine use for either the treatment or prevention of MDR-TB. For MDR-TB prevention, the risk/benefit ratio needs to be even more carefully considered, given the fact that well children are treated to prevent future MDR-TB disease. Although the QT interval-prolonging effect of levofloxacin appears to be less of a concern, the very high peak concentrations achieved with the simulated once-daily doses in children might still pose a risk of QT prolongation that would need to be assessed carefully. Splitting of the total daily dose into twice-daily doses would be a way to preserve the AUC without producing such a high C_{max}; however, in this situation, the balance between potentially (but perhaps only modestly) improved safety and the burden to drug treatment programs and/or the impact on adherence to the treatment regimen must be considered. The relationship between pharmacokinetic parameters (C_{max} versus AUC) and other toxicities, such as the CNS effects, has not been characterized. Therefore, splitting of the daily dose to reduce the expected C_{max} may not eliminate the risk of adverse

effects if they are, in fact, more closely associated with the AUC. Exploration of the use of higher doses of levofloxacin would therefore need to be carried out thoughtfully with careful safety monitoring. It should also be noted that the use of higher levofloxacin doses for the treatment of MDR-TB in adults is being explored in a phase II trial (the Opti-Q trial; ClinicalTrials.gov registration number NCT01918397); depending on the results from that study, target levofloxacin exposures may be higher than the current target exposures, which would impact dosing in the pediatric population.

Favorable outcomes are reported in 75 to 90% of children with MDR-TB (29, 30). This is much better than that in adults with MDR-TB, where globally only 50% are successfully treated (31). However, there are a number of reasons for the further optimization of levofloxacin doses in children with MDR-TB disease. First, there is scope for improving successful MDR-TB treatment outcomes further in children beyond the current 75 to 90% rate. Second, increasing evidence shows that low drug exposures are associated with poor TB treatment outcomes (including death and treatment failure) in children (32, 33), and low drug exposures may increase the risk of acquired drug resistance (34). Third, tuberculous meningitis is a relatively frequent form of TB in young children and is associated with devastating morbidity and mortality. A recently published analysis showed that doses of levofloxacin considerably higher than those currently used in children, approximately 19 to 33 mg/kg, are needed to obtain optimal concentrations in the CSF (35). Lastly, revised WHO guidance provided in 2016 recommended avoidance of the use of injectable medications in children with clinically diagnosed MDR-TB; optimization of the exposures to the fluoroquinolone in the regimen is especially important to ensure the potency of injectable-sparing MDR-TB treatment regimens (16) Additionally, the extrapolation of efficacy to children from the results of ongoing or planned trials of treatments for TB in adults, many of which contain levofloxacin, is, in principle, dependent on achieving similar drug exposures in children (36, 37).

There are limitations to the approach of using adult exposures as targets to define pediatric doses for anti-TB drugs. However, in the absence of other more optimal, validated approaches to defining doses in children, we believe that it remains a useful strategy. The use of pharmacokinetic (PK)/pharmacodynamic (PD) targets, such as the AUC/MIC, is an option. However, exposures in plasma may not reflect exposures at the site of disease. Differences in drug penetration into lesions or macrophages, which may not be reflected in the levels of plasma exposure, may have important implications for efficacy. These are potentially important limitations to the targeting of PK/PD indices. Interesting work on the development of hollow-fiber models of pediatric TB to establish drug targets has recently been undertaken (38); however, in the absence of validation of the exposure targets generated by these hollow-fiber models, we believe that it is premature to define doses on the basis of that work. Therefore, despite the limitations, the use of exposures in adults associated with efficacy remains a reasonable approach to establishing pediatric doses for anti-TB drugs. We would argue that TB disease in children differs from that in adults both in severity and in type (extrapulmonary versus pulmonary). Although a small proportion of children, mostly older children and adolescents, develop cavitating, adult-type TB, the majority of children develop less severe, paucibacillary disease, which is likely to be more responsive to treatment. It is therefore possible that children may be successfully treated with less intense treatment. However, what less intense treatment means needs to be carefully defined. We can be confident that the doses in children resulting in exposures approximating those seen with efficacious doses in adults would be as efficacious or more efficacious in children. It may be that exposures lower than those needed in adults with TB may still be efficacious in children; however, in the absence of a clear definition of "lower," targeting of adult exposures remains a reasonable approach. One approach would be to maintain good exposures early in treatment to ensure a rapid response to treatment and a rapid reduction in organism burden but then to reduce the intensity of treatment by shortening the treatment duration in children. Such a strategy is being evaluated in the SHINE trial (International Standard Randomized Controlled Trial number ISRCTN63579542), which is a study of 4 versus 6 months of treatment for nonsevere intrathoracic TB in children.

A limitation of our study was the lack of older children, as moxifloxacin was routinely used in children 8 years of age and older in the local setting. However, pharmacokinetics is easier to predict in older children than in younger children. Another limitation is the lack of clear documentation of previous dosing times prior to the day on which samples were collected for pharmacokinetic analysis. Incomplete data on creatinine values, which were not collected for all children, limited our ability to assess whether renal function could explain some of the variability in clearance. Finally, we cannot explain the differences between our data and those published previously, such as higher values for clearance, and we cannot rule out the possibility of a contribution of the formulation used. We were unable to include different levofloxacin formulations in this study. This may need further evaluation, and the levofloxacin doses derived from our simulations should not be used routinely in children before additional careful evaluations have been completed.

In the large cohort of young South African children treated for MDR-TB described here, levofloxacin doses of 15 to 20 mg/kg resulted in exposures (C_{max} and AUC) lower than those seen in adults receiving the standard 750-mg daily dose for the same indication, even in those very young children with immature renal function. Considerably higher levofloxacin doses will thus be needed for children. Model-derived dose recommendations are provided and should be explored prospectively, with special attention being given to safety.

MATERIALS AND METHODS

Study design. This was a prospective observational intensive pharmacokinetic study.

Setting and population. The study was performed in Cape Town, South Africa. Children were eligible if they were 0 to 14 years of age, were routinely treated with levofloxacin for the treatment of pulmonary or extrapulmonary MDR-TB disease or exposure, had been on TB treatment and antiretroviral therapy (ART) for at least 2 weeks if they were HIV infected, and provided written informed consent and assent. Children with body weights of <5 kg or with hemoglobin concentrations of <8 g/dl were excluded or enrollment was deferred. Children were diagnosed with confirmed or probable TB on the basis of signs, symptoms, microbiology, and radiography. In addition, the following was required: isolation from the child of an *M. tuberculosis* strain with resistance to both isoniazid and rifampin (i.e., confirmed MDR-TB disease), exposure to a case of infectious MDR-TB (probable MDR-TB), or clinical evidence of the failure of adherent first-line TB treatment (possible MDR-TB) (39). Children with MDR-TB disease were treated according to local and international recommendations with at least four drugs confirmed or likely to be effective against the infecting strain plus pyrazinamide and ethambutol. Local guidelines recommended the use of levofloxacin for children <8 years of age and moxifloxacin for adults and children >8 years of age on the basis of the available drug formulations (250 mg levofloxacin; Austel, South Africa). In our setting, high-risk contacts of an infectious MDR-TB source case, including children aged <5 years and HIV-infected children aged <14 years, without evidence of TB disease were prescribed preventive therapy against MDR-TB consisting of levofloxacin, ethambutol, and high-dose isoniazid daily for 6 months. All children in the study were routinely tested for HIV by an HIV-specific enzyme-linked immunosorbent assay if they are ≥18 months of age and by a PCR for HIV DNA if they are <18 months of age. All HIV-infected children in the study received ART on the basis of local recommendations, including lopinavir-ritonavir (children <3 years of age) or efavirenz (children >3 years of age) in combination with two nonnucleoside reverse transcriptase inhibitors, usually lamivudine (3TC) with abacavir or zidovudine.

Levofloxacin dosing and pharmacokinetic sampling. Children received standard doses of 10 to 15 mg/kg once daily during the beginning of the study (2012 and 2013), according to the dose used for routine care at the time. Following interim analyses showing low drug exposures with these doses, the dose used for routine care was increased to 15 to 20 mg/kg once daily (2013 to 2017) (12). Pharmacokinetic sampling was performed between 2 and 16 weeks after the start of the anti-TB treatment. On the day of sampling, after an overnight fast, a levofloxacin dose of 15 mg/kg (during 2012 and 2013) or 20 mg/kg (from 2013 to 2015) (exact milligram-to-kilogram dosing to 0.1 mg adjusted to body weight) was administered by the study team together with all other anti-TB medications in the child's MDR-TB regimen. Medications were administered either as whole tablets (for older children able to swallow pills) or as tablets crushed and dissolved in water and given either orally or via a nasogastric tube (NGT), depending on what the child would tolerate. One hour after the anti-TB medication was dosed, a standard meal was offered. For HIV-infected children, antiretrovirals were also administered at that time. Blood samples were collected predose and then at 1, 2, 4, 6, and 8 h after levofloxacin dosing.

Levofloxacin assay. A method for the quantification of levofloxacin in plasma was validated and consisted of protein precipitation using acetonitrile, followed by high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) detection. The calibration curve fit a linear (weighted by 1/concentration) regression over the range of 0.0781 to 5.00 mg/liter on the basis of the peak area ratios for levofloxacin. Levofloxacin-d8 was used as the internal standard. The lower limit of quantification (LLOQ) was set at the concentration of the lowest validated standard for levofloxacin, 0.0781

mg/liter. Inter- and intraday coefficients of variation were below 7% for all quality control samples. Dilutions were performed using a validated dilution procedure for samples with concentrations greater than the upper limit of the assay.

Population pharmacokinetic model development. The data were interpreted using nonlinear mixed-effects modeling (NONMEM software, version 7.3) (40) and an algorithm with first-order conditional estimation with a eta-epsilon interaction. Summary statistics were calculated using the R program, and model development was assisted and graphic diagnostics were generated using the R program (41) and the Perl-Speaks-NONMEM, Xpose4, and Pirana programs (42). Several structural models were tested, including models with 1- and 2-compartment dispositions with first-order elimination and first-order absorption with and without the use of a lag time or a chain-of-transit compartment to model the onset of absorption (43). Random between-subject variability (BSV) and between-occasion variability (BOV) were assumed to have a lognormal distribution, and the error model included both an additive component and a proportional component. Concentrations below the LLOQ of the assay were imputed as LLOQ/2, as suggested in the M6 method of Beal (44), and the additive error component was constrained to be at least 20% of the LLOQ. The effect of differences in body size on the pharmacokinetic parameters was described using allometric scaling, with exponents fixed to 3/4 for clearance parameters and 1 for volumes of distribution (45), while the effect of growth and development was tested with a maturation function using postmenstrual age (i.e., postnatal age plus the estimated gestational age) (46), as shown in the following equation: $\text{maturation} = [\text{PMAGE}^{\gamma}/(\text{PMAGE}_{50}^{\gamma} + \text{PMAGE}^{\gamma})]$, where PMAGE denotes postmenstrual age, PMAGE_{50} is the value of PMAGE at which 50% of the maturation is complete, and γ is a parameter changing the shape of the relationship. Since no information on the actual gestational age of the children was available, the postmenstrual age was assumed to be the postnatal age plus 9 months.

Additionally, other covariate effects were explored, including HIV infection status, MDR-TB disease versus exposure status, ethnicity, undernutrition, the method of drug administration (crushed tablets via an NGT, crushed tablets orally, whole tablets orally), and coadministration of antituberculosis or antiretroviral drugs (the regimens are shown in Table S1 in the supplemental material). Creatinine clearance (estimated using the serum creatinine concentration and the revised formula of Schwartz et al. [47]) was also explored as a covariate; however, it was available only for children receiving MDR-TB treatment and not those receiving preventive therapy. Additionally, since administration of the dose 1 day prior to the day of sampling for pharmacokinetic analysis was not observed by the study team and the exact time and method (crushed versus whole tablets) of dose administration were not always accurately recorded, the inclusion of a larger BOV for the absorption and bioavailability pharmacokinetic parameters was tested for the pharmacokinetic profiles following a nonobserved dose (i.e., all the predose concentrations) (Table 2). Model development was guided by the use of the change in the objective function value (ΔOFV), considering decreases of 3.84 points to be significant at a P value of <0.05 for the inclusion of one additional parameter (denoted as the number of degrees of freedom [df]), by inspection of goodness-of-fit plots, and by the use of visual predictive checks (48). The precision of the final parameter estimates was evaluated using a nonparametric bootstrap with replacement ($n = 500$).

Levofloxacin dosing simulations. Simulations from the final model were used to optimize the doses across different weight bands. To account for the effect of age (in addition to weight), simulations were performed using *in silico* patients with combinations of age and weight relevant for the population for whom the dosing guidelines are designed. To increase the granularity of the simulations and reflect the fact that children affected by TB generally have weights lower than those of children in the general population, we used a model of weight for age developed with children with TB to generate *in silico* patients: 100 males and 100 females at each 1-month age step from 0 to 18 years of age (49). Those in the weight range of 4 to 35 kg were retained for the simulations. All *in silico* children were assumed to be born at term, to be HIV uninfected, and to be dosed every 24 h with whole or crushed tablets without an NGT. Values of AUC and C_{max} were collected.

The pharmacokinetic target was the AUC expected in adults with normal renal function treated for TB with levofloxacin at the currently recommended 750-mg daily dose. The AUC target was set at 96.8 mg · h/liter, a value that was obtained after rescaling the median AUC reported by Peloquin et al. (15) from 1,000 mg (reported in the study) to a 750-mg daily dose, since the pharmacokinetics of levofloxacin has been reported to be linear in this dose range (50). The study by Peloquin et al. (15) was chosen as it provides the only current data on levofloxacin pharmacokinetics in adults treated for TB. Reassuringly, the AUC rescaled to 750 mg is similar to the value reported to be obtained with a 750-mg dose in healthy adults without TB (mean AUC, 101 mg · h/liter) (10). As there may be implications for toxicity, we compared the simulated C_{max} levels with the original C_{max} values from the same study (median C_{max}, 15.55 mg/liter) (15), without any rescaling of the dose, since these adult TB patients received 1,000 mg and levofloxacin was reported to be well tolerated during a 7-day dosing period. The optimized dosing regimen was designed to achieve a median AUC in each 1-kg weight band within a 20% tolerance of the target while ensuring that the median C_{max} would not exceed by 20% the value previously observed in adults receiving the well-tolerated 1,000-mg dose. Finally, the doses in each weight band were adjusted to account for the fact that levofloxacin is currently available as a 250-mg tablet, which can be split in half or into quarters, if needed.

Ethical considerations. Informed consent was provided by the parent(s) or legal guardian of all participants, and assent was provided by all participants aged 7 years and older. Ethics approval for the study was provided by the Health Research Ethics Committees of Stellenbosch University (N11/03/59) and the University of Cape Town (397/2011).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01521-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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Chapter 4: Clinical and cardiac safety of long-term levofloxacin in children treated for multidrug-resistant tuberculosis

Rationale

The long-term use of fluoroquinolones for TB in children has traditionally been limited to date due to safety concerns, based largely on studies in juvenile animals which showed that fluoroquinolones were associated with a destructive arthropathy (66, 92). Extensive experience with the short-term use of fluoroquinolones in children has however not found a meaningful risk of similar events (55, 93), and the fluoroquinolones, including levofloxacin, are routinely recommended for use in children for specific clinical indications such as MDR-TB (126). However, persistent concerns among healthcare providers remained a barrier to using fluoroquinolones in children with MDR-TB in many settings, and there was limited safety data for levofloxacin at the doses and durations currently used for MDR-TB. QT-interval prolongation is a class-wide adverse effect of the fluoroquinolones that has not been well characterized in children. Data was, however, urgently needed as novel and repurposed antituberculosis drugs (clofazimine, bedaquiline, delamanid) also prolong the QT-interval. Therefore, in addition to establishing the optimal paediatric dose of levofloxacin for children with MDR-TB, its long-term safety at current doses should also be demonstrated to inform current treatment as well as future research evaluating levofloxacin used in regimens with other QT prolonging medications.

Study aims

The aim of this study was to characterize the safety of levofloxacin in children enrolled in the previously described prospective cohort study (Chapter 3).

Methods

Children were enrolled as a part of the MDRPK1 study. Children who were a part of the previously described levofloxacin pharmacokinetics study (Chapter 3, aged less than 15 years and routinely treated with levofloxacin) and who received levofloxacin (10-20 mg/kg/day) as a component of the routinely used 6-7 drug treatment regimen for MDR-TB disease had longitudinal follow-up to document safety (i.e. not those treated with preventive therapy for MDR-TB exposure who only contributed cross-sectional

pharmacokinetic data). Standard clinical and laboratory monitoring was completed 1-2 monthly, and 12-lead electrocardiograms (ECGs) were done on pharmacokinetic sampling days pre-dose and at 2 hours post-levofloxacin dose (expected maximum plasma concentration) and QT-interval estimated using the Fridericia correction (QTcF) by one of two paediatric cardiologists. ECGs were only implemented later during the study, based on emerging data from adults, so not all children contributed to the analysis on the QT-interval. All adverse events were recorded, assessed for attribution to levofloxacin, and graded according to standard criteria and summarized using descriptive statistics. Multivariable linear regression was used to characterize the association between QT-interval and levofloxacin concentrations (from the pharmacokinetic study in Chapter 3).

Results

Seventy children (median age 2.1 years, range 0.4 to 7.3) were included in this analysis and were observed for a total person time of 68.5 years (median 11.6 months, IQR 9.2 to 14.7); 41 children contributed ECG data (median age 2.1 years, range 0.2 to 4.8). There were no Grade 4 or serious adverse events, and levofloxacin was not permanently discontinued for any adverse events. There were few and only mild and self-limited musculoskeletal events. No child had a QTcF >450 ms, and 5 (13%) and 1 (3%) had a change in QTcF from pre-dose to 2 hours post-dose of 30 to 60 ms or >60 ms respectively. In multivariable linear regression, only age (p=0.028), and not change in levofloxacin concentration, was associated with change in QTcF from pre-dose to 2 hours post-dose.

Conclusions and recommendations

Levofloxacin was safe and well tolerated in this cohort of children treated for MDR-TB. Although the levofloxacin-associated risk of some events, such as sleep disturbance, may have been underestimated and the risk of some non-specific events such as vomiting, alanine aminotransferase (ALT) elevation, overestimated, this study provides important and robust evidence on the safety of levofloxacin with currently used doses and for long durations. The lack of substantial QT-interval prolongation in these children is reassuring considering plans to combine levofloxacin with other QT

prolonging drugs in novel MDR-TB regimens. This study provides important data to guide current and future MDR-TB treatment regimens in children.

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Title: Clinical and cardiac safety of long-term levofloxacin in children treated for multidrug-resistant tuberculosis

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Running title: Levofloxacin safety in children with MDR-TB

Key words: Levofloxacin, safety, children, MDR-TB, QT prolongation

Abstract

Safety concerns persist for long-term pediatric fluoroquinolone use. Seventy children (median age 2.1 years) treated with levofloxacin 10-20 mg/kg once daily for multidrug-resistant tuberculosis (median observation time 11.8 months) had few musculoskeletal events, no levofloxacin-attributed serious adverse events, and no QTcF >450 ms. Long-term levofloxacin was safe and well tolerated.

Introduction

Levofloxacin is a key component of multidrug-resistant tuberculosis (MDR-TB) treatment regimens in children, typically for 9-18 months duration [1]. Levofloxacin is also used as preventive therapy for MDR-TB in children in some settings for 6 months or longer. Fluoroquinolones cause a destructive arthropathy in juvenile animals, which had traditionally limited their use in children [2]. In addition, the fluoroquinolones may cause the following: Achilles tendon rupture; nausea, vomiting, diarrhea; central nervous system effects such as hyperactivity, insomnia, hallucinations, and raised intracranial pressure; dysglycemia; and QT-interval prolongation [3].

Despite these historical concerns, accumulating data has not demonstrated serious arthropathy, tendinopathy, or other serious safety concerns in children over short durations (7-14 days) [2, 4, 5]. Fluoroquinolones are now recommended by the World Health Organization (WHO) and others for use in children where there are limited treatment options, including for MDR-TB [6]. However, there is a paucity of data in children on levofloxacin safety and tolerability over long durations and at the higher doses currently used for MDR-TB treatment. Levofloxacin's QT-interval prolonging effects in children have also not yet been well described, however this information is needed as levofloxacin is increasingly being combined in treatment regimens with novel TB drugs, which also cause QT-interval prolongation [7].

We aimed to characterize the safety and tolerability of levofloxacin in children routinely treated for MDR-TB.

Patients and Methods

Study design, setting and population: We have previously described the design of this prospective observational pharmacokinetics study in Cape Town, South Africa, in detail [8]. Briefly, children were included in this study if they were <15 years of age, >5 kg body weight, and routinely treated for MDR-TB with levofloxacin. In this setting, children with MDR-TB are treated with 6-7 drug regimens, which usually contain a fluoroquinolone, amikacin, ethionamide, terizidone, high-dose isoniazid, pyrazinamide, ethambutol, and occasionally para-aminosalicylic acid, linezolid and clofazimine. Levofloxacin (250 mg tablets) was the recommended fluoroquinolone for children with

MDR-TB <8 years of age due to challenges with administering the moxifloxacin 400 mg tablet formulation used in children >8 years of age and adults. Levofloxacin routine dosing in our setting changed from 10-15 mg/kg once daily to 15-20 mg/kg once daily during the study. Children are often hospitalized for 1-6 months at the beginning of MDR-TB treatment, and then complete their treatment as outpatients.

Parents or legal guardians provided informed consent. The Health Research Ethics Committees of Stellenbosch University provided study approval (N11/03/059).

Data collection: Standard clinical and laboratory assessments [alanine aminotransferase (ALT), bilirubin, creatinine, potassium] were done 1-2 monthly throughout treatment. All adverse events were recorded, assessed for attribution to levofloxacin, and graded for severity (DAIDS Grading Table, Version 1.0, August 2009) [9]. Twelve-lead electrocardiograms (ECGs) were performed in triplicate on pharmacokinetic sampling days just prior to the pharmacokinetic blood draws pre-dose and at 2 hours post-dose (expected maximum levofloxacin plasma concentration). ECGs were only started later during the study and were interpreted by one of two pediatric cardiologists; the measured QT-interval was corrected using the Fridericia correction (QTcF) and the mean of the triplicate QTcF values used for analysis. Pre-dose and 2 hour levofloxacin concentrations were obtained according to previously described methods [8]; concentrations below the limit of quantification (BLQ) were assigned a value of zero for this analysis.

Analysis: Demographic and clinical characteristics, and QTcF results were summarized using descriptive statistics. The frequency of adverse events was reported by grade for all events, and also for events that were possibly, probably or definitely related to levofloxacin. Person time was calculated from the baseline study assessment until the treatment completed or the last available study visit. Event rates were reported per 100 person-years.

Multivariable linear regression was done to characterize the association between the change in QTcF with the change in levofloxacin concentration, controlling for gender, HIV status, and age. The standard errors were adjusted to account for one patient with two sets of levofloxacin concentatration and ECG data from different days. Stata/SE 14.0 was used to analyze the data (StataCorp. 2105. *Stata Statistical Software: Release 15.* College Station, TX: StataCorp LP).

Results

Seventy children (median age: 2.1 years, range 0.4, 7.3) were included in the safety analysis; 38 (54%) were male and 12 (17%) were HIV-infected (see Supplemental Table 1). These children were observed for a total duration of 68.5 person-years (median 11.6 months, interquartile range [IQR] 9.2-14.7). Table 1 shows all adverse events and those at least possibly related to levofloxacin. There were no Grade 4 or any serious adverse events attributed to levofloxacin, and no adverse event resulted in permanent levofloxacin discontinuation.

ECG results were available in 41 children, median age 2.1 years (range: 0.2-4.8 years); 20 (49%) were male and 10 (24%) were HIV-infected. All HIV-infected children were on antiretroviral therapy; 9 were on lopinavir/ritonavir with two nucleoside reverse transcriptase inhibitors (NRTIs) and 1 was on efavirenz with two NRTIs. Three patients had one ECG at 2 hours only and two patients had one ECG at both 0 and 2 hours. There were 38 pre-dose (0 hour) ECGs, and 41 2-hour ECGs, with 37 children contributing 38 paired results. The mean [standard deviation (SD)] QTcF was 359 ms (21.0) at 0-hours and 365.4 ms (26.6) at 2-hours; no QTcF was >450 ms. The mean (SD) change in QTcF from 0 to 2 hour reading was 4.7 ms (27.3). Five (13%) had a change in QTcF of 30 to <60 ms from 0 to 2 hour readings, and 1 (3%) had a change >60ms. For the children with paired ECG results, the mean (SD) levofloxacin concentration pre-dose was 0.33 μg/mL (0.61) and at 2 hours was 8.57 μg/mL (2.55); 11 (28.9%) pre-dose concentrations were BLQ. Figure 1 shows the change in QTcF vs. change in levofloxacin concentrations from 0 to 2 hours. In multivariable linear regression, only age (p=0.028) was significantly associated with change in QTcF from 0 to 2 hours, with every 1 year increase in age associated with a 7.36 ms increase in QTcF change (Supplemental Table 2). The one patient treated with clofazimine, known to prolong the QT-interval, had a QTcF change of 44 ms.

Discussion

In this cohort of children with MDR-TB, long-term levofloxacin treatment was safe and well-tolerated. The few musculoskeletal complaints (pain, arthralgia) were mild and

self-limited. Mild musculoskeletal complaints and those in young children may have been underestimated, however it is unlikely that more severe events were missed, such as those having objective signs of arthritis or those resulting in gait abnormalities or failure to bear weight. This should be reassuring to clinicians and TB programs, some of whom are still hesitant to treat children affected by TB with fluoroquinolones.

Hyperactivity and sleep disturbances have been well described in children treated with fluoroquinolones [10], however we observed few such events. These may be underestimated due to children being admitted early in their treatment to the TB hospital without their caregivers, which may have obscured reported changes in behavior and sleep patterns.

The most common events overall were non-specific, such as rash, nausea, vomiting, and ALT elevation. These likely represent overestimates of the rate of these events due to levofloxacin; more likely these were due to other medications such as isoniazid, pyrazinamide (ALT elevation) and ethionamide (nausea, vomiting). We erred on the side of attributing these events at least possibly to levofloxacin, unless there was strong evidence of the relationship with another medication. The poor palatability of levofloxacin formulation, especially when crushed, may have contributed to some of the nausea and vomiting.

No child had a QTcF >450 ms, and few had a change >30 ms from pre-dose to 2 hours. We did not observe a relationship between QTcF and levofloxacin concentration. Fluoroquinolone-associated QT prolongation is mediated through dose-dependent inhibition of cardiac potassium channels that varies by agent, with moxifloxacin, gatifloxacin having a more potent effect than levofloxcin, ciprofloxacin and ofloxacin [11]. In previous adult studies, 1000 mg levofloxacin resulted in a mean change in QTc of 3.5-4.8 ms compared to placebo [12], and doses as high as 1500 mg in adults had a minimal impact on QTc [13]. This is consistent with our findings. The association of older age with QTcF change in our cohort needs further evaluation. A limitation to our study is that these children did not have true pre-treatment QTcF values for comparison, as all had already been on levofloxacin for atleast one week at the time ECGs were completed. However, the pre-dose concentrations were generally low, including many that were BLQ, so the change in QTcF from pre-dose remains a useful evaluation. This data therefore also provides support for using levofloxacin in

combination with other QT-prolonging TB medications such as clofazimine, bedaquiline and delamanid. It also establishes a baseline for QT-intervals in children treated with levofloxacin-containing MDR-TB regimens for interpreting cardiac safety results of ongoing pediatric bedaquiline and delamanid trials.

A limitation of our study is the lack of children >8 years of age who may have a different adverse event profile, would likely be able to report subjective symptoms better, and may have different QT effects, and should be included in future studies.

In summary, levofloxacin, at doses up to 20 mg/kg once daily, was safe and well-tolerated and should remain a mainstay of pediatric MDR-TB treatment.

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Conflict of Interest

All authors have no conflicts of interest to report.

Table 1. Adverse events in children (n=70) treated for multidrug-resistant tuberculosis with levofloxacin

Adverse Event	All adverse event by grade							Adverse effects possibly, probably, definitely attributed to levofloxacin by grade						
	# of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	total # of events	Event Rate (per 100 person-years)	# of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	total # of events	Event Rate (per 100 person-years)
Arthralgia	3	3	0	0	0	3	4.4	2	2	0	0	0	2	2.9
Arthritis	0	0	0	0	0	0	--	0	0	0	0	0	0	--
Pain other than trauma	11	11	0	0	0	11	16.1	4	4	0	0	0	4	5.8
Headache	4	4	0	1	0	5	7.3	2	1	0	1	0	2	2.9
Neurosensory alteration	1	1	0	0	0	1	1.5	0	0	0	0	0	0	--
Insomnia	1	0	1	0	0	1	1.5	1	0	1	0	0	1	1.5
Fatigue/malaise	1	1	0	0	0	1	1.5	0	0	0	0	0	0	--
Nausea	12	13	0	0	0	13	19.0	8	9	0	0	0	9	13.1
Vomiting	19	23	1	0	0	24	35.1	14	16	0	0	0	16	23.4
Anorexia	11	8	5	0	0	13	19.0	7	4	3	0	0	7	10.2
Cutaneous reaction	12	8	6	0	0	14	20.4	7	3	4	0	0	7	10.2
Pruritus	13	16	1	0	0	17	24.8	7	7	1	0	0	8	11.7
ALT elevation	22	17	3	2	5	27	39.4	16	16	2	0	0	18	26.3
Bilirubin elevation	0	0	0	0	0	0	--	0	0	0	0	0	0	--

Total person time of observation = 68.5 years

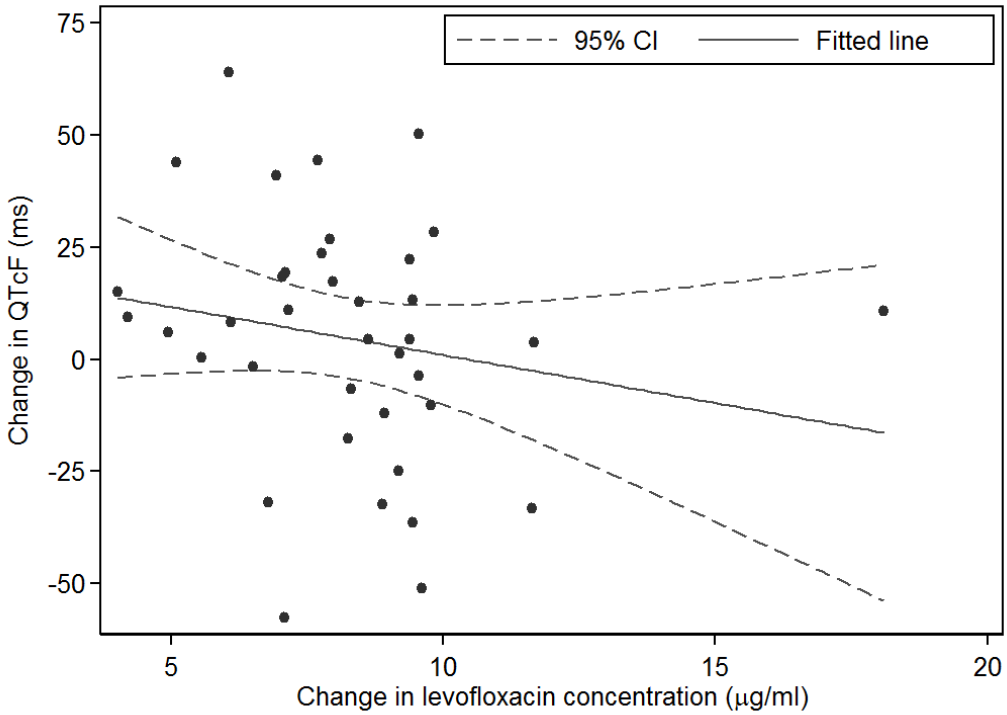

Figure 1. Change in QTcF versus change in levofloxacin concentration in children treated for multidrug-resistant tuberculosis

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Supplemental Table 1. Demographic and clinical characteristics of children with multidrug-resistant tuberculosis treated with levofloxacin (n=70)

Age at enrollment (%)	
0-2 years	31 (44.3)
2-5 years	35 (50.0)
6-15 years	4 (5.7)
Male sex (%)	38 (54.3)
Ethnicity (%)	
Black	43 (61.4)
Mixed race	27 (38.6)
Certainty of TB diagnosis (%)	
Bacterioloical confirmation	22 (31.4)
Probable TB	26 (68.6)
TB disease type (%)	
PTB only	54 (77.1)
EPTB only	4 (5.7)
PTB and EPTB	12 (17.1)
HIV-infected (%)	12 (17.1)
If HIV-infected, ART regimen [n=12]	
Efavirenz + 2 NRTIs	3 (25%)
Lopinavir/ritonavir + 2 NRTIs	9 (75%)
Weight-for-age-Z-score <-2.0 (%) [n = 69][a]	16 (23.2)
Height-for-age-Z-score <-2.0 (%) [n = 69][a]	24 (34.8)

[a]The sample size is less than 70 due to missing data
NRTI = nucleoside reverse transcriptase inhibitor

Supplemental Table 2. Univariable and multivariable linear regression of change in QTcF prior to and 2 hours post-levofloxacin-dose in children with multidrug-resistant tuberculosis (N=38)

	Univariable		Multivariable	
	Coefficient β (95% CI)	p-value	Coefficient β (95% CI)	p-value
Change in levofloxacin concentration (µg/mL)	-2.14 (-5.56 to 1.28)	0.212	-0.38 (-2.19 to 1.43)	0.673
Age in years	9.18 (3.60-14.77)	0.002	7.36 (0.86 - 13.87)	0.028
HIV status	-20.01 (-40.03 to 0.04)	0.050	-12.15 (-36.53 to 12.23)	0.319
Male gender	9.07 (-8.15 to 26.28)	0.292	4.54 (-11.47 to 20.54)	0.569

*R^2 = 0.238; QTcF= QT interval corrected using the Fridericia method

Chapter 5: Pharmacokinetics, safety and dosing of novel levofloxacin dispersible tablets in children with exposure to multidrug-resistant tuberculosis

Rationale

The TB-CHAMP trial (ISRCTN92634082) is a phase III cluster randomised placebo-controlled trial to assess the efficacy of levofloxacin vs. placebo for prevention of TB in child household contacts of adult MDR-TB source cases. For the trial, a levofloxacin 100 mg dispersible tablet was developed for the trial by Macleods Pharmaceuticals (Mumbai, India). In order to characterize exposures with this novel formulation in the target population (young children below 5 years of age) prior to utilizing it in this large phase 3 efficacy trial (n=1556), a lead-in pharmacokinetic study was designed. Macleods have since submitted this formulation for WHO Prequalification and is making it commercially available, so this study would potentially inform its use in routine care in addition to informing its use in the TB-CHAMP trial. This work builds directly on the work from Chapter 3, in which we identified questions regarding the potential effects of formulation on the levofloxacin exposures observed in that study.

Study Aims

The aim of this study was to characterize the pharmacokinetics, safety and optimal dosing of a novel 100 mg dispersible tablet formulation of levofloxacin in children with MDR-TB exposure, to inform its use in the context of a large phase 3 preventive therapy trial.

Methods

This was an open-label pharmacokinetic lead-in study to the TB-CHAMP trial. Children less than 5 years of age who were household contacts of an adult MDR pulmonary TB index case diagnosed during the previous 6 months were eligible. Exclusion criteria included prevalent TB disease at enrolment, receipt of preventive therapy with isoniazid or a fluoroquinolone for ≥ 16 weeks, or known concurrent exposure to an isoniazid-susceptible source case. Levofloxacin 100 mg dispersible tablets were prescribed according to weight bands (15-20 mg/kg once daily). Doses were dispersed

in 2.5-10 mL of clean water in a syringe and cup, and administered. The dosing containiner was then rinsed with clean water and administered. Pharmacokinetic sampling was performed 7-14 days after starting levofloxacin, with samples taken just prior to, and at 1, 2, 4, 6, and 8 hours after an observed dose. Assays were performed at the University of Cape Town Division of Clinical Pharmacology according to the same method described in Chapter 3 (LC MS/MS). NLME modelling was used to analyse data from the current study pooled with the levofloxacin data reported in Chapter 3 of this dissertation from children receiving the standard adult 250 mg tablet formulation, as the population, site, sampling schema, sampling procedures and laboratory assays were similar to this study. After refitting the model with the new data, pharmacokinetic parameters were re-estimated. Optimal weight-banded doses for the 100 mg dispersible tablet were estimated using clinical trial simulations targeting the same AUC as in Chapter 3 (96.8 mg·h/L). Any adverse events occurring during the time the child was on the study drug were reported, attribution to levofloxacin assessed and graded according to standard Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (Corrected version 2.1, July 2017) (127).

Results

Twenty-four children (median age 2.1 years; IQR 1.2 to 2.7) completed the study; none were HIV-infected. The levofloxacin pharmacokinetic model structure was the same and the parameter values not significantly different from the previous analysis, except for the bioavalability of the new dispersible tablets. When the new dispersible tablet was chosen as reference for biavailability (F=1), the standard 250 mg adult tablets used in the previous study were found to be 41% less bioavailable. The value of CL/F for a typical 2-year old, 12 kg child was 2.8 L/h. After adjusting for full maturation and using allometric scaling to resize to a 70-kg adult, the value of CL/F from this study for a typical 2-year old (2.8 L/h) becomes 11.4 L/h, which is much more consistent with scaled values from published adult (CL/F 8.35-9.16) and pediatric studies (CL/F 11.6-15.4) compared to the value from the study in Chapter 3 (18.8 L/h) (128). Optimal weight-banded doses with the dispersible tablet formulation were simulated; median mg/kg doses for weight bands varied from 14.3 mg/kg in the 3 to 4 kg weight band up to 25 mg/kg from 7 to <11 kg weight band. No patients had any grade 3 or 4 adverse events or any serious advents.

Conclusions and recommendations

Exposures in children receiving this novel 100 mg dispersible levofloxacin tablet formulation were much higher than previously observed in children receiving a standard 250 mg adult tablet, due to higher bioavailability of the new formulation. Our proposed weight-banded dosing can be used for this novel levofloxacin formulation for the TB-CHAMP trial and also by TB programmes as this formulation becomes increasingly available and used in routine care settings. This study highlights the importance of carefully investigating formulation-related factors in paediatric antituberculosis therapeutics.

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Title: Pharmacokinetics, safety, and dosing of novel pediatric levofloxacin dispersible tablets in children with multidrug-resistant tuberculosis exposure

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Running title: Dispersible levofloxacin PK

Key words: Levofloxacin, pharmacokinetics, dispersible tablets, children, bioavailability

Abstract

Background: Levofloxacin is used for prevention and treatment of multidrug-resistant tuberculosis (MDR-TB) in children.

Objectives: To characterize the pharmacokinetics, optimal paediatric dosing and safety of a novel 100 mg scored dispersible levofloxacin formulation (Macleods Pharmaceuticals) in children.

Patients and methods: In this open-label pharmacokinetic study, children aged <5 years with household MDR-TB exposure received daily weight-banded doses of levofloxacin 15-20 mg/kg using 100 mg tablets dispersed in water. After 7-14 days, children were sampled pre-dose and at 1, 2, 4, 6 and 8 hours post-dose. Nonlinear mixed-effects modelling was used to analyse data, which was pooled with previously published data from 109 children with MDR-TB receiving routinely available adult (250 mg) levofloxacin tablets. Weight-band dosing was optimised using simulations targeting the levofloxacin adult exposure achieved with 750 mg. Adverse events were systematically recorded.

Results: 24 HIV-uninfected children completed pharmacokinetic sampling, median (IQR) age 2.1 years (1.2 to 2.7). Levofloxacin pharmacokinetic parameters were similar to those previously published, except for 41% lower bioavailability of standard adult 250 mg tablets compared to the novel dispersible tablets. The CL/F for a typical 2-year old, 12 kg child was 2.8 L/h, consistent with previously reported adult values, after adjusting for body size with allometric scaling. There were no grade 3 or 4 adverse events, nor any serious events.

Conclusions: Exposures in children receiving the 100 mg dispersible levofloxacin tablets were much higher than previously observed due to higher bioavailability of this formulation. Our proposed weight-banded dosing can be used for this novel levofloxacin formulation.

Introduction

Levofloxacin is an increasingly important medication for the treatment and prevention of multidrug-resistant (MDR) tuberculosis (TB) in adults and children. Levofloxacin is well-absorbed after oral administration and primarily eliminated unchanged in the urine.[1] Its pharmacokinetics in children have now been evaluated in multiple studies with somewhat variable results.[2-5] A major barrier to its use in young children to date has been the lack of a widely available child-friendly formulation. In most high TB burden settings, adult 250 mg levofloxacin tablets are administered to young children after splitting or crushing and mixing with water or food. In addition to inaccurate dosing and poor palatability, the effect of this formulation manipulation on levofloxacin exposures is unknown.

A 100 mg scored dispersible taste-masked tablet has been developed (Macleods Pharmaceutical, Ltd., Mumbai, India) and has now received World Health Organization (WHO) Pre-qualification, with an expected increase in routine use for TB. The TB-CHAMP trial (ISRCTN92634082) is a phase III cluster randomised placebo-controlled trial to assess the efficacy of levofloxacin vs. placebo for prevention of TB in child household contacts of adult MDR-TB source cases, and plans to use this novel 100-mg levofloxacin formulation.

As part of the TB-CHAMP trial, an open-label lead-in study was undertaken to characterize the pharmacokinetics and short-term safety of the levofloxacin 100-mg dispersible tablet in children less than 5 years of age, the intended target population for the TB-CHAMP trial.

Patients and Methods

Study design: Prospective descriptive pharmacokinetic and safety study.

Setting and population: The study was undertaken in Cape Town, South Africa. Children less than 5 years of age were eligible for this lead-in study if they were a household contact of an adult MDR pulmonary TB index case diagnosed during the previous 6 months, and written informed consent was provided by the parent or legal guardian. Exclusion criteria in children included prevalent TB disease at enrolment, receipt of preventive therapy with isoniazid or a fluoroquinolone for ≥ 16 weeks, TB treatment in

the previous 12 months, known concurrent exposure to an isoniazid-susceptible source case, or known myasthenia gravis or Guillain-Barré syndrome.

Levofloxacin dosing and pharmacokinetic sampling: Levofloxacin 100 mg dispersible tablets were prescribed according to weight-bands, targeting a dose of 15-20 mg/kg (Supplemental Table 5.1). Doses were prepared by placing the tablets in a plastic dosing cup or syringe, adding 2.5-10 mL of clean water, swirling the cup or shaking the syringe until the tablets were fully dispersed, and then it was administered. An additional 2.5-10 mL of clean water was added to the dosing container, swirled or shaken to suspend any remaining medication particles, and administered. Pharmacokinetic sampling was performed 7-14 days after starting levofloxacin. An observed levofloxacin dose was given after an overnight fast, and samples taken just prior to, and at 1, 2, 4, 6, and 8 hours after the dose. A standard breakfast was offered 1 hour post-dose.

Levofloxacin assay: Levofloxacin concentrations were quantified by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) using a validated method as previously described.[5]

Safety: All treatment emergent adverse events, i.e. adverse events that occurred during the time the child was receiving the study levofloxacin formulation, were recorded, assessed for attribution to levofloxacin, and graded for severity according to standard DAIDS Grading.[6]

Population pharmacokinetic model and dosing simulations: Nonlinear mixed-effects modelling implemented in NONMEM (version 7.4)[7] was used to analyse data from the current study pooled with our previously published levofloxacin data.[5] The previous study included 109 children with MDR-TB receiving the routinely available standard adult 250 mg solid tablet formulation (Austell, Johannesburg, South Africa), administered either as tablets crushed, mixed in water and administered orally or by nasogastric tube if the child refused to swallow it, or swallowed whole. The study population, site, sampling schema, sampling procedures and laboratory assays were very similar to the current study. The pharmacokinetic model from the previous study was used as a starting point, but the model structure was revisited and the model parameters re-estimated when fitting the new data. Improvements in objective function value (ΔOFV) and goodness of fit plots were used for model development. The

final model was then used to optimise weight-band dosing targeting the levofloxacin AUC achieved in adults with TB with normal renal function receiving a 750 mg daily dose, set as 96.8 mg·h/L based on rescaling previously reported values in adults with TB receiving a 1000 mg dose.[5, 8]

Ethical considerations: Informed consent was provided by the parent/s or legal guardians. Ethics approval for the study was provided by the Health Research Ethics Committees of Stellenbosch University (M16/02/009).

Results

Study population: Twenty-eight children were enrolled. One child was withdrawn prior to pharmacokinetic sampling; 27 children contributed to safety data, however 3 children were unable to complete pharmacokinetic sampling due to difficulties with phlebotomy, resulting in 24 completing the study. The median age of children completing pharmacokinetic sampling (n=24) was 2.1 years (interquartile range 1.2 to 2.7); none were HIV-infected, 3 (13%) had a WAZ less than -2 (complete baseline characteristics are shown in Supplemental Table 5.2).

Pharmacokinetic model: A total of 144 levofloxacin concentrations were available for analysis, with two pre-dose samples below the limit of quantification. The levofloxacin pharmacokinetic model structure was the same and the parameter values not significantly different from the previous analysis, as shown in Table 5.1, except for the bioavailability of the new dispersible tablets. When the new dispersible tablet was chosen as reference for biavailability (F=1), the tablets used in the previous study were found to be 41% less bioavailable (ΔOFV=132, 1 extra degree of freedom, $p<10^{-6}$). Visual predictive checks are provided in Figure 5.1, showing how the model suitably fits the data of both the previous and the current study, and the large difference in exposure due to the change in bioavailability. The value of CL/F for a typical 2-year old, 12 kg child was 2.8 L/h. After adjusting for full maturation and using allometric scaling to resize to a 70-kg adult, the value becomes 11.4 L/h, which is more consistent with scaled values from published adult (CL/F 8.35-9.16) and pediatric studies (CL/F 11.6-15.4) that we previously summarised compared to the standard 250 mg adult tablet we previously studied (CL/F 18.8 L/h).[5] Interestingly, administration with nasogastric tube

was not found to affect the speed of absorption of the dispersible tablets, unlike with the standard 250 mg tablets in the previous study for which the absorption lag time was shortened when nasogastric tube was used for drug administration.

Simulated exposures: Proposed weight-banded dosing is shown in Table 5.2. Simulated maximum plasma concentrations (C_{max}) and area under the concentration time curve (AUC_{0-24}) with this dosing approach are shown in Figure 5.2.

Safety: Two participants had three adverse events that were at least possibly related to levofloxacin, including vomiting (grade 1), difficulty sleeping (grade 1), and anorexia (grade 2). All adverse events and those at least possibly related are shown in Supplemental Tables 5.2 and 5.3. No patients had any grade 3 or 4 adverse events, any serious advents and none discontinued levofloxacin because of potentially drug-related adverse events.

Conclusions

This novel pediatric levofloxacin dispersible tablet resulted in substantially higher exposures in children relative to the adult 250 mg solid tablets used in previous pediatric pharmacokinetic studies by our group,[5] with bioavailability primarily accounting for this difference. Our previously published work included children receiving levofloxacin either for MDR-TB treatment, often in combination with at least five other TB medications, or for MDR-TB preventive therapy, given in combination with isoniazid and ethambutol. We cannot rule out an interaction with other TB drugs that might explain the difference in exposures between the two studies, however this is unlikely, considering no such interactions have been previously described. This may be worth evaluating in future studies. The difference in bioavailability we describe is likely due to the effect of formulation. The effect of formulation is increasingly being recognized as an important contributor to drug exposure, and the effect of formulation may differ between children and adults.[9, 10] The value for levofloxacin's apparent oral clearance (CL/F) with the 100 mg dispersible tablets, after adjusting for body size with allometric scaling, is lower than we described previously with the 250 mg immediate release tablets, but consistent with most previously published values.[5] Moreover, when choosing the bioavailability of the new formulation as reference (value of 1), and thus

rescaling the bioavailability of the previous study to 0.587, the disposition parameters of the previous analysis also become consistent with the historical reports on levofloxacin pharmacokinetics. This suggests the routinely used levofloxacin formulation used in our previous work (and in routine TB care in South Africa) had unexpectedly low bioavailability. Additional work to understand potential formulation effects are warranted given the widespread global use of the 250 mg levofloxacin tablets in children with TB.

The lack of safety concerns in this study is reassuring and consistent with previous reports.[11] Careful safety monitoring should however continue in children receiving levofloxacin, particularly with longer treatment durations; the main TB-CHAMP trial will contribute such data.

The levofloxacin formulation reported on here is expected to be used widely by national TB progammes for treatment of children in routine settings. Our proposed weight-banded doses can be adopted by programmes for dosing in children with this formulation. International dosing recommendations for children with TB should be updated for levofloxacin taking into consideration emerging data and new formulations for levofloxacin, as is needed for other second-line antituberculosis medications.

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Transparency declarations: All authors, nothing to declare.

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Table 5.1. Pharmacokinetic parameter values of levofloxacin in children with multidrug-resistant tuberculosis disease or exposure

PK parameter	Typical value (95% CI)		Variability (95% CI)	
	Current pooled analysis (n=24 + 109)	*Previous study* (n=109)*	Current pooled analysis (n=24 + 109)	*Previous study* (n=109)*
CL (L/h)[a]	HIV-: 2.80 (2.43, 3.19) HIV[d]+: -16.0% (-26.3%, -4.39%)	***HIV-:*** *2.76 (2.57, 2.94)* ***HIV+:*** *-15.9% (-26.6%, -5.93%)*	**BSV:** 14.3% (10.3%, 16.3%)	***BSV:*** *15.2% (10.6%, 19.0%)*
Vc (L)[a]	12.0 (10.1, 13.7)	*11.3 (6.40, 12.8)*		
Q (L/h)[a]	0.227 (0.109, 0.616)	*0.467 (0.195, 2.79)*		
Vp (L)[a]	2.08 (1.55, 15.2)	*2.00 (1.49, 22.7)*		
Tlag (h)	Oral Dosing: 0.183 (0.0114, 0.465) NGT[e]: -71.0% (-99.3%, -22.6%)	***Oral dosing:*** *0.242 (0.0385, 0.654)* ***NGT:*** *-85.6% (-99.3%, -34.6%)*	**BOV:** 116% (44.3%, 349%)	***BOV:*** *130% (30.9%, 303%)*
ka (1/h)	1.86 (1.45, 2.85)	*1.61 (0.855, 2.78)*	**BOV:** 66.4% (54.9%, 79.5%)	***BOV:*** *64.8% (43.4%, 80.9%)*
F (-)	**Dispersible tablet:** 1 FIXED (reference) **Standard tablet:** -41.3% (-49.9%, -33.3%)	***Standard tablet:*** *0.587 FIXED*	**BOV:** 23.2% (16.5%, 29.4%)	***BOV:*** *21.8% (13.7%, 28.4%)*

Other parameters (95% CI)	Current pooled analysis	*Previous study**	Other parameters (95% CI)	Current pooled analysis	*Previous study**
Scaling of BOV in F for unobserved doses (-)[b]	4.28-fold (3.46, 6.02)	*4.48-fold (3.31, 7.08)*			
PMAGE50 (months)	10.7 (8.20, 13.0)	*10.6 (7.55, 12.9)*	**γ (-)**	3.53 (1.94, 4.99)	*3.39 (1.42, 4.98)*
Additive error (mg/ L)[c]	0.0160 FIXED (20% of LLOQ)	*0.0160 FIXED (20% of LLOQ)*	**Proportional error (%)**	12.7% (11.2%, 14.2%)	*11.6% (10.0%, 12.7%)*

[a] All clearance and volume parameters have been scaled with allometric scaling. The typical values reported here refer to a 12-kg child, aged 2 years. Age affects clearance, since maturation was used. At 2 years after birth, maturation is predicted to be 97.9% complete.

[b] This is a multiplicative factor increasing the BOV in bioavailability for pre-dose concentrations following an unobserved dose

[c] The estimate of the additive error was hitting the stipulated lower boundary (20% of LLOQ), so it was fixed to this value.

[d] The effect of HIV could not be evaluated in the children in the current study, since none was HIV-infected.

[e] The effect of nasogastric tube was only observed with standard 250 mg tablet formulation, and it does not apply to the new dispersible tablet formulation.

*These values refer to the previous study performed with standard adult 250 mg solid dosage formulation. For ease of comparison, the original value of the disposition parameters (CL, Vc, Q, and Vp) has been rescaled to the estimated bioavailability of the standard 250 mg tablet (0.587) when compared to the one used on the current study.

Values in parentheses are empirical 95% confidence intervals, obtained with a 500-sample nonparametric bootstrap.

The PK parameter variability was included either as between-subject (BSV) or between-occasion (BOV) variability, assuming a lognormal distribution. It is reported here as approximate %CV.

The estimate of the additive component of the error was not significantly different from its lower boundary of LLOQ/2, so it was fixed to this value.

CL: clearance, Vc: central volume of distribution, Q: inter-compartmental clearance, Vp: peripheral volume of distribution, Tlag: absorption lag time, ka: absorption rate constant, F: bioavailability, PK: pharmacokinetic, PMA50: Post-menstrual age at which 50% of maturation is reached, γ : shape factor for the maturation function, BSV: between-subject variability, BOV: between-occasion variability, HIV+/- HIV-infected or uninfected, NGT: drug administration with naso-gastric tube

Table 5.2. Weight-banded dosing of levofloxacin 100 mg scored dispersible tablets to approximate exposures in adults with a 750 mg dose

Weight band (kg)	Number of 100 mg tablets per dose	Daily dose (mg)	Average daily dose (mg/kg)
3 – <4	0.5	50	14.3
4 - <5	0.75	75	16.7
5 - <6	1	100	18.2
6 - <7	1.5	150	23.1
7 - <9	2	200	25
9 - <11	2.5	250	25
11 - <16	3	300	22.2
16 - <22	4	400	21.1
22 - <28	5	500	20.0
28 - <35	6	600	19.1

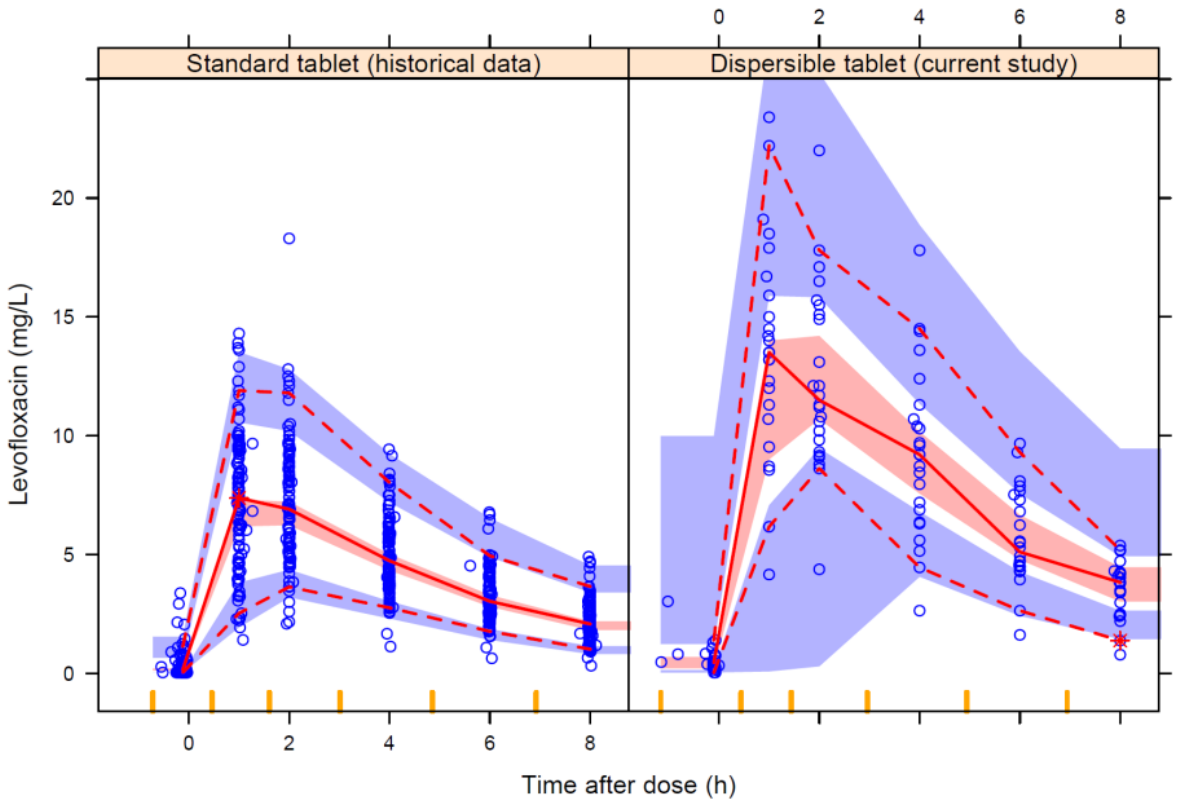

Figure 5.1. Visual predictive check of the levofloxacin concentration versus time after dose for the historical 109 controls receiving adult 250 mg standard levofloxacin tablets (Denti P, et al. 2018) (left panel) vs. the 24 patients on the pediatric 100 mg scored dispersible tablets (right panel). The solid and dashed lines represent the 50th, 5th, and 95th percentiles of the observed data, while the shaded areas represent the model-predicted 95% confidence intervals for the same percentiles. The dots are the observed concentrations.

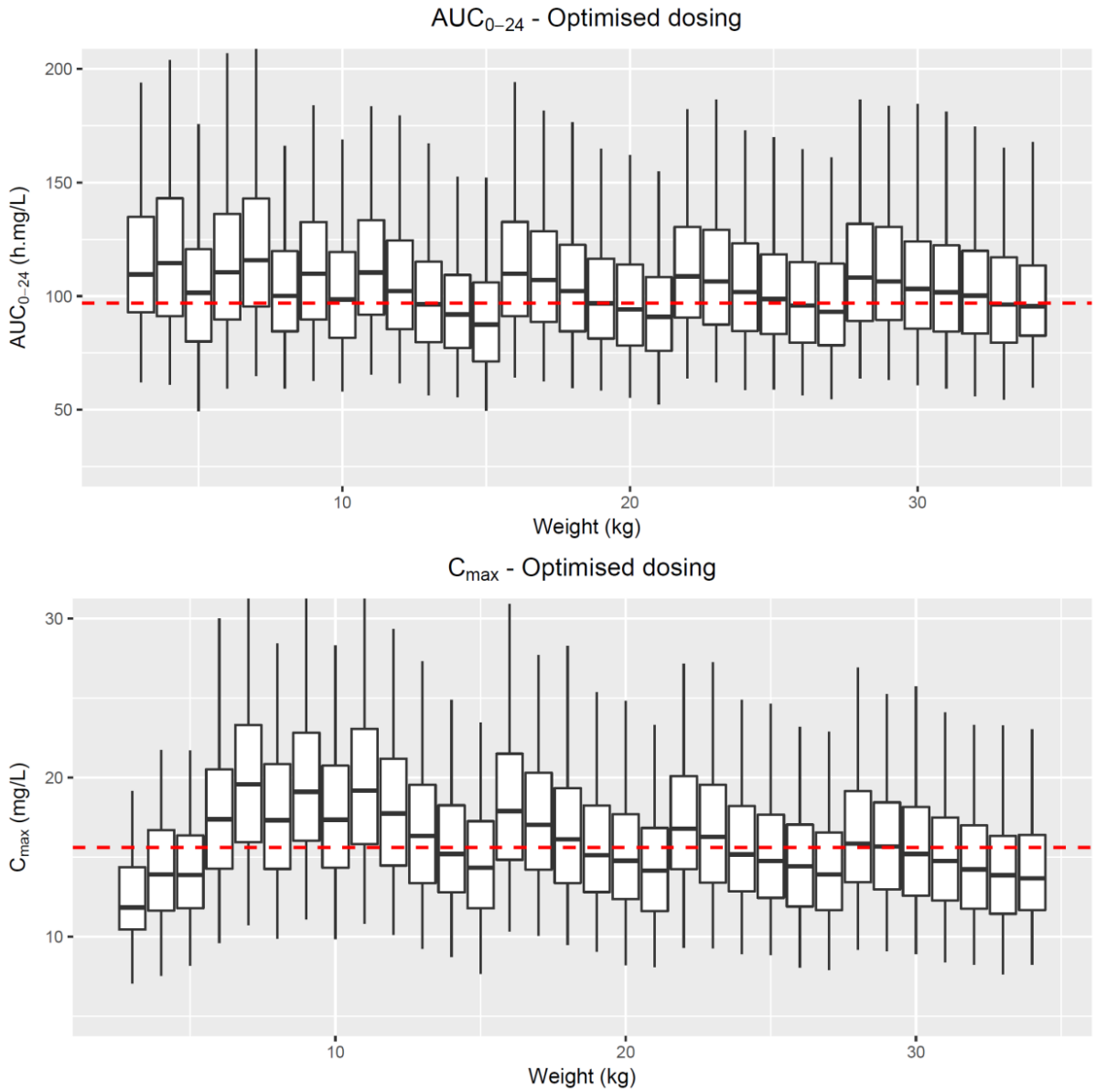

Figure 5.2. Simulated steady state levofloxacin C_{max} and $AUC_{0\text{-}24}$ versus body weight using weight-banded dosing of pediatric levofloxacin 100-mg scored dispersible tablets. The dashed line for C_{max} (15.55 mg/liter) is the median C_{max} from adults with TB receiving 1000 mg daily in Peloquin et al.[8] ; the dashed line for AUC (96.8 mg·h/liter) is the median AUC from the same study after rescaling the dose from 1000 mg to 750 mg daily.

Supplemental Table 5.1. Weight-banded dosing for levofloxacin 100 mg dispersible tablets used in TB-CHAMP lead-in pharmacokinetic study

Weight bands	# of Levofloxacin 100 mg tablets	Range of mg/kg doses within weight bands	
3.0-4.9 kg	0.5	10	17
5.0-6.9 kg	1	14	20
7.0-9.9 kg	1.5	15	21
10.0-11.9 kg	2	17	20
12.0-15.9 kg	2.5	16	21
16.0-19.9 kg	3	15	19
20.0-24.9 kg	4	16	20
25.0-29.9 kg	5	17	20

Supplemental Table 5.2. Demographic and clinical characteristics of children receiving levofloxacin 100 mg dispersible tablets in TB-CHAMP lead-in pharmacokinetic study

	Participants completing PK study (n=24)	Participants contributing safety data (n=27)
Median age at enrollment (IQR)	2.1 (1.2, 2.7)	2.1 (0.8, 2.7)
Male gender (%)	15 (63)	16 (59.3)
Ethnicity (%)		
Black	7 (29)	8 (30))
Mixed-race	17 (71)	19 (70)
Median weight in kg (IQR)	12.3 (8.6, 13.5)	12.2 (8.2, 13.3)
Median height in cm (IQR)	87.2 (74.5, 93.3)	87.0 (71.0, 92.4)
Weight-for-age Z-score <-2* (%)	3 (13)	3 (11.1)
Height/length-for-age Z-score <-2* (%)	5 (21)	5 (18.5)
HIV-infected (%)	0 (0.0)	0 (0.0)
Received levofloxacin via nasogastric tube on pharmacokinetic sampling day (%)	4 (17)	--

IQR= interquartile range; *calculated based on WHO reference values.

Supplemental Table 5.3. Summary of all adverse event among children receiving levofloxacin 100 mg dispersible tablets in TB-CHAMP lead in pharmacokinetic study (n=27)

	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)	Total N (%)
Number of patients with any Grade 1 or Grade 2 AE (%)*	**8 (28.6)**	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**8 (28.6)****
Gastrointestinal disorders (%)	**2 (7.1)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**2 (7.1)**
Constipation (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)
Diarrhoea (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)
Vomiting (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)
Metabolism and nutrition disorders (%)	**0 (0.0)**	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**1 (3.6)**
Anorexia (%)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	1 (3.6)
Respiratory, thoracic, mediastinal disorders (%)	**2 (7.1)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**2 (7.1)**
Upper respiratory tract infection (%)	2 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.1)
Skin and subcutaneous tissue disorders (%)	**4 (14.3)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**4 (14.3)**
Diaper dermatitis	2 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.1)
Rash	2 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.1)
General (%)	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**1 (3.6)**
Difficulty sleeping (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)

*The total number of patients equals 8 as one patient reported a Grade 1 and a Grade 2 AE
AE= adverse event

Supplemental Table 5.4. Summary of adverse events possibly, probably or definitely study-drug related, among children receiving levofloxacin 100 mg dispersible tablets in TB-CHAMP lead in pharmacokinetic study (n=27)

	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)	Total N (%)
Number of patients with Grade 1 or Grade 2 AE possibly, probably or definitely related to study drug (%)*	**2 (7.1)**	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**2 (7.1)****
Gastrointestinal disorders (%)	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**1 (3.6)**
Vomiting (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)
Metabolism and nutrition disorders (%)	**0 (0.0)**	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**1 (3.6)**
Anorexia (%)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	1 (3.6)
General (%)	**1 (3.6)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**1 (3.6)**
Difficulty sleeping (%)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)

*The total number of patients equals 2 as one patient reported a Grade 1 and a Grade 2 AE
AE= adverse event

Chapter 6: Linezolid for the treatment of drug-resistant tuberculosis in children: a review and recommendations

Rationale

Treatment options for MDR-TB, especially with additional resistance such as pre-XDR and XDR-TB, are limited. At the time this review was undertaken in 2013-14, the novel TB drugs bedaquiline and delamanid were still in being evaluated in adults and not yet available for use outside of clinical trials. Other existing medications were being explored as repurposed treatments for use in MDR/XDR-TB. Linezolid, an oxazolidinone antibiotic, had shown potent antimycobacterial activity *in vitro* and *in vivo* (129, 130). It was being increasingly used in adults, especially those with pre-XDR- and XDR-TB, but there were serious concerns about its safety with long-term use. Although there were children with MDR/XDR-TB who stood to benefit from linezolid treatment, there was minimal formal paediatric data or guidance about specific indications, doses, and safety monitoring.

Study Aims

The aim of this study was to systematically review the literature on the use of linezolid in adults and children for the treatment of MDR-TB in order to formulate practical paediatric guidance and to identify key questions for future research.

Methods

A structured review of the literature on linezolid for MDR-TB was undertaken. Pubmed was searched up through December 31, 2012 using a broad search strategy to identify relevant studies of the efficacy and safety of linezolid, and a second specific search for pharmacokinetic data. Key evidence on linezolid's efficacy for TB treatment, safety and pharmacokinetics was summarized. Data on children treated with linezolid for MDR-TB was pooled in order to characterize the state of knowledge on its efficacy and safety for this indication. Based on an overall assessment of the data from the review, practical recommendations were made for the use of linezolid in MDR-TB and key questions for future research identified.

Results

Multiple studies were identified that evaluated linezolid efficacy against *M. tuberculosis* in different *in vitro* and *in vivo* conditions. A number of key publications were identified demonstrating the efficacy of linezolid for DR-TB. Linezolid had a modest EBA_{0-2}, of 0.26 and 0.18 for 600 mg twice and once daily respectively, compared to 0.67 for isoniazid 300 mg (131). Two systematic reviews primarily including observation data from adults routinely treated with linezolid for MDR- or XDR-TB reported successful outcomes in 68% and 82% of patients (107, 132), which suggested efficacy of linezolid in these difficult to treat patients. A single clinical trial in highly treatment experienced adults with chronic MDR-TB who were randomized to immediate or delayed (after 2 months) linezolid along with an optimized background regimen reported culture conversion at 4 months of 79% in the immediate group vs. 35% in the delayed group; a remarkable 87% of patients had culture converted by 6 months (97). In all of these studies, frequent serious and treatment limiting dose and duration-dependent adverse effects were report. Roughly 60% of those included in each of the above systematic reviews experienced a linezolid-associated adverse effect, with the most common being anaemia and neuropathy (96, 107). In one of these reviews, 69% of events required linezolid discontinuation or dose adjustment (96). Three studies of linezolid pharmacokinetics in adults with TB were identified, which could provide target exposures for paediatric dose selection (133-135). There were data on linezolid pharmacokinetics in children, but none in children with TB (103). There was limited published experience with linezolid use in children with MDR-TB. Eight reports including 18 children were identified (136). Outcomes were good, with 15 of 18 (83%) having successful treatment outcomes despite many with prolonged previous culture positivity and extensive resistance. Nine of the 18 (50%) had a reported linezolid-related adverse event, with 5 (28%) requiring a dose-reduction and 2 (11%) permanently discontinuing linezolid. Linezolid should be used in children with XDR-TB, or who are failing MDR-TB treatment, and should be considered in those with MDR-TB meningitis. Linezolid doses of 10 mg/kg twice daily for those <10 years of age and once daily for those >10 years of age should be considered for MDR-TB treatment in children until more data is available. Careful monitoring for adverse effects, in particular anaemia and peripheral neuropathy, is critically important.

Conclusions and recommendations

Linezolid appears to be an effective antituberculosis drug and an important treatment for MDR-TB considering the limited options, however it is associated with frequent, serious dose and duration dependent adverse effects. Based on this evidence review, practical recommendations for linezolid use in children with MDR-TB were made and future questions for research identified, including: identifying the optimal dose of linezolid in adults with TB (that balances efficacy and safety); characterizing the pharmacokinetics of linezolid in children with TB and its optimal paediatric dose; describing the safety of long-term linezolid in children with MDR-TB.

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REVIEW

Linezolid for the treatment of drug-resistant tuberculosis in children: A review and recommendations

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SUMMARY

Options for the treatment of children with drug-resistant tuberculosis (DR-TB) are limited. Emerging evidence in adults from systematic reviews and a randomized trial has shown good efficacy of linezolid in difficult cases of DR-TB but with frequent serious adverse effects. Published data in children are limited and we are unaware of formal guidelines for linezolid in treatment of paediatric DR-TB, though it will likely be an important component of DR-TB treatment for a growing number of children. We performed a structured review of existing literature on the efficacy, adverse effects, pharmacokinetics and pharmacodynamics of linezolid in DR-TB, highlighting the key evidence from the adult literature and systematically evaluating published paediatric data. Our search identified 8 reports of 18 children receiving linezolid for difficult to treat DR-TB. All 18 had culture conversion and 15 of 18 had successful long-term treatment outcomes. Adverse events were reported in 9 of 18; a linezolid dose reduction was required in 5 of 18, and 2 of 18 permanently discontinued linezolid because of adverse events. We make specific recommendations for the use of linezolid in children with DR-TB, and identify priority questions for future research. For children with multidrug-resistant (MDR)-TB with additional resistance or with extensively drug-resistant (XDR)-TB, linezolid may make the difference between a successful or poor outcome, and until newer antituberculosis agents with better efficacy and safety become available in children, linezolid will be an important component of treatment for children with the worst forms of DR-TB.

1. Introduction

Children account for an estimated 10–15% of the global burden of disease caused by *Mycobacterium tuberculosis* (*Mtb*) with conservative estimates of 490,000 reported cases and 64,000 deaths among HIV-negative children in 2011 [1,2]. Multidrug-resistant tuberculosis [MDR-TB; i.e. *Mtb* resistant to at least both rifampicin (R; RMP) and isoniazid (H; INH)] is increasing worldwide, with an estimated 630,000 prevalent cases in 2011 [2]. There is a growing recognition of the importance of drug-resistant TB (DR-TB) in younger ages. A recent systematic review identified 8 cohorts with 318 children with MDR-TB and reported a pooled estimate for treatment success of 81.7% [3]. Extensively drug-resistant TB (XDR-TB; i.e. resistance to isoniazid, rifampicin, a fluoroquinolone, and one of the second-line injectable drugs) has been identified in 84 countries and accounts for 9.0% of MDR-TB cases globally [2,4]. A systematic review reported successful outcomes in only 43.7% of adults with XDR-TB [5]. There is little published data or guidance on best management of children with XDR-TB.

The World Health Organization (WHO) categorizes linezolid in Group 5, an antituberculosis agent with unclear efficacy or concerns regarding usage [6]. There is an increased interest in linezolid for DR-TB treatment, especially in XDR-TB, and recent systematic reviews and a randomized controlled trial have added substantially to the adult literature. There is little published data about linezolid use in children with DR-TB, though it will likely be an important component of DR-TB treatment for a growing number of children given the lack of availability of new TB drugs in children. This paper reviews the existing knowledge about the efficacy, adverse effects, pharmacokinetics and pharmacodynamics of linezolid in DR-TB, highlighting the key evidence from the adult literature and systematically evaluating published paediatric data. We also make

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Table 1
Minimum inhibitory concentrations (MICs) (in μg/ml)*, epidemiologic cut-off (ECOFF), and proposed critical concentrations (in μg/ml) for linezolid against *Mycobacterium tuberculosis*.

	Mtb strains	Middlebrook 7H10	Middlebrook 7H11	Bactec460	MGIT960
Published reports of MICs					
Zurenko GE et al., 1996 [20]	Clinical isolates, DS	0.5†	–	–	–
	Clinical isolates, DR	0.5–2.0‡	–	–	–
Rodriguez JC et al., 2002 [19]	Clinical isolates, mostly DS	–	0.5, 1.0	–	–
Alcala L et al., 2003 [21]	Clinical isolates, DS and DR	0.5, 1.0	–	–	–
Erturan Z et al., 2005 [22]	Clinical isolates, DR	–	–	4.0, 8.0	–
Sood R et al., 2005 [23]	Clinical isolates, DR	–	1.0, 32.0	–	–
Tato M et al., 2006 [24]	Clinical isolates, DS and DR	0.25, 0.5	–	–	–
Yang C et al., 2011 [26]	Clinical isolates, DS and DR	0.125, 0.5	–	–	–
Epidemiologic cut-off					
Schon T et al., 2011 [30]	–	–	0.5	–	–
Proposed critical concentrations					
Rusch-Gerdes S et al., 2006 [25]	–	–	–	1.0	1.0
WHO 2008 [28]	–	–	–	1.0	1.0
WHO 2012 [29]	–	–	–	–	1.0

Mtb = *Mycobacterium tuberculosis*; DS = drug-susceptible; DR = drug-resistant, to at least isoniazid or rifampicin, or both; WHO = World Health Organization; MGIT = Mycobacterial Growth Indicator Tubes.
* Expressed as MIC_{50}, MIC_{90}, respectively, unless otherwise specified.
† Inhibited all strains.
‡ Range of MICs.

recommendations for the use of linezolid in children with DR-TB, and identify specific questions for future study.

2. Methods

2.1. Structure of review

Because the evaluation of antituberculosis drug efficacy relies on microbiologic endpoints, which are challenging to evaluate in children, there are few trials of antituberculosis drug efficacy in children with TB disease, but no reason to presume that agents efficacious in adults will not also be in children, who typically have paucibacillary TB [7,8]. Clinicians managing children with DR-TB should be aware of the adult literature for drug efficacy. There may be considerable age-related variation in severity and frequency of adverse effects of drugs, so the safety profile of drugs should be specifically evaluated in children [7–9]. We summarize the key evidence on the efficacy and safety of linezolid in adults receiving the prolonged courses of linezolid used in DR-TB treatment, and report all the paediatric experience identified in our search.

The pharmacokinetics of many antituberculosis drugs differ in children and adults [10–12] due mostly to age-related changes in drug absorption, distribution, metabolism, and excretion [9,13,14]. Paediatric antituberculosis drug doses should be used that result in the same drug exposure as that of efficacious recommended doses in adults [7,9]. We therefore also present studies of the pharmacokinetics of linezolid in adults with TB, and in children with TB or other conditions.

2.2. Search

We searched Pubmed through December 31, 2012, using a broad search strategy and a second specific search for pharmacokinetic information, using the terms described in Supplemental Table 1. We also reviewed the bibliographies of key articles and reviews, and surveyed experts in the field. We systematically extracted information on the outcomes and adverse effects for all children treated with linezolid for DR-TB. We used key articles from our search to describe the efficacy, adverse effects, and pharmacokinetics of linezolid for DR-TB treatment in adults and children.

3. Overview

Linezolid belongs to the oxazolidinone class of antibiotics, which bind to the 50S ribosomal subunit, inhibiting formation of the initiation complex and preventing translation and protein synthesis [15–17]. This novel mechanism of action limits cross-resistance with other protein-synthesis inhibitors and makes it attractive for drug-resistant infections [15]. Linezolid has been approved by the U.S. Food and Drug Administration for treatment of susceptible strains of some microorganisms, most commonly resistant Gram-positive bacteria, for nosocomial pneumonia, and for skin and skin structure infections, but is used off-label for drug-resistant TB [18]. Patent coverage of linezolid in the U.S. and other countries, along with a lack of quality-assured alternative producers, has resulted in prohibitively high costs of linezolid in many settings [18]. Linezolid is available as 600 mg tablets and as 100 mg/5 ml powder for suspension.

4. Efficacy of linezolid against *M. tuberculosis*

4.1. Activity in vitro and in animals

The *in vitro* activity of linezolid against *Mtb* has been consistently demonstrated, and minimum inhibitory concentrations (MIC) from published studies are listed in Table 1 [19–26]. The critical concentration of a drug is defined as the 'lowest concentration of drug that will inhibit 95% of wild strains of *M. tuberculosis* that have never been exposed to drugs, while at the same time not inhibiting clinical strains of *M. tuberculosis* that are considered to be resistant' [6,27], and the epidemiological cut-off (ECOFF) is defined as the highest MIC among the wild-type MIC distribution [27]. Currently proposed [25] and WHO recommended critical concentrations [28,29], and a proposed ECOFF for linezolid are listed in Table 1 [30]. A single study showed a trend towards higher linezolid MICs in MDR isolates over 10 years despite a lack of linezolid exposure, which was associated with resistance to the fluoroquinolones (except levofloxacin) and to kanamycin; the explanation for these findings is not clear [31]. Using a test concentration of 6 μg/ml linezolid on 295 MDR clinical isolates including 9 which were XDR, only 2 isolates were found to be resistant [32]; however the clinical relevance of that breakpoint is not clear.

In a study assessing *in vitro* combinations of drugs against *Mtb*, linezolid showed synergistic activity with rifampicin but not the fluoroquinolones [33]. Linezolid had intracellular activity against *Mtb* in a murine macrophage model [23], but against non-replicating *Mtb* in a latent growth phase only the highest concentrations showed any bactericidal activity, suggesting limited sterilizing ability [34].

In one of the first *in vivo* evaluations, linezolid showed dose-dependent activity in a murine model of *Mtb*, based on lung and spleen colony forming units (CFUs) in comparison to untreated controls [35]. Subsequent studies in mice were less encouraging, showing limited bactericidal activity at doses approximating the clinically relevant exposure in humans [36], antagonistic activity when it was added to isoniazid, rifampicin, and pyrazinamide, [37] and no increased activity of linezolid and moxifloxacin over moxifloxacin alone [38]. We found no reports evaluating the combination of linezolid with pyrazinamide, although the related compound PNU-100480 showed augmented activity when combined with pyrazinamide [39].

4.2. Activity in adults

A single study reported a modest early bactericidal activity (EBA) for linezolid at doses of 600 mg once and twice daily [40]. The EBA for days 0–2 (EBA_{0-2}) was 0.26 for linezolid 600 mg twice daily and 0.18 for 600 mg once daily, compared to 0.67 for INH 300 mg [40]. The values for the extended EBA for days 2–7 (EBA_{2-7}) were 0.09 for twice daily and 0.04 for once daily linezolid, and 0.16 for isoniazid [40]. The differences in EBA of linezolid 600 mg once and 600 mg twice daily were small and not statistically significant [40]. There was no correlation between area under the concentration time curve (AUC)/MIC or %T > MIC with linezolid EBA in this study, which may be related to the relatively favourable pharmacodynamics at both doses [40]. These data provide some evidence for the effectiveness of once daily dosing, though the small sample size limited the ability to detect small differences. The low EBA_{2-7} may suggest minimal sterilizing activity, though this is an imperfect marker of sterilizing activity, and pyrazinamide, which is known to have potent sterilizing activity, also has a limited EBA_{2-7}.

In one of the first clinical studies of linezolid in DR-TB, three adults with MDR-TB and resistance to other second-line agents had successful outcomes with linezolid use [41]. Multiple other small case series and observational studies reported similar results, with good outcomes in patients with substantial drug resistance and limited treatment options, but with frequent adverse effects [42–56]. These and other reports were synthesized in two systematic reviews published in 2012 evaluating the safety and efficacy of linezolid for DR-TB in adults [57,58]. The first included 11 studies representing 148 patients [57]. The pooled percentage of patients with treatment success was 68.0% (95% CI 58.0–79.0) and culture conversion was 97.9% (95% CI 95.2–100%) [57]. There was no significant difference in pooled treatment success in studies with a mean duration of treatment >7 months versus ≤7 months, or for studies that used >600 mg daily versus ≤600 mg daily [57]. The second systematic review included 207 patients in 12 studies, including many but not all the same studies as the first review, and reported similar findings [58]. Of 121 patients with definite treatment outcomes, 82% (95% CI 74–88%) had successful treatment outcomes, with 93% (95% CI 87–97%) having culture conversion [58]. A subgroup analysis found no significant differences in outcomes between those receiving ≤600 mg daily versus >600 mg [58].

A single clinical trial evaluated linezolid in 39 highly treatment-experienced patients with chronic XDR-TB in which patients were randomized to immediate versus delayed addition of linezolid to their existing failed background regimen [59]. By 4 months, 79% in the immediate group compared to 35% in the delayed group had culture conversion ($p = 0.001$), and by 6 months 87% of all the patients had culture converted [59]. At the time of study publication, 8/38 patients had withdrawn from the study due to treatment failure ($n = 4$), personal reasons ($n = 1$), and adverse events ($n = 3$), while 17/38 were still receiving the study treatment [59]. Thirteen had successfully completed treatment with no relapse to date, suggesting sterilizing potential for linezolid [59]. Although the numbers are small, these results are much better than existing reported outcomes for XDR-TB and provide evidence for linezolid efficacy in these patients [59]. Of the 4 patients who did not have culture conversion, all acquired linezolid resistance, with increased MICs by a factor of 8–32 from baseline and known mutations identified by gene sequencing [59]. This demonstrates that resistance can emerge during treatment, despite a low mutant-prevention concentration ($MPC_{90} = 1.2$ μg/ml) comparable to that of moxifloxacin [60] and *in vitro* evidence that it is difficult to induce linezolid resistance in *Mtb* [34].

Only 5% [57] and 8.7% [58] of patients were HIV-infected in the two systematic reviews, and HIV infection was an exclusion criteria in the above clinical trial [59], so caution should be taken in extrapolating these results to HIV-infected persons.

4.3. Activity in children

There is substantial evidence of the effectiveness of short courses (less than 28 days) of linezolid in children for complicated bacterial skin and soft tissue infections, nosocomial and community-acquired pneumonia, and resistant Gram-positive infections, including four clinical trials [61]. Experience with linezolid in children with DR-TB is limited, and our search identified 8 reports including 18 children [one patient was included in two reports [62,63]] treated with linezolid for DR-TB [43,54,62–67], with results summarized in Table 2. All 18 patients had culture conversion, most within 1–3 months, and 15 of 18 (83%) had a successful long-term outcome, with 1 lost-to-follow-up and 2 deaths. The deaths were due to respiratory failure in one, and Stage 3 tuberculous meningitis and liver failure in a second, and both patients were culture-negative at the time of death [43,67]. In many of these patients, the good outcomes were despite extensive disease, substantial drug resistance, and prolonged culture positivity and failed treatment with other second-line drugs prior to linezolid use for periods as long as 9 months [54], 7 months [62], and 6–12 months [63].

Despite the small numbers and all patients were identified from case reports or small series, the outcomes described in children on linezolid are good. The proportions of children with culture conversion and successful treatment are similar to those reported for adults. This provides some evidence for the utility of linezolid in children with DR-TB, including those with XDR-TB.

4.4. Safety and tolerability

Although well tolerated in short courses, linezolid is associated with important dose- and time-dependent adverse effects [68,69]. In general, adverse effects are reported less in linezolid treated children than adults [61,70]. Inhibition of mitochondrial protein synthesis by linezolid may be the cause of many of these adverse effects [68].

4.5. Gastrointestinal adverse effects

Gastrointestinal adverse effects are commonly associated with linezolid, but rarely require alteration or discontinuation of the

Table 2
Demographics and treatment outcomes for children (<18 years) treated with linezolid for drug-resistant tuberculosis.

Published report	Age (yrs) and gender	HIV	TB resistance profile	Dose and duration of linezolid treatment	Culture conversion	Treatment outcome
Park IN et al., 2006 [43]	17 F	Neg	H, R, E, CS, KM, OFX, PAS, PTH	600 mg OD, 8 months	Yes, 147 days	Death (respiratory failure)
Condos R et al., 2008 [54]	10 F	Pos	H, R, E, Z, S, CIP, AM, AUG, RB, PAS, CAP	600 mg OD, 25 months	Yes, 29 days	Successful
Schaaf HS et al., 2009 [62] and Rose PC et al., 2012 [63]	0.9 F	Neg	H, R, E, OFX, AM	10–12 mg/kg BD, 19 months	Yes, 23 days	Successful
Pinon M et al., 2010 [64]	1.9 F	Neg†	(H, R, E, Z, S, KM)*	10 mg/kg BD, 13 months	Yes, 1 month	Successful
	0.9 M	–	(H, R, E, Z, S, ETH, PAS, CS)*	10 mg/kg BD, 3 months	Yes, 2 months	Lost-to-follow-up
Dauby N et al., 2011 [65]	14 F	Neg	H, R, RB, E, OFX, Z, AM, CS, PTH	600 mg OD, 8 months	Yes, 11 weeks	Successful
Kjollerstrom P et al., 2011 [66]	14 M	Neg	H, R, Z, E, S, RB, ETH, CAP, AM	600 mg BD, 9 months	Yes, 12 weeks	Successful
	12 F	Neg	H, R, Z, S, RB, ETH, CS, PAS, KM, OFX	600 mg BD, 4 months; 300 mg OD 2 months	Yes, 6 weeks	Successful
	4 F	Neg	H, R, S, ETH	10 mg/kg BD, 1 month; half dose for 5 months	Yes, 12 weeks	Successful
	17 M	Pos	H, R, Z, E, S	600 mg BD, 11 months	Yes, 12 weeks	Successful
Rose PC et al., 2012 [63]	13 M	Neg	H, R, AM	300 mg OD, 23 months	Yes, 3 months	Successful
	10 M	Pos	H, R, E, AM, OFX	300 mg OD, 20 months	Yes, 4 months	Successful
	13 F	Neg	H, R, E, AM, ETH, OFX	300 mg OD, 15 months	Yes, 2.5 months	Successful
	0.6 M	Neg	H, R, E, AM, OFX	10 mg/kg BD, 15 months‡	Yes, 3 months	Successful
	10 F	Pos	H, R, E, ETH, KM, S	300 mg BD, 24 months; 200 mg BD, 3 months‡	Yes, 18 months	Successful
	5 F	Pos	H, R, E, KM, S, OFX	300 mg OD, 7 months	NA (negative prior to linezolid)	Successful
Katragkou A et al., 2013 [67]	2.5 F	Neg	H, R, E, Z, LFX, AM, CAP	10 mg/kg TD, 7 months, 7 mg/kg TD 3 months‡	NA (negative prior to linezolid)†	Successful
	1.5 M	Neg†	H, R, Z, E, AM	10 mg/kg TD, 6 months	Yes, 1 month	Death (Stage 3 TBM, liver failure)

H = isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide, ETH = ethionamide, PTH = prothionamide, PAS = para-aminosalicylic acid, KM = kanamycin, AM = amikacin, CAP = capreomycin, OFX = ofloxacin, LFX = levofloxacin, CIP = ciprofloxacin, RB = rifabutin, AUG = augmentin, CS = cycloserine, OD = once daily, BD = twice daily; TD = thrice daily; F = female; M = male; NA = not applicable; TBM = tuberculous meningitis.

* Resistance profile of source case reported.

† not in original publication, but provided by authors.

‡ Treatment ongoing at time of report.

Table 3
Adverse events among children (<18 years) treated with linezolid for drug-resistant tuberculosis.

Published report	Age (yrs) and gender	HIV	Dose and duration of linezolid treatment	Adverse event/s	Action and outcome
Park IN et al., 2006 [43]	17 F	Neg	600 mg OD, 8 months	None	
Condos R et al., 2008 [54]	10 F	Pos	600 mg OD 25 months	None	
Schaaf HS et al., 2009 [62] and Rose PC et al., 2012 [63]	0.9 F	Neg	10–12 mg/kg BD, 19 months	None	
Pinon M et al., 2010 [64]	1.9 F	Neg†	10 mg/kg BD, 13 months	None	
	0.9 M	–	10 mg/kg BD, 3 months	None	
Dauby N et al., 2011 [65]	14 F	Neg	600 mg OD, 4 months, 300 mg OD, 4 months	Moderate peripheral neuropathy after 4 months	Improved with dose reduction to 300 mg once daily
Kjollerstrom P et al., 2011 [66]	14 M	Neg	600 mg BD, 9 months	Severe progressive peripheral neuropathy after 9 months	Completely resolved after discontinuation of linezolid
	12 F	Neg	600 mg BD, 4 months; 300 mg OD 2 months	Peripheral neuropathy after 4 months	Responded to dose reduction to 300 mg once daily
				Severe anaemia requiring transfusion	Anaemia attributed to linezolid and comorbid sickle cell disease; linezolid continued
	4 F	Neg	10 mg/kg BD, 1 month; half dose for 5 months	Urticarial rash	Attributed to linezolid hypersensitivity; resolved after dose reduced to half
	17 M	Pos	600 mg BD, 11 months	None	
Rose PC et al., 2012 [63]	13 M	Neg	300 mg OD, 23 months	None	
	10 M	Pos	300 mg OD, 20 months	Pancreatitis at 8 months	Attributed to d4T, anticonvulsant, high-fat diet, and possibly linezolid; linezolid continued
	13 F	Neg	300 mg OD, 15 months	None	
	0.6 M	Neg	10 mg/kg BD, 15 months*	None	
	10 F	Pos	300 mg BD, 24 months, 200 mg BD, 3 months*	Peripheral neuropathy at 24 months	Linezolid dose reduced, d4T changed to ABC, terizidone dose reduced, pyridoxine increased; symptoms resolved
				Mild anaemia and leukopaenia at 25 months,	Anaemia, leukopaenia attributed to HIV
	5 F	Pos	300 mg once daily, 7 months	Severe pancreatitis and lactic acidosis requiring ICU admission at 7 months	Attributed to linezolid which was discontinued, fully recovered
Katragkou A et al. (2013) [67]	2.5 F	Neg	10 mg/kg TD, 7 months, 7 mg/kg TD 3mnths*	Mild neutropaenia after 7 m of linezolid	Attributed to linezolid, but did not improve after reduction of linezolid dose
	1.5 M	Neg†	10 mg/kg TD, 6 months	Liver failure, resulting in death	Cause unknown, unlikely related to linezolid

OD = once daily, BD = twice daily; TD = thrice daily; F = female; M = male; NA = not applicable.
* Treatment ongoing at time of report.
† Not in original publication, but provided by authors.

drug [69]. In phase III clinical trials in adults, the most common drug-related adverse events were nausea (3.4%) and diarrhoea (4.3%) [69]. In a review of clinical trials of short durations of linezolid in children, diarrhoea (3.8–9.1%) and vomiting (1.2–4.2%) were the most common adverse effects, though there was no difference in frequency between linezolid and the comparators (cefadroxil and vancomycin) [71].

4.6. Hematologic adverse effects

Both dose and time-dependent myelosuppression were noted in pre-clinical evaluations of linezolid in animals [69]. A review of adult clinical trial data of linezolid courses <28 days showed no statistical difference in haematologic adverse effects between linezolid and comparator groups, although there was a trend towards increased mild anaemia and thrombocytopaenia in the linezolid group for those treated for more than 2 weeks [69,72]. Anaemia is more frequent in longer courses of linezolid, thought to be related to a bone marrow suppression due to inhibition of mitochondrial protein synthesis [69]. Studies have been variable in adults, but suggest a slight risk of thrombocytopaenia that is increased with longer duration of linezolid, but reversible with drug cessation [69]. The exact mechanism of thrombocytopaenia is unknown, but an immune-mediated phenomenon has been proposed [69]. Reversible leukopaenia and pancytopaenia have been described but are rare [69]. A single report of two adult patients suggested that linezolid-associated cytopaenias may respond to vitamin B6 (pyridoxine) supplementation [73], but was followed by other observational studies in adults which showed no effect of pyridoxine 125 mg daily [74] or 200 mg daily [75] on the risk of anaemia or thrombocytopaenia. Pyridoxine supplementation would not be expected to impact on the proposed mechanisms for cytopaenias described above. The risk of cytopaenias with prolonged linezolid treatment in DR-TB is discussed below.

Paediatric data from clinical trials of short courses of linezolid showed a trend towards mild reversible thrombocytopaenia in children treated >14 days but no statistical difference in hematologic adverse events between the linezolid and comparator groups [76].

4.7. Neurologic adverse effects

Peripheral neuropathy was not noted in clinical trials of linezolid, but has been well described during prolonged courses [69,77]. It usually presents as paraesthesia and numbness in distal extremities in a "stocking and glove" distribution, with lower extremities affected more commonly than upper [69]. In adults, peripheral neuropathy is not responsive to vitamin B6 [73], and is usually not reversible, but may improve slowly in some cases after linezolid discontinuation [69,77]. Linezolid also causes toxic optic neuropathy, with painless, bilateral central vision loss, often of sudden onset, and gradual progressive loss of colour vision and visual acuity [69]. Onset of symptoms is from 3 to 12 months, and existing evidence suggests optic neuropathy will improve with discontinuation of linezolid, but can result in permanent visual deficits [69]. The risk of neuropathy with prolonged treatment is discussed below.

In addition to the cases of peripheral neuropathy among linezolid-treated children with DR-TB described below, a recent review identified 8 cases of neuropathy in children − 5 with peripheral neuropathy alone, 1 with optic neuropathy, and 2 with both peripheral and optic neuropathy [78]. Seven of 8 were on prolonged courses with a range of 4 weeks to 7 months at the time of onset [78]. As opposed to adults, 5 of 5 in which the outcome was reported had improvement or resolution of symptoms after discontinuation of linezolid [78]. A single case of possible auditory nerve neuropathy has been described in a neonate [79].

There is little information on the impact of co-treatment of linezolid with isoniazid or cycloserine/terizidone on peripheral neuropathy. High-dose isoniazid causes neuropathy due to Vitamin B6 depletion, and pyridoxine supplementation greatly reduces this risk [80]. Cycloserine and terizidone may also cause peripheral neuropathy by a Vitamin B6 related mechanism, though this is controversial [81,82]. Even though the likely mechanism of linezolid-induced neuropathy by mitochondrial protein synthesis inhibition is distinct from that of isoniazid or cyloserine and terizidone, close monitoring of co-treated patients is warranted. The nucleoside reverse transcriptase inhibitor (NRTI) class of antiretrovirals (ARVs) also causes peripheral neuropathy by mitochondrial protein synthesis inhibition [83] and there is a potential for increased risk of neuropathy when used concomitantly with linezolid in HIV-infected children, but little data reported to date. With the exception of symptomatic management, the lack of effective treatments for ARV-induced neuropathy makes it less likely that pyridoxine or other existing medications will be effective for linezolid-induced neuropathy [84]. Additional evidence is needed and close monitoring of such patients is indicated.

4.7.1. Other

Linezolid-associated hyperlactatemia and lactic acidosis have been described, with a 2009 review identifying 9 adult cases [69]. Patients may be asymptomatic or have non-specific symptoms, with nausea and vomiting commonly reported [69]. Hyperlactatemia resolves over the course of 1–2 weeks after linezolid discontinuation [69]. Metabolic acidosis was reported in 2 of 79 (2.5%) children receiving linezolid in a randomized trial, though both had other comorbidities [85]. Three additional cases were described in children with liver disease and other comorbid illnesses [86], and more recently a case was described in an HIV-infected child receiving ARVs and long-term linezolid for DR-TB [63].

Rhabdomyolysis has been reported in an adult on linezolid for DR-TB [87]. Linezolid is a weak monoamine oxidase inhibitor (MAOI), and in combination with other drugs such as selective serotonin reuptake inhibitors (SSRIs) may rarely precipitate serotonin syndrome [69]. A single suspected case has been described in a child [88].

4.8. Adverse events in DR-TB treatment regimens

In the first systematic review of linezolid for DR-TB, the pooled percentage of adverse events was 61.5% (95% CI 40.2–80.8%), with pooled percentages of neuropathy of 36.1% (95%CI 19.1–53.2) and bone marrow suppression of 28.5% (95%CI 14.8–42.1), and with 36.2% (95%CI 20.7–51.8) stopping linezolid because of adverse events [57]. There was a trend towards increased risk of adverse events for linezolid doses >600 mg [49.9% (37.3–62.4)] versus ≤600 mg [34.4% (95%CI 23.0–45.8)] ($p = 0.07$), and a statistically significant difference in those discontinuing linezolid because of adverse events for doses >600 mg [60.8% (95%CI 42.7–78.8)] versus ≤600 mg [29.5% (95%CI 3.2–55.7)] ($p = 0.05$) [57].

In the second systematic review, 59% (95% CI 49–68%) had an adverse event, of which 69% (95% CI 58–79%) required linezolid discontinuation or dose adjustment [58]. The most common adverse events were anaemia (38.1%), peripheral neuropathy (47.1%), gastrointestinal disorder (16.7%), optic neuritis (13.2%), and thrombocytopaenia (11.8%) [58]. There was a statistically increased risk of adverse events for those receiving >600 mg daily (74.5%) versus those receiving ≤600 mg daily (46.7%) [58]. The higher dose was also associated with statistically increased risk of some specific adverse events, including anaemia (60% vs. 2.5%), leukopaenia (17.1% vs. 2.0%), and gastrointestinal symptoms (29.4% vs. 8.0%) despite a much shorter duration of treatment in the higher dose group [58]. In the clinical trial of linezolid for chronic XDR-TB, 33 of 38 (87%) of the patients had a clinically significant adverse event, of which 31 were possibly or probably related to linezolid [59]. After a second randomization in this study to continuation with 300 mg versus 600 mg linezolid, the 600 mg group was 2.7 times (95% CI 1.1–6.5) more likely to experience an adverse event compared to the 300 mg group, though adverse events were still common in the 300 mg group [59]. The lack of HIV-infected persons in these studies makes extrapolation of these results to this important subgroup difficult.

Table 3 lists the adverse events among published reports of children on linezolid for DR-TB. At least one adverse event was reported for 9 of 18 children (50%) with 5 of 18 (28%) requiring a linezolid dose reduction, and 2 of 18 (11%) permanently discontinuing linezolid. Peripheral neuropathy was the most common, occurring in 4 of 18 (22%), but was reported to resolve after dose reduction or discontinuation of linezolid in each case. The association of linezolid with anaemia reported in 2 of 18 (11%) is unclear, as one episode was attributed to linezolid and a vaso-occlusive crisis in a child with comorbid sickle cell disease, and in the second a bone marrow biopsy showed dyserythropoeisis possibly due to HIV. The single life-threatening adverse event was a case of severe pancreatitis and lactic acidosis [63]. Three of 5 (60%) known HIV-infected children experienced adverse events, compared to 5 of 12 (42%) known HIV-uninfected. In our limited personal clinical experience, 3/3 HIV-infected children had adverse events but 0/4 HIV-uninfected children [63]. As the NRTI class of ARVs also can inhibit mitochondrial DNA, there is a theoretical basis for increased risk of toxicity in HIV-infected persons taking NRTIs [89]. These numbers are too small to draw any robust conclusions about different risk between the two groups, but very close monitoring of

Table 4
Results of pharmacokinetic studies of linezolid in adults with tuberculosis, and children (concentrations in μg/mL, area under the concentration time curve (AUC) in μg h/mL, time in h).

Study	Methods	Age (in years or specified)	*N*	Dosage	T_{max}	*t*1/2	C_{max}	C_{min}	AUC 0–24
Adults with tuberculosis (none known HIV-infected)									
Dietze R et al., 2008 [40] *	HPLC	45.0 (39.0–48.0)	9	600 mg twice daily	1.0 (1.0–4.0)	4.56 (2.1–7.0)	19.4 (11.8–24.9)	–	232.9 (100.8–394.4)
		33.5 (23.0–42.0)	10	600 mg once daily	1.5 (1.0–4.0)	3.20 (1.5–5.0)	15.0 (11.9–21.3)	–	96.9 (47.8–143.7)
Koh WJ et al., 2009 [44] †	HPLC	–	10	300 mg once daily	–	–	11.6 (4.4)	2.1 (1.3)	–
Alffenaar JW et al., 2010 [99] *	LCMS/MS assay	28 (26–38)*	8	300 mg twice daily	1.2 (0.5–1.2)	5.6 (3.0–6.4)	9.5 (7.7–10.1)	1.9 (0.6–2.2)	115.2 (77.0–128.4)¶
			8	600 mg twice daily	1.4 (0.8–1.4)	5.8 (4.7–6.0)	20.4 (16.3–21.9)	5.8 (2.7–6.8)	291.6 (202.4–321.8)¶
Children without tuberculosis (none known HIV-infected)									
Jungbluth GL et al., 2003 [91] †	HPLC	Newborn, preterm,§ <1 week of age	9	10 mg/kg	–	5.6 (2.4–9.8)	12.7 (9.6–22.2)	–	108 (41–191)‡
		Newborn, full term, <1 week of age	10	10 mg/kg	–	3.0 (1.3–6.1)	11.5 (8.0–18.3)	–	55 (19–103)‡
		Newborn, full term ≥1week ≤28 days	10	10 mg/kg	–	1.5 (1.2–1.9)	12.9 (7.7021.6)	–	34 (23–50)‡
		Infants >28 days to <3 months	12	10 mg/kg	–	1.8 (1.2–2.8)	11.0 (7.2–18.0)	–	33 (17–48)‡
		Young children, 3 months to 11 years	59	10 mg/kg	–	2.9 (0.9–8.0)	15.1 (6.8–36.7)	–	58 (19–153)‡
		Adolescents 12–17 years	36	10 mg/kg or 600 mg	–	4.1 (1.3–8.1)	16.7 (9.9–28.9)	–	95 (32–178)‡

T_{max} = Time to reach maximum concentration; $t_{1/2}$ = elimination half-life; C_{max} = maximum serum concentration; C_{min} = minimum serum concentration; AUC = area under the concentration–time curve; HPLC = high-performance liquid chromatography; LCMS/MS = liquid chromatography-tandem mass spectrometry; TB = tuberculosis; MDR = multidrug-resistant; XDR = extensively drug-resistant.

* All values in this study reported as median and interquartile range.

† All values in this study reported as mean and range.

‡ AUC 0 to ∞.

§ Preterm considered<34 weeks gestation.

¶ Originally reported as AUC 0–12, but values doubled here to generate AUC 0–24 to facilitate comparisons between studies.

Table 5
Recommendations for the use of linezolid in children with drug-resistant tuberculosis.

Indications	
XDR-TB	Should be used routinely in all cases
Pre-XDR-TB, failed treatment with second-line drugs	Should be used routinely in all cases
Pre-XDR-TB, meningitis	Consider, depending on severity of illness, extent of disease, other available drugs, response to treatment
Pre-XDR-TB, standard cases	Consider, depending on severity of illness, extent of disease, other available drugs, response to treatment
MDR-TB, failed treatment with second-line drugs	Should be used routinely in all cases
MDR-TB, meningitis	Consider, depending on severity of illness, extent of disease and other available drugs
MDR-TB, standard cases	Not routinely recommended
Dosing	
<12 years of age	10 mg/kg twice daily
≥12 years of age	10 mg/kg once daily up to 300 mg
Monitoring	
Full blood picture – monthly	Dose reduction for cytopaenias
Active clinical monitoring for peripheral neuropathy	Dose reduction for peripheral neuropathy; discontinuation if no improvement
Monitoring visual acuity where able; challenge with such monitoring in young children should not limit linezolid use when otherwise indicated	Discontinuation if any signs of optic neuropathy
Monitoring for lactic acidosis, rhabdomyolysis, other rare adverse effects only if clinically indicated	Dose reduction or discontinuation depending on severity

XDR-TB = extensively drug-resistant tuberculosis; Pre-XDR-TB = multidrug-resistant tuberculosis with additional resistance to either a fluoroquinolone or a second-line injectable drug; MDR-TB = multidrug-resistant tuberculosis.

HIV-infected persons on linezolid is warranted until additional data are available.

These data show a substantial number of children treated with linezolid for DR-TB will have adverse effects. Though this appears to be less than in adult reports, the small number of paediatric cases makes it difficult to say with certainty. The majority of adverse effects responded to dose reduction, including neuropathy. Children on long-term linezolid should have close monitoring for any toxicity, with a dose reduction for any non-life-threatening adverse effects.

5. Pharmacokinetics and pharmacodynamics

5.1. Pharmacokinetics

Linezolid is well absorbed in both the oral suspension and tablet formulation, with oral availability approaching 100% [15,90]. In healthy volunteers the time to maximum concentration (T_{max}) is 0.5–2 h. Co-administration with a high fat meal may delay the T_{max} and slightly reduce the maximum plasma concentration (C_{max}), but does not affect the (AUC) [90]. Protein binding is reported to be 31% [15,90]. Linezolid has complex metabolism with two primary and multiple minor metabolites [90]. The rate-limiting step in linezolid clearance is the non-enzymic formation of the primary metabolite, and both renal and non-renal routes are involved in elimination [90], with non-renal elimination accounting for roughly 65% [91]. In healthy volunteers the mean C_{max} after steady state dosing with 600 mg varies from 16.3 to 21 μg/ml and the mean AUC_{0-12} from 107 to 138 μg h/ml [90]. Increased clearance, decreased AUC, and substantial inter-patient variability have been noted in ill patients relative to healthy volunteers [92,93]. Linezolid has good tissue penetration [90,94], including into lung and epithelial lining fluid [95,96]. Penetration into cerebrospinal fluid (CSF) is good, with reported CSF-to-plasma ratios of 0.7 [90] and 0.66 [97] and PK parameters in the CSF of adult neurosurgery patients [97] and ventricular fluid of children and adolescents [98] suggesting excellent pharmacodynamics. Meningeal inflammation did not appear to influence CSF penetration.

Our search identified 3 studies of linezolid pharmacokinetics in adults with TB, with results reported in Table 4 [40,44,99,100]. The trial of linezolid for chronic XDR reported mean AUC_{0-24} of 91.1 μg h/ml for 300 mg once daily, and 180.4 μg h/ml for 600 mg once daily [59]. In the same study, in all those taking 600 mg daily the serum concentration exceeded the MIC for the entire dosing period, but the trough was below the MIC for 9 of 16 taking 300 mg once daily, including 2 patients who developed linezolid resistance [59].

Our search did not identify any studies of linezolid pharmacokinetics in children with TB. A review summarized the paediatric pharmacokinetic data on linezolid from four clinical trials including over 180 children (Table 4) [91]. In newborns linezolid clearance approximates that in adults, but increases to 2–3 times adult values by the first week of life, gradually declining over time until around 12 years of age when it and other PK parameters approximate that of adults [91]. The increased clearance results in shorter serum half-life ($t_{1/2}$) and smaller AUCs relative to adults [91]. It was recommended that in order to approximate the adult dose of 600 mg twice daily for Gram-positive infections, to give a dose of 10 mg/kg 8 hourly in children <12 years of age, and for adolescents ≥ 12 years of age to give adult doses [91].

Based on published pharmacokinetics, a dose of 10 mg/kg in children 3 months to <12 years of age will approximate the C_{max} of a 600 mg dose in adults. Because of the increased clearance, the exposure (AUC) of a 10 mg/kg dose in the same age group will approximate that of a 300 mg dose in adults, so twice daily dosing would be expected to provide similar coverage as a 600 mg adult dose. A dose of 10 mg/kg twice daily for those <10 years, and 10 mg/kg once daily for those ≥10 years has been suggested [63], and is the dose most commonly used in published linezolid-treated paediatric DR-TB cases to date.

Clinicians should be aware of drug interactions with clarithromycin, also a WHO Group 5 antituberculosis drug which may be given as part of a treatment regimen for XDR-TB patients. In adults, co-administration with 500 mg clarithromycin increased linezolid exposure by 44% [101], which theoretically could increase the risk of adverse effects.

5.2. Pharmacodynamics

Linezolid appears to have both time and concentration dependent killing, with both the AUC/MIC ratio and percent time above MIC (%T > MIC) correlated with linezolid activity against Gram-positive bacteria [102,103]. Suggested targets for Gram-positive bacteria are AUC/MICs>80–120 and %T > MIC of 100% [100,102]. Specific targets for *Mtb* have not been established, though its much slower doubling time relative to Gram-positive bacteria means lower targets may still be effective [104]. A moderate post-antibiotic effect for linezolid, reported to be 4 h in a single study [105], would support maintaining concentrations above the MIC throughout the entire treatment period, though the clinical importance of this in *Mtb* is not known.

Excellent values have been reported for both free AUC/MIC and %T > MIC for 600 mg once and twice daily dosing, though there was no correlation between either of these measures and the EBA_{0-2} or EBA_{2-7} in the study [40]. Favourable pharmacodynamic parameters were also described for both linezolid 600 mg twice daily ($AUC_{0\text{-}24}$/MIC 243.2, and %T > MIC 100.0), and 600 mg once daily ($AUC_{0\text{-}24}$/MIC 116.2, and %T > MIC 62.8) [100]. A linezolid dose of 300 mg twice daily resulted in an $AUC_{0\text{-}24}$/MIC from 167 to 667 for 7 of 8 patients with a ratio >100 and %T > MIC of 100% for all patients, suggesting that lower doses may maintain efficacy while hopefully limiting toxicity [99]. A higher %T > MIC of 100% for a 300 mg twice daily dose [99] compared to 62.8% for a 600 mg once daily dose [100] may reflect differences between the two studies in both the MICs of the *Mtb* isolates and in the reported linezolid pharmacokinetics. The 300 mg twice daily dose resulted in higher exposures, which may be related to differences in the pharmacokinetic assay methodology between studies or to individual participant variability in these small samples, though real differences due to dose-related alterations in linezolid elimination cannot be excluded. In the single linezolid clinical trial for XDR-TB, neither C_{max} nor trough concentration was associated with time to culture conversion [59].

6. Recommendations for the use of linezolid in children with DR-TB

The WHO 2008 guidelines recommend the use of Group 5 drugs, including linezolid, only when a regimen containing 4 drugs with likely activity cannot be created from Groups 1–4, though no other specific recommendations regarding linezolid were made [6]. The recommended dosage is 600 mg twice daily for 4–6 weeks, then 600 mg once daily [6]. The WHO 2011 guidelines update did not specifically address linezolid [106]. We are unaware of any other formal recommendations for the use of linezolid in children with DR-TB, in these or other documents [6,106].

In the absence of existing recommendations, Table 5 summarizes our working recommendations for the use of linezolid in children with DR-TB.

6.1. Indications for use in children with DR-TB

Because of the high cost, considerable toxicity, and good outcomes with current treatment regimens, existing evidence does not support the routine use of linezolid for children with MDR-TB. We recommend linezolid for use in children with XDR-TB or for those who have failed treatment for MDR-TB with or without additional drug resistance. Linezolid is likely to be the most active drug for such children and could make the difference between treatment success and failure. Linezolid should be considered for children with MDR-TB with additional fluoroquinolone or second-line injectable resistance (Pre-XDR-TB), especially those who have extensive disease or meningitis. Linezolid should also be considered for children with MDR-TB meningitis, especially those who have had a slow or poor response to standard treatment. The good CSF penetration of linezolid makes it particularly useful for DR-TB meningitis, as there are few second-line agents with potent antituberculosis activity and good CSF penetration.

6.2. Dosage

There remains uncertainty about the optimal dose of linezolid in adults with DR-TB, which balances efficacy and toxicity [107–109]. Currently most adults will start with a dose of linezolid 600 mg once daily for the intensive phase of treatment, though some would advocate for a 300 mg dose. In the continuation phase adults will complete their treatment with either a dose of 600 mg or 300 mg once daily, though many of those using 600 mg will switch to 300 mg due to adverse effects.

Generally children ≥12 years of age should receive the same dose as adults, and we have had success using a dose of 10 mg/kg once daily up to 300 mg for children ≥12 years of age, as in our cases included in this report [62,63]. For children 3 months to 12 years we recommend a dose of 10 mg/kg twice daily. For children with extensive disease or TB meningitis it may be advisable to use up to a higher total daily dose of 600 mg, at least initially.

6.3. Monitoring

For children on linezolid we recommend monitoring of full blood counts monthly. We also recommend active monitoring for signs of peripheral neuropathy. Children who develop signs of peripheral neuropathy should initially have a linezolid dose reduction, as many will respond to this. The decision to reduce the linezolid dose should be made considering the severity of the adverse effects, severity of the TB disease, and other available treatment options. Adults using 600 mg once daily usually reduce the dose to 300 mg once daily when necessary. In children we recommend reducing the dose by 1/3 or 1/2, however there is little evidence for this, and close monitoring for persistence or worsening of the adverse effects, or recrudescence or worsening of the TB disease is important. Thrice weekly dosing of linezolid seemed to reduce adverse effects in a small number of adult patients [110]. This is a potential approach for those with few other treatment options, and further evaluation of this strategy in adults and children would be useful. If peripheral neuropathy persists then linezolid may need to be discontinued. The cumulative evidence, though of low quality, suggests no effect of vitamin B6 (pyridoxine) supplementation on the risk of linezolid-related adverse effects, and routine supplementation is not warranted; however patients receiving high-dose INH or cycloserine/terizidone should receive pyridoxine supplementation as currently recommended.

In settings where resources and expertise for ophthalmologic assessments are available, routine eye exams in children on long-term linezolid are warranted. Considering the challenges of ophthalmologic assessments in young children, this is unlikely to be feasible in resource-limited settings, and referral for ophthalmologic examination or discontinuation of linezolid for possible optic neuropathy may be best indicated by any signs of decreasing visual acuity. Because of the reported rarity of optic neuropathy, the limited treatment options and the importance of linezolid to the antituberculosis drug regimen in children with extensive resistance, we strongly recommend that the inability to perform routine eye exams in young children should not limit the use of linezolid when it is otherwise indicated. Any signs of deteriorating visual acuity without other explanation should prompt a thorough ophthalmologic examination and discontinuation of linezolid. Though routine monitoring for rare adverse effects such as lactic

acidosis or rhabdomyolysis is unnecessary, clinicians should be aware of the potential for these effects should patients develop consistent signs or symptoms.

7. Questions for future study

The optimal dosing of linezolid in adults and children remains unclear. Once an adult dose has been established, a more formal recommendation can be made for paediatric dosing that gives a similar drug exposure. We are unaware of published linezolid pharmacokinetic data in children with TB, though such data would be important for guiding appropriate dosing for this indication. An ongoing study in Cape Town, South Africa is evaluating the pharmacokinetics, safety, and tolerability of second-line antituberculosis drugs in children, including linezolid, when used in children with DR-TB.

Little data exist on linezolid use in HIV-infected adults or children with DR-TB. Because of a potential increased risk of toxicity related to co-administration with ARVs and limited published data to date, additional information about the efficacy and safety in HIV-infected persons is needed. Additional evidence on the impact of linezolid co-treatment with high-dose INH and cycloserine/terizidone, as well as the impact of pyridoxine supplementation on adverse effects in this context would be useful.

Biomarkers for treatment response or other improved surrogate endpoints for trials in both adults and children with drug-susceptible and drug-resistant tuberculosis are urgently needed and would greatly facilitate individualized management of children with drug-resistant tuberculosis and the rational use of drugs like linezolid.

Considering what appears to be potent activity of linezolid in difficult DR-TB cases, exploration of treatment intensification with a short course of linezolid in children with severe DR-TB disease may be warranted. The second-line injectable agents (amikacin, kanamycin, and capreomycin) are considered key drugs for DR-TB treatment, but must be given by painful intramuscular injections and are associated with permanent sensorineural hearing loss in as many as 24% of children when given long term [111]. Substitution of the second-line injectables by linezolid in the intensive phase of treatment is an approach that warrants study.

A search of www.clinicaltrials.gov and the WHO International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) revealed no registered studies of linezolid in children with TB, and future large trials of linezolid in children with DR-TB are unlikely. We would encourage clinicians using linezolid for DR-TB in children to systematically record key information about each case, documenting degree of drug resistance, dosing, treatment response including culture conversion and outcome, and any adverse effects, and to report these cases as widely as possible.

The oxazolidinone antibiotic PNU-100480 has shown better efficacy against *Mtb* than linezolid in pre-clinical studies [37], and further study and development of it and other novel agents will be important to improving treatment options for adults and children with DR-TB. The inclusion of children with DR-TB in such trials is of critical importance.

8. Conclusion

Despite modest activity of linezolid against *Mtb in vitro* and in animal models, emerging data in adults have shown it to be effective in difficult cases of DR-TB. These benefits are currently offset by its high cost, and frequent and often severe time- and dose-dependent toxicity. Though data are limited, the efficacy and adverse effects of linezolid in treatment of children with DR-TB reported to date are similar to adults. For children with MDR-TB with additional resistance or with XDR-TB, linezolid may however make the difference between a successful or poor outcome, as demonstrated in many of the paediatric cases described to date. Because of its good CSF penetration, linezolid may also be an important option for children with MDR-TB meningitis, for which outcomes are often poor and other drugs with potent antituberculosis activity and good CSF penetration are limited. We would support calls for lowering linezolid costs and making it available, including in suspension form, for children with these indications [18]. Until newer antituberculosis agents with better efficacy and safety become available, linezolid will be an important component of treatment for children with the worst forms of drug-resistant tuberculosis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version at http://dx.doi.org/10.1016/j.tube.2013.10.003.

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Chapter 7: Pharmacokinetics and safety of linezolid in children treated for multidrug-resistant tuberculosis

Rationale

As identified in the review in Chapter 6, linezolid has potent antimycobacterial activity and is an increasingly important antituberculosis drug for the treatment of MDR-TB in adults and children. There are no linezolid pharmacokinetic data available yet in children with TB and there is limited guidance on its dose for long-term treatment of MDR-TB. Linezolid's dose and duration-dependent adverse effects have not been prospectively and systematically described in children treated long-term for MDR-TB.

Study aims

The objective of this study was to characterize the pharmacokinetics, safety and optimal dose of linezolid in HIV-infected and –uninfected children treated for MDR-TB.

Methods

The MDRPK2 study (*Optimizing and operationalizing pediatric drug-resistant tuberculosis treatment*, PI Garcia-Prats, Savic HD083047) was an NIH RO1 funded study targeting 100 HIV-infected and uninfected children receiving one of three key second-line antituberculosis drugs (levofloxacin, moxifloxacin or linezolid) for pharmacokinetic sampling and longitudinal follow-up for safety and treatment outcomes. This study was a direct follow-up to the MDRPK1 study (see previous chapters), and built on MDRPK1 study methods and data. The primary objectives of the MDRPK2 study were to characterize the pharmacokinetics and safety of levofloxacin, moxifloxacin and linezolid using weight-banded optimized doses based on data from MDRK1, among HIV-infected and –uninfected children routinely treated for MDR-TB. The study was open from 2016 and enrollment and follow-up are ongoing. As for MDRPK1, children with MDR-TB are recruited from Tygerberg Hospital and Brooklyn Hospital for Chest Diseases, Cape Town as well as Brewelskloof Hospital, Worcester.

Children were eligible for MDRPK2 if they were 0 to <18 years of age, HIV-infected or –uninfected, routinely treated with second-line antituberculosis drugs including at least one of levofloxacin, moxifloxacin or linezolid for treatment of MDR-TB for <12 weeks. Exclusion criteria included laboratory documented anaemia (haemoglobin <8 g/dL),

other serious clinical comorbidity, or weight <2.5 kg. Children in the study had semi-intensive pharmacokinetic sampling (pre-dose, then 1, 4, and 10 hours post-dose), and were followed longitudinally for safety and TB treatment outcome. Children in MDRPK2 not receiving linezolid as a component of their routine treatment regimen were prescribed a single dose on the day of pharmacokinetic sampling, and so contributed cross sectional pharmacokinetic data but not long-term safety data.

As linezolid is expensive and associated with frequent adverse events, it is reserved in Cape Town for children with MDR-TB who have additional resistance to, toxicity or intolerance to additional second-line antituberculosis drugs, so relatively few children receive it in their regimens. Therefore, children from both MDRPK1 and MDRPK2 who received linezolid were included in this analysis. All children had pharmacokinetic sampling between 2 and 12 weeks of MDR-TB treatment. The routinely available formulations of linezolid were used (600 mg tablets, 20 mg/mL granules for suspension, Pfizer, Sandton, South Africa). Children receiving linezolid as a component of their routine treatment regimens were generally prescribed 10 mg/kg/dose given twice daily if <10 years of age or once daily if ≥10 years of age (maximum daily dose of 600 mg). On the day of pharmacokinetic sampling, linezolid was administered as an exact 10 mg/kg dose in MDRPK1, and as a weight banded dose approximating 10 mg/kg in MDRPK2. Linezolid was administered either as whole tablets, suspension or occasionally as crushed tablets, and either orally or by nasogastric tube, depending on what the child was able to tolerate.

Linezolid plasma concentrations were measured using a validated liquid chromatography tandem mass spectrometry (LC MS/MS) assay at the University of Cape Town Division of Clinical Pharmacology. Linezolid pharmacokinetic parameters were calculated, and associations with covariates of interest assessed, using non-linear mixed effects modeling. The final model was used to simulate weight banded doses resulting in exposures in children across ages that approximated the target seen in adults with MDR-TB receiving 600 mg once daily (110 mg/L·h). Children receiving linezolid as a component of their routine treatment regimen had 1-2 monthly clinical and laboratory safety monitoring. All adverse events were reported, and assessed for severity and attribution to linezolid. In MDRPK1, events were graded for severity using standard Division of AIDS criteria (DAIDS Table for Grading the Severity of Adult and

Pediatric Adverse Events version 1.0, December 2004, updated August 2009) (122). In MDRPK2, events were graded according to the updated DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Corrected version 2.1, July 2017) (127). Frequency and rates of adverse events were reported by grade.

Results

Forty-eight children, mean (range) age 5.9 years (0.6 to 15.3), were included in this study. Three were HIV-infected. Nine children were from MDRPK1, and 39 from MDRPK2, the majority of whom (n=31) received single dose linezolid. The final pharmacokinetic model consisted of a one compartment model characterized by clearance (CL) and volume (V) parameters which included allometric scaling to account for weight. No other evaluated covariates contributed to the model. Simulations using the model estimated that once daily doses from 14.5 mg/kg (children 5 to <7 kg body weight) down to 8.2 mg/kg are needed (children 43 to <56 kg body weight) to achieve target exposures. Ten of 17 children followed long-term had a linezolid-related adverse event, including 5 with a Grade 3 or 4 event, which were all anaemia. These adverse events frequently resulted in linezolid dose reductions (n=4), temporary interruptions (n=5) or permanent discontinuations (n=4).

Conclusions and recommendations

In this first clinical study of linezolid pharmacokinetics and safety in children treated for MDR-TB, linezolid exposures were adequate, and higher than expected considering previous linezolid data in children treated for non-TB infections. The frequent, serious adverse events highlight the importance of careful monitoring for children receiving linezolid. Although the cumulative incidence of events increased with longer treatment durations, events, including some grade 3 and 4, were seen even in the first two months of treatment. The use of shorter duration of linezolid may reduce but not eliminate the risk of adverse events, and careful safety monitoring remains important regardless of the duration. Simulated doses achieving target exposures were lower than are currently being used in many routine settings. These doses may reduce the risk of adverse events. The impact of reduced doses on efficacy and risk of acquired resistance should be considered, especially in older children, although a 600 mg dose in adults appears to be efficacious with a low risk of acquired resistance, and there is no reason to expect this to differ in children achieving the same exposures. These newly proposed

linezolid doses should be evaluated prospectively in children to characterize the pharmacokinetics, safety and efficacy of these doses.

Citation: This study has been prepared as a manuscript but has not yet been submitted. Author contribution is as described in Appendix 1.

Title: Pharmacokinetics, optimal dosing and safety of linezolid in children with multidrug-resistant tuberculosis

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Running title: Linezolid PK and safety in children with TB

Key words: Linezolid, pharmacokinetics, MDR-TB, children, safety

Abstract

Background: Linezolid is an increasingly important antituberculosis drug for the treatment of multidrug-resistant tuberculosis (MDR-TB) in adults and children. However there are no linezolid pharmacokinetic data in children with TB, and its adverse effects have not been prospectively described in children treated long-term for MDR-TB. The objective of the study was to characterize the pharmacokinetics, safety and optimal dose of linezolid in children treated for MDR-TB.

Patients and methods: Data from two observational pharmacokinetic studies in South Africa were included. Children routinely treated for MDR-TB had pharmacokinetic sampling after either a single dose or multiple doses of linezolid (at steady state). Linezolid pharmacokinetic parameters, and associations of these with covariates of interest, were described using non-linear mixed effects modelling. Children receiving long-term linezolid as a component of their routine treatment had regular clinical and laboratory monitoring. All adverse events were graded for severity and assessed for attribution to linezolid. Simulations with the final pharmacokinetic model were used to estimate weight banded doses resulting in exposures in children that approximate those in adults receiving linezolid 600 mg once daily.

Results: Forty-eight children were included (mean age 5.9 years, range 0.6 to 15.3); 2 were HIV-infected, 31 received a single dose of linezolid and 17 received multiple doses. The final pharmacokinetic model consisted of a one compartment model characterized by clearance (CL) and volume (V) parameters which included allometric scaling to account for weight; no other evaluated covariates contributed to the model. Simulated weight-banded once daily doses estimated that doses from 14.5 mg/kg (children 5 to <7 kg body weight) to 8.2 mg/kg are needed (children 43 to <56 kg body weight). Ten of 17 children followed long-term had a linezolid-related adverse event, including 5 with a Grade 3 or 4 event, which were all anaemia. These adverse events resulted in linezolid dose reductions (n=4), temporary interruptions (n=5) or permanent discontinuations (n=4).

Conclusions: Linezolid exposures were satisfactory in this cohort of children with MDR-TB, compared to current adult target exposures. Linezolid-related adverse effects were frequent and occasionally severe, and careful safety monitoring is required. Compared to doses currently being used in children in many settings for MDR-TB

treatment, lower doses may approximate target exposures and should be evaluated in children.

Introduction

Multidrug-resistant (MDR) tuberculosis (TB) continues to threaten global TB control, with an estimated 490,000 incident cases worldwide in 2016 [1]. Treatment options remain limited, especially in children. Linezolid, an oxazolidinone antibiotic that binds to the 50s ribosomal subunit inhibiting protein synthesis [2], is increasingly being used for MDR-TB treatment, particularly when there is additional resistance to second-line antituberculosis medications, such as for extensively drug-resistant (XDR) TB (i.e. MDR-TB with additional fluoroquinolone and second-line injectable resistance). In routine use in adults with MDR-TB, linezolid has been associated with good outcomes, with 68% and 82% of patients successfully treated in two systematic reviews, respectively [3, 4].

Revised 2016 WHO antituberculosis drug groupings for MDR-TB treatment have given a higher priority to linezolid, moving it to Group C, from Group 5 in the older grouping [5, 6]. Interest in linezolid has grown further based on the preliminary results of the Nix-TB trial (NCT02333799), a single-arm open-label phase 3 study which evaluated adults with XDR-TB or MDR-TB treatment intolerance, or failure, with a three-drug regimen of bedaquiline, pretomanid and linezolid (1200 mg given once daily) for six months [7]. At the time of interim analysis, 61 adult participants had been enrolled (49% HIV-infected, 74% with XDR-TB) [7]. Of these 61, 4 had died and of the surviving patients, 45 (74%) were culture negative by 8 weeks of treatment. By 4 months of treatment, all patients were culture negative; 34 of 61 (56%) had completed 6 months of treatment, 20 (33%) had completed 6 month post-treatment follow-up and only one patient has had microbiological relapse to date [7]. Linezolid is also a component of multiple novel, shortened MDR-TB treatment regimens currently under evaluation in adults [8].

In short courses (<28 days), linezolid is safe and well tolerated, but with the longer treatment durations being used for MDR-TB treatment (typically 6 months or longer in adults), it is associated with frequent, serious, dose and duration-dependent adverse effects, including anaemia, neutropaenia, thrombocytopaenia, peripheral neuropathy, and more rarely, optic neuropathy, lactic acidosis, pancreatitis and rhabdomyolosis [9]. In the same systematic reviews of linezolid-treated adults with MDR-TB described earlier, linezolid-related adverse events were reported in 61% and 59% of patients, respectively [3, 4], with 69% of these requiring linezolid dose-adjustment or

discontinuation in one review [3]. A linezolid dose of >600 mg daily was associated with an increased risk of adverse effects in these reviews [3, 4]. In the Nix-TB study, 27% of participants experienced a serious adverse event; 71% of participants had at least one linezolid treatment interruption, due mostly to myelosuppression or peripheral neuropathy [7].

There is a substantial burden of MDR-TB in children, with an estimated 25,000-32,000 incident cases globally each year [10, 11], many of whom could benefit from linezolid treatment given considerable delays in the evaluation of novel effective drugs like bedaquiline in children. The evidence base for linezolid use in children with MDR-TB is currently limited. A 2014 review identified only case reports and small case series in the literature that described 18 children with MDR-TB treated with linezolid [9]. As in adults, treatment outcomes were good with 15 of 18 children (83%) successfully treated, but 9 (50%) experienced a linezolid-related adverse event, with 5 (28%) requiring dose adjustment and 2 (11%) discontinuing linezolid [9]. This limited early data was also retrospective, and had variable reporting of key information. High quality prospective data is needed to better characterize the incidence, severity, and timing of adverse events among children with MDR-TB receiving long-term linezolid.

The optimal dose of linezolid for treatment of adults with MDR-TB is not yet certain. However 600 mg once daily is currently the most frequently used routine dose in adults. The dose of linezolid in children needed to approximate target exposures in adults with MDR-TB receiving a 600 mg dose has not been characterized. Linezolid is well absorbed after oral administration, with bioavailability of nearly 100% [2, 12]. Linezolid has complex metabolism with the primary metabolite formed through a non-enzymatic mechanism, with the major pathway of excretion being the urine [12]. A benefit of linezolid is its good penetration into tissues, including cerebrospinal fluid, where its exposure is 70-98% of plasma exposure [12-14]. Linezolid pharmacokinetics have been studied extensively in adults; studies to date have shown substantial variability between patients, patient populations and studies [15-17].

Linezolid pharmacokinetics have been studied in children with Gram-positive infections and resulted in dosing recommendations of 10 mg/kg three times daily for children <10 years of age, and 10 mg/kg twice daily for children ≥10 years of age to approximate exposures in adults receiving 600 mg twice daily [18]. A previous attempt at linezolid

dose selection for TB treatment in children used simulations with this paediatric data and targeted a linezolid area under the concentration time curve (AUC) to a minimum inhibitory concentration (MIC) ratio (AUC/MIC) of >62 mg/L·h to 93.4 mg/L·h [19]. The suggested doses were 15 mg/kg for children <3 months and 10 mg/kg for children 3 months to 11 years of age [19]. However, this evaluation was limited by the lack of clinical studies of linezolid pharmacokinetic data in children with TB and requires clinical evaluation. Dosing guidance should ideally be based on data from the target population, particularly as linezolid pharmacokinetics is known to vary considerably across disease states [17]. Additionally, the validity of the proposed targets using AUC/MIC are uncertain. Understanding the optimal dose of linezolid for MDR-TB treatment in children is critical, as too high a dose may increase the risk of serious adverse effects, while underdosing may reduce the efficacy of this drug that is often playing a critical role in regimens where treatment options are very limited, such as resistance (such as XDR-TB), toxicity, or intolerance to other second-line antituberculosis drugs, and may also lead to the development of resistance to linezolid [20, 21]. Practical, weight-banded paediatric dosing guidance for linezolid based on its pharmacokinetics in children with TB is urgently needed.

The objective of this study was to characterize the pharmacokinetics and safety of linezolid in children routinely treated for the spectrum of MDR-TB, and to estimate optimal weight-banded doses that approximate robust adult target exposures for MDR-TB treatment.

Patients and Methods

Study design, setting and population: The data described here are combined from two prospective observational pharmacokinetic studies implemented in Cape Town, South Africa, which used standard clinical study measures, the same clinical personnel, identical drug formulations and laboratory methods. The first study (MDRPK1) enrolled HIV-infected and -uninfected children from 0 to <15 years of age (n=173), routinely treated with second-line antituberculosis drugs for probable or confirmed drug-resistant TB, who were followed until the end of MDR-TB treatment, from 2011 to 2015. Children from this cohort routinely treated with linezolid were included in the current analysis. The second study (MDRPK2), was a direct follow-up study to

MDRPK1, and enrolled HIV-infected and -uninfected children from 0 to <18 years of age (n=64), routinely treated for MDR-TB with levofloxacin, moxifloxacin or linezolid, in the same setting, from 2016. Enrolment and follow-up for MDRPK2 are ongoing. In MDRPK2, children not prescribed linezolid as a component of their routine MDR-TB care received a single dose of linezolid on the day of pharmacokinetic sampling and therefore contributed data to pharmacokinetic analyses only, but not to long-term safety.

Children with MDR-TB were treated according to national and international guidelines with a minimum of four confirmed or likely effective drugs, usually with the addition of pyrazinamide and ethambutol, generally for 12-18 months' duration [6, 22, 23]. Due to its cost and associated adverse effects, linezolid is currently reserved in Cape Town for MDR-TB patients with: 1) probable or confirmed additional fluoroquinolone resistance, including those with XDR-TB; 2) MDR-TB meningitis; 3) or intolerance to other second-line antituberculosis medications. Management of linezolid-associated adverse events were individualized depending on the type and severity of the event, the severity of the child's TB disease, and the availability of other treatment options. For common, non-severe events such as low grade anaemia, linezolid would be temporarily interrupted and then restarted at half the dose. For more severe events, linezolid may have been permanently discontinued, depending on other treatment options, and the child's clinical status including their response to TB treatment at the time.

Linezolid dosing and pharmacokinetic sampling: The routinely prescribed dose of linezolid in the study setting was 10 mg/kg/dose twice daily for children <10 years of age and 10 mg/kg/dose once daily for children >10 years of age, up to maximum total daily dose of 600 mg. Linezolid was available as 600 mg unscored tablets and as a 20 mg/mL suspension (Pfizer, Sandton, South Africa). There is currently no global evidence-based dosing recommendation for linezolid for TB treatment in children.

In the MDRPK1 study, on pharmacokinetic sampling days, an exact 10 mg/kg was prepared and administered along with other antituberculosis medications in the regimen. Samples were drawn just prior to, and then at 1, 2, 4, 8 and either 6 or 11 hours after the antituberculosis medications' dose. In MDRPK2, a weight-banded dose (approximately 10 mg/kg) was adminstered on pharmacokinetic sampling days along with other routine antituberculosis medications in the regimen. The exact linezolid

dose given on the day was documented. Samples were drawn just prior to, and at 1, 4 and 10 hours after the observed dose. For both MDRPK1 and MDRPK2, whole blood samples were centrifuged, and plasma separated and frozen at -80 C within 30 minutes of phleobotomy. For both studies, medications were administred on an empty stomach after an overnight fast, and given either as whole tablets if the child was able to swallow, as suspension if available, or crushed tablets mixed in the smallest volume of water possible, if the child was unable to swallow and the suspension was unavailable. On occasssion, all antituberculosis medications were adminstered via nasogastric tube on pharmacokinetic sampling days if the child refused to swallow them orally (e.g. very young children). One hour after the antituberculosis medication dose, antiretrovirals were administered if relevant, and a standard breakfast offered.

Laboratory assays: Pharmacological assays were performed at the Unversity of Cape Town Division of Clincal Pharmacology using a validated liquid chromatography tandem mass spectometry (LC MS/MS) method. This LC MS/MS method involves a simple protein precipitation using 20 µl of plasma, followed by isocratic separation on a Poroshell 120EC-C184.6 X 50mm, 2.7 um column. A deuterated internal standard, Linezolid-d3, is used to monitor the method across a calibration range of 0.100 µg/ml (LLOQ) to 30 µg/ml (ULOQ). The method performed well over the period of analysis with an accuracy ranging from 96.6-98.7% and a precision estimate of less than 7.2% (% CV) over all three control concentrations.

Safety: All children had regular clinical and laboratory safety assessments, including a full blood count, completed at the National Health Laboratory Services (NHLS), Cape Town, monthly for the first 6 months, then every two months or as clinically indicated, until completion of treatment. All adverse events were recorded, assessed for attribution to linezolid, and graded for severity. In MDRPK1, events were graded according to standard Division of AIDS criteria (DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 1.0, December 2004, updated August 2009) [24]. In MDRPK2, events were graded according to the updated DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Corrected version 2.1, July 2017) [25].

Statistical analysis: Baseline demographic and clinical characteristics were presented with descriptive statistics. Weight-for-age and height-for-age z-scores were calculated

using British Reference values, as WHO references only include children <10 years of age [26]. Only children receiving linezolid as a component of their routine DR-TB treatment regimen were included in this safety analysis (i.e. not children only receiving single dose linezolid). Adverse events at least possibly related to linezolid were presented by grade and the rate of events per person-time of observation calculated. The median (interquartile range [IQR]) time without linezolid-related adverse events was estimated using Kaplan-Meier plots; patients were censored at the time of the relevant linezolid-related event or when linezolid was discontinued.

Population pharmacokinetic model and dosing simulations: Pharmacokinetic data were characterized based on the population nonlinear mixed effects modeling approach using the software NONMEM 7.41 (ICON Development Solutions, Ellicot City). The method of estimation used was FOCE with the option INTERACTION. Regarding the statistical part of the model, between subject variability (BSV) was modeled exponentially, and the residual error was described using a combination of an additive and proportional error model. Model building was performed in two stages, with the structural model developed first and the covariate analysis done subsequently. The main covariates evaluated during the analysis were weight, height, age, sex and race. HIV status , linezolid administration method (oral vs. nasogastric tube), formulation (whole vs. crushed vs. suspension), linezolid given as single dose vs. steady state, and pharmacokinetic interactions with concomitant drugs. Covariate identification was done using the stepwise covariate modeling (SCM) through the PsN software (v4.6.0). This method consists of the stepwise testing of linear and non-linear covariate-parameter relationships with forward inclusion and backward exclusion approaches with significance levels of 0.05 and 0.01, respectively. The final inclusion of the identified significant covariates was done taking into account scientific plausibility, statistical significance and clinical relevance. Both stages of model building were evaluated by the likelihood ratio test, goodness of fit plots, and visual predictive checks (VPC). R software (version 1.1.383) was used to make the graphical representation, using the Xpose package (v4.6.1) for dataset checkout and graphical evaluation of the results.

The final model was used to simulate different linezolid doses depending on children's weights. The target linezolid exposure used for dosing simulations was the steady-state

$AUC_{0\text{-}24}$ ($AUC_{0\text{-}24,ss}$) in adults with TB after a 600 mg once daily dose (110 mg/L·h). This target is based on as-yet unpublished data on linezolid pharmacokinetics from two ongoing adult MDR-TB trials (personal communication, R. Savic). Doses were calculated by dividing the target AUC by the CL, which is a function of weight. Two hundred simulations of the pharmacokinetic profile of a paediatric population with weights ranging from 5 to 56 kg, receiving the calculated weight-banded dose regimen, were performed in NONMEM, and their AUCs reported.

Ethical considerations: Informed consent was provided by the parent/s or legal guardians, and informed assent by participants ≥7 years of age. Ethical approval for the study was provided by the Health Research Ethics Committee of Stellenbosch University (N11/03/059 for MDRPK1 and N15/02/012 for MDRPK2).

Results

Study population: Forty-eight children were included in the study, 9 from MDRPK1 and 39 from MDRPK2. The mean (range) age overall was 5.9 years (0.6 to 15.3). Three patients (6.3%) patients were HIV-infected. Baseline clincial and demographic characteristics by study (MDRPK1, MDRPK2) are shown in Table 7.1.

Pharmacokinetic model: Of the 48 children included, five participants contributed data from more than one pharmacokinetic sampling occasion. Four patients from MDRPK2 contributed full profiles from two occassions, and one patient from MDRPK1 contributed one full profile and two partial profiles. The final pharmacokinetic model consisted of a one compartment disposition model characterized by clearance (CL) and volume (V) parameters. CL and V included allometric scaling, using the exponents 0.75 and 1, respectively, to account for changes in weight. The rate of absorption (K_a) was constrained to be faster than the rate of elimination (CL/V) in order to avoid flip-flop kinetics during model estimation. BSV was estimated on both CL and V. No covariates significantly improved the model fit for any of the pharmacokinetic parameters, after accounting for the effect of weight, already included allometrically in CL and V. Table 7.2 describes the final model parameters. The final model fit the observed data well, as shown in the VPC (Figure 7.1). Calculated AUC and maximum plasma concentrations from our study population are described in Table 7.3.

Simulated exposures: The proposed weight-banded doses across weights that approximated the emerging AUC targets reported in adults with MDR-TB receiving a 600 mg once daily dose are shown in Table 7.4. The expected linezolid exposures ($AUC_{0\text{-}24ss}$) across weights from simulations using the final model and this weight banded dosing strategy are shown in Figure 7.2.

Safety: Seventeen children were included in the safety analysis (mean age 6.1 years, range 0.6 to 15.3) and followed for a median duration of 17.7 months on linezolid (IQR 7.5 to 19.7). Ten patients (59%) experienced at least one adverse event possibly attributed to linezolid, the most frequent being anaemia, with 12 events in 10 patients (see Table 7.5). There were three grade 3 and two grade 4 events in five (23%) participants, all anaemia. The linezolid dose was adjusted or linezolid was temporarily interrupted and restarted after 4 (23.5%) and 5 (29.4%) events, respectively, and 4 (23.5%) participants permanently discontinued linezolid due to adverse events. For the single episode of peripheral neuropathy, linezolid was temporarily interrupted, and the patient was treated with gabapentin, resulting in symptomatic improvement. The linezolid was restarted at a lower dose after symptoms resolved with no sequelae and symptoms did not recur at the lower dose. We did not identify any events of optic neuropathy.

The median (IQR) time to adverse events of any grade at least possibly related to linezolid was 3.2 months (1.8 to 13.9), with the Kaplan Meier curve shown in Figure 7.3. The median (IQR) time to grade 3 or 4 events at least possibly related to linezolid was 2.4 months (1.8 to 4.7), with the Kaplan Meier curve shown in Figure 7.4.

Discussion

This study provides the first data on the pharmacokinetics of linezolid in children with TB, and the first prospective data on the safety of long-term linezolid in children treated for MDR-TB.

The linezolid plasma AUCs seen in our cohort were higher than expected compared to previously published values in children (58 mg/L·h for children 3 months to 11 years of age, and 95 mg/L·h for 12 to 17 years of age after a 10 mg/kg dose) [18]. This could be related to previously unidentifed drug-drug interactions with other antituberculosis

drugs. This was explored in the analyses and no association was found between any pharmacokinetic parameters and concomitant drugs, making this less likely. Other causes of reduced clearance in our cohort relative to previously reported studies would also explain these differences. Linezolid has complex metabolism, and as previously noted, there is substantial variability in linezolid pharmacokinetics between individuals and by disease state [17, 27-29]. Differences in bioavailability, including formulation effects, should be considered. However the previously published paediatric data reported on intravenous doses only. The formulations used in our study were all non-generic formulations (Pfizer), as used in most adult studies to date, and no differences were observed in pharmacokinetic parameters by formulation administration (whole vs. crushed vs. suspension) in our cohort, making this less likely. Additional data on linezolid pharmacokinetics in children treated for MDR-TB would be useful to confirm our findings.

This is also the first prospective data on the safety of long-term linezolid use in children with MDR-TB. At the linezolid doses used in this study, 10 of 17 children treated long-term experienced a linezolid-related adverse event, all of whom had anamia in addition to some other less frequent events, approximately similar to a previous summary of highly variable published case series and case reports in which 9 of 18 children had a linezolid-related event [9]. In our study, regular monitoring, at least monthly, was able to identify anaemia at relatively mild grades in most children. The approach of temporarily interrupting the linezolid until the haemoglobin had improved, followed by re-introduction at a lower dose (usually half the previous dose), was generally safe. Five children experienced a grade 3 or 4 anaemia, four of whom permanently discontinued linezolid. Although all patients recovered from their anaemia without sequelae, the frequency and severity of these events highlights the importance of careful monitoring of haematological parameters during long-term linezolid treatment. In our study, once haemoglobin values began to drop, they often fell rapidly, so the first signs of a falling haemoglobin should prompt more frequent testing and there should be a low threshold for temporarily interrupting doses. The importance of the contribution of the linezolid to the efficacy of the regimen must also be considered. The cumulative incidence of adverse events increased with longer duration of treatment, however there were events even in the first 60 days, including grade 3 and 4 events. Reducing the duration of linezolid treatment would likely reduce but not eliminate the risk of adverse events, and

careful safety monitoring will be necessary in future studies and routine care, regardless of the linezolid duration.

We identified few events of thrombocytopaenia, and it was difficult to rule out linezolid as a potential cause. Only one child experienced peripheral neuropathy, which improved with gabapentin treatment and ultimatley resolved without sequelae after reducing the linezolid dose. It is possible other peripheral neuropathy events were missed, particularly in young children who were unable to report subjective symptoms of neuropathy, but we did not find other children with objective evidence of neuropathy. We did not identify any serious, more rare events such as optic neuropathy or lactic acidosis. This should be interpreted cautiously, as the number of children included here is small, and formal ophthalmologic examinations were not feasible to perform. Lactate levels and pacreatic enzymes were not monitored, which is also a limitation. However, no children had clinical symptoms suggestive of optic neuropathy, lactic acidoses or pancreatitis, so the risk is low that clinically significant condtions were missed.

Using the final model, our simulations identified weight-banded doses that would achieve the proposed target exposure, based on emerging data in adults with MDR-TB receiving a 600 mg dose (Figure 7.2, Table 7.3). These proposed doses are lower than those used in this study and in routine care in many settings. Lower doses in children would likely reduce the risk of adverse events, which were frequent in this cohort, although this would need to be confirmed in further studies. However, lower doses in children, despite paucibacillary disease in many children, may potentially impact negatively on the efficacy of linezolid. As currently used, linezolid is most often used in regimens where there is MDR-TB with additional resistance, such as fluoroquinolone-resistance, and so is relied on heavily in these regimens. Lower doses of linezolid may potentially also increase the risk of acquisition of drug resistance, as has been previously shown in adults treated with 300 mg daily doses [21], although the risk of this would likely be low in most children, who usually have paucibacillary TB. Older children and adolescents however frequently develop adult-type, cavitating pulmonary TB, where both efficacy and the emergence of drug resistance, would be a potential concern. Despite these limitations, as exposures with these doses in children would be expected to approximate those in adults after a 600 mg dose, the efficacy would be

expected to be as good or better in most compared to adults receiving this dose. Whether to use the proposed doses in the routine care of children with MDR-TB should be considered carefully, weighing the potential risks and benefits. Careful further evaluation of the pharmacokinetics, safety and treatment outcomes in children treated with these proposed weight banded doses of linezolid is therefore warranted.

There were few young and small children included in this analysis, and none under 6 months of age. There is data in neonates and young infants that clearance of linezolid is associated with post-natal age, and children <8 days of age had lower clearance than those 8 days to 12 weeks of age (i.e., clearance rapidly increased in the first week of life) (137). Gestational age was a predictor of volume of distribution (Vd). This data suggests that the approach to dosing for infants >7 days post-natal age matched that for older infants and young children, but differed for younger infants. Our proposed doses for smaller children should therefore be interpreted with caution, as young (<6 months) and small children did not contribute to the model. This is particularly true for infants <8 days of age, for whom clearance is known to differ from older children. As few children younger than 6 months of age are treated for MDR-TB it is difficult to generate data in this age group. However, it also means this will not likely impact many patients. For clinicians in the field responsible for caring for the occasional young, small child with MDR-TB, some practical guidance on dosing, even with inherent limitations, is still of value.

The exposure targeted clearly has an impact on paediatric dosing studies and recommendations. The target exposure chosen here (110 mg/L·h) is based on as-yet unpublished data from two adult MDR-TB trials. The target was chosen as it was taken from recent studies that were linked to efficacy and toxicity data. The optimal linezolid target exposure is not yet fully established, nor is the optimal dosing strategy for linezolid in adults. An ongoing phase 3 trial (ZeNix, NCT03086486) is evaluating multiple doses and durations of linezolid in combination with bedaquiline and pretomanid in adults with MDR-TB. This trial will provide important data to inform linezolid dosing recommendations. Proposed pharmacokinetic-pharmacodynamic targets for linezolid and Gram-positive organisms have been suggested in adults, and a free-AUC/MIC ratio of >100 has been proposed for efficacy based on hollow fibre model data [30, 31]. A linezolid free-AUC/MIC ratio of <96 or a trough <2 mg/L have been

proposed as targets to reduce the risk of adverse effects [30-32]. However more data is needed to understand how relevant these targets are in patients, including in children of different ages. Additionally, there is a growing awareness of the importance of drug exposures at the site of disease, which is influenced by penetration of drugs into different areas of TB lesions [33]. Targeting plasma AUC/MIC does not take this key aspect into consideration. Targeting drug exposures that are linked with efficacy in TB patients with the spectrum of TB disease and lung pathology does incorporate this aspect, and in our estimation, is a more appropriate target given the current state of knowledge. Regardless, paediatric linezolid dosing recommendations may need to evolve as additional data on optimal dosing in adults becomes available.

Conclusions

This study provides the first data on linezolid pharmacokinetics and prospectively observed safety data in children with TB. Linezolid exposures were higher than expected, and adverse events were common and frequently severe. Based on these data, we have proposed, for the first time, paediatric weight banded doses approximating a 600 mg adult dose for further prospective evaluation. Clear guidance on the optimal dose of linezolid for children with TB is urgently needed given its growing importance in MDR-TB treatment regimens.

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Table 7.1. Baseline demographic and clinical characteristics of children with MDR-TB receiving linezolid in two observational pharmacokinetic studies

	MDRPK1 (n=9)	MDRPK2 (n=39)	Combined (n=48)
Mean age in years (range)	5.0 (0.6 - 13.8)	6.1 (1.2 - 15.3)	5.9 (0.6 - 15.3)
Male gender(%)	6 (66.7)	18 (46.2)	24 (50.0)
Ethnicity (%)			
Black	4 (44.4)	21 (53.9)	25 (52.1)
Mixed race	5 (55.6)	18 (46.2)	23 (47.9)
HIV-infected (%)	1 (11.1)	2 (5.1)	3 (6.3)
WAZ <-2 (%)	3 (33.3)	6 (15.4)	9 (18.8)
Administration on day of PK sampling			
NGT (%)	6 (66.7)	6 (15.8)	12 (25.5)
Oral (%)	3 (33.3)	32 (84.2)	35 (74.5)
Formulation (%)			
Whole tablet (%)	1 (11.1)	5 (12.8)	6 (12.5)
Crushed tablet (%)	1 (11.1)	8 (20.5)	9 (18.8)
Suspension (%)	7 (77.8)	26 (66.7)	33 (68.8)
Single-dose linezolid only (%)	0 (0.0)	31 (79.5)	31 (64.6)

WAZ = weight-for-age Z-score; PK = pharmacokinetic; NGT = nasogastric tube

Table 7.2. Parameter values for modelled linezolid pharmacokinetics in children with multidrug-resistant tuberculosis (n=48)

Parameter	Estimate	RSE (%)
K_a (h^{-1}) θ_K_a+(CL /V)	θ_K_a=0.77	25
CL/F (L/h) =θ_CL×〖"(WT/70")〗^0.75	θ_CL=4.73	7
V/F (L) =θ_V×("WT/70")	θ_V=54.8	6
$BSV_{CL/F}$ (%)	37	16
$BSV_{V/F}$ (%)	32	23
Additive error (mg/L)	0.78	91
Proportional error (CV%)	25	25

K_a = absorption rate constant; CL = clearance; F = bioavailability; θ = typical parameter; WT= weight in kg; V = volume of distribution; BSV = between subject variability; RSE = relative standard error; CV = coefficient of variance

Table 7.3. Proposed weight-banded once daily doses of linezolid for children treated for multidrug-resistant tuberculosis approximating the exposure in adults with MDR-TB receiving a 600 mg once daily dose ($AUC_{0\text{-}24ss}$ 110 mg·h/L)

Weight band (kg)	Daily dose (mg)	Daily dose (mg/kg)
5 to <7	80	14.5
7 to <9	100	13.3
9 to <11	120	12.6
11 to <15	150	12
15 to <19	180	10.9
19 to <25	220	8.9
25 to <33	270	9.5
33 to <43	330	8.8
43 to <56	400	8.16

Table 7.4. Calculated linezolid $AUC_{0\text{-}24,ss}$ and C_{max} from children with multidrug-resistant tuberculosis (n=48) stratified by age and daily dose

Age	Dose	Number of children	$AUC_{0\text{-}24,SS}$ (mg/L·h)		C_{max} (mg/L)	
			Median	[Min-Max]	Median	[Min-Max]
<10 years	<10 mg/kg	16	78.5	56.63-158.09	7.35	5.49-9.16
	10-20 mg/kg	16	127.52	59.23-243.23	10.1	6.95-15.8
	>=20 mg/kg	5	189.8	139.3-215.9	11.193	9.36-14.1
≥10 years	<10 mg/kg	7	98.9	75.27-321.90	7.306	5.10-21.2
	10-20 mg/kg	4	113.7	102.2-271	11.175	9.87-19.5
	>=20 mg/kg	0	-	-	-	-

Table 7.5. Adverse events in children treated for multidrug-resistant tuberculosis with linezolid (n=17)

	Adverse events possibly, probably or definitely attributed to linezolid by grade						
Adverse Event	# of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	Total # of events	Event Rate (per 100 person-yrs)
Peripheral neuropathy	1	1	0	0	0	1	4.8
Low haemoglobin	10	5	2	3	2	12	57.2
Low platelets	2	1	1	0	0	2	9.5
Low white blood cell count	1	1	0	0	0	1	4.8

17 patients followed for a median time of 17.7 months (IQR: 7.5 - 19.7 months); total person years = 21.0.

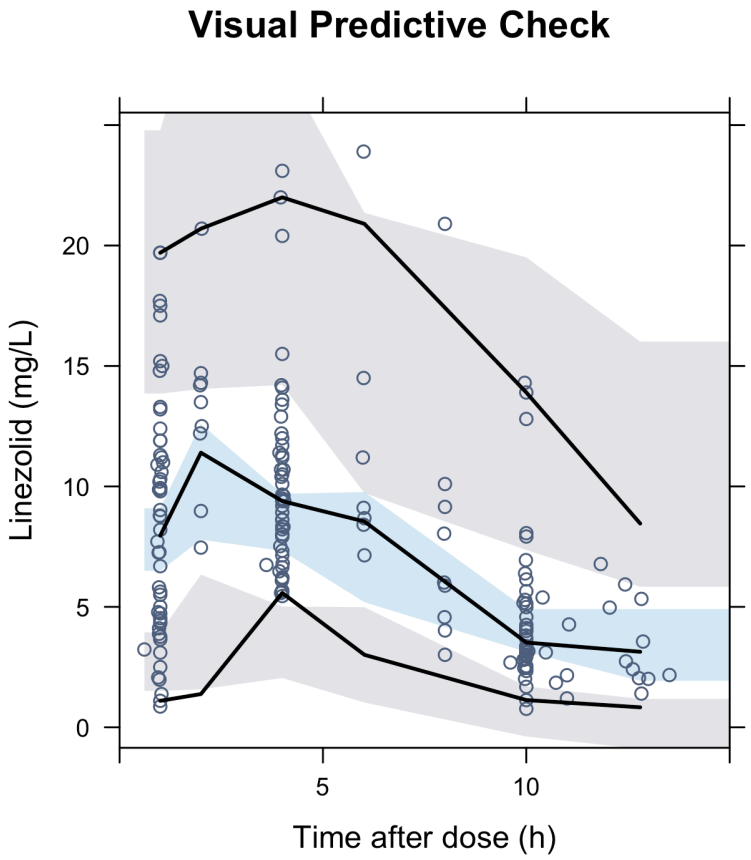

Figure 7.1. Visual predictive checks corresponding to final linezolid pharmacokinetic model. Dots represent the observed linezolid concentrations; the solid lines correspond to the 5th, 50th and 95th percentiles of these observations. Shaded areas are the model-predicted 95% confidence intervals for the median (blue), and 5th and 95th (grey) percentiles obtained from 500 simulated datasets.

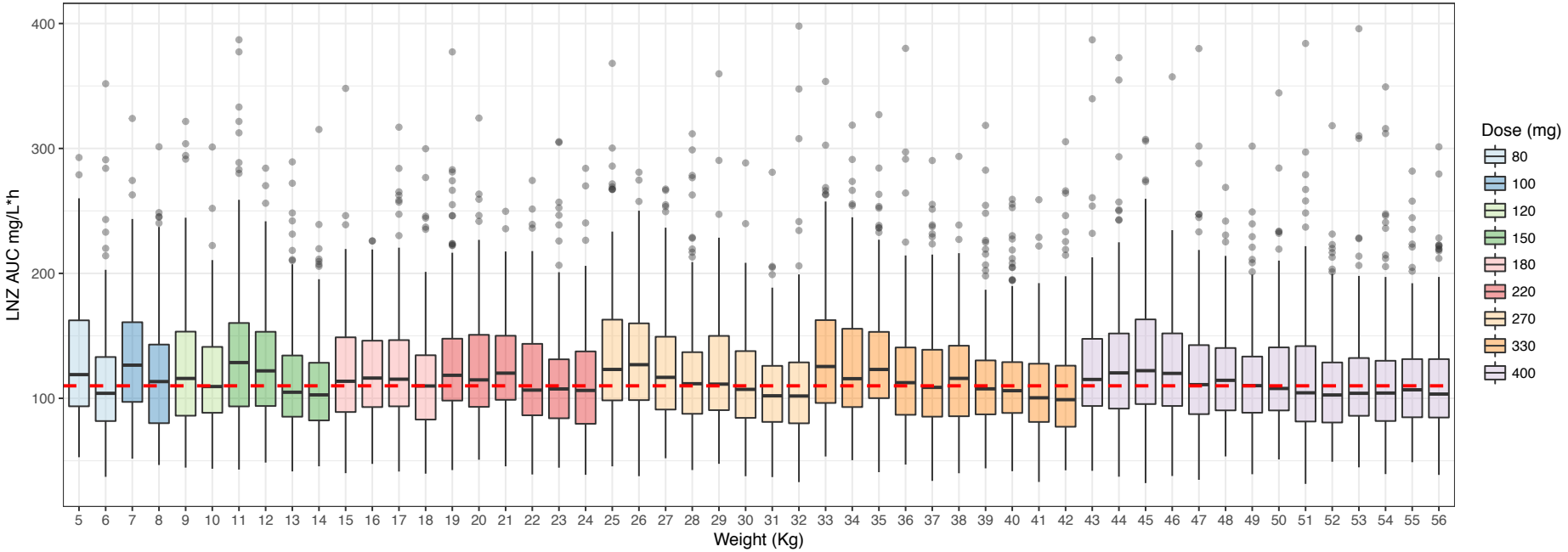

Figure 7.2. Simulated linezolid area under the concentration time curve from 0-24 hours versus body weight using the linezolid doses listed. The red hashed line indicates the adult target exposure ($AUC_{0\text{-}24ss}$ 110 mg/L·h). LNZ=linezolid; AUC=area under the concentration time curve.

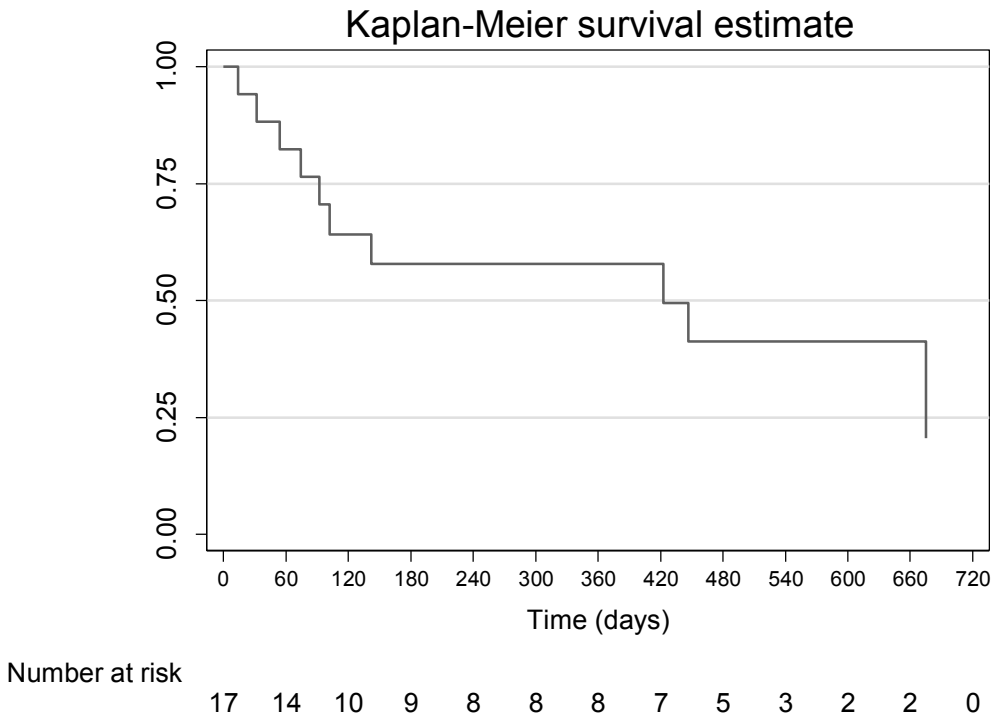

Figure 7.3. Kaplan-meier estimates of the time (in days) on linezolid without an adverse event of any grade at least possibly related to linezolid.

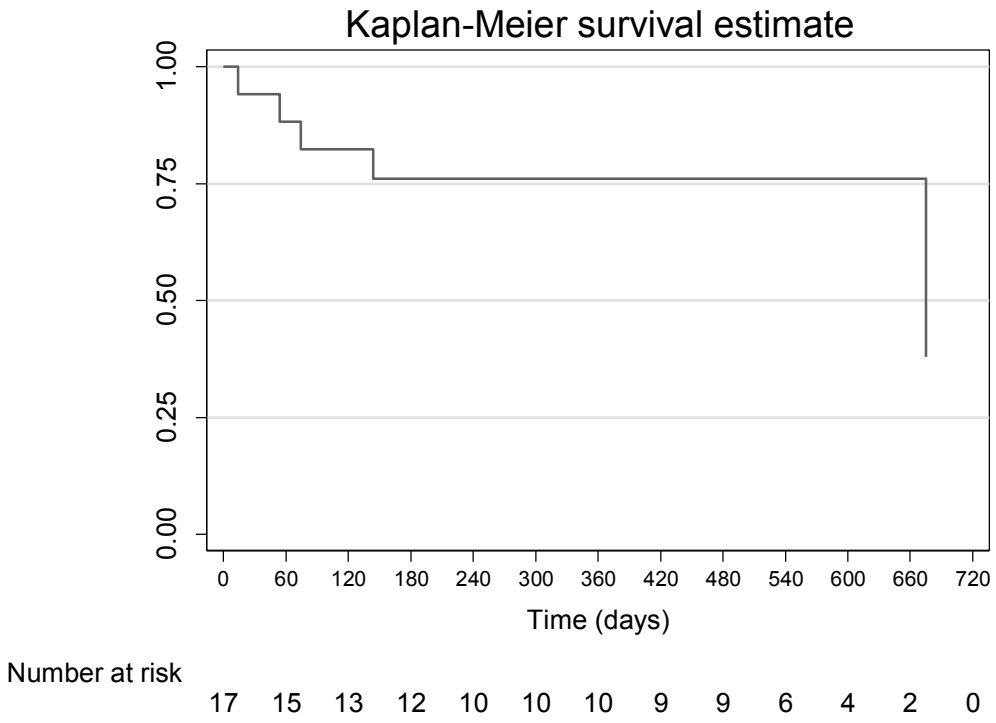

Figure 7.4. Kaplan-meier estimates of time (in days) on linezolid without a Grade 3 or 4 adverse event at least possibly related to linezolid

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Chapter 8: Effect of coadministration of lidocaine on the pain and pharmacokinetics of intramuscular amikacin in children with multidrug-resistant tuberculosis: a randomized crossover trial

Rationale

Current guidelines continue to recommend a second-line injectable antituberculosis medication (amikacin, kanamycin or capreomycin) for 4-6 months for most children treated for MDR-TB (18). This long duration of second-line injectable treatment is associated with permanent sensorineural hearing loss in up to 24% of children (45). In addition to this high risk of ototoxicity, the injectables are required to be given parenterally. Long-term indwelling catheters are not feasible in most high TB burden settings and pose a high risk of infection, so the majority of MDR-TB patients receive these medications as daily intramuscular injections, which are very painful and a source of significant distress (59). Treatment regimens that no longer rely on injectable medications are urgently needed. However, in the meantime temporizing strategies to improve the tolerability of the required intramuscular injections would greatly benefit MDR-TB patients. The addition of the anaesthetic agent lidocaine (lignocaine) to intramuscular injections of other antibiotics has been shown to reduce the associated pain without affecting the medication's pharmacokinetics (116, 117), but this has not been evaluated with the second-line injectables.

Study Aims

The aim of the study was to evaluate the effect of coadministering lidocaine on the pain and pharmacokinetics of intramuscular amikacin in children with multidrug-resistant tuberculosis.

Methods

This randomized double-blinded two-period crossover study was a sub-study nested in the MDRPK1 study. Children were eligible for this sub-study if they were enrolled in MDRPK1, were 8 to 18 years of age, were routinely receiving amikacin as part of treatment for MDR-TB, and provided informed consent and assent for participation in this sub-study. Amikacin was routinely given as part of the recommended MDR-TB

treatment regimen at a dose of 15-20 mg/kg once daily 6 days per week, for 2-6 months duration. As part of the study, each participant received two treatments on a single occasion each: Treatment A – amikacin administered with lidocaine; Treatment B – amikacin administered without lidocaine. Participants were randomly assigned to receive Treatment A first or Treatment B first (sequence 1 or 2 respectively). Amikacin, available as 500 mg/2 mL vials (Fresenius, Midrand, South Africa), was administered as an exact 15 mg/kg dose on pharmacokinetic sampling day by intramuscular injection (21 gauge, 1.5 inch needle) to the dorsogluteal area on the opposite side of the previous day's injection. Lidocaine, available as 2% (20 mg/mL) lidocaine solution for injection (Fresenius, Midrand, South Africa), was drawn up with the amikacin into the syringe and administered at a pre-specified weight-banded dose (within a range of 0.2-0.4 mg/kg/dose). The randomization was generated from a computer generated random number list, in blocks of four, and the allocations placed in consecutively numbered, sealed, opaque envelopes. The participants and all team members were blinded to the allocation, other than two study team members who prepared the injections but did not otherwise interact with the participants. On study days, pharmacokinetic sampling was done just before and 1, 2, 4, 6, and 8 hours after an exact 15 mg/kg amikacin dose with or without lidocaine. Amikacin plasma concentrations were measured using a commercial Particle Enhanced Turbidimetric Inhibition Assay at the University of Cape Town Division of Clincial Pharmacology. Pharmacokinetic measures were estimated using NCA. Pain was assessed using the validated Wong-Baker FACES pain scale, just prior to the injection and then immediately, 30 and 60 mins after the injection. All adverse events were reported using the approach described in previous chapters. The median and IQR for each pharmacokinetic measure and for adjusted post-injection pain scores were reported by whether lidocaine was given, and comparisons made using the Wilcoxon matched-pairs signed-rank test.

Results

Between July 2103 and August 2015, 12 participants (median age 11.5 years, IQR 9.9 to 13.4) were enrolled and randomized. The median adjusted pain score immediately after the injection was lower when administered with lidocaine (1.0 [IQR 0.5-2.0] vs. 2.5 [1.0-4.0] without lidocaine (p=0.004); scores at 30 and 60 minutes post-injection were not significantly different. There were no statistical differences in AUC_{0-8}, AUC_{0-inf}, or

C_{max} when administered with or without lidocaine. No adverse events were reported during the study pharmacokinetic sampling days.

Conclusions and recommendations

This trial demonstrated that the coadministration of lidocaine with intramuscular amikacin injections in children with MDR-TB was safe, reduced immediate post-injection pain and did not significantly affect amikacin pharmacokinetic measures. This is an important, simple, inexpensive strategy to improve the tolerability of second-line injectable treatment until effective injectable-free regimens become available.

Citation (this study was accepted for publication and is currently in press):

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Title: Effect of co-administration of lidocaine on the pain and pharmacokinetics of intramuscular amikacin in children with multidrug-resistant tuberculosis: a randomised crossover trial

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Competing interests: Nothing to disclose.

Key words: multidrug-resistant tuberculosis, injectable, lidocaine, amikacin, children

Abbreviated title: Lidocaine administration with IM amikacin in pediatric MDR-TB

Running head: Lidocaine coadministration with IM amikacin

Abstract

Background: Currently recommended treatment for multidrug-resistant (MDR) tuberculosis (TB) includes 4-8 months of an injectable medication, which is poorly tolerated. We evaluated the impact of co-administering lidocaine on pain and pharmacokinetics of intramuscular injections of amikacin in children with MDR-TB.

Methods: Children 8-18 years of age, receiving amikacin for MDR-TB treatment in Cape Town, South Africa, were eligible for this randomized crossover trial. Participants received a 15 mg/kg dose of intramuscular amikacin with and without additional lidocaine (0.2-0.4 mg/kg) on different days, and were randomized to the order of the treatments (the sequence). Participants and staff completing evaluations were blinded to sequence. Samples were drawn pre-dose, and at 1, 2, 4, 6 and 8 hours post-dose for measurement of plasma amikacin concentrations. Pain was assessed by participants using the Wong Baker FACES pain scale (0 to 5) pre-dose, immediately after the injection and then at 30 and 60 minutes. Pharmacokinetic measures were calculated using noncompartmental analysis.

Results: Twelve children were included, median age 11.5 years (IQR 9.9-13.4y). Participant-reported pain scores immediately after the amikacin injection were lower when lidocaine was co-administered: 1.0 (IQR 0.5-2.0) with lidocaine vs. 2.5 (1.0-4.0) without lidocaine (p=0.004). The median area under the concentration time curve $(AUC)_{0\text{-}8}$ and median maximum plasma concentration (C_{max}) of amikacin were 109.0 µg*h/mL (IQR 84.7-121.3) and 36.7 µg/mL (IQR 34.1-40.5) with lidocaine compared to 103.3 µg*h/mL (IQR 81.7-135.0; p=0.814) and 34.1 µg/mL (IQR 35.6-46.4; p=0.638) without lidocaine, respectively.

Conclusions: The co-administration of lidocaine resulted in reduced pain immediately after the injection and did not alter amikacin AUC or C_{max}.

Introduction

Treatment outcomes for children with multidrug-resistant (MDR) tuberculosis (TB), defined as resistance to at least both isoniazid and rifampicin, are good, with more than 80% of children successfully treated (1). However, current MDR-TB treatment regimens are long, requiring 9 to 18 months of treatment, are poorly tolerated, and are associated with frequent and important adverse effects (2). The second-line injectable antituberculosis drugs, including amikacin, kanamycin, and capreomycin, have been considered a key component of MDR-TB treatment, with guidelines recommending 4-8 months of an injectable agent (3-5). However their use can result in nephrotoxicity, electrolyte abnormalities, and is associated with a risk of permanent sensorineural hearing loss, in up to 25% of children (6). Recent guidance from the World Health Organization (WHO) opened the possibility of limiting injectable use in children with less severe TB. However the newly recommended 9-12 month shortened regimen for MDR-TB includes injectables for at least 4 months, indicating that they will likely remain in use for the near future (5).

In addition to the substantial risk of adverse effects with long-term injectable treatment, their use is complicated by the requirement for parenteral administration. Long-term indwelling catheters, such as portacaths or peripherally inserted central-venous catheters are not feasible in most settings with a high burden of MDR-TB. With constrained health care resources, the risk of catheter infection is problematic, and access to rapid, effective treatment of such infections limited. Hence, the vast majority of children with MDR-TB receive these agents as daily intramuscular injections. The injections are painful for adults and children, and have been cited as one of the worst aspects of MDR-TB treatment (7). They are a source of substantial distress for children, their parents and caregivers, as well as for health care workers who are tasked with delivering this painful intervention for months. Strategies to reduce this injection pain and improve the tolerability of the injectables are urgently needed.

Lidocaine, also known as lignocaine, is a local anaesthetic agent, which blocks nerve conduction, producing rapid local anaesthesia lasting 1-3 hours, with a maximum effect within minutes (8). Adverse effects are generally mild. However serious systemic adverse effects may rarely occur if there is inadvertent intravascular injection, including central nervous system effects (paraesthesias, visual disturbance, seizures) and

cardiovascular effects (hypotension, bradycardia, arrhythmia, cardiovascular collapse) (8). When co-administered with intramuscular injections of ceftriaxone (9, 10) and penicillin (11) lidocaine reduced injection pain without affecting the antibiotics' pharmacokinetics. However this strategy has not been evaluated with the second-line injectable antituberculosis medications. The injectables are rapidly absorbed after intramuscular injection and are renally eliminated unchanged (12, 13). Compared to intravenous injection, there may be increased variability in the rate and degree of absorption of intramuscularly injected aminoglycosides (14). The impact of lidocaine on the pharmacokinetics of the injectables needs to be considered, as these injectable medications have concentration-dependent activity against *Mycobacterium tuberculosis*, with the maximum plasma concentration most closely associated with efficacy (15). Ototoxicity is associated with cumulative drug exposure (16).

The objective of this study was to evaluate the impact of co-administering lidocaine on the pain and pharmacokinetics of intramuscular injections of amikacin in children and adolescents routinely treated for MDR-TB.

Materials and methods

Trial design

This was a randomized double-blinded two-period crossover trial.

Participants

Children were eligible to the study if they were 8 to 18 years of age, routinely treated for MDR-TB with a regimen including amikacin, and had received amikacin for at least 14 days. Exclusion criteria included acute illness (enrolment could be deferred), neurologic disability that may have prohibited reporting of pain, or a haemoglobin < 8 g/dL. Consecutively eligible children were recruited from the Brooklyn Chest Hospital, a provincial TB hospital that provides long-term care of children with drug-resistant and other complicated forms of TB in Cape Town, South Africa. A sample size of 12 participants was primarily based on pragmatic considerations of the expected number of eligible children. The treatment of MDR-TB was consistent with local and international guidance, and generally included 6-7 antituberculosis medications given for 12-18 months duration. Amikacin, a second-line injectable antituberculosis

medication, was included in the regimen of most children with MDR-TB, and was given as an intramuscular injection 6 days each week for 2-6 months. Amikacin is recommended for MDR-TB treatment in adults and children, but is used off-label for this purpose.

Interventions

Each participant received two treatments, each on a single occasion. In treatment A, amikacin was administered without lidocaine; in treatment B, amikacin was administered with lidocaine. Participants were assigned 1:1 to receive treatment A or treatment B first (sequence 1 or sequence 2, respectively). Amikacin was available in 2 mL vials as a 500 mg/2 mL solution for injection (Fresenius, Midrand, South Africa), and was administered as an exact 15 mg/kg dose on the day of sampling. A pre-specified weight-banded dose of lidocaine (2% lidocaine solution for injection [20 mg/mL], Fresenius, Midrand, South Africa), within the range of 0.2-0.4 mg/kg/dose was drawn up into a syringe along with the amikacin, to be co-administered (see Table 1); this is well below the maximum safe dose of lidocaine for anaesthesia of 3-4 mg/kg. Intramuscular injections were administered with a 21 gauge 1.5 inch needle in the dorsogluteal area on the opposite side as the previous day's injection, according to standard local practice.

Randomisation and blinding

The randomization was generated by the study statistician using a computer-generated list of random numbers with a permutated fixed block randomization having a block size of 4 to assign the order of injections. Allocations were placed in consecutively numbered, sealed, opaque envelopes (17). Upon enrolment of a participant, two unblinded study team members reviewed the allocation, and on the days of pharmacokinetic sampling, they prepared the amikacin injections with or without lidocaine according to the allocation.

Only these two study team members responsible for preparing the injections and the study statistician were unblinded. None of these unblinded team members participated in administration of the injections or in pain assessments. After preparation of the injections, opaque tape was placed around the syringe to ensure that small differences in volume of the injection would not be visible, to further ensure integrity of the

blinding. The study participant and caregivers, and the remainder of the study team were blinded to the allocation.

Pharmacokinetic sampling

Pharmacokinetic sampling was completed from 2-16 weeks after starting treatment. On the day of sampling the amikacin dose was administered by the study team together with all the other oral TB medications in the child's MDR-TB regimen. One hour after TB medication was dosed HIV-infected children were given their antiretroviral drugs. Blood samples were collected pre-dose and then at 1, 2, 4, 6, and 8 hours after amikacin dosing into an EDTA-containing tube and placed on ice. Blood samples were centrifuged and plasma separated and frozen at -80 degrees Celsius within 30 minutes.

Amikacin plasma concentrations were measured using a commercial Particle Enhanced Turbidimetric-Inhibition Immunoassay (PETINIA) (Architect ci4100, Abbott Laboratories, Diagnostics Division, Abbott Park, IL.). The assay was valid over the range 2.0 – 50 µg/ml and quality controls were run daily to monitor the assay performance.

Data collection

Pain was assessed using the Wong-Baker FACES pain scale, a 5-point hedonic scale, which has been extensively validated for assessing pain in children older than 7 years of age (18, 19). The pain scale was translated into Afrikaans and Xhosa, the most frequently used local languages. Using this scale, the children were asked by a study team member to rate the pain in the dorsogluteal area on the side of the day's IM injection. This was done before the injection, to account for pre-existing pain from previous injections, immediately after the injection, and then at 30 and 60 minutes post-injection. In order to account for pre-existing pain from previous daily injections, adjusted pain scores were calculated by subtracting the pain score taken just prior to the injection, from the pain score immediately after injection, and from the scores at 30 and 60 minutes after the injection. HIV status was determined by HIV ELISA testing in children >18 months of age, and HIV DNA PCR in those <18 months. Weight-for-age z-scores were calculated using the 1990 British growth curves (20). The study team monitored for adverse events related to the injections during the 60 minutes post-injection. Adverse events were graded according to standard grading criteria (21) and attribution assessed by the study investigator.

Statistical methods

Demographic and clinical characteristics were summarized using descriptive statistics. Pharmacokinetic measures were estimated using non-compartmental analysis (NCA). Observed maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were recorded directly from the concentration-time data. The area under the concentration time curve from 0-8 hours (AUC_{0-8}) was calculated using the linear trapezoidal rule. The $AUC_{(0-\infty)}$ was calculated using an exponential extension to the $AUC_{(0-8)}$. Half-life ($t_{1/2}$) was denoted as $\ln(2)/k_{el}$, where k_{el} (elimination rate constant) was the negative slope of the log-linear regression of the three final data points of the concentration-time curve. Pre-dose drug concentrations below the lower limit of quantification (BLQ) (2.0 µg/mL) were set to zero in the analysis. For post-dose concentrations that were BLQ, the first was set to ½ of the lower limit of quantification (LLOQ), and any subsequent were set to zero.

The primary outcome was adjusted pain scores post-injection. Secondary outcomes were C_{max}, $AUC_{(0-8)}$, $AUC_{(0-\infty)}$ and the number of adverse events at least possibly related to the injection.

The median and interquartile range (IQR) for adjusted pain scores immediately, 30 and 60 minutes after injection and pharmacokinetic parameters (C_{max}, $AUC_{(0-8)}$, $AUC_{(0-\infty)}$, T_{max}, and $t_{1/2}$) were reported by whether lidocaine was given. Comparisons for each variable (adjusted pain scores and pharmacokinetic measures) by lidocaine status were made using the Wilcoxon matched-pairs signed-ranks test. Geometric means, the exponentiated arithmetic means of log-transformed values, are often better estimators for comparing pharmacokinetic parameters, which are frequently positively skewed and log-normally distributed. Geometric mean ratios (GMR) were reported with 90% confidence intervals and p-values to determine if treatment status was associated with C_{max}, $AUC_{(0-8)}$, $AUC_{(0-\infty)}$ or T_{max}. A test drug is considered to be bioequivalent to a reference drug if the 90% confidence interval of the GMR of the AUC and C_{max} between the test and reference fall within 80%-125%. Carryover effects for pain and pharmacokinetic measures were assessed statistically using accepted methods, by comparing the mean and median values for the pharmacokinetic parameters and the adjusted pain score outcomes between the two sequences (AB vs. BA) using t-tests and Wilcoxon rank sum tests, respectively (22). Pharmacokinetic parameters and other data

analysis were performed using Stata 14.1 (StataCorp, 2015. *Stata Statistical Software Release 14.* College Station, TX: StataCorp LP.)

This study was approved by the Health Research Ethics Committee of Stellenbosch University (M12/08/043) and the University of Cape Town. Informed consent was provided by the parent or legal guardian and informed assent by the participant. The trial was registered with the Pan-African Clinical Trials registry, registration number PACTR201401000670381.

Results

Between July 2013 and August 2015, 18 participants were screened, with 12 enrolled and randomized (see Figure, Supplemental Digital Content 1). Overall, the median age was 11.5 years (IQR: 9.9 – 13.4); other baseline characteristics are shown by sequence in Table 2. All randomized participants successfully completed the trial.

Adjusted pain scores are shown in Table 3. The median adjusted pain score immediately after the injection was lower when administered with lidocaine added: 1.0 (IQR 0.5-2.0) with lidocaine vs. 2.5 (1.0-4.0) without lidocaine (p=0.004); no significant carryover effects were detected. The median adjusted pain scores 30 and 60 minutes after the injection with lidocaine added were zero, however this was not statistically different compared with injections without lidocaine.

Summary pharmacokinetic measures are shown in Table 4 and Figure 1. All 24 pre-dose concentrations were BLQ, and sixteen 8-hour concentrations were BLQ. One participant had both 6-hour and 8-hour concentrations that were BLQ. There were no statistical differences in $AUC_{0\text{-}8}$, $AUC_{0\text{-}\infty}$ or C_{max} when administered with or without lidocaine (Table 3); no significant carryover effects were detected. GMRs for pharmacokinetic measures of interest are shown in Table 5. The 90% confidence intervals (CIs) for the GMRs are outside of what would be considered bioequivalence (0.80-1.25). Two participants had amikacin exposures that were statistical outliers (one for C_{max}, one for $AUC_{0\text{-}8}$); there was no clinical or laboratory explanation for these extreme values. No adverse events during the two pharmacokinetic sampling days were reported.

Discussion

In this trial we have shown that the addition of lidocaine to amikacin injections in children and adolescents reduced immediate injection pain, was safe, and did not have substantial effects on amikacin pharmacokinetics.

Given previous research with lidocaine co-administered with ceftriaxone and penicillin, it is not surprising that pain was reduced with the addition of lidocaine. In those previous studies, however, lidocaine was used as a diluent to powder for injection, so the final volume for injection was not altered. The amikacin formulation routinely available in our study setting comes as a prepared solution for injection, so the volume for injection was larger when lidocaine was added. Although these additional volumes were relatively small, this may have reduced the anaesthetic effect, as larger volumes would be expected to be associated with increased pain. When lidocaine is used as a diluent instead, it may be that the pain would be reduced even further. Some of the reported pain is likely related to the needle penetrating the skin. In this study we did not use a topical anaesthetic agent, however it is possible that this would further reduce pain and would be an additional measure to improve the tolerability of these intramuscular injections. Although we did not include children aged <8 years in this study, there is no specific reason to believe that the addition of lidocaine to amikacin injections would not also reduce pain in younger children.

Pain at 30 and 60 minutes post-injection was not statistically different with lidocaine. It may be the study was under-powered to detect a difference. However, it is notable that the median adjusted pain score at these time points was zero on the occasion when administered with lidocaine, meaning that pain was no different than prior to the injection, and that in a proportion of the patients the adjusted pain score was less than zero, meaning that after the injection pain was lower than before. It may be that the local anaesthetic reduced the pre-existing pain related to past injections.

There were no significant differences between either the C_{max} or AUC with or without lidocaine. Establishing amikacin bioequivalence between the two treatments was not a pre-specified aim and the study was not powered to formally assess this. The 90% CIs for the GMRs did not fall between the targets for bioequivalence, which may be due to the disproportionate effect of a few outlier concentrations. Substantial between occasion variability of the rate and extent of absorption of injectable medications has

been described (23, 24), and may explain some of the differences we observed between the two treatments in our small sample. Additional work, powered to demonstrate bioequivalence, would confirm more definitively that the addition of lidocaine does not significantly affect amikacin pharmacokinetics. However it is reassuring that there was not a statistical difference in key pharmacokinetic measures in our study.

Carryover effects were expected to be minimal both for pharmacokinetics, as amikacin is rapidly absorbed and eliminated, and for pain, as injections were always given on the alternate side as the previous day's injection and lidocaine has only an intermediate duration of action (hours).

There were no adverse effects noted on the two study days related to the injections. As severe systemic adverse effects are associated with intravascular injection, careful adherence to IM injection administration practices is important. This includes choosing an appropriate injection site and ensuring that the needle is not in a vascular structure after penetration of the needle through the skin and prior to injection. The study was not designed to evaluate the safety of long-term daily intramuscular lidocaine administration. However to our knowledge there is no reason to suspect such adverse effects.

Based on these results, we would argue that the co-administration of lidocaine should become routine practice with intramuscular injections of the second-line antituberculosis drugs in both children and adults with MDR-TB. Lidocaine should be widely available, as it is used routinely for local anaesthesia and is included in the WHO Model List of Essential Medicines. This is likely to be a minimal additional expense for TB programs, particularly given the potential of the intervention to reduce patients' pain and improve the tolerability of this treatment. Although elimination of the need for injectable treatment remains a longer-term priority, this is a safe, feasible and clinically impactful intervention that could immediately be implemented, which substantially reduces the pain associated with these very large number of injections, and could potentially improve the tolerability of MDR-TB treatment.

Acknowledgments

We thank the children who participated in this study and their caregivers.

Table 1. Weight-banded doses and volumes of lidocaine co-administered with amikacin intramuscular injections in children with multidrug-resistant tuberculosis

Body weight	2% lidocaine volume (mg) added	Lidocaine mg/kg dose, range	Amikacin (15 mg/kg) volume, range	Volume of combined injection, range
10 - <20kg	0.2 mL (4 mg)	0.2 – 0.4 mg/kg	0.6 – 1.2 mL	0.8 – 1.4 mL
20 - <30kg	0.3 mL (6 mg)	0.2 – 0.3 mg/kg	1.2 – 1.8 mL	1.5 – 2.1 mL
30 - < 40kg	0.4 mL (8 mg)	0.2 – 0.27 mg/kg	1.8 – 2.4 mL	2.2 – 2.8 mL
40 - <50kg	0.5 mL (10 mg)	0.2 – 0.25 mg/kg	2.4 – 3 mL	2.9 – 3.5 mL
≥50kg	0.5 mL (10 mg)	<0.2 mg/kg	3 mL	3.5 mL

Table 2. Baseline characteristics by sequence for children routinely treated with amikacin for multidrug-resistant (MDR) tuberculosis (TB)

	Sequence 1 (Without lidocaine then with lidocaine) (n=6)	Sequence 2 (With lidocaine then without lidocaine) (n=6)
Median age at enrolment (IQR)	10.3 (9.8-11.4)	13.4 (11.7-14.5)
Male gender (%)	6 (100)	2 (33)
HIV-infected (%)	3 (50)	0 (0)
WAZ < -2 (%)	3 (50)	1 (17)
Median weeks on MDR-TB treatment (IQR)	6.4 (3.0-15.9)	9.5 (5.9-11.7)

IQR=interquartile range, WAZ=weight-for-age z-score

Table 3. Adjusted pain scores among children with multidrug-resistant tuberculosis (n=12) receiving amikacin injections with and without added lidocaine

	Treatment A (without added lidocaine)	Treatment B (with added lidocaine)	p-value*
Median adjusted pain score, immediately after injection (IQR)	2.5 (1 to 4)	1 (0.5 to 2)	0.004
Median adjusted pain score, 30 minutes post-injections (IQR)	1 (0 to 1.5)	0 (-0.5 to 0.5)	0.107
Median adjusted pain score, 60 minutes post injections (IQR)	1 (0 to 1)	0 (-1 to 0.5)	0.075

IQR = interquartile range
* Wilcoxon matched-pairs signed-ranks test

Table 4. Summary statistics for amikacin (15 mg/kg) pharmacokinetic parameters with and without lidocaine among children with multidrug-resistant tuberculosis (n=12)

Pharmacokinetic parameter	Treatment A Without lidocaine, median (IQR)	Treatment B With lidocaine, median (IQR)	p-values*
AUC_{0-8} (µg*h/mL)	103.3 (81.7-135.0)	109.0 (84.7-121.3)	0.814
$AUC_{0-\infty}$ (µg*h/mL)	107.5 (83.8-136.8)	111.3 (86.8-126.4)	0.754
C_{max} (µg*h/mL)	34.1 (25.6-46.4)	36.7 (34.1-40.5)	0.638
$t_{1/2}$ (h)	1.28 (1.14-1.49)	1.50 (1.27-1.62)	0.182
T_{max} (h)	1 (1.0-1.0)	1 (1.0-1.0)	0.157

*Wilcoxon matched-pairs signed-ranks test
AUC=area under the concentration time curve, C_{max}=maximum plasma concentration, $t_{1/2}$=half-life, T_{max}=time to C_{max}

Table 5. Geometric means with and without lidocaine, geometric mean ratio and 90% confidence interval for amikacin (15 mg/kg) pharmacokinetic parameters (n=12)

Pharmacokinetic parameter	Geometric mean (GM)		GM Ratio (90% CI)*
	Treatment A Without lidocaine	Treatment B With lidocaine	
AUC_{0-8}	93.8	110.6	1.18 (0.89-1.56)
$AUC_{0-\infty}$	96.2	114.3	1.19 (0.90-1.57)
C_{max}	31.8	37.6	1.18 (0.92-1.52)

* Treatment B/Treatment A

AUC=area under the concentration time curve; C_{max}=maximum plasma concentration; CI=confidence interval

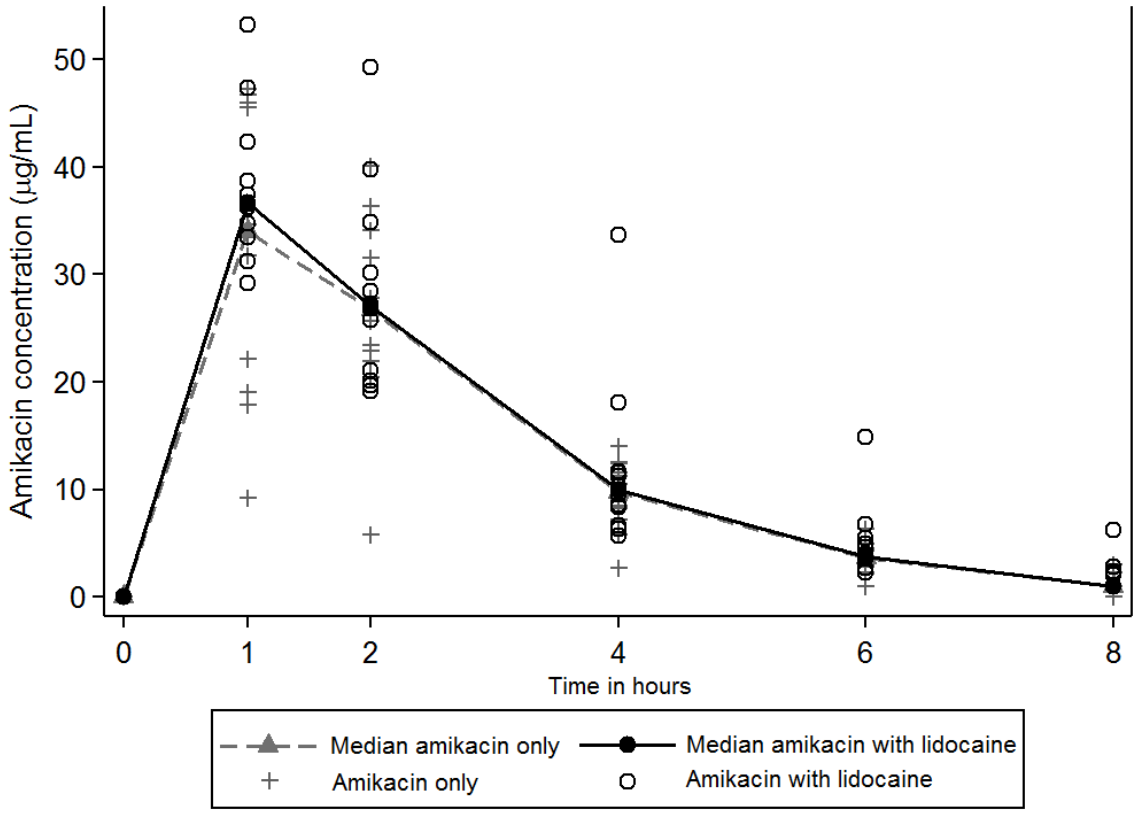

Figure 1. Concentration-time profiles of intramuscular amikacin with and without lidocaine among children (n=12) treated for multidrug-resistant tuberculosis

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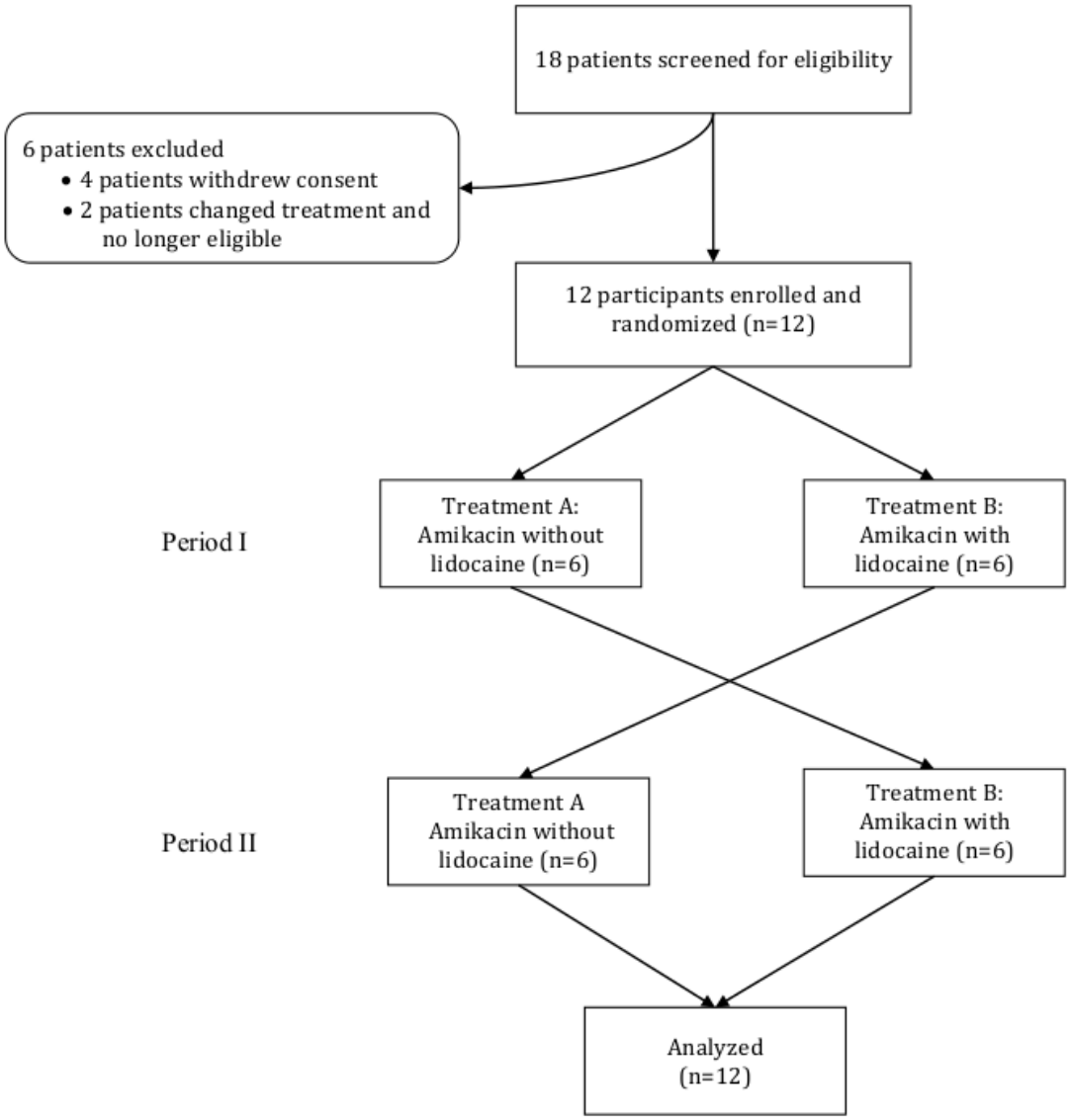

Supplemental Digital Content 1. Figure (Consort flow diagram)

Chapter 9: The relative bioavailability of adult bedaquiline tablets suspended in water: implications for dosing in children

Rationale

The novel antituberculosis drug bedaquiline is increasingly important for the treatment of MDR-TB. Following on its accelerated approval from the U.S. FDA in 2012, it is being rolled out globally for adults with MDR-TB and is being evaluated in multiple clinical trials in adults as a component of shortened treatment regimens (57, 118). The Janssen Pharmaceutical sponsored trial (C211) and the U.S. NIH (NIAID, NICHD) sponsored trial (IMPAACT P1108) have been very delayed, opening only in 2016 and 2017 respectively. These trials are expected to identify the optimal dose and safety of bedaquiline in HIV-infected and -uninfected children. Although a paediatric formulation has been developed for the C211 trial, it was not clear whether this formulation would be available for the IMPAACT P1108 trial or when it would be accessible for routine use in high TB burden settings. The adult formulation is widely available and could be manipulated (crushed, suspended in water) for administration to young children. However it is unclear whether or how this formulation manipulation might affect bedaquiline's bioavailability.

Study Aims

The aim of the study was to evaluate the relative bioavailability, short-term safety, acceptability and palatability of suspended bedaquiline tablets compared to whole tablets, in order to explore the possibility of using the existing tablet formulation in young children.

Methods

This randomized, open-label, two-period crossover study was conducted in Cape Town, South Africa. As bedaquiline and its M2 metabolite accumulate in cells and tissues, resulting in long terminal elimination half-lives (bedaquiline 164 days, M2 159 days), a crossover study using a traditional non-compartmental analysis would have required an exceedingly long washout period to avoid carryover into the second period. Alternatively a parallel design could be employed, but would require a much larger sample size. Therefore a model-based analytic approach was utilized for the study,

which is able to account for the carryover effect, allowing a more efficient crossover design. The sample size, length of the washout period and sampling schema were selected using an existing, published bedaquiline population pharmacokinetic model and clinical trial simulations (138). With the selected design (24 participants receiving two single doses separated by a 14 day washout period), the trial had 87% power to demonstrate bioequivalence. Healthy adult volunteers, ages 18-55 years were eligible for the study. All participants received two single doses of 400 mg bedaquiline, including one dose administered as 4 x 100 mg tablets swallowed whole with 240 mL water, and one dose administered as a bedaquiline suspension, prepared by adding 4 x 100 mg tablets to 30 mL clean water in a plastic dosing cup, using a metal spoon to stir and break up the tablets over 2 minutes and followed by two small rinses. Participants were randomized 1:1 to the order of the treatments. The randomization was generated using a computer generated random numbers in a single block, and allocations were concealed using sealed, consecutively numbered, opaque envelopes. Pharmacokinetic samples were drawn just before and at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48 and 336 hours after each dose. All adverse events were recorded and assessed for attribution to the study medication and for severity using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, dated November 2014. A palatability/acceptability questionnaire was administered to each participant within one hour of each dose. Bedaquiline and M2 plasma concentrations were measured using a validated LC MS/MS assay at the University of Cape Town, Division of Clinical Pharmacology. Non-linear mixed effects models were used to estimate the pharmacokinetic parameters, and secondary pharmacokinetic metrics (area under the concentration curve until 48 and 336 hours after dose, i.e. $AUC_{0\text{-}48h}$ and $AUC_{0\text{-}336h}$, and time and magnitude of peak concentrations, i.e. T_{max} and C_{max}) were derived from the final model. The bioequivalence of bedaquiline tablets swallowed whole vs. suspended in water, based on the primary pharmacokinetic parameter affecting the extent of absorption, i.e. the bioavailability, would be assessed.

Results

Twenty-four adult volunteers were enrolled, randomized and all completed the study. The existing bedaquiline model described the data well. The difference in bioavailability between suspended and whole tablets was not statistically significant

(p=0.92). The nonparametric 95% confidence interval (CI) of the relative bioavailability of suspended bedaquiline tablets was 94-108% relative to that of whole bedaquiline tablets; hence the predefined bioequivalence criteria (80-125%) were fulfilled. The mean absorption time was slightly longer for suspended tablets, +23% (95% CI 2.1-48%, p=0.03). There were no Grade 3 or 4 or serious treatment emergent adverse events. The majority of participants were either neutral to or liked most individual aspects of acceptability, and twenty-three of 24 participants (96%) reported the suspension to be acceptable overall.

Conclusions and recommendations

Bedaquiline tablets suspended in water were bioequivalent to bedaquiline tablets swallowed whole, and were acceptable to this group of healthy adult volunteers. This suggests that the bedaquiline formulation, increasingly widely available, could be used to treat MDR-TB in children, to bridge the gap between when paediatric dosing regimens have been established and when a paediatric dispersible formulation becomes routinely available, improving access for children to this much needed treatment in the near future. This should not be used to delay or negatively affect timely roll-out of the paediatric formulation as soon as possible. Replication of this pragmatic approach should be considered for other medications, such as delamanid and other antituberculosis drugs where access to child-friendly formulations is unlikely in the short-term. Additionally, this study demonstrated the value of pharmacometric methods, which were critically important for this crossover study with a long half-life drug, but which is also valuable in the design and analysis of other paediatric trials.

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Relative bioavailability of bedaquiline tablets suspended in water: implications for dosing in children

Running title: Bioavailability of suspended bedaquiline

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Abstract

Objectives: Bedaquiline is an important novel drug for treatment of multidrug-resistant tuberculosis, but no paediatric formulation is yet available. This work aimed to explore the possibility of using the existing tablet formulation in children by evaluating the relative bioavailability, short-term safety, acceptability and palatability of suspended bedaquiline tablets compared to whole tablets.

Methods: A randomized, open-label, two-period cross-over study was conducted in 24 healthy adult volunteers. Rich pharmacokinetic sampling over 48 hours was conducted at two occasions 14 days apart in each participant after administration of 400 mg bedaquiline (whole or suspended in water). The pharmacokinetic data were analysed with nonlinear mixed-effects modelling. A questionnaire was used to assess palatability and acceptability.

Results: There was no statistically significant difference in the bioavailability of the suspended bedaquiline tables compared to whole. The nonparametric 95% confidence interval of the relative bioavailability of suspended bedaquiline tablets was 94-108% of that of whole bedaquiline tablets; hence the predefined bioequivalence criteria were fulfilled. There were no Grade 3 or 4 or serious treatment emergent adverse events recorded in the study and no apparent differences between whole tablets and suspension regarding taste, texture or smell.

Conclusions: The bioavailability of bedaquiline tablets suspended in water was the same as for tablets swallowed whole and the suspension was well tolerated. This suggests that the currently available bedaquiline formulation could be used to treat MDR-TB in children, to bridge the gap between when paediatric dosing regimens have been established and when a paediatric dispersible formulation is routinely available.

Keywords: bedaquiline, paediatric dosing, bioavailability, suspended tablets, population PK

What is already known about this subject

- There is currently no paediatric formulation available of the novel anti-tuberculosis drug bedaquiline

- Important absorption properties such as the bioavailability can be altered if a drug formulation is manipulated to enable administration to children, for example through crushing or suspending the tablets

What this study adds

- A precise estimate of the relative bioavailability of bedaquiline tablets suspended in water compared to when administered whole
- Support for the use of suspended bedaquiline tablets as an option for treatment of multidrug-resistant tuberculosis in children

Introduction

Bedaquiline, the first novel antituberculosis drug developed in decades, is increasingly used for treatment of multidrug-resistant (MDR) tuberculosis (TB). Based on phase II trial data it received accelerated approval from the U.S. Food and Drug Administration in 2012 and is included on the World Health Organization (WHO) list of essential medicines. Bedaquiline is being rolled out for programmatic use globally (1). Janssen Pharmaceuticals has developed a paediatric dispersible formulation of bedaquiline and paediatric bedaquiline trials are now underway (Janssen C211, NCT02354014 and IMPAACT P1108, NCT02906007). However, considerably more time is needed for this formulation to become available for widespread routine care, limiting bedaquiline's immediate potential for use in young children.

Bedaquiline is a diarylquinoline that inhibits mycobacterial ATP-synthase, resulting in potent antimycobacterial activity (2). The recommended adult dose is 400 mg daily for two weeks, then 200 mg thrice weekly for 22 weeks. In adults, the time to maximum bedaquiline serum concentrations is 4-6 hours, with 2.0-2.4 fold increased absorption when administered together with food (3). Absolute bioavailability, *i.e.* the fraction of the total dose administered which is absorbed to the systemic blood circulation, for bedaquiline is not known (4). Bedaquiline is primarily metabolized by CYP3A4 to M2 (3). This leads to drug-drug interactions with several antiretroviral and anti-TB compounds which induces or inhibits CYP3A4 (5–7). Both bedaquiline and its M2 metabolite have cationic amphiphilic properties. They bind to phospholipids and accumulate in cells and tissues, resulting in long terminal elimination half-lives (bedaquiline 164 days; M2 159 days) due to the slow release from tissues (3).

Bedaquiline 100 mg tablets are increasingly available for the treatment of adults with MDR TB, with global access improving rapidly (8,9). These tablets could potentially be used for treatment of children. However administration of these tablets to young children who cannot swallow tablets may require suspending or crushing the adult formulation. Such formulation manipulation for paediatric administration, which is commonly done for most second-line TB drugs given in children, may affect the bioavailability (10). Characterizing the effect of suspending bedaquiline tablets on the bioavailability would inform the safe and effective use of this formulation in young

children, potentially accelerating access to this much needed medication, given limited treatment options and availability of child-friendly formulations in children.

The primary objective of this study was to evaluate the bioequivalence of bedaquiline tablets swallowed whole vs. suspended in water, based on the primary pharmacokinetic parameter affecting the extent of absorption, i.e. the bioavailability. Secondary objectives were to determine the impact of suspending the tablets on the time-course of absorption, and to describe the palatability, acceptability and safety of whole vs. suspended bedaquiline.

Methods

Study design and participants

This was a randomized, open-label, two-period cross-over study. Healthy male and female adults were eligible if they were 18-55 years of age and weighed between 40-90 kg. Exclusion criteria included a history or clinical evidence of any of the following: QT prolongation, dysrhythmia, or other significant cardiac conditions; other serious comorbid illness including but not limited to liver disease, kidney disease, HIV infection, Hepatitis B or C infection, hypothyroidism; use of QT prolonging medications or CYP3A4 inducers or inhibitors; suspected or documented current active tuberculosis or recent household tuberculosis exposure. The study was conducted in Cape Town, South Africa, from November to December 2016.

The sample size, the length of the washout-period and the number and timing of pharmacokinetic samples were selected with help of clinical trial simulations. These were conducted with stochastic simulation and re-estimation (SSE) procedures, using a published population model of bedaquiline and M2 pharmacokinetics developed on data from healthy volunteers (5). The designs evaluated included washout-periods between 2 and 28 days long, and 11 to 17 samples per dosing occasion. One hundred virtual trials were simulated for each design, assuming no difference in absorption characteristics for whole and suspended tablets. Model parameters were re-estimated including factors allowing for differences in bioavailability, and delay and rate of absorption, between whole and suspended tablets. Effects on distribution- and elimination-related parameters were not evaluated, as these cannot be affected by the

drug formulation. The power to show bioequivalence under the suggested design given that the two forms are truly equal, were evaluated by calculating in how many of the 100 trials the 95% confidence interval of the factors describing a difference between whole and suspended tablets were fully contained within the bioequivalence criteria, defined as 80-125% of the expected value (11). The selected design (described below) was estimated to have an 87% power (95% confidence interval 80-94%) to fulfil the formal bioequivalence criteria for bioavailability.

Intervention and randomization

The selected design included 24 participants who received a single dose of each treatment (formulation), with a washout period of 14 days between the two dosing occasions. Each bedaquiline tablet (Sirturo™, Janssen Pharmaceuticals) contains 120.89 mg of bedaquiline fumarate drug substance, which is equivalent to 100 mg of bedaquiline. As tablets, 400 mg bedaquiline was administered as 4 x 100 mg tablets swallowed whole with 240 mL water. A bedaquiline suspension was prepared by adding 4 x 100 mg tablets to 30 mL clean water in a plastic dosing cup, using a metal spoon to stir and break up the tablets over 2 minutes. The suspension was administered within 5 minutes of adding the tablets to the water. An additional 20 mL of water was added to the dosing cup to rinse any residual medication from the cup and stirrer, and administered to the participant. Lastly, another 10 mL of water was added to the cup for a final rinse and then administered to the participant. All doses were administered within 30 minutes after a standardized breakfast consisting of approximately 670 kcal with at least 33% fat content.

Participants were randomized 1:1 to having bedaquiline administered as whole tablets at the first occasion, and then as tablets suspended in water on the second occasion, or the reverse. The randomisation scheme was created using computer generated random numbers in a single block to ensure equal numbers of participants were assigned to each treatment sequence. Consecutively numbered, sealed, opaque envelopes were prepared by the study pharmacist and stored in a secure location at the site. After enrolment of a participant, the research pharmacist opened the next consecutively numbered envelope in order to determine the participant's allocation.

Data collection

Pharmacokinetic samples were drawn just before and at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48 and 336 hours after each dose (the 336 hour sample for the first occasion also serving as the predose sample for the second). Whole blood samples were drawn into EDTA-containing tubes and immediately placed on ice. Samples were centrifuged at 1500-2000g within one hour, plasma was separated and stored at -80 C until bioanalysis was performed.

All treatment emergent laboratory and clinical adverse events were recorded by the study team, and assessed for attribution to the study medication and for severity using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, dated November 2014 by the site investigators. Twelve-lead electrocardiograms (ECGs) were done at screening and on dosing days, just prior to the dose and at 4 hours post-dose. The QT interval was calculated using the Frederica correction and captured along with other clinically significant abnormalities. Safety lab monitoring included haematology, liver function tests, and lactate.

A palatability/acceptability questionnaire was administered to each participant within one hour of each dose. The questionnaire utilized a 5-point facial hedonic scale to assess taste, smell, visual appearance, texture, size/volume, and overall acceptability of both treatments.

Bioanalysis

Bedaquiline and M2 concentrations were determined using a validated LC-MS/MS assay developed in the Division of Clinical Pharmacology, University of Cape Town, South Africa, validated according to FDA and EMA guidelines, as previously described (12) and further detailed in the online supplementary material. The assay was validated over the concentration range of 0.01 – 5 µg/ml for BDQ and 0.01 – 0.5 µg/ml for M2 (lower and upper limit of quantification). During sample analysis, the accuracies (% Nom) for bedaquiline were 102.6%, 102.0% and 99.3% at the high (4 µg/ml), medium (2 µg/ml) and low (0.024 µg/ml) QC levels respectively with precision (% CV) less than 10% across all three levels. The accuracies for M2 were 99.1%, 99.3% and 96.7% at the high

(0.40 µg/ml), medium (0.20 µg/ml) and low (0.024 µg/ml) QC levels, respectively, with precision (% CV) less than 7%.

Pharmacokinetic analysis

Nonlinear mixed-effects models, able to characterize both typical parameter values and distributions of random inter-individual and inter-occasion variability (IIV and IOV), as well as unexplained residual variability, were employed for the analysis. The previously developed population pharmacokinetic model utilized in the clinical trial simulations was used as a starting point (5). This model included three distribution compartments for bedaquiline and two for M2. Each bedaquiline dose was defined as a separate occasion. IIV and IOV were implemented with log-normal distributions. A correlation between the residual errors for observations at the same time point was included. Concentration measurements below the limit of quantification were excluded from the analysis. Model selection was based on maximum likelihood ratio test (95% significance level) and goodness-of-fit plots, including visual predictive checks based on simulations from the final model (n=1000). Secondary PK metrics (area under the concentration curve until 48 and 336 hours after dose, i.e. $AUC_{0\text{-}48h}$ and $AUC_{0\text{-}336h}$, and time and magnitude of peak concentrations, i.e. T_{max} and C_{max}) were derived from the final model.

Data management, post processing of results and plotting were performed in R (R Foundation for Statistical Computing, Vienna, Austria)(13). The modelling and simulations were performed in NONMEM 7.3 (Icon Development Solutions, Ellicott City, MD, USA)(14), aided by PsN (Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden) and Pirana (Pirana Software & Consulting, San Francisco, CA, USA)(15). Parameter uncertainty was obtained from the covariance step in NONMEM. Additionally, log-likelihood profiling (as implemented in PsN) was used to obtain non-parametric confidence intervals for the parameters describing potential effects of suspending tablets.

Ethics

This study was approved by the Pharma-Ethics Independent Research Ethics Committee of South Africa (#141110730). All participants provided written informed consent. The trial was registered at clinicaltrials.gov with identifier NCT03032367.

Results

Study participants and data

All 24 participants completed the study. A summary of the demographic characteristics can be found in Table 1. Study investigators noted that the tablets did suspend in this volume of water over 2 minutes, although some amount of stirring and breaking up the tablets with the spoon handle were required. Some visual particles usually remained, which were successfully suspended by the described rinses. There were 552 concentration observations each for bedaquiline and M2 available. All predose observations at the first sampling occasion as well as five postdose bedaquiline and 81 postdose M2 observations were below the limit of quantification. The average concentration of bedaquiline and M2 per nominal time point is shown in supplemental figure S1.

Pharmacokinetic analysis

The starting model generally described the data well, and only a few modifications were required. A six hour maximum limit for the mean absorption time (i.e. the typical time to when 90% of the dose is absorbed) was added to the flexible transit compartment model describing absorption, consistent with another recently published model of bedaquiline pharmacokinetics (16). The absorption model was simplified without a statistically significant loss of fit by making the rate of absorption from the last transit compartment the same as the rate of transfer between the transit compartments.

The difference in bioavailability between suspended and whole tablets was not statistically significant (p=0.92). The nonparametric 95% confidence interval (CI) of the relative bioavailability of suspended bedaquiline tablets was 94-108% relative to that of whole bedaquiline tablets; hence the predefined bioequivalence criteria (80-125%) were fulfilled. Inter-individual variability in bioavailability was not significantly different between whole and suspended tablets. The mean absorption time was slightly longer for suspended tablets, +23% (95% CI 2.1-48%, p=0.03). In the final model, only the formulation effect on mean absorption time was included. Parameter estimates with uncertainty are reported in Table 2, and the NONMEM control stream detailing the parametrization is included in the online supplementary material. The fit of the model to the observed data per formulation and sequence are shown in Figure 1 and 2 for

bedaquiline and M2, respectively. The typical pharmacokinetic profile after a single 400 mg dose bedaquiline administered either as whole or suspended tablets are demonstrated in Figure 3. Secondary PK metrics ($AUC_{0\text{-}48h}$, $AUC_{0\text{-}336h}$, C_{max} and T_{max}) are reported in Table 3 and Supplemental Table S1 to facilitate comparison with other clinical studies.

Safety, palatability and acceptability

There were no Grade 3 or 4 or serious treatment emergent adverse events, nor any treatment adverse events leading to withdrawal from the study. All adverse events and all potentially bedaquiline-related treatment emergent adverse events are shown in Supplemental Tables S2 and S3 by grade of severity. The most frequent event (n=7) was mild or moderate headache. No participant had a QTcF >450 ms at any point during the study. No lactate levels above 3 mmol/L were found. Table 4 shows results of the acceptability and palatability questionnaire by formulation (whole tablets vs. suspension). The large majority of participants (88-100%) were either neutral to or liked most aspects of the bedaquiline suspension palatability, such as taste, smell, texture. Twenty-three of 24 participants (96%) reported the suspension to be acceptable overall.

Discussion

This study demonstrates that a 400 mg dose of bedaquiline given as 100 mg tablets suspended in a small volume of water had equivalent bioavailability to bedaquiline administered as 100 mg tablets swallowed whole. The suspended bedaquiline tablets were considered by the majority of participants to be palatable and acceptable.

The mean absorption time for suspended bedaquiline was found to typically be 23% longer for suspended tablets. This translates to a delay in the time to peak bedaquiline concentrations from 4.3 to 5.2 hours, and a decrease in typical maximal concentrations of 5%, but no change in the average concentration. Given that average rather than peak concentrations have been linked to bedaquiline efficacy (17), we do not expect the effect of suspending on mean absorption time to be clinically relevant. The bedaquiline exposures observed in this study (see Table 3) were somewhat lower compared to other studies in healthy volunteers with similar design and the same dose. Dooley *et al.*

reported bedaquiline $AUC_{0\text{-}336h}$ of 58200 (42200–78200) ng/mL*h (18), and Winter *et al.* reported 67200 (standard deviation 20200) ng/mL*h (19), while the median in this study was 44000 ng/mL*h. This might be explained by the larger proportion of black subjects included here (88%) compared to in the studies by Dooley and Winter (22% and 6%, respectively), since black race has been associated with higher bedaquiline clearance (16).

Our findings have important implications for the clinical use of bedaquiline 100 mg tablets in young children, who will likely not have access to paediatric bedaquiline formulations in routine care settings in the near future. Data on the safety and dosing of bedaquiline from paediatric trials across the age range (0-17 years) is expected to be available long before the paediatric formulation used in the studies is registered and widely available, as there are many barriers preventing access to TB medications in children (20), including the development, manufacture, licensure, procurement and uptake of paediatric drug formulations. Given no other choice due to the lack of child friendly formulations, adult formulations manipulated either by splitting, crushing, dissolving or suspending are frequently used in paediatric TB care, especially for MDR-TB (10). However the impact of such manipulation on drug exposures or formulation acceptability is often unknown. Data from our study addresses these questions for bedaquiline and will facilitate the use of bedaquiline in children with the already widely available adult 100 mg tablets, once paediatric dosing and safety is established, and until the paediatric formulation becomes widely available in the field. It is reassuring that the vast majority of adults found the suspended tablets to be palatable and acceptable, a critical consideration for children's medication and adherence to long-term treatment. Although children may have different perceptions of palatability and acceptability, the data suggests that poor palatability or acceptability are unlikely to be major barriers to use of suspended bedaquiline tablets in children.

The design of this study was supported by clinical trials simulations to ensure adequate statistical power. The data was analysed with a model-based approach to handle the extremely long terminal half-life of bedaquiline and M2 and expected carry-over between the sampling occasions, avoiding the risk of bias associated with non-compartmental analysis in such cases (21). However, the final model has some limitations. Individual bedaquiline profiles showed a tendency towards having dual

peaks. This is not accounted for in the structure of the final model, but simply handled by the larger residual error estimated for the absorption phase (first 6 hours after dose). An expanded structural model including enterohepatic circulation linked to meal times was evaluated, but did not improve the model fit to the data. Furthermore, concentration observations below the limit of quantification were excluded in this analysis. For bedaquiline, the proportion of samples below limit of quantification was very low (<1%), hence the exclusion is not expected to impact our results. For M2, the proportion of samples below the limit of quantification was larger (15%) and 95% of occurred within the first 4 hours after dose administration. This may have influenced the M2 parameter estimates. However, predictions from the final model at the time points of samples with below limit of quantification results were in 77 of 81 cases below two times the quantification limit, indicating a reasonable description also at low concentration levels.

This study demonstrating bioequivalence of 100 mg bedaquiline tablets suspended in water vs. swallowed whole will support the use of bedaquiline for MDR TB in children. Similar work would be beneficial for other novel TB medications in the future. While this data addresses an immediate gap in medication formulation availability for children, it should not result in delays or limit the development and availability of an affordable child-friendly formulation of bedaquiline, preferably a palatable dispersible scored formulation. Equitable access to child-friendly formulations of life-saving medications must continue to be a priority for the TB community.

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Conflict of interest

AD, BS, EMS, JB, JN, LW, RK, SN, AGP and VRJ have no conflict of interest to declare. ACH chairs the IMPAACT P1108 study protocol (NCT02906007).

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Table 1. Summary of the demographic characteristics of the study participants.

Characteristics [unit]	**All participants Median (range)/n (%)**	**Whole tablets first Median (range)/n (%)**	**Suspension first Median (range)/n (%)**
N	24	12	12
Weight [kg]	63.4 (45.6, 88.5)	66.15 (45.6, 84.7)	61.85 (53.3, 88.5)
Age [years]	23.5 (19, 37)	22 (19, 26)	24.5 (20, 37)
Female sex	15 (62.5%)	8 (66.7%)	7 (58.3%)
Race Black Mixed race	 21 (87.5%) 3 (12.5%)	 11 (91.7%) 1 (8.3%)	 10 (83.3%) 2 (16.7%)

Table 2. Parameter estimates for the final model including uncertainty.

Structural parameters	**Parameter value**	**Relative standard error**
MAT [h]	2.63	5.0%
NN	4.00	10.9%
CL_{BDQ}/F [L/h]	5.67	10.1%
V_{BDQ}/F [L]	130	6.1%
$Q_{BDQ,1}$/F [L/h]	6.33	9.6%
$VP_{BDQ,1}$/F [L]	3020	28.0%
$Q_{BDQ,2}$/F [L/h]	4.83	15.5%
$VP_{BDQ,2}$/F [L]	64.5	13.1%
CL_{M2}/F/fm [L/h]	17.2	11.8%
V_{M2}/F/fm [L]	1380	9.2%
Q_{M2}/F/fm [L/h]	126	12.9%
VP_{M2}/F/fm [L]	3450	11.7%
Weighting residual error samples 0-6h	1.67	5.6%
Effect of suspending on MAT [%]	23	43.0%
Variability between individuals and occasions		
IOV F	9.1%	23.8%
IIV F	22.6%	12.8%
IOV MAT	66.3%	9.4%
IIV CL_{BDQ}	17.1%	23.2%
Correlation IIV CL_{BDQ}-CLM2	8.5%	5.4%
IIV CL_{M2}	20.5%	23.4%
IIV V_{BDQ}	28.3%	15.5%
IIV $Q_{BDQ,1}$	17.3%	25.7%
IIV V_{M2}	26.3%	25.3%
IIV VP_{M2}	22.3%	35.6%
Residual variability		
Proportional error BDQ	23.1%	4.0%
Correlation error BDQ-M2	53.1%	11.7%
Proportional error M2	11.4%	3.7%

Abbreviations: BDQ, bedaquiline; M2, metabolite M2; MAT, mean absorption time; NN, number of transit compartments; CL, clearance; V, volume of distribution central compartments; Q, intercompartmental clearance; VP, volume of distribution peripheral compartments; F, bioavailability; IOV, inter occasion variability; IIV, inter individual variability

Table 3. Summary of secondary PK metrics after two single doses of 400 mg bedaquiline administered 14 days apart. The numbers represent geometric mean and range of individual exposure estimates from the final model.

	First dose, whole (n=12)	**First dose, suspended (n=12)**	**Second dose, whole (n=12)**	**Second dose, suspended (n=12)**
Bedaquiline				
$AUC_{0\text{-}48h}$ [ng/mL*h]	31900 (18600, 51600)	32900 (25600, 43800)	35900 (29600, 49100)	34700 (20700, 54000)
$AUC_{0\text{-}336h}$ [ng/mL*h]	43500 (24900, 69300)	45900 (34300, 60700)	56700 (44600, 75400)	53400 (31100, 89700)
C_{max} [ng/mL]	2400 (1410, 3660)	2260 (1750, 3280)	2500 (2030, 3820)	2460 (1490, 3910)
T_{max} [h]	4.3 (2.8, 5.6)	4.9 (2.8, 6.9)	4.1 (2.8 ,6.7)	4.9 (3.3, 7.3)
M2 metabolite				
$AUC_{0\text{-}48h}$ [ng/mL*h]	1650 (818, 3110)	1780 (1270, 2580)	2800 (1990, 4140)	2520 (1360, 4380)
$AUC_{0\text{-}336h}$ [ng/mL*h]	8450 (4520, 15400)	9340 (6480, 13600)	14900 (10400, 22000)	13400 (7750, 22300)
C_{max} [ng/mL]	44.3 (22.0, 81.2)	47.9 (33.4, 72.2)	69.5 (50.7, 106)	63.6 (33.9, 109)
T_{max} [h]	14.4 (10.1, 19.6)	14.8 (11.4, 18.1)	14.0 (10.9, 17.3)	14.8 (11.0, 20.0)

Abbreviations: AUC, area under the concentration curve; C_{max}, maximal concentration, T_{max}, time of maximal concentration

Table 4. Results of palatability and acceptability assessments in healthy adults receiving suspended vs. whole bedaquiline tablets (n=24)

	Whole formulation		Suspended formulation	
	Dislike very much or dislike	**Neutral, Like or Like very much**	**Dislike very much or dislike**	**Neutral, Like or Like very much**
How did you feel about the **visual appeal** of the formulation? (did the formulation look acceptable to you?)	1 (4%)	23 (96%)	3 (13%)	21 (88%)
How did you feel about the **smell** of the formulation?	0 (0%)	24 (100%)	1 (4%)	23 (96%)
How did you feel about the **taste** of the formulation?	3 (13%)	21 (88%)	3 (13%)	21 (88%)
How did you feel about the **texture** of the formulation? (how did the formulation feel in your mouth?)	5 (21%)	19 (79%)	2 (8%)	22 (92%)
How did you feel about the **size/amount** of the formulation (volume of liquid or size of tablet)?	5 (21%)	19 (79%)	0 (0%)	24 (100%)
How did you feel about the **OVERALL acceptability** of the formulation?	0 (0%)	24 (100%)	1 (4%)	23 (96%)
If a **child** is required to take this formulation, how do you think they would feel about the **taste** of the formulation?	5 (21%)	19 (79%)	2 (8%)	22 (92%)
If a **child** is required to take this formulation, how do you think they would feel about the **size/amount** of the formulation (volume of liquid or size of tablet)?	6 (25%)	18 (75%)	1 (4%)	23 (96%)
If a **child** is required to take this formulation, how do you think they would feel about the **OVERALL acceptability**?	3 (13%)	21 (88%)	3 (13%)	21 (88%)

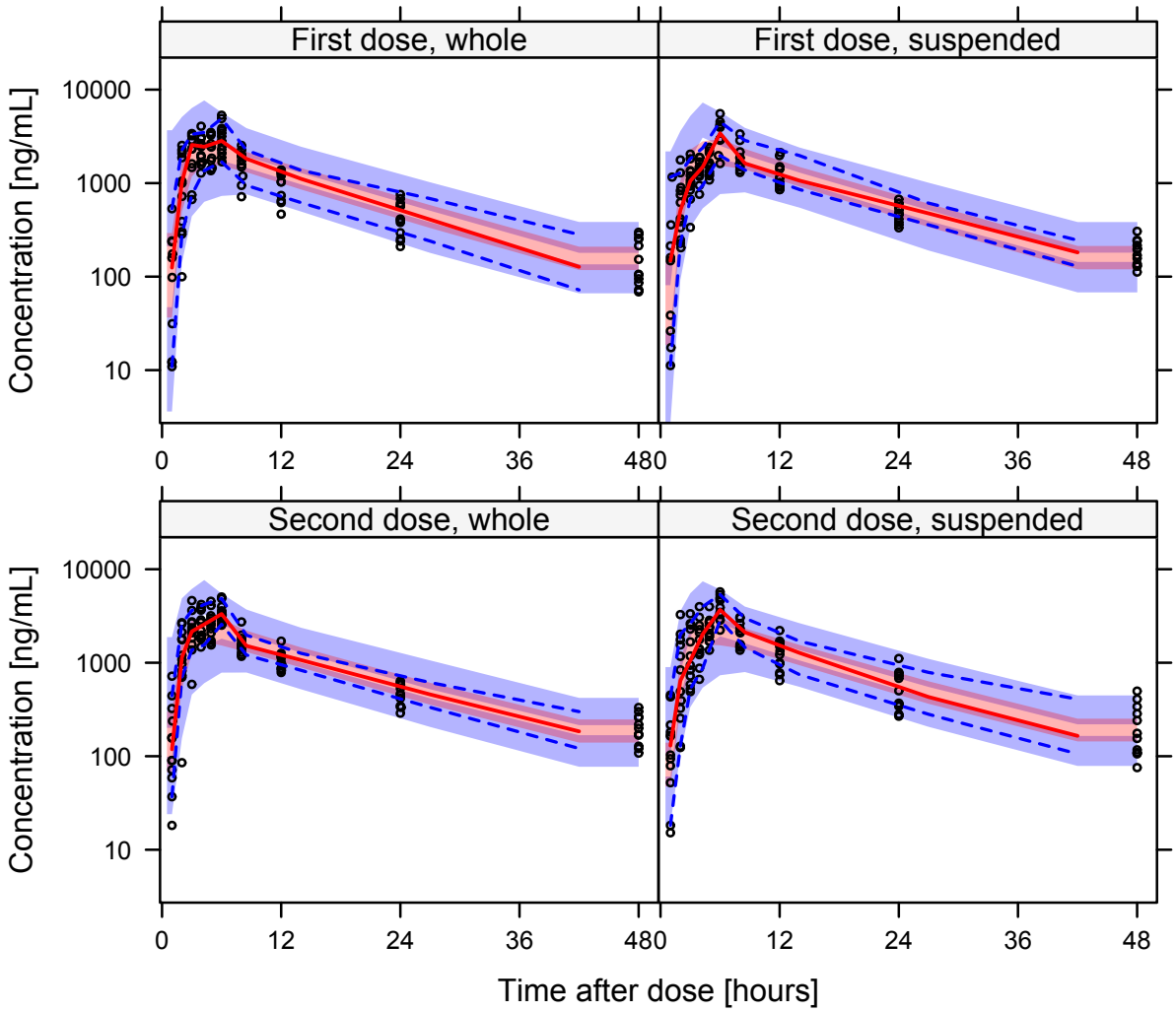

Figure 1. Visual predictive check showing the 5th, 50th, and 95th percentiles (lines) of observed bedaquiline concentrations (open circles) over time after dose, per formulation (whole or suspended tablets) and dose. The shaded areas represent the 90% confidence intervals for the same percentiles calculated from model-simulated data.

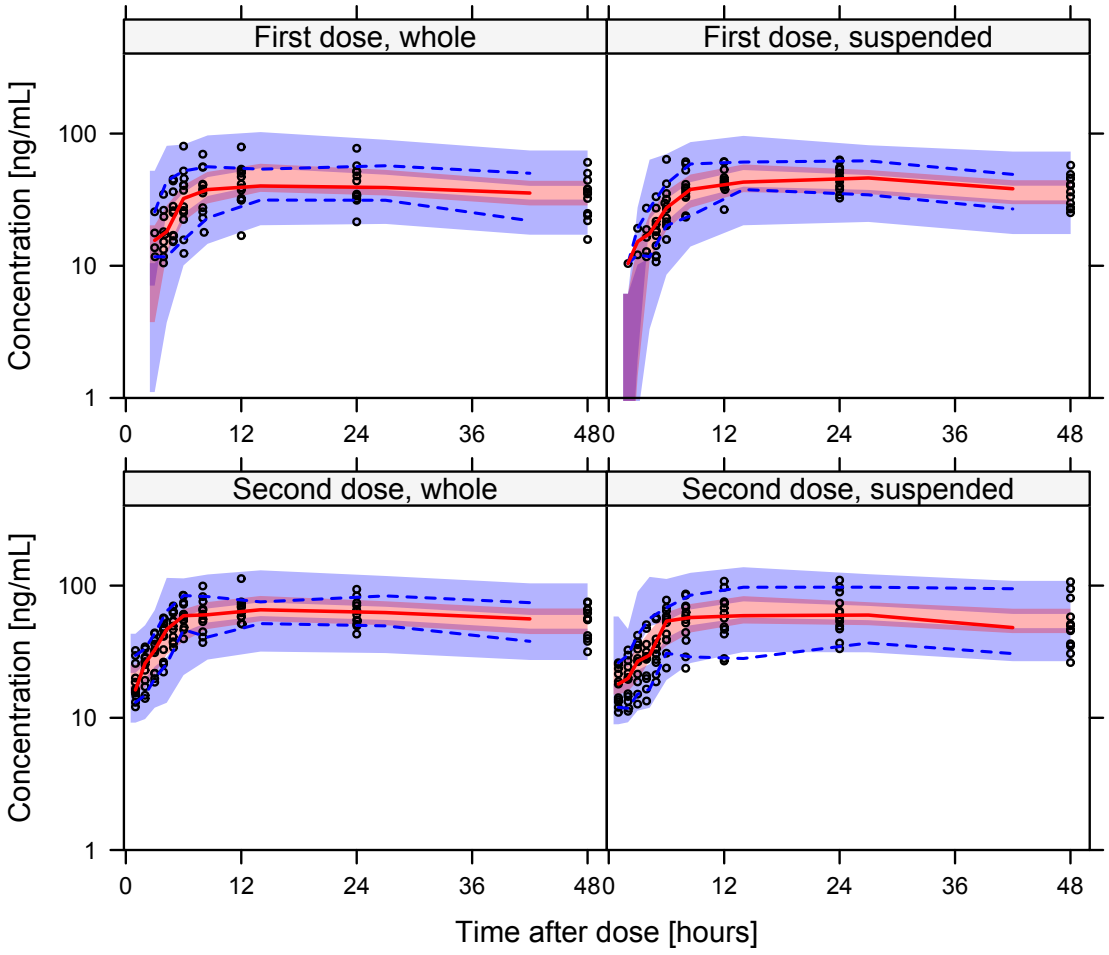

Figure 2. Visual predictive check showing the 5th, 50th, and 95th percentiles (lines) of observed M2 concentrations (open circles) over time after dose, per formulation (whole or suspended tablets) and dose. The shaded areas represent the 90% confidence intervals for the same percentiles calculated from model simulated data.

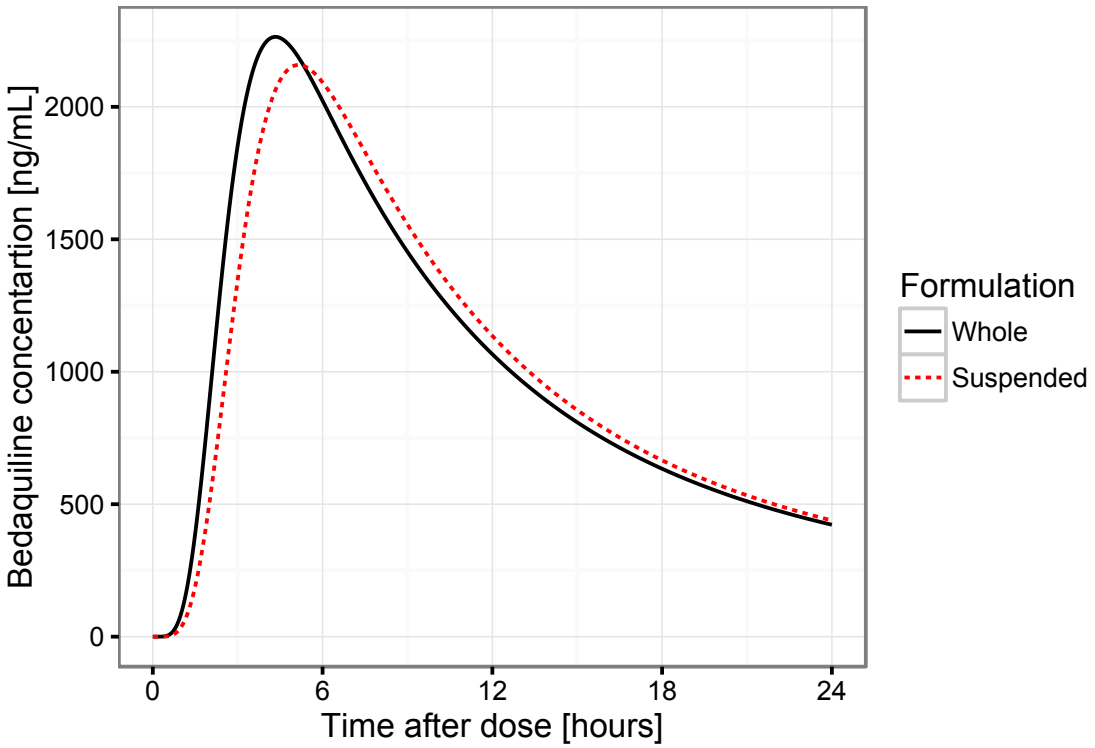

Figure 3. Typical pharmacokinetic profile after a single 400mg dose bedaquiline administered either as whole (solid line) or suspended (broken line) tablets, based on final model parameters.

Chapter 10: Discussion

10.1 Discussion

The overall objective of this doctoral research was to characterize the pharmacokinetics, including the effects of formulation, the optimal dose, safety, and the tolerability of key second-line and novel antituberculosis drugs in children, in order to inform their use in the safer and effective treatment of children with MDR-TB. As currently recommended treatment regimens for MDR-TB in children are long, toxic and poorly tolerated, the ultimate goal of this research was to inform the development of treatment regimens for children with MDR-TB that are highly effective, shorter, child-friendly (their use is child-friendly and formulations are acceptable), safer and better tolerated (specifically avoiding the use of injectable medications). The three-pronged approach used to achieve this goal was to optimize the use of 1) existing and 2) novel antituberculosis drugs in children by characterizing their pharmacokinetics, optimal dose, and safety, including consideration of formulation, and 3) to combine these medications into better regimens. Not all issues could be addressed in this doctoral dissertation. However, I identified the following priority questions: characterizing the pharmacokinetics and safety of currently recommended doses of key "second-line" antituberculosis drugs including ofloxacin, levofloxacin, and linezolid in children with MDR-TB, investigating the impact on pharmacokinetics and pain of administration practices for intramuscular injections of amikacin in children with MDR-TB, and characterizing the effects of formulation and/or formulation manipulation on the pharmacokinetics of levofloxacin and bedaquiline.

Below, I discuss the results of this doctoral research in relation to its objective, how this research is influencing policy and practice of paediatric MDR-TB care (Section 10.2), and future research that is emerging and is building on research from my doctoral dissertation (Section 10.3), and other considerations (Section 10.4).

10.1.1 Fluoroquinolone studies (Chapters 2, 3, 4, and 5)

In Chapter 2, I described a prospective observational study of the pharmacokinetics and safety of ofloxacin in children routinely treated for MDR-TB disease or exposure (139). At the time the study was conceived and implemented, ofloxacin was the only

recommended fluoroquinolone for MDR-TB treatment in children. Although this is no longer true, as levofloxacin and moxifloxacin have now become the fluoroquinolones of choice for MDR-TB treatment, this study, the largest study of ofloxacin pharmacokinetics in children to date, made a number of valuable contributions. It importantly identified that at the recommended dose of ofloxacin for children (15-20 mg/kg), drug exposures were well below the target exposure in adults receiving the recommended 800 mg once daily dose. Although ofloxacin is unlikely to be used frequently for MDR-TB treatment in the future, this finding has a number of important implications. It confirms that mg/kg extrapolation of doses from adults to children should not be relied on, as has been done for many second-line antituberculosis drugs. This limitation of mg/kg extrapolation and the concept of allometric scaling and its importance for drug dosing in children is well known among clinical pharmacologists, but not well enough among general TB clinicians and policy makers. However, it is important for this concept to be better known and better communicated, as it has critical impact on policy and clinical practice. Well-designed pharmacokinetic studies of antituberculosis drugs should be undertaken in children across the age spectrum to identify paediatric doses that achieve target exposures. Additionally, it is interesting to note that despite exposures of ofloxacin that were well below target levels, children in the same setting as this study, treated with these doses of ofloxacin, expected to be the key, most bactericidal drug in the treatment regimen, had excellent outcomes in observational studies (17). In a prospective cohort study of 137 children with probable or confirmed MDR-TB treated in Cape Town between January 2009 and December 2010, 96.4% were treated with ofloxacin at recommended doses, and 92% of children were successfully treated (cure or probable cure) (17). As children with MDR-TB in this cohort had excellent outcomes even with a fluoroquinolone, the most important drug in the regimen, which was less potent than fluoroquinolones currently in use and that was likely underdosed, there may be scope for treating children with less intensive treatment regimens in future. Optimizing the dose of fluoroquinolones, given its critical role, could make such an approach even more likely to be effective.

Additionally, the study documented that ofloxacin at the recommended doses was safe and well tolerated for the long durations required for MDR-TB treatment. Despite clear recommendations for the use of fluoroquinolones in children for some indications, including for MDR-TB, some clinicians and TB programmes remain hesitant.

Although ofloxacin is unlikely to be widely used in the future, and there may be variations in the safety profile of different fluoroquinolones, this is reassuring for long duration fluoroquinolone use in children. There were few musculoskeletal events in this cohort. It is possible that mild, subjective musculoskeletal complaints were under-reported, especially in young children. However, it is unlikely that overt arthritis or more serious arthralgia events that would have affected weight-bearing or functional activities were missed. As described in the publication, sleep disturbance and hyperactivity are well described with fluoroquinolones in children (87, 88) but were reported infrequently in this cohort. These events may have been underreported as most children in the cohort were hospitalized early in treatment, potentially obscuring these types of behavioural changes. Future studies among children treated primarily as outpatients should be certain to observe carefully for these events. As these children were on multidrug regimens, with at least 5-7 other antituberculosis drugs, assessing the attribution of events to individual medications was challenging, and should be interpreted cautiously. It is likely that many of the non-specific events reported in the study as possibly related to ofloxacin were more likely related to other drugs in the regimen, such as vomiting with ethionamide. Although the safety profile of ofloxacin in this study was reassuring, it must be considered in the context of the low drug exposures shown. Should ofloxacin paediatric doses be increased to more closely approximate adult target exposures, then safety should be carefully reassessed.

In Chapter 3, I reported on the results of the largest study of the pharmacokinetics of levofloxacin in children to date. Similarly to the study of ofloxacin, levofloxacin exposures were well below targets with the formulation studied (the formulation routinely available in the TB programme in Cape Town) with the doses most widely used currently for MDR-TB (15-20 mg/kg once daily). This has important implications considering that the fluoroquinolones are the most important component of existing MDR-TB treatment regimens. Optimizing their bactericidal activity is important for rapidly reducing the burden of metabolically active mycobacteria early in treatment, and for protection against acquisition of resistance in companion drugs. Doses that are likely to achieve target exposures with the formulation studied were estimated from the resultant model using clinical trial simulations. These doses were much higher than currently recommended, up to nearly 40 mg/kg in some weight bands. Additionally the estimated doses varied substantially across weights, from nearly 40 mg/kg down to 18

mg/kg at the lowest weights (youngest ages), clearly demonstrating the problem with recommending a single mg/kg dose to be used across children of all sizes and ages. The formulation used is an important consideration for paediatric medicines, and in this study the effect of formulation manipulation was assessed. Administration of levofloxacin by nasogastric tube was associated with an increased speed of absorption, but had no effect on overall bioavailability. Nasogastric tubes are rarely used in routine practice as this is not feasible. However in this study it was at times necessary to ensure the medication doses were completely ingested in a short amount of time in young children, many of whom would refuse due to the large pill burden (most were taken with 5-7 other antituberculosis drugs) and poor palatability of many of the second-line antituberculosis drugs. The bioavailability of levofloxacin administered as crushed tablets did not differ from whole tablets. This finding was limited by the small number of children in this study who were able to swallow whole tablets, and also that the administration method (crushed vs. whole) was highly associated with age. Nevertheless, this is reassuring, as until very recently this was the only option for administering levofloxacin to young children in most settings. Interestingly, when scaling the clearance (CL/F) from this study and other published levofloxacin studies, it can be seen as clearly higher than both previous adult and paediatric studies (Chapter 3, table 4). The published manuscript describes the scaled value as CL, rather than as CL/F, in error. Regardless, the explanation for this difference is not clear, however it was hypothesized that this was related to the formulation studied. Formulation would not be expected to affect CL, but could plausibly affect bioavailability (F) and thus CL/F. This appears to be likely considering the findings from the study in Chapter 5. It may be that this was an issue with the individual formulation studied in Chapter 3, or it may be an issue more broadly with adult formulations administered to children. As young children may continue to receive adult levofloxacin tablets in many settings, this question has high practical importance and deserves future study.

In Chapter 4, I reported on the clinical and cardiac safety of levofloxacin among a subset of the cohort reported on in Chapter 3. I reported a very similar safety profile as for the ofloxacin-treated children in Chapter 2, which adds to the confidence in these findings. Again, the study may have underestimated some levofloxacin-associated events such as mild musculoskeletal complaints, sleep disturbance, mild behavioural changes, and overestimated the contribution of levofloxacin to non-specific events such as nausea,

vomiting, and ALT elevation. Overall however, this is reassuring for the safety of levofloxacin at these doses and resulting exposures. Although not reported in this cohort, a child with MDR-TB in a separate concurrent study was incidentally noted to have papilloedema on an ophthalmologic exam and a high opening pressure on lumbar puncture (>50 cm H_2O) and was subsequently diagnosed with levofloxacin-associated intracranial hypertension (140). This has been previously but very rarely described with levofloxacin and other fluoroquinolones (141-143) and highlights the importance of ongoing vigilance when evaluating the safety of antituberculosis drugs in children. A concern for intracranial hypertension should not limit fluoroquinolone use in children where it is needed, however future studies may consider more comprehensive ophthalmologic assessments to ascertain whether intracranial hypertension is more frequent than previously understood.

The report of QT-interval prolongation in this study was one of the first detailed assessments of this fluoroquinolone class-wide adverse effect in children with MDR-TB. A previous study, which I contributed to, showed that among children (n=23) treated for MDR-TB disease or exposure with ofloxacin (20 mg/kg) or levofloxacin (15 mg/kg) the mean QTcFs were 361 ms (SD 37) and 369 ms (SD 22) respectively, and no child had a QTcF >450 ms (85). This study was limited by its size, lower levofloxacin dose than currently in use and limited ability to evaluate associations between levofloxacin concentration and QT-interval because of the timing of ECGs (85). The study in *Chapter 4* again reported minimal QT-interval prolongation, with mean QTcFs of 359 (SD 21.0) just prior to the levofloxacin dose and 364 (SD 26.6) at 2 hours post-levofloxacin dose, with no QTcF >450 ms. This provides important evidence to support the use of levofloxacin in combination with other QT-interval prolonging drugs, such as bedaquiline, delamanid and clofazimine. Additionally, this data provides a benchmark for QT-interval among children treated with levofloxacin-containing regimens, with which to contextualize findings on QT prolongation in the paediatric trials of bedaquiline and delamanid, which do not have control groups. The lack of association seen between levofloxacin concentration and QT-interval is slightly different than what is reported in adults, where there is a relationship between levofloxacin concentration and QT interval. However, this paediatric data is consistent with what has been published in adults in which there was little clinically important QT prolongation (i.e. significantly prolonged QT interval that would be a risk for arrhythmia) even with

levofloxacin doses as high as 1500 mg (91). The effect on QT-interval should still be carefully assessed if higher levofloxacin doses or exposures are evaluated in future. Additionally, the study was limited by the lack of children >8 years of age, who may have a different adverse event profile and different effects of levofloxacin on the QT-interval compared to younger children. Older children should be included in future levofloxacin studies.

In Chapter 5, I reported on the pharmacokinetics of a novel levofloxacin 100 mg dispersible tablet in children with MDR-TB exposure. When pooled together in a model with the data from the study in Chapter 3, models parameters were unchanged, except for bioavailability, with the standard adult 250 mg tablet studied in Chapter 3 having 41% lower bioavailability compared to the dispersible tablet. When the typical CL/F from these studies was scaled to a 70 kg adult, values for the dispersible tablet formulation (11.6 L/h) were much more consistent with published adult and paediatric literature compared to the standard adult 250 mg tablet studied in Chapter 3 (18.8 L/h). This highlights the importance of evaluating the effects of formulation and formulation manipulation on pharmacokinetics in children. Differences in bioavailability for different formulations have been well described for other antituberculosis drugs, notably rifampicin (61). Different excipients can affect bioavailability, and these effects can differ between adults and children (60). It is not clear whether the lower bioavailability in the standard adult 250 mg tablet studied in Chapter 3 is inherent to this specific formulation (formulation quality) or may be seen with other formulations. Although we did not find an effect on overall bioavailability of crushing the adult tablet in Chapter 3, that analysis had some limitations (confounding by age, few children swallowing whole tablets). As there currently are few child-friendly formulations of second-line antituberculosis drugs, off-label use of the adult formulations in children requires crushing or splitting tablets. This practice is routine and the assumption is that this has minimal effects on bioavailability, however this is not always true, and the effects of this manipulation should be studied if the expectation is that children will be using adult formulations. It is of clear practical importance to understand these formulation effects for levofloxacin given the magnitude of the difference in exposures for this key medication for MDR-TB treatment and preventive therapy, and follow-up studies are planned (see Section 10.3).

There are a number of other overarching issues raised by the studies in Chapters 2-5 together. Selection of targets for establishing paediatric doses is challenging. The current approach of approximating the AUCs observed in adults receiving the efficacious, safe, recommended doses of the drug of interest may still be the best approach, but has a number of limitations. One challenge is that the optimal adult dose has not been established for several key drugs. At the time ofloxacin was the recommended fluoroquinolone for MDR-TB, the sufficiency of the recommended 800 mg dose in adults was already being questioned. Based on data from South African adults with MDR-TB, the probability of attaining the pharmacodynamic target for ofloxacin (AUC/MIC of at least 100) was only 0.45, which is clearly not optimal (144). Although levofloxacin is more likely to attain pharmacodynamics targets with the currently used 750-1000 mg dose, the optimal doses has similarly not been well established. The Opti-Q trial (NCT01918397) is a phase 2 trial in adults with MDR-TB evaluating four doses of levofloxacin, up to 20 mg/kg, in combination with an optimized background regimen to determine the AUC/MIC that achieves the shortest time to sputum culture conversion, the highest AUC that is safe and well tolerated, and the dose that achieves the targeted AUC. Preliminary pharmacokinetic results from this study demonstrated that higher doses of levofloxacin resulted in higher exposures, up to a median $AUC_{0\text{-}24}$ (IQR) of 207 mg/L*h (143 to 534) with a 20 mg/kg levofloxacin dose (145). The safety and efficacy of these doses have not yet been reported from this trial, but the pharmacokinetic data may provide target exposures to be evaluated in children should higher doses be safe and efficacious in adults (145). It is thus possible that the optimal levofloxacin dose in adults with MDR-TB may be changing. Doses of levofloxacin and other antituberculosis drugs in children need to adapt as targets evolve over time based on new data. Population pharmacokinetic models can be used to simulate doses that will result in similar exposures to new adult targets, however actual drug exposures and careful safety evaluations would need to be performed in children. Other novel approaches to establish dosing targets for childhood TB are being investigated, including with hollow fiber models (146). However, these remain exploratory and would need validation before being applied to clinical dose selection. The mutant prevention concentration (MPC), defined as the concentration that is able to suppress the growth of the least susceptible single-step mutant, has also been proposed as a way to select target exposures for TB drugs (147). Achieving this concentration

would ensure that growth of even resistant mutants would be suppressed, so acquisition of resistance during treatment would be unlikely. One current limitation to using the MPC as targets for dose selection is that the MPC has not been well characterized for most antituberculosis drugs, and for those drugs for which it has been studied, there is up to 10-fold variability across studies (147). It is additionally not clear whether exceeding the MPC or maintaining concentrations above the MPC, as suggested by animal data, is required for preventing mutation amplification, which would result in very different dosing requirements (148). The value of achieving the MPC for a single drug in strong multidrug regimens is also not clear, as companion drugs protect other drugs in the regimen against resistance acquisition. Lastly, for children who usually have paucibacillary TB and thus a low risk of acquisition of resistance, the importance of achieving concentrations above the MPC is not clear. Nonetheless, the MPC may still contribute to choices of target concentrations for paediatric dose selection as additional information about its value becomes available.

Although not discussed at length in the studies in this dissertation, this body of research on the fluoroquinolones has highlighted the importance of acceptability and palatability in paediatric therapeutic research. The fluoroquinolones are bitter compounds, and the very poor palatability of moxifloxacin is an important reason why levofloxacin has been the fluoroquinolone of choice for young children. Acceptability and palatability can impact on children's and their caregivers' experience of treatment (149), and can significantly affect adherence (150), an important factor for the success of TB treatment. As such, considerations of the acceptability and palatability are increasingly a component of ongoing and future studies informed by this doctoral research (see 10.3).

The research in this thesis has focused on ofloxacin and then levofloxacin, which was and remains the fluoroquinolone of choice for children with TB, primarily due to its ease and feasibility of use (i.e. tablet strength and palatability) relative to moxifloxacin. As previously noted, moxifloxacin is the most potent fluoroquinolone against *M. tuberculosis* (66), and is used as the first choice in adults and older children with MDR-TB. Moxifloxacin may have advantages over levofloxacin related to increased uptake into macrophages and higher bactericidal activity that may confer it improved overall efficacy for TB treatment (151). Levofloxacin may have safety advantages over moxifloxacin related to reduced QT prolongation (68, 89), which may allow for

levofloxacin to be both used at higher doses relative to moxifloxacin and used more safely in combination with other key antituberculosis drugs that also prolong the QT interval, such as bedaquiline, delamanid and clofazimine. There have been few head-to-head comparisons of the two medications to date to better understand their relative importance in MDR-TB treatment. As currently it is unclear whether levofloxacin or moxifloxacin may ultimately be the fluoroquinolone of choice for TB treatment, or whether both may have a role, it is important to ensure the dosing and safety are well characterized for both medications. I have previously contributed to a study of moxifloxacin in children with MDR-TB which characterized its pharmacokinetics and safety in 23 children 8 years of age and older, and I am involved with ongoing research on moxifloxacin in children (see Section 10.3).

An additional highly instructive aspect of this research was the value of pharmacometrics as a tool for the design and analysis of paediatric pharmacokinetic studies. Pharmacometrics is the application of mathematical models to physiology, pharmacology and disease to understand the interactions of drugs and patients (152). The non-linear mixed effects modeling techniques employed can more easily account for the known effects of size on drug pharmacokinetics using allometric scaling, and can better characterize the effects of age (153). In addition to other analytical benefits, clinical trial simulations using these models can be used for selecting optimal doses in children of different age and weights (152, 153), as was done in Chapters 3 and 5. Limitations to pharmacometrics are that it is highly specialized and less accessible and understandable to the general clinician than traditional non-compartmental analysis, and that it requires specialized expertise that is in limited supply. However, these challenges are outweighed by the benefits, and the technique is being increasingly used in both design (see Section 10.1.4) and analysis in paediatric studies of antituberculosis drugs, as evidenced in this dissertation.

10.1.2 Linezolid (Chapters 6, 7)

In Chapter 6, I performed a scoping review of the literature on linezolid for use in MDR-TB treatment. The review highlighted that the data, although of relatively low quality, suggested that linezolid was a highly effective antituberculosis drug, but associated with frequent serious dose and duration dependent adverse effects. It also highlighted the

paucity of data on linezolid in children with TB. The review guided our approach to using linezolid in children with MDR-TB at the time, and also identified priority research questions that informed the work in Chapter 7.

In Chapter 7, I describe the results of an analysis combining linezolid pharmacokinetic and safety data from two similar observational studies (MDRPK1 and MDRPK2) of children routinely treated for MDR-TB. To my knowledge, this is the first linezolid pharmacokinetic data in children with TB. At the time these studies were ongoing, linezolid was important as one of the few highly effective antituberculosis medications for children with highly resistant forms of MDR-TB, such as XDR-TB. Currently, linezolid may have an even more important role in the rapidly evolving MDR-TB treatment landscape. The Nix-TB trial (NCT02333799) has demonstrated the excellent efficacy of linezolid (1200 mg once daily) in adults with very difficult to treat MDR-TB in a 6-month three-drug regimen (100). Although the risk of frequent serious linezolid-related adverse events in this trial is not acceptable for children with MDR-TB, who generally have good outcomes already, these were generally manageable in adults in this trial (100). A follow-up study, ZeNix (NCT03086486), is evaluating alternative linezolid dosing approaches, including lower doses and shorter durations, in combination with bedaquiline and pretomanid treatment in adults with MDR-TB, and will likely inform linezolid dosing for MDR-TB in future. Other adult trials are also evaluating other regimens that include linezolid in multiple other combinations (152). More information on the optimal use of linezolid for MDR-TB is expected in the future. The reduced doses of linezolid in our proposed weight banded dosing compared to the most commonly used dosing currently, would almost certainly reduce the risk of linezolid-related adverse effects that were described in our paediatric study, although this would need to be confirmed. There is however a concern that the lower linezolid doses would reduce its efficacy and increase the risk of resistance acquisition, which has been seen with lower doses in adults (300 mg once daily) (97). However there are a number of reasons to think this will not be the case. First, the proposed weight banded doses should achieve exposures that approximate those seen in adults receiving a 600 mg once daily dose. This dose is the most commonly used dose in routine care in adults, and has been shown to be highly effective and to have a minimal risk of resistance acquisition (97, 154). There is thus no reason to think that children, who tend to have paucibacillary disease and which should respond to treatment as well as or better than

in adults, would not respond to these doses. Secondly, linezolid is increasingly being combined with other effective antituberculosis drugs, such as clofazimine, bedaquiline, and delamanid, that were not available until recently, but that now provide additional efficacy to the regimen and potentially protection of other companion drugs in the regimen (including the linezolid) from acquisition of resistance.

There is currently a strong interest in moving away from the use of the injectable drugs to all-oral regimens for MDR-TB treatment for adults and children. South Africa has recently announced a change to national MDR-TB treatment guidelines that will now recommend substitution of bedaquiline for the injectable drug in most regimens for patients aged 12 years and older (see Section 10.3) (155). Due to the lack of paediatric data on the dosing and safety of bedaquiline and delamanid, younger children would still need to use other medications as substitutes for the injectable in order to benefit from all-oral regimens. Linezolid is an option for this strategy. However, safety concerns in addition to cost considerations, have led to caution in using linezolid routinely as a substitute for the injectable. However, the lower doses proposed in our study would likely result in fewer adverse events and preserve substantial efficacy, making linezolid a more attractive option for use as a substitute for the injectable in MDR-TB treatment regimens. Even with the proposed lower doses, the ability to carefully monitor safety, especially haematologic indices, should be a requirement for long-term linezolid use.

The current reality is that access to linezolid remains limited in most settings, due its high cost. Although a suspension exists, availability in routine settings is lower than for the tablet formulation. The adult 600 mg tablet formulation is unscored, and difficult to manipulate for use in children. A reasonably priced generic linezolid dispersible tablet formulation is urgently needed to improve access for children to this increasingly important medication for MDR-TB treatment.

10.1.3 Second-line injectables (Chapter 8)

In Chapter 8, I described a small randomized blinded two-period crossover study that demonstrated that adding lidocaine to amikacin injections was safe and reduced early post-injection pain without significantly affecting amikacin pharmacokinetics or causing additional adverse effects. A recently published study evaluating a comparable strategy in adults with MDR-TB treated with kanamycin reported similar findings (156). The

priority is to develop MDR-TB treatment regimens that do not require the second-line injectable drugs, and the other studies in this doctoral research aim to contribute to this goal. However, in the meantime, the injectables remain components of most current MDR-TB treatment regimens, and the study in Chapter 8 identifies a feasible, inexpensive and safe strategy for improving the tolerability of the injectables. The results of the study support the practice of routinely adding lidocaine to intramuscular injections of the second-line injectable drugs when they are required to be used (Section 10.2).

In addition to the specific findings, the study was an excellent learning opportunity for me to be involved with the design, implementation, analysis and write-up of a small randomized crossover study such as this. I was able to think carefully about design elements to reduce bias in trials, such as the details of the randomization, allocation concealment, blinding, and others, and how to report the results of a trial consistent with international consensus for trial reporting (CONSORT) (157). Addtionally, the trial provided an opportunity to better understand the concept of bioequivalence. Although the study was not powered to demonstrate bioequivalence of amikacin with and without lidocaine, this was evaluated in the study. The analysis followed the accepted approach to establish average bioequivalence, in which the measure of interest (AUC, C_{max}) is log transformed, means are obtained, and the means antilogged, to then generate the geometric mean ratios with 90% confidence intervals. Bioequivalence is demonstrated when these 90% confidence intervals fall within 80-125%. This was especially valuable experience for subsequent trials I have been involved with (e.g. Chapter 9 and others outlined in Section 10.3).

10.1.4 Bedaquiline (Chapter 9)

In Chapter 9, I described a randomized two-period crossover study in healthy adult volunteers in which it was demonstrated that 100 mg (adult formulation) bedaquiline tablets administered suspended in water was bioequivalent to 100 mg (adult formulation) tablets swallowed whole. The suspended tablets were also found to be acceptable and palatable to the majority of participants, an important finding considering that crushing or suspending some tablets, such as the fluoroquinolones, reduces their acceptability substantially.

State of the art pharmacometrics was critical to the design and analysis of this study, as bedaquiline and its primary metabolite, M2, have very long half-lives (bedaquiline 164 days, M2 159 days) (158). This traditional approach to assessing bioavailability uses a crossover design and non-compartmental analysis to compare pharmacokinetic measures (AUC, C_{max}) in individuals receiving both treatments separated by a washout period (159). However, the long half-life of bedaquiline complicates this approach, as even with a long washout period there is likely to be some carryover (138, 159, 160). An option is to utilize a parallel group design, but this requires a larger sample size and is less efficient. A modeling approach is able to handle the carryover effect and characterizes the primary bioavailability parameter, allowing the more traditional, efficient crossover approach (159). This is a relatively new statistical approach to evaluating bioequivalence, and there is less familiarity and guidance from regulatory authorities in this regard. However, for long half-life drugs it is a critical tool to evaluating bioequivalence. Modeling and simulations in this study also informed the sample size calculations and the sampling schema, as described in Chapter 9, to ensure the most efficient study design while preserving the study's precision for key parameters. As a clinical trials investigator in therapeutics, it is increasingly important to understand the benefits and limitations of pharmacometrics in order to work with pharmacometricians to optimally design and analyze trials relevant to children.

The study was originally envisioned to support the P1108 trial, which evaluates the pharmacokinetics and safety of bedaquiline in HIV-infected and –uninfected children, and which may not have access to the paediatric formulation. However, it was also informed by an improved understanding of the barriers and challenges to making a paediatric formulation accessible for treatment of children in routine care settings, given the expectation that paediatric bedaquiline and delamanid formulations are not likely to be widely available for some time. Research gaps, such as a lack of data on the pharmacokinetics, optimal dose and safety of medications, are critical and are being addressed by phase 1/2 trials. However, there are multiple other barriers to access much-needed medications in children, including both downstream (pre-approval) and upstream (post-approval) barriers (161, 162). In addition to research gaps that are described in this dissertation, other downstream barriers include *policy gaps* such as a lack of consensus prioritization of TB formulations for children and lack of evidence-based antituberculosis medication dosing guidance, *development (formulation) gaps*

such as poor acceptability of existing key second-line antituberculosis drugs and persistent critical gaps in available child-friendly formulations, and challenges with *regulatory approvals* by stringent regulatory authorities (64, 161, 162) and production of formulations. Upstream barriers include challenges with *national regulatory approvals*, such as South Africa, and issues with *procurement*, *introduction* and *pharmacovigilanc*e (161, 162). The very small, fragmented market for second-line antituberculosis drugs, exacerbated by the small proportion (<5%) of children with MDR-TB globally each year who are diagnosed and treated (163), further complicates these problems. Pragmatic solutions, such as this study, which will allow for the rational use of the widely available adult formulation to accelerate paediatric access, are an important part of the response and can temporarily address issues around formulation development. However, these barriers must be addressed by a broad array of partners and advocates, often at the level of national and international policy. This work has stimulated an interest in broader paediatric drug development issues and has resulted in collaborations with other groups and partners outside of the traditional paediatric TB research community (see Section 10.5).

10.2 Impact on policy and practice

As the studies in this doctoral thesis were designed to answer therapeutic questions that are currently directly relevant to the clinical care of children, they have already had substantial impact on current policy and practice of caring for children with MDR-TB in the study setting and beyond. Although fluoroquinolones are recommended for use in children with MDR-TB, there is hesitance to use them among many clinicians due to persistent concerns about safety. The data on ofloxacin and levofloxacin safety in Chapters 2 and 4 should provide reassurance to clinicians and programmes providing paediatric MDR-TB care and should improve the uptake of fluoroquinolone-containing treatment or preventive therapy. Formal WHO guidance, which is currently under review again, still recommends levofloxacin to be given at 15-20 mg/kg daily divided into two doses in children <5 years and 10-15 mg/kg once daily for children >5 years (124). This is likely to underexpose many children. The levofloxacin pharmacokinetic data presented in Chapter 3 has already influenced local practice in Cape Town, where levofloxacin is now being dosed at 15-25 mg/kg once daily for MDR-TB treatment in

children. Data from this research is hoped to influence ongoing and future WHO and other international dosing recommendations. Given concerns about the levofloxacin formulation studied and the much higher doses of levofloxacin estimated by this study needed to meet target exposures, further evaluation, especially of safety at higher doses, should be undertaken before these doses are recommended, especially in healthy children who are at risk of developing future MDR-TB. However this data, along with that from Chapter 5, and other previously published data certainly call into question currently recommended levofloxacin doses. As previously described, the levofloxacin dispersible tablets studied in Chapter 5, and which development was supported by the TB-CHAMP trial, have now been WHO prequalified, are available through the Global Drug Facility for procurement and will begin to be used in eligible TB programmes. The weight banded dosing in Chapter 5 will directly inform dosing of this formulation for routine programmatic use.

Given that there was little formal guidance on linezolid use in children with MDR-TB, the review in Chapter 6 provided a suggested practical approach, that considered the available evidence despite its relatively low quality, and was directed at clinicians and TB programmes. The dosing guidance, despite its limitations, has informed local practice and what has been advised by other partners such as the Sentinel Project in its weight banded dosing chart (http://sentinel-project.org/2014/06/21/dosing-chart-second-edition-2/), to which I contributed. However, higher quality data on the optimal linezolid dose in children for MDR-TB and its long-term safety was clearly needed.

This has been addressed in part in Chapter 7, where I showed that at current linezolid doses used most commonly in routine care for MDR-TB treatment in children, drug exposures were good, but that there were frequent, serious adverse events, primarily anaemia. Weight banded doses achieving target exposures in adults after a 600 mg once daily dose were proposed, and were lower than the most commonly used doses currently. This data has the potential to inform international paediatric dosing guidelines for linezolid for MDR-TB treatment, as there is currently minimal formal dosing guidance. The choice to recommended the proposed weight banded doses for routine care of children with MDR-TB would need to carefully consider the implications of the lower doses for efficacy and risk of acquisition of drug resistance. These weight

banded doses will inform the design of future paediatric trials (see IMPAACT 2020, Section 10.3.3)

Chapter 8 demonstrated that co-administration of lidocaine with IM injections of amikacin reduces injection pain, did not significantly affect amikacin concentrations, and was safe. These findings provided evidence for what was already the practice at the Brooklyn Chest Hospital paediatric wards, where the majority of children with MDR-TB in the Cape Town area are cared for. The City of Cape Town TB programme is reviewing this evidence and will consider whether to make this a policy for the DR-TB programme, including for adults. The results of the study have already been disseminated through organization such as the Sentinel Project and Treatment Action Group's annual Pipeline Report, with the intent to influence policy and practice at an international level.

The results of the data from Chapter 9 demonstrating bioequivalence of bedaquiline tablets suspended in water compared to tablets swallowed whole, has already had important impacts. The ongoing IMPAACT P1108 paediatric bedaquiline trial may not have access to the paediatric bedaquiline dispersible tablet formulation, so adult tablets suspended in water will be used in the younger age cohorts (less than 6 years of age), informed by this research. The results from Chapter 9 will also have important implications for policy and practice. As widespread access to the paediatric bedaquiline formulation (which, like delamanid, is still a trial formulation and not licensed) is expected to lag far behind data on the dosing and safety of bedaquiline in children of all ages, the data from Chapter 9 will allow the rational use of the manipulated adult 100 mg tablet formulation in young children (and older children and adults) who are unable to swallow tablets whole. This will accelerate access to bedaquiline for young children, a much needed treatment option. Emerging data and recent policy changes have made access to bedaquiline an even greater priority. Recently published evidence from a South African bedaquiline access programme for adults with MDR-TB reported that bedaquiline-treated patients with MDR-TB and XDR-TB had reductions in all-cause mortality by 65% and 74% respectively compared to standard regimens (120). On the strength of this evidence, the South African National Department of Health announced that national MDR-TB treatment guidelines would now recommend that bedaquiline be given to all patients 12 years of age and older with MDR-TB as a substitute for the second-line injectable drug in an injectable-free regimen in all MDR-TB cases (155).

Children under 12 years of age, however, cannot currently benefit from this policy due to the lack of bedaquiline dosing and safety data. This policy change highlights the importance of rapid access to bedaquiline for children, and the practical importance of the results from Chapter 9.

In addition to impact on current policy and practice, the data has also impacted substantially on the research agenda in children with MDR-TB, as discussed in Section 10.3

10.3 Other related research and future directions

This doctoral research has made important direct contributions towards the goal of developing effective, shorter, safer, better tolerated and child-friendly regimens for MDR-TB treatment in children. It has also informed and complemented other ongoing or planned research across the three-pronged approach to achieve this goal, as described below.

10.3.1 Optimizing the use of existing second-line TB medications

My research has focused on levofloxacin and linezolid as key existing second-line medications requiring additional optimization. The study MDRPK2 (see Chapter 9) of which I am the Co-Principal Investigator, is building on the levofloxacin data described here, and is evaluating the pharmacokinetics and safety of higher doses of levofloxacin (20-25 mg/kg) in children using the routinely available standard adult 250 mg levofloxacin formulation. Other priority second-line drugs, not evaluated in this thesis, include moxifloxacin and clofazimine (see Section 1.5, Table 3). Building on moxifloxacin research from MDRPK1, the MDRPK2 study is also evaluating the pharmacokinetics and safety of higher doses (10-15 mg/kg) of moxifloxacin, including in young children <8 years of age, which is a major gap (68). Clofazimine, a rhiminophenazine antibiotic that disrupts the mycobacterial respiratory chain (164) and may destabilize the mycobacterial membrane (165), has been used for decades in leprosy treatment, but has only recently been explored for MDR-TB because of the severely limited treatment options. It is an increasingly important antituberculosis medication as it is a component of the newly WHO-recommended shortened regimen now being widely used in adults and children with MDR-TB (18). However, there is no

available paediatric pharmacokinetic data, and the existing soft-gel capsule formulation is inflexible and problematic for young children. The enrollment of children into MDRPK2, who are now being routinely treated with clofazimine, represents an opportunity to generate much needed paediatric data on this medication. Stored samples from MDRPK2 will have clofazimine assays done at the University of Cape Town Division of Clinical Pharmacology, and analysis is planned with MDRPK2 Co-Principal Investigator Rada Savic (University of California San Francisco) during 2018. In addition, I have contributed to a proposal for a prospective study of the pharmacokinetics and safety of clofazimine in children with MDR-TB that Novartis, the manufacturers of clofazimine, has agreed to fund.

The studies in this thesis have highlighted the importance of formulation for the pharmacokinetics and acceptability of antituberculosis drugs in children (see Section 10.4) and future planned research will include careful consideration of formulation. MDRPK2 also includes a crossover component in which children able to swallow whole tablets will have an additional pharmacokinetic sampling occasion on which they will received crushed tablets, to better characterize the effect of formulation manipulation on the pharmacokinetics of levofloxacin and moxifloxacin. This added crossover component of the study design will address some of the limitations in analyses on formulation effects described in Chapters 2 and 3, including the small number of children swallowing whole tablets and the difficulty in separating the effect of age from crushing of tablets. In addition, I have led the development of a recently submitted proposal that among other activities aims to: 1) characterize the effects of formulation and formulation manipulation on levofloxacin pharmacokinetics (following on the results described in Chapters 3 and 5; 2) characterize the pharmacokinetics, safety and acceptability of other novel second-line dispersible tablet formulations developed by Macleods, including moxifloxacin; 3) partner with the TB Alliance, a product development partnership, to develop paediatric formulations of key second-line antituberculosis drugs that are not yet available (linezolid, clofazimine).

MDRPK1 is evaluating the pharmacokinetics and safety of all the existing second-line antituberculosis drugs in children, and I am contributing to ongoing analyses of ethionamide, terizidone, high-dose isoniazid, amikacin, and PAS.

Along with the research presented in this thesis, the ongoing and planned research described here will substantially contribute to optimizing our use of these key existing second-line antituberculosis drugs in children.

A finding across these studies was the high degree of variability in pharmacokinetics within the study population. It is worth considering whether individualization of doses, which may reduce some of this variability may be beneficial. Therapeutic drug monitoring (TDM) is one approach that has the potential to reduce the variability of drug exposures between patients. It remains to be seen whether such a strategy will be valuable or feasible for paediatric MDR-TB treatment. Characteristics of individual drugs that make them good candidates for TDM include substantial interindividual variability, limited interoccasion variability, well characterized exposure or concentration targets for efficacy and/or safety, narrow therapeutic window, and stability and type of samples required for drug assays. Few drugs used in the context of MDR-TB treatment in children meet these criteria. Linezolid may be an exception, as it is associated with clinically important adverse events that are clearly dose and duration related. TDM may provide an opportunity to individualize drug doses to maintain sufficient concentrations for efficacy but to limit high exposures and reduce the risk of adverse events. This may be a topic for further exploration, particularly as linezolid becomes more widely used for MDR-TB. However, constrained TB programmes in high burden settings may not have the capacity to implement TDM and individualized drug dosing. It would be important to evaluate the feasibility and cost-effectiveness of such a strategy before implementing it at scale.

10.3.2 Optimizing the use of novel TB drugs

Novel regimens for MDR-TB, and also potentially drug-susceptible TB, will almost certainly rely on novel antituberculosis medications. The paediatric trials to characterize the pharmacokinetics, optimal dose and safety of bedaquiline and delamanid were beyond the scope of this doctoral research. However, they have been ongoing in parallel and I have made meaningful contributions to some of these studies.

IMPAACT P1108 is the NIH-sponsored phase 1/2 trial of the pharmacokinetics and safety of bedaquiline in HIV-infected and -uninfected children with MDR-TB treated

with an optimized background regimen. The results from the study in Chapter 9 on suspended bedaquiline tablets directly informed the design of P1108, which will be evaluating suspended tablets in the younger cohorts. The trial opened in Quarter 4 of 2017, and I have been giving input as a member of the core protocol team. As the Medical Director of the Desmond Tutu TB Centre's site at Brooklyn Chest Hospital, a site for P1108 and the NIH-funded IMPAACT network, I have been overseeing our day-to-day implementation of the trial.

The Otsuka-sponsored phase 1 (242-12-232) (NCT01856634) and phase 2 (242-12-233) (NCT01859923) trials of delamanid in children with MDR-TB are age de-escalation trials that aim to characterize delamanid pharmacokinetics and safety over 10 days (trial 232) and 6 months of dosing (trial 233). These trials opened in 2013, and enrolled at two sites – one in the Philippines and the second at the Desmond Tutu TB Centre site in Cape Town. Since 2016, I have been the site Principal Investigator, overseeing the site's implementation of the trial, providing support as needed to the protocol team, and giving input on interpretation of trial results. Group 1 (ages 12-17 years, n=7), Group 2 (ages 6-11 years, n=6) and Group 3 (ages 3-5 years, n=12) completed enrolment and long-term follow-up. Group 4 (ages 0-2 years, n=12) has completed enrolment and long-term follow-up is expected to complete in early 2019. Based on preliminary results from these trials, the WHO issued recommendations for delamanid use in children ages 6-17 years with MDR-TB and limited treatment options (121). Data from these trials is critical for informing delamanid use in children and ultimately ensuring it is accessible to children in the field.

I am also the protocol Co-Chair of the NIH-sponsored IMPAACT 2005 protocol, a phase 1/2 open-label single-arm multi-centre study to evaluate the pharmacokinetics, safety and tolerability of delamanid with an optimized background regimen in HIV-infected and –uninfected children with MDR-TB (NCT03141060). The trial will include up to 48 HIV-infected and –uninfected children 0 to <18 years of age with MDR-TB. The primary objectives of the study are to evaluate the 1) pharmacokinetics and 2) safety of delamanid when added to an optimized background regimen at doses determined most likely to achieve exposures achieved in adults receiving 100 mg twice daily. Data from the 232/233 trials will be modeled and optimized doses estimated and then evaluated in this trial. Key secondary objectives include to characterize the impact of HIV-

coinfection and/or cotreatment on delamanid drug disposition, to characterize delamanid acceptability, and to characterize treatment outcomes, as children in the trial will be receiving an injectable-sparing regimen. As the protocol Co-Chair I have led the protocol development, will oversee its overall implementation and lead the interpretation and dissemination of the trial results, as well as leading the trial implementation on-site. This trial will further contribute to the optimal use of delamanid, including in HIV-infected children, and will provide preliminary evidence on the efficacy of an injectable-sparing delamanid-containing regimen.

As described above, the delamanid paediatric trials are nearing completion and there will soon be dosing and safety information across ages. Although Otsuka has developed two paediatric delamanid formulations (5 mg and 25 mg dispersible tablets), these were trial formulations and it is likely that these formulations would not be commercially available for some time (possibly years). The adult formulation is becoming much more widely available, and could be used in young children. However the effect of formulation manipulation (crushing or suspending the adult tablets in water) on its bioavailability is not known. This key question could be addressed by a study similar in design to that in Chapter 9 with bedaquiline. I have led a funding proposal for such a study. As for the study of the relative bioavailability of suspended bedaquiline tablets, such a study with delamanid would inform the rational use of the adult formulation in young children, accelerating access to this much needed treatment.

The novel antituberculosis medication pretomanid, a nitroimidazole antibiotic like delamanid, is being evaluated in multiple novel regimens in adults, however safety concerns have delayed its evaluation in children, and paediatric trials are only at the earliest stages. I have been contributing to initial work to develop a paediatric investigational plan (PIP) for the EMA for pretomanid, which TB Alliance is leading.

10.3.3 Combining existing and novel TB drugs into effective shorter, safer and child-friendly treatment regimens in children

As referred to in Section 1.4, traditionally, the efficacy of TB treatment regimens has been extrapolated to children from adult studies. Therefore pharmacokinetics and safety of individual TB drugs have been considered the priority research area in children (166). However, children tend to have paucibacillary TB (approximately 30-40% culture-confirmed and <10% sputum smear-positive for acid-fast bacilli in

pediatric pulmonary TB under well investigated conditions) and could reasonably be expected to respond to TB treatment better than adults. This is consistent with the much better treatment outcomes in children with MDR-TB (75-90% successfully treated) (17, 23) compared to adults (50% successfully treated) (167). Additionally, children may suffer more damaging consequences of adverse effects of these drugs like permanent hearing loss, separation from caregivers during critical periods of attachment and interruption of education, all of which have drastic implications for neurodevelopment and long-term functioning when TB occurs in early childhood. Taken together, children with MDR-TB may suffer disproportionately more from some adverse effects, and are also more likely than adults to respond to shorter, less intensive treatment for MDR-TB.

With this rationale, and considering the availability of novel oral drugs like delamanid and repurposed oral drugs like clofazimine and linezolid, a phase 3 efficacy trial of a shortened, all-oral treatment regimen for children with at least rifampicin-resistant TB has been considered a high priority (168). I am chairing this protocol called IMPAACT 2020, sponsored by the U.S. NIH through the IMPAACT network, which has been in development since late 2017. As initially envisioned, this partially randomized multicentre phase 3 trial would enroll HIV-infected and –uninfected children <15 years of age with probable or confirmed at least rifampicin-resistant TB (including RMR-, MDR-, preXDR- and XDR-TB) pulmonary or extrapulmonary TB (other than TB meningitis or osteoarticular TB). Children with rifampicin resistance without additional injectable or fluoroquinolone resistance would be randomized to the primary intervention arm (Arm 1, 26 weeks of bedaquiline, delamanid and levofloxacin with addition of linezolid to the first 8 weeks) or to the control arm (Arm 2, the WHO 9-12 month shortened regimen). Children with additional injectable or fluoroquinolone resistance would be assigned to intervention arms depending on their resistance patterns (Arms 3 or 4). In addition to a number of secondary and exploratory objectives, the primary objectives of the trial were to evaluate whether the primary intervention arm (Arm 1) had non-inferior efficacy (Objective 1) and superior safety (Objective 2) compared to the control arm (Arm 2). My doctoral research presented here, specifically Chapters 2-5 on levofloxacin, Chapters 7-8 on linezolid, and potentially Chapter 9 on bedaquiline, was directly informing the design of the intervention arm and other aspects of the trial.

Considering the recent policy change in South Africa to substitute bedaquiline for the injectable drug in MDR-TB treatment regimens, it is no longer acceptable to randomize children to an injectable-containing control arm. The trial is now being reconsidered as a smaller phase 2 study, with the objectives to characterize the safety, pharmacokinetics and treatment outcomes among children with probable or confirmed rifamipicin-resistant TB treated with two intervention regimens: Arm 1 for fluoroquinolone-susceptible rifampicin-resistant TB cases (Arm 1, 26 weeks of bedaquiline, delamanid and levofloxacin with addition of linezolid to the first 8 weeks); Arm 2 for fluoroquinolone-resistant rifampicin-resistant TB cases (Arm 1, 26 weeks of bedaquiline, delamanid and clofazimine with addition of linezolid to the first 8 weeks). This is still informed by my doctoral research, and the trial may still eventually inform a future phase 3 trial in children, depending on evolving data from the adult trial landscape. Data from this doctoral dissertation remains critical to informing the IMPAACT 2020 trial design.

10.3.4 Cross-cutting issues

Medication acceptability and palatability is a cross-cutting issue highlighted by this doctoral research, and is an area of ongoing and future research. This is often accomplished using a mixed methods approach, utilizing both quantitative and qualitative methods. As part of the TB-CHAMP lead-in pharmacokinetics study (Chapter 5), assessments were done of the acceptability of the novel levofloxacin formulation, for which the analysis and write-up are ongoing. Characterizing the acceptability of the second-line antituberculosis drugs is one of the aims of the MDRPK2 study and will involve quantitative observations and survey questions paired with in-depth interviews performed by qualitative researchers. Objectives to assess acceptability are also included in Otsuka 232/233 paediatric and IMPAACT 2005 delamanid trials.

10.4 Other relevant considerations

The doctoral research presented here has contributed to the strengthening or initiation of numerous local, national and international collaborations. I have worked closely with the University of Cape Town Division of Clinical Pharmacology on multiple studies described here including laboratory, pharmacology and pharmacometrics experts. I

worked closely with pharmacometricians at the University of California San Francisco (Rada Savic) and Upssala University (Elin Svensson), both of whom I am collaborating with now on multiple other projects.

During the time of my doctoral research I have had many opportunities to provide leadership, contribute to capacity building, and give input on policy on MDR-TB treatment in children, at local, regional and international levels. I am involved with and have contributed to a number of policy statements from the Sentinel Project. I am a Paediatric Expert for the WHO-European Respiratory Society TB Consilium, in which capacity I provide expert clinical advice on difficult paediatric MDR-TB cases through the WHO-ERS on-line consultation platform. I have lectured and contributed to multiple capacity building efforts through the Desmond Tutu TB Centre, including at the annual International Child TB Training Course, and through other partners including Médecins Sans Frontières (MSF), the KNCV Tuberculosis Foundation (KNCV), the International Union Against Tuberculosis and Lung Diseases (IUTLD), and the WHO Africa Regional Office (AFRO). I have regularly contributed to local MDR-TB policy for the City of Cape Town and the Western Cape Department of Health. I am a member of the WHO AFRO Childhood TB Task Force (2016-current). I am an investigator within the TB Scientific Committee of the IMPAACT network, where I am leading or involved with multiple protocols (see Section 10.3), and am helping shape the TB Scientific Committee's agenda. I am an Associate Editor for the journals Public Health Action (2017-current) and BMC Infectious Diseases (2018-current). I have represented the childhood TB community at international forums on paediatric drug development, including at meetings of the WHO's Paediatric Antiretroviral Drug Optimization (PADO) group and the Global Accelerator for Paediatric Formulations (www.gap-f.org). I look forward to continuing to leverage the skills and experience I have developed during the time of this doctoral research to have a global impact on childhood TB and MDR-TB treatment.

10.5 Conclusions

In conclusion, this doctoral research has addressed a number of important key knowledge gaps related to the optimal paediatric MDR-TB treatment. This research has raised a number of follow-up questions that in addition to other key knowledge gaps, discussed above, myself and colleagues are working to address in order to continuing

advancing the field towards a goal of effective, safe, shorter MDR-TB treatment for children.

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Appendix 1 Author contributions

For Chapters 2, 3 and 4, I contributed to the design, led the implementation of the study as the lead clinician and co-investigator for MDRPK1, contributed to the analytical plan, and led the interpretation of the results and write-up of these manuscripts. For Chapter 5, I led the design, oversaw the implementation, contributed to the analytical plan, and led the interpretation of the results and write-up of the manuscript. For Chapter 6, I designed and performed the review, and led the write-up of the manuscript. For Chapter 7, I designed the study, oversaw its implementation as lead clinician for MDRPK1 and Principal Investigator for MDRPK2, contributed to the analytical plan, and led the interpretation of the results and write-up of the manuscript. For Chapter 8, I contributed to the design of the study, led the implementation, contributed to the analytical plan, led the interpretation of the results and the write-up of the manuscript. For Chapter 9, I led the design of the study as the protocol co-Chair, contributed to the analytical plan, co-led the interpretation of the results and write up of the manuscript.

Appendix 2 Funding

The studies in Chapters 2, 3, 4 and 8 primarily included data from the MDRPK1 study, an NIH R01-funded study primarily supported by The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) of the National Institutes of Health under award number R01HD069169 (AC Hesseling, HS Schaaf). The study in Chapter 5 was undertaken as a component of the TB-CHAMP trial, which is funded by the Joint Global Health Trials Scheme of the Department for International Development, UK (DFID), the Wellcome Trust and The Medical Research Council (MRC UK) (Grant # MR/M007340/1) and the South African Medical Research Council (SA MRC) Strategic Health Innovation Partnerships (SHIP) (PI: AC Hesseling). The study in Chapter 7 included some data from MDRPK1 and the MDRPK2 study, an NIH R01-funded study primarily supported by The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) of the National Institutes of Health under award number R01HD083047 (PIs AJ Garcia-Prats, R Savic). The study in Chapter 9 was funded by the IMPAACT network, which receives funding from the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) with co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH).

During the time of this doctoral research I received funding from the NIH as a co-investigator from MDRPK1, as the Principal Investigator for MDRPK2, from IMPAACT as the co-Chair for the BDQ CRUSH study, from BMRC as a co-investigator for TB-CHAMP. I received additional funds from other work not contributing directly to this doctoral dissertation, including as a Co-investigator (Opti-Rif trial), Principal Investigator (Otsuka 232/233 trials) and protocol Co-Chair (IMPAACT 2005) on other TB trials.

Appendix 3

3.1 Other contributing works (full texts included)

1. Harausz EP, **Garcia-Prats AJ**, Law S, Schaaf HS, Kredo T, Seddon JA, Menzies D, Turkova A, Achar J, Amanullah F, Barry P, Becerra M, Chan ED, Chan PC, Ioana Chiotan D, Crossa A, Drobac PC, Fairlie L, Falzon D, Flood J, Gegia M, Hicks RM, Isaakidis P, Kadri SM, Kampmann B, Madhi SA, Marais E, Mariandyshev A, Méndez-Echevarría A, Moore BK, Nargiza P, Ozere I, Padayatchi N, Ur-Rehman S, Rybak N, Santiago-Garcia B, Shah NS, Sharma S, Shim TS, Skrahina A, Soriano-Arandes A, van den Boom M, van der Werf MJ, van der Werf TS, Williams B, Yablokova E, Yim JJ, Furin J, Hesseling AC; Collaborative Group for Meta-Analysis of Paediatric Individual Patient Data in MDR-TB. Treatment and outcomes in children with multidrug-resistant tuberculosis: A systematic review and individual patient data meta-analysis. P*LoS Med*. 2018 Jul 11;15(7):e1002591.

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PLOS | MEDICINE

RESEARCH ARTICLE

Treatment and outcomes in children with multidrug-resistant tuberculosis: A systematic review and individual patient data meta-analysis

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Abbreviations: ADR, adverse drug reaction; AFB, acid-fast bacilli; aOR, adjusted odds ratio; ART, antiretroviral treatment; CI, confidence interval; DST, drug susceptibility test; ECDC, European Centre for Disease Prevention and Control; IPD, individual patient data; LILACS, Latin American and Caribbean Health Sciences Literature; MDR-TB, multidrug-resistant tuberculosis; MeSH, Medical Subject Headings; MSF, Médecins Sans Frontières; MTB, *Mycobacterium tuberculosis*; NIH, National Institutes of Health; PMTCT, prevention of mother to child transmission; pre-XDR-TB, pre-extensively drug-resistant tuberculosis; TB, tuberculosis; US CDC, United States Centers for Disease Control and Prevention; WHO, World Health Organization; XDR-TB, extensively drug-resistant tuberculosis.

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Abstract

Background

An estimated 32,000 children develop multidrug-resistant tuberculosis (MDR-TB; *Mycobacterium tuberculosis* resistant to isoniazid and rifampin) each year. Little is known about the optimal treatment for these children.

Methods and findings

To inform the pediatric aspects of the revised World Health Organization (WHO) MDR-TB treatment guidelines, we performed a systematic review and individual patient data (IPD) meta-analysis, describing treatment outcomes in children treated for MDR-TB. To identify eligible reports we searched PubMed, LILACS, Embase, The Cochrane Library, PsychINFO, and BioMedCentral databases through 1 October 2014. To identify unpublished data, we reviewed conference abstracts, contacted experts in the field, and requested data through other routes, including at national and international conferences and through organizations working in pediatric MDR-TB. A cohort was eligible for inclusion if it included a minimum of three children (aged <15 years) who were treated for bacteriologically confirmed or clinically diagnosed MDR-TB, and if treatment outcomes were reported. The search yielded 2,772 reports; after review, 33 studies were eligible for inclusion, with IPD provided for 28 of these. All data were from published or unpublished observational cohorts. We analyzed demographic, clinical, and treatment factors as predictors of treatment outcome. In order to obtain adjusted estimates, we used a random-effects multivariable logistic regression (random intercept and random slope, unless specified otherwise) adjusted for the following covariates: age, sex, HIV infection, malnutrition, severe extrapulmonary disease, or the presence of severe disease on chest radiograph. We analyzed data from 975 children from 18 countries; 731 (75%) had bacteriologically confirmed and 244 (25%) had clinically diagnosed MDR-TB. The median age was 7.1 years. Of 910 (93%) children with documented HIV status, 359 (39%) were infected with HIV. When compared to clinically diagnosed patients, children with confirmed MDR-TB were more likely to be older, to be infected with

HIV, to be malnourished, and to have severe tuberculosis (TB) on chest radiograph ($p < 0.001$ for all characteristics). Overall, 764 of 975 (78%) had a successful treatment outcome at the conclusion of therapy: 548/731 (75%) of confirmed and 216/244 (89%) of clinically diagnosed children (absolute difference 14%, 95% confidence interval [CI] 8%–19%, $p < 0.001$). Treatment was successful in only 56% of children with bacteriologically confirmed TB who were infected with HIV who did not receive any antiretroviral treatment (ART) during MDR-TB therapy, compared to 82% in children infected with HIV who received ART during MDR-TB therapy (absolute difference 26%, 95% CI 5%–48%, $p = 0.006$). In children with confirmed MDR-TB, the use of second-line injectable agents and high-dose isoniazid (15–20 mg/kg/day) were associated with treatment success (adjusted odds ratio [aOR] 2.9, 95% CI 1.0–8.3, $p = 0.041$ and aOR 5.9, 95% CI 1.7–20.5, $p = 0.007$, respectively). These findings for high-dose isoniazid may have been affected by site effect, as the majority of patients came from Cape Town. Limitations of this study include the difficulty of estimating the treatment effects of individual drugs within multidrug regimens, only observational cohort studies were available for inclusion, and treatment decisions were based on the clinician's perception of illness, with resulting potential for bias.

Conclusions

This study suggests that children respond favorably to MDR-TB treatment. The low success rate in children infected with HIV who did not receive ART during their MDR-TB treatment highlights the need for ART in these children. Our findings of individual drug effects on treatment outcome should be further evaluated.

Author summary

Why was this study done?

- Treatment for multidrug-resistant tuberculosis (MDR TB) affects 32,000 children per year, requires longer treatment with much more toxic medications than drug-susceptible tuberculosis. Unfortunately, little is know about the optimal treatment for children with MDR TB.
- This study reviewed treatment and outcome data from children around the world in order to better understand the management of MDR-TB in children.
- This study also sought to understand the risk factors for poor treatment outcomes in children with MDR-TB.
- This study informed the World Health Organization guidelines on treatment of MDR-TB in children.

What did the researchers do and find?

- We performed a systematic review and individual patient data meta-analysis on clinical characteristics and treatment outcomes on 975 children from across 18 countries.

- Children were analyzed in two separate groups, those with bacteriologically confirmed MDR-TB and those who were clinically diagnosed with MDR-TB.
- We found that, in general, children do well when treated with the second-line MDR-TB medications (78% overall had successful treatment outcomes), despite the fact that there was a high burden of severe disease.
- Malnutrition and not being treated for HIV (if the child was HIV-positive) during TB treatment significantly increased the risk of poor outcomes.
- Second-line injectable agents and high-dose isoniazid were associated with treatment success. However, a high proportion of children with non-severe disease who received no second-line injectable agents still did well; therefore, children with non-severe disease may be able to be spared from these toxic medications.

What do these findings mean?

- Consideration should be given to using high-dose isoniazid in treatment regimens, and if children have non-severe disease, the possibility of excluding second-line injectable agents from the treatment regimen should be considered.
- HIV treatment should be started as soon as is possible, and malnutrition should be aggressively treated.

Introduction

Almost 500,000 people developed multidrug-resistant tuberculosis (MDR-TB) (defined as *Mycobacterium tuberculosis* with resistance to at least isoniazid and rifampin) in 2015 [1]. Despite the fact that as many as 32,000 children younger than 15 years of age develop MDR-TB globally each year, little is known about the optimal treatment for children with MDR-TB [2]. The diagnosis and treatment of MDR-TB in children is challenging: it can be difficult to bacteriologically confirm the diagnosis because of difficulties in collecting respiratory samples in younger children and in children who frequently have paucibacillary (smear- or culture-negative) disease [3].

Treatment of MDR-TB is difficult as well, requiring the use of second-line medications in regimens much longer (often lasting 18–20 months) than those for drug-susceptible disease [4]. These regimens are frequently hard to tolerate, particularly in children, due to the length of treatment, drug toxicity, and the lack of child-friendly formulations. However, unlike adults, children have a wide spectrum of tuberculosis (TB) disease, from limited disease with low bacillary burden, to more severe adult-type disease (e.g., cavitating pulmonary TB) with a higher bacillary load [3]. Children with less severe disease may not require a treatment regimen as intensive as children with more severe disease or adults. Therefore, they may be able to be spared from these long, toxic regimens [5].

A previous systematic review of 315 children from eight cohorts from five countries reported successful treatment outcomes in 82% of children with clinically or bacteriologically diagnosed MDR-TB [6]. Clinical or treatment factors associated with outcomes in children

with MDR-TB have been evaluated in a few individual studies but have not been well characterized in large, geographically diverse cohorts. The small size of individual published cohorts limits the precision in assessing the impact of clinical factors on outcomes, and their lack of geographic diversity restricts the generalizability of findings. Study-level meta-analyses limit the useful inferences that may be drawn on the associations between outcomes and the treatment, and limit the adjustment for confounding or interaction that would be possible from the analysis of individual patient data (IPD). A more rigorous evidence base is needed to help guide the management of MDR-TB treatment in children globally. Given the paucibacillary nature of TB in most children and the potential for certain children to receive less intensive and less toxic treatment regimens, an understanding of risk factors for poor outcomes across settings is important for designing future treatment regimens.

In order to provide this stronger evidence base and to inform the revised 2016 World Health Organization (WHO) MDR-TB treatment guidelines, we undertook a systematic review and IPD meta-analysis to describe treatment outcomes among children with confirmed or clinically diagnosed MDR-TB, and to characterize demographic, clinical, and treatment factors associated with treatment outcomes [4].

Methods

Eligibility criteria

A cohort was eligible for inclusion in this IPD meta-analysis if it included a minimum of three children (aged <15 years) within a defined treatment cohort who were treated for bacteriologically confirmed ("confirmed") or clinically diagnosed pulmonary or extrapulmonary MDR-TB, and for whom treatment outcomes were reported, using standard 2014 WHO MDR-TB outcome definitions (Table 1) [7,8]. All cohorts containing children included in a previous, largely adult systematic review and IPD meta-analysis of MDR-TB were also considered eligible; those data were accepted as one data set, even if that data set contained fewer than three children from a defined geographic area [9]. In order to be defined as having "confirmed" MDR-TB, there needed to be bacteriological confirmation with documented resistance to both isoniazid and rifampicin on genotypic or phenotypic testing. The basis for a clinical diagnosis of TB is described in Table 1. Eligibility criteria were applied at the individual level, so that studies reporting on both adults and children could be considered eligible if they otherwise met the specified criteria. Both published and unpublished data were included, without date restrictions. Eligible study designs included controlled and noncontrolled retrospective and prospective studies and case series. Reports written in English, Spanish, French, Dutch, and Russian were included. Studies that used only combinations of rifampin, isoniazid, pyrazinamide, ethambutol, or streptomycin to treat MDR-TB were excluded, as this is considered inadequate therapy.

Identifying primary reports

In order to identify eligible reports, we searched PubMed, LILACS (Latin American and Caribbean Health Sciences Literature), Embase, The Cochrane Library, PsychINFO, and BioMedCentral databases through 1 October 2014, with a search strategy using a combination of the search terms "tuberculosis," "multidrug resistance," "MDR-TB," "multidrug-resistant," and "children," both as exploded MeSH (Medical Subject Headings) headings and free-text terms, and without language restriction. The specific search strategies for Pubmed and Embase are presented in S1 Table. We also reviewed conference abstracts from the annual World Lung Health Conferences of the International Union Against Tuberculosis and Lung Diseases (The Union).

Table 1. Tuberculosis case definitions and treatment outcome definitions.

TB case definitions	
Bacteriologically confirmed TB	A case of TB in a patient from whom a biological specimen was positive by smear microscopy[1], culture, or WHO-approved rapid diagnostics (including GeneXpert MTB/RIF).
Clinically diagnosed TB	A case of TB in a patient who does not fulfill the criteria for bacteriological confirmation but who has been diagnosed with TB disease by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of radiographic abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation.
TB source case	A case of infectious TB (usually sputum smear- or culture-positive) in a person who transmits infection to one or more other individuals.
MDR-TB outcome definitions	
Cured	Treatment completed as recommended by the national policy without evidence of failure, and three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase of treatment.
Treatment completed	Treatment completed as recommended by the national policy without evidence of failure, but no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase of treatment.
Treatment failed	Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of • lack of conversion by the end of the intensive phase, • bacteriological reversion in the continuation phase after conversion to negative, • evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs, or • ADRs.
Died	A patient who dies for any reason during the course of treatment.
Lost to follow-up	A patient whose treatment was interrupted for two consecutive months or more.
Not evaluated	A case of TB in a patient for whom no treatment outcome is assigned. This includes patients "transferred out" to another treatment unit and whose treatment outcome is unknown.
Treatment success	The sum of cured and treatment completed.

Adapted from: Guidance for national tuberculosis programmes on the management of tuberculosis in children: second edition. WHO, 2014 [7].

[1]For this study, participants had to have MDR-TB confirmed by culture or WHO-approved rapid diagnostics; smear microscopy was not sufficient to be classified as bacteriologically confirmed.

Abbreviations: ADR, adverse drug reaction; MDR-TB, multidrug-resistant tuberculosis; MTB/RIF, *Mycobacterium tuberculosis*/rifampin; TB, tuberculosis; WHO, World Health Organization.

https://doi.org/10.1371/journal.pmed.1002591.t001

To identify additional published and unpublished data, we contacted experts in the field of pediatric MDR-TB. We also requested data through other routes, including at national and international conferences and training events, and through international and in-country organizations working in pediatric MDR-TB, including the Sentinel Project on Pediatric Drug-Resistant Tuberculosis, the WHO Childhood TB Sub-Group, Médecins Sans Frontières (MSF), the United States Centers for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC), The Union, and the National Institutes of Health (NIH).

Report selection and review

All abstracts were screened by EPH and a researcher with Cochrane South Africa to select full text reports to review. All full text reports were reviewed independently by two reviewers (EPH, AJGP, HSS, JF, ACH) to assess for eligibility, except reports in Russian, French, Dutch,

and Spanish, which were reviewed by a single reviewer (AT, EPH, ACH, and JF, respectively). Disagreements about study selection were resolved by a third reviewer. If eligibility was unclear because of missing information, two attempts were made to contact authors of the primary report. After two unsuccessful attempts, reports were excluded.

The quality of individual studies was described using a modified version of the Newcastle-Ottawa approach for cohort studies adapted for use in pediatric MDR-TB [10]. An example of our grading approach is provided in S2 Table, followed by the grading of the individual studies.

Data sharing and abstraction

The authors of all eligible studies and cohorts were contacted to request IPD. De-identified IPD were included following written agreement from the original authors, or the lead clinical physician in the case of unpublished data.

Data were requested on factors that could influence treatment decision and outcome, including:

Participants: age, sex, nutritional status, HIV status and antiretroviral treatment (ART), adult MDR-TB source case information, bacteriologically confirmed versus clinical diagnosis (Table 1), disease site (pulmonary or extrapulmonary), severity of disease on chest radiograph, drug susceptibility test (DST) results, and history of previous TB episodes.

Intervention: use of individual drugs and the duration of drug use in the treatment regimen.

Outcomes: acid-fast bacilli (AFB) smear microscopy (smear) and culture conversion, adverse events, and WHO-defined treatment outcomes, including cure, treatment completion, treatment failure, loss to follow-up, not evaluated, and death (see Table 1 for definitions) [7].

Severity of TB disease on the chest radiograph was graded independently by two reviewers (EPH, ACH) as either severe or non-severe, based on the reported chest radiographic findings using adapted Wiseman criteria [11]; disagreements were arbitrated by a third reviewer (HSS). Malnutrition was defined as being severely underweight for age (weight-for-age-adjusted z-score of less than −3) or malnourished, as per attending clinician's clinical assessment, including the presence of nutritional edema. Severe extrapulmonary TB was defined as TB meningitis, miliary TB, abdominal, osteoarticular, or "disseminated TB disease" (this diagnosis was given in 11 children, without further details). The primary authors of all included reports were contacted to resolve any queries.

Although we collected information on adverse effects, we were unable to complete formal analyses given the limitations in the data.

Statistical analysis

Data from children with confirmed pre-extensively drug-resistant (pre-XDR)-TB (MDR-TB with additional resistance to either a fluoroquinolone or a second-line injectable agent) were combined with those from children with MDR-TB. Children with confirmed extensively drug-resistant TB (XDR-TB, defined as MDR-TB with additional resistance to both a fluoroquinolone and a second-line injectable agent) were excluded from analysis and will be reported on separately. This was done because patients with XDR-TB generally have very different treatment requirements, such that their outcomes would not be representative of the MDR-TB and pre-XDR-TB cohorts. When analyzing the association between individual drug use and treatment success, treatment outcomes were dichotomized as either successful or unsuccessful and stratified by confirmed versus clinically diagnosed MDR-TB. Successful treatment outcome was defined as cure or treatment completion (Table 1). Unsuccessful outcome was defined as

treatment failure or death. Children who were lost to follow-up or not evaluated were excluded from analyses.

With respect to the analysis of the estimates of the association between individual antituberculosis medications (used as part of a multidrug regimen) and treatment success, in order to obtain adjusted estimates, we used a random-effects multivariable logistic regression (random intercept and random slope, unless specified otherwise) with adaptive quadrature approximation. Our models for predictors of drug effect on TB treatment outcome only included those children with bacteriologically confirmed MDR-TB. Patients were considered to be clustered within cohorts such that intercepts and slopes of the main exposure variables were allowed to vary across cohorts. This was done to account for unmeasured differences in patient populations and other site-specific characteristics. Estimates were adjusted for the following covariates: age (as a continuous variable), sex, HIV infection, malnutrition, severe extrapulmonary disease, or the presence of severe disease on chest radiograph. There were nine sites that did not report any information on patients' nutritional status ($n = 44$ children) and one site that did not report on the severity of extrapulmonary disease ($n = 2$ children). For the patients at sites that had no data from which to estimate a mean value, their missing values were replaced in the analysis by the mean value of the variable in the entire sample. At sites where most patients had these values reported, for individual patients who had missing data on HIV status ($n = 65$), sex ($n = 3$), severe extrapulmonary disease ($n = 68$), and malnutrition ($n = 43$), missing values were replaced by their respective site's mean value of the variable. Children from countries with very low HIV prevalence who did not have an HIV test were assumed to be HIV uninfected following consultation with site investigators. A p-value of 0.05 was taken as the limit of statistical significance. All statistical analyses were performed using SAS software (version 9.3, SAS Institute, Cary, NC).

Ethical considerations

A protocol prespecified the study rationale and methods (S2 Text). Ethics approval was provided by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University (reference number X14/09/020). The collection of the original data and sharing of those data was approved by the appropriate oversight body at each contributor's local institution, including the use of unpublished data, prior to release of data to the team.

For IPD contributed by the US CDC, Atlanta, GA, Institutional Review Board approval was obtained from the South African Medical Research Council Ethics Committee and the Human Research Ethics Committee of University of the Witwatersrand in Johannesburg and was determined to be routine disease surveillance by the US CDC.

Results

Search results and report selection

The search results and report selection are summarized in Fig 1 (see S1 Text for PRISMA checklist). The search yielded 2,772 reports; after screening of abstracts and review of the full texts, 33 studies were eligible for inclusion, with IPD provided for 28 of these [9,12–33] (Fig 1). Overall, the studies were noted to be of low quality, given the lack of any randomized controlled trials (see S3 Table for overview of studies). Although we are unable to quantify the pediatric data that were not included, we believe they were minimal. The largest group of studies from which data were excluded were the studies in which there was no reply from the authors, so it is unknown exactly how many children may have been excluded. However, these

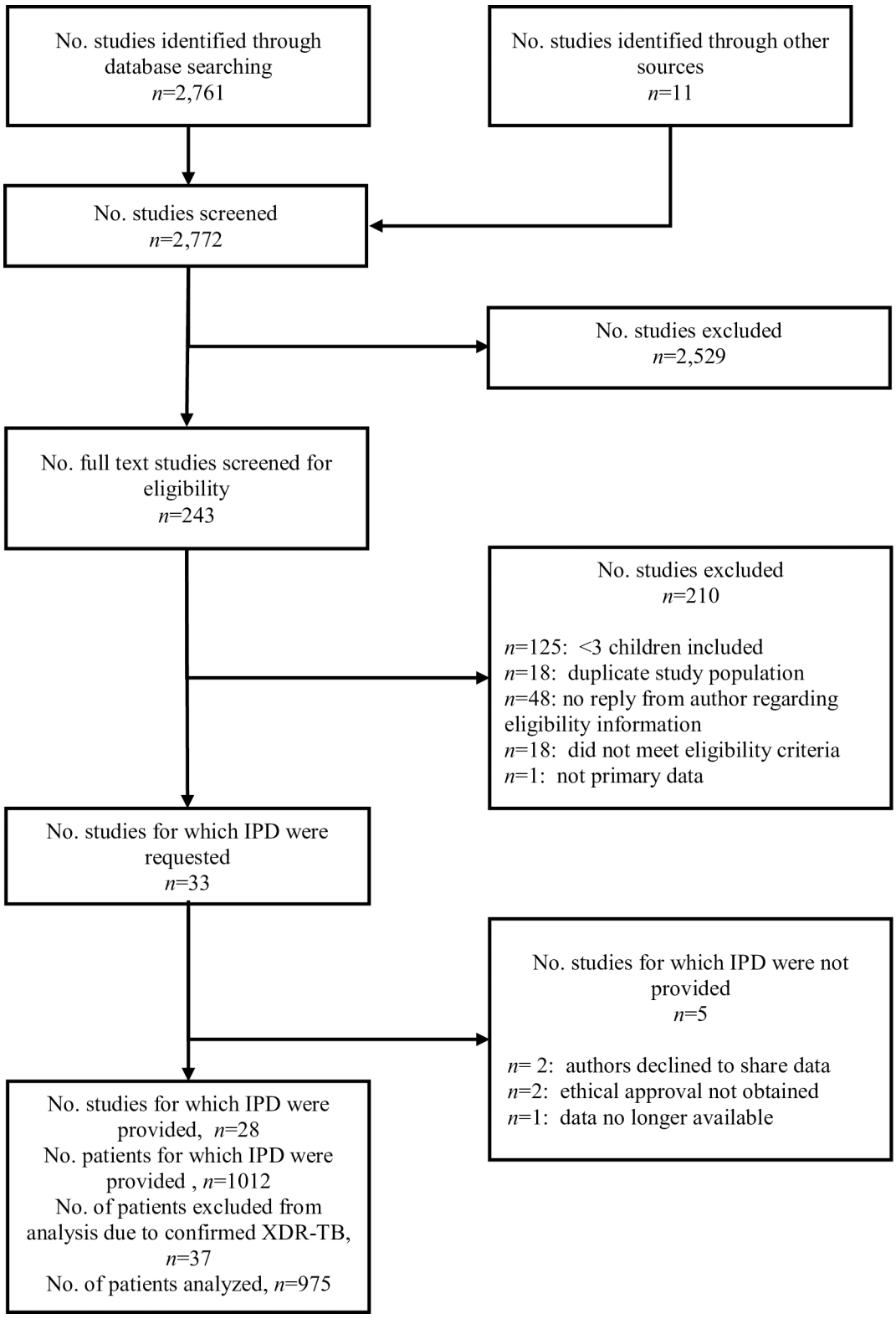

Fig 1. Study selection. IPD, individual patient data; XDR-TB, extensively drug resistant tuberculosis.

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were almost all studies with mainly adult patients, but inclusion of children could not be ruled out with certainty based on reading these articles.

Report characteristics

Data sets were received from sites in 18 countries with a broad geographic distribution (Fig 2), with the majority from Africa. Nine countries (Belarus, India, Pakistan, Peru, Russian Federation, South Africa, Tajikistan, Ukraine, Uzbekistan) were among the 30 high-burden MDR-TB countries [1]. Details of published and unpublished cohorts included are presented in S3 Table. Eight percent of children were treated between 1990 and 2004, 67% of children were treated between 2005 and 2009, and 25% were treated from 2010 and later.

Summary patient data and outcomes

The 28 cohorts included data from 1,012 patients. All data were from observational studies in which children were treated with the local standard of care. Thirty-seven children with confirmed XDR-TB were excluded from analyses; therefore, data from 975 children were analyzed. Of the 975 children, 731 (75%) had confirmed MDR-TB by DST and 244 (25%) had clinically diagnosed MDR-TB. Most data predated the rollout of GeneXpert MTB/RIF; the vast majority of confirmed patients were diagnosed using culture. Of the 731 children with a confirmed diagnosis, 68 (9.3%) had pre-XDR-TB (36 with MDR-TB with additional fluoroquinolone resistance, and 32 with additional second-line injectable resistance). It should be noted that 68% of confirmed patients had no additional information regarding resistance to fluoroquinolones and/or second-line injectable agents. Of the 244 children with a clinical diagnosis of MDR-TB, 164 (67%) had clinical TB with a known close source case with DST-confirmed MDR-TB, 14 (6%) had failure of first-line therapy (first-line oral anti-TB drugs are isoniazid, rifampin, ethambutol, pyrazinamide, rifabutin, and rifapentine [34]) despite documented good adherence, and one child had a clinical diagnosis of TB with a known MDR-TB source case, in addition to treatment failure on first-line TB drugs despite good adherence. The basis for the clinical diagnosis of MDR-TB was not specified in 65 patients (27%).

Key demographic and clinical characteristics, stratified by confirmed versus clinically diagnosed MDR-TB, are shown in Table 2. The median age was 7.1 years (IQR 2.6–11.7); 429 (44%) were males. HIV status was documented in 910 (93%) children, of whom 359 (39%) were infected with HIV. Children with confirmed MDR-TB were more likely to be older, to be infected with HIV, to be malnourished, and to have severe TB on chest radiograph.

TB treatment outcomes are summarized in Table 3. Overall, 764 of 975 (78%) had a successful treatment outcome, with successful treatment outcomes in 548/731 (75%) of bacteriologically confirmed children and 216/244 (89%) in clinically diagnosed children. Treatment success rates did not differ among those children with pre-XDR, although numbers were small. Across all sites, most children had successful treatment outcomes, particularly those with a clinical diagnosis (Fig 3). There was considerable heterogeneity between sites with regard to treatment outcomes among confirmed cases, and less heterogeneity among clinical cases. Treatment outcomes among children infected with HIV assessed by the time of initiation of ART relative to MDR-TB therapy initiation are shown in Table 4. These data are presented by site in Fig 4. Treatment outcomes were worse in children infected with HIV who were never on any ART during their MDR-TB therapy, compared to children who received ART (either started before or concurrent with MDR-TB treatment). Among children with confirmed MDR-TB, treatment was successful in 56% (15/27) of children infected with HIV who did not receive any ART during MDR-TB therapy, compared to 82% (149/182) in

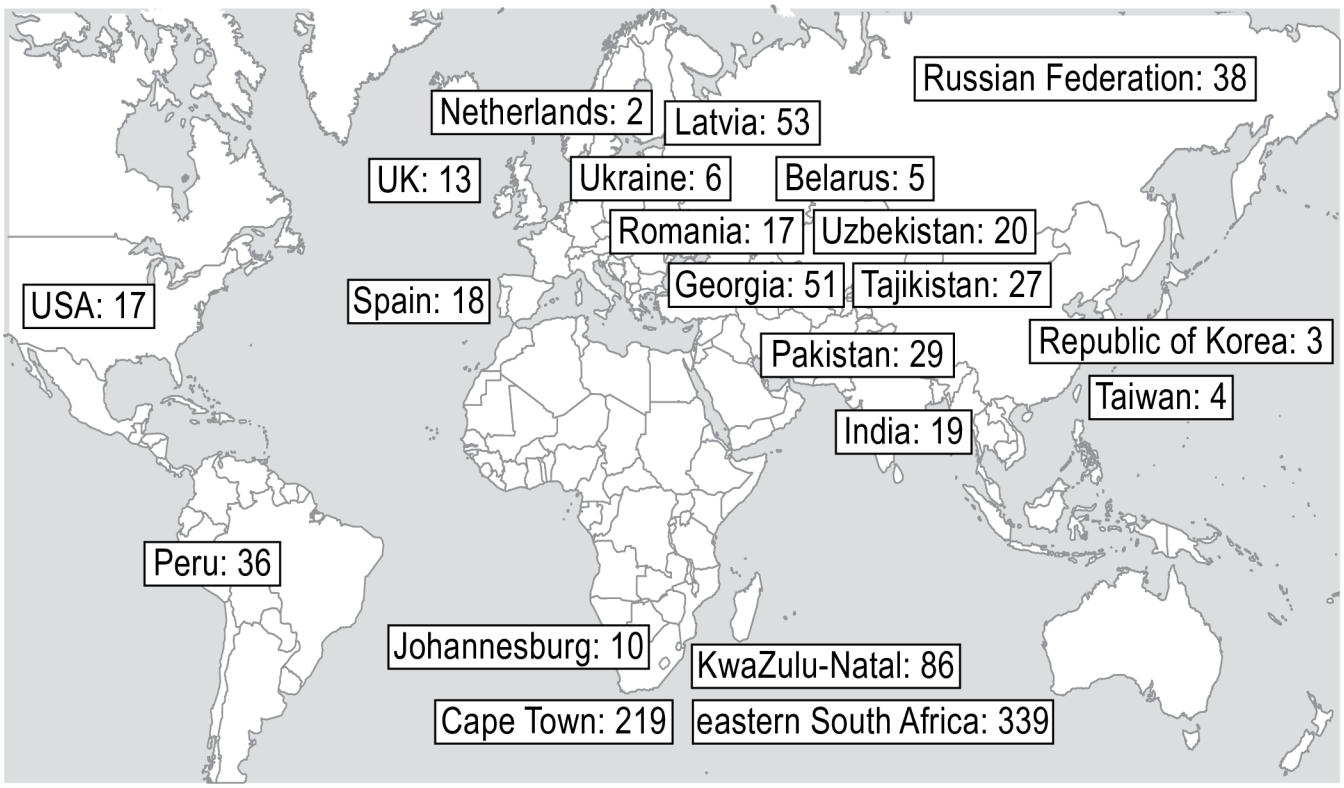

Fig 2. Geographic distribution of patients. Locations of patients included in the individual patient data meta-analysis. The number indicates the number of participants included from each location.

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children who received ART during MDR-TB therapy. Among children not infected with HIV, 93% with confirmed MDR-TB had successful treatment outcomes.

In multivariable analysis, among children with confirmed MDR-TB, HIV infection (adjusted odds ratio [aOR] 0.3, 95% CI 0.2–0.6) and malnutrition (aOR 0.3, 95% CI 0.2–0.6) were associated with reduced odds of successful treatment outcome versus treatment failure or death (Table 5). Malnutrition was an independent risk factor from HIV infection for poor treatment outcome. This analysis did not adjust for the regimen used or individual drugs used. Among children with clinically diagnosed MDR-TB, no assessed covariates predicted treatment outcome.

Treatment outcomes and specific drug therapy

We also conducted analyses to assess estimates of the association between individual antituberculosis medications, used as part of a multidrug regimen, and treatment success (Table 6) to inform the WHO treatment guideline process. This analysis was restricted to patients with bacteriologically confirmed MDR-TB. Although children who were lost to follow-up were excluded from the analysis, among children with confirmed MDR-TB, there were no differences in the following key variables between children who were lost to follow-up versus those with a known outcome: age, HIV status, sex, the presence of severe TB or severe extrapulmonary TB, previous TB treatment, or nutritional status (S4 Table). In children with confirmed MDR-TB, the second-line injectable agents and high-dose isoniazid (15–20 mg/kg/day) were associated with treatment success. Ninety-eight percent (130/133) of these children treated

Table 2. Demographic and clinical characteristics among children with MDR-TB.

Characteristic	All children *n* = 975 (%)	Bacteriologically confirmed MDR or Pre-XDR-TB *n* = 731 (%)	Clinically diagnosed MDR-TB *n* = 244 (%)	*p*-value[d]
Age (years)				
<5	399 (41)	240 (33)	159 (65)	<0.001
5 to <10	234 (24)	178 (24)	56 (23)	
10 to <15	342 (35)	313 (43)	29 (12)	
Median age	7.1, IQR 2.6–11.7	8.5, IQR 3.4–12.2	3.6, IQR 1.9–7.0	
Sex				
Female	543 (56)	414 (57)	129 (53)	0.570
Male	429 (44)	315 (43)	114 (47)	
Unknown	3 (0.3)	2 (0.3)	1 (0.4)	
HIV status				
Infected with HIV	359 (37)	323 (44)	36 (15)	<0.001
Not infected with HIV	551 (57)	356 (49)	195 (80)	
Unknown	65 (7)	52 (7)	13 (5)	
Malnourished[a]				
Yes	332 (34)	276 (38)	56 (23)	<0.001
No	556 (57)	381 (52)	175 (72)	
Unknown	87 (9)	74 (10)	13 (5)	
Severe disease on chest radiograph				
Yes	474 (49)	407 (56)	67 (28)	<0.001
No	294 (30)	168 (23)	126 (52)	
Unknown	207 (21)	156 (21)	51 (21)	
Severe extrapulmonary disease[b]	127 (13)	103 (14)	24 (10)	0.031
Site of disease				
Pulmonary only	710 (73)	526 (72)	184 (75)	0.002
Extrapulmonary only	99 (10)	67 (9)	32 (13)	
Both extrapulmonary and pulmonary	152 (16)	130 (18)	22 (9)	
Unknown	14 (1)	8 (1)	6 (3)	
Extrapulmonary disease sites[c]				
Meningitis	34 (14)	23 (12)	11 (20)	
Miliary	34 (12)	29 (15)	5 (9)	
Bone/joint (including spine)	25 (10)	20 (10)	5 (9)	
Pleural	19 (8)	15 (8)	4 (7)	
Urogenital	1 (0.4)	1 (0.5)	0	
Abdominal	53 (21)	48 (24)	5 (9)	
Skin	1 (0.4)	1 (0.5)	0	
Disseminated disease not otherwise specified	11 (4)	10 (5)	1 (2)	

[a]Malnourished was defined as being underweight or malnourished by clinical diagnosis, having nutritional edema, or having low weight for age (weight-for-age-adjusted z-score of less than −3).

[b]Severe extrapulmonary disease was defined as meningitis, miliary, abdominal, osteoarticlar, and disseminated disease not otherwise specified.

[c]Disease sites are not mutually exclusive; one child could have multiple disease sites. Denominator is children with only extrapulmonary and with both pulmonary and extrapulmonary disease sites.

[d]*p*-value represents differences in characteristics between clinically diagnosed and bacteriologically confirmed cohorts.

Abbreviations: MDR-TB, multidrug-resistant tuberculosis, pre-XDR-TB, pre-extensively drug-resistant tuberculosis; XDR-TB, extensively drug resistant tuberculosis.

https://doi.org/10.1371/journal.pmed.1002591.t002

Table 3. Summary of treatment outcomes for children treated for MDR-TB.

MDR-TB treatment outcome	Bacteriologically confirmed MDR-TB *n* = 731 (%)	Clinically diagnosed MDR-TB *n* = 244 (%)	Absolute difference (95% CI)[a]	*p*-value	All children *n* = 975 (%)
Cured/completed treatment	548 (75)	216 (89)	14% (8%–19%)	<0.001	764 (78)
Death	77 (11)	8 (3)	8% (4%–11%)	<0.001	85 (9)
Failed treatment	16 (2)	0	2% (0%–4%)	0.044	16 (2)
Lost to follow-up/not evaluated	90 (12)	20 (8)	4% (0%–9%)	0.100	110 (11)

[a]The overall chi-squared *p*-value treatment outcomes between clinically diagnosed and bacteriologically confirmed cohorts is <0.001.

Abbreviation: MDR-TB, multidrug-resistant tuberculosis.

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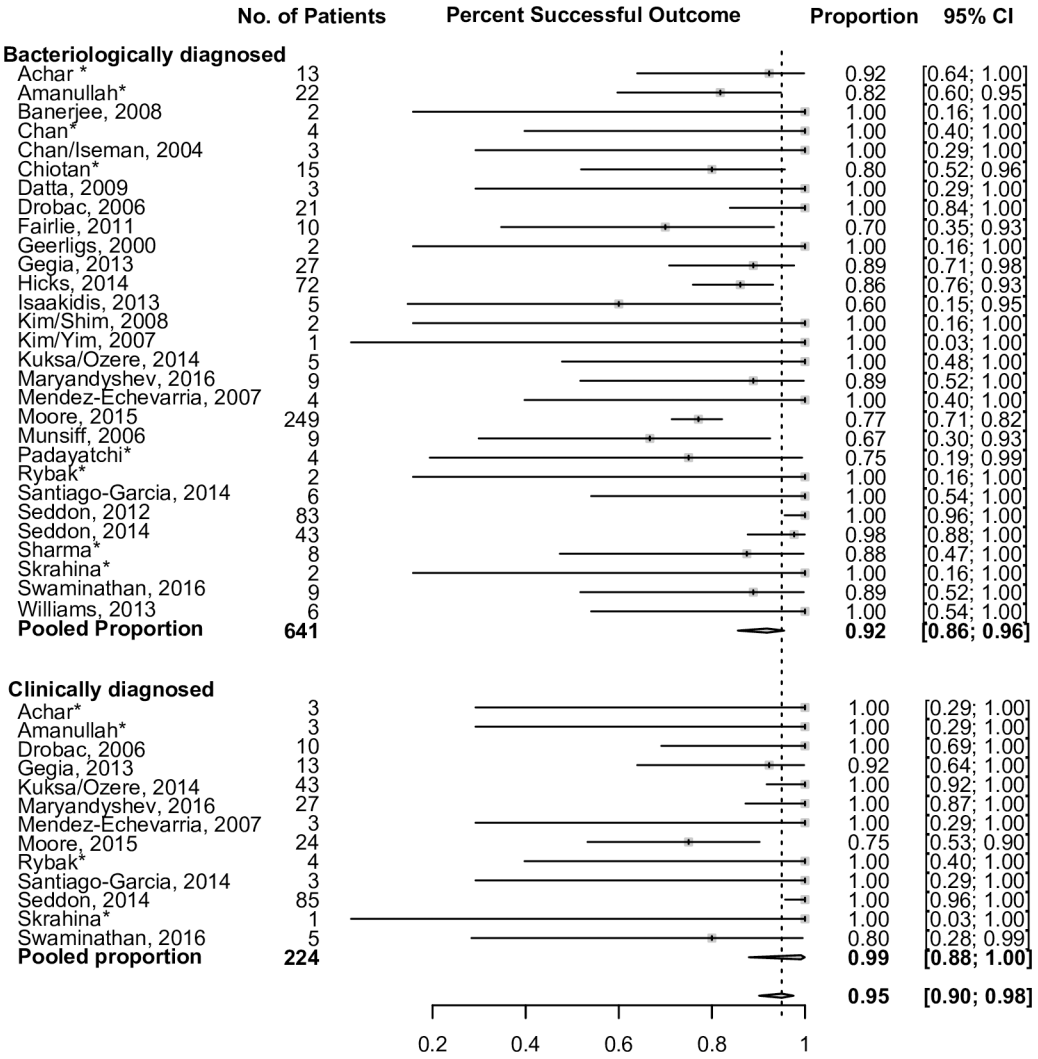

Fig 3. Proportion of patients achieving successful treatment outcomes, stratified by method of diagnosis, by site. Excludes lost to follow-up and cases that did not have an outcome. Results estimated via random effects modeling to account for clustering by cohort. The 95% confidence limits were estimated using exact (Clopper-Pearson) method.
*Unpublished data.

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Table 4. Comparing treatment success rates among children with MDR-TB infected with HIV, by timing of initiation of ART and by HIV status[a].

Characteristics	No ART during MDR-TB treatment, number treatment success[a]/*N* (%)	ART during MDR-TB treatment, number treatment success[a]/*N* (%)[b]	Absolute difference in proportion (ART versus No ART) (95% CI)	No data on ART, number treatment success/*N* (%)	Absolute difference in proportion (ART versus No data on ART) (95% CI)	*p*-value	All children infected with HIV, number treatment success[a]/*N* (%)	All children not infected with HIV, number treatment success[a]/*N* (%)	Absolute difference in proportion (HIV+ versus HIV−) (95% CI)
Bacteriologically confirmed MDR-TB	15/27 (56)	149/182 (82)	26% (5%–48%)	57/78 (73)	9% (−3%–21%)	0.006	221/287 (77)	291/312 (93)	16% (10%–22%)
Clinically diagnosed MDR-TB, number treatment success/N (%)	1/4 (25)	21/21 (100)	75% (18%–100%)	3/5 (60)	40% (−15%–95%)	<0.001	25/30 (83)	180/183 (98)	15% (0%–30%)

[a]Treatment success is defined as cured or completed treatment, lost to follow-up excluded. The overall chi-squared *p*-values comparing treatment success rate by ART status among bacteriologically confirmed and clinically diagnosed children infected with HIV, respectively, are $p = 0.006$ and $p < 0.001$.

[b]Either on ART before beginning MDR-TB treatment or ART started during MDR-TB treatment.

Abbreviations: ART, antiretroviral treatment; MDR-TB, multidrug-resistant tuberculosis.

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with high-dose isoniazid were from South Africa. When the association of individual drugs with treatment success was examined in South African data only, the CI widened but the point estimate remained the same (S5 Table). Among clinically diagnosed children, multivariable models were too unstable to provide reliable estimates.

Among the clinically diagnosed children (who tended to have less severe disease on chest radiograph and lower rates of malnutrition and HIV), 60 (25%) received no injectable agent or less than 1 month of injectable agent. Fifty (83%) of these children had successful treatment outcomes.

Later generation fluoroquinolones were defined as moxifloxacin, gatifloxacin, sparfloxacin, or high-dose levofloxacin. Only 63/885 (7%) children in total were treated with moxifloxacin (44/641 [7%] in the confirmed and 19/244 [9%] in the clinically diagnosed groups). One child with confirmed disease received both gatifloxacin and sparfloxacin. Information on the dose of levofloxacin was available in only 93/885 (11%) of children. This infrequent use precluded useful analysis of the clinical impact of fluoroquinolones on treatment outcome.

Discussion

This systematic review and IPD meta-analysis of pediatric MDR-TB represents a global collaborative effort of the pediatric TB community. It has for the first time, to our knowledge, generated a data set of children treated for MDR-TB in multiple countries, which has informed the revised 2016 WHO MDR-TB treatment guidelines [4].

A striking finding is the high proportion (78%) of successful treatment outcomes amongst all children, with 75% success in children with confirmed MDR-TB and 89% in those with clinically diagnosed disease. These good outcomes are despite the high prevalence of comorbidities (37% infected with HIV; 34% malnourished), severe pulmonary (49%) and extrapulmonary (13%) TB, and the abscence of the newer, safer, and more effective drugs. A previous systematic review, without IPD, reported a similar proportion of children with MDR-TB successfully treated (82%) [6]. These outcomes are considerably better than the treatment success rates (54%) reported in adults [9].

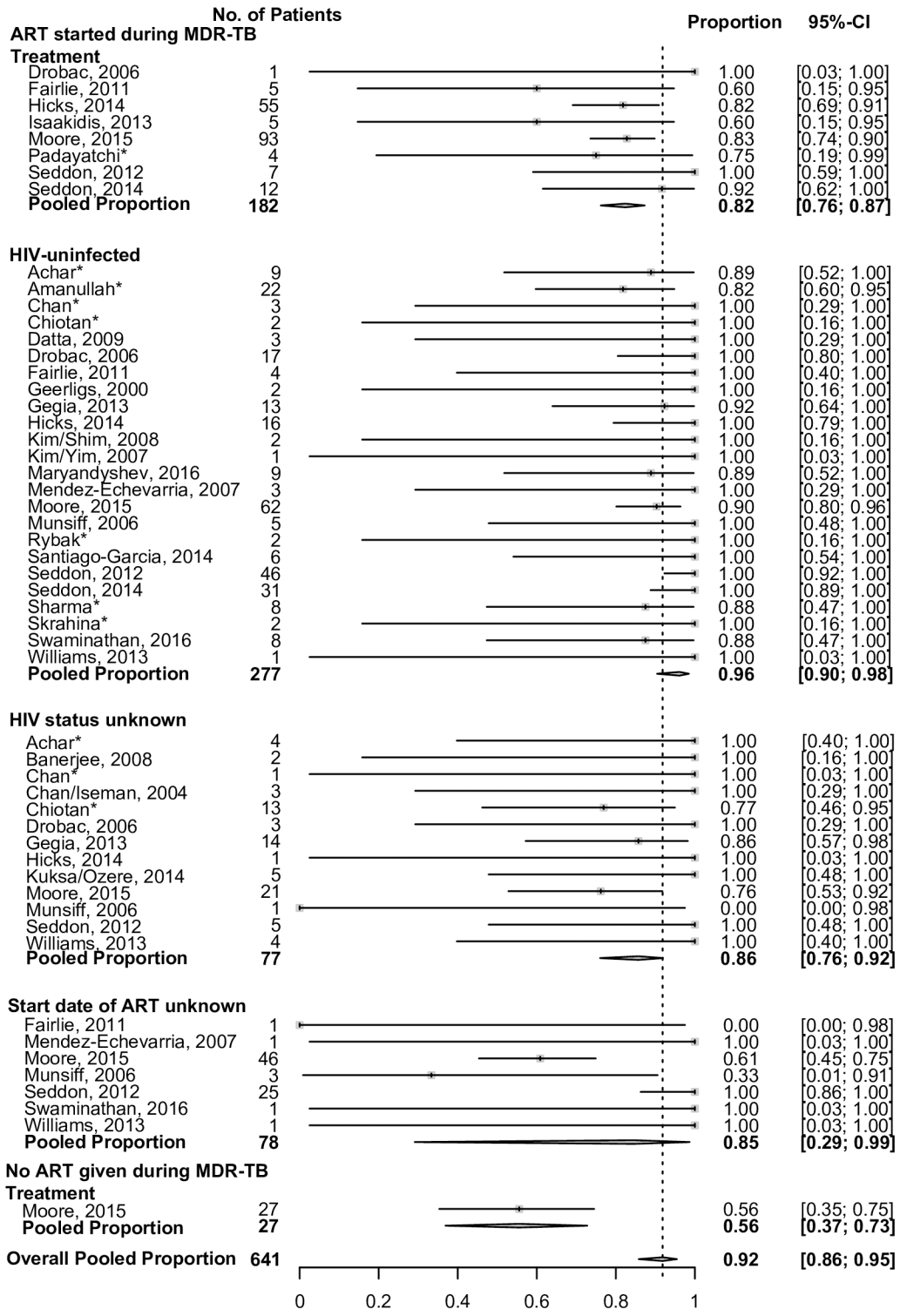

Fig 4. Proportion of bacteriologically confirmed MDR-TB patients achieving successful treatment outcomes, stratified by HIV infection and HIV ART. Data are stratified by HIV and ART status and by site. The 95% CIs were estimated using exact (Clopper-Pearson) method. Children from countries with very low HIV prevalence who did not have an HIV test were assumed not to be infected with HIV following consultation with site investigators. *Unpublished data. ART, antiretroviral treatment; MDR-TB, multidrug-resistant tuberculosis.

https://doi.org/10.1371/journal.pmed.1002591.g004

Table 5. Clinical variables associated with treatment outcome in children with MDR-TB: *N* = 975.

Characteristics	Treatment success versus Failure/Death[a]					
	Bacteriologically confirmed *n* = 641[b]			Clinically diagnosed *n* = 224[b]		
Clinical variable	**Adjusted OR**	**95% CI**	***p*-value**	**Adjusted OR**	**95% CI**	***p*-value**
Age ≥5 years	1.36	0.78–2.37	0.191	9.64	0.53–176.98	0.969
Male	0.72	0.44–1.18	0.321	0.63	0.04–11.22	0.267
Infected with HIV[e]	**0.30**	**0.15–0.63**	<0.001	0.49	0.02–14.97	<0.001
Severe disease on chest radiography	0.66	0.32–1.34	0.001	0.07	<0.01–5.76	0.042
Malnutrition[c, e]	**0.33**	**0.19–0.60**	<0.001	0.01	<0.01–1.92	<0.001
Severe extrapulmonary disease[d]	0.60	0.32–1.13	0.026	0.09	<0.01–2.68	0.013

[a]Estimated using random effects models (random intercept and slope) with quadrature approximation. Missing values for sex, HIV, and severe extrapulmonary disease, severe disease on chest radiography, and malnutrition variables were imputed using the mean value of each variable for each site.

[b]Lost to follow-up was excluded from analysis.

[c]Malnutrition is defined as being underweight or malnourished by clinical diagnosis, having nutritional edema, or having low weight for age (weight-for-age-adjusted z-score of <−3).

[d]Severe extrapulmonary disease is defined as meningitis, miliary, abdominal, osteoarticlar, or disseminated disease not otherwise specified.

[e]Bolded results met the prespecified criteria for statistical significance.

Abbreviations: MDR-TB, multidrug-resistant tuberculosis; OR, odds ratio.

https://doi.org/10.1371/journal.pmed.1002591.t005

Table 6. Summary of association of use of individual drugs with treatment success in children treated for confirmed MDR-TB (*n* = 641)[a,b,c,d].

Drug used	*N* (%)	aOR[e]	95% CI	*p*-value
Pyrazinamide	599 (93)	1.63[g]	(0.41–6.56)	0.484
Second-line injectable agents[f, j]	**584 (91)**	**2.94[g]**	**(1.05–8.28)**	**0.041**
Ethionamide/ prothionamide	590 (92)	2.19	(0.42–11.54)	0.332
Cycloserine/ terizidone	356 (56)	1.66[h]	(0.91–3.05)	0.104
Clofazimine	23 (4)	0.55	(0.02–19.20)	0.714
High-dose isoniazid[j]	**133 (21)**	**5.86[g]**	**(1.68–20.51)**	**0.007**
Para-aminosalicylic acid	147 (23)	0.70[i]	(0.25–1.96)	0.483
Clarithromycin	32 (5)	0.29[i]	(0.05–1.53)	0.132

Treatment success was compared to Failure/Death by drug use.

[a]Adjusted estimates for the clinically diagnosed children were not possible due to very low rates of failure.

[b]Lost to follow-up was excluded from analysis.

[c]All random effects (random intercept and random slope) models used maximum likelihood estimation with quadrature approximation and were specified with an unstructured variance–covariance matrix parameterized through its Cholesky root unless otherwise stated.

[d]Too few children were treated with late-generation fluoroquinolones, carbapenems, and linezolid to be analyzed. No children in these cohorts were treated with bedaquiline or delamanid.

[e]aOR, for use of drug, with nonuse as reference category. Adjusted for age, sex, HIV infection, malnutrition, severity of disease on chest radiograph, and severity of extrapulmonary disease.

[f]Second-line injectable agents are amikacin, kanamycin, and capreomycin.

[g]Random-slope only model without random intercept, specified with standard variance components.

[h]Random-intercept only model without random slope, specified with standard variance components.

[i]Model specified with standard variance components (not unstructured).

[j]Bolded results met the prespecified criteria for statistical significance.

Abbreviations: aOR, adjusted odds ratio; MDR-TB, multidrug-resistant tuberculosis.

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The association of HIV infection and malnutrition with reduced odds of successful TB treatment is consistent with the published literature [24,40]. Children infected with HIV but not receiving ART during their MDR-TB treatment were less likely to have successful treatment compared to children who were on ART at some point during their MDR-TB treatment episode. The 2016 WHO ART guidelines recommend that ART should be started as soon as possible and at least within 8 weeks of starting TB treatment in all TB patients infected with HIV, irrespective of CD4 count, with the possible exception of TB meningitis [41–44]. All data on children infected with HIV but not receiving ART came from a single study from South Africa (Fig 4) [21]; the data were collected from several public clinics throughout South Africa and are representative of the care children were receiving. Although the data all coming from a single study is a limitation, the finding that the lack of ART is associated with worse outcomes in people coinfected with TB and HIV has been well established [41, 45]. Our data support the importance of ART in children with TB who are infected with HIV, and highlight the need to aggressively treat children infected with HIV for both TB and HIV and to include nutritional support as part of standard care.

In this data set, children infected with HIV were more likely to have confirmed MDR-TB, despite reports of higher rates of paucibacillary TB disease in adults infected with HIV. This may be because the majority (95%) of children infected with HIV were from South Africa, where diagnostic tools and approaches may differ. There may also be hesitancy to start MDR-TB treatment in addition to ART; thus, treatment delays may lead to children having more severe TB disease, resulting in more bacteriologically confirmed disease. Children infected with HIV also tended to be older, as prevention of mother to child transmission (PMTCT) initiatives were rolling out during the study period in South Africa; this may have contributed to more children infected with HIV having a confirmed diagnosis.

A prominent finding was the benefit of high-dose (15–20 mg/kg/day) isoniazid. Twenty-one percent of children with confirmed MDR-TB received high-dose isoniazid; however, the majority of these children were from Cape Town. Despite attempts to statistically control for site-specific effect, it is possible that other unmeasured site-related effects, including the quality of clinical care, could have contributed to this finding. However, the fact that the point estimate for the benefit of high-dose isoniazid remained unchanged when South African data were examined demonstrates that this benefit remains, despite possible site-specific effect. When high-dose isoniazid is considered as part of MDR-TB treatment but the mutation conferring isoniazid resistance is unknown, the local epidemiology of isoniazid resistance mutations should be considered (high-level isoniazid resistance is conferred by the *katG* mutation and lower level resistance by the i*nhA* mutations) [46]. High-dose isoniazid can overcome an i*nhA* mutation's low-level isoniazid resistance but is unlikely to overcome the high-level isoniazid resistance due to a *katG* gene mutation [47]. The Western Cape Province, South Africa (which includes Cape Town), has high rates of *inhA* mutations (61% of the isoniazid resistant mutations among pediatric patients [46]), which may have contributed to this high-dose isoniazid treatment benefit. However, none of the children underwent testing for isoniazid mutation and were therefore treated in a standard manner at the time the data were generated. Despite these caveats, our observations are consistent with other reports that suggest that high-dose isoniazid may have an important role in MDR-TB treatment as a component of shorter treatment regimens and as an add-on agent to the longer MDR-TB treatment regimens [1,48–50].

Among children with confirmed MDR-TB, the use of second-line injectable agents was associated with successful treatment versus failure/death. Although we found improved outcomes in children with confirmed disease when treated with injectable agents, we saw high levels of treatment success in children with clinically diagnosed MDR-TB who received no

second-line injectable agents or who had received these parenteral antibiotics for less than 1 month. Eighty-three percent (50/60) of the children with clinically diagnosed disease (who tended to have less severe disease, with lower rates of HIV and malnutrition and less severe disease on chest radiography) who received no or less than 1 month's treatment with a second-line injectable agent had successful treatment outcomes. These data need to be interpreted with caution and the use of second-line injectable agents in children needs more study. We did not collect data on the reason for using an injectable-free regimen, but possibilities include less severe disease, the presence of resistance to an injectable agent (although not all sites had access to extended drug-susceptibility testing), and perceived and actual injectable-associated adverse events.

Nonetheless, our findings lend support to not using second-line injectable agents in children with non-severe MDR-TB [6,18,51]. A previous study found hearing loss in 24% of children treated for MDR-TB with a second-line injectable agent [51]. Hearing loss can be devastating in children, impacting language acquisition and schooling, and administration of injectables is painful and resource intensive, frequently requiring daily visits to healthcare facilities. Avoiding second-line injectable agents should also be seen in the context of current and future MDR-TB treatment options. The new and repurposed MDR-TB drugs—bedaquiline, delamanid, linezolid, late-generation fluoroquinolones—were not used enough to be evaluated, but emerging evidence suggests they could be as effective and safer than the injectable agents [5,52].

Our results were used to inform the revision of the 2016 WHO MDR-TB treatment guidelines, which state that the harms associated with second-line injectable agents may outweigh potential benefits, and therefore injectable agents may be excluded from the treatment regimens of children with non-severe forms of MDR-TB disease [4].

A strength of our study was the inclusion of children with both bacteriologically confirmed and clinically diagnosed TB. Because of the paucibacillary nature of pediatric TB and the challenges of obtaining clinical samples, approximately 60%–70% of children treated globally for TB will be clinically diagnosed [25,35]. The inclusion of clinically diagnosed children makes our findings relevant to a broader range of children treated for MDR-TB, even if the proportion of children with clinically diagnosed MDR-TB (25%) was lower in our study than global values cited elsewhere. Notably, the low proportion of children in this study with clinically diagnosed MDR-TB may indicate potentially more severe pulmonary disease in our cohort, because bacteriological yield correlates with chest radiographic findings in children with TB [36]. The proportion of patients with bacteriologically versus clinically diagnosed TB differed by site (Fig 3). The reasons for this are likely varied; in some places, healthcare workers are reluctant to treat MDR-TB without bacteriological confirmation, and in some countries it is national policy that a bacteriological diagnosis be made in order for a child to be treated for MDR-TB, thereby excluding children with a clinical diagnosis from treatment. Some sites are MDR-TB specialty sites, so these may be better equipped to obtain samples and complete laboratory testing. Additionally, by the time children were evaluated at these tertiary centers, many probably had more advanced disease, which is more likely to be bacteriologically confirmed. Age may also explain some of our cohort's attributes: some clinicians are less comfortable diagnosing TB in younger children, in whom the diagnosis is less likely to be confirmed and symptoms are frequently nonspecific. There are also likely publication biases involved here, whereby studies may have been more likely to have been published if they included more bacteriologically confirmed cases. However, in an attempt to combat this publication bias, we actively sought out unpublished data.

A criticism of studies that include clinically diagnosed children is that some of these children may not have MDR-TB and so treatment outcomes may not reflect true MDR-TB

disease. In this study, among children with a clinical diagnosis, 67% had clinical TB with a known MDR-TB source case, and another 6% had failure of first-line therapy despite good adherence. Given that studies have shown that children are most likely to share the TB strain of their household contact, it is likely that the majority of children in this cohort did in fact have MDR-TB [37–39].

Limitations

The most significant limitation of this study is that the estimated treatment effects of individual drugs should be interpreted with caution because the analysis could not isolate the benefit of each drug. We could only compare regimens with and without each drug. If particular drugs were used or not used together—because of disease severity or resistance profiles or toxicity—there is potential for confounding by indication that would produce biased effect estimates for each drug. Also, treatment decisions were based on the clinician's perception of illness, with resulting potential for bias.

Furthermore, we were not able to compare specific regimens given the large variation in drugs used and treatment duration as part of individualized treatment. All studies included were retrospective or prospective observational cohort studies; there were no clinical trials. However, given that the study was an IPD, some adjustments could be made for covariates at the individual level, which is not possible in study-level meta-analyses. This worked to mitigate the risk of confounding and other potential biases. The sample size was modest compared to adult IPD data sets, and estimates were frequently imprecise, while some associations were not estimated because of limited data. There were no data available yet on the novel drugs, delamanid and bedaquiline, and data on linezolid were insufficient to include in this analysis. Data on late-generation fluoroquinolones and clofazimine were sparse. Children who were lost to follow-up and not evaluated (11% overall) were not included in this analysis, because their treatment outcome was unknown. Relapse was also not evaluated, as data on outcomes after completing antituberculosis treatment were rarely reported. This could have resulted in overestimation of favorable treatment outcome.

A further limitation is that, apart from DST for isoniazid and rifampicin, data about a strain's susceptibility to other drugs (especially the fluoroquinolones and second-line injectable agents) were frequently not available for analysis; therefore, we could not assess the association between combinations of likely effective drugs (based on DST results) and outcomes. This may have also led to an underestimate of the benefit of drugs to which the strain was susceptible. Additionally, data on adverse events were incompletely reported and could not be included in the analysis. This scarcity of the reporting of adverse events also contributed to us being unable to distinguish treatment failure due to lack of efficacy from treatment failure due to drug intolerance.

Data were limited regarding the duration of posttreatment follow-up or posttreatment outcomes; therefore, we could not evaluate recurrence, relapse, or the effect of total duration of treatment on MDR-TB outcomes. Data on history of previous TB treatment were sparse and could not be analyzed.

All studies used consecutive sampling of children enrolled in MDR-TB treatment, which should have minimized selection bias. However, it is possible that the diagnosis of MDR-TB could have been missed in some children due to passive case finding at most study sites and general difficulties in diagnosing TB and MDR-TB in children. Children in whom the diagnosis may have been missed, often children with clinically diagnosed TB, who would usually have less severe, more paucibacillary disease, might have had different clinical characteristics and different outcomes than those included in the selected studies and therefore may have better

treatment outcomes if they had in fact been enrolled. Thus, the likelihood of selection bias cannot be ruled out.

An additional limitation of this study was that the literature published after October 2014 was not included. In order to determine what effect this may have on our findings, we did a search of the pediatric MDR-TB literature from October 2014 to the present. The studies were reviewed, either full studies or abstracts, and details of studies that had the potential to be included in our study are included in S6 Table. As per the reasons listed in S6 Table, it is unlikely that including these in our study would have changed our findings.

Conclusions

Children treated for MDR-TB have good treatment outcomes—even those with comorbidities and severe disease. That children tend to have much better treatment outcomes than adults raises the possibility that children may be specifically suited to do well with shorter, less intense treatment regimens. This deserves further study. Our study provides evidence that a substantial proportion of children with clinically diagnosed MDR-TB treated without second-line injectable agents have successful outcomes and that high-dose isoniazid may improve treatment success in some settings. Given the limitations of the current study, these findings should be interpreted cautiously but deserve further evaluation in subsequent studies. The high mortality and low treatment success rates among children infected with HIV who were not on ART during TB treatment demonstrates the importance of testing children with TB for HIV and initiating ART as soon as possible.

Data regarding the treatment outcomes and pharmacokinetic and safety profiles of bedaquiline, delamanid, later generation fluoroquinolones, linezolid, and clofazimine in children are urgently needed in children with MDR-TB, including in children infected with HIV. Future research is also needed on pediatric outcomes involving the shorter MDR-TB regimen [49,50]. Consensus on standardized reporting of demographic, clinical, and treatment characteristics for childhood MDR-TB would enhance the value of observational data and improve the comparability of results in published literature. It is notable that after extensive efforts to identify all possible pediatric cohorts, including unpublished cohorts, over a period of 24 years, fewer than 1,000 children with MDR-TB could be included in this analysis. Given that as many as 32,000 children develop MDR-TB each year [2], this represents a small fraction of children with MDR-TB. This paucity of literature highlights the fact that children with MDR-TB have been largely disregarded and underreported and that urgent attention needs to be paid to diagnose, treat, and report on this neglected population.

Supporting information

S1 Table. Search strategies for Pubmed and Embase.
(DOCX)

S2 Table. Newcastle-Ottawa approach for grading quality cohort studies used in this review.
(DOCX)

S3 Table. Overview of included studies and cohorts.
(DOCX)

S4 Table. Key clinical variables of children lost to follow-up versus those with known treatment outcomes.
(DOCX)

S5 Table. Summary of association of use of individual drugs with treatment success in children treated for confirmed multidrug-resistant tuberculosis in South African study sites.
(DOCX)

S6 Table. Overview of recent studies that may have been eligible for inclusion in this manuscript from October 2014 to present.
(DOCX)

S1 Text. PRISMA checklist.
(DOC)

S2 Text. Study protocol.
(DOCX)

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REVIEW

Challenges of using new and repurposed drugs for the treatment of multidrug-resistant tuberculosis in children

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ABSTRACT

Introduction: New and repurposed antituberculosis drugs are urgently needed to more safely and effectively treat multidrug-resistant (MDR) tuberculosis (TB) in children. Multiple challenges limit timely access to new MDR-TB treatments in children.

Areas covered: Diagnosis of MDR-TB in children remains a barrier, with few children with MDR-TB diagnosed and treated. Other barriers to timely access to new and repurposed drugs are discussed, and include delayed initiation of paediatric trials, limited funding for paediatric drug development, fragmented regulatory systems and operational challenges. The status of access to current repurposed and novel drugs is presented.

Expert commentary: More timely initiation of paediatric trials is needed and paediatric work should happen and be funded in parallel with each phase of adult trials. Better quality data, increased regulator resources and expertise, harmonization of regulatory requirements across borders/organisations and registration fee waivers would improve registration timelines. Improved diagnosis, recording and reporting will establish better demand. Improved systems for procurement and supply chain management would reduce in-country operational barriers to getting medications to children. The challenges must be addressed to ensure timely and equitable access to new drugs and regimens that are urgently needed for effective, safe and shorter treatment of children with MDR-TB.

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1. Introduction

Tuberculosis (TB) in children accounted for approximately 1 million (10%) of the estimated 10.4 million new TB cases globally in 2015 of which only 38% were reported [1]. There were an estimated 580,000 cases of multidrug-resistant (MDR)-TB (i.e. disease caused by *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin) in 2015; only 125,000 (20%) accessed appropriate MDR-TB treatment with only 52% successfully treated [1]. Model-based estimates suggest 25,000–32,000 new cases of MDR-TB in children annually [2,3]; however, the true burden is not known, as recording and reporting of drug-resistant (DR)-TB in children is poor [4,5]. MDR with additional resistance, such as extensively drug-resistant (XDR)-TB (i.e. MDR plus resistance to a fluoroquinolone and a second-line injectable agent) and pre-XDR-TB (i.e. MDR plus resistance to a fluoroquinolone or a second-line injectable agent), is increasingly recognized in children [6,7].

New anti-TB drugs to more safely and effectively treat MDR-TB are as much needed in children as in adults; however, children, and especially very young children who develop serious forms of disseminated TB, such as miliary TB and TB meningitis, are always last to gain access to these drugs. In the last decade, a number of new and repurposed drugs have entered adult MDR-TB trials from phase 1 to 3. Bedaquiline and delamanid have received accelerated or conditional approvals in different countries for use in MDR-TB treatment regimens, while clofazimine, linezolid, and the later-generation fluoroquinolones are used off-label for MDR-TB treatment in adults and children. Pretomanid has only been used in new trial regimens for both drug-susceptible (DS-TB) and DR-TB in adults and has not yet been registered.

In this review, we will highlight clinical, research, regulatory, and operational challenges to the use of new TB treatments in children with MDR-TB, and propose potential solutions.

2. Challenges to using new and repurposed drugs for children with MDR-TB

2.1. Diagnostic challenges

A major challenge for using new TB drugs in children is the underdiagnosis of pediatric MDR-TB. DR-TB and DS-TB in children are clinically indistinguishable. Bacteriological confirmation of TB, and therefore also drug susceptibility testing, in children remains a challenge due to difficulty in obtaining suitable specimens, poor sensitivity of available bacteriological tests due to low organism burden of TB in children (paucibacillary disease in most young children), and a lack of adequate

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laboratory facilities in many settings [8,9]. TB is often not considered in young children and specimens are frequently not collected. Despite mounting evidence that children with TB who are in contact with infectious DR-TB source cases have the same DR *M. tuberculosis* strain [10,11], some national TB programs (NTPs) and clinicians remain reluctant to start appropriate DR-TB regimens in children without bacteriologic confirmation [12]. This hesitance is likely due to longer duration of treatment with more toxic and expensive regimens needed to treat these children for MDR-TB. New, safer, shorter, and less expensive regimens may reduce the hesitation of clinicians to treat presumed DR-TB cases. Although the number of children diagnosed, recorded, reported, and treated for MDR-TB is unknown, it is suspected that <5% of pediatric cases are identified globally each year. Children cannot benefit from new TB medications if they are never identified as having probable or confirmed MDR-TB, and in the absence of much improved case finding, much of the benefit of new medications in children will remain unrealized.

2.2. Challenges in pediatric TB drug development

The challenges for developing new and investigational drugs in children lie at several levels. A recent consensus statement on early inclusion of children in TB drugs trials recommends that pharmacokinetic and safety studies in children should start as soon as phase 2b studies have established appropriate dosing, and therefore pharmacokinetic targets and safety, in adults [13]. Studies of new drugs in children, including development of child-friendly formulations should not wait for the results of the long phase 3 clinical trials in adults. This remains a challenge, as pharmaceutical companies are reluctant or slow to develop such pediatric investigational plans (PIPs) and implement pediatric trials, despite incentives and a mandate from regulatory authorities in the United States and Europe, respectively.

Efficacy trials for single new drugs are not necessary in children unless these drugs are for specific pediatric indications, as efficacy in adult TB cases infers efficacy in children. Clinicians are aware of this caveat. Therefore, if dose finding and safety studies in children are not done early in new drug evaluations, clinicians may start using these drugs without properly established dosing and without evaluation of its safety in children. An example is the Centers for Disease Control and Prevention recommendation that bedaquiline can be used in children if no alternative options are available, but no bedaquiline doses in children have been established nor any dosing guidelines provided [14]. Pharmacometric modeling, using available pharmacokinetic data at established effective doses in adults assists in determining likely effective doses in children, taking into account physiological changes that occur in children at different ages. However, pharmacokinetic studies in children are still necessary to establish whether these doses achieve adult targets.

The milligram strength, formulation, and taste (palatability) of drugs are important considerations in using drugs in children. Small children often need fractions of adult-sized tablets, and breaking and crushing or dissolving tablets in solutions may lead to changes in bioavailability or inaccurate dosing (too high or too low). Furthermore, there may be huge differences in taste/palatability between a coated tablet swallowed whole by an adult or an adolescent and crushed/suspended tablets needed to be taken by young children. It is therefore imperative that child-friendly formulations that consider milligram strength, size, formulation, and palatability should be developed for all new and investigational drugs.

2.3. Financial and market barriers to pediatric TB drug development

The cost of bringing a novel drug to market is at least $110–170 million [15]. As the vast majority of TB patients live in poor countries, national health systems and individual patients have limited ability to pay the substantial costs that would allow drug companies to recoup these research and development (R&D) investments. The markets for DR-TB are even smaller, and, for children with DR-TB, smaller yet. A market-based approach to drug development, based on the private sector investing in R&D with the aim of later recouping costs through sales is unlikely to be successful for the development of new drugs for TB. However, other options exist.

Various push-and-pull mechanisms have been proposed to incentivize the development of drugs for neglected diseases [16–18]. Push mechanisms generally provide funds to support the earlier stages of drug development and promote the identification of novel entities that can enter the drug development pipeline. These allow investigators and industry to reduce the costs and risks associated with development and include direct funding mechanisms and tax credits on R&D. Pull mechanisms allow guaranteed or increased returns on products when they get to market. These usually act further along the drug and regimen development pipeline, supporting and encouraging early- and later-stage clinical trials. These mechanisms include market guarantees (such as advanced purchase commitments), prizes, orphan drug program incentives/exemptions, patent extensions, priority review vouchers, and patent buyouts. A recent approach, advocated by Médecins Sans Frontières (MSF), the Life Prize, formerly the 3P project (Push, Pull, and Pool), formalizes some of these mechanisms for TB drug development with the ultimate goal of de-linking the cost of R&D from that of the end product [19].

2.4. Pediatric TB drug R&D funding

In 2015, funders invested $620.6 million in TB R&D of which $26.7 million went to pediatric research. $231.9 million overall was provided for TB drug development, and of that $16.1 went to pediatric TB drug R&D [20]. The overall amount of funding for TB R&D is far less than the estimated need according to the Stop TB Partnership's 2011–2015 Global Plan to Stop TB; within this inadequate budget, the proportion of the TB R&D budget that is being allocated for childhood TB is very small. As 10% of the global burden of TB is in children (<15 years) [1], it would seem appropriate that a similar proportion of the R&D budget were allocated to this population.

The money for TB R&D comes from a number of sources [20]. The majority (63% for all TB R&D) comes from the public

sector. This includes government scientific agencies, national development agencies, disease control agencies, regional political entities, and publically funded trials networks. Further funding comes from philanthropic organizations (20%), the private sector (14%), and multilateral organizations (such as Unitaid; 3%). The money is disbursed directly to researchers and developers, through grants, as well as to Product Development Partnerships (collaborations between academics, the public sector, and industry that work together to develop new medicines, vaccines, or diagnostics). In 2013, TB Alliance was awarded $16.7 million from Unitaid to catalyze the market introduction of child-friendly formulations of first-line TB medicines, a grant named STEP-TB. This led to the development of appropriately dosed, dispersible formulations for treating DS-TB in children. While second-line drugs were not considered for inclusion under STEP-TB, Macleods has developed WHO-prequalified pediatric dispersible tablets of ethionamide, levofloxacin, moxifloxacin, and a mini capsule of cycloserine; WHO-prequalified pediatric formulations of linezolid and clofazimine are also forthcoming.

2.5. Regulatory challenges

In addition to the R&D necessary to determine appropriate doses and formulations acceptable for children, regulatory and procurement policies are critically important to facilitate access to new and repurposed drugs for the treatment of children with DR-TB.

2.5.1. Existing regulatory pathways and requirements

Regulatory authorities require data demonstrating the efficacy and safety of new medicines in adults and often accept that these efficacy data are applicable to children with the same disease [21]. Only pediatric pharmacokinetic and safety data are then necessary for regulatory authorities to include children in the population for which the new drug they are reviewing will be indicated, or to expand the indication for an existing drug to include children. Regulatory pathways and requirements for the registration of new and repurposed drugs for use in children and for pediatric formulations vary by country.

For pediatric formulations, many regulatory authorities require demonstration (in healthy adults) of bioequivalence between the pediatric and registered adult formulations. However, some regulatory authorities require that these studies be conducted locally, adding a significant amount of work for the drug or formulation sponsor. In the absence of a TB indication, as is the case for repurposed drugs that are used off-label for the treatment of DR-TB, including linezolid and clofazimine, it is not possible to register a pediatric formulation in the absence of a reference product without also submitting data demonstrating safety and efficacy for the desired indication. However, these data are often not available given the expectation that efficacy is demonstrated in adult populations, and generics manufacturers don't typically have the capacity or experience necessary to conduct safety and efficacy studies.

2.5.2. Access in the absence of local registration

In the absence of in-country registration, and where country import and other policies allow, adults and children can access new drugs through company compassionate use or other preapproval access programs. However, not all sponsors have preapproval access programs, and those that do, often limit the eligible population to exclude children. Preapproval access programs are often conducted on a named patient basis, which can be onerous for clinicians requesting the product and often require weeks to get through necessary sponsor and regulatory approval requirements, jeopardizing clinical care. Furthermore, not all countries have legal or regulatory mechanisms for preapproval use. Import waivers can be used to facilitate access to repurposed drugs that aren't registered locally for TB but have received approval from a Stringent Regulatory Authority (SRA) and/or the WHO Prequalification (PQ) Programme. Preapproval access programs and import waivers are critically important mechanisms, but given the significant amount of additional effort and paper work they require from already overburdened TB programs, and frequent limitations on number of treatment courses allowed to be imported under the waiver or length of the waiver, when possible, local registration is preferable. Local registration is becoming an increasingly important issue given anticipated shifts in the global donor landscape expected to affect country procurement practices; however, local registration in countries with smaller markets is often not of interest to drug sponsors or manufacturers.

The Global Fund requires funding recipients to purchase all DR-TB medications through the Global Drug Facility (GDF). This policy was designed to ensure quality, stabilize supply, and reduce prices and lead times for DR-TB medications, which have a smaller market than first-line medicines. The GDF facilitates obtaining import waivers to supply countries with DR-TB medicines that have SRA approval or WHO PQ, but that are not locally registered for TB. Without such a procurement policy, as is the case for medications for DS-TB, countries follow their domestic procurement policies, which often require programs to buy drugs from domestic sources using a local tendering process.

The Global Fund is in the process of transitioning a larger proportion of TB, HIV, and malaria programs in many countries to domestic financing. Without intervention pre-transition to assist countries in finding ways to continue to purchase medicines from quality-assured sources, or interventions to ensure that products for more fragile markets such as DR-TB medicines are not transitioned from donor funding, countries will likely have to revert back to buying DR-TB medications from domestic sources. As a result, programs will no longer benefit from the lower drug prices and stable supply the GDF has been able to negotiate using its market share, and will also have to take on the burden of applying on their own for waivers in order to purchase medicines that have not been locally registered if that is even possible.

The WHO PQ Programme has a collaborative registration process, whereby the 31 participating country regulatory authorities – mostly in Eastern Europe and Africa – agree to review dossiers within 90 days of their approval by the WHO

PQ Programme. National regulatory authorities, especially in countries with small markets for TB medicines, should subscribe to the WHO PQ Programme's collaborative registration process to expedite review timelines and overcome the issues that come up in the absence of local registration. Generics manufacturers often prefer WHO PQ to SRA approval because it has to date been a less expensive and onerous process, though historically WHO PQ timelines have been unpredictable and at times lengthy.

Recently announced changes to the WHO PQ fee structure may make this pathway less attractive to manufacturers of TB medicines for small, fragmented, and non-lucrative markets, like pediatric DR-TB, whose sales may not be sufficient to cover fees and even marginal requisite profits. However, the WHO created a waiver process that is hoped to keep suppliers engaged in these low-volume markets. Tracking the impact of new fees on engaging suppliers in the pediatric DR-TB market will be critical.

2.6. Operational challenges

The small number of children currently being treated for MDR-TB produces a vicious cycle throughout the supply chain. Manufacturers are unwilling to produce large volumes of medications unless demand is assured. NTPs are unwilling to purchase appropriate stock unless they are confident that drugs will be used. Finally, more regional pharmacies are not keen to stock sufficient numbers of individual drugs as each have limited shelf-lives and a mismatch between supply and demand leads to wastage. This is compounded by inaccurate forecasting, relatively short drug shelf-lives, long lag times between ordering and production, long shipping and importation times, and frequently, complex internal distribution networks [22].

Introduction of new treatments requires substantial effort for NTPs. Guidelines must be reviewed and updated. Program monitoring tools, such as forms and registers, often must be adapted to accommodate new treatments or regimens. Healthcare workers must then be trained on the new treatments and guidelines. All of this means there will necessarily be delays in getting new treatments taken up in routine program settings. Additionally, many pediatric trials to date have been age de-escalation trials in which older children are first enrolled, and after demonstrating safety and optimal dose, progressively younger children are enrolled. This means data likely to influence practice may emerge sporadically over the course of the trials. The WHO and NTPs should develop processes to rapidly incorporate new data into practice, so that children can benefit from new treatments as quickly as possible.

3. Individual repurposed drugs

3.1. Clofazimine

The rhiminophenazine antibiotic clofazimine (Lamprene; Novartis Pharma AG, Basel, Switzerland) has been used for decades in leprosy treatment, but has only recently been studied for the treatment of MDR-TB, because of limited existing treatment options. It has activity against nonreplicating and actively growing bacteria [23]. It is reduced by NADH dehydrogenase (NDH-2) to release reactive oxygen species upon reoxidation by O_2 [24]. It competes with menaquinone (MK-4), a key cofactor in the mycobacterial electron transfer chain, for its reduction by NDH-2.

A clofazimine-containing MDR-TB regimen was able to shorten treatment duration to 9–12 months with more than 80% cure in adult MDR-TB cases [25]. Further observational studies in adults confirmed this outcome [26,27]. This shorter regimen is now recommended by the WHO for pulmonary rifampicin mono-resistant and MDR-TB without second-line drug resistance in adults and children [28]. Preliminary data of a randomized controlled trial of this shorter regimen compared to the 20–24-month regimen (STREAM) in adults showed 78.1% vs. 80.6% favorable outcome, respectively, for these regimens [29].

Although clofazimine is only registered for use in leprosy, it recently has been used off-label as a second-line anti-TB drug in many MDR-TB regimens, including in children, and WHO currently classifies clofazimine as a group C (other core second-line agents) MDR-TB drug [28].

Clofazimine is a highly lipophilic drug; despite high variability in absorption, it can be administered orally. Available evidence suggests that clofazimine should be administered with food to enhance its absorption. It seems to be highly protein bound [30]. Clofazimine has a large volume of distribution, concentrating in fatty tissues and macrophages, the latter of which may be therapeutically advantageous [23]. It is metabolized in the liver with minimal renal excretion. The elimination half-life of clofazimine is long (25–70 days); therefore, it takes months before plasma concentrations reaches steady state. Early bactericidal activity studies found no activity over the first 14 days [31], but delayed bactericidal effect was found with clofazimine in mice [32]. In experimental studies, clofazimine seems to be important in possible shortening of MDR-TB treatment regimens [30]. Dosing of clofazimine in MDR-TB is uncertain, but in the shorter MDR-TB regimen 100 mg daily is recommended in adults. Doses of 200–300 mg of clofazimine were previously used in MDR-TB patients with relatively few adverse effects [33,34].

The main adverse effect of clofazimine is skin discoloration or darkening – in our experience occurring in the majority of children receiving the drug. This may be distressing to patients and lead to stigmatization. However, in our experience, it did not lead to discontinuation of the drug. Discoloration may improve even while on treatment and in most it clears within months of stopping clofazimine. Other adverse effects noted are ichthyosis and gastrointestinal disturbance. Clofazimine may cause dose-related QTc-interval prolongation, so caution is advised when using clofazimine in combination with other drugs with QT prolonging effects.

There is only limited pharmacokinetic study of clofazimine in adults and none in children. Despite the lack of pharmacokinetic data, clofazimine has become an important component of MDR-TB regimens in adults and children. Clofazimine is inexpensive but it currently seems difficult to procure especially given increased global demand with the rollout of the shorter MDR-TB regimen in many countries. Recommended

doses for children are 2–3 mg/kg/day and some experts recommend up to 5 mg/kg/day because of the absence of child-friendly formulations. In many settings, only 50 or 100 mg gel capsules are available which cannot be opened or dissolved in water. Smaller children are therefore dosed every second or third day depending on body weight and available formulation. This could be acceptable due to the drug's long elimination half-life. However, there is an urgent need for pharmacokinetic data, as well as child-friendly formulations, to take this drug forward in MDR-TB treatment shortening trials. Luna Innovations has done initial stability testing on a clofazimine gummy formulation for children [35]. Macleods Pharmaceuticals is developing a 50 mg dispersible clofazimine tablet.

3.2. Linezolid

The oxazolidinone class antibiotic, linezolid, has a novel mechanism of inhibiting protein synthesis, making cross-resistance with other protein-synthesis inhibitors unlikely. Linezolid is active against gram-positive organisms, including resistant gram-positive infections, but does not have an official indication for treatment of DR-TB [36]. However, the oxazolidinones – including linezolid and more recently, sutezolid – have shown excellent antimycobacterial activity [36,37], and linezolid has been effective for treating MDR-TB in adults in multiple observational studies and in a delayed-start randomized controlled trial [38–40]. Linezolid is currently used in the NiX XDR-TB treatment salvage and shortening trial [41,42].

Since 2006, the WHO has recommended the use of linezolid in the treatment of MDR/XDR-TB and it is classified as a group C MDR-TB drug [28]. Linezolid has a narrow therapeutic window. The optimal dose that maximizes linezolid's efficacy while minimizing the substantial dose and duration toxicity has not yet been determined, thus limiting its potential impact [43].

Linezolid is well absorbed (almost 100%) after oral administration, with equally good absorption with the oral suspension and tablet formulation. Coadministration with a high fat meal may delay absorption but does not affect exposure. Linezolid has complex metabolism with two primary and multiple minor metabolites. The primary route of elimination is nonrenal, accounting for approximately 65%. Increased clearance and decreased drug exposure has been noted in ill patients relative to healthy volunteers, as well as substantial inter-patient variability, which raises the question of the role of therapeutic drug monitoring [36].

Although there are pharmacokinetic data on linezolid used as a short-term antibiotic in children, there are no data for long-term TB treatment. Although optimal dosing in adults is not yet known, the current target for pharmacokinetic studies in children is to approximate exposures achieved in adults with a 600 mg once-daily dose [44]. A linezolid suspension exists, but it is expensive and access in resource-constrained settings is limited. The adult 600 mg tablet is not suitable for dosing young children due to the large tablet size and strength, difficulty in suspending, and lack of data on impact on crushing or suspending on bioavailability. A child-friendly dispersible tablet formulation at reasonable cost is needed [45]. The WHO has approved a call for the development of a 150 mg dispersible tablet and Macleods is expected to file for WHO PQ soon [46]. A further challenge to its more widespread use in children for MDR-TB is linezolid's toxicity profile, including bone marrow suppression, peripheral neuropathy, and optic neuritis among others. This calls for close monitoring of children treated with linezolid [47]. There is a need for pediatric formulation development, and pharmacokinetic and safety studies in children of sutezolid, a less toxic oxazolidinone which is now entering phase 2b studies in adults. Finally, although linezolid has been registered in many countries for use as an antibiotic in children, it is currently used off-label for TB.

3.3. Fluoroquinolones (levofloxacin and moxifloxacin)

The later-generation fluoroquinolones levofloxacin and moxifloxacin have become such an integral part of current MDR-TB regimens that they are hardly considered as repurposed drugs, but they were originally developed as antibacterial drugs. They inhibit DNA gyrases, preventing bacterial DNA synthesis [48]. A huge body of evidence for their effectiveness in MDR-TB in adults and children, despite the lack of randomized controlled trials, their good bactericidal and sterilizing activity and their favorable pharmacokinetics and toxicity profile have made them the most important component of existing MDR-TB treatment regimens [49]. However, there remain a number of unresolved issues with fluoroquinolone use in both adults and children, including the optimal dose of any given fluoroquinolone, the optimal duration of therapy, and the preferred fluoroquinolone, especially with other new and repurposed drugs also having the potential of QT-interval prolongation [48]. Further, there is concern about increased fluoroquinolone resistance due to uncontrolled use in several developing countries [50].

Characterization of the pharmacokinetics of levofloxacin and moxifloxacin in children with TB is improving although there remain important knowledge gaps. Levofloxacin is well absorbed after oral administration and primarily eliminated unchanged by the kidneys. The WHO dosage recommendations in children are 15–20 mg/kg/day divided twice daily in children aged ≤5 years, and 10–15 mg/kg/day once daily in children aged >5 years [51]. More recent data have shown that doses closer to 20 mg/kg/day in children are required to approximate target exposures in adults receiving a 750 mg dose [52,53]. Ongoing pharmacokinetic and safety studies (MDRPK2, TB-CHAMP lead-in PK study) [52,53], including with a novel dispersible formulation, are expected to provide additional much needed information. There is less data on moxifloxacin pharmacokinetics in children. Moxifloxacin has >90% oral bioavailability, with roughly 50% metabolized in the liver and the remainder eliminated unchanged in the feces and urine [54,55]. The current WHO-recommended dose in children is 7.5–10 mg/kg once daily; however, this dose results in exposures in young children well below targets seen in adults receiving the usual 400 mg daily dose [56]. HIV infection is associated with lower moxifloxacin exposures, due either to the effects of HIV infection itself or drug–drug interactions

(DDIs) with antiretrovirals [56]. The ongoing MDRPK2 study [56] is expected to inform optimal moxifloxacin dosing across ages.

Despite the good clinical toxicity profile documented in children regarding musculoskeletal adverse effects [49], neither levofloxacin nor moxifloxacin has been licensed for TB in children <18 years of age by the US FDA [57,58]. In the United Kingdom, the fluoroquinolones are not licensed for TB in either adults or children. To date, safety concerns remain the biggest challenge in the appropriate use of the fluoroquinolones for MDR-TB treatment and prevention in children, as was experienced by the V-Quin MDR-TB prevention trial in Vietnam in 2016 not being able to get approval for levofloxacin use as preventive treatment in children [56].

The WHO recommends the use of levofloxacin and moxifloxacin for treatment of DR-TB in children. They have put out a call for development of dispersible child-friendly formulations of both drugs [46]. A levofloxacin 100 mg dispersible tablet (Macleods, India) has already been manufactured and bioavailability studies completed as part of TB-CHAMP trial (MDR-TB prevention in children <5 years in South Africa). Macleods has also produced a 100-mg dispersible moxifloxacin tablet [41].

4. Novel TB drugs

4.1. Bedaquiline

The diarylquinoline bedaquiline (Sirturo; Janssen Therapeutics, New Brunswick, NJ, USA), previously known as TMC207, kills both dormant and actively replicating mycobacteria by a unique mechanism of inhibiting mycobacterial adenosine triphosphate (ATP) synthase [59]. It was the first of the current new anti-TB drugs to receive accelerated approval in December 2012 by the FDA for the treatment of MDR-TB, based on phase 2b trial data in adults. At the time there was concern about significantly more deaths occurring in the bedaquiline arm of a phase 2 study [60], which led to a black box warning in the packet insert. Bedaquiline has since then also been approved for MDR-TB by the European Medicines Agency (EMA) and the South African Medicines Control Council [61]. It is also included in the complementary list (essential medicines for priority diseases, for which specialized diagnostic or monitoring facilities, and/or specialist medical care, and/or specialist training are needed) of WHO Model List of Essential Medicines (March 2017). Further phase 3 studies and compassionate use programs have shown fewer deaths in MDR-TB patients receiving bedaquiline compared to those not receiving bedaquiline in their MDR-TB treatment regimens [62].

According to the 2016 WHO DR-TB guidelines on bedaquiline and in the context of the new WHO recommendations for the use of a shorter regimen to treat MDR-TB, bedaquiline is indicated for MDR-TB patients who are NOT eligible for the shorter regimen, that is, in case that a minimum of five effective TB drugs regimen cannot be composed with agents from groups A to C and the addition of pyrazinamide. This means bedaquiline continues to be indicated for the treatment of adult MDR-TB patients with (i) confirmed additional resistance to fluoroquinolones or second-line injectable drugs; (ii) patients with XDR-TB; (iii) patients with known adverse drug effects, poor tolerance, or contraindication to any component of the WHO-recommended longer regimen; or (iv) in case of unavailability or lack of guaranteed supply of a medicine being part of the WHO-recommended longer regimen.

Pediatric pharmacokinetic and safety studies of bedaquiline have been much delayed despite increasing routine access for adults [63]. Two pediatric trials have started enrolment. The Janssen-sponsored C211 trial (NCT02354014) is a phase 2 trial of bedaquiline pharmacokinetics and safety in combination with an optimized background regimen (OBR) in HIV-uninfected children with MDR-TB. This age de-escalation trial began in 2016 and has completed enrolment (15 adolescents) in the 12- to <18-year age-group; enrolment in the 6- to <12-year age-group is progressing, but no results are yet available. The second trial, sponsored by the U.S. National Institute of Allergy and Infectious Disease Infant Maternal Pediatric Adolescent AIDS Clinical Trial network (IMPAACT) (P1108; NCT02906007), will study pharmacokinetics and safety of bedaquiline in combination with OBR in both HIV-infected and -uninfected children with MDR-TB and opened September 2017.

Because the pharmacokinetics of adolescents are similar to that of adults and the need for new drugs in difficult-to-treat MDR-TB cases, this has led to bedaquiline being recommended by the Sentinel Project (on DR-TB in children) in children as young as 12 years of age and weighing >33 kg in the absence of any studies and despite WHO recommendations of use of bedaquiline only in adults >18 years of age [47,64]. The delay in these pediatric dose finding and safety studies has prevented access of younger children to this effective new anti-TB drug other than off-label use through organizations such as MSF for individual cases [65].

Although bedaquiline is well absorbed (improved by a fatty meal) and peak plasma concentrations are reached within 4–5 h, steady-state conditions takes >7 days of treatment to be reached in adults. Both bedaquiline and its M2 metabolite have very long terminal half-lives in adults of 164 (range 62–408) and 159 (range 69–407) days, respectively, most likely due to its redistribution from tissue compartments [59]. This complex pharmacokinetic behavior and toxicity risk makes extrapolation of the adult dose (400 mg daily for 2 weeks and then 200 mg three times weekly for 22 weeks) to children difficult.

Although Janssen Therapeutics has developed a dispersible child-friendly bedaquiline formulation that will be evaluated in pediatric trials, this formulation may not be available for routine care in many settings for some time. The current adult formulation is available as 100 mg tablets, is palatable and can be suspended in water. A bioavailability study of whole vs. suspended adult formulation tablets (TASK-002, NCT03032367; sponsored by IMPAACT) has been recently completed. Bioavailability was similar for crushed/suspended tablets compared to whole tablets in healthy adult volunteers. This means that once data on bedaquiline dosing in children are available even if the pediatric formulation is not, young children can use crushed or suspended adult 100-mg tablets off-label without concern that this formulation manipulation will affect bedaquiline's bioavailability [66].

4.2. Delamanid

The nitroimidazo-oxazole, delamanid (Deltyba, Otsuka Novel Products GmbH, Munich, Germany), previously known as OPC-67683 is another new drug shown to be effective when added to an MDR-TB regimen in a randomized, placebo-controlled phase 2b trial [67]. Delamanid is the first compound from a new drug class (nitro-dihydro-imidazooxazoles) that is bactericidal and specific to *M. tuberculosis*, including MDR strains. Following bioreduction within *M. tuberculosis* by the mycobacterial F420 system (as it is thought to be a prodrug), it inhibits mycolic acid biosynthesis. It has activity against both growing and dormant mycobacteria. A phase 3 randomized controlled trial of delamanid vs. placebo with an OBR for MDR-TB has recently been completed (NCT01424670) [41]; provisional results presented at the 48th Union World Conference on Lung Health in Guadalajara, Mexico, showed similar outcomes with or without delamanid under strict trial conditions, though shorter times to culture conversion were observed among those treated with delamanid. In April 2014, delamanid received conditional approval by the EMA based on phase 2b data – since then it has also been approved in Hong Kong, Japan, South Korea, Turkey, The Philippines, China, Indonesia, and India and filed in Peru and South Africa; dossiers are also reportedly being prepared for registration in Vietnam and Russia. It is also included in the complimentary list (with age restriction >6 years) of the WHO Model List of Essential Medicines (March 2017), the most effective and safe medicines needed in a health system.

Delamanid T_{max} is 4–6 h after oral administration and absorption is increased 2–2.4-fold when it is taken with a meal. Delamanid is converted by albumin to its primary metabolite DM-6705, which is further metabolized by CYP3A4 [68,69]. Both delamanid and DM-6705 have extremely long half-lives, with median (range) $t_{1/2}$ of 30–38 and 150–600 h respectively [70]. A DDI study in healthy adult volunteers showed that administration of delamanid with first-line antituberculosis drugs resulted in a nearly 50% reduction in delamanid exposures which was hypothesized to be due altered delamanid absorption; this has resulted in the recommendation to give delamanid at least 1 h apart from other TB medications [71]. Delamanid exposures are not proportional to dose, with exposures plateauing at a dose of 300 mg and higher due most likely to saturated dissolution [71]. The WHO-recommended adult delamanid dose is 100 mg twice daily [72]. The Otsuka-sponsored delamanid phase 3 trial will study a dose of 100 mg twice daily for 2 months, then 200 mg once daily for an additional 4 months. Once-daily dosing is much easier for patients and programs, and is beneficial for adherence. The ACTG-IMPAACT led PHOENIx Trial (A5300B/12003B/PHOENIx) will include a lead-in pharmacokinetic study evaluating the pharmacokinetics and safety of once-daily delamanid dosing.

Otsuka-sponsored Phase 1 (242-12-232, NCT01856634) and Phase 2 (242-12-233, NCT01859923) pediatric delamanid age de-escalation trials started in 2013 in The Philippines and South Africa [41]. Pharmacokinetic data from the first two groups (Group 1: ages 12 to <18 years, adult dose and Group 2: ages 6 to <12 years, half adult dose) of these trials showed that delamanid exposures in children were within the range seen in adults. On the basis of this data, WHO adapted their delamanid policy guidance to include children from 6 to 17 years of age: recommended dose of delamanid in children with MDR-TB (aged 6 to <12 years) is 50 mg BID for 6 months, and in adolescents (aged 12 to <18 years) it is 100 mg BID for 6 months given with food [73]. Pharmacokinetic data from the third group (ages 3 to <6 years) have already been collected and final results are awaited; enrolment in the fourth age-group (ages <3 years) has begun. Although clinically significant DDIs with antiretrovirals have not been seen to date, coadministration with lopinavir–ritonavir results in 20% higher delamanid exposures. The trial IMPAACT 2005 expects to open enrolment early 2018, and plans to address knowledge gap on delamanid pharmacokinetics and safety in HIV-infected children. Otsuka has successfully developed dispersible, taste-masked pediatric formulations in two strengths, which are being evaluated in younger age groups (<6 years of age) in its pediatric trials, and will also be used in the IMPAACT 2005 study [45].

Despite the above advances, the registration and resulting uptake of delamanid has been slow and few children have managed to access it to date [74]. A compassionate use program for children is available from Otsuka through approval processes such as European Respiratory Society/WHO TB Consilium and MSF, but the process is unfamiliar and cumbersome for most potential users [74]. Although in South Africa a Delamanid Clinical Access Programme was launched World TB Day (24 March) 2017, this access program is aimed at treating adults, and although children 6–18 years are supposed to be included, this has not become available to clinicians managing children <12 years of age (awaiting Medicines Control Council approval) [75].

4.3. Pretomanid

The bicyclic nitroimidazole-like molecule (a nitroimidazopyran) pretomanid, also known as PA-824, was developed by Pathogenesis Corporation and later transferred to the TB Alliance. Pretomanid is the second nitroimidazole to enter phase 3 clinical trials. It has a mechanism of action similar to delamanid and is active against DS- and DR-TB, both replicating and hypoxic, nonreplicating *M. tuberculosis*. In *M. tuberculosis*, the prodrug pretomanid is activated by F420-dependent glucose-6-phosphate-dehydrogenase pathway [76]. Activation of pretomanid within the mycobacteria results in production of reactive nitrogen species (including nitric oxide) which leads to nonspecific damage of intracellular macromolecules, a decrease in intracellular ATP and anaerobic killing. Pretomanid also kills aerobically by inhibiting cell wall mycolic acid biosynthesis [76,77].

Pretomanid has been well tolerated in adults with constant pharmacodynamic parameters when given once daily; T_{max} is 4–5 h following oral administration. The mean half-life varies from 16.1 to 23.8 h. Pretomanid has a large volume of distribution, indicating that the drug concentrates in tissues [78]. In adults, the drug appeared safe and efficacious when given between 100 and 200 mg once daily; it may induce a mild QT-

interval prolongation as well as a transient increase in creatinine levels [25]. Pretomanid has mainly been used in phase 2b and 3 trials in combination regimens with other novel (e.g. bedaquiline) and repurposed (e.g. moxifloxacin, linezolid) drugs with or without pyrazinamide for both DS-TB and DR-TB. These regimens are studied with the prospect of shortening both DS-TB and DR-TB treatment durations.

The NiX-TB trial (NCT02333799), an open-label phase 3 study sponsored by TB Alliance of bedaquiline, pretomanid, and linezolid for 6–9 months in patients with XDR-TB and failed MDR-TB, has shown promising preliminary results [41,42]. In preliminary data on 40 patients who had finished treatment, 31 had finished 6 months of follow-up; only 2 of these 31 have relapse or reinfection [35].

Pretomanid has not yet been approved by any regulatory authority, but there may be pressure on TB Alliance to work with other stakeholders to arrange an access program before regulatory approval is granted [59]. Although the use of pretomanid had setbacks in some phase 3 trials, the promising results of the NiX-TB trial should make pharmacokinetic and safety studies in children a priority. Concern regarding possible testicular toxicity observed in rodents in preclinical studies stalled pediatric pretomanid trials. Male reproductive hormone data collected from participants in phase 2 and 3 studies by TB Alliance have been submitted to the FDA to demonstrate safety and allow children to be dosed with pretomanid. TB Alliance plans to submit a PIP to the EMA during 2017 and has already developed a child-friendly dispersible formulation [35].

5. Challenge of HIV infection and DDIs

DDIs between anti-TB and antiretroviral drugs are common, and these should be carefully considered when using these drugs in HIV coinfected children. Possible DDIs, based on studies in adults only, are summarized in Table 1. Some DDIs, such as between bedaquiline and the protease inhibitors, may be barriers to using bedaquiline in some HIV-infected children.

6. New regimens, future directions, and new compounds

Combinations of new and investigational drugs have the potential to greatly improve the treatment of MDR-TB, and even potentially DS-TB. The goal is TB treatment regimens which are shorter, better tolerated, and do not include an injectable drug. The adult MDR-TB trial landscape is rapidly evolving, with multiple late phase trials evaluating novel, shorter regimens, many of which contain only orally administered medications. New medications may also play a role in preventive therapy. Delamanid is going to be evaluated in the PHOENIx trial for prevention of TB in high-risk MDR-TB exposed adults and children.

To date, pediatric TB drug trials have focused on dose finding and safety for individual medications, with efficacy extrapolated from adult studies. However, children have much better outcomes than adults with MDR-TB (successful outcomes in 75–90% of children vs. 50% of adults) most likely due to the paucibacillary, less severe nature of pediatric TB disease [79]. This raises the potential that children could do equally well with even shorter, less intense treatment regimens. There is now international consensus that an efficacy trial of a shortened regimen is needed in children with MDR-TB, and such a trial is in development through the IMPAACT network [80]. SMaRT Kids is a multicenter phase 3 non-inferiority trial among children <15 years of age with probable or confirmed MDR-TB, which will compare a 6-month, all-oral regimen of delamanid, clofazimine, linezolid, levofloxacin, and pyrazinamide vs. the 9–11-month shortened regimen recommended by the WHO [28] (Personal communication AJ Garcia-Prats). This study is early in development and unlikely to open until 2019.

Additionally, there are a number of new compounds in phase 1 and 2 clinical development. This includes the novel oxazolidinones sutezolid and LCB01-0371; the DprE1 inhibitors OPC-167832, PBTZ-169, and TBA-7371; the rhiminophenazine TBI-166; and agents with other mechanisms of action including Q203, nitazoxanide, and GSK070 [81]. Pediatric development programs should occur in parallel with adult development, as described elsewhere [56], to avoid the delays seen with bedaquiline, pretomanid and to a lesser extent delamanid.

7. Expert commentary

New and investigational drugs are urgently needed for children with MDR-TB, and are likely to be increasingly important depending on results of ongoing or planned trials. However,

Table 1. Possible drug–drug interactions between new and repurposed antituberculosis drugs and antiretroviral drugs.

Anti-TB drug	Antiretroviral drug	Drug–drug interaction
Bedaquiline (BDQ) (and its M2 metabolite)	Lopinavir–ritonavir (boosted protease inhibitor; PI) [83]	Increase in BDQ and M2 exposure due to reduced clearance; may lead to increased toxicity. Dose adaptation required
BDQ (and its M2 metabolite)	Nevirapine (NVP) [83]	No significant interaction
BDQ	Efavirenz (EFV) [84]	About a 50% reduction in BDQ exposure when chronically coadministered with efavirenz
Delamanid	EFV, NVP, Tenofovir and boosted PI [85,86]	No relevant DDIs with any ARVs, as no induction or inhibition of CYP450 system
Pretomanid (PA824)	EFV [87]	CYP3A minor metabolic pathway for PA824. Reduces PA824 exposure by 35%
Pretomanid (PA824)	Boosted PI (Lpv/r) [87]	CYP3A minor metabolic pathway for PA824. Reduces PA824 exposure by 17%
Clofazimine		No known DDIs with ARVs, but studies lacking.
Linezolid	Antiretroviral drugs [88]	No known DDIs, but possible increase risk of mitochondrial optic neuropathies with nucleotide reverse transcriptase inhibitors (NRTIs)
Fluoroquinolones		No known DDIs with ARVs

ARVs: antiretroviral drugs; DDI: drug–drug interactions; TB: tuberculosis.

at each step their use is obstructed or delayed by clinical research, drug development, financing, regulatory, and operational barriers. For most of these barriers, potential solutions are known or have been proposed. One of the most important changes needs to be more timely initiation of pediatric trials for novel drugs. As described in Section 2.2, pediatric work should happen in parallel at each phase of adult trials. In order to facilitate timely pediatric work, other contributing barriers must be addressed.

Additional funding and novel mechanisms are needed to finance pediatric TB drug development, as there is little profit incentive for pharmaceutical companies to do so. The Life Prize, formally known as 3P, is a project that attempts to speed TB drug development through push-and-pull mechanisms to incentivize different phases of drug development, and through pooling of intellectual property [82].

Regulatory authorities, charged with protecting the public, are often forced to walk a difficult line between ensuring sufficient evidence of safety and efficacy while facilitating access to new medications. This is especially true in the case of medications for unmet medical needs or for conditions for which the existing standard of care is suboptimal, like for DR-TB. Generally, better quality data, increased regulator resources and expertise, and harmonization of regulatory requirements would greatly improve registration timelines. For children, harmonization across regulatory authorities regarding pediatric data requirements, and further, the establishment of an expedited review pathway for pediatric formulations would greatly improve the speed at which pediatric investigations and regulatory reviews are conducted. Additionally, clear and harmonized regulatory guidance regarding the pathway and data requirements necessary to support a supplementary TB indication for repurposed drugs are needed. Registration fee waivers could be used to incentivize more local registrations of products that have limited market potential, like pediatric formulations for DR-TB medicines. Similarly, the impact of WHO PQ fee exemptions for products for children with DR-TB and other fragile markets should be monitored to ensure that manufacturers will continue to pursue WHO PQ status for their products. Finally, policy change at the country level to enable procurement using domestic resources for quality-assured medicines at the lowest price possible (i.e. via the GDF or enabling GDF to bid on national tenders) would help to prevent further market fragmentation and to maintain the availability of good quality medicines at low prices.

If regimens for MDR-TB treatment in children were more standardized, then forecasting would be more straightforward. Mechanisms for increasing demand would include more extensive training, more inclusive guidelines, currently available diagnostic tests being more utilized in children, and the development of better pediatric diagnostic tests. A more streamlined approach to drug distribution, from manufacturer to patient, would help significantly and the use of a centralized procurement agency, such as the GDF, would allow small regional demands to be aggregated and managed on a global level.

Improved capacity and systems for procurement and supply chain management would reduce in-country operational barriers to getting medications to children in the field. Systems within TB programs for the rapid introduction and scale-up of new medications, as well as other evidence-based policies and other tools such as diagnostics, are needed. Lastly, efforts to scale up the diagnosis and programmatic management of children with MDR-TB must begin in earnest. This must include improved recording and reporting of pediatric MDR-TB cases, with pediatric data reported by the WHO alongside adult data.

8. Five-year view

Clinicians managing MDR-TB in children are eager to use the new drugs, especially delamanid and bedaquiline, in treatment regimens, and to eliminate the injectable agents altogether. Delamanid dose and safety data in young children should be available in the next 1–2 years, while data on dose and safety of bedaquiline should be available within the next 5 years. Although child-friendly formulations have been developed for both these drugs, there remains uncertainty about their market introduction and availability, but adult formulations in crushed and dispersed forms will be available and bioavailability studies are being performed. Repurposed drugs are already in use, but more data will be known on optimal dosing in children at different ages, and child-friendly formulations should be available. We are optimistic that with all the treatment-shortening trials ongoing in adults and planned for children that we will have a shorter 6-month, all-oral MDR-TB treatment regimen for children. The challenge of diagnosing more children with MDR-TB given the limits of existing diagnostic tools remains an urgent priority. The lessons learned in developing delamanid and bedaquiline in children need to be applied to other new compounds as they undergo evaluation, to avoid the same pitfalls and ensure equitable access of important treatment advances for children.

Key issues

- New antituberculosis drugs to more safely and effectively treat MDR-TB are needed as much for children as for adults; however, children are always last to gain access to these drugs
- Confirmation of MDR-TB in children is challenging therefore currently only a small proportion of MDR-TB cases in children are diagnosed. Children cannot benefit from new TB medications if they are not diagnosed as having probable or confirmed MDR-TB, and in the absence of much improved case finding or more sensitive diagnostic tests, much of the benefit of new medications in children will remain unrealised.
- Efficacy trials for new drugs are not necessary in children unless these drugs are for specific pediatric indications, as efficacy in adult TB cases infers efficacy in children. Dose finding and safety studies in children should be done early in new drug evaluations otherwise clinicians may start using these drugs without properly established dosing and without evaluation of their safety in children.
- Because of the lack of market incentive for antituberculosis drugs, various push and pull mechanisms have been proposed to incentivise the development of new drugs.

- In addition to the research and development necessary to determine appropriate doses and formulations acceptable for children, improved regulatory and procurement policies are critically important to facilitate access to new and repurposed drugs for the treatment of children with MDR-TB.
- Operational challenges should be addressed by organisations, such as the World Health Organization and National TB Programmes, developing processes to rapidly incorporate new data and tools into practice, so that children can benefit from new treatments as quickly as possible.
- Data is emerging from ongoing studies on the pharmacokinetics and safety of new (bedaquiline, delamanid and pretomanid) and repurposed (fluoroquinolnes, clofazimine and linezolid) drugs.
- We are optimistic that with the available arsenal of newer, repurposed, and existing drugs, and ongoing and planned treatment shortening trials, that a 6-month, all-oral MDR-TB treatment regimen for children will be possible within five years.

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Current status of pharmacokinetic and safety studies of multidrug-resistant tuberculosis treatment in children

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SUMMARY

After decades of neglect, data are finally becoming available on the appropriate, safe dosing of key second-line anti-tuberculosis drugs used for treating multidrug-resistant tuberculosis (MDR-TB) in children, including levofloxacin (LVX), moxifloxacin (MFX), linezolid (LZD) and delamanid (DLM). Much needed data on some novel and repurposed drugs are still lacking, including for bedaquiline (BDQ), pretomanid (PTM) and clofazimine (CFZ). We review the status of pharmacokinetic (PK) and safety studies of key anti-tuberculosis medications in children with MDR-TB, identify priority knowledge gaps and note ongoing work to address those gaps, in the context of planning for an efficacy trial in children with MDR-TB. There is international consensus that an efficacy trial of a novel, all-oral, shortened MDR-TB treatment trial in children is both needed and feasible. Key novel and repurposed second-line anti-tuberculosis drugs include BDQ, DLM, PTM, MFX, LVX, CFZ and LZD. The rapidly emerging PK and safety data on these medications in children with MDR-TB from studies that are underway, completed or planned, will be critical in supporting such an efficacy trial. Commitment to addressing the remaining knowledge gaps, developing child-friendly formulations of key medications, improving the design of paediatric PK and safety studies, and development of international trial capacity in children with MDR-TB are important priorities.

KEY WORDS: MDR-TB; children; PK; trials; safety

CHILDREN REPRESENT a substantial proportion of the global burden of multidrug-resistant tuberculosis (MDR-TB), defined as TB resistant to at least both isoniazid and rifampicin, with an estimated 25 000–32 000 incident cases globally each year.[1,2] Recent years have seen a rapidly evolving research landscape for MDR-TB treatment in adults, which has included the development and conditional registration of multiple novel anti-tuberculosis medications, the increased use of repurposed medications and the evaluation of shorter, better-tolerated regimens. Children also stand to benefit substantially from these advances in treatment strategies for MDR-TB; however, there have been delays in evaluating these new and repurposed medications in paediatric studies.

Understanding the pharmacokinetics (PK) and safety of anti-tuberculosis medications in children, and ensuring the availability of child-friendly formulations, are critically important to providing access to safe and effective treatment of MDR-TB in human immunodeficiency virus (HIV) infected and non-infected children. Furthermore, there is international consensus that an efficacy trial of a shortened, all-oral regimen is needed in children with MDR-TB.[3] An understanding of optimal, safe doses of medications to be included in Phase III trial regimens is critically important. We highlight here the status of PK and safety studies of key novel and repurposed second-line anti-tuberculosis medications in children with MDR-TB, including bedaquiline (BDQ), delamanid (DLM), pretomanid (PTM), moxifloxacin (MFX), levofloxacin (LVX), clofazimine (CFZ) and linezolid (LZD), and identifying priority knowledge gaps and ongoing work to address those gaps, in the context of planning for an efficacy trial in children with MDR-TB.

APPROACH TO DESIGN OF TRIALS OF THE PHARMACOKINETICS AND SAFETY OF ANTI-TUBERCULOSIS DRUGS IN CHILDREN

Well-designed and executed paediatric PK and safety studies are central to anti-tuberculosis drug development in children. The first step in the currently accepted approach to determining the types of studies

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required for drug development in children is to assess whether it is reasonable to extrapolate a drug's efficacy from adult trials to children.[4] This depends on whether, compared to adults, children could reasonably be expected to have a similar disease progression and a similar relationship between drug exposure and response to the relevant treatment.[4] In the case of TB, the consensus has been that children would be expected to have at least as good a response to treatment as adults, given the paucibacillary nature of TB disease in children.[5,6] The priority then for paediatric trials of anti-tuberculosis drugs is to perform PK studies to identify the doses in children that result in exposures approximating those in adults receiving the recommended dose, and to establish safety in children at those doses.[5,6]

Although common in routine clinical practice, paediatric drug doses cannot reliably be directly extrapolated from the mg/kg of body weight dose in adults. This is because of both size-related and age-related differences in PK. Size-related changes can be described using allometry, which is the study of how biological processes, volumes, body parts or organs scale with body size.[7] Many biological processes, including clearance, which is a primary driver of drug exposure, scale with body size at a less-than-proportional rate, with the generally accepted 3/4 allometric scaling approach.[8] The linear extrapolation of mg/kg doses from adults to children therefore results in lower drug exposures in children, with the worst underexposure in the smallest children. Like many other body volumes, volume of distribution, also a key driver of drug exposures, scales more proportionally with body size, although body composition differences alter this in the smallest infants.[8]

In addition to considerations of size, age-related factors also affect anti-tuberculosis drug PK in children.[9] Most biological processes, many of which have an important influence on drug disposition, continue to mature and develop after birth. This includes, but is not limited to, changes in gastrointestinal function and gastric pH, changes in renal function, alterations in body composition, such as proportion of body fat and body water, and development of the capacity of enzymes responsible for drug metabolism.[9] The most dramatic changes occur in the first weeks and months of life, and most processes have reached near or full maturity by 2 years of age.

To establish the appropriate paediatric doses of TB medications, carefully designed trials are thus needed that include children across all relevant age groups, with a particular focus on children aged <2 years, where the influence of size and age on PK is most pronounced. Given the potential for drug-drug interactions, it is also critically important that HIV-infected children are included in these studies.

CONSIDERATIONS FOR ASSESSING SAFETY IN CHILDREN

Adverse effects of anti-tuberculosis drugs are often reported to be less common in children than in adults.[10,11] Although this observation may be correct, it may be at least partly due to lower drug exposures at the same mg/kg bodyweight doses in children than in adults, as has been shown with the fluoroquinolones (FQs).[12–14] Also complicating interpretation of this is the distinct lack of prospective studies accurately documenting adverse effects in children. Due to the difficulty in assessment in children, some adverse effects may be under-recognised, especially those based on subjective report such as peripheral neuropathy, pain and vision impairment, or those requiring specialised testing such as hearing loss. The majority of reported adverse effects in children are mild or moderate and of little clinical importance. However, these may still be unacceptable to children or care givers/parents, leading to social stigmatisation (e.g., skin discolouration with CFZ) or non-adherence to medication (e.g., due to nausea and vomiting), which may negatively impact treatment outcomes.

Data in juvenile animal studies and from trials in adults should provide some additional insight into anticipated adverse effects in children. It is tempting to assume that adverse effect profiles in children are similar to those in adults, given similar drug exposures. Although they may be, this assumption must be carefully tested in paediatric studies.[15,16] Adverse effects seen in juvenile animal models or in adults may not be seen in children at clinically relevant doses, although such medications may be perceived as poor candidates for paediatric treatment. A relevant example is the persistent reluctance to use FQs in children with MDR-TB in some settings due to the arthropathy seen in juvenile animals,[17] although several human studies have documented the safety of long-term FQ use in children.[12,18] On the other hand, children as developing organisms with developing systems may be at risk for a spectrum or severity of adverse effects not seen in adults. The classic example is chloramphenicol, which was effectively and safely used widely in adults with penicillin-resistant infections, but caused 'gray-baby syndrome' in infants due to the immaturity of the hepatic uridine 5′-diphospho-glucuronyl transferase enzymes in children, which hampered metabolism of the drug.[19] The safety of new medications or repurposed medications with new doses must be carefully evaluated, particularly in young children.

Monitoring safety in studies of MDR-TB treatment has its own inherent challenges. Given that these multidrug regimens include as many as 5–7 different medications, with potentially overlapping adverse effects, assessing the attribution of individual drugs for certain adverse effects is challenging. This may be

Table 1 Status of PK and safety data on novel anti-tuberculosis medications in children and ongoing or planned paediatric studies

	Current data in children	Key knowledge gaps	Ongoing or planned studies
BDQ			
PK	No data in children	Data on PK and optimal paediatric doses in all ages	Janssen C211: BDQ PK and safety in non-HIV-infected children with MDR-TB aged 0–17 years ($n = 60$)
Safety	No data in children	Data on safety at paediatric doses in all ages	IMPAACT P1108: BDQ PK and safety in HIV-infected and non-infected children with MDR-TB aged 0–17 years ($n = 72$); includes HIV-infected children on selected ARVs
Drug-drug interactions	Important interactions with ARVs expected; no data in children	Data on interactions with ARVs in HIV-infected children	
DLM			
PK	Data on PK in non-HIV-infected children aged 6–17 years ($n = 13$); drug exposure in children within adult range	Data on PK and optimal doses in children aged <6 years; potential for once daily dosing (twice daily dosing being studied)	Otsuka 232/233: DLM PK and safety in children with MDR-TB aged 0–17 years ($n = 36$)
Safety	Data on safety in non-HIV-infected children aged 6–17 years ($n = 13$); safe with minimal QT prolongation	Data on safety at paediatric doses in children aged <6 years	IMPAACT 2005: DLM PK and safety in HIV-infected and non-infected children with MDR-TB aged 0–17 years ($n = 36$–48)
Drug-drug interactions	Important interactions with ARVs not expected; no data in children	Data on interactions with ARVs in HIV-infected children	
Pretomanid			
PK	No data in children	Data on PK and optimal paediatric doses in all ages	None
Safety	No data in children	Data on safety at paediatric doses in all ages	
Drug-drug interactions	Important interactions with some ARVs expected; no data in children	Data on interactions with ARVs in HIV-infected children	

PK = pharmacokinetic; BDQ = bedaquiline; HIV = human immunodeficiency virus; MDR-TB = multidrug-resistant tuberculosis; ARV = antiretroviral; IMPAACT = International Maternal Pediatric Adolescent AIDS Clinical Trials; DLM = delamanid.

more challenging in HIV-infected children who will also be taking antiretrovirals. Even where the adverse effects of individual drugs are described, it may be difficult to predict whether there will be an additive risk of adverse effects where there is overlap. QT interval prolongation, a known adverse effect of BDQ, DLM, CFZ, and the FQs, is such an example. For repurposed medications for which there may be some paediatric experience, such as for LZD, the long duration of treatment for TB may result in a different risk of adverse effects compared to short-term treatment of non-tuberculosis infections. Particularly for the novel medications, the number of adults or children who have ever been treated with these medications remains small. An efficacy trial enrolling a large number of children would therefore need to carefully monitor for previously unreported adverse effects.

The adverse effects of the priority anti-tuberculosis medications listed above have been described in detail elsewhere;[12] key safety considerations and knowledge gaps are shown in Tables 1 and 2.

DEVELOPMENTS IN PHARMACOKINETIC TRIAL DESIGN IN CHILDREN WITH TUBERCULOSIS

The study of the PK of anti-tuberculosis drugs in children has historically often been through observational studies, with sample size and sampling schedule primarily pragmatically based on available resources and experience from adult studies. More recently, the use of model-based clinical trial simulations using pharmacometrics methodology has been introduced; this ensures sufficient statistical power while simultaneously minimising the burden on the participating children and the resources used.[20] Pharmacometrics is a rapidly advancing field, aimed at developing and applying mathematical models to represent physiology, pharmacology and disease progression. Because of the insight pharmacometrics provides into PK-pharmacodynamic relationships, it has been demonstrated to be highly useful in drug development.[20,21] There is clear regulatory guidance on the expectations for paediatric PK studies which states that paediatric studies should be designed to have at least 80% power to fulfil defined precision criteria for key PK parameters.[22] Clinical trial simulations using appropriately adjusted pharmacometric models developed from adult data were used in designing the above-mentioned IMPAACT (International Maternal Pediatric Adolescent AIDS Clinical Trials) sponsored paediatric studies of BDQ (P1108) and DLM (P2005). By evaluating the likelihood of fulfilling these precision criteria for a diverse range of design options and sample sizes, the simulations played an integral part in the design decision-making process for these key paediatric PK studies.

For novel medications not previously used in children, an age de-escalation approach, starting enrolment with a cohort of older children and subsequently moving down in age, has traditionally been used. Information gathered in the oldest children can be added to the existing knowledge from adults to generate more confidence in the

Table 2 Status of PK and safety data on key re-purposed anti-tuberculosis medications in children and ongoing or planned paediatric studies

	Current data in children	Key knowledge gaps	Ongoing or planned studies
LVX			
PK	Data in children with MDR-TB across all ages ($n = 73$), limited in children aged <2 years; low exposures at currently used doses	Data on PK in young children, and optimal paediatric doses in all ages	MDRPK1: PK and safety of second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–14 years with MDR-TB (data expected on >100 children on LVX)
Safety	Some data across all ages at currently used doses; generally safe and well tolerated at currently used doses	Data on safety at optimal paediatric doses in all ages	MDRPK2: PK and safety of model-optimised doses of key second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–17 years (data expected on 80–100 children on LVX)
Drug-drug interactions	Important interactions with ARVs not expected; limited data in children	Confirm no important interactions with ARVs in HIV-infected children	
MFX			
PK	Data in children with MDR-TB aged 7–14 years ($n = 23$) showing low exposures at current doses; no data for ages <7 years	Data on PK in children aged <7 years, and optimal paediatric doses in all ages	MDRPK1: PK and safety of second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–14 years with MDR-TB (data expected on $n = 40$ on MFX)
Safety	Data in children with MDR-TB aged 7–14 years ($n = 23$), safe at current doses; no data for ages <7 years	Data on safety at optimal paediatric doses in all ages	MDRPK2: PK and safety of model-optimised doses of key second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–17 years (data expected on $n = 80$–100 on MFX)
Drug-drug interactions	Some evidence for possible interactions with ARVs; limited data in children	Data on interactions with ARVs in HIV-infected children	
LZD			
PK	Data in children with non-tuberculous infections; no published data in children with TB	Data on PK and optimal paediatric doses for anti-tuberculosis treatment in all ages	MDRPK1: PK and safety of second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–14 years with MDR-TB (data expected on <10 on LZD)
Safety	Limited data in children treated for long courses (>28 days) for MDR-TB	Data on safety at current and optimal paediatric doses used long-term for anti-tuberculosis treatment	MDRPK2: PK and safety of model-optimised doses of key second-line anti-tuberculosis drugs in HIV-infected and non-infected children aged 0–17 years (data expected on LZD PK in $n = 80$–100 children, long-term safety in 10–20)
Drug-drug interactions	Important interactions not expected	Data on overlapping toxicity with ARVs in HIV-infected children	
CFZ			
PK	No data in children	Data on PK and optimal paediatric doses for anti-tuberculosis treatment in all ages	No planned trials Small amount of opportunistic PK data possible from MDRPK1 and MDRPK2
Safety	Some experience in children treated for leprosy	Data on safety at optimal paediatric doses; safety data when combined with other QT prolonging anti-tuberculosis drugs	
Drug-drug interactions	Interactions possible with ARVs, but limited data in adults or children	Data on interactions with ARVs in adults and children	

PK = pharmacokinetic; LVX = levofloxacin; MDR-TB = multidrug-resistant tuberculosis; ARV = antiretroviral; MFX = moxifloxacin; HIV = human immunodeficiency virus; LZD = linezolid; CFZ = clofazimine.

prediction of doses to be tested in the next, younger cohort. A model-based approach leveraging data from adults and older children may provide the best chance of selecting adequate doses for further study.[23] The age de-escalation approach, however, is very time consuming and cumbersome, with many interim trial stoppages for analysis and dose adjustment, and is not a formal requirement for paediatric trials by stringent regulatory authorities. Modified approaches, such as enrolling children aged >3 years in parallel, or using single-dose followed by multiple-dose trials in all ages concomitantly, have been proposed and are being considered.[24]

Historically, a lack of capacity to implement PK studies in children with MDR-TB has been a limitation to efficient anti-tuberculosis medication evaluation in children. Until recently there was no support for or recognition of the need for such studies, and high MDR-TB burden settings frequently lacked sufficient clinical, research or regulatory capacity for them. This situation is now rapidly evolving, with much needed clinical trial capacity being built, partly due to the opening of the paediatric trials sponsored by Otsuka (Tokyo, Japan) and Janssen (Beerse, Belgium). Furthermore, the IMPAACT network is beginning to leverage its existing,

Table 3 Current status of paediatric formulation development for novel and key repurposed anti-tuberculosis medications for multidrug-resistant tuberculosis treatment

	Adult formulation	Paediatric formulation
Bedaquiline	100 mg unscored tablets	Dispersible paediatric tablets developed by Janssen (Beerse, Belgium) for C211 trial
Delamanid	50 mg unscored tablets	Dispersible paediatric tablets developed by Otsuka (Tokyo, Japan) for 232/233 trials
Pretomanid	100 mg, 200 mg tablets (trial formulations)	Dispersible paediatric tablets developed by Dr Reddy's Laboratories (Hyderabad, India), TB Alliance (Geneva, Switzerland)
Levofloxacin	250 mg tablets	Paediatric suspension exits, but not widely available and not preferred formulation; dispersible tablets developed by Macleods (Mumbai, India) for TB-CHAMP trial
Moxifloxacin	400 mg tablets	Gummy formulation being developed by Luna Innovations (Roanoke, VA, USA)
Linezolid	600 mg tablets	Paediatric suspension exists, but prohibitively expensive and not widely available
Clofazimine	50 mg and 100 mg gel capsules	Gummy formulation being developed by Luna Innovations

TB-CHAMP = Tuberculosis CHild and Adolescent Multidrug-resistant Preventive.

experienced network of trial sites, which to date have been more focused on HIV, to study paediatric TB and MDR-TB. These efforts, with continued investment of resources, are important to ensure timely access to children of advances in MDR-TB treatment and prevention, and to support increased international collaboration and advocacy.

CURRENT STATE OF KNOWLEDGE, GAPS AND PLANNED OR ONGOING PAEDIATRIC STUDIES

The landscape of trials evaluating novel medications and regimens in adults with MDR-TB is rapidly evolving. To ensure that children will be able to benefit from MDR-TB regimens found to be efficacious and/or safer in adult trials, paediatric PK and safety studies should focus on the anti-tuberculosis drugs that are being prioritised in adult studies and that are likely to play key roles in future TB regimens.[25] An examination of ongoing or planned trials in adults with MDR-TB shows that the following novel and repurposed anti-tuberculosis medications are key components of many MDR-TB treatment regimens under evaluation: BDQ, DLM, PTM, MFX, LVX, CFZ and LZD.[26] This group of drugs should therefore be the focus of future paediatric studies.

Ensuring the development and availability of child-friendly formulations is another key consideration. Child-friendly characteristics include small tablet/capsule size and strength (in mg), ability to be mixed with juice or water, measurability even at very small doses, and flavour/palatability. The lack of many of these characteristics in currently available formulations creates the need for imprecise manipulations such as splitting or crushing adult tablets, dissolving them in water or mixing them with food. This impairs accurate dosing, particularly in the youngest children, introduces complexity in administration for parents and health care workers, reduces medication palatability, and may affect bioavailability in unknown ways.[27,28] These myriad effects have the potential to adversely influence adherence, which is vitally important for ensuring optimal treatment outcomes.[29,30] The acceptability of formulations for children and their care givers must be considered and should be formally evaluated in studies of anti-tuberculosis drugs. Novel approaches, such as the development of gummy formulations, are being explored and deserve consideration.[31]

Specific PK characteristics of anti-tuberculosis medications in children have been reviewed elsewhere, and such a discussion is beyond the scope of this article.[32] We summarise below the current state of paediatric knowledge and key gaps, and ongoing or planned trials for priority anti-tuberculosis medications for MDR-TB (in Tables 1 and 2). Much of the PK and safety data described, other than from the multicentre trials, are from a single site in South Africa. Data from diverse settings and populations are needed to better account for pharmacogenetic and other potential differences in PK and safety of these medications. Table 3 shows the current status of paediatric formulation development for these same medications.[31] It should be noted that delays in studying these medications and developing paediatric formulations are in part related to regulatory and economic barriers. A detailed consideration of these issues is beyond the scope of this review, but they have been discussed elsewhere.[24] Although the need for improved treatment among affected children is great, the market is small. Innovative solutions will be needed to accelerate this important work to ensure equitable access to anti-tuberculosis treatment in children.

Bedaquiline

The opening of paediatric BDQ trials has been much delayed. Despite global roll-out and increasing access to BDQ for adults, even in routine care settings,[33] to date there are no PK data in children. Two paediatric trials will begin to fill this gap. The Janssen-sponsored

C211 paediatric trial (NCT02354014) is a Phase II study of BDQ PK and safety in combination with an optimised background regimen (OBR) in children with MDR-TB; only non-HIV-infected children are included. Enrolment in this age de-escalation trial began in 2016. The P1108 trial (NCT02906007) sponsored by the US National Institute of Allergy and Infectious Disease (NIAID; Bethesda, MD, USA) Infant Maternal Pediatric Adolescent AIDS Clinical Trial (IMPAACT) network will also evaluate the PK and safety of BDQ in combination with OBR in both HIV-infected and non-infected children with MDR-TB; the trial is expected to open in early 2017. Janssen has developed a dispersible paediatric BDQ formulation, which will be evaluated in their trial; however, this formulation may not be available in the field for some time. The IMPAACT network has sponsored a trial comparing the bioavailability of whole and dissolved BDQ (TASK-002, NCT03032367), which will inform the use of the dissolved adult formulation for children who cannot swallow whole tablets. It is also conceivable that local economic or regulatory barriers will limit the widespread availability of the paediatric dispersible tablets for some time. Bioavailability data on crushed adult tablets will help bridge the expected time gap when data on BDQ dosing in children are available but the BDQ paediatric formulation is not.

Delamanid

The Otsuka-sponsored Phase I (242-12-232, NCT01856634) and Phase II (242-12-233, NCT01859923) paediatric DLM trials opened in 2013. DLM PK data from Group 1 (ages 12–17 years, adult dose) and Group 2 (ages 6–11 years, half adult dose) of these age de-escalation trials showed that exposure in children was within the range seen in adults. This led the World Health Organization (WHO) to recommend doses and indications for DLM use for children aged 6–17 years with MDR-TB.[34] PK data from Group 3 (ages 3–5 years) and Group 4 (ages 0–2 years) from this trial are expected in 2017. HIV-infected children were excluded from Groups 1–3, but will be allowed in Group 4. IMPAACT 2005 is a planned trial on DLM PK and safety in both HIV-infected and non-HIV-infected children with MDR-TB to address this gap, and is expected to open in 2017. Otsuka has developed a dispersible, taste-masked paediatric formulation in two strengths, which is being evaluated in Groups 3 and 4 of its paediatric trials, and will also be used for the youngest children (age <6 years) in the IMPAACT 2005 study.

Pretomanid

There are no PTM pharmacokinetic data in children, and no ongoing or immediately planned paediatric trials. This delay is at least in part due to concerns with PTM safety. Testicular toxicity was seen in some animal models, as it has been with other nitroimidazole compounds such as metronidazole;[35] however, the clinical significance in humans is not entirely clear. Moreover, the Shortening Treatment by Advancing Novel Drugs (STAND) trial evaluating a regimen comprising PTM-MFX-pyrazinamide for both drug-susceptible and MDR-TB in adults (NCT02342886) was temporarily put on hold due to hepatotoxicity in the experimental arm; the trial has been cleared by the Data Safety Monitoring Committee to re-start.[26] A substudy within the STAND trial aims to evaluate the presence of any testicular toxicity.[36] Particularly given emerging evidence on the success of PTM-containing regimens in adult Phase II and III trials,[37,38] the paediatric trials should start as soon as safety concerns have been sufficiently alleviated. A prototype paediatric formulation of PTM has been developed.[39]

Moxifloxacin

The paediatric development of MFX for non-tuberculosis bacterial indications was limited, at least partly due to its bitterness and poor palatability. The only published PK data are for 23 children aged 7–15 years treated for MDR-TB,[14] from a US National Institutes of Health (NIH; Bethesda, MD, USA) funded observational PK study in Cape Town, South Africa (MDRPK1, Pharmacokinetics and toxicity of second-line anti-tuberculosis drugs in HIV-infected and -uninfected children). This study showed low MFX exposures in children who received the recommended paediatric doses (10 mg/kg) relative to exposures seen in adults after a 400 mg dose.[14] PK data in children aged <7 years and analysis to determine more optimal doses across all ages are thus urgently needed. An NIH-funded study of the PKs of model-based doses of key second-line TB medications in children routinely treated for MDR-TB in Cape Town opened in 2015, and should address some of these knowledge gaps (MDRPK2, Optimizing and operationalizing pediatric drug-resistant tuberculosis treatment). There is no child-friendly formulation of MFX, and the existing adult formulation is an exceedingly large tablet, difficult to split and bitter when crushed, making accurate and tolerable dosing in children problematic. A taste-masked dispersible tablet with lower strength is needed for reliable paediatric dosing.

Levofloxacin

A growing body of observations in children receiving LVX for MDR-TB has shown that currently recommended paediatric doses for MDR-TB (15–20 mg/kg once daily) result in exposures well below those in adults receiving the most frequently used dose of 750 mg once daily.[40,41] Optimal paediatric doses of LVX, and safety at those doses, remain to be established; the MDRPK2 study aspires to address this gap.

Although an LVX suspension does exist, it is not available in most high TB burden settings, and suspensions are not preferred either by health systems, because of challenges with storage and shelf-life, or by patients and care givers.[42] A 100 mg dispersible LVX tablet (Macleods Pharmaceuticals Ltd, Mumbai, India) has been developed for a paediatric trial of LVX for MDR-TB preventive therapy (Tuberculosis CHild and Adolescent Multi-drug-resistant Preventive [TB-CHAMP] therapy trial, ISRCTN92634082), and could potentially become available more widely after the trial has been completed. The PK of this formulation is being evaluated in a lead-in phase of that trial.

Clofazimine

Although CFZ has been used for decades for the treatment of leprosy in children, there are no published, or to our knowledge unpublished, paediatric PK data available. There are no ongoing or planned trials of CFZ PKs in children with TB. Limited data from a few patients in the MDRPK1 study are available, and there may be the possibility to opportunistically study CFZ in the MDRPK2 study as the routine use of CFZ becomes more widespread in response to new guidelines from the WHO.[43] CFZ is only available in 50 mg and 100 mg gel capsules. These are not flexible formulations, cannot be split or opened, and may be challenging for young children to swallow. Given the potentially important role in future TB treatment regimens, CFZ PK, safety and acceptability studies in children, and the development of a child-friendly formulation, are therefore a priority.

Linezolid

As LZD has been used for the treatment of resistant Gram-positive bacterial infections, there are data on its PK and safety in children with non-tuberculous infections for short-term use (<28 days).[44] However, there are no published PK data in children with TB, and the doses needed in children to approximate exposures in adults after the 600 mg once daily dose used for TB are not yet known.[45] The MDRPK2 study is aimed at evaluating LZD PKs in children with MDR-TB; combined with data from MDRPK1, this will provide much-needed information on optimal and safe LZD paediatric dosing for TB treatment. An LZD suspension exists, but its use for long courses for MDR-TB treatment is cost-prohibitive, and access in many settings is limited. Manipulation of the adult 600 mg tablet for paediatric dosing may be problematic and is not a long-term solution, due to the large tablet size and strength, difficulty in suspending and lack of data on impact on bioavailability. A reasonably priced flexible solid-oral dosage formulation for children is needed.

IMPLICATIONS FOR AN EFFICACY TRIAL IN CHILDREN WITH MDR-TB

There is a critical need for shorter duration, injectable-sparing, safe, acceptable and efficacious regimens for children, and there is consensus that an efficacy trial is also urgently needed. A clearer understanding of the safety and appropriate dosing of medications to be included in a Phase III paediatric MDR-TB efficacy trial is required. The lack of comprehensive data or knowledge of perfect doses should not be used to delay such a trial going forward, in which dosing based on the best available evidence should be used. Data from ongoing studies are expected to inform paediatric dosing of MFX, LVX, LZD and DLM in the near future. The lack of planned paediatric studies of CFZ is worrying. Although it is already recommended and being used in the field for MDR-TB treatment in children, and it is reassuring that there is substantial experience supporting its safety in children treated for leprosy, pharmacokinetic studies of CFZ in children with MDR-TB should be prioritised. The lack of paediatric-specific data on BDQ and PTM will likely preclude their use in a paediatric efficacy trial in the immediate future.

It is also worth noting that existing paediatric PK data clearly add support for an efficacy trial in children. The FQs, with their potent bactericidal activity, are considered the most important component of existing MDR-TB regimens. Alarmingly, published data have shown that children with MDR-TB treated with currently recommended doses of ofloxacin, LVX and MFX have plasma FQ exposures well below those in adults.[14,40,41] However, despite these low exposures, treatment outcomes in children are much better than in adults with MDR-TB, with 80–90% of children successfully treated globally compared to 50% of adults in most high-burden settings.[46,47] Low drug exposures in children, combined with already improved outcomes compared to adults, clearly call into question the assumption that children have a similar disease progression and response to treatment to that of adults; these factors combined provide a clear justification for an efficacy trial in children. Improved paediatric dosing of these medications is still important in promoting optimal outcomes with a shortened regimen in such a trial.

CONCLUSION

Although much remains to be done, there has been substantial progress in recent years in studying and understanding second-line and novel anti-tuberculosis medications in children. Precisely because they differ from adults with MDR-TB, children represent a group that is uniquely poised to benefit from shorter,

less toxic and less burdensome treatments for MDR-TB. It is imperative that the rigorous investigation of such treatments continues and accelerates. Expanding global clinical trial capacity for MDR-TB in children, and closer collaboration between researchers, industry, funders and affected communities, will be required to ensure that the progress made thus far is not lost, and that children with MDR-TB continue to benefit.

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RÉSUMÉ

Après des décennies de négligence, nous disposons finalement de données relatives à la posologie appropriée et sûre des principaux médicaments antituberculeux de deuxième ligne utilisés pour le traitement de la tuberculose multirésistante (TB-MDR) des enfants, dont la lévofloxacine (LVX), la moxifloxacine (MFX), le linézolide (LZD) et le délamanide (DLM). Mais on manque toujours de données relatives à certains médicaments nouveaux et recyclés, incluant la bédaquiline (BDQ), le prétomanide (PTM) et la clofazimine (CFZ). Nous revoyons le statut des études de pharmacocinétique (PK) et de sécurité des principaux médicaments antituberculeux chez les enfants atteints de TB-MDR, identifions les lacunes prioritaires en matière de connaissances et notons le travail en cours pour affronter ces lacunes, dans le contexte de la planification d'un essai d'efficacité chez des enfants atteints de TB-MDR. Il y a un consensus international sur le fait qu'un essai d'efficacité d'un traitement de TB-MDR nouveau, exclusivement oral et raccourci chez des enfants est à la fois nécessaire et faisable. Les médicaments antituberculeux de deuxième ligne principaux, nouveaux et recyclés, incluent la BDQ, le DLM, le PTM, la MFX, le LVX, la CFZ et le LZD. Les données, en rapide expansion, de PK et de sécurité relatives à ces médicaments chez les enfants atteints de TB-MDR, émanant d'études qui sont en cours, achevées ou prévues, seront cruciales pour soutenir un tel essai d'efficacité. Un engagement à affronter les lacunes résiduelles de connaissances, l'élaboration de formules des principaux médicaments adaptées aux enfants, l'amélioration de la conception des études pédiatriques de PK et de sécurité et le développement d'une capacité internationale de réalisation d'essais parmi les enfants atteints de TB-MDR sont des priorités majeures.

RESUMEN

Tras decenios de negligencia, por fin se han publicado datos sobre la posología apropiada y segura de los principales fármacos antituberculosos de segunda línea en el tratamiento de la tuberculosis multirresistente (TB-MDR) de los niños, que incluyen el levofloxacino (LVX), el moxifloxacino (MFX), el linezolid (LZD) y el delamanid (DLM). Aún faltan datos muy necesarios sobre algunos fármacos nuevos y fármacos a los cuales se ha asignado una nueva utilización, como la bedaquilina (BDQ), el pretomanid (PTM) y la clofazimina (CFZ). En el presente artículo se analiza la situación de los estudios farmacocinéticos (PK) y de toxicidad de los medicamentos antituberculosos en los niños con TB-MDR, se definen las prioridades en las carencias de conocimientos y se mencionan los trabajos en curso que abordan estas lagunas en el contexto de la planificación de un ensayo clínico de eficacia en niños con TB-MDR. Existe una opinión internacional unánime sobre la necesidad y la factibilidad de un ensayo clínico de eficacia de un nuevo tratamiento de la TB-MDR pediátrica que sea corto y de administración oral exclusiva. Entre los fármacos antituberculosos de segunda línea nuevos y con nuevas indicaciones se cuentan los siguientes: BDQ, DLM, PTM, MFX, LVX, CFZ y LZD. Los numerosos datos PK y de toxicidad que surgen en la actualidad sobre la utilización de estos fármacos en la TB-MDR de los niños, provenientes de estudios en curso, estudios finalizados o ensayos planificados serán esenciales para fundamentar el ensayo clínico de eficacia antes mencionado. Otras prioridades importantes se relacionan con el estudio de los vacíos que persisten en los conocimientos, el desarrollo de presentaciones de los principales fármacos que sean adecuadas para los niños, el perfeccionamiento del diseño de los estudios PK y de toxicidad en pediatría y el refuerzo de la capacidad para realizar ensayos internacionales en los niños con diagnóstico de TB-MDR.

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Review

Pediatric multidrug-resistant tuberculosis clinical trials: challenges and opportunities

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SUMMARY

On June 17, 2016, RESIST-TB, IMPAACT, Vital Strategies, and New Ventures jointly hosted the Pediatric Multidrug Resistant Tuberculosis Clinical Trials Landscape Meeting in Arlington, Virginia, USA. The meeting provided updates on current multidrug-resistant tuberculosis (MDR-TB) trials targeting pediatric populations and adult trials that have included pediatric patients. A series of presentations were given that discussed site capacity needs, community engagement, and additional interventions necessary for clinical trials to improve the treatment of pediatric MDR-TB. This article presents a summary of topics discussed, including the following: current trials ongoing and planned; the global burden of MDR-TB in children; current regimens for MDR-TB treatment in children; pharmacokinetics of second-line anti-tuberculosis medications in children; design, sample size, and statistical considerations for MDR-TB trials in children; selection of study population, design, and treatment arms for a trial of novel pediatric MDR-TB regimens; practical aspects of pediatric MDR-TB treatment trials; and strategies for integrating children into adult tuberculosis trials. These discussions elucidated barriers to pediatric MDR-TB clinical trials and provided insight into necessary next steps for progress in this field. Investigators and funding agencies need to respond to these recommendations so that important studies can be implemented, leading to improved treatment for children with MDR-TB.

1. Introduction

The global epidemic of multidrug-resistant tuberculosis (MDR-TB), i.e., *Mycobacterium tuberculosis* that is resistant to isoniazid and rifampicin, is a major threat to human health.[1] In the past decade, there have been substantial improvements in our ability to diagnose and treat MDR-TB; however efforts have mainly focused on MDR-TB in adults. MDR-TB also has a substantial impact in children; currently, most MDR-TB (and drug-susceptible TB) treatment guidelines for children are extrapolated from adult data and rely on clinical experience instead of controlled trials. However, differences in the pathophysiology, diagnosis, and treatment of childhood TB relative to TB in adults are well described, and have limited the benefit children have received from recent advances in adult MDR-TB care.[2] There are relatively few trials that have focused specifically on childhood TB. In order to address this deficit and begin the process of developing a science-based framework on which to base recommendations, the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) and Research Excellence to Stop TB Resistance (RESIST-TB) networks organized a meeting to bring together investigators and clinicians working in this field. The aim was to summarize the current status of knowledge, identify important areas of research, and develop plans for future research for pediatric MDR-TB. This report summarizes the results of this meeting – the Pediatric MDR-TB Landscape Meeting, held June 17, 2016, in Washington DC.

2. Update on current pediatric MDR-TB studies in progress

Prior to 2008, no clinical trials of MDR-TB treatment had ever been performed in adults or children, and treatment was based entirely on clinical opinion.[3] Since then, phase 2 clinical trials have demonstrated the efficacy of three new anti-TB drugs –bedaquiline, delamanid, and pretomanid – for the treatment of MDR-TB and extensively drug-resistant (XDR-TB).[4] Moreover, linezolid,

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Table 1
PK/safety studies in children with MDR-TB disease

Study	Intervention	Design	Target population	Aim	Sample size
MDR-PK 1	PK and safety of levofloxacin, moxifloxacin, ofloxacin, amikacin, high-dose isoniazid, ethionamide, para-aminosalicylic acid, terizidone, and cycloserine at routine doses	Observational cohort	Children 0–2 years, 3–5 years, 6–15 years old; all enrolled simultaneously	PK, drug–drug interactions, and safety in children treated for MDR-TB with/without HIV co-infection	318
MDR-PK 2	Moxifloxacin, levofloxacin, and linezolid	Observational cohort	Children 0–<2 years, 2– <6 years, 6–<12 years, 12–<18 years old	PK, drug–drug interactions, and safety in children treated for MDR-TB with/without HIV co-infection	100
Jansen C211	Bedaquiline in combination with other second-line agents	Open-label, single-arm	HIV-uninfected children ages 0–1 year, 2–4 years, 5–11 years, 12–17 years old	PK, safety, and anti-mycobacterial activity of bedaquiline in combination with other second-line drugs	60
IMPAACT P1108	Bedaquiline in combination with other second-line agents	Open-label, single-arm	HIV-infected and uninfected children with MDR-TB under an FDA IND; children 6–17 years currently enrolled; children 3–5 years and 0–2 years to follow in parallel	Dose-finding and safety study of bedaquiline in combination with other second-line drugs	Not yet enrolling
Otsuka 232 and 233	Delamanid	Open-label, multiple-dose	HIV-uninfected children ages 0–2 years, 3–5 years, 6–11 years, 12–17 years	PK, safety, and tolerability of delamanid	36
IMPAACT 2005	All-oral, injectable-sparing, delamanid-based MDR-TB regimen	Open-label, single-arm clinical trial	Children 0–<3 years, 3–<6 years, 6–<12 years, 12–<18 years old	PK, safety, and tolerability of intervention regimen; assess effect of HIV and ART on delamanid PK	Not yet enrolling

PK, pharmacokinetic; MDR-TB, multidrug-resistant tuberculosis; FDA IND, Food and Drug Administration Investigational New Drug; ART, antiretroviral therapy.

clofazimine, and meropenem (existing drugs not previously used for MDR-TB) have been recognized to have activity against *M. tuberculosis* and therefore to be potential companion agents in new regimens for MDR-TB treatment.[4] This has led to a long-overdue increase in MDR-TB treatment trials. By 2016, four phase 2 and one phase 3 MDR-TB treatment trials had been completed,[5–9] and an additional eight phase 2 and eight phase 3 trials were under way.[10] While this represents a welcome increase in activity that will hopefully expand treatment options for MDR-TB, only two of these trials are enrolling children under the age of 12 years, while one is enrolling adolescents aged 13–17 years. Thus, there is a substantial unmet need for data that will guide the treatment of children with MDR-TB. This meeting reviewed clinical trials and observational cohort studies of pediatric MDR-TB to identify knowledge gaps and generate momentum for new studies to address those gaps.

Planned and ongoing pediatric MDR-TB studies can be divided into two groups. The first is treatment studies in which the pharmacokinetics (PK) and safety in children with MDR-TB are characterized. The goal of these studies is to define the optimal doses for children with TB, taking into account efficacy–toxicity tradeoffs (shown in Table 1). The second is studies of preventive therapies, in which pediatric household contacts of MDR-TB patients are treated to prevent disease (shown in Table 2).

Missing from these tables are studies specifically evaluating the efficacy of novel regimens for the treatment of pediatric MDR-TB; none are ongoing. It is hoped that a better understanding of the PK and safety of new and existing drugs will lead to the rational design of trials to evaluate optimized regimens specifically tailored to pediatric patients.

3. Global burden of MDR-TB in children

TB remains substantially under-diagnosed among children due to challenges with microbiological confirmation,[11] a dearth of good diagnostics, and limitations in the recording and reporting of pediatric TB.[12] These challenges are further exacerbated in children with MDR-TB. Until 2012, the World Health Organization (WHO) did not provide estimates of the burden of pediatric TB. Two recent studies have provided evidence that the proportion of children with MDR-TB reflects the proportion of new (i.e., never previously treated for TB) adult TB cases with MDR-TB in the same setting.[13,14] The first estimate of pediatric MDR-TB incidence, published in 2014 by Jenkins et al., was 32 000 annual incident cases (3.2% of their overall TB incidence estimate).[14] In 2016, Dodd et al. published an extension of their mathematical model to estimate the number of children with several different forms of

Table 2
Trials of MDR-TB preventive therapy in children

Study	Intervention	Design	Target population	Aim	Sample size
V-QUIN	Levofloxacin, 6 months	Randomized, double-blind, placebo-controlled phase 3 trial	Adult, child, and adolescent household contacts of pulmonary MDR-TB patients	Evaluate intervention compared to placebo for prevention of MDR-TB in household contacts	Not yet enrolling children
TB-CHAMP	Levofloxacin, 6 months	Randomized, double-blind, placebo-controlled phase 3 trial	Children <5 years old who are household contacts of pulmonary MDR-TB patients	Evaluate efficacy and safety of levofloxacin compared to placebo for prevention of MDR-TB	Not yet enrolling
A5300/P2003	Delamanid	Open-label, phase 3 trial	Adult and child (0–17 years) household contacts of MDR-TB patients	Evaluate efficacy and safety of delamanid compared to standard-dose isoniazid for TB prevention	Not yet enrolling

MDR-TB, multidrug-resistant tuberculosis.

drug-resistant TB.[15] They estimated that 24 800 children developed MDR-TB annually (i.e., 2.9% of incident TB cases).

The proportion of children with MDR-TB who are diagnosed, and the proportion of those children who receive appropriate treatment, is also unknown. However, it is likely a very small proportion of the 25 000–32 000 children who develop MDR-TB annually. Although children who are diagnosed and receive treatment for MDR-TB are likely to recover and have good treatment outcomes,[16] those who remain undiagnosed have a high risk of death. A recent literature review from the pre-treatment era demonstrated high mortality in children who did not receive treatment for TB. Given the high number of children with MDR-TB who are untreated, mortality is likely to be significant.[17]

4. Regimens for MDR-TB treatment in children: preclinical–clinical translation?

To assess whether or not preclinical models can help inform clinical assessments of anti-TB drugs for children, the characteristics of TB disease in children must first be understood. Pediatric and adult TB are very different. The clinical manifestations of pediatric TB are highly variable and roughly correlate with age; very young children more commonly develop disseminated disease than older children and adults, and children aged 2–12 years commonly have paucibacillary, non-cavitary disease limited to the lung or lymph nodes, without caseous necrosis (see Figure 1). Children over the age of 12 years can present with adult-like pulmonary disease, often with lung cavitation and high bacterial burden.[2] Since younger children tend to have paucibacillary TB (approximately 30% culture-confirmed and <10% sputum smear-positive) they can reasonably be expected to respond to treatment better than adults. Improved treatment outcomes amongst children with MDR-TB compared to adults are already achieved despite substantially lower drug exposures in children for many key second-line drugs. However, this variability in disease severity, pathology, and mycobacterial burden (10^4 in paucibacillary versus 10^7 to 10^9 in cavitary disease)[18] presents a challenge for the selection of a single regimen and treatment duration to test for 'pediatric MDR-TB'.

A critical concern for successful TB treatment is delivery of effective drugs at adequate concentrations to the site of disease. Penetration of TB drugs into macrophages, the central nervous system, lymph nodes, lung parenchyma, and cavitary contents may be needed for the treatment of pediatric MDR-TB, depending on the age of the child and his or her associated TB-related pathology. Penetration coefficients of drugs into these different compartments vary widely.[19] Studies assessing the spatial distribution of anti-TB drugs in relevant preclinical models may help inform the selection of drugs and/or drug combinations for further testing in specific populations (e.g., children with disseminated intracellular disease, or lymphadenitis or meningitis). Drugs also differ in their ability to protect each other against the emergence of resistance. In patients with a high bacillary load, chromosomally mediated resistance is invariably present in a subpopulation of organisms, so drugs must be given in combination to prevent the emergence of these pre-existing resistant strains. So for adolescents with cavitary disease, it is likely that drugs must both penetrate into cavitary contents and achieve concentrations sufficient to protect companion drugs against the emergence of resistance in that compartment. For children with paucibacillary disease, the number of drugs needed to prevent the emergence of resistance is unknown, but may be fewer than in adults.

Pulmonary disease

Age (yrs)	Ghon/LN	Bronchial	Effusion	"Adult-type"
<1	X	X		
1-2	X	X		
2-5	X	X		
5-10	X	X	X	X
>10			X	X

* Disease in children aged 5-10 years of age is generally rare.

Figure 1. Manifestations of pediatric tuberculosis, by age, adapted from Marais et al.[2] Disease in children aged 5–10 years of age is generally rare.

There is no single best animal model for pediatric TB disease. In the 'standard' mouse TB treatment model in BALB/c mice, the disease is largely intracellular, and the mice do not typically develop caseous necrosis or cavities, and thus their pathology is similar to that seen in young children.[20] Animal models that develop necrotic lesions and/or cavitary disease (e.g., so-called Kramnik (C3HeB/FeJ) mice or select rabbit models) may be more akin to, and informative of, adolescent TB disease. Thus, no single animal model has been validated as a pediatric TB treatment model. Indeed, given the wide spectrum of disease burden and manifestations, a one-size-fits-all approach to regimen composition, dosing, and treatment duration for pediatric MDR-TB in both practice and clinical trials may result in under- or over-treatment of many children.

5. Pharmacokinetics of second-line anti-TB medications in children

The approach to studying individual anti-TB medications in children has been to perform PK and safety studies, to establish doses in children that achieve exposures similar to those in adults receiving standard doses, and evaluate safety at those doses. Extrapolation of mg/kg doses directly from adults to children is often inappropriate because of age-related changes in drug disposition and metabolism, also known as 'developmental pharmacology'. Specific studies are therefore needed in children across the age spectrum (with a particular focus on very young children in whom drug handling is rapidly changing), and many important knowledge gaps remain.[21] Emerging evidence on fluoroquinolone PK in children with MDR-TB has shown much lower exposures in children relative to adults with currently recommended doses.[22–24] Age-specific PK data for ethionamide, terizidone, and para-aminosalicylic acid (PAS) are expected soon (MDR-PK1 study).

Research priorities should be centered on those medications expected to be components of novel MDR-TB regimens; this includes levofloxacin, moxifloxacin, linezolid, clofazimine, and the novel medications bedaquiline and delamanid. Work on optimizing pediatric doses of levofloxacin, moxifloxacin, and linezolid is ongoing (MDR-PK2). Data on the PK and safety of delamanid in children aged 6–17 years have been disseminated, with work ongoing in younger children, including with a pediatric formulation. Pediatric bedaquiline studies are just starting. Clofazimine PK is poorly understood in adults, and no data for children are available, representing an important gap. Of note, PK parameters and values associated with optimal efficacy for second-line drugs are poorly defined for adults, so PK targets for children are not well established. In general, dose-finding studies aim to identify doses that give equivalent exposures in adults and children. However, despite 'low' drug exposures of key medications like the fluoroquinolones, outcomes in children with MDR-TB are good relative to adults.[16] This suggests that children may need less intense treatment and provides justification for an efficacy trial of a shortened regimen in children with MDR-TB. Few child-friendly formulations of second-line anti-TB medications exist; however

they are urgently needed to allow accurate and acceptable dosing to children in the field.

6. Design, sample size, and statistical considerations for MDR-TB trials in children

As with other aspects of TB trials, there are similarities and differences between studies of children and adults. Phase 3 studies of TB regimens are typically designed as superiority or non-inferiority trials. Although a number of design innovations have been proposed to increase information gained and/or efficiency, specifically multi-arm multi-stage (MAMS) designs[25,26] and adaptive randomization,[27] these designs are dependent on an easily identifiable intermediate outcome measure such as 2-month sputum culture conversion. Since this endpoint cannot be measured in many children, the usefulness of such innovations in trial design for studies in children may be limited.

A design issue that is of greater relevance in children is that of stratification by factors that are likely to influence treatment outcomes. Since age, extent or type of disease, and severity of disease are variable in children, these factors should be controlled for by stratification. If regimen effectiveness is expected to vary by these factors, it may be necessary to perform separate sample size calculations for each stratum. In some situations a factorial design may be employed to achieve greater efficiency, but this depends on effects being similar across strata.

An issue that is more prominent in pediatric trials is the presence of imperfect final stage outcomes. By this we are referring to the lack of clarity about whether a patient's TB has been cured. If the diagnosis was clinical (i.e., not confirmed microbiologically), or if a microbiologically confirmed diagnosis required invasive procedures to establish, it may not be possible to confirm that the disease has been eradicated; a long post-treatment observation period without relapse may increase certainty, but at the cost of a prolonged study timeframe and consequent delay in determining the success of the investigational treatment. A final, more practical issue faced in TB trials is the inability to blind the study or provide placebo control for some study agents. For example, replacing an injectable agent with an equally effective oral drug is highly desirable; however, an injectable placebo raises ethical issues, and it would likely be unacceptable to patients and families.

7. Selection of study population, design, and treatment arms for a trial of a novel pediatric MDR-TB regimen

7.1. Which children to include?

Consideration could be given to treating all children less than 18 years of age (the near universal age of majority), which includes adolescents, who are frequently neglected and for whom safety is rarely established. Alternatively, one might include all children less than 15 years of age, to align with the age brackets used by the WHO for reporting TB statistics. Finally, a younger age cut-off could be considered to try to capture those children whose pathophysiology (and drug disposition and metabolism) is most different from adults. Including all children, irrespective of extent of disease, is more inclusive and representative. However, specific issues exist around the treatment of children with more limited, paucibacillary disease, where shorter, less intensive regimens may be possible and for whom there are clear differences in response to treatment compared to adults. A useful classification system has been proposed by Wiseman et al. which provides guidance on how to classify children as having severe vs. non-severe disease.[28] It may be appropriate to include only children with a confirmed diagnosis (i.e., microbiological confirmation of the presence of *M. tuberculosis* shown to be resistant by genotypic or phenotypic testing), as this gives an unambiguous entry point and allows changes in microbiological status to provide microbiological endpoints. However, this excludes the majority of children with MDR-TB for whom the diagnosis is made clinically. A trial that included only microbiologically confirmed cases (in whom disease severity or bacterial burden is often higher) would not be representative of all children with MDR-TB. Regarding the drug resistance profile, it may be appropriate to only include children with MDR-TB with preserved susceptibility to the fluoroquinolones and injectables, as this is a more homogeneous population, and regimens (both control and intervention) could be standardized.

7.2. Trial design

It may be appropriate to use the same control and intervention regimens for all children in the trial, as this will provide simplicity, improved power to determine endpoints, and transferability into practice. However, it would likely mean that many children will be over-treated (children with limited disease and less extensive resistance) and some may be undertreated (children with extensive disease and more extensive resistance). Alternatively, it may be possible to divide children in the trial into different categories (based on resistance profile, extent of disease, or whether the diagnosis is microbiologically confirmed or not) and provide different intervention and control arms to each.

7.3. Composition of regimens

For the control arm, a number of options are available. First, a standard-duration, traditional WHO-recommended regimen could be selected, where all children in the trial receive the same drugs for the same duration. Standard treatment includes up to 6 months of an injectable and a total duration of 18 months of therapy. A second option is for all children to have an individualized control regimen whose component drugs and treatment duration is designed based on each patient's disease severity, drug resistance profile, and response to treatment. Third, a number of distinct, pre-defined control regimens could be used based on resistance profile or severity. Finally, the new WHO-endorsed shortened regimen could be used. This has the advantage of being a 9–12-month regimen, which may be more desirable for patients and also for standardization of study endpoints. However, there is limited experience using this regimen in children, and it is currently only recommended for patients who have TB caused by isolates that are known to be susceptible to fluoroquinolones and injectable agents, or for whom resistance to these drug classes is unlikely.

When designing the intervention regimen it is important to construct a combination regimen that includes drugs that, together, achieve the following goals: (1) good early bactericidal activity, (2) potent sterilizing activity, (3) robustness to resistance, and (4) adequate penetration into relevant sites of disease. Regimens with limited drug–drug interactions, both with companion TB drugs and also with antiretroviral drugs, are also highly desirable. Finally, it is important to consider how easy the regimen would be to use programmatically, in terms of procurement, formulations, requirement for laboratory or safety testing, shelf life, etc. A fluoroquinolone (likely levofloxacin, because it has a limited effect on the QT interval) plus a novel drug (delamanid or bedaquiline), together with linezolid and clofazimine provides a potential core set of drugs in such a regimen. The fluoroquinolone provides potent bactericidal activity and reduces bacterial burden quickly, the novel drugs have good sterilizing activity, linezolid has a high barrier to resistance and protects companion drugs, while clofazimine has good sterilizing activity.[29–31] The addition of other drugs, such as ethionamide, cycloserine, pyrazinamide, and/or high-dose isoniazid can be considered following careful assessment of the potential

benefits versus safety risks. The duration of therapy in the intervention arm would need to be considered. With multiple active drugs, some with good sterilizing efficacy, a shorter duration of therapy is a realistic possibility. Also, given that children frequently have paucibacillary disease, a shortened treatment of as little as 6 months may be more likely to be successful in children than adults.

8. Practical aspects of a pediatric MDR-TB treatment trial

For pediatric MDR-TB research, disease severity must be carefully collected and documented, as disease severity will assuredly influence treatment outcomes. End-points for such trials should include sub-analyses of patients with culture-confirmed disease looking at bacteriological cure, even if the main study outcome is favorable versus unfavorable outcomes. Other measures of treatment response may include weight gain, clinical improvement (symptoms/physical signs), radiological improvement, and changes in potential biomarkers. Given that the adverse effects (AEs) associated with individual drugs are fairly well-described and standard treatment commonly causes significant toxicity, it is especially important to carefully measure and report safety outcomes for new versus control regimens in all pediatric MDR-TB trials. Lastly, every effort should be made to confirm the presence of MDR-TB in enrolled patients (to avoid misdiagnosis or misclassification), by employing multiple diagnostic methods, including culture and phenotypic drug susceptibility testing, as well as molecular methods such Xpert and line probe assay .

9. Integrating children into adult TB trials

Despite substantial urging by pediatricians, clinical trialists, and regulatory authorities, subjects under the age of 18 years are rarely included in phase 3 clinical trials of TB. A recently completed trial of treatment of TB infection, the PREVENT TB Trial, was successful in enrolling adults and children as young as 2 years of age and provides an instructive example of both the challenges and some potential solutions to this problem.[32] PREVENT TB was a randomized, open-label, non-inferiority trial of once-weekly, directly-observed rifapentine + isoniazid for 3 months (3HP) compared to daily self-administered isoniazid for 9 months (9H) for the treatment of latent TB infection (LTBI) in high-risk tuberculin skin test (TST) reactors. The target population comprised TST-positive close contacts of a culture-confirmed TB case, TST-converters, HIV-infected persons with a positive TST or close contacts to a TB case regardless of TST, and TST-positive persons with fibrosis on chest radiography consistent with prior untreated TB. The primary aim of the study was to evaluate the effectiveness of weekly 3HP versus daily 9H in preventing progression to TB disease.

The study started enrolling adults and children aged 12–17 years in 2001, as there were no PK data available to guide dosing in younger children. Doses were subsequently established for younger children in PK/safety studies, and in 2005 the protocol was amended to include children aged 2–11 years. Final accrual of children was achieved by 2010, and collaboration with a pediatric clinical trials network (IMPAACT) facilitated enrolment of a large number of children. The study found 3HP to be as well-tolerated and as effective as 9H for preventing TB in children; 3HP had significantly higher treatment completion rates and was less hepatotoxic. Revision of the Centers for Disease Control and Prevention (CDC) LTBI guidelines to allow 3HP for children ages 2–11 years is now under consideration. Ideally, children should be included from the outset. However, if this is not feasible, it may be possible to start the trial in adults but with a clear plan to gather PK and safety data while the trial starts, and then subsequently include children when PK data are available. It would also be possible to do age de-escalation, where adults are initially included, with older children then younger children included later. There is little reason to exclude persons >12 years old from any adult trial.

10. Conclusions

The topics identified in this report identify the critical issues in pediatric MDR-TB that need to be addressed and provide a blueprint for moving forward. Investigators and funding agencies need to respond to this agenda so that important studies can be implemented, leading to improved treatment for children with MDR-TB.

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New and Repurposed Drugs for Pediatric Multidrug-Resistant Tuberculosis

Practice-based Recommendations

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Abstract

It is estimated that 33,000 children develop multidrug-resistant tuberculosis (MDR-TB) each year. In spite of these numbers, children and adolescents have limited access to the new and repurposed MDR-TB drugs. There is also little clinical guidance for the use of these drugs and for the shorter MDR-TB regimen in the pediatric population. This is despite the fact that these drugs and regimens are associated with improved interim outcomes and acceptable safety profiles in adults. This review fills a gap in the pediatric MDR-TB literature by providing practice-based recommendations for the use of the new (delamanid and bedaquiline) and repurposed (linezolid and clofazimine) MDR-TB drugs and the new shorter MDR-TB regimen in children and adolescents.

Keywords: multidrug-resistant tuberculosis; *Mycobacterium tuberculosis*; child; adolescent; pediatric

Each year, approximately 33,000 children develop multidrug-resistant tuberculosis (MDR-TB, *Mycobacterium tuberculosis* with *in vitro* resistance to at least isoniazid and rifampin), but diagnosis and treatment remain problematic (1, 2). Although children have better treatment outcomes than adults with MDR-TB, global treatment success rates still remain unacceptably low and children suffer from the serious side effects of the older second-line MDR-TB drugs (3, 4). Unfortunately, children have limited access to the new drugs bedaquiline and delamanid and to the repurposed drugs clofazimine and linezolid that have shown improved interim outcomes and acceptable safety profiles in adults (5, 6). Children also have limited access to the

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shorter MDR-TB treatment regimen[1] that has shown promise in adult and adolescent patients (7, 8) and has been recommended by the World Health Organization (WHO) for selected individuals with MDR-TB (9).

This differential standard of care is due, in part, to the exclusion of children from most TB clinical trials (10). There is some consensus that adolescents should be included in adult TB efficacy trials, but it may not be necessary to repeat efficacy trials in younger children. It is important, however, that children of all ages be included in both pharmacokinetic and safety studies of TB drugs and new regimens, to ensure there is appropriate pediatric dosing and safety information (11). Such inclusion should be done quickly to prevent unnecessary delays in pediatric access to therapeutic advances.

While awaiting data from studies, front-line providers must make decisions about how to treat children with MDR-TB, including how to use the new and repurposed drugs. Despite positive experiences with these interventions in adults, there are no recommendations regarding their clinical use in children (12). This review fills a gap in the pediatric MDR-TB literature by providing practice-based recommendations for the use of new and repurposed drugs and novel regimens.

Some of the results of these studies have been previously reported in the form of abstracts (13–17).

Methods

Articles identified from a comprehensive literature review were consulted using a methodology previously described (18). Briefly, articles from a systematic review were complemented via a comprehensive literature search using PubMed and a review of recent WHO guidelines. A writing committee (E.P.H. and J.F.) wrote the initial draft and then incorporated comments from the remaining coauthors. In accordance with recommendations from an expert panel on inclusion of children in TB clinical studies, assessments of efficacy in adults were applied to children, although there may be limitations to this approach (11). However, data on safety and dosing in children were reviewed and considered separately from these data in adults. This was done as it is accepted that adult efficacy data can be applied to children; however, safety and dosing data need to be pediatric specific. Because there are limited published data on the use of new drugs and regimens in children, expert consensus was reached where evidence was lacking. The consensus was generated by clinical health professionals working within the Sentinel Project on Pediatric Drug-Resistant Tuberculosis (19). The process differed from that used by some normative bodies (e.g., the WHO) as it considered practice-based experience, and its target audience is clinical providers. The authors of this review have more than two decades of experience in caring for children with MDR-TB in the international context, including the use of new and repurposed drugs.

Treatment Recommendations

Summary recommendations are provided in Table 1. Clinical studies of new and repurposed drugs are summarized in Table 2. Information on formulations and procurement is summarized in Table 3. Because of length constraints, recommendations for the short MDR-TB regimen are available as an online supplement. For every medication and regimen discussed, it is recommended that informed consent be obtained, active pharmacovigilance be performed, and monitoring of patients be performed according to WHO recommendations for adult patients (20). Children should also receive adherence counseling, social support, and nutritional supplementation as recommended for the treatment of children with MDR-TB (21, 22).

Delamanid

Efficacy and safety in adults. Delamanid (Otsuka Pharmaceutical, Tokyo, Japan) is a nitroimidazole shown to be effective when added to an MDR-TB regimen in a randomized, placebo-controlled phase IIb trial (23). In this phase IIb trial, patients were randomized to receive an optimized background regimen (OBR) with either placebo or delamanid added for 8 weeks. After the initial 8 weeks, there was a 4-week period of continued OBR treatment, and then all participants were offered open-label delamanid for an additional 24 weeks. When participants who received at least 6 months of delamanid were compared with participants who received less than 2 months of delamanid, there were statistically significant differences seen in the time to culture conversion, the rates of culture conversion at both 8 and 24 weeks, and treatment success, with those who had at least 2 months of therapy doing better (24). The drug was well tolerated with mild to moderate QTc prolongation (12.1 ms) observed but no clinical cardiac complications (25). This study led to conditional marketing approval for delamanid in the European Union and Japan in 2013 and later in South Korea (26). A phase III randomized, placebo-controlled trial of delamanid has completed enrollment and the period of study drug administration; treatment outcome results are expected in 2017–2018 (27).

Delamanid was recommended by the WHO in 2014 for treatment of adults with MDR-TB. WHO guidelines state that delamanid may be given to adults with MDR-TB in whom a four-drug regimen plus pyrazinamide cannot be constructed due to resistance or significant intolerance to other medications and that delamanid be given to adults at "high risk" of poor treatment outcomes. These guidelines stress that delamanid should be used as part of programmatic management of MDR-TB under the following conditions: (*1*) proper patient inclusion; (*2*) close clinical and programmatic monitoring; (*3*) used as part of a regimen that follows WHO guidelines; (*4*) due process followed for informed consent; and (*5*) active monitoring and management of adverse events. The WHO guidelines offer no recommendation on the use of delamanid in persons less than 18 years old, noting that there was insufficient evidence to make such a recommendation at the time of review in 2014 (28).

Dosing and safety in children. Otsuka has led a pediatric development program for delamanid, with enrollment in phase I and II pediatric trials beginning in 2013. Since the WHO interim guidelines were published in 2014, two studies of pharmacokinetics and safety in HIV-uninfected children as young as 6 years old have been presented at international meetings (13, 14). These studies follow an age de-escalation protocol in which the drug is first given to older

[1] "Shorter regimen" refers to the 9- to 12-month regimens that have been assessed under operational research conditions and are often referred to as the "Bangladesh" or "9-month" regimen.

Table 1. Summary Recommendations for New and Repurposed Drugs and Regimens for Multidrug-Resistant Tuberculosis in Children and Adolescents

Delamanid

Recommended dose

>35 kg: 100 mg twice daily
20–34 kg: 50 mg twice daily
<20 kg: consult with expert
Duration: 24 wk; longer duration could be considered on a case-by-case basis (no alternative drug option)

Indications for use: *children ⩾6 yr old and ⩾20 kg*

- Confirmed MDR-TB when a four-drug regimen plus pyrazinamide cannot be constructed owing to resistance or significant intolerance
- Probable MDR-TB with a source case with known or suspected additional resistance to second-line agents
- Confirmed or probable MDR-TB with a high risk of treatment failure

Indications for use: *children <6 yr old and <20 kg*

It is recommended that consultation with expert clinicians be sought before administering delamanid to children in this age range via consultation with the European Respiratory Society–hosted TB Consilium (https://www.tbconsilium.org) or the Sentinel Project on Pediatric Drug-Resistant Tuberculosis (tbsentinelproject@gmail.com)

Contraindications

- Baseline QTc interval greater than 500 ms that does not correct with medical management
- Allergy to delamanid or metronidazole
- Prior treatment with nitroimidazole agents (i.e., pretomanid/PA-824)

Monitoring

Baseline: ECG to assess QTc interval and albumin in addition to standard MDR-TB assessments
Follow-up: Monthly ECG to assess for QTc prolongation (although less frequent monitoring could be considered after 8 wk in children with a normal baseline and follow-up QTc intervals if access to electrocardiographic monitoring is a challenge) in addition to standard MDR-TB assessments

Bedaquiline

Recommended dose

Adolescents ⩾12 yr old who weigh 33 kg or more: 400 mg daily for 14 d followed by 200 mg given three times weekly for an additional 22 wk
Duration: 24 wk; longer duration could be considered on a case-by-case basis

Indications for use: *children ⩾12 yr old and who weigh >33 kg*

- Confirmed MDR-TB in whom a four-drug regimen plus pyrazinamide cannot be constructed because of resistance or significant intolerance and where delamanid is not available
- Probable MDR-TB with a source case with known or suspected additional resistance to second-line agents and where delamanid is not available

Indications for use: *children <12 yr old or who weigh <33 kg*

It is recommended that consultation with expert clinicians be sought before administering bedaquiline to children in this age range via consultation with the ERS-hosted TB Consilium (https://www.tbconsilium.org) or the Sentinel Project on Pediatric Drug-Resistant Tuberculosis (tbsentinelproject@gmail.com)

Contraindications

- Baseline QTc interval greater than 450 ms that does not correct with medical management
- Patient or family history of cardiac arrhythmias
- Severe cardiac disease
- Allergy to bedaquiline

Monitoring

Baseline: ECG to assess QTc interval in addition to standard MDR-TB assessments
Follow-up: Monthly ECG to assess for QTc prolongation in addition to standard MDR-TB assessments

Linezolid

Recommended dosing

Children ⩾12 yr: 10 mg/kg once daily
Children <12 yr of age: 10 mg/kg twice daily
Duration: entire course of treatment as long as the child tolerates it

Indications for use

- Confirmed or probable MDR-TB as part of the core second-line regimen
- If adverse events cannot be monitored, linezolid is best used in patients with additional resistance or intolerance to other second-line medications

Contraindications and monitoring

- Avoid in children with significant anemia, leukopenia, or thrombocytopenia
- Avoid in children with significant peripheral neuropathy
- Monthly screening for peripheral neuropathy and monthly complete blood counts should be assessed while the child is receiving linezolid

Clofazimine

Recommended dosing

2–3 mg/kg given daily for a maximum daily dose of 100 mg or every other day in smaller children (gelcaps cannot be split)
Duration: entire course of treatment as long as the child tolerates it

(Continued)

Table 1. (*Continued*)

Indications for use
• Confirmed or probable MDR-TB as part of the core second-line regimen
Contraindications and monitoring
• Avoid in children with a baseline QTc interval greater than 500 ms that does not correct with medical management
• Baseline and monthly ECGs to assess QTc interval
Shorter MDR-TB regimen ("9- to 12-mo regimen")
Recommended doses
As per published guidelines. Of note, there are no pediatric safety data yet on the doses of moxifloxacin (12 mg/kg) used in this study
Duration: 9–12 mo, depending on culture conversion and/or clinical response
Indications for use
• Probable or confirmed MDR-TB in which resistance to second-line drugs is unlikely
• Probable or confirmed MDR-TB with no previous second-line drug treatment in the child or source case
Contraindications and monitoring
• Avoid in children with known resistance to any component of the shortened regimen except isoniazid
• Avoid in children whose source cases have known resistance to any component of the shortened regimen except isoniazid
• Avoid in children with an allergy to any of the medications in the shortened regimen
• Baseline and monthly ECG to assess QTc interval

Definition of abbreviations: ERS = European Respiratory Society; MDR-TB = multidrug-resistant tuberculosis; TB = tuberculosis.

children and, when shown to be safe with established dosing, it is given to younger children. Although age bands were used to design the study, the ultimate dosing recommendations are based on weight, and weight should guide the dose selection for children. For children at least 13 years of age and weighing at least 35 kg, a dose of 100 mg twice daily was safe and achieved adequate serum concentrations. For children 6–12 years of age and weighing 20–34 kg, a dose of 50 mg of delamanid twice daily achieved adequate serum concentrations. A safety and pharmacokinetics study of delamanid in children 3–5 years old is currently enrolling patients; when dosing and safety are established, then children and infants less than 3 years of age will be evaluated. A pediatric dispersible formulation of delamanid is being assessed as part of these trials. In addition, studies in children who are HIV coinfected are planned. Delamanid is available for pediatric patients at least 6 years of age and with a body weight greater than 20 kg via compassionate use from Otsuka. Of note, there are more pediatric data on the safety and dosing of delamanid than for some other second-line drugs that are frequently used to treat children for MDR-TB, such as cycloserine and clofazimine (29).

Recommendations for delamanid use in children. Delamanid may be included in the treatment regimens of children at least 6 years old and who weigh at least 20 kg for the same indications as for adults: those with MDR-TB in whom a four-drug regimen plus pyrazinamide cannot be constructed due to resistance or significant intolerance *or* those with a high risk of treatment failure. Because MDR-TB may be difficult to bacteriologically confirm in children, delamanid may be used in children if they have an MDR-TB source case with known or suspected resistance to second-line agents. Children at high risk for treatment failure include those with immunocompromising conditions (e.g., HIV, diabetes, malnutrition) or those with extensive disease (defined as extrapulmonary TB other than isolated lymphadenitis; or pulmonary TB with bilateral infiltrates and/or cavities) (4). Children in this age and weight group receiving standard MDR-TB therapy and who develop significant toxicity in response to any of their medications should have the causative agent discontinued and delamanid started as a substitute. Avoid adding a single drug to a failing regimen.

Delamanid can be considered on a case-by-case basis in children less than 6 years old and who weigh less than 20 kg, if the children meet the criteria previously described and no suitable alternatives are available. Although studies of delamanid in this age group are ongoing, there may be cases in which the benefits of including delamanid outweigh the risk (30). As with other second-line drugs, the delamanid dose for this age and weight range would have to be extrapolated from the dosages used in older children and adults (31). Until the dispersible tablet is available, administration of delamanid to younger children might involve crushing and mixing the drug, which could affect its stability and bioavailability, although this is true for most second-line drugs. It is recommended that consultation with expert clinicians be sought before administering delamanid to children in this age range. Such consultation is available through the European Respiratory Society–hosted TB Consilium (https://www.tbconsilium.org) (32) or through the Sentinel Project on Pediatric Drug-Resistant Tuberculosis (tbsentinelproject@gmail.com).

As with adults, programs treating children with delamanid should meet the five conditions recommended by the WHO for its use in adults (28). All children should undergo the standard baseline testing for MDR-TB treatment. They should also undergo a baseline ECG to assess the QTc interval[2] and a baseline serum albumin level. Children with a baseline QTc interval greater than 500 milliseconds should not be started on delamanid until the interval is corrected by medical management (33). Delamanid is metabolized by albumin and there may be higher rates of adverse events in children with hypoalbuminemia; therefore children with serum albumin levels less than 2.8 g/dl should receive protein supplementation during delamanid

[2]The QTc interval is a measure of cardiac myocyte readiness for depolarization. A prolonged QTc interval (defined as greater than 500 ms) may be a risk factor for the development of fatal cardiac arrhythmias. The most commonly used correction for the QT interval in children is by the Fridericia correction (QTcF).

therapy. Children with an allergy to delamanid or metronidazole or who have been treated with other nitroimidazole drugs (i.e., pretomanid/PA-824) should not be treated with delamanid. While receiving delamanid, children should undergo a monthly ECG to assess for QTc prolongation, although less frequent monitoring after 8 weeks could be considered in children with a normal baseline and follow-up QTc intervals if access to electrocardiographic monitoring is a challenge. Combination with drugs that also prolong the QTc interval should be done with caution, and substitution of levofloxacin (which has less effect on the QTc interval) for moxifloxacin is advised. Official recommendations are for delamanid to be given for 24 weeks total, although longer courses of therapy have been given to adults and children with limited therapeutic options. In the initial phase IIb trials in adults, a substantial number of individuals (192 of 421) received 8 months of delamanid without any additional safety signals (23). Phase I studies in adults show that delamanid did not have any significant drug–drug interactions with antiretroviral therapy, including tenofovir, efavirenz, and lopinavir–ritonavir (15).

Replacement of the injectable agent with delamanid? Children tend to tolerate second-line medications better than adults do; however, the second-line injectable medications are problematic (34). Although there are currently no data to support the routine substitution of delamanid for a second-line injectable agent within the MDR-TB regimen, providers could consider using delamanid instead of the injectable drug in the initial regimen in children with MDR-TB. This substitution could substantially benefit children given the risk of permanent sensorineural hearing loss in children (reported in up to 25% of children) due to the second-line injectable agents and the pain and hospitalization requirements that are associated with daily intramuscular injections (34). These drugs have been assessed only when given in combination with other agents; it is therefore challenging to tease out the individual contribution of specific drugs in an MDR-TB regimen (35, 36). However, there is higher quality evidence for the inclusion of delamanid than there is for the inclusion of the injectable given that delamanid has been assessed in randomized placebo-controlled clinical trials for MDR-TB whereas the injectable agents have not. There also exists substantial clinical experience in treating children with nonsevere MDR-TB disease with an injectable-sparing regimen, with good results (3).

Bedaquiline

Efficacy and safety in adults. Bedaquiline (Janssen Pharmaceuticals, Beerse, Belgium) is a diarylquinoline that was effective when added to an MDR-TB regimen in a randomized, placebo-controlled phase IIb trial (37). In this phase IIb trial, patients were randomized to receive an OBR with either placebo or bedaquiline for 24 weeks. When compared with placebo, bedaquiline was associated with significantly reduced time to culture conversion, increased rates of culture conversion at 24 weeks, and increased cure rates (38). The drug was associated with moderate QTc prolongation (15.7 ms), and although there were higher rates of cure and lower rates of failure and loss to follow-up in the bedaquiline group, there was also a significantly higher rate of all-cause mortality (although the number of deaths was small) in the bedaquiline group compared with the placebo group (39). This study led to conditional approval of bedaquiline for the treatment of MDR-TB by the U.S. Food and Drug Administration (FDA) in 2012 and subsequently by a number of other stringent regulatory authorities in the European Union, South Africa, and India (40). In 2013, the WHO and the U.S. Centers for Disease Control and Prevention (CDC) published interim guidelines on the programmatic use of bedaquiline in specified adult patients with MDR-TB (41, 42). The STREAM-II (Evaluation of a Standard Treatment Regimen of Antituberculosis Drugs for Patients with MDR-TB) trial, which is a phase III randomized, placebo-controlled trial of bedaquiline, began enrolling patients in May of 2016 (27). A number of observational cohort studies confirming the efficacy and safety of bedaquiline have been reported from a variety of settings (5, 6). There are currently more than 3,000 individuals receiving bedaquiline worldwide (43).

The WHO and CDC guidelines state that bedaquiline may be offered to adults with MDR-TB in whom a four-drug regimen plus pyrazinamide cannot be constructed for reasons of resistance or significant intolerance to other medications. The WHO guidelines also stress that bedaquiline should be used as part of programmatic management of MDR-TB under the same five conditions as delamanid, although they specify the need for a signed informed consent for bedaquiline. The WHO guidelines state there was not sufficient evidence to make a recommendation regarding bedaquiline use in persons less than 18 years old at the time the evidence was reviewed in 2013 (41). The CDC also stated that there is insufficient evidence to provide guidelines for the use of bedaquiline in children, but that its use can be considered on a case-by-case basis, given the high mortality and limited treatment options for MDR-TB (42).

Dosing and safety in children. Bedaquiline has not yet been formally assessed in children less than 18 years old. However, a Janssen Pharmaceuticals safety and pharmacokinetics study in children (ages 5–11 yr) and adolescents (ages 12–18 yr) opened for enrollment in South Africa in May of 2016 (27). Younger children will be included in this trial, based on the data obtained from older children and following an age de-escalation protocol. A 20-mg dispersible tablet formulation will be assessed in this trial. A second trial of bedaquiline in HIV-infected and uninfected children sponsored by the U.S. National Institutes of Health (NIH) IMPAACT (International Maternal Pediatric Adolescent AIDS Clinical Trials) network, is being planned and may begin enrolling in 2016 (44). A compassionate use protocol for children is also being developed by Janssen Pharmaceuticals.

There has been some programmatic experience using bedaquiline in adolescents not exceeding 12 years of age (16, 17). When adolescents weighing at least 33 kg have been given bedaquiline, they have received the same dosages that are given to adults: 400 mg daily for 14 days followed by 200 mg three times weekly for 22 weeks.

Recommendations for bedaquiline use in children. Bedaquiline could be considered for the treatment of children at least 12 years old and weighing at least 33 kg for the same indications extrapolated from adults: those with MDR-TB in whom a four-drug regimen plus pyrazinamide cannot be constructed due to resistance or significant intolerance. Bedaquiline may be included in treatment regimens for children with

Table 2. Summary of Ongoing, Planned, or Completed Pediatric Studies Involving New Drugs, Repurposed Drugs, and New Regimens*

Drug/ Regimen	Study/Reference Number	Design	Status of Study	Findings
Bedaquiline	Janssen-sponsored PK, safety, and tolerability trial in children and adolescents/ NCT02354014	A phase II, open-label, multicenter, single-arm study to evaluate PK, safety, tolerability, and antimycobacterial activity of TMC207 in combination with a background regimen of MDR-TB medications for the treatment of children and adolescents 0 mo to <18 yr of age who have confirmed or probable pulmonary MDR-TB	Enrolling 11–17 yr; will begin enrolling 5–10 yr with IRB approval; will enroll younger cohorts once data available from older cohorts	Pending
	IMPAACT PK and safety/P1108	Phase I/II, open-label, single-arm study to evaluate PK, safety, and tolerability of bedaquiline in combination with optimized individualized MDR-TB therapy in HIV-infected and HIV-uninfected infants, children, and adolescents with MDR-TB disease	Not yet open to enrollment	Pending
	NiX trial/NCT02333799	A phase III open-label trial assessing the safety and efficacy of bedaquiline plus PA-824 plus linezolid in subjects with pulmonary infection of either XDR-TB or treatment-intolerant/nonresponsive MDR-TB (NiX-TB)	Enrolling, includes adolescents ages 14 yr and older	Pending
	Observational cohorts	Bedaquiline is being given to adolescent patients as part of programmatic management of MDR-TB, compassionate use protocols, and operational research projects in multiple countries	Cohort data collection and analysis is ongoing	Pending
Delamanid	Otsuka-sponsored long-term safety, efficacy, and pharmacokinetic study of delamanid in pediatric patients with MDR-TB/NCT01859923	Phase II, open-label, multiple-dose trial to assess the safety, tolerability, PK, and efficacy of delamanid in pediatric patients with MDR-TB and receiving therapy with an optimized background regimen of anti-TB drugs over a 6-mo treatment period	Enrollment completed for cohorts age 6+ yr; enrollment open for cohort age <6 yr	Plasma concentrations in the pediatric patients were within the range observed in the open-label trial in adults. Delamanid was well tolerated after 6 mo of therapy in the 12-to 17-yr age group
	Otsuka-sponsored short-term pharmacokinetic and safety trial of delamanid to determine the appropriate dose for pediatric patients with MDR-TB	Phase I, open-label, multiple-dose, and age de-escalation trial to assess the PK, safety, and tolerability of delamanid (OPC 67683) in pediatric patients with MDR-TB receiving therapy with an optimized background regimen of anti-TB drugs	Enrollment completed for cohorts aged 6–11 and 12–17 yr; enrollment for cohort aged 3–5 yr open	Median delamanid exposures were higher in the population of patients ages 6–17 yr compared with adults but within the ranges observed in the adult population. Delamanid was well tolerated in the short term in these cohorts

(Continued)

Table 2. (*Continued*)

Drug/ Regimen	Study/Reference Number	Design	Status of Study	Findings
	Observational cohorts	Delamanid is being given to pediatric patients as part of compassionate use protocols and operational research projects in multiple countries	Cohort data collection and analysis is ongoing	Early results from 19 pediatric patients receiving delamanid as part of multidrug therapy for MDR-TB show that the drug is well tolerated and appears to be efficacious
Linezolid	Pharmacokinetics and toxicity of the second-line anti-TB drugs in HIV-infected and uninfected children	Prospective, longitudinal, hospital-based, observational PK study in HIV-infected and uninfected children aged 0–15 yr who are routinely receiving chemotherapy or chemoprophylaxis for the treatment or prevention of DR-TB	By the end of 2014, a total of 230 participants had already been enrolled; target enrollment is 318 children	Pending
	NiX trial/NCT02333799	A phase III open-label trial assessing the safety and efficacy of bedaquiline plus PA-824 plus linezolid in subjects with pulmonary infection with either XDR-TB or treatment-intolerant/nonresponsive MDR-TB (NiX-TB)	Enrolling, includes adolescents ages 14 yr and older	Pending
	Observational cohorts	Linezolid is being given to pediatric patients as part of programmatic management of MDR-TB and operational research projects in multiple countries	Cohort data collection and analysis is ongoing	
Clofazimine	Pharmacokinetics and toxicity of the second-line anti-TB drugs in HIV-infected and uninfected children	Prospective, longitudinal, hospital-based, observational PK study in HIV-infected and uninfected children aged 0–15 yr who are routinely receiving chemotherapy or chemoprophylaxis for the treatment or prevention of DR-TB	By the end of 2014, a total of 230 participants had already been enrolled; target enrollment is 318 children	Pending
	Observational cohorts	Clofazimine is being given to pediatric patients as part of programmatic management of MDR-TB and operational research projects in multiple countries	Cohort data collection and analysis is ongoing	Pending
Shorter regimens	Observational cohorts	Shortened regimens are being given to pediatric patients as part of programmatic management of MDR-TB and operational research projects in multiple countries	Cohort data collection and analysis is ongoing	Case reports show that the shortened regimens are safe and effective in the limited number of pediatric patients who have received them

Definition of abbreviations: DR-TB = drug-resistant tuberculosis; IMPAACT = International Maternal Pediatric Adolescent AIDS Clinical Trials; IRB = institutional review board; MDR-TB = multidrug-resistant tuberculosis; PK = pharmacokinetics; TB = tuberculosis; XDR-TB = extensively drug-resistant tuberculosis.

*Adapted from the RESIST-TB site (http://www.resisttb.org/?page_id=1602) and from the 2016 TAG Pipeline Report (http://www.pipelinereport.org/2015/tb-pediatrics).

probable MDR-TB if they have a source case who meets these criteria. Children in this age group receiving standard MDR-TB therapy and who develop clinically significant toxicity in response to any of their medications should have the causative agent discontinued and bedaquiline started as a substitute, but it should not be added as a single drug to a failing regimen. The adult dose of bedaquiline should be given (400 mg daily for 14 d and then 200 mg three times per week for 22 wk).

Bedaquiline could be considered in children less than 12 years old if the children meet the criteria described previously and no suitable alternatives are available. Delamanid, however, is the preferred novel agent in this population, given that there

Table 3. Formulations and Procurement of New and Repurposed Drugs for Multidrug-Resistant Tuberculosis

Drug	Formulation	Procurement	Cost and Funding	Comments
Delamanid	50-mg tablet	GDF	1,700 USD for 6-mo course	This price is available for GF-eligible countries GF grants will cover the cost of GDF-procured delamanid Countries not eligible for GF can obtain delamanid from Otsuka Single-patient compassionate use still available from Otsuka*
Bedaquiline	100-mg tablet	GDF	USAID donation for GF-eligible countries. Tiered pricing for non–GF-eligible countries: 6-mo course of 188 tablets is 900 USD for low-income countries and 3,000 USD for middle-income countries	Information on the USAID donation program can be found at http://www.stoptb.org/news/stories/2014/ns14_025.asp
Linezolid	600-mg tablet; 20-mg/ml suspension	GDF; country-specific	2.50–6.00 USD for each 600-mg tablet (64); country-specific	GF will support the procurement of linezolid
Clofazimine	50-mg gelcaps; 100-mg gelcaps	GDF	1.10 USD for each 100-mg tablet; 0.55 USD for each 50-mg tablet	GF will support the procurement of clofazimine
Shortened regimen	All components except gatifloxacin are available via the GDF			

Definition of abbreviations: GDF = Global Drug Facility; GF = Global Fund for AIDS, Tuberculosis, and Malaria; USAID = U.S. Agency for International Development; USD = U.S. dollars.
*For additional information on compassionate use of delamanid, contact Alexandra Martin at amartin@otsuka-onpg.com.

are more data on safety and dosing of delamanid. As with other second-line drugs, the bedaquiline dosage for these lower age and weight ranges would have to be extrapolated from adult dosages. Furthermore, there is no currently available pediatric formulation of bedaquiline, and administration of this drug to younger children would involve crushing and mixing of the drug, which could affect stability and bioavailability. It is recommended that consultation with expert clinicians be sought before administering bedaquiline to children in this age range.

As with adults, programs treating children with bedaquiline should meet the five conditions recommended by the WHO (41). All children being considered for bedaquiline should undergo the baseline testing normally undertaken before MDR-TB treatment but should also undergo a baseline ECG to assess the QTc interval. Contraindications for bedaquiline use are a baseline QTc interval greater than 450 milliseconds that does not correct with medical management, a patient or family history of arrhythmia, or severe cardiac disease. While receiving bedaquiline, children should undergo a monthly ECG to assess for QTc prolongation and should also have monthly potassium levels determined. As with delamanid, caution should be used when other QTc-prolonging medications are used and levofloxacin should be substituted for moxifloxacin. Guidelines recommend that bedaquiline be given for 24 weeks total, although longer courses have been given to individuals with limited therapeutic options (45).

Studies in adults show that bedaquiline should not be given with efavirenz, as efavirenz reduces bedaquiline levels. Therefore, children receiving antiretroviral therapy and for whom bedaquiline is being considered should be switched to a regimen containing nevirapine or raltegravir for the duration of bedaquiline therapy (46). If a nevirapine- or raltegravir-containing regimen is not appropriate, a triple nucleoside reverse transcriptase inhibitor (NRTI) regimen could be considered, keeping in mind the lower potency of such regimens. Of note, lopinavir–ritonavir is used in adults receiving bedaquiline, but two- to threefold higher concentrations of bedaquiline are predicted and have been observed when the two are given together (47). Although the clinical implications of this are unknown, this combination should be avoided if possible in children until more data and experience are available.

Regimens combining bedaquiline and delamanid. Case reports of successful use of the combination of bedaquiline and delamanid in adults are emerging (48, 49). Treatment regimens containing both bedaquiline and delamanid in children could be considered on a case-by-case basis, if no other treatment options exist, in consultation with expert clinicians. The use of combined delamanid and bedaquiline in this population should follow more recently proposed guidelines, which recommend it be used only when an effective regimen cannot otherwise be designed; the clinical center has expertise with treatment of MDR-TB; the patient and caregivers are counseled as part of informed consent for both drugs;

pharmacovigilance is in place; and an independent, qualified organization considers the use of both drugs to be appropriate (50).

Linezolid

Linezolid is an oxazolidinone antibiotic indicated for the treatment of gram-positive bacteria that has been effective for treating MDR-TB in adults in multiple observational studies (51, 52) and in a delayed-start randomized controlled trial (53). The main factor limiting wider use is the drug's toxicity profile, especially bone marrow suppression, and that an optimal dose has not been established in adults (54, 55). Bone marrow suppression is reversible on cessation of linezolid. The outcome of peripheral neuropathy on drug cessation is more variable; it may not be reversible, although it is unclear whether resolution may be seen with longer follow-up.

There have been studies of linezolid in children with MDR-TB showing the drug is effective, but safety issues have been reported with longer durations and higher doses, including hematologic toxicity and peripheral neuropathy (56–58). Linezolid has been recommended in children when close clinical monitoring is possible, and the WHO recommends that linezolid be included in the treatment regimens of children with confirmed or probable MDR-TB as part of the "other core second-line agents" that can be used to build a treatment regimen of at least four effective drugs (9, 59). However, because of the risk of adverse events, if the patient cannot be monitored, linezolid is best used in patients with additional possible resistance or intolerance to other second-line medications. Linezolid has excellent penetration into the cerebrospinal fluid, and thus should be considered for MDR-TB meningitis. Dosages of 10 mg/kg once daily for children at least 12 years old and of 10 mg/kg twice daily for children less than 12 years are recommended because younger children have increased metabolism of the drug (not to exceed a maximum dose of 600 mg daily). Children should be closely monitored for adverse events, especially for peripheral neuropathy, anemia, thrombocytopenia, lactic acidosis, and optic neuropathy. Linezolid should be given for the entire duration of therapy or for as long as it is tolerated. It can be safely given with antiretroviral therapy, although there should be close monitoring due to potential for overlapping toxicity if used with NRTIs, given the potential for both linezolid and NRTIs to inhibit mitochondrial protein synthesis (60).

Clofazimine

Clofazimine is a lipophilic riminophenazine antibiotic, traditionally used for leprosy treatment. It was effective for treating MDR-TB in adults in observational studies and in a nonplacebo randomized controlled trial (61, 62). There is a resurgence of interest in it, given its use in shorter MDR-TB regimens. Although there have been no formal studies of clofazimine in children with TB, there is substantial experience in using it to treat children with leprosy. In a leprosy trial of 422 children in China and India, clofazimine was well tolerated (63). The main adverse events associated with clofazimine include prolongation of the QTc interval, and reversible skin pigmentation. The WHO has included clofazimine as part of the "other core second-line agents" that can be used to build a treatment regimen of at least four effective drugs (9). A dose of 2–3 mg/kg per day (maximum dose, 100 mg daily) is recommended for children; however, there is limited published pharmacokinetics to support this dose. Clofazimine comes in 50- and 100-mg gelcaps that cannot be split, and therefore if lower doses are needed children could be given doses every other day because of the drug's long half-life. Children receiving clofazimine should have monthly ECGs assessed when possible, especially when more than one QTc-prolonging agent is used. Children and their caregivers should be advised about associated skin color changes, which may take a long time to resolve. Clofazimine should be given for the entire duration of therapy or as long as it is tolerated. It can be safely given with antiretroviral therapy.

Conclusions

The use of new and repurposed drugs for the treatment of MDR-TB has improved interim outcomes and helped manage toxicity in adults. Years after they have been recommended for adults, children facing the same challenges have not benefited from these therapeutic advances. This has caused a concerning disparity, in which vulnerable children with MDR-TB are left behind. This is occurring even in the setting of a robust pediatric drug development program for delamanid and multiple calls to action for inclusion of children in clinical research for MDR-TB. These practice-based recommendations can assist front-line providers treating children with MDR-TB and provide a base for national TB programs and the donor community to further support the use of new and repurposed drugs in the pediatric population, especially as additional data on safety and optimal dosing of these drugs in children continue to emerge. Children and adolescents with MDR-TB have had to endure the problems with the current treatment regimen for far too long, and it is time they too benefit from these exciting developments. ■

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The safety and tolerability of the second-line injectable antituberculosis drugs in children

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REVIEW

The safety and tolerability of the second-line injectable antituberculosis drugs in children

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ABSTRACT

Introduction: A growing number of children globally are being treated for multidrug-resistant tuberculosis (MDR-TB). The second-line injectable antituberculosis medications amikacin, kanamycin and capreomycin, traditionally a mainstay of MDR-TB treatment, cause important adverse effects including permanent sensorineural hearing loss, nephrotoxicity, electrolyte abnormalities, injection pain and local injection site complications.

Areas covered: To characterize the safety and tolerability of the second-line injectables in children treated for MDR-TB, we reviewed data on the mechanism of injectable associated adverse effects, risk factors for their development, and the incidence of injectable-associated adverse effects in adults and children treated for MDR-TB.

Expert opinion: Despite a substantial evidence base in adults demonstrating the frequent and potentially serious adverse effects of second-line injectables, important knowledge gaps remain. Improved characterization of the incidence of injectable-associated adverse effects will inform rational guidance on monitoring children with TB on injectables. Eliminating the need for injectables in MDR-TB treatment regimens is a high priority, and will rely on the use of novel antituberculosis TB drugs. Strategies to reduce the risk of adverse effects of injectables, if used, deserve evaluation. This includes evaluation of potentially otoprotective medications N-acetylcysteine or aspirin, high frequency hearing screening for earlier detection of ototoxicity and therapeutic drug monitoring.

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1. Introduction

There is a substantial global burden of multidrug-resistant tuberculosis (MDR-TB), defined as TB resistant to at least both isoniazid and rifampicin. Accurate figures of cases in children are lacking, however, two models have provided similar estimates of 32,000 pediatric cases of MDR-TB in 2010 [1] and 27,500 cases in 2014 [2]. Although a small percentage of children with MDR-TB are appropriately diagnosed and treated, there are ongoing efforts to reduce and eliminate this 'treatment gap' [3]. Thus, more children will be accessing MDR-TB treatment in coming years.

The treatment of MDR-TB relies on the so-called second-line antituberculosis medications. Current guidance from the World Health Organization (WHO) on MDR-TB treatment recommends building a regimen with at least four TB medications confirmed or likely to have activity, which should include a second-line injectable medication for a minimum duration of 8 months [4]. The second-line injectable antituberculosis drugs collectively refer to the aminoglycosides amikacin, kanamycin, and the cyclic polypeptide capreomycin. These are considered together because of their similar characteristics, including mechanism of action of protein synthesis inhibition, pharmacokinetics, administration requirements, and adverse effect profiles [5]. All of these medications are rapidly degraded when given orally, so must be administered intravenously or by intramuscular injection. Given the current limited existing treatment options for MDR-TB, despite recent registration of the novel drugs bedaquiline and delamanid for use in adults with MDR-TB, the injectables have traditionally been considered key components of current regimens [4]. Even a shortened 9-month regimen which has demonstrated good outcomes in observational studies in adults, and is being evaluated in an ongoing clinical trial, still includes an injectable drug for 4 months [6].

As opposed to adults for whom MDR-TB treatment outcomes remain poor, outcomes in children with MDR-TB are generally good, with successful outcomes reported in 80% of children and higher in some cohorts [7,8]. However, current treatment regimens are associated with frequent and potentially severe adverse effects [8]. The second-line injectables can cause a number of serious adverse effects including nephrotoxicity, electrolyte abnormalities, vestibular toxicity, and most importantly permanent sensorineural hearing loss [5]. Additionally, their requirement to be given by intramuscular injection in many settings makes them poorly tolerated, resulting in substantial pain, distress, and local injection-related complications. An overview of the second-line injectables is shown in Table 1. A thorough understanding of the adverse effects of the second-line injectables is important for

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Article highlights

- Permanent sensorineural hearing loss occurs in at least 20% of children treated long term with the second-line injectable medications amikacin, kanamycin, and capreomycin, and is associated with cumulative drug exposure
- There is limited data on electrolyte abnormalities and renal dysfunction in children treated with the second-line injectables, but the risk appears to be low; there may be a higher risk with capreomycin use so closer monitoring may be warranted
- Local injection site adverse effects have been poorly described, however the injections are painful and poorly tolerated by adults and children
- The carbapenems are generally well-tolerated agents, but their safety with long-term use for MDR-TB in adults or children has not been well characterized
- Given the adverse effect profile of the second-line injectables, developing efficacious all-oral regimens that don't require their use is an urgent priority
- Potential strategies deserving evaluation for reducing second-line injectable adverse effects include the use otoprotective agents, addition of local anesthetic to reduce injection pain, therapeutic drug monitoring, and improved audiologic monitoring.

This box summarizes key points contained in the article.

healthcare workers caring for children with MDR-TB, and for persons designing and evaluating novel MDR-TB treatment regimens.

The carbapenem antimicrobials meropenem, imipenem, and ertapenem, when combined with a β-lactamase inhibitor such as clavulanate, have demonstrated antimycobacterial activity. However, because of the limited efficacy data in humans, high cost, and the need for multiple daily intravenous doses, these medications have been mostly used in salvage regimens for patients with very limited treatments options in higher-resourced settings. There may be renewed interest in the carbapenems as antituberculosis medications, as a recent study has now shown a substantial early bactericidal activity of meropenem/amoxicillin-clavulanate [9].

The objective of this review is to examine current knowledge of the adverse effects of the second-line injectable antituberculosis medications and the carbapenems in children, and to highlight knowledge gaps and priority questions for future research.

2. Ototoxicity and vestibulotoxicity

2.1. Overview of ototoxicity

Ototoxicity is a well-known complication of the second-line injectables, and aminoglycoside-induced ototoxicity has been studied in depth. An understanding of the pathophysiology helps provide improved understanding of many of the clinical findings of ototoxicity in injectable-treated patients.

Very early after administration, the injectable antituberculosis medication enters the cochlear hair cells through a membrane channel [10]. This channel acts like a one-way valve, trapping the injectable medication in the hair cell where they are not metabolized; the half-life for disappearance of aminoglycosides from hair cells appears to be >30 days [11]. This results in accumulation of injectable medication in the hair cells over time, and explains why hearing loss can occur or progress after their discontinuation. Once in the hair cells, the injectable medications form complexes with iron (Fe), generating reactive oxygen species; along with direct interactions between the injectable medication and hair cell mitochondrial rRNA; this results in disruption of mitochondrial integrity which ultimately results in apoptotic cell death [10]. This cell death begins at hair cells responsible for recognizing higher-frequency sounds, above the speech threshold, and progresses to lower frequencies [10]. This has important implications for clinical management, as hair cell damage may occur prior to any perceived loss of hearing by participants. As such, subjective patient report cannot be relied on for detection of ototoxicity; audiological testing which includes high frequencies, at least 6000–8000 kHz or higher, is therefore important for early identification of ototoxicity and prompt management.

Studies exploring potential risk factors for aminoglycoside-associated ototoxicity have reported conflicting results. Trough concentrations of injectables have been associated with ototoxicity in some studies, but not in others; older age, duration of use, and cumulative dose are associated with hearing loss in some, but not in others [12–15]. A number of genetic mutations have been identified which increase the risk of injectable-associated ototoxicity [10,16–18]. These mutations are in the gene (*MT-RNR1*) encoding the mitochondrial 12 s rRNA, making it more similar to bacterial rRNA and increasing its affinity for aminoglycosides, with a resultant

Table 1. Overview of the second-line injectable antituberculosis medications.

Medications	• Amikacin and kanamycin (aminoglycosides) • Capreomycin (cyclic polypeptide)
Key characteristics	• Administration intravenously or intramuscular injection • All have similar pharmacokinetics and similar spectrum of adverse effects
Current WHO-recommended role in multidrug-resistant tuberculosis treatment	• To be included in all treatment regimens in adults and children for at least 4 months
Key adverse effects and risk factors	• Ototoxicity, resulting in permanent sensorineural hearing loss • Vestibular toxicity, resulting in vertigo and balance problems • Nephrotoxicity, resulting in oliguric renal failure • Electrolyte abnormalities, hypokalemia, hypomagnesemia most frequent • Injection pain and local injection site complications

decrease in mitochondrial protein synthesis [10,16–18]. When present, severe hearing loss can occur rapidly, even after a few doses. However, these mutations are relatively infrequent in the general population, present in anywhere from 0–1.8% of populations representing a broad geographic distribution; no specific populations to date have been identified with a particularly high prevalence of these mutations [10,16,19]. In a cohort of South African adults with MDR-TB, none of 153 patients with hearing loss had any of these known mutations, demonstrating that the overall contribution of these genetic predisposition to ototoxicity in MDR-TB treatment is likely small [16,19].

The most important risk factor for ototoxicity appears to be cumulative drug exposure. A number of elegant studies in guinea pigs provided some important insights into this relationship, which appears to be consistent with experience in humans [20,21]. In guinea pigs treated with continuous amikacin infusions at various rates, ototoxicity was closely associated with total dose and total cumulative drug exposure (total area under the time–concentration curve [AUC]) [20,21]. The infusion rate and plasma concentrations were not associated with ototoxicity and did not affect the total dose or total AUC at which ototoxicity occurred. This may explain the limited value of peak and trough measurements as predictors of ototoxicity. This also has important implications for dosing strategies, and suggests that intermittent dosing (thrice weekly versus daily) will not impact on the risk of ototoxicity. In addition to an association with incidence of ototoxicity, the total dose and total AUC were also associated with the magnitude of hearing loss. There was also a significant negative relationship between total dose/total AUC with the hearing frequency at which hearing loss occurred, meaning ototoxicity occurs at higher frequencies at a lower total dose/total AUC, and progresses to lower frequencies as the total dose/total AUC increases. This is consistent with clinical experience in humans. The relationship between total dose/total AUC and ototoxicity fit a sigmoid curve which had a very steep slope, which could result in large differences in incidence and magnitude of ototoxicity over relative small ranges in total dose/total AUC, and could partly explain the large variability and unpredictability of ototoxicity in humans. The proposed explanation for all of these findings was that aminoglycosides enter hair cells at a rate that is linearly related to the plasma concentration through an essentially one-way process, accumulating over time and resulting in ototoxicity once a certain threshold concentration is reached [20,21].

These guinea pig studies are consistent with and explain many of the findings in human studies. In a study comparing the impact of dosing strategy on ototoxicity in adults, there was no difference in risk of ototoxicity between persons treated with a daily dose of streptomycin, kanamycin, or amikacin of 15 mg/kg/dose (5 days a week) compared to those receiving a three times weekly dose of 25 mg/kg/dose [22]. Neither maximum plasma concentration nor trough concentration were associated with risk of hearing loss. However older age, total dose and the related duration of treatment, were associated with ototoxicity, with a 6.9-fold increase in ototoxicity for every 10-fold increase in total dose received [22]. These confirm the limited value of intermittent dosing and measuring peak/trough concentrations for reducing ototoxicity risk, and confirm the importance of cumulative dose. In a cohort of 28 adults with MDR-TB in Botswana treated with amikacin 750–1000 mg/day, 11 (39%) had hearing loss [23]. Amikacin peak and trough concentrations were not associated with hearing loss, however cumulative days of therapy and cumulative amikacin AUC were predictive of hearing loss. As in the animal studies, the relationship between cumulative AUC and cumulative days of therapy was a sigmoid curve; the modeled estimated risk of hearing loss began to sharply increase around 6 months of cumulative therapy and was nearly 100% at 9 months of therapy.

The relative risk of ototoxicity between the different second-line injectables has not been well characterized, but could potentially be clinically relevant. Although not a second-line injectable, streptomycin was found to be less ototoxic than kanamycin or amikacin in one adult study [22]. In a retrospective review of adults with MDR-TB treated with either amikacin or kanamycin in routine care, the risk of hearing loss was higher with amikacin, with an adjusted odds ratio (aOR) of 2.3 (95% CI 1.0–5.4); the association of amikacin with more severe hearing loss was even higher, with an aOR of 4.0 (95% CI 1.5–10.8) [24]. Duration of treatment and total dose were not reported or controlled for in this study, however, these results are suggestive of a higher ototoxicity risk with amikacin. In some sources, it has been stated that capreomycin is less ototoxic than amikacin or kanamycin, however, evidence for this assertion is limited and somewhat conflicting. In a cohort of adults with MDR-TB in the UK, 11 of 29 (38%) treated with amikacin had ototoxicity, however, none of 11 treated only with capreomycin as their injectable agent experienced ototoxicity [25]. In a small Irish cohort, 1 of 4 (25%) adults treated with capreomycin had hearing loss, compared to 7 of 34 (20.6%) treated with amikacin and 4 of 26 (15.4%) treated with kanamycin [26]. These reports should be interpreted with caution given the small numbers and retrospective observational design. We have observed clinicians substituting capreomycin for other injectables in MDR-TB patients once early hearing loss has been detected, or suggesting a preferential use of capreomycin presuming a reduced risk of ototoxicity; we would suggest caution in using such approaches in the absence of better evidence, as there is still a risk of ototoxicity with capreomycin, and other serious adverse effects may be related to capreomycin use as discussed below [27]. The injectables have concentration-dependent activity against *Mycobacterium tuberculosis*, with the ratio of the maximum plasma concentration (Cmax) to the minimum inhibitory concentration (MIC) being the pharmacodynamics measure most closely associated with efficacy [5]. Amikacin has been consistently shown to be the more potent second-line injectable against *M. tuberculosis in vitro*, with a lower MIC compared to kanamycin and capreomycin [5], and for that reason may be the injectable of choice for MDR-TB treatment. This is a consideration that should be weighed against a potentially higher risk of ototoxicity with amikacin.

Other factors have been shown to increase the risk of ototoxicity, and clinicians should at least be aware of them. The concomitant use of loop diuretics has been shown to

increase aminoglycoside ototoxicity in animals, and there are descriptions in adults [28–30]. Although the risk in adults and children has been poorly characterized, clinicians should at least be aware of the theoretical increased potential for ototoxicity with co-treatment with these two classes of medications. Concomitant administration of iron also increases ototoxicity risk in gentamicin-treated guinea pigs; it was postulated that this was related to the role of Fe in the generation of reactive oxygen species and free radicals [31]. The contribution of these factors relative to other risk factors, like cumulative drug exposure, is not entirely clear

2.2. Incidence of ototoxicity in second-line injectable-treated adults and children with MDR-TB

The incidence of hearing loss reported among adults with MDR-TB treated with second-line injectables is quite variable; this may be related at least in part to the inconsistency in the quality of reporting, methods of assessments, and definitions of ototoxicity [32]. In a 2012 systematic review of hearing loss in drug-resistant TB patients, the percent of reported hearing loss ranged from 2.6–61.5% [32]. In cohorts reported after this systematic review, the stated risk of ototoxicity remains quite variable. However, among studies in which comprehensive audiology assessments are done and reported, the risk of hearing loss is high and quite concerning. In a South African cohort of adults, 57% developed hearing loss [19]. HIV-infected persons in this study had a higher risk of hearing loss; HIV-infected persons might receive longer injectable treatment because of more severe disease or slow treatment response, however, duration of injectable treatment was not controlled for in this analysis. In 12 adults with MDR-TB in the UK treated with amikacin (15 mg/kg once daily) who received careful audiology assessments, 7 (58%) had documented hearing loss, 3 reported tinnitus; 8 (67%) had their amikacin interrupted because of these abnormalities, and 4 patients had further progression of hearing loss after this interruption [33]. A large cohort of adults with MDR-TB treated with amikacin (15–25 mg/kg daily, maximum 1000 mg) in Botswana reported similar results, with 270 of 437 (62%) reporting hearing loss, with 147 (54%) of these confirmed by audiology; dose and duration of treatment were associated with hearing loss [34]. A similar risk was reported in a Namibian adult cohort, with overall 58% having any hearing loss, with 10% of these having severe and 15% having profound hearing loss [24].

Given the prolonged half-life of the injectables in cochlear hair cells, recent previous treatment with streptomycin, as in patients receiving a WHO Category II regimen, would be expected to increase the risk of hearing loss with subsequent injectable-containing MDR-TB treatment. This has not been well quantified, but clinicians should be aware of this potential increased risk.

Data on ototoxicity in children treated for MDR-TB is more limited. This may in part be due to the challenges with accurately assessing hearing in young children, and the limited published reports of pediatric MDR-TB in the literature. Young children often cannot cooperate with the pure tone audiometry (PTA) assessments used in adults and older children. Alternative methods of hearing testing are available for younger ages, including otoacoustic emission (OAE) testing, which is feasible with the appropriate equipment in most settings. Other audiology testing used in young children, such as auditory brainstem evoked response (ABER), play audiometry, and conditioned response audiometry requires more specialized training and equipment, and access may be limited in many settings. A detailed discussion of these different testing modalities, approaches to audiological assessment in different ages, interpretation of results and classification of hearing loss is beyond the scope of this review, but important and has been previously written in about in detail [32,35,36].

The lack of capacity for audiological testing in many high-burden settings, particularly for children, is problematic. In a 2009 survey of 18 sub-Saharan African countries, only South Africa, had more than 1 audiologist per 100,000 population, and many had no audiologist. Only 2 of 18 countries had an audiology training program, and access to services such as basic audiology, ABER, and routine hearing screening was rated as nil to poor in most countries and where present was centralized and not widely accessible [37]. This contributes to the limited data, and results in inadequate monitoring of children treated with the second-line injectables, putting them at risk for more severe hearing loss.

Taking a conservative approach and using a strict definition of hearing loss, 24% of children in a retrospective cohort of children treated for MDR-TB had confirmed hearing loss [38]. A high proportion of children with hearing loss was reported in the same setting in a follow-up prospective cohort study [8]. In this study, 25 of 142 (17.6%) children treated for MDR-TB had hearing loss; however in this cohort, only 94 (66%) children (those with more severe MDR-TB disease) were treated with an injectable, and the median duration of injectable use was only 4 months (interquartile range 4–6). This therefore likely underestimates the risk of hearing loss in children receiving the current routinely recommended 6 months of injectable treatment. Two other pediatric cohorts reported a lower risk of ototoxicity, with hearing loss in 2 of 38 (6.7%) and 1 of 10 (10%), however, audiological monitoring was not well described in these studies [7,39,40].

Children are known to have a higher clearance of aminoglycosides, resulting in lower drug exposure relative to adults given the same mg/kg dose [5]. If cumulative drug exposure is the most predictive risk factor for injectable-associated ototoxicity, then children would be expected to have a lower risk compared to adults treated for the same duration and same mg/kg dose of injectable. However, children younger than 6 months of age may have a reduced aminoglycoside clearance relative to older children and adults due to immature renal function, resulting in higher drug exposures and a potentially higher risk of ototoxicity. Although devastating at any age, hearing loss in young children is particularly harmful as it occurs during critical periods of neurodevelopment and may have a profound impact on speech development.

2.3. Otoprotective strategies

Understanding the cellular and molecular pathogenesis of ototoxic drug-induced hair cell loss, has led to exploration of a number of potential antidotes or preventive treatments,

mainly focusing on counteracting reactive oxygen species and free radicals [10]. Iron chelators such as deferoxamine may inhibit formation of reactive oxygen species, and in animals such agents reduce aminoglycoside-induced ototoxicity [10,41,42]. Acetylsalicylate has the dual benefit of being an iron chelator and an antioxidant, and reduces gentamicin-induced ototoxicity in animals [10]. Many other antioxidants, including D-methionine, glutathione, N-acetylcysteine (NAC) have also demonstrated benefit in animal models [10]. There is some evidence for the benefit of a number of these agents in humans for prevention of ototoxicity. In a randomized controlled trial in adults receiving short courses of gentamicin for acute infections, ototoxicity was shown in 13% receiving placebo and 3% of those receiving aspirin (3 grams daily, divided doses) [43,44]. This study acts as a proof of concept, and demonstrates the potential benefits of coadministration with the widely available aspirin. Concerns, however, particularly with long-term high-dose aspirin treatment, include the risk of tinnitus from aspirin itself, an association of Reye syndrome with aspirin use in children with viral infections, and a risk of gastrointestinal ulcerations and bleeding. A recent systematic review assessed the safety and otoprotective potential of NAC [45]. There is no data in MDR-TB; however, in this review, aminoglycoside-treated dialysis patients receiving NAC had a substantially reduced risk of ototoxicity, though numbers were small and overall quality of evidence was low. The safety of NAC when given for at least 6 weeks duration was also evaluated, and it was found that abdominal pain, nausea, vomiting, diarrhea, and arthralgia were more common in NAC-treated groups compared to controls, however, severe adverse events were limited. NAC may be an attractive otoprotective agent in MDR-TB and should therefore be evaluated in children with MDR-TB.

2.4. Vestibulotoxicity

The vestibular system located in the inner ear and composed of the utricle and sacule which detect linear movement, and the semicircular canals which detect rotational movement, is responsible for balance and spatial orientation. Treatment with the injectables can also result in vestibulotoxicity through effects on the hair cells of the vestibular system, however, this has been less studied than ototoxicity. It may occur in the absence of ototoxicity. It may present with feelings of dizziness, vertigo, lightheadedness, which may be subtle at the onset [46]. In adults treated for MDR-TB, vestibulotoxicity is variably reported. In one study of adults receiving long-term injectable treatment, objective measures of vestibular dysfunction were documented in 8 of 87 (9%); there may be some reversibility of symptoms over time, but changes may persist [22]. Subjective vestibular symptoms were much more common in this cohort, with 41% reporting subjective balance changes and 47% reporting dizziness; these were mostly mild, associated with the injectable infusion and resolved over time [22]. In a cohort of 53 South African adults treated for MDR-TB, vestibular complaints were common, with vertigo reported in 45%, sensation of falling in 38% and imbalance in 26%; information about progression or resolution over time was not provided [47]. There is little data on vestibulotoxicity in children treated for MDR-TB. In our experience, we have not seen clinically apparent vestibulotoxicity, however, this may be related to difficulties in eliciting symptoms in young children and it is possible that this is an under-recognized adverse effect.

3. Nephrotoxicity and electrolyte abnormalities

3.1. Nephrotoxicity

The second-line injectables are well known for their potential to cause renal impairment. The injectables elicit renal injury primarily by causing renal tubular dysfunction [48,49]. After glomerular filtration, the injectables enter renal tubular cells and accumulate there, ultimately resulting in renal tubular cell death via apoptosis or necrosis through an as yet unspecified mechanism. In addition to tubular dysfunction, the injectables have also been shown to induce altered glomerular filtration and reduced renal blood flow [49]. The clinical result of these effects is oliguric renal failure, however, this may be preceded by more subtle evidence of tubular dysfunction such as mild wasting of glucose, protein, and electrolytes [48,49]. This renal injury is generally reversible after discontinuation of the injectable.

Uptake of the injectables into renal tubular cells is a saturable phenomenon at clinically relevant concentrations [48]. This phenomenon has been exploited through a strategy of higher less-frequent dosing to potentially reduce nephrotoxicity. In a randomized trial of once vs. twice daily dosing of aminoglycosides in adults, nephrotoxicity occurred in 15% of adults receiving twice-daily therapy, and in 0% of those receiving once-daily therapy; the dosing strategy was closely associated with occurrence of nephrotoxicity, as was AUC [50]. Meta-analyses in adults have shown no difference in efficacy with once- vs. multiple-daily dosing strategies of aminoglycosides, however, there are conflicting results with regard to nephrotoxicity, with one showing a reduced incidence with extended interval dosing [51] but others showing no difference [52,53]. A meta-analysis of extended interval dosing in children found no difference in nephrotoxicity [54,55]. Given the programmatic simplicity and benefits for cost and adherence, in MDR-TB treatment the injectables are therefore given as once-daily or thrice-weekly doses.

The proportion of adults treated for MDR-TB with reported renal impairment ranges from <1% to 9.8% [27,56–60]; this may reflect the heterogeneity in definitions, monitoring, and treatment approaches between studies. The risk of nephrotoxicity in children has not been well described, but appears to be low. In a systematic review of children treated for MDR-TB, only 1 of 182 children was reported to have any renal impairment, an asymptomatic elevation of creatinine [7]. In our personal experience, renal impairment is quite uncommon in children with once-daily administration of the recommended doses of injectables. In a prospective observational cohort study of children treated for MDR-TB [8], no cases of abnormal renal function were seen among injectable-treated children, although this was not specifically reported in the paper (personal communication, H.S. Schaaf).

There are no direct comparisons of the relative risk of nephrotoxicity between the second-line injectables, however, the limited existing evidence does point to a higher risk with capreomycin. In a cohort of 151 patients in South Africa with extensively drug-resistant TB (XDR-TB), 61 (58%) experienced a total of 161 adverse events during treatment [27]. In total, 6 of the 161 adverse events resulted in death, and all 6 were due to capreomycin (5 cases of renal failure, 1 case of hypokalemia); 5 of these 6 were in HIV-infected persons. The deaths occurred at a median of 14 days after starting capreomycin (range 9–73) [27]. This high risk of death due to capreomycin-associated renal failure is concerning, and there should be close monitoring of renal function when capreomycin use is required.

3.2. Electrolyte abnormalities

Hypokalemia, hypomagnesemia, and hypocalcemia are well-described adverse effects of the injectables, most likely due to renal electrolyte wasting, although other mechanisms may also contribute [56,61]. Reported risk factors for the development of electrolyte abnormalities include cumulative dose, low body weight, and choice of injectable. The available evidence suggests a substantially higher risk of electrolyte abnormalities with capreomycin [61–63]. The most comprehensive description in MDR-TB treatment is from a cohort of 115 adults in Peru, where 34.8% had an electrolyte abnormality detected [61]. Hypokalemia was the most frequent, found in 31.3%, with hypomagnesemia detected in 15.7% and with 12.2% of patients having both abnormalities; capreomycin-treated patients had a higher risk, with 68.2% having hypokalemia. In multivariable analysis, hypokalemia was associated with capreomycin use and low body weight. Capreomycin-attributed hypokalemia was responsible for one death in a cohort of South African XDR-TB patients, and in this cohort capreomycin was the most frequent drug discontinued because of adverse effects [27].

A systematic review of children treated for MDR-TB reported only one episode of electrolyte abnormality in 182 children [7]. This is consistent with our experience; our practice is routine monitoring of electrolytes every 4–8 weeks in children with MDR-TB during their injectable treatment, however, we have identified few abnormalities. Given the concerns in adults, particular care may be warranted in children treated with capreomycin, with more frequent monitoring of electrolytes prudent (every 2–4 weeks if feasible).

4. Injection site adverse events

Local site adverse effects of the injectable agents, including infection, pain, subcutaneous and muscle abscess formation, muscle contracture and fibrosis, and neurovascular injury have been poorly reported to date. Although the risk of an adverse effect after a single injection is low, given the very large total number of injections over the treatment course, adverse effects would be expected to occur regularly [64]. A painful, inflamed injection site was reported as an adverse effect in 4% of an adult MDR-TB cohort, with abscess formation in <1% [58], but most published MDR-TB cohort data does not report on such effects. This has similarly not been well described in children. The smaller size and lower muscle mass introduces additional challenges in children, and some injection-related adverse effects would be expected to be more common in children, such as neurovascular injury. Suggested best practices for intramuscular injections in children exist, but this has not carried over into MDR-TB treatment recommendations [64]. This is a common challenge for frontline healthcare workers, and guidance would be valuable given the large number of required injections and age-related changes in optimal injection site.

The pain associated with the intramuscular injections is an important source of distress for MDR-TB patients, and is often mentioned by patients as one of the worse aspects of treatment [65]. Strategies to reduce the pain associated with daily intramuscular injections of the second-line injectables are needed. The addition of a local anesthetic has been shown to reduce the pain of intramuscular injections of penicillin and ceftriaxone without substantially altering their pharmacokinetics [66–68]. A similar strategy is being evaluated in a randomized blinded crossover study of the effect of added lignocaine on pain and pharmacokinetics of intramuscular amikacin in older children and adolescents with MDR-TB (PACTR201401000670381), with results expected soon. Implanted catheters or peripherally inserted central venous cannulas may be used in some settings, and would clearly eliminate the need for intramuscular injections. However, this approach has its own risks, including complications of line placement and line infection. In most high TB-burden settings, the regular use of such long-term venous access is not feasible due to a lack of capacity for line placement and the difficulty in caring for lines.

5. Carbapenems for TB treatment

The carbapenems are generally well tolerated, with the most common adverse events being diarrhea, rash, and nausea/vomiting, which are mostly mild [69–71]. As with other β-lactams, there is a risk of hypersensitivity (anaphylactic) reactions that may be severe. Imipenem in particular has been associated with seizures, with a higher risk in those with existing central nervous system disease or meningitis, although the absolute risk is low [70,72,73]. A systematic review of carbapenem-containing regimens for MDR-TB identified nine eligible studies; carbapenem-attributed adverse events were reported in 0–13.5% of patients depending on the cohort, with 0–40% of these adverse events resulting in at least temporary interruption of the carbapenem [74]. This review is limited by the high variability in treatment regimens, disease and drug-resistance spectrum, specific carbapenem used and dose. These cohorts only included two children, so no conclusions can be drawn regarding long-term carbapenem safety in children with MDR-TB from the existing data. In addition to data on the safety of carbapenems, adverse effects related to the need for long-term vascular access in children would also need to be considered given the lack of a current orally administered carbapenem and the need for multiple-daily dosing for most of these agents. Complications from central lines or peripherally inserted central venous catheters are not infrequent in children [75].

6. Conclusion

The second-line injectable antituberculosis medications remain a component of currently recommended treatment regimens for MDR-TB. Their clinically important adverse effects include ototoxicity, vestibulotoxicity, nephrotoxicity, electrolyte abnormalities, and injection site complications. However, the incidence of these adverse effects and risk factors for their occurrence have not been well described in children with MDR-TB. Clinicians caring for children with MDR-TB should be aware of these potential adverse effects, so that appropriate screening and management can occur, to ensure that MDR-TB treatment is as safe as possible.

7. Expert opinion

Although there is a substantial body of evidence on the safety of the second-line injectable antituberculosis medications, mostly in adults, a number of priority areas remain for future research, including improved characterization of adverse effects in children treated with injectables for MDR-TB, development of strategies to reduce or eliminate the need for injectables in MDR-TB treatment, and evaluation of strategies to reduce the risk of injectable-related adverse effects when they are truly required to be used in MDR-TB regimens (Table 2).

There is limited high-quality data on the risk of adverse effects due to the second-line injectables in children treated for MDR-TB. There is likely systematic under-detection and under-reporting in children of some injectable-related adverse effects, such as ototoxicity, local injection site complications, and pain and distress due to injections; this may be partly related to challenges in assessing some of these effects in children. For other potential adverse effects, such as nephrotoxicity and electrolyte abnormalities, the risk in children may be relatively low. Improved data would help inform practical guidance for monitoring of adverse effects in children treated for MDR-TB, would make a case for devoting resources for appropriate safety monitoring in children, such as equipment and training for audiological monitoring in young children, and ultimately could provide motivation for urgently identifying MDR-TB treatment strategies that exclude the use of injectables.

The most obvious approach to reducing the risk of second-line injectable-related adverse effects is to limit or eliminate their use in MDR-TB treatment altogether. As described above, children with non-severe MDR-TB can be successfully treated without injectable treatment or at a minimum, with reduced duration of injectables [8]. Such a strategy should be employed wherever possible, and updated treatment recommendations should reflect the potential of such a strategy when carefully employed. This would rely on early identification of MDR-TB in children before progression to more severe disease, which is much more likely when children are identified by active contact investigation of infectious MDR-TB cases. In adults, multiple trials are underway to evaluate novel regimens for MDR-TB treatment, most of which include one of the new TB medications bedaquiline or delamanid, and many of which do not use an injectable medication. However, efficacy data on these regimens is unlikely to be available soon. A shortened, all oral MDR-TB treatment regimen for children is a research priority, but data on such a regimen would likewise be unavailable for some time. In the interim, an important way forward may be the careful exploration of whether another TB drug or drugs, such as delamanid, bedaquiline, or even linezolid, could be substituted for the injectable in pediatric MDR-TB treatment regimens.

In addition to efforts to eliminate or reduce second-line injectable use, strategies to reduce the risk of injectable-related adverse effects should be pursued in parallel. It is likely that the injectables will continue to play a role in MDR-TB treatment for the foreseeable future, while data, experience with, and access to novel regimens accumulate. Additionally, resistance to novel agents may develop with their more widespread use [76], and the injectables may again become important agents in future salvage regimens. Improved risk reduction approaches aimed at making the injectables safer and more tolerable when their use is required, should be pursued in parallel to strategies of eliminating or reducing their use. Many such risk reduction strategies are relatively

Table 2. Summary of research priorities related to the safety and tolerability of the second-line injectable antituberculosis medications in children with multidrug-resistant tuberculosis (MDR-TB).

Improved characterization of incidence of injectable-associated adverse effects in children with MDR-TB
• Cohort data on incidence and severity of adverse effects in children
Strategies to eliminate or reduce the duration of injectable treatment of MDR-TB
• Trials of all oral MDR-TB regimens
• Omission of injectable use in children with non-severe MDR-TB
• Reduced duration of injectable use in children with non-severe MDR-TB
• Substitution of injectable with novel or repurposed TB drugs in otherwise standard MDR-TB treatment regimens
Reduce the risk of adverse effects when injectables are used for MDR-TB
• Formal guidance on safe intramuscular injection in children, including age-appropriate injection site selection
• Addition of local anesthetic (lignocaine) to intramuscular injections for pain reduction
• Use of potentially otoprotective medications such as N-acetylcysteine, aspirin
• Therapeutic drug monitoring
• Inhaled injectable treatment

simple and straightforward. Formal guidance is needed on safe intramuscular injection techniques in children. The addition of local anesthetic to intramuscular injections as the potential to greatly reduce injection pain, and data is urgently needed. Strategies to reduce the risk of ototoxicity, the most serious and concerning injectable-related adverse effect, are urgently needed. The use of NAC, aspirin, or other antidotes deserves evaluation in trials in adults and/or children, and could be done within the context of other planned studies. Audiological screening of high- and ultra-high frequency hearing, could potentially lead to earlier detection of ototoxicity and discontinuation of injectables prior to more severe hearing loss that would be more likely to affect speech thresholds. Improved capacity to deliver basic audiology services in high TB burden countries is needed. Strategies that utilize age-appropriate testing and can be delivered in a decentralized fashion by non-audiologists with minimal training, such as tele-audiology and the use of smart phone technology, should be explored [77–79]. Although traditional peak and trough measurements are unlikely to predict ototoxicity, appropriately timed therapeutic drug monitoring could potentially help predict the risk of ototoxicity and inform a safer duration of treatment [80]. Inhaled therapy with the second-line injectables may also be a way to deliver these medications to the site of disease while limiting systemic exposures and thus reducing adverse effects [81]. Information on the safety and efficacy of inhaled injectable treatment is relevant, however, the feasibility of delivering inhaled therapy at a programmatic level could be an important limitation. Inhaled therapy targeted to achieve similar systemic exposures as parenteral therapy would negate many of the benefits of inhaled delivery [82].

Work to address these priority issues would greatly improve the overall safety and tolerability of MDR-TB treatment in children.

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REVIEW

Adverse effects of oral second-line antituberculosis drugs in children

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ABSTRACT

Introduction: Increasing numbers of children with drug-resistant tuberculosis are accessing second-line antituberculosis drugs; these are more toxic than first-line drugs. Little is known about the safety of new antituberculosis drugs in children. Knowledge of adverse effects, and how to assess and manage these, is important to ensure good adherence and treatment outcomes.

Areas covered: A Pubmed search was performed to identify articles addressing adverse effects of second-line antituberculosis drugs; a general search was done for the new drugs delamanid and bedaquiline. This review discusses adverse effects associated with oral second-line antituberculosis drugs. The spectrum of adverse effects caused by antituberculosis drugs is wide; the majority are mild or moderate, but these are important to manage as it could lead to non-adherence to treatment. Adverse effects may be more common in HIV-infected than in HIV-uninfected children.

Expert opinion: Although children may experience fewer adverse effects from oral second-line antituberculosis drugs than adults, evidence from prospective studies of the incidence of adverse events in children is limited. Higher doses of second-line drugs, new antituberculosis drugs, and new drug regimens are being evaluated in children: these call for strict pharmacovigilance in children treated in the near future, as adverse effect profiles may change.

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1. Introduction

Children with drug-resistant tuberculosis (DR-TB) are increasingly accessing care. An estimated 850,000 children develop tuberculosis (TB) every year; of these, approximately 25,000 will have multidrug-resistant tuberculosis (MDR-TB; i.e., resistance to at least isoniazid and rifampicin) [1]. Although the proportion of children accessing appropriate second-line antituberculosis treatment for mainly MDR-TB is still relatively small, the number of children treated is increasing due to changes in global guidelines for diagnosis and treatment. MDR-TB is treated with combinations of the second-line antituberculosis drugs, which the World Health Organization (WHO) recently reorganized into four groups (Table 1) [2]. Two novel TB drugs, bedaquiline and delamanid, now included in WHO Group D2, have recently received conditional approval for use in adults with MDR-TB and will become more frequently used components of MDR-TB treatment in the near future. Currently recommended MDR-TB treatment regimens in children typically include five to seven medications for as long as 18 months. Second-line antituberculosis drugs have more adverse effects than the first-line drugs. This, combined with the large number of medications used for long duration, lead to frequently observed adverse effects in children on MDR-TB treatment. However, there is a paucity of high-quality prospective data on the safety and tolerability of these drugs in children.

There are a number of reasons why the safety of the second-line antituberculosis drugs should be reviewed in children. First, children may experience a different spectrum, frequency, or severity of adverse effects compared to adults, and although efficacy trials of antituberculosis drugs or regimens are usually not required in children with tuberculosis, dose finding, safety, and tolerability studies of these drugs remain critically important in children [3]. However, few such studies have been done to date. Adverse antituberculosis drug effects are often reported to be less common in children compared to adults; possible reasons may be lower concentrations of drugs at the same mg/kg body weight doses in children compared to adults and the difficulty in assessing adverse effects in children.

Second, health-care providers caring for children with MDR-TB should have a detailed knowledge of the possible adverse effects of the second-line antituberculosis drugs in order to safely and successfully manage children's treatment. Antituberculosis treatment includes a number of drugs, which all have potential adverse effects, which may be more severe if certain drugs are combined, and children with MDR-TB may have comorbid conditions, such as HIV coinfection, requiring medications which could have similar adverse effects to the antituberculosis drugs. In current complicated multidrug regimens, it can be difficult to determine the culprit drug(s). It is also important to do baseline testing (before starting treatment), as abnormalities from other causes may already be present.

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Article highlights

- The number of children accessing second-line antituberculosis drugs is increasing and new antituberculosis drugs are entering the child tuberculosis treatment armamentarium
- There is a lack of high quality prospective data on the safety and tolerability of second-line antituberculosis treatment in children
- Health care providers managing children with drug-resistant tuberculosis should have detailed knowledge of possible adverse effects of second-line antituberculosis drugs
- In designing new multidrug-resistant tuberculosis regimens for children, the safety of component drugs must be a key consideration
- Some adverse effects, such as hypothyroidism, are more common in HIV-infected children compared to HIV-uninfected children
- Mild to moderate adverse events, such as abdominal disturbance, pruritis and skin rash are common, and may lead to non-adherence. However, severe adverse events, such as severe cutaneous adverse drug effects and hepatotoxicity leading to interruption or permanent discontinuation of drugs, are relatively rare

This box summarizes key points contained in the article.

Severe adverse effects, such as severe hepatotoxicity or severe cutaneous adverse effects, should lead to immediate discontinuation of the likely drug(s) responsible, as there is a high risk of mortality. Even so, with some severe adverse effects (e.g. hepatotoxicity), patients can be rechallenged with the likely responsible drug(s), while in others, a rechallenge with the responsible drug may be fatal. Some adverse effects are not fatal but could cause irreversible morbidity. Although many adverse events are mild or moderate and do not require stopping or changing of drugs, if these are not addressed by the health-care workers, adverse events may lead to non-adherence because they are unpleasant or unacceptable to the patient or their family [4,5]. Health-care workers managing patients with MDR-TB should also be aware of possible rare but severe adverse effects, which can occur with antituberculosis drugs. In some cases, there may be no alternative drug options to those drugs causing adverse effects, and it may be necessary to weigh the risks of tolerating potentially serious adverse effects versus poor or failed response to treatment.

Finally, MDR-TB treatment outcomes in children are usually better than that in adults, with more than 80% successfully treated [6,7]. However, improving the safety and tolerability of treatment while maintaining the efficacy is an important priority in children. In designing novel MDR-TB regimens for children, the safety of component drugs will be a key consideration. An in-depth understanding of individual drugs and any potential overlapping or synergistic adverse effects will be important for constructing future safe and well-tolerated MDR-TB regimens in children.

In this review, we provide a brief description of the more important adverse effects of the currently available oral second-line antituberculosis drugs (injectable agents are reviewed separately in this journal). We also include the new antituberculosis drugs soon to be available in children: delamanid and bedaquiline.

Table 1. World Health Organization drug groups for second-line drugs recommended for the treatment of rifampicin resistant and multidrug resistant.

Drug groups	Drug names	Abbreviations
Group A. Fluoroquinolones	Levofloxacin	Lfx
	Moxifloxacin	Mfx
	Gatifloxacin	Gfx
Group B. Second-line injectable agents	Amikacin	Am
	Capreomycin	Cm
	Kanamycin	Km
	(Streptomycin – only if susceptible in special cases)	(S)
Group C. Other core second-line agents	Ethionamide/prothionamide	Eto/Pto
	Cycloserine/terizidone	Cs/Trd
	Linezolid	Lzd
	Clofazimine	Cfz
Group D. Add-on agents (not part of the core multidrug-resistant TB regimen)	D1 Pyrazinamide	Z
	Ethambutol	E
	High-dose isoniazid	H^h
	D2 Bedaquiline	Bdq
	Delamanid	Dlm
	D3 *p-Aminosalicylic acid*	PAS
	Imipenem–cilastatin	Ipm
	Meropenem	Mpm
	Amoxicillin–clavulanate (only used with imipenem or meropenem)	Amx-Clv
	(Thioacetazone – only for HIV-negative cases)	(T)

TB: tuberculosis.
Data taken from Ref. [2].

2. Methods

We searched PubMed without date or language restrictions, using the following search terms: 'tuberculosis, second-line drugs, adverse effects or side-effects, child*'; 'tuberculosis, multidrug-resistant, treatment, adverse effects or side-effects, child*'; and 'tuberculosis, drug-resistant, treatment, toxicity, child*'; many abstracts were not applicable to the review, and there was a great overlap between search results. We also searched adverse effects or toxicity for each individual drug in children and used a general search where no published data were available in children, such as with the new drugs. Abstracts were reviewed and full-text articles retrieved for studies with relevant information. The reference lists of identified articles were searched for additional relevant reports.

3. Definitions

Published literature on toxicity of drugs often uses different terms inconsistently. Therefore, we clarify our use of terms for this review as follows: an adverse (drug) effect (adverse drug reaction) is an unintended symptom, sign, condition, or abnormal laboratory test finding caused by taking a specific drug (or in some cases, a combination of two or more drugs). An adverse event refers to any unfavorable and unintended symptom, sign, condition, abnormal laboratory finding, or disease temporally associated with the use of a drug regardless of whether it is considered related to the drug [8]. An adverse effect is a special type of adverse event in which a causative relationship can be shown [8].

Standard grading of many adverse events is available, such as in the Division of AIDS table of adverse events, and can assist in decisions of whether to discontinue treatment or not and for safety monitoring purposes in clinical research [8].

4. Prevalence of adverse events from second-line antituberculosis drugs in children

Children, especially those younger than 10 years of age, seem to tolerate second-line combination antituberculosis therapy better than adults [9,10]. There are few studies of MDR-TB in children which have monitored adverse events closely [7,9]. In a systematic review and meta-analysis, adverse events were documented in 39.1% (95% confidence interval [CI]: 28.7–49.4) of HIV-infected and HIV-uninfected children treated for DR-TB [6]. However, in a small cohort of adolescents coinfected with HIV, adverse effects were documented in 8/11 (72%) cases, and in a systematic review and meta-analysis of MDR-TB in HIV-infected adults and children, adverse events were documented in 92.5% (95% CI: 83.7–100) of HIV-coinfected children [11,12]. Our own experience in children is also that HIV-infected patients have higher rates of adverse events compared to HIV-uninfected children [13,14]. However, the majority of adverse events in children are mild to moderate, not necessitating interruption or complete cessation of treatment, and even with the few severe adverse events, permanent discontinuation of drugs is rarely necessary [7,9].

5. Adverse effects associated with the second-line antituberculosis drugs

Table 2 summarizes the most common adverse effects with the drugs that could be responsible and how to monitor for these adverse effects. Table 3 summarizes adverse effects by antituberculosis drug, including rare adverse effects.

5.1. Fluoroquinolones (WHO Group A)

The fluoroquinolones used for antituberculosis therapy are ofloxacin (Ofx) and, more frequently, its L-isomer levofloxacin (Lfx) and moxifloxacin (Mfx). The fluoroquinolones inhibit the mycobacterial DNA gyrase (topoisomerase II) leading to disruption of bacterial DNA synthesis, resulting in cell death [15]. The fluoroquinolones have bactericidal activity against metabolizing bacilli, with a relative potency against *Mycobacterium tuberculosis* of Mfx>Lfx>Ofx [16]. They are also active against dormant mycobacteria [17]. Fluoroquinolones are well absorbed after oral administration and distribute widely in the body including in cerebrospinal fluid (CSF).

The most frequent adverse effects of fluoroquinolones are gastrointestinal disturbances (0.9–4.7%) including loss of appetite, nausea and vomiting, abdominal discomfort, and anorexia [18,19]. In rare cases, fluoroquinolones have been associated with *Clostridium difficile* infections causing mild diarrhea to life-threatening pseudomembranous colitis [20].

Central nervous system (CNS) adverse effects occur in up to 5% of patients, although less than 0.5% are serious [21,22]. These are often dose dependent and generally reversible after cessation of the fluoroquinolone [18,19]. Dizziness, headache, and drowsiness are the most common complaints, while more severe effects such as hallucinations, agitation, anxiety, restlessness, paranoia, depression, suicidal ideation, psychosis, and convulsions are rare [18,19,23]. Cases of peripheral neuropathy, Guillain–Barré syndrome, and secondary intracranial hypertension associated with fluoroquinolone use have been reported [19,24,25]. In children receiving Ofx, mood/sleep disturbances have been reported and may be underrecognized [18].

Dermatological problems occur in less than 0.5% of patients. Photosensitivity, usually mild but which may be severe, is a fluoroquinolone class effect, but the phototoxic potential of Ofx, Lfx, and Mfx seems to be minimal [22,26]. Cutaneous hypersensitivity reactions can present as rash, pruritus, urticaria, erythema, and angioedema [22,27]. Although rare, severe reactions such as toxic epidermal necrolysis or Stevens–Johnson syndrome have been reported in adults and children following fluoroquinolone therapy [28–30].

Concern about musculoskeletal adverse effects, particularly chondrotoxicity, has historically limited the widespread fluoroquinolone use in children. In studies on juvenile animals, fluoroquinolones as a class exhibit a potential to induce irreversible cartilage damage on weight-bearing joints [31]. Nevertheless, data from multiple observational studies concluded that there is no documentation of sustained injury on bone or joint growth or severe or irreversible arthropathy in children although there may be some association between fluoroquinolones and reversible arthralgia [19,32,33].

Data on the risk of arthropathy in children on long-term fluoroquinolone therapy, as for antituberculosis treatment, are limited. In children receiving Ofx for between 6 and 18 months, Grade 1 to 2 musculoskeletal disorders [8] occurred in 3–9% of patients with no signs of severe arthropathy [7,18,34]. In a small case series on five children on Lfx-containing antituberculosis regimens, two children experienced joint pain necessitating discontinuation of Lfx in one [35]. The arthropathic potential of long-term Mfx therapy in children is difficult to assess due to limited reports; of 23 children 7–15 years of age in one study, five reported arthralgia, which resolved spontaneously without discontinuation of Mfx [36]. Tendonitis and Achilles tendon rupture are extremely rare in children. We could not find a single case report of tendon rupture in children; this agrees with our clinical experience, having treated hundreds of children with fluoroquinolones for long durations (6–24 months).

Fluoroquinolones, especially Mfx, may cause QT-interval prolongation [19]. Studies in children receiving Mfx (n = 32) and Lfx (n = 23) for DR-TB treatment found no evidence of QT prolongation >450 ms [36–38]. We did not identify any report on arrhythmia or sudden death associated with fluoroquinolone use in children. Nevertheless, clinicians should be aware of this possible adverse effect especially when

Table 2. An alphabetical list of more common adverse effects due to drugs used in multidrug-resistant tuberculosis treatment, likely drugs that could be responsible, and how to monitor for these adverse effects.

Adverse drug effect	Possible causative drugs	How to monitor	Frequency of tests[a]
Arthralgia/arthritis	FQNs, Z (especially in combination), Rfb	Painful joints, watch gait, examine joints Uric acid levels (Z)	Routine follow-up[a] Uric acid levels (not done routinely)
Blood dyscrasias	H, Eto/Pto, Rfb, Z, E, FQNs, PAS, Cm, Cla	Clinically – anemia, petechiae Full blood count when suspected	Routine follow-up
Central nervous system toxicity: headache, drowsiness, seizures, weakness, insomnia, and hallucinations	FQNs (insomnia and hallucinations), H, Eto/Pto, Cs/Trd, Cla	History from parent/patient	Routine follow-up
Depression/psychosis	H, Eto, Cs/Trd	History and observation	Routine follow-up
Endocrine effects			
- hypothyroidism	Eto/Pto, PAS	TSH/fT4/fT3	2 to 3 monthly
- gynecomastia	Eto/Pto, H	Physical examination	Routine follow-up
Flu-like symptoms	Rfb, PAS	Clinical examination/history	Routine follow-up
GIT disturbances: nausea, vomiting, abdominal pain, and diarrhea	Very common, mainly Eto/Pto, E, PAS, FQNs, Cfs, Lzd, Bdq	Mainly history of abdominal complaints. With new-onset vomiting or abdominal pain, consider hepatitis	Routine follow-up Alanine transferase and bilirubin if new-onset symptoms
Hearing impair/ototoxicity	Am, Km, Cm, S	Audiology	Monthly while on SLID and 6 months after completion of SLID
Hair loss	H, Eto/Pto	History/clinical examination	Routine follow-up
Intracranial hypertension	FQNs	History/ophthalmoscopy and CT scan/LP when suspected	Routine follow-up
Jaundice/hepatotoxicity	H, Z, Eto/Pto, PAS, FQNs (Mfx more likely)	New-onset vomiting, abdominal pain, and jaundice	Alanine transferase and bilirubin – usually as clinically indicated but can do 2-monthly
K^+ (potassium) loss	Cm, PAS	K^+ levels	2-monthly
Lactic acidosis	Lzd	Clinical examination and lactate levels if suspected	Routine follow-up
Myelosuppression	Lzd	Full blood count	Monthly
Nephrotoxicity	Cm, Am, Km, S	Serum creatinine	2-monthly
Optic neuritis: vision acuity/color blindness	E, Lzd, H, Eto/Pto, PAS	Vision and color blindness testing	Routine follow-up
Peripheral neuropathy	Lzd, H, Eto/Pto, Cs/Trd	History of pain in hands/feet, watch mobility and gait, test sensation, position	Routine follow-up
Pancreatitis	Lzd	Abdominal pain/clinically, serum amylase if suspected	Routine follow-up
QTc-interval prolongation	Bdq, Dlm, FQN (Mfx), Cfz, Cla	ECG	Currently not routinely done except with Dlm and Bdq
Rashes/cutaneous adverse drug effects including pruritus	Z, FQNs, Cs/Trd, PAS, and many other	History and clinical examination	Routine follow-up
Skin discoloration – red skin	Cfz	Clinical examination	Routine follow-up
Tendinitis/tendinopathy	FQNs	Clinical examination	Routine follow-up
Uveitis	Rfb	Examination of eyes	Routine follow-up
Vasculitis	H, FQNs	Clinical examination	Routine follow-up
Vestibular toxicity	S, Am, Km, Cm	History, clinical observation	Routine follow-up

Am: amikacin, Bdq: bedaquiline; Cfz: clofazimine: Cla: clarithromycin; Cm: capreomycin; Cs: cycloserine; Dlm: delamanid; E: ethambutol; Eto: ethionamide; FQNs: fluoroquinolones; H: isoniazid; Km: kanamycin; Lzd: linezolid; Mfx: moxifloxacin; PAS: para-aminosalicylic acid; Pto: prothionamide; Rfb: rifabutin; S: streptomycin; Trd: terizidone; Z: pyrazinamide; CT: computed tomography; ECG: electrocardiogram; GIT: gastrointestinal; LP: lumbar puncture; QTc: corrected QT; SLID: second-line injectable drug; TSH: thyroid-stimulating hormone.
[a]Routine follow-up would be at least monthly initially and then 2-monthly.

fluoroquinolones are used with other drugs with QT-prolonging potential (e.g. delamanid, bedaquiline, clarithromycin, or clofazimine).

Disordered glucose regulation associated with fluoroquinolones, especially gatifloxacin, has been reported in adults (not in children) with differences between the fluoroquinolones and very low risk for Lfx and Mfx [19]. Further rare adverse effects of the fluoroquinolones include hemolytic anemia, hepatotoxicity, pancreatitis, interstitial nephritis, and ophthalmological problems [19]. None of these adverse events have been reported in children. However, ongoing surveillance is critically important as more children will receive long-term fluoroquinolone therapy as part of MDR-TB regimens in future.

5.2. Thioamides (WHO Group C)

The thioamides, ethionamide (Eto), and its propyl analog prothionamide (Pto) form important components in the treatment of MDR-TB and drug-susceptible disseminated TB (e.g. TB meningitis). Eto and Pto are prodrugs that, following enzymatic activation by mycobacterial EthA, inhibit InhA, a target shared with isoniazid [39], resulting in inhibition of mycolic acid biosynthesis and cell lysis [40]. Eto and Pto are bacteriostatic drugs but can be bactericidal at higher doses [41].

The main adverse effects of thioamide are gastrointestinal disturbances, resulting in nausea, abdominal discomfort, vomiting, diarrhea, and, as a result of these symptoms, weight loss [42]. Pto was developed to improve tolerability, but gastrointestinal adverse effects still occur in the majority of patients. Gastrointestinal intolerance is dose dependent, generally improves after the first weeks of therapy, and can often be reduced by dose ramping (increasing the dose over a few days) or giving divided daily doses at the beginning of therapy [43]. In children, we do not recommend antiemetics, although in adolescents this may be an option.

Table 3. Adverse effects (AEs) associated with second-line antituberculosis drugs in children.

Antituberculosis drugs	Adverse effects (AEs)
Fluoroquinolones - Moxifloxacin - Levofloxacin - Ofloxacin	Common: Gastrointestinal disturbance; central nervous system (CNS) AEs (e.g. dizziness, headache, drowsiness, and hallucinations); arthralgia; cutaneous AEs (usually mild, e.g., pruritus, rash, and photosensitivity) Rare: Arthritis; tendonitis; QT prolongation (Mfx>Lfx/Ofx); severe cutaneous AEs; hemolytic anemia; hepatotoxicity; pancreatitis; secondary intracranial hypertension; and ophthalmologic problems
Thioamides - Ethionamide - Prothionamide	Common: Gastrointestinal disturbances; hepatotoxicity; and hypothyroidism Rare: CNS AEs (peripheral neuritis, seizures, and psychosis); gynecomastia; alopecia; hypoglycemia; pellagra-like rash; and blood dyscrasias
Cycloserine/terizidone	Common: CNS AEs (dizziness, headache, tremor, insomnia, anxiety, lethargy, inability to concentrate, and depression) Rare: severe CNS AEs (peripheral neuropathy, suicidal ideation, psychosis, seizures, and encephalopathy); severe cutaneous AEs
Para-aminosalicylic acid	Common: Gastrointestinal disturbances; hypothyroidism Rare: Hepatotoxicity; hypokalemia; hypersensitivity reactions (fever and maculopapular rash); severe cutaneous AEs; and blood dyscrasias
Linezolid	Common: Myelosuppression (anemia, thrombocytopenia, and leucopenia); peripheral neuropathy Rare: Pancreatitis; vision loss (optic neuritis); hyperlactatemia and lactic acidosis, and rhabdomyolysis
Clofazimine	Common: Gastrointestinal disturbances; red–brown discoloration of the skin Rare: QT prolongation
Delamanid	Little experience in children. QT prolongation; hepatotoxicity in adults
Bedaquiline	No experience in children. QT prolongation; gastrointestinal AEs in adults

During therapy with Eto and Pto, asymptomatic elevation of liver transaminases frequently occurs in adults and children, and hepatotoxicity has been reported in about 2% of adult patients [44]. Following cessation of thioamide therapy, liver transaminases generally normalize, and it has been shown that thioamides can be restarted without recurrence of hepatotoxicity in children [45].

Thioamides inhibit thyroid hormone synthesis by blocking organification and uptake of iodine into thyroid cells. During long-term therapy, hypothyroidism occurs frequently (20–70%) in adults and in children [13,46,47]. HIV infection and concomitant treatment with para-aminosalicylic acid (PAS) are associated with an increased risk for hypothyroidism [13,48]. Although the clinical significance of hypothyroidism due to thioamide therapy has not yet been evaluated, our practice is to supplement thyroxine in children with elevated thyroid-stimulating hormone (TSH) and low free thyroxine levels, given the potential impact of hypothyroidism on neurodevelopment in young children [7,13].

Thioamide use has also been associated with CNS toxicity including peripheral neuropathy, psychosis, behavioral disorders, seizures, or pellagra-like encephalopathy in adults, but there are no reports in children [42]. Niacin or pyridoxine supplementation might be beneficial in case of nervous system toxicity [49].

Other rare adverse effects of thioamides include gynecomastia, alopecia, hypoglycemia, pellagra-like rash, and blood count alterations [42].

5.3. Cycloserine/terizidone (WHO Group C)

Cycloserine is an analog of the amino acid D-alanine and inhibits enzymes needed for synthesis of peptidoglycan, a key component of the *M. tuberculosis* cell wall [50]. Terizidone is a molecule formed by the combination of two molecules of D-cycloserine [51]. Cycloserine and terizidone are bacteriostatic drugs with relatively weak antimycobacterial activity at currently recommended doses, yet are still currently routinely used for MDR-TB treatment in adults and in children. They do have the advantage of good CSF penetration, which is important for the treatment of tuberculous meningitis.

Cycloserine is known to exert CNS effects as an agonist of N-methyl-D-aspartate receptors and at low doses is being explored as a potential treatment for a number of neuropsychiatric conditions [52]. At the much higher doses used in TB treatment, cycloserine has well-known CNS adverse effects. These are highly variable, ranging from relatively mild effects including dizziness, headache, tremor, slurred speech, insomnia, anxiety, lethargy, inability to concentrate, to more serious effects including severe depression, suicidal ideation, psychosis, seizures, and encephalopathy [53,54]. In a small study, when cycloserine dosing was targeted to maintain peak concentrations between 20 and 40 µg/ml, only 4 of 60 participants experienced CNS adverse effects; all four had concentrations >40 µg/ml [55]. This suggests that these effects are dose related and concentration related, and symptoms are reversible upon cycloserine discontinuation or dose reduction. Cycloserine may lead to peripheral neuropathy via direct antagonistic effects on pyridoxine and by increasing its renal elimination; pyridoxine should be coadministered to reduce this risk [56]. Other more rare adverse effects, including Stevens–Johnson syndrome have been reported [57]. There is limited published information on terizidone. It has been suggested that terizidone has fewer adverse effects than cycloserine; however, there are no published data to our knowledge to support this assumption.

A recent systematic review and meta-analysis attempted to describe the frequency of cycloserine- and terizidone-associated adverse effects in adults treated for MDR-TB [58]. This review included 27 studies reporting cycloserine safety data, which included 2164 patients, and 10 studies reporting terizidone safety data, which included 707 patients. The pooled frequency of any adverse effect due to cycloserine was 9.1% (95% CI: 6.4–11.7), with the majority of these being psychiatric adverse effects. Reporting of terizidone safety was highly variable, with serious adverse effects described in 0–31% of participants in these studies. In adults, there is a large burden of preexisting and incident mental illness during MDR-TB

treatment, which complicates attribution of these psychiatric events; aggressive management with psychiatric medications appears to be very effective and may limit the need for cycloserine or terizidone dose adjustment [59]. There are limited published safety data for either cycloserine or terizidone in children with MDR-TB. A systematic review of children treated for MDR-TB reported 6 of 182 children had cycloserine-associated adverse effects, which included depression, anxiety, hallucinations, psychosis, and blurred vision [6]. Neurologic and psychiatric symptoms may be more difficult to elicit especially in young children, and a high index of suspicion is required. In our experience in children younger than 14 years of age, severe adverse effects with terizidone are rare.

5.4. Linezolid (WHO Group C)

Linezolid, an oxazolidinone class antibiotic, selectively inhibits bacterial protein synthesis. Its high durable efficacy against extensively drug-resistant (XDR)-TB (i.e. MDR-TB plus resistance to a fluoroquinolone and a second-line injectable agent) [60,61] and good CSF penetration have made it an increasingly important antituberculosis drug [62]. It is being evaluated as a component of multiple novel TB regimens, including treatment shortening regimens, currently under evaluation; however, severe toxicity remains a concern, and it is currently often reserved for the difficult-to-treat MDR-TB and XDR-TB cases in children.

Overall, adverse effects with linezolid are reported to be less frequent in children than in adults [63]. The mechanisms of most adverse effects are thought to be due to the inhibition of mitochondrial protein synthesis [64]. Gastrointestinal disturbances, such as vomiting and diarrhea, are the most common. These are usually mild, occurring before 1 month, and rarely require treatment modification [61].

Neurologic adverse effects are well described with linezolid in adults and children and are an important concern [65]. Peripheral neuropathy is the most frequent, occurring among patients on prolonged durations of linezolid therapy. It presents as paresthesia and numbness of the distal extremities in a 'stocking and glove' distribution and is not responsive to pyridoxine; although reversible, the improvement may be slow [66,67]. Optic neuropathy presents as painless, progressive loss of color vision and visual acuity, with onset generally after 3–12 months of linezolid treatment; it may improve after linezolid discontinuation but can result in permanent visual deficits [66].

Hematological adverse effects of linezolid are not uncommon and include reversible anemia, thrombocytopenia, and leucopenia [66,68]. These should be screened for routinely and usually respond to dose reduction. Other adverse effects are less common, but potentially serious and important to be aware of. Linezolid-associated hyperlactatemia and lactic acidosis have been described, which usually resolve over 1 to 2 weeks after linezolid discontinuation [66]. This has been reported in children, most frequently in association with liver disease or other serious comorbidities [69–71]. Rhabdomyolysis [72] and serotonin syndrome in patients receiving a serotonin agonist drug have rarely been reported with linezolid [66,73]. The majority of these adverse effects appear to be dose and time dependent, with higher doses and longer treatment durations greatly increasing the risk; however, the specific risks have not been well described.

In a systematic review including 107 adults treated with linezolid for MDR-TB/XDR-TB, 59% experienced an adverse effect, of which 69% required linezolid discontinuation or dose adjustment. The most common adverse effects were peripheral neuropathy (47%), anemia (38%), gastrointestinal disorders (17%), optic neuritis (13%), and thrombocytopenia (12%) [74]. Subsequent studies confirmed these findings [75]. There is a significant increase in risk of experiencing an adverse effect when using a dose >600 mg daily [74].

A 2014 literature review identified 18 children from eight different reports treated with linezolid for MDR-TB [76]. Fifty percent experienced an adverse effect, with peripheral neuropathy the most common (4/18, 22%); 2 (11%) had linezolid permanently discontinued, and 5 (28%) had a dose reduction. Gastrointestinal disturbances, hepatotoxicity, anemia, and thrombocytopenia were also reported. Subsequent reports have shown similar findings [77,78]. The single life-threatening adverse event was a case of severe pancreatitis and lactic acidosis [71]. Other antituberculosis drugs have overlapping toxicities and may have contributed to some of these adverse events. Peripheral neuropathy may be caused by high-dose isoniazid, ethionamide, and cycloserine/terizidone; therefore, close monitoring of these patients is especially important. HIV-infected children appear to be at higher risk for adverse events, possibly due to overlapping toxicities with the nucleoside reverse transcriptase inhibitor class of antiretrovirals which also inhibit mitochondrial DNA synthesis [79]. A recent study in 86 HIV-uninfected children with TB meningitis found no significant increase in frequency of adverse events when receiving additional linezolid for 1–4 months (linezolid group 33.3% [n = 36] versus control group 32%) [62].

Substantial inter-patient variability in linezolid clearance occurs, which could justify the role of therapeutic drug monitoring (TDM) in future. A center conducting TDM for linezolid in adults has reported a 0% adverse event rate [75].

5.5. Clofazimine (WHO Group C)

Clofazimine is a riminophenazine compound that has been used for the treatment of leprosy but is only now being explored for use in antituberculosis treatment. To date, its mechanism of action remains unclear. Clofazimine is a prodrug, which is reduced by type 2 NADH-quinone oxidoreductase (NDH-2), which then releases reactive oxygen species upon spontaneous reoxidation by O_2 [80]. It competes with menaquinone, the only quinone present in mycobacteria, and a key electron acceptor, for its reduction by NDH-2 [80].

To date, clofazimine has been used mostly in salvage antituberculosis treatment regimens, but the success of a shortened clofazimine-containing regimen for MDR-TB has generated interest in its more widespread use [81]. Clofazimine is now included in the WHO–recommended 9–12-month shortened treatment regimen for MDR-TB [2] and is

being evaluated as a component of multiple novel TB regimens in adults.

The most frequently reported adverse effects attributed to clofazimine are gastrointestinal and dermatologic problems. The most common gastrointestinal complaints include nausea, vomiting, diarrhea, and abdominal pain [82]. These are usually mild but can occasionally be severe [83]. Dermatologic adverse effects are more frequent and concerning. The majority of clofazimine-treated patients will develop reddish-black or orange discoloration of the skin, eyes, and body fluids [84]. This resolves slowly over the course of months, but can persist for longer [85]. Ichthyosis, a skin disorder with thickened, dry, scaly skin which can be quite distressing to patients, may occur in up to a quarter of patients treated with clofazimine [82]. Clofazimine is known to prolong the QT-interval; however, this has been poorly characterized. This is of particular concern if clofazimine is to be combined with Mfx and the novel TB drugs bedaquiline, delamanid, or pretomanid, all of which also cause QT-interval prolongation.

Most clofazimine safety data are from leprosy patients, and there is relatively little experience with clofazimine in the treatment of MDR-TB or in children. In a systematic review of the safety of clofazimine in MDR-TB and XDR-TB treatment, including five studies with 602 clofazimine-treated patients, the proportion experiencing a clofazimine-attributed adverse effect ranged from 0 to 11%; however, only 0.1% overall discontinued clofazimine [86]. Data from more recent studies have been somewhat variable. In a randomized controlled trial in 105 adults with MDR-TB who received an optimized background regimen (OBR) with or without clofazimine, in clofazimine-treated patients skin discoloration was reported in 94.3% and ichthyosis in 47.2%; there was no difference in gastrointestinal adverse effects between the two groups [87]. However, in a South African cohort, dermatologic adverse effects were reported less frequently, with a dermatologic reaction reported in only 6 of 42 (14%); only three participants in this cohort discontinued clofazimine [88]. There are limited data on children with MDR-TB treated with clofazimine. In a recently reported case series, seven children with MDR-TB were treated with clofazimine for a median of 20-month duration [78]. Clofazimine-attributed adverse effects were reported in four children, including one with transient gastritis, one with ichthyosis, one with red skin discoloration, and one with ichthyosis and red skin discoloration; clofazimine was not interrupted or discontinued in any case. Additional data in children are important, given the potentially increased use of clofazimine for MDR-TB treatment.

5.6. High-dose isoniazid (WHO Group D1)

Isoniazid is a bactericidal drug in drug-susceptible *M. tuberculosis*. It is a prodrug that is converted by a mycobacterial catalase-peroxidase to an active metabolite. Following activation, isoniazid inhibits the biosynthesis of mycolic acids in the mycobacterial cell wall [89]. Resistance to isoniazid is conferred mainly by mutations in either *katG* gene (usually intermediate- to high-level resistance) or InhA promoter region (usually low-level resistance) [90]. Patients infected with low-level isoniazid-resistant *M. tuberculosis* isolates may benefit from treatment with high-dose isoniazid [91].

Isoniazid is arguably the most used antituberculosis drug, and adverse effects are well-characterized. Hepatotoxicity is most serious: slight elevation of liver enzymes (less than three times upper limit of normal [ULN]) occurs in up to 40% with preventive therapy [92]; in this study, 16/227 (7%) had transaminase levels greater than three times ULN – four were switched to rifampicin preventive therapy. In an extensive review, Donald [93] showed that there is no significant influence of dose regarding those with increased serum transaminases; increased transaminase levels were reported in 141/1762 (8%) children receiving isoniazid at 10 mg/kg compared to 139/1406 (9.8%) children receiving doses of 10–20 mg/kg. However, persons with increased isoniazid concentrations or its metabolites, as found in slow acetylators of isoniazid, seemed more likely to experience rise of transaminase levels above normal [93]. New-onset vomiting and abdominal pain are early warning signs of significant hepatotoxicity; jaundice is a late sign, in which case all hepatotoxic drugs should immediately be discontinued, as continuation of these drugs, including isoniazid, could lead to rapidly fatal hepatic failure. Once transaminases and bilirubin levels have returned to normal, isoniazid can be reintroduced, but with high-dose isoniazid in an isoniazid-resistant case, the benefit of this should be weighed against the risk.

Dose-related neurotoxicity, such as peripheral neuropathy, seizures, psychosis, ataxia, and optic neuritis, is rare in children and can be prevented in high-dose isoniazid by adding pyridoxine to the regimen. Very rare hematological adverse effects, also responsive to pyridoxine, are sideroblastic anemia, hemolytic anemia, thrombocytopenia, and aplastic anemia. Skin rashes are common; in adolescents, acneiform eruptions are common [94].

5.7. Delamanid (WHO Group D2)

Delamanid (Deltyba®), previously OPC-67683, is a new antituberculosis drug derived from the nitro-dihydro-imidazooxazole class of compounds that inhibits mycolic acid synthesis [95]. The drug has been approved by the European Medicines Agency (EMA) and several other countries for treatment of MDR-TB in adults and is now also available through the Global Drug Facility. It is not yet approved by the United States Food and Drug Administration (FDA). It has been available through compassionate use from the manufacturer to children older than 12 years of age and more recently for children down to 6 years of age and more than 20 kg body weight [96].

Delamanid is likely a prodrug activated by mycobacterial F420-dependent Ddn coenzymes and is eliminated directly from plasma. It is not excreted in the urine but metabolized largely by plasma albumin. It is largely converted to its primary metabolite, DM-6705, which is thought to be mainly responsible for its effect of QTc prolongation [97].

Adverse events were similar in a study comparing delamanid 100 mg twice-daily, 200 mg twice-daily, and placebo group in patients on background MDR-TB treatment except for QTc prolongation, which differed significantly between the

three groups in pairwise comparisons (highest in the delamanid 200-mg twice-daily group). No patients with prolonged QTc interval were symptomatic, and no patient stopped delamanid due to QTc prolongation [95]. Administration of concomitant drugs with QT prolongation effect or CYP3A4 inhibitors (as the delamanid metabolite DM-6705 is metabolized by CYP3A4) and hypoalbuminemia may be associated with an increased risk of QTc prolongation [95,97].

Hepatotoxicity was found at a rate of 3% across all three adult MDR-TB groups in the study by Gler et al. [95]. However, a single report of severe hepatotoxicity in a 25-year-old male was reported to the EMA [98]. The EMA therefore advises to avoid delamanid in patients with moderate-to-severe hepatic impairment [97].

Two studies, a Phase 1, open-label, multiple-dose, age de-escalation trial to assess the pharmacokinetics, safety, and tolerability and a Phase 2 open-label, multiple-dose trial to assess the safety, tolerability, pharmacokinetics, and efficacy of delamanid in children with MDR-TB, are ongoing [99]. Provisional results have been presented in older children: of 13 HIV-uninfected children 6–17 years of age (six children <12 years of age), none experienced any serious adverse events, and no child had an absolute corrected QT by Fridericia's formula (QTcF) of >500 ms or an increase in QTcF from baseline >60 ms [100]. Although delamanid therefore seems safe and well tolerated in children, more data on dose and safety in children are required, especially in HIV-infected and in younger children; younger children are currently being enrolled.

5.8. Bedaquiline (WHO Group D2)

Bedaquiline (TMC 207) is a diarylquinoline, a new class of antituberculosis drug and the first new drug to be approved for tuberculosis treatment since rifampin in 1971 [101]. It is an adenosine triphosphate synthase inhibitor specific for *M. tuberculosis* and some nontuberculous mycobacteria; it kills both dormant and actively replicating mycobacteria [101]. Bedaquiline received accelerated approval by the FDA in 2012 for adults >18 years of age with MDR-TB [102]. WHO does not recommend using bedaquiline in children since there are no pediatric data available [103]; however, the Centers for Disease Control and Prevention has allowed for bedaquiline to be used off-label on a case-by-case basis in children [104]. No reports of bedaquiline use in children have yet been published. Two studies on dose finding, safety, and tolerability in children with MDR-TB and with and without HIV coinfection are planned – Janssen Therapeutics C211 (in HIV-uninfected children) has recently started enrolling children >11 years of age, and IMPAACT P1108 (HIV-infected and uninfected children) is scheduled to open late 2016 [105].

Nausea was the only adverse effect more common in the bedaquiline plus OBR MDR-TB treatment group compared to the control OBR MDR-TB treatment group in adults [106]. Other frequent adverse effects noted were headache and arthralgia [107]. QTc-interval prolongation is a known adverse effect, which usually is minimal, but in one study, 7/35 (20%) experienced a ≥60-ms increase in QT interval, leading to bedaquiline discontinuation in 2/35 (6%) cases [108]. Caution is therefore advised in using bedaquiline with other antituberculosis drugs with the potential of QT prolongation.

A higher mortality rate was found in the bedaquiline group in one randomized controlled trial comparing placebo plus OBR with bedaquiline plus OBR for treatment of MDR-TB. In this adult Phase 2 study (C208), 10 of 79 in the bedaquiline group vs. 2 of 81 in the placebo group died; only one death occurred within the 24-week trial period while receiving bedaquiline. No specific association with bedaquiline was identified in any of the other deaths, which occurred long after stopping bedaquiline [109]. This led to a black box warning and was included in the adverse effects section of the bedaquiline (Sirturo) product insert [102,110]. However, subsequent studies in adults under more programmatic conditions have not found increased mortality with bedaquiline [106].

5.9. PAS (WHO Group D3)

PAS was the first effective antituberculosis drug to be used in 1944; however, its mechanism of action remains unclear. Mutations in the thymidylate synthase gene, found in PAS-resistant *M. tuberculosis* strains, imply that PAS may inhibit thymidylate synthesis, and it could also interfere with the organism's acquisition of iron [111]. PAS is primarily considered a bacteriostatic drug, but a single high daily dose PAS might have bactericidal activity [112]. PAS is now mainly used as an enterically coated granular formulation.

PAS is often poorly tolerated mainly because of its gastrointestinal adverse effects. The granular PAS formulation seems to be better tolerated than older formulations; nevertheless, half of 12 patients reported mild nausea, vomiting, diarrhea, or bloating [113]. A recent study of once-daily compared to twice-daily dose of PAS granules showed little difference in tolerance in adult MDR-TB patients [114]. Gastrointestinal intolerance led to withdrawal of PAS in 5–7% of patients in two studies in adults [115,116]. No specific data are available in children, but in our experience, gastrointestinal disturbances, in particular diarrhea, are common, but usually resolve after 1 to 2 weeks.

Hypothyroidism, a common adverse effect associated with PAS, is caused by inhibition of thyroid peroxidase [13]. It is more frequent when PAS is used together with the thioamides and in HIV-infected children. In one small study, 15 of 19 (79%) children with a high rate of HIV infection developed hypothyroidism while on both PAS and ethionamide [117]. Our practice is to do thyroid function tests at baseline to exclude disease-related thyroid dysfunction and thereafter every 2 to 3 months. If both the TSH is raised and the free T4 is low, we supplement children with levothyroxine until the end of antituberculosis treatment [13]. Symptomatic hypothyroidism and goiter in children are rare.

Although hypokalemia is not specifically associated with PAS, it may occur in children who are severely malnourished and have diarrhea, especially in HIV-infected children [117].

Hepatotoxicity may occur in 0.3–0.5% of patients on PAS [94]. Although PAS and its metabolite are eliminated mainly in the urine, PAS is acetylated in the liver and intestines to N-acetyl-PAS; the latter is an inactive but hepatotoxic

metabolite [118]. If discontinuation of hepatotoxic drugs is required, as is the case with moderate or severe hepatotoxicity, PAS may be rechallenged once hepatic enzymes have normalized.

Hypersensitivity reactions, mainly fever and maculopapular rash and a case of drug reaction with eosinophilia and systemic symptoms syndrome, have been described with PAS, but these are rare [119,120].

The specific role of PAS as the cause of adverse effects in MDR-TB regimens has not often been studied. In a study from Korea, PAS was associated with adverse effects in 47/192 (24%) patients, the majority of which were gastrointestinal [121]. No such studies are available in children.

5.10. Amoxicillin/clavulanate (WHO Group D3; to be used with meropenem or imipenem)

The combination of a beta-lactam and a beta-lactamase inhibitor (clavulanate) has its effect on the mycobacterial cell wall [122]. *M. tuberculosis* produces a chromosomally encoded beta-lactamase enzyme, and therefore, the use of clavulanate is essential for killing *M. tuberculosis*. The efficacy of these combinations against *M. tuberculosis* is controversial, especially in cases where this is most needed, such as XDR-TB [122–125]. However, a few clinical studies have shown some positive effect [126].

Common adverse effects are gastrointestinal disturbances, including diarrhea, and skin rashes. Hypersensitivity reactions are known with amoxicillin, but can also be associated with clavulanate [127]. Severe hypersensitivity reactions or anaphylaxis should lead to discontinuation of the drug. Hepatotoxicity has been associated with amoxicillin/clavulanate, although a recent systematic review and meta-analysis has shown a low incidence of drug-induced liver injury with amoxicillin/clavulanate [128]. A number of cases have been described in children [129].

6. Conclusion

An increasing number of children with drug-resistant TB are being treated with second-line antituberculosis drugs, and new drugs are starting to be introduced. The second-line drugs are generally seen as more toxic, and they have many additional adverse effects compared to first-line drugs, which the health-care worker managing children with drug-resistant TB should be aware of. As children often cannot effectively communicate drug adverse effects, caregivers and health-care workers have a special responsibility to evaluate for these effects. Fortunately, the majority of currently known adverse effects are mild to moderate, but some adverse effects may be severe and irreversible and thus important to diagnose and to act upon early.

7. Expert opinion

The spectrum of adverse events due to second-line antituberculosis drugs in children is wide. It is often difficult to tease out the role of individual drugs as cause of specific adverse events, since antituberculosis drugs have overlapping toxicities and they are always used in combination regimens. Identifying the culprit drug is even more difficult if TB treatment is used with other medications, such as antiretroviral drugs. In this review, we have not addressed drug–drug interactions in depth, which could complicate matters even further.

Although it is frequently anecdotally reported that children experience fewer adverse events than adults on antituberculosis treatment, there is limited evidence from rigorous prospective clinical studies. Retrospective studies rely on health-care workers accurately documenting adverse events, which are frequently omitted. Furthermore, adverse events are difficult to monitor in children: a history of symptoms may be difficult to obtain in children; clinical examination for adverse events such as peripheral neuritis, hearing or vision loss, and depression or other CNS features is complicated and often not possible in young children; and special investigations such as laboratory tests and age-appropriate ophthalmological or hearing evaluation are rarely available in resource-limited settings where the majority of children with MDR-TB are treated. High-quality prospective studies in children of the safety of second-line containing MDR-TB treatment regimens are needed; such data are important for informing safety monitoring recommendations and for informing future regimen design.

There are currently few widely available child-friendly formulations of the second-line drugs, which results in children receiving split or crushed adult tablets or capsules. There is a potential for inaccurate dosing, which may increase the risk of adverse effects if this results in higher than recommended doses being given. Additionally, crushing or splitting adult tablets can worsen palatability and can contribute to drug-associated vomiting. The development of child-friendly formulations that are more palatable and allow more accurate dosing may improve the safety and tolerability of MDR-TB treatment in children.

The observed lower rate of adverse events in children may also be due to lower drug exposure in children than in adults using the currently recommended doses of second-line drugs, as has been found in several studies [34,36,38,130]. Adverse events may therefore increase as higher TB drug doses are recommended because many adverse events are dose related; this should be prospectively monitored. The aim is to dose a drug at its maximum effective concentration, but keeping adverse events to a minimum; to determine this optimal benefit vs. risk ratio, careful long-term prospective clinical studies of safety and tolerability and pharmacokinetic/pharmacodynamics in children are needed.

New and innovative methods may be necessary to better evaluate adverse events of antituberculosis drugs in children. Body mapping (drawing a life-size 'self' and writing on it what children experience) was used in a recent study with older children and teenagers to express what they feel; this helped children to articulate adverse events and experiences while on MDR-TB therapy [131]. Exploration of these and other innovative methods of adverse event assessment in children may improve our understanding of the safety profile of these drugs.

Regimens for DR-TB are changing; new drugs are becoming available, and new combinations to shorten treatment duration and exclude the injectable agents are currently being evaluated in adults and will soon follow in children. Careful pharmacovigilance is needed to document adverse events, of which some may be unexpected or new. Special investigations such as electrocardiogram monitoring and additional

laboratory tests will be required to determine optimal and safe drug use in new MDR-TB regimens in children.

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Paediatric formulations of second-line anti-tuberculosis medications: challenges and considerations

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SUMMARY

There is a growing number of children worldwide accessing second-line anti-tuberculosis drugs for multi-drug-resistant tuberculosis (TB); however, there are very few child-friendly formulations. For paediatric use, dispersible tablets offer distinct advantages over liquid formulations and other approaches. This is particularly relevant for TB, where stability, long shelf-life and reduced manufacturing, transport and storage costs are all critical to ensuring that drugs are accessible and affordable. In addition, fixed-dose combinations that reduce the pill burden and provide adequate taste masking may promote long-term adherence to anti-tuberculosis treatment and prevention regimens likely to last many months in children. Partial adherence may result in treatment failure and the further selection and spread of resistant mycobacteria. Unfortunately, no second-line TB paediatric drugs exist in dispersible formulations. We discuss here the main obstacles to developing such tablets and present strategies for overcoming them. We also advocate for timely anticipation of paediatric use when new TB drugs are being developed, and for the development of child-friendly anti-tuberculosis formulations in general.

KEY WORDS: CMC; TB; FDC; rifampicin; linezolid

TUBERCULOSIS (TB) is a serious health problem among children worldwide, causing an estimated 136 000 deaths and at least 1 million new cases each year.[1] Moreover, there appears to be an increase in the number of children with multi- (MDR) and extensively drug-resistant TB (XDR-TB).[2] Unfortunately, the development of paediatric TB medications trails behind those made available to adults. As the orally formulated, immediate-release, adult-strength tablets available were generally not designed for flexible/alternate dosing, they are difficult to adapt to redosing based on age and/or weight, which is customary in children.

One common makeshift solution is to crush tablets normally intended for adults and administer a portion of the powder to the child, a procedure that comes with a number of problems: inaccurate dosing (due to lack of information of what is appropriate), disruption of the coating (potentially affecting exposure and worsening palatability), improper/imprecise dose administration and a potential waste of the active pharmaceutical ingredient (API). These problems may worsen adherence, as these drugs are generally to be taken over a period of many months, often at home.

As an alternative, some of the second-line TB drugs are provided as liquid solutions or in suspension. However, these come with their own problems: the containers that contain these are bulky; liquids are generally less stable even when refrigerated; taste-masking is difficult; storage, packaging and safe transportation are expensive; and care givers prefer tablet formulations over suspensions for chronic conditions such as HIV.[3]

A third, and now preferred option, as outlined in the World Health Organization (WHO)/United Nations Children's Fund (UNICEF) new 2008 children's medicine guide is dispersible tablets,[4] which are dispersed in water before intake. Their main advantages are ease of administration, transport, storage and opportunities for taste masking. One successful example of taste masking in a drug for another condition is Coartem® Dispersible (Novartis, Basel, Switzerland), an anti-malarial developed especially for children as a sweet-tasting, cherry-flavoured tablet (www.mmv.org). Another example of taste-masking for paediatric use is the development of a quinine formulation with decreased solubility that ensures that the bulk of this bitter API is not released in the mouth but further down in the gastrointestinal tract.[5]

Another advantage of dispersible tablets is that

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Table 1 Paediatric formulations of second-line anti-tuberculosis medications: overview of challenges, ongoing work and recommendations

	LVX	MFX	Ethionamide
Existing formulations	250 mg scored tablets 500 mg scored tablets Suspension/solution of 25 mg/ml (not available via GDF) Note: some film-coated 250 mg tablets are not scored	400 mg non-scored, film-coated tablet	250 mg film coated, non-scored tablet; non-film coated tablet
PK work in children (summary of data/ ongoing work)	10–20 mg dose needed, with higher doses needed for MIC of >0.25 μg/ml[6] Levofloxacin dose 15–20 mg/kg in children of all ages[7] Ongoing work in Cape Town evaluating dosing of 15–20 mg/kg	Dose of 7.5–10 mg/kg in children of all ages[7] No published PK data in children aged <7 years Ongoing work in Cape Town	15–20 mg/kg is appropriate dose[8] Ongoing work in Cape Town unlikely to result in dose change
Dosing recommended for children[14]	WHO: 7.5–10 mg/kg once daily Expert opinion: 15–20 mg/kg once daily.	WHO: 7.5–10 mg/kg once daily	WHO: 15–20 mg total daily dose given twice daily; Expert opinion: 15–20 mg/kg total daily dose, given once daily twice daily if not tolerated
Challenges with existing formulations	Difficult to crush and mix; may reduce exposure when mixed with certain food and drinks	Not scored, difficult to crush, may reduce exposure when mixed with certain foods and drinks	Not scored, difficult to crush, may reduce exposure when mixed with certain foods and drinks
Increasing demand, clinical	Given to almost all children treated for DR-TB Use can be promoted by global networks providing training of treatment and prevention of DR-TB	Given to children treated for DR-TB; needed for XDR-TB regimens Use can be promoted by global networks	Given to almost all children treated for DR-TB. Use can be promoted by global networks.
Potential for inclusion in future research studies	MFX or LVX essential part of most backbone regimens Essential in preventive therapy trials	MFX or LVX essential part of backbone regimen Could be part of preventive therapy trials	Needs to be part of most backbone regimens

LVX = levofloxacin; MFX = moxifloxacin; PAS = para-aminosalicylic acid; GDF = Global Drug Facility; MIC = minimum inhibitory concentration; MDR-TB = multidrug-resistant TB; PK = pharmacokinetic; TB = tuberculosis; WHO = World Health Organization; DR-TB = drug-resistant TB; XDR-TB = extensively drug-resistant TB.

different pill strengths can be produced in order to finetune dosing by age and/or weight. These tablets can be scored (shaped with break-marks to allow easy division into two or four equal-sized parts) to provide further dosing flexibility. Unfortunately, no second-line dispersible anti-tuberculosis tablets exist today (Table 1). We review here the main obstacles that prevent the development of such formulations and how these problems could be successfully tackled.

DEFINING THE NEEDS

Policy and dosing guidelines

One of the key difficulties in developing dispersible tablet formulations of second-line TB medications is the absence of paediatric dosing guidance. Simple allometric scaling and extrapolating adult exposure to children usually will not suffice due to adult/child differences in gastric emptying and pH, gastrointestinal tract permeability in absorption surface area, expression of some drug transporters, biliary function, drug metabolism and renal clearance.[16] The required absolute drug dose in children may thus vary 100-fold,[17] and it is dangerous to assume that exposure will be comparable (can simply be scaled) even between older and younger children. Because of well-established differences between older and younger children and infants, the International Conference of Harmonization has recommended that childhood be divided into five stages with respect to clinical drug use.[18] Establishing paediatric exposure will therefore require dedicated clinical studies.

A summary of the pharmacokinetic data that are available to date is presented in Table 1, and a detailed discussion on paediatric dose-finding is provided in another article in this Supplement.[19] Once age-dependent dosing requirements have been established, dispersible tablets can be scored or manufactured in a small set of easily distinguishable shapes and strengths to accommodate dosing requirements. Established dosing guidelines are essential before the regulatory authorities can approve the

Table 1 (continued)

Cycloserine	PAS	Linezolid	Clofazimine
250 mg film coated hard caplet 250 mg capsule	Granules in sachets of 4 g or mixed; dosing spoon	600 mg non-scored tablets Suspension at 20 mg/ml (not available via GDF)	50 mg, 100 mg soft capsules
Limited work; 15–20 mg/kg is likely appropriate dose[9] Ongoing work in Stellenbosch University, on terizidone; unlikely to result in dose change	150 mg/kg total daily dose given once or twice daily in 10 children with MDR-TB[10] Ongoing work in Cape Town	Some work in children with cystic fibrosis shows need for higher dosing <10 years[11] Additional work needed,[12] as even adult dose not yet well established[13] No data on children with TB; ongoing work in Cape Town	No published PK in children
WHO: 10–20 mg/kg once daily	WHO: 150 mg/kg total daily dose given as split dose twice or thrice daily. Expert opinion: 150 mg/kg total daily dose given once or twice daily	WHO: None Expert opinion[14]: 10 mg/kg per dose given twice daily for children <10 years; 300 mg daily for children >10 years	WHO: none Expert opinion: 2–3 mg/kg or 3–5 mg/kg given once daily; if calculated mg/kg dose is lower than tablet/soft-gel formulation, could be given every other day[15]
Unable to be split, must open capsules and mix 'compound', may reduce exposure when mixed with certain foods and drinks	Dosing spoon limited at lower weight bands	Not scored, difficult to crush; suspension not widely available	Cannot be crushed or split
Given to children treated for DR-TB. Use can be promoted by global networks.		Given to children treated for DR-TB; needed for XDR-TB regimens. Use can be promoted by global networks.	Important for a growing number of children with XDR-TB; may become very important if the 9 month regimen/STREAM trial shows non-inferiority. Use can be promoted by global networks
Could be part of backbone regimen		Component of some oral, injectable-sparing shorter regimens being tested in future regimens	Is being tested in STREAM part 2 trial; may become part of standard of care

submitted information package for the dispersible tablets for registration and approval. Dosing guidelines have recently been specified for first-line anti-tuberculosis drugs for drug-susceptible TB; however, to date there have been no such recommendations for MDR-TB.

Stability and compatibility challenges with dispersible formulations

A dispersible tablet has to meet the specifications of the disintegration test (dispersal under 3 min) and 'fineness of dispersion test' (dispersed powder should pass through a 710 μM pore aperture sieve).[20] A common approach to generating dispersible tablets is the inclusion of high amounts of disintegrants such as sodium starch glycollate, microcrystalline cellulose, crospovidone and/or croscarmellose, excipients that swell upon contact with moisture and help disintegrate the tablet. The problem with many of these excipients is that they are highly hygroscopic (moisture absorbing), resulting in spontaneous softening and swelling of the tablets over time. Moreover, storage conditions in most of the high TB burden countries have high temperature and humidity. Dedicated manufacturing procedures and special packaging can reduce the risks of moisture ingress, using, for example, double-aluminium blisters. When flavourings and sweeteners are added, it is important to exclude the risk of interaction with the API(s); this means that long-term stability studies are necessary. Finally, all other excipient components in the mix should be thoroughly evaluated in combination for compatibility issues with the API. For example, the binding of quinolone antibiotics to divalent cations has been well-documented.[21]

Taste masking

The second-line anti-tuberculosis drugs available today comprise only a small set of chemical classes, and most have a highly bitter and obnoxious taste, for which children in particular have low tolerance. It is critical that these medicines, as used for children, are effectively taste masked and in addition contain child-adapted flavours. Poor palatability of medications has been shown to negatively impact treatment adherence in children,[22] which in the case of TB

could result in treatment failure, promote the emergence and spread of MDR-TB and waste resources. Today's anti-tuberculosis APIs are soluble and rapidly released, which tends to overwhelm taste masking and flavouring efforts. One strategy to mask the drug's taste involves adsorbing the drug onto a complex forming agent.[23,24] This can be achieved using ion-exchange resins that release their ligands at increased pH (upon entry in the duodenum). However these resins tend to display an unreliable ligand-release rate, complicating drug exposure. The API may also be complexed with cyclodextrins, but as these are required in large quantities, this leads to increased tablet size and manufacturing costs. Finally, barrier coating can be used to minimise the contact between the API and taste receptors with the help of insoluble polymers. Various manufacturing techniques are available to achieve such a formulation, for example spray drying, bottom spray, solvent evaporation, etc. These techniques require the use of specialised equipment to achieve a good barrier coat; moreover, the coatings may affect the API's release and exposure. Taste masking therefore needs to be balanced with effective drug release, and this process requires extensive experimentation; in most cases, changes in exposure that result from different formulations will need to be formally evaluated in pharmacokinetic studies in children.

Work on taste masking should be planned for and performed during the product development stage. Once a product is approved, significantly changing the composition requires extensive stability testing ($\geqslant$2 years). The early use of tools such as the 'artificial tongue'[25] and well-planned taste testing panels (involving children) would pay generous dividends.

Bioequivalence studies

Dispersible tablets represent an alternative solid dosage form to the existing immediate-release tablets. As previously mentioned, dispersible tablets contain different excipients and tend to release the API earlier. Bioequivalence studies are therefore required to characterise the rate and extent of API absorption and compare it with a comparator product representing the currently available dosage form. The paediatric dispersible tablet will contain a substantially lower amount of API than the currently available adult formulation. Multiple dispersible tablets will therefore need to be administered and compared with a single adult immediate-release tablet, or in some cases with multiple immediate-release tablets to achieve dose equivalence. The difference in release characteristics of the APIs and the formulations, combined with the different number of tablets, result in a complex bioequivalence evaluation scenario. Failure to properly evaluate all these parameters may trigger the need for further clinical efficacy studies conditional to successful registration. Timely development and acceptance of dosing guidelines and a decision on the appropriate comparator products may facilitate the process. Funding these important studies is problematic. As bioequivalence studies are typically performed in adults, funding sources that focus on paediatric applications usually do not pay for them, and because the results are applied to children, sponsors who primarily address TB in adults do not pay for them either.

Fixed-dose combinations

All TB regimens today involve multiple drugs; combining these as fixed-dose combinations (FDCs) is highly desirable, mostly for reasons of patient adherence. However, the development of FDCs further complicates the previously described challenges, making this task even more formidable for most combinations. Another problem is that the combination ratio is indeed 'fixed'; if the planned combination consists of one drug that is given at a single dose to all populations and a second one that requires finely tuned age- or weight-based dosing adjustments, then the required FDC cannot be designed (or multiple FDCs may have to be designed wherein one drug is kept at a constant dose while the other is varied).

Ideally, FDC dispersible tablets are manufactured so that they can be dosed in multiples to meet the requirements of different age groups. Dosing guidelines become a critical factor in deciding the quantities of each API in dispersible tablets. Even in its simplest form, formulating a FDC tablet in which the APIs are uniformly distributed is challenging, and is further complicated by the need to use suitable taste-masking strategies. There is also the risk that the APIs themselves may interact. Physical interactions between the first-line anti-tuberculosis drugs have been well documented.[23,24] This can be overcome by designing bilayered tablets, i.e., FDC tablets in which the APIs are deposited in layers.[26] However, the manufacture of bilayered tablets with stable formulations is challenging and may require special equipment and multiple trials.

Finally, bioequivalence must be demonstrated for each API and compared to the individual pharmacokinetics observed in clinical efficacy studies; this may fail if the drugs have different release profiles. Given the rapidly evolving anti-tuberculosis treatment landscape, pursuing paediatric FDCs of second-line drugs may be challenging.

Regulatory challenges

One of the main problems in providing worldwide patient access to new, innovative formulations is that registration, a lengthy, bureaucratic process, needs to occur on a country-by-country basis for the most

part, with requirements that vary by country. Examples of these differences include batch size, number of batches, how and how many stability studies were performed, disagreement over dissolution profile interpretation, bioequivalence study design, and choice and formulation of the comparator in the studies.

Some countries may refuse on principle to accept a new dispersible tablet on the basis of bioequivalence, requiring instead the demonstration of equivalent efficacy in clinical studies. Other countries do not use any FDCs. In some cases, it is mandatory that the reference product be sourced from within the country where approval is sought or the bioequivalence study be conducted in that country. Advances in this area include the WHO Prequalification Programme, which facilitates a joint review process and comprises an expanding set of countries and the introduction of the 'biowaiver procedure'.[27] The WHO Prequalification Programme is a 'quiet revolution' that addresses drug quality problems in countries with weak regulatory and legal monitoring.[28] The biowaiver implies that bioavailability and/or bioequivalence can in some cases be based on dissolution tests instead of new in vivo studies.

Supply management

The demand for second-line anti-tuberculosis drugs is very limited compared to first-line drugs. Manufacturers generally tend to maintain a low inventory to avoid expiration-related losses. Batch sizes are typically determined by production (equipment) capacity, shelf life and market demand. As a rule, batch manufacturing is to be initiated when 80% of the batch is covered by existing orders. In the subsequent steady-state marketing situation, batches are to be shipped with no less than 75–85% of shelf life remaining. In practice, however, the initial required order volume is often not reached, and an exhibit batch size is kept that is as small as possible, normally 125 000–200 000 tablets. A frequent problem is mass drug expiration. There is clearly room for suppliers, procurement agencies, customers and donors to overcome this waste by streamlining the production-to-patient flow. Stocking of these drugs through a central procurement mechanism such as the Global Drug Facility is another possible solution.[25] This option would ensure the ready availability of the product as needed.

LESSONS FROM DEVELOPMENT OF DISPERSIBLE AND FIXED-DOSE COMBINATIONS FOR FIRST-LINE PAEDRIATIC ANTI-TUBERCULOSIS DRUGS

For first-line treatment of paediatric TB, dispersible tablets and FDCs have already been developed, overcoming the challenges listed above. Most of the dispersible FDCs include rifampicin (RMP). One of the biggest hurdles in developing these was the stability problems associated with the combination of RMP and isoniazid (INH), and these APIs tend to generate a complex that results in a reduction in RMP bioavailability.[23,24] Problems associated with other APIs stem from hygroscopicity (a tendency to absorb water) of ethambutol, problematic divisibility of scored INH/RMP dispersible tablets and other difficulties. Other challenges included taste masking and bioequivalence when comparing with reference (comparator) capsules or film-coated tablets.

Progress in this area of development was made thanks to the productive collaboration between funders, regulators, manufacturers and the TB Alliance. Creative solutions such as adding RMP later in the blend of ingredients to minimise contact with INH and moisture and the judicious selection of packaging materials have helped create elegant drug products. These products are tested to very high standards under varying storage conditions. Many of the hard-earned lessons learned in the above undertakings will instruct future efforts in developing paediatric dispersible tablets for second-line anti-tuberculosis medications.

PROGRESS IN DEVELOPING DISPERSIBLE AND FIXED-DOSE COMBINATIONS FOR SECOND-LINE PAEDIATRIC ANTI-TUBERCULOSIS DRUGS

Table 1 shows key APIs that are being considered for second-line paediatric TB treatment regimens, along with their key characteristics important for formulation-related decisions, and demonstrates the many knowledge gaps that must be addressed to move each of these forward as dispersible tablets. Many of the existing drugs and formulations have clearly been developed without considering their potential use in children; for example, some tablets are not scored. A more serious and recurring problem is that the pharmacokinetics/pharmacodynamics relation has not been established for children. Some prototypes of child-friendly formulations are early in development (Macleods Pharmaceuticals Ltd), including levofloxacin 100 mg dispersible tablets, moxifloxacin 100 mg dispersible tablets, ethionamide 125 mg dispersible tablets, cycloserine 125 mg capsules, and linezolid 150 mg dispersible tablets. However, substantial additional work would be needed on these formulations to bring them to market, including work on bioequivalence, quality assurance and regulatory approvals. These formulations are therefore unlikely to be available in the field in the short term; they are, nevertheless, an important step forward.

Table 2 summarises challenges faced in developing dispersible formulations of existing second-line anti-tuberculosis drugs, and some potential solutions. Of note, the newly approved drug delamanid has an

Table 2 Challenges with the development of child-friendly formulations of second-line anti-tuberculosis medications in children

	Challenges	Way forward
Policy and dosing guidelines	Currently a lack of existing dosing guidelines for existing second-line anti-tuberculosis medications Lack of guidelines inhibit regulatory agencies from approving paediatric formulations	Support PK studies of existing second-line anti-tuberculosis drugs in children of all ages International paediatric dosing recommendations based on PK data in children
Stability and compatibility challenges with dispersible tablets	Dispersible tablets use excipients which are highly moisture absorbing, and sensitive to high temperature and humidity conditions; long-term stability studies may be necessary Excipients in final formulation must be evaluated together for interactions with the API	Dedicated manufacturing procedures, such as use of double-aluminium blister packs, can limit the risk of moisture absorption
Taste masking	Existing second-line drugs are mostly bitter compounds, requiring substantial taste masking Taste-masking may impact drug exposure, increase tablet size, require specialised manufacturing techniques and increase manufacturing costs	Work on taste masking should be anticipated and performed early in product development Well planned tasting panels, including children where appropriate, and newer tools such as the 'artificial tongue' should be used
Bioequivalence studies	Bioequivalence studies are required for dispersible tablets Limited funding, as studies are for paediatric products but typically performed in adults	Ensure appropriate funding or incentives for these important studies
Fixed-dose combinations	Challenges with possible interactions between multiple APIs and excipients; may affect drug release, impacting bioequivalence May require specialised manufacturing techniques	Anticipate challenges and ensure appropriate funding and incentives Ensure PK of APIs are well described
Regulatory challenges	Need for country-by-country registration Regulatory processes may differ by country Limited market, so no financial incentive to register	Use and expand the WHO Prequalification Programme Use biowaivers to limit the need for bioequivalence studies, simplifying registration
Supply management	Limited demand results in challenges for production, storage and supply	Streamline production-to-patient flow of medication Use central procurement mechanisms, such as the Global Drug Facility

PK = pharmacokinetic; API = active pharmaceutical ingredient; WHO = World Health Organization.

advanced paediatric development programme, has completed pharmocokinetic testing in children as young as 6 years, is enrolling children aged <6 years in current PK studies, and has developed a scored, dispersible tablet of DLM (https://clinicaltrials.gov/show/NCT01856634). The other recently approved drug, bedaquiline, is just beginning to undergo pharmocokinetic testing in older children (Table 3).

An important final consideration is the price of the product. Efforts must be made to ensure that the cost of developing a paediatric formulation does not result in a product that is too expensive for TB programmes to procure and use in the field.

CONCLUSIONS

While challenging, much can be learned from earlier reformulation successes for first-line TB treatments, and this can be used to guide development of subsequent formulations; their successful development also illustrates how the process can be driven by productive partnerships. However, many gaps in knowledge in the use of most of second-line anti-tuberculosis drugs in children remain, especially regarding optimal dosing.[29] It is of the greatest importance that existing clinical trials and formulation efforts already take into account how new regimens can be administered to children, and that, at the very least, adequate data on adult drug exposure are available to guide subsequent paediatric formulation development. The need for paediatric formulations and, in particular, the development of dispersible tablets for the treatment of TB in children is a medical necessity.[30] Because most TB APIs have an unpleasant taste but must be taken over a long period of time, the importance of developing taste-masked dispersible tablets for children remains a high priority, forming an essential link in the chain that runs from patient detection and diagnosis and drug access to adherence and treatment success.

Table 3 Delaminid and bedaquiline: two recently approved drugs

	Delamanid	Bedaquiline
Existing formulations	50 mg tablet	100 mg non-scored tablet
PK work in children (summary of data/ ongoing work)	Ongoing work in children aged <6 years (the Philippines, South Africa) PK studies completed in children aged ⩾6 years	Early PK work starting in older children
Dosing recommended for children[14]	For children aged ⩾13 years, the dosing recommendation is the same as in adults (100 mg by mouth twice daily)	No recommendation
Challenges with existing formulations	Current tablet is film-coated, but company has developed a scored, dispersible tablet	Current tablet is not scored; development of paediatric formulation planned
Increasing demand, clinical	Access to this drug outside of clinical trials has been very limited; Has been given to children as young as 13 years on compassionate grounds; Important in children with resistance to FQs, injectables or both	Adult doses used in children as young as 16 years Important in children with resistance to FQs, injectables or both and where delamanid is not available
Potential for inclusion in future research studies	Awaiting results of PK and safety trials in children Could be important in injectable-free regimens for children Possible role in preventive therapy trials	Additional data on safety and dosing in children needed

PK = pharmacokinetic; FQ = fluoroquinolone.

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RESUME

Il y a dans le monde un nombre croissant d'enfants ayant accès aux médicaments antituberculeux de deuxième ligne pour traiter une tuberculose (TB) multirésistante, mais il y a peu de formulations vraiment pratiques pour les enfants. En médecine pédiatrique, les comprimés dispersibles ont des avantages nets sur les formes liquides et d'autres approches. Ceci est particulièrement pertinent en matière de TB, où une bonne stabilité, une longue durée de conservation et des coûts réduits de fabrication, de transport et de stockage sont tous cruciaux pour s'assurer que les médicaments sont accessibles and abordables. De plus, les combinaisons à dose fixe qui réduisent le nombre de comprimés et masquent suffisamment leur goût contribuent à une bonne adhésion à long terme aux protocoles de traitement et de prévention de la TB pour les enfants, car ces protocoles peuvent durer de longs mois. Une adhésion partielle peut aboutir à un échec du traitement et à davantage de sélection et de propagation de mycobactéries résistantes. Malheureusement, aucun traitement de TB pédiatrique de deuxième ligne n'existe sous forme dispersible. Nous discutons ici des principaux obstacles à l'élaboration de tels comprimés et présentons des stratégies sur la manière dont on pourrait les surmonter. Nous plaidons également pour une anticipation précoce de l'utilisation pédiatrique lors de l'élaboration de nouveaux médicaments antituberculeux et pour l'élaboration de formulations anti-tuberculeuses en général acceptables par les enfants.

RESUMEN

Cada vez un mayor número de niños en el mundo tiene acceso a los medicamentos de segunda línea contra la tuberculosis (TB) multidrogorresistente, pero existen muy pocas formulaciones de estos medicamentos que sean adaptadas al uso pediátrico. Los comprimidos dispersables, cuando se utilizan en pediatría, ofrecen ventajas claras con respecto a las presentaciones líquidas y otras preparaciones. Esta ventaja adquiere un interés especial en el caso de la TB, donde la estabilidad adecuada, un período de conservación prolongado y los bajos costos de producción, transporte y almacenamiento son características primordiales que favorecen la accesibilidad y la asequibilidad de los medicamentos. Además, las asociaciones en dosis fijas que disminuyen la cantidad de comprimidos y enmascaran el sabor facilitan el cumplimiento del tratamiento antituberculoso y los regímenes preventivos, que suelen durar muchos meses. Un cumplimiento terapéutico parcial puede llevar a un fracaso y favorecer una mayor selección y la diseminación de las micobacterias resistentes. Desafortunadamente, no se cuenta aun con tratamientos antituberculosos de segunda línea en forma dispersable. En el presente artículo se analizan los principales obstáculos al desarrollo de este tipo de comprimidos y se proponen estrategias encaminadas a superarlos. Se preconiza también la anticipación oportuna del uso pediátrico de los medicamentos antituberculosos que están en vía de desarrollo y la obtención de formulaciones mejor adaptadas al uso pediátrico en general.

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