Drug-drug interactions between antiretrovirals and bedaquiline

by

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For submission as a mini-dissertation toward completion of Master of Public Health to the University of Cape Town.

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DECLARATION

I, *…Mishal Pandie……*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Tuberculosis (TB) is a leading cause of morbidity and mortality worldwide. People living with HIV are particularly susceptible to TB infection, and treatment of HIV-TB co-infection is challenging for multiple reasons, including potential drug-interactions.

Drug-resistant TB is difficult to treat and is associated with high treatment failure rates, mainly because the antimycobacterial drugs currently available are ineffective against drug-resistant TB. Bedaquiline is a new antimycobacterial drug which has shown great promise through its excellent efficacy for treating drug-resistant TB. Being a new drug, however, potential drug interactions with antiretrovirals are a major concern.

Bedaquiline is metabolized in the liver by an enzyme called cytochrome P450 3A (CYP3A). The antiretrovirals nevirapine, efavirenz, and lopinavir/ritonavir (LPV/r) can affect the activity of this enzyme, and consequently affect the concentration of bedaquiline in the patient's blood. Nevirapine and efavirenz increase the activity of CYP3A, which may result in increased metabolism of bedaquiline, thus decreasing the concentration of bedaquiline, with consequent risk of treatment failure or the further development of drug-resistance. LPV/r inhibits the CYP3A enzyme, which may result in decreased bedaquiline metabolism, thus causing high concentration of bedaquiline in the blood, with consequent risk of toxicity.

We conducted a pharmacokinetic study in 43 adult patients with drug-resistant TB to evaluate the drug-interactions between bedaquiline and the antiretrovirals nevirapine and LPV/r. We did serial measurements of the bedaquiline concentration in their plasma over 48 hours, and compared these concentrations in patients who were on antiretroviral and those who were not on antiretrovirals.

Our results showed that nevirapine had no significant effect on bedaquiline concentrations, while patients on LPV/r had bedaquiline concentrations 2 fold higher than patients not on antiretrovirals. We could not determine the clinical significance of this, but recommend that

patients receiving LPV/r and bedaquiline in combination must be closely monitored for side-effects.

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Table of Contents

Part A: STUDY PROTOCOL

Title:

Drug-drug interactions between bedaquiline and antiretrovirals.

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SUMMARY

The treatment of multi-drug resistant (MDR-) and extensively-drug resistant tuberculosis (XDR-TB) are associated with poor success rates: 48% for MDR-TB, and 33% for XDR-TB according to the World Health Organization (WHO, 2013). Patients in whom second-line regimens fail have limited treatment options, and have a very poor prognosis.

Bedaquiline is a novel antimycobacterial drug active against drug-sensitive and drug-resistant TB (Andries et al. 2005). Phase 2 clinical trials of bedaquiline have shown great promise, with faster time to culture conversion in MDR-TB patients when added to standard therapy (Diacon et al. 2009)

Bedaquiline does not currently have regulatory approval or marketing authorization in South Africa, but has been made available through a national Bedaquiline Clinical Access Programme (BCAP) for inclusion in individually tailored treatment regimens for patients with pulmonary infection due to pre-XDR-TB or XDR-TB.

Bedaquiline is largely metabolized by cytochrome P450 (CYP) 3A to a less active N-monodesmethyl metabolite (M2). Current evidence of potential drug interactions with antiretrovirals is limited to small, healthy volunteer drug-interaction studies using single-dose bedaquiline. Nevirapine and LPV/r (but not efavirenz) are recommended for co-administration with bedaquiline.

Nevirapine is an inducer of CYP3A, and has not been shown to reduce bedaquiline concentrations significantly (van Heeswijk et al. 2011). This may however be an underestimation of the potential drug-interaction, which may result in sub-therapeutic bedaquiline concentrations if co-administered with nevirapine, with consequent failure of therapy, and potential development of further drug resistance.

Ritonavir is an inhibitor of CYP3A, shown to increase bedaquiline concentrations by 22% in a single dose study, but the inhibitory effect may be more substantial at steady state (van Heeswijk et al. n.d.). Higher concentrations of bedaquiline may result in increased toxicities, the most concerning of which are QT prolongation and elevated hepatic transaminases.

We propose to study the pharmacokinetics of bedaquiline when administered with nevirapine or lopinavir/ritonavir in patients with tuberculosis. Our study design will be observational: we propose to conduct pharmacokinetic sampling in participants enrolled into the BCAP. We will use intensive pharmacokinetics sampling and analysis techniques to describe and compare bedaquiline pharmacokinetics in patients receiving and not receiving antiretrovirals.

CONTENTS

1. ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
ART	Antiretroviral treatment
BCAP	Bedaquiline Clinical Access Programme
BMI	Body mass index
CI	Confidence interval
Cmax	Maximum concentration
Cmin	Minimum concentration
CYP	Cytochrome P450 isoenzyme
EDTA	Ethylenediaminetetraacetic acid
Hr	Hour
HIV	Human Immunodeficiency virus
LC-MS	Liquid chromatography – tandem mass spectrometry
LPV/r	Lopinavir/ritonavir
M2	N-monodesmethyl metabolite
M3	N-didesmethyl metabolite
NLME	Nonlinear mixed-effects
NNRTI	Nonnucleoside reverse transcriptase inhibitor
NRTI	Nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
PK	Pharmacokinetic/s
RCT	Randomized control trial
ULN	Upper limit of normal

2. STUDY RATIONALE

The estimated global incidence of tuberculosis (TB) in 2011 was 8.7 million new cases, with South Africa accounting for the 3rd highest at approximately 500 000 incident cases per year (World Health Organization, 2013).

The rising prevalence of drug-resistant TB is of great concern. In South Africa, the estimated proportion of new TB cases with MDR-TB is 1.8%, and 6.7% of previously treated TB cases (World Health Organization, 2013).

Multi-drug resistant TB (MDR-TB) is defined as resistance to both rifampicin and isoniazid, the 2 key drugs in the drug-sensitive TB regimen. Extensively-drug resistant TB (XDR-TB) is resistance to isoniazid, rifampicin, a fluoroquinolone and an injectable 2nd line TB drug. Pre-XDR-TB is resistance to isoniazid, rifampicin, and either a fluoroquinolone or an injectable 2nd line TB drug.

The second-line treatment regimens used for the treatment of MDR-TB and XDR-TB are more toxic, more expensive, and less effective than those used to treat drug-sensitive TB. WHO estimates treatment success of 48% for MDR-TB, and 33% for XDR-TB (World Health Organization, 2013). The treatment failure rate for XDR-TB is very high, as evidence by a retrospe2ctive study of 174 patients treated in South Africa which showed a 6-month sputum-culture conversion rate of only 19% (Dheda et al. 2010).

Pre-XDR and XDR-TB is treated with individualized treatment regimens designed according to drug-sensitivity testing results and the history of previous drugs used. A meta-analysis of outcomes in MDR-TB reported that success rates were higher if regimens included at least 4 effective drugs, which is very difficult to achieve in pre-XDR and XDR-TB (Ahuja et al. 2012). Patients in whom these second-line regimens fail have limited treatment options, and have a very poor prognosis.

Bedaquiline, previously known as TMC207, is a diarylquinoline antimycobacterial drug active against drug-sensitive and drug-resistant TB (Andries et al. 2005). Bedaquiline has the novel mechanism of action of inhibiting ATP synthase. Bedaquiline has no cross-resistance with

existing anti-TB agents. Phase 2 clinical trials have shown faster time to culture conversion in MDR-TB patients (Diacon et al. 2009).

On 28th December 2012, the U.S. Food and Drug Administration approved Sirturo (bedaquiline) as part of combination therapy to treat adults with multi-drug resistant pulmonary tuberculosis (TB) when other alternatives are not available (Food and Drug Administartion, 2012).

Bedaquiline does not currently have regulatory approval or marketing authorization in South Africa. However, a national Bedaquiline Clinical Access Programme (BCAP) has been established to provide access to bedaquiline for inclusion in individually tailored treatment regimens for patients with pulmonary infection due pre-XDR-TB or XDR-TB.

Patients with confirmed pulmonary pre-XDR or XDR-TB in whom standard therapy is not effective are eligible for bedaquiline use in a tailored treatment regimen. In addition to bedaquiline, this regimen must contain at least 3 anti-tuberculosis drugs to which the patient is known to be susceptible (based on recent drug susceptibility testing) or likely to be susceptible (based on known treatment history).

The South African National TB Cluster anticipate approximately 200 patients will be enrolled in this programme per year. Currently, 4 sites have been approved for the programme (Table 1), and more sites may be added in the future.

Table 1. Sites approved for bedaquiline Clinical Access Programme

Province	**MDR unit**
Gauteng	Sizwe Tropical Disease Hospital
Kwazulu Natal	King George V Hospital
Northwest	Klerksdorp/Tsepong Hospital
Western Cape	Khayelitsha sub-district, City of Cape Town

2.1. Dose and Pharmacokinetics of Bedaquiline

Bedaquiline is well absorbed after oral administration with food, and reaches Cmax at a median of 4 to 5 hours.(Andries et al. 2005) Bedaquiline is largely metabolized by cytochrome P450 (CYP) 3A to a less active N-monodesmethyl metabolite (M2). It undergoes triphasic elimination, and although the average half-life over a dosing interval is approximately 24 hours, its terminal half-life is approximately 5 months (Andries et al. 2005).

The recommended dose of bedaquiline is given as a loading dose of 400mg once daily for 2 weeks, followed by maintenance doses of 200mg 3 times per week for 22 weeks. It should be taken with food, as this increases bioavailability by 95%.

Table 2. Bedaquiline dosing schedule

	Week 1 and 2	Week 3 to week 24
Dose	400mg daily	200mg three times per week

In a double-blind randomized placebo-controlled trial of 47 patients with newly diagnosed multi-drug resistant pulmonary tuberculosis, 23 patients received bedaquiline (400mg daily for 2 weeks, followed by 200mg three times per week for 6 weeks) and 24 patients placebo, in combination with a standard five-drug second-line antituberculosis regimen (Diacon et al. 2009). The bedaquiline group had an increased proportion of conversion of sputum culture to negative at 8 weeks (48% vs. 9%).

Serial bedaquiline plasma concentrations were measured during the loading dose period at week 2 (over 24 hours) and during the maintenance dose period at week 8 (over 48 hours).

Table 3. Bedaquiline plasma concentrations. Adapted from Diacon et al. (Diacon et al. 2009)			
	Mean (±SD) Peak (ng/ml)	Mean (±SD) Minimum (ng/ml)	Mean (±SD) Steady-state (ng/ml)
Week 2	3270 ±1144	956 ±557	1770 ±701
Week 8	1659 ±722	620 ±466	902 ±535

The majority of patients achieved average steady-state plasma concentrations above the target of 600ng/ml. There was no significant difference in steady-state plasma concentrations between patients who did and did not have sputum-culture conversion. By week 8, steady-state plasma trough concentrations were reached 36 to 48 hours post-dose.

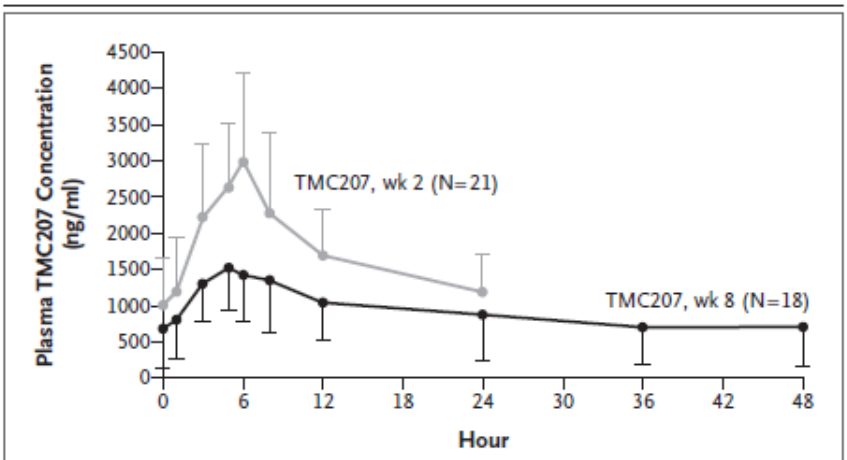

Figure 1. Mean (±SD) Plasma Concentration–Time Profiles for TMC207 at Week 2 and Week 8.
Dosing was selected to maintain plasma TMC207 concentrations above a target average steady-state plasma concentration of 600 ng per milliliter. T bars indicate standard deviations.

from Diacon et al. NEJM 2009. (Diacon et al. 2009)

2.2. Toxicity

Potential toxicities associated with bedaquiline include QT prolongation, hyperuricaemia, phospholipidosis, elevation of liver enzymes, nausea, joint pain, headache, chest pain, and haemoptysis (Janssen Therapeutics, 2012).

In the BCAP, the site investigator at each site takes full responsibility for the appropriate use of the investigational product including appropriate safety monitoring, as well as complying with safety reporting requirements to the Medicines Control Council, local health authorities and ethics committees.

2.3. Drug-interactions with ARVs

Of the 500 000 incident TB cases per year in South Africa, an estimated 330 000 (66%) are co-infected with HIV (WHO, 2013). Antiretroviral drugs used in the first line regimen induce CYP3A, while protease inhibitors, used in second and third line regimens, inhibit CYP3A. It is therefore important to study the effect of antiretrovirals (ARVs) on bedaquiline pharmacokinetics (PK).

The evidence for the use of nevirapine and LPV/r with bedaquiline is limited to small drug-interaction studies done in healthy volunteers with single-dose bedaquiline, and requires further evaluation at steady-state bedaquiline maintenance dosing.

Drug-interaction studies have explored the effect of CYP3A inducers (rifampicin, nevirapine, and efavirenz) and inhibitors (ritonavir) on bedaquiline plasma concentrations. These studies were, however, done with a single dose of bedaquiline, not at steady state. In addition, these studies were done in healthy subjects and had small sample sizes. They are therefore likely to be poor estimates of the drug-interactions patients are likely to experience and require further evaluation at steady-state bedaquiline maintenance dosing.

Rifampicin reduces both the area under the plasma concentration curve (AUC) and maximum concentration (Cmax) of bedaquiline by approximately 58% (Everitt et al. 2012).

Efavirenz is a non-nucleotide reverse transcriptase inhibitor (NNRTI) and an inducer of CYP3A. In a phase 1 drug interaction study in 33 healthy subjects, single-dose bedaquiline AUC was reduced by a median of 20% when administered with steady-state efavirenz (Dooley et al. 2012). The authors considered this unlikely to be clinically significant, but subsequent population pharmacokinetic modeling estimated that the reduction in steady state bedaquiline concentrations could be as high as 50% (Svensson et al. 2013). In addition, the Cmax of the less-active, toxic bedaquiline metabolite M2 was increased by 89% when co-administered with efavirenz. The AUC of M2 did not differ significantly. Efavirenz is therefore not recommended for concomitant use with bedaquiline in the BCAP.

Nevirapine is also an inducer of CYP3A. In a phase 1 drug-interaction study in 16 HIV-1 infected participants, single-dose bedaquiline AUC was not affected by steady-state nevirapine, but bedaquiline Cmax was reduced by 20% (van Heeswijk, et al. DATE). The ratios of the Least Squares means of bedaquiline Cmax was 0.80 (90% CI 0.62-1.04) and AUC was 1.03 (90% CI 0.87-1.22). The M2 C_{max} and AUC were not affected during co-administration of nevirapine.

Without data with both bedaquiline and nevirapine at steady-state, it is difficult to be sure that co-administered nevirapine will not significantly decrease the bedaquiline concentration and have a consequent negative effect on bedaquiline efficacy. Nevirapine may also increase the AUC or Cmax of the metabolite M2, and result in toxicity.

Ritonavir is an inhibitor of CYP3A, and is used in combination with lopinavir as the first choice protease inhibitor in South Africa. In a randomized open-label, cross-over trial conducted in 16 healthy subjects, single-dose bedaquiline was co-administered with LPV/r (van Heeswijk et al. DATE). Bedaquiline AUC increased by 22%, and there was no effect on bedaquiline Cmax. The ratio of the Least Squares means of bedaquiline C_{max} was 0.99 (95% CI 0.88-1.12) and AUC was 1.22 (95%CI 1.11-1.34). The M2 C_{max} and AUC were reduced by 51% and 41%, respectively when co-administration of LPV/r.

The clinical relevance of this interaction is unclear. If the steady state concentration of bedaquiline is significantly increased in patients on LPV/r, it may result in an increased risk of toxicity.

The BCAP recommends 3 options for ART in patients receiving bedaquiline:

- Discontinue or defer ART while patient is receiving bedaquiline and up to one month thereafter.
- A triple NRTI regimen of zidovudine/lamivudine/abacavir.
- A nevirapine or LPV/r containing regimen (in combination with appropriate NRTIs).

Clinicians are more likely to favour the nevirapine or LPV/r containing regimens. Clinicians are unlikely to defer ART for 24 weeks of bedaquiline plus an additional month thereafter, given the very poor prognosis of XDR-TB in patients with HIV infection. Clinicians are also unlikely to select the triple NRTI combination, as randomised controlled trials show that they are inferior to standard ART regimens, especially in patients with high viral loads (Gulick et al. 2004).

2.4. Proposed study

We will study the pharmacokinetics of bedaquiline when administered in HIV-uninfected and HIV-infected patients (with or without ARVs) treated for pre-XDR or XDR-TB nested in the national BCAP. We will use intensive PK sampling to better define the pharmacokinetics of bedaquiline in patients by HIV status, and to study the potential ARV-bedaquiline drug interactions.

We will collect data on bedaquiline and its metabolite M2 data using intensive PK sampling in the maintenance phase of treatment (after week 2). To assess for potential drug-interactions between bedaquiline and ARV (specifically LPV/r and nevirapine), we will compare data for patients on ART to those not on ART. Where possible, we will sample HIV positive patients pre- and post-ART initiation.

We will submit the study to the Human Research Ethics Committee of the University of Cape Town and the University of the Witwatersrand for approval, and will conduct the study in accordance with ICH GCP and the Declaration of Helsinki.

3. STUDY OBJECTIVES

3.1 To describe the steady state pharmacokinetics of bedaquiline in the study population.

3.2 To describe the difference in the pharmacokinetics of bedaquiline in patients not on ART to those who are on ART (specifically LPV/r and nevirapine).

4. STUDY DESIGN

The study is an observational, prospective pharmacokinetic study in patients on bedaquiline.

4.1. Endpoints

4.1.1. The steady state pharmacokinetics of bedaquiline in the study population.

4.1.2. The difference in area under the plasma concentration-time curve (AUC_{0-48}) of bedaquiline in participants who are on ARVs and those who are not on ARVs (LPV/r and nevirapine).

4.2. Sample size calculation

The sample size calculations are based on PK data from a study by Diacon et al. using bedaquiline in 23 patients with MDR-TB.(Diacon et al. 2009) In the study, the mean steady state plasma concentration of bedaquiline at 8 weeks was 956 ng/ml, with a standard deviation of ±535 ng/ml. Our sample size was calculated so that we would detect a 50% change in steady-state AUC with 80% power and 5% significance. As we would likely be

using non-parametric statistical testing, we added an additional 15% to the sample size calculated. We would therefore require a sample size of 16 participants in each of three treatment groups (no ART, nevirapine-based ART and lopinavir/ritonavir-based ART).

The exploratory analysis will include the data from 16 individuals (undergoing intensive PK sampling) in each of the 3 groups described above to evaluate the effects of nevirapine and lopinavir/ritonavir on the AUC (of primary interest), Cmax and trough concentrations of bedaquiline and its M2 metabolite derived using non-compartmental analysis.

4.3. Intensive pharmacokinetic sampling

Our aim is to perform intensive PK sampling on 16 patients in each of the following sub-groups:

1. Patients not on ARVs
2. Patients on steady-state nevirapine
3. Patients on lopinavir-ritonavir

Participants will undergo intensive pharmacokinetic sampling once they are in the maintenance dose phase (200mg 3 times per week) of their bedaquiline treatment. To ensure steady-state, this will be conducted after at least 1 week on the maintenance dose. To ensure standardisation, patients will be given a standardised meal, and all sampling will be done relative to the Wednesday dose (assuming dosing is Monday, Wednesday, Friday.)

Sampling will be done at 9 time-points: pre-dose, and at 1, 3, 4, 5, 6, 8, 24, and 48 hours post-dose (Table 3).

Table 3. Intensive PK sampling (option B – 9 samples)

PK sample	Number	1	-	2	3	4	5	6	7	8	9
	Time from dose	-5min	Dose given	1 hr	3 hrs	4 hrs	5 hrs	6 hrs	8 hrs	24 hrs	48 hrs
Example	Time	08h55	09h00	10h00	12h00	13h00	14h00	15h00	17h00	09h00	09h00
	Day	Day 1								Day 2	Day3

In HIV positive patients not yet on ARVs, our aim is to do intensive sampling before ARV initiation, and again after ARVs have been initiated. To ensure maximal CYP induction, patients on nevirapine must have completed at least 2 weeks of nevirapine and had their dose increased to 200mg twice a day. To ensure maximal CYP inhibition, patients must have been on LPV/r for at least 2 days.

5. STUDY METHODS FOR INTENSIVE PK SAMPLING

5.1. Study participants

Patients receiving bedaquiline as part of the national BCAP for the treatment of tuberculosis will be approached for enrolment into the study.

5.2. Inclusion criteria

5.2.1. All patients entered into the national BCAP for the treatment of tuberculosis.

5.2.2. HIV positive and negative adults (≥18 years).

5.2.3. Able to provide full informed consent.

5.3. Exclusion criteria

5.3.1. Active alcohol consumption in excess of 2 units/day or 14 units/week.

5.3.2. Gastrointestinal disease that may interfere with the pharmacokinetics of the drugs studied.

5.3.3. Receiving drugs known to induce or inhibit CYP3A4 or alter the pharmacokinetics of bedaquiline (not including ART) 30 days prior to sampling.

5.4. Study participant instructions and restrictions

As participants will be inpatients, drug administration and documentation of time ingested will be recorded by hospital staff. Patients must not ingest food and beverages which may affect drug metabolism (induction or inhibition of the CYP3A4).

5.5. Study treatments

As this is an observational study, all treatment will be provided as per the BCAP. Additional treatment, including ART and timing of initiation, will be decided by the bedaquiline site investigator along with the physician caring for the patient. All potentially eligible candidates for bedaquiline are reviewed by a national Clinical Advisory Committee and the DR-TB treatment and ARV regimens are agreed upon by this committee.

6. CLINICAL PROCEDURES FOR INTENSIVE PK SAMPLING

6.1. Screening

Patients receiving bedaquiline will be approached for enrolment into the bedaquiline/PK study.

6.2. Enrolment

Eligible participants will be enrolled after obtaining written informed consent. We will familiarize participants with study procedures during enrolment, and keep a log of all enrolled participants.

Data collection at enrolment visit will include:

- Date of birth
- Sex
- Race
- Height and weight
- Details of TB treatment history (microbiology, drug-sensitivity testing, previous drugs used)
- HIV status
- Antiretrovirals and concomitant drugs
- Date/s of starting ART and relevant ART regimens
- Routine baseline blood results: ALT, Haemoglobin, creatinine, and creatinine clearance, viral load, and CD4+ count. If not done by the physician caring for the patient in the preceding month, these tests will be repeated (except viral load and CD4+ count, within the last 6 months).

The above data (as well as additional variables) will be collected as part of the BCAP. Additional data on toxicities, sputum conversion, and clinical outcomes will be prospectively collected.

6.3. PK sampling procedure

6.3.1. Blood sampling

A venous blood sample of 4ml blood collected in a K3 EDTA tube will be collected for bedaquiline analysis at the time-points specified above.

Each sample will be labeled with the participant's study code, and the date and time the sample was taken. The exact times (to the nearest minute) of blood sampling will be noted in the case record form (CRF), as well as the exact time of dose ingestion prior to sampling.

6.3.2. Sample processing, storage and transport

The tubes will be pre-chilled on ice prior to use. Blood samples will be kept on ice and plasma will be separated and stored from the remaining blood within 1 hr of sample collection by centrifugation at 1500 – 2000 G for 10 minutes. Immediately after centrifugation, the plasma will be removed from cells and transferred into polypropylene tubes for storage at approximately -70°C. A duplicate sample will be kept for each time point. The samples will not be thawed after freezing prior to thawing for analysis. The time of sampling, storing and any sign of haemolysis will be recorded.

Samples will be stored at approximately -70°C. Once a batch of samples has been collected, they will be couriered on dry ice to the University of Cape Town (UCT) Division of Pharmacology laboratory in Cape Town.

6.5. Safety monitoring

Safety monitoring will be carried up by the physician caring for the patient (overseen by the BCAP site investigator) as part of the BCAP.

6.6. Data recording and reporting

All data will be recorded in the study specific CRF of each participant. Results of high bedaquiline concentrations which may be associated with high risk of drug toxicity will be reported to the BCAP site investigator and the physician caring for the patient.

7. ANALYSIS

7.1. Determination of drug-concentrations

Validated liquid chromatography – tandem mass spectroscopy (LC-MS/MS) methods will be used to determine bedaquiline and M2 concentrations in the plasma samples in the UCT Division of Pharmacology's laboratory, which is ISO17025 accredited for this purpose. Concentrations below the limits of quantification will be reported as such.

Bioanalysis of bedaquiline and M2 will be performed using a validated method consisting of a protein precipitation, using acetonitrile spiked with a mixture of 2 stable isotope labeled internal standards (TMC07-d6 and M2-d3), followed by high performance liquid chromatography with MS/MS detection using an ABSCIEX API4000. 20 µL aliquots of plasma are extracted and chromatographic separation is achieved using a Gemini-NX C18, 50 x 2 mm, 5 µm analytical columns with a mobile phase of 0.1% formic acid/acetonitrile at a flow rate of 0.5 mL/min. Detection is by tandem MS with ion spray operated in the positive ion mode. Analytes and internal standards are monitored at mass transitions of the protonated precursor ions m/z 555.2, m/z 541.1, m/z 561.1, and m/z 545.3 to the product ions m/z 58.1, m/z 480.1, m/z 64.3, and m/z 480.1 for bedaquiline (TMC207), M2, TMC07-d6 and M2-d3, respectively. The calibration curve fits a quadratic (weighted by 1/concentration^2) regression over the ranges 0.00977 – 5 µg/ml for Bedaquiline (TMC207) and 0.00313 – 0.2 µg/ml for M2.

7.2. PK analysis and statistics

The primary analysis will be performed on the intensive sampling PK data using non-compartmental analysis (NCA) to determine the following PK measures for bedaquiline:

- Cmin (pre-dose sample concentration)
- Cmax (peak plasma concentration)
- Tmax (the time to reach Cmax after drug ingestion)
- $AUC_{0\text{-}48}$ (the area under the concentration-time curve from 0-48 hours after drug ingestion)
- Half–life

The bedaquiline PK measures generated by NCA from the data in intensively-sampled patients will be compared in parallel groups of patients on LPV/r versus bedaquiline only, and nevirapine versus bedaquiline only, using a t-test for individual groups (or unpaired data), or equivalent nonparametric analyses for skewed data (eg. Mann-Whitney U-test).

NCA will be performed using the latest version of the software package STATA (StataCorp, Texas, US).

8. ETHICAL AND REGULATORY ASPECTS

8.1. Informed consent

The investigator or designated study team member will explain the nature, conditions and consequences of the study to the study participants. When necessary, a competent translator familiar with the study will assist in the informed consent process. A witness independent of the study will countersign the consent documents of illiterate patients. Each participant will be given a copy of the signed consent document. No participants will be enrolled without their written consent.

8.2. Data management and storage

A CRF will be compiled for each participant to collect subject-specific details and the exact times of PK sampling and drug dosing relevant to those samples. The raw data relating to the drug concentrations will be kept in a study-specific document. Duplicate plasma samples will be kept at -80^{o}C until one year after study completion.

8.3. Compensation for participation

Patients will be compensated for their time. They will receive R250 for the 3 day intensive sampling period.

8.4. Withdrawal

Participants may withdraw from the study at any time, without prejudice to them and with no effect on their rights to care. Participation in the study may be discontinued by the BCAP site investigator and/or attending physician if he/she deems it to be in the best interest of the patient, or if there is poor compliance or non-adherence to the study-related treatment.

8.5. Ethical and regulatory considerations

The risks and inconveniences of the study will be fully explained to each patient. No subject will be enrolled in the study before they have been fully informed about the study verbally and by means of a subject information document, with the aid of a translator if necessary and signed consent has been obtained on the study-specific consent form.

The study documents will be submitted to the Research Ethics Committee of the University of Cape Town. Approval of the protocol and any amendments will be obtained before implementation. The study will be conducted in accordance with the Declaration of Helsinki 2008, International Guidelines for Good Clinical Practice (ICH 1997) and according to national guidelines for Good Clinical Practice (National Department of Health 2006).

REFERENCES

Ahuja, S.D., Ashkin, D., Avendano M., Banerjee R., Bauer M., Bayona, J.N., Becerra, M.C., Benedetti A. et al. 2012. Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. *PLoS medicine*. 9(8), p.e1001300.

Andries, K., Verhasselt, P., Guillemont, J., Göhlmann, H.W.H., Neefs, J.M., Winkler, H., Van Gestel, J., Timmerman, P. et al. 2005. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. *Science*. 307(5707):223–227.

Dheda, K., Shean, K., Zumla, A., Badri, M., Streicher, E.M., Page-Shipp, L., Wilcox, P., John, M.A. et al. 2010. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. *Lancet*, 375(9728):1798–807.

Diacon, A.H., Pym, A., Grobusch, M., Patientia, R., Rustomjee, R., Page-Shipp, L., Pistorius, C., Krause, R. et al. 2009. The Diarylqunoline TMC207 for Multidrug-Resistant Tuberculosis. *The New England Journal of Medicine*, 360(23): 2397–2405.

Dooley, K.E., Park, J.G., Swindells, S., Allen, R., Haas, D.W., Cramer, Y., Aweeka, F., Wiggins, I. et al. 2012. Safety, tolerability, and pharmacokinetic interactions of the antituberculous agent TMC207 (bedaquiline) with efavirenz in healthy volunteers: AIDS Clinical Trials Group Study A5267. *Journal of acquired immune deficiency syndromes*. 59(5):455–462.

Everitt, D. Winter, H., Egizi, E. 2012. Pharmacokinetic interaction between the investigational anti-tuberculosis agent TMC207 and rifampicin or rifapentin. Proceeding of the 19th International AIDS Conference. Washington, DC. Abstract no. MOAB0304.

Food and Drug Administartion, 2012. *News Release*, Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm.

Gulick, R.M. Ribaudo, H.J., Shikuma, C.M., Lustgarten, S., Squires, K.E., Meyer, W.A., Acosta, E.P., Schackman, B.R. et al. 2004. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. *The New England journal of medicine*. 350(18):1850–1861.

Janssen Therapeutics. 2012. Sirturo information leaflet. Ittusville, NJ. Page 1–9.

van Heeswijk, R., Vandevoorde, A., Meyvisch, P., De Marez, T., McNeeley, D., Hoetelman, R. 2011. The effect of nevirapine on the pharmacokinetics of TMC207, an investigational antimycobacterial agent, in HIV-1-infected subjects. Proceeding of the *6th International AIDS Conference*. 17-20 July 2011. Rome, Italy.

van Heeswijk, R., Vandevoorde, A., Meyvisch, P., De Marez, T., De Beule, K., Mc Neeley, D, Hoetelman, R. 2010. The effect of lopinavir/ritonavir on the pharmacokinetics of TMC207, an investigational antimycobacterial agent. Proceeding of the *18th International AIDS Conference*. 18-23 July 2010. Vienna, Austria.

Svensson, E.M., Aweeka, F., Park, J.G., Marzan, F., Dooley, K.E., Karlsson, M.O. 2013. Model-based estimates of the effects of efavirenz on bedaquiline pharmacokinetics and suggested dose adjustments for patients co-infected with HIV and tuberculosis. *Antimicrobial agents and chemotherapy*. 57(6):2780-2787.

WHO. 2012. Global Tuberculosis Report 2012. *Geneva, Switzerland.*

APPENDICES

Appendix 1: Participant informed consent for intensive PK sampling form

PARTICIPANT INFORMED CONSENT for INTENSIVE PK SAMPLING

DATE OF FIRST INFORMED CONSENT DISCUSSION: ☐☐ / ☐☐ / ☐☐ ☐☐ *(dd/mm/yyyy)*

INTRODUCTION:

You are invited to take part in the research study, entitled: ***Drug-drug interactions between bedaquiline and antiretrovirals: a population pharmacokinetic approach*** (BDQ.ART study).

(*Blood levels of bedaquiline when given with or without antiretroviral drugs in patients with tuberculosis*)

RESEARCH INSTITUTION:

Division of Clinical Pharmacology, K-floor Old Main Building, Groote Schuur Hospital, Observatory.

Tel: 021 406 6008

Fax: 021 448 1989

STUDY DOCTOR:

Dr Mishal Pandie Tel: 021 406 6353 Cell: 082 874 1708

Person conducting informed consent interview: _____

Before you agree to take part in this study, you need to understand why the research is being done and what participation will involve. Please ask us if anything is not clear or if you need further information. It is important that you have enough time to decide. Do not agree to take part until you are satisfied that you fully understand what will happen, the potential benefits and the risks of the study.

Why have you been invited to take part?

You have been approached because you are being treated with the antituberculosis drug bedaquiline. We need to do tests of the new drug in HIV positive and HIV negative patients.

What is the purpose of the research?

Bedaquiline is a new drug used to treat tuberculosis. Previous studies have shown that bedaquiline is effective in treating tuberculosis. However, we do not know whether it is safe or effective to use bedaquiline at the same time as certain antiretroviral treatments. One of the concerns is that certain antiretrovirals may increase the blood levels of bedaquiline, and thus increase the risk of developing side-effects. Conversely, certain antiretrovirals may decrease the blood levels of bedaquiline, and thus may make bedaquiline less effective in treating the tuberculosis. If you are HIV positive, we want to study whether the antiretrovirals you are using, will increase or decrease the levels of bedaquiline. If you are HIV negative, or HIV positive and not yet on antiretrovirals, we want to compare your blood levels of bedaquiline to patients who are on antiretrovirals.

What are your options?

You do not have to take part in this study. It is your decision whether to take part or not.

If you decide to take part, you will be asked to sign that you consent to take part. You will be given a copy of this informed consent form to keep.

If you decide to take part, you can change your mind at any stage and withdraw before the study starts, or during the study. Not taking part or withdrawing from the study will not affect the standard of medical care that you receive.

What will happen to you if you take part?

You will be asked to sign this form to indicate that you understand what the study is about and that you agree to take part in the study. Study staff will have access to your medical records with regard to your tuberculosis treatment.

Study treatments: This study will not affect the treatments you are receiving. During the study period you will continue to take your regular treatment for tuberculosis, and antiretrovirals if you are HIV positive.

Blood samples: You will have blood taken for measurement of the amount of bedaquiline in your blood.

On the first day of blood sampling, we will put a cannula (a flexible plastic needle like those used to give fluids to hospitalized patients) into one of your arm veins. We will take blood samples from it 7 times during the day. The total amount of blood taken will be about 30 milliliters or 6 teaspoons. Each sampling will take approximately 20 minutes. You will need to be available for blood sampling from 8am to 5pm. The cannula will then be removed.

On the 2nd day, we will take another single blood sample, and on the 3rd day, another single blood sample. Each of these sampling procedures will take approximately 20 minutes.

If you are HIV positive, and not on antiretrovirals when the above 3 days of blood sampling takes place, we will repeat the 3 days of blood sampling once you are on antiretrovirals.

What will we use your blood for?

To measure the amounts of bedaquiline in your blood.

What will happen to the blood we take from you?

The samples used to measure the drug levels will be destroyed 1 year after the end of the study. The samples will not be used for any purposes other than those stated in this document.

What are the risks of taking part?

We will not change your tuberculosis or antiretroviral treatment. We will be taking blood to measure the amount of drug in your blood. The blood-taking process may cause discomfort or bruising, but these will be minimized because experienced health professionals will take the blood samples.

What if something goes wrong?

The sponsor of the study is the University of Cape Town. The university has taken out an insurance policy to compensate you for reasonable medical expenses related to injury or illness which result from taking part in the study (Internationally accepted guidelines will be used, as laid down by the Guidelines for Good Clinical Practice in the Conduct of Clinical Trials in Human Participants in South Africa). The insurance company will compensate you where the injury probably resulted from the blood-taking procedure. We would not be bound by these guidelines to pay compensation where the injury resulted from a drug or procedure outside the study protocol, or if the protocol was not followed.

What are the possible benefits of taking part?

You are unlikely to benefit personally from taking part in this study. The results of the study will benefit future patients who need treatment for tuberculosis and HIV at the same time. However, if we find any abnormalities from the blood tests that we do during the study, we will inform your doctor, so that your treatment can be adjusted if necessary.

Will your privacy be protected?

Yes, as far as is possible. Your medical records will be seen by some of the study staff, and by authorized persons who check to see that the research is being carried out correctly. Your records may also be seen by representatives of the South African regulatory authority (MCC) or the UCT Human and Research Ethics Committee. All those people are obliged to maintain your confidentiality. Your identity will not be given in any publication of the research findings.

Will you be compensated for your travel costs, time and inconvenience?

Yes. You will receive R250 as compensation for the time and inconvenience of participating in the study.

What will happen with the results of the research?

The results of the study will be published in a medical journal so that they are available to the medical profession throughout the world. No patients will be identified individually.

What organizations are supporting the study?

The study is supported and organized by the University of Cape Town.

What if you have complaints about the study?

If you want any information regarding your rights as a research participant, or have complaints regarding this research, you may contact Prof. Marc Blockman, the **Chairperson of the Research Ethics Committee** at the University of Cape Town (021 406 6492). If after you have consulted your doctor or the ethics committee and they have not provided you with answers to your satisfaction, you should write to: The Registrar, South African Medicines Control Council (MCC), Department of Health, Private Bag X 828, PRETORIA 0001.

INFORMED CONSENT FORM

……*(STUDY DOCTOR / DESIGNATED STUDY TEAM MEMBER)* has provided me with a copy of this informed consent form regarding the study, *Drug-drug interactions between bedaquiline and antiretrovirals: a population pharmacokinetic approach (BDQ.ART study)* and has fully explained to me the nature, risks, benefits and purpose of the study.

- I have been given the opportunity to ask any questions concerning the research study procedures, the potential benefits, and the risks.
- It has been explained to me that I am free to withdraw from the study at any time, without any disadvantage to my future care.
- I understand everything that has been explained to me and I consent to take part in this research study.

Participant name	*Signature / Thumbprint*	*Date*
Study doctor / designated study team member	*Signature / Thumbprint*	*Date*
Translator / person explaining informed consent	*Signature / Thumbprint*	*Date*
Witness if participant is unable to sign	*Signature / Thumbprint*	*Date*

Appendix 2: Patient record form

PATIENT RECORD

Patient name		Hospital/Clinic number	
Date of birth		Study site name	
Investigator name		Study ID number	

Demographic details					
Sex	Female ☐		Male ☐		
Race	Black African ☐	Coloured ☐	Indian/Asia ☐	Caucasian ☐	Other _____

Study details		
Sampling date	Type of sampling (Intensive/Sparse)	Patient weight

HIV details			
HIV status	Positive ☐	Negative ☐	Unknown ☐
Date HIV diagnosed:			
CD Nadir	Count:	Date:	

CD4 and viral load history and follow-up			
Date	CD4 count	Viral load	
ARV regimens			
Date started	Regimen	Date Stopped/Changed	Reason for stop/change

TB details		
Date of TB diagnosis:		Date pre-XDR/XDR confirmed:
Reason for bedaquiline: Pre-XDR ☐ XDR ☐		
Bedaquiline		
Date started	Date stopped	Reason for stop

TB drug regimens			
Date started	Regimen	Date stopped/changed	Reason for change

Additional notes/Comments

Monitoring

Sputum monitoring chart																	
Sample date	Lab number	Pos (+) ; Neg (-)		Resistant (R) ; Sensitive (S) ; No result – leave blank													
DD/MM/YY		Smear	Culture	LPA	LPA	Rif	H low	H high	PZA	Eth	Str	Ami	Km	Cm	Ofl	Mfx	Eto	Ter
		+/-	+/-	Rif	INH													

Blood test baseline and monitoring					
Date	K^+	Creatinine	GFR	ALT	Hb

QT baseline and monitoring		
Date	QTinterval (ms)	QTc interval (ms)

Additional notes/Comments

Appendix 3: Intensive sampling record form

INTENSIVE SAMPLING RECORD

Patient name		Study ID number	
Hospital number		Study site name	
Investigator name		Patient weight (kg)	
Last bedaquiline dose	Date: DD / MM / YYYY Time: HH : MM Dose in mg: _____		

Sample number	Time relative to dose	Planned sample time	Actual sample time	Sampler initials	Label details	Comments
Day 1: DD / MM / YYYY						
01	Pre-dose					
Time of observed dose: HH : MM Dose in mg: _____						
02	+1hr					
03	+3hrs					
04	+4hrs					
05	+5hrs					
06	+6hrs					
07	+8hrs					
Day 2: DD / MM / YYYY						
08	+24hrs					
Day 3: DD / MM / YYYY						
09	+48hrs					

Appendix 4: Standard operating procedure for intensive PK sampling

BDQ.ART Study: Standard Operating Procedure for intensive PK sampling

Preparation before PK sampling

i. Confirm patient consent form(s) completed.
ii. Label and chill K3EDTA tubes.
iii. Prepare phlebotomy consumables (cannulas, alcohol swabs, cottonwool, tape) and equipment (tourniquet, sharps container).
iv. Select the appropriate sampling form: intensive or sparse sampling.
v. Document: Patient name, folder number, study number.
vi. Document: Date and time of last dose of bedaquiline.

PK sampling:

Day 1

1. Insert cannula into an arm vein.
2. Draw pre-dose PK sample (document date and time of sample) and genetic sample (if patient consented).
3. Patient should have breakfast before ingestion of bedaquiline.
4. Observe patient taking dose of bedaquiline (document time).
5. Centrifuge bedaquiline sample at 1500 – 2000G for 10 minutes.
6. Remove plasma from cells and transfer with pipette to 2 polypropylene tubes.
7. Place polypropylene tube samples in freezer or dry ice.
8. Place EDTA genetic sample in freezer.
9. Set out schedule for the rest of the PK sampling based on the time of observed dose of bedaquiline. (+1hr, +2hrs, +3hrs, +4hrs, +5hrs, +6hrs, +7hrs, +8hrs, +24hrs, +48hrs)
10. Take samples at follow-ups as per PK sampling schedule. Remember to document the actual time the sample was taken.
11. After the 8hr sampling, confirm time for the next day's sampling with patient (the +24hrs sample).
12. Transport bedaquiline samples to facility with -70^{o}C fridge. (Can keep in -20^{o}C freezer, and transfer all specimen together on day 3).
13. Keep the genetic sample in a -20^{o}C freezer.

Day 2

14. Draw 24hr sample, document date and time, then follow instructions 5 to 7.
15. After the 24hr sampling, confirm time for the next day's sampling with the patient (the +48hrs sample).
16. Transport samples to facility with -70^{o}C fridge. (Or keep until day 3)

Day 3

17. On day 3: draw 48hr sample, document date and time, then follow instructions 5 to 7.
18. Transport samples to facility with -70^{o}C fridge.

Part B: LITERATURE REVIEW

Contents

Objectives of literature review:

- To understand the role of bedaquiline in tuberculosis therapy.
- To review the pharmacokinetics of bedaquiline and how antiretrovirals may affect the pharmacokinetics of bedaquiline.
- To review the current literature regarding drug-drug interactions between bedaquiline and antiretrovirals.
- To identify gaps in the literature.

Literature search strategy

The literature review included searching databases Pubmed and Google Scholar to identify relevant articles using the keywords BEDAQUILINE and TMC207 (the name by which bedaquiline was previously known). Because bedaquiline is a relatively new drug, this broad search strategy yielded a manageable list of publications which could be searched through manually.

The search was conducted on the 1st June 2015. Relevant scientific articles published in English in peer-reviewed journals were included. Furthermore, the reference lists within these identified articles were manually reviewed to identify further relevant literature. I also consulted with experts in the fields of pharmacology, tuberculosis and HIV, to identify appropriate articles and authoritative texts on the subject.

Summary and interpretation of literature

Introduction

The WHO estimates that there were 9.6 million new cases of tuberculosis (TB) in 2014 (World Health Organization, 2015). Of major concern is the rising numbers of patients with drug-resistant TB. Drug-resistant TB is associated with poor outcomes, mainly because of a lack of effective drugs (Calligaro et al. 2014). Bedaquiline is a new anti-TB drug shown to be effective in the treatment of drug-resistant TB (Diacon et al. 2014). HIV-TB co-infection is common, and the use of bedaquiline in patients co-infected with HIV is difficult because of potential drug interactions between bedaquiline and antiretrovirals.

This literature review aims to explore the current knowledge on bedaquiline pharmacokinetics and drug-interactions with antiretrovirals, and thus identify gaps in current literature so as to guide further research which will enable safe co-administration of bedaquiline and antiretrovirals.

The role of bedaquiline in TB treatment

Drug-sensitive TB is treated with a regimen of 4 anti-TB drugs for 6 months (World Health Organization 2010). The 2 most important drugs in this regimen are rifampicin and isoniazid. Multi-drug resistant TB (MDR-TB) is defined as resistance to both rifampicin and isoniazid (World Health Organization, 2010). Extensively-drug resistant TB (XDR-TB) is defined as resistance to isoniazid, rifampicin, a fluoroquinolone, and an injectable 2nd line TB drug. In 2014, there were 480 000 cases of MDR-TB globally, and 190 000 deaths from MDR-TB (World Health Organization, 2015). The WHO further estimates that 9.7% of cases with MDR-TB have XDR-TB.

The second-line anti-TB drugs in the 5 drug regimens used to treat MDR-TB and XDR-TB are more toxic, more expensive, and less effective than those used to treat drug-sensitive TB, and are a minimum of 18 months in duration (Calligaro et al. 2014). As a result, treatment failure and default rates are high in patients with drug-resistant TB. The WHO estimates treatment success rates of 50% for MDR-TB, and 26% for XDR-TB (World Health Organization 2015).

XDR-TB is treated with individualized treatment regimens designed according to drug-susceptibility testing and the history of previous drug use. Patients in whom second line regimens fail have limited treatment options, and a very poor prognosis. There is therefore a dire need for new effective drugs with better adverse effect profiles to treat DR-TB.

Bedaquiline, previously known as TMC207, is the first in a new class of antimycobacterial agents known as the diarylquinolones, and is active against both drug-sensitive and drug-resistant TB (Singh et al. 2013). Bedaquiline showed impressive efficacy in phase 2 clinical trials, and because of the dire need for new effective treatments for drug-resistant TB, was given accelerated approval by the U.S. Food and Drug Administration for the treatment of adults with MDR-TB in December 2012. Bedaquiline is now one of the most important drug available for the treatment of XDR-TB.

HIV-TB Co-infection

Tuberculosis is a contagious bacterial infection caused by *Mycobacterium Tuberculosis*. Individuals living with HIV are particularly susceptible to TB infection, and are estimated to be 26 times more likely to develop TB than HIV-negative individuals (World Health Organization 2015). The WHO estimates that 1.2 million of the 9.6 million new cases of TB in 2014 were co-infected with HIV (World Health Organization 2015).

Current international recommendations are for all patients with TB-HIV co-infection to be started on antiretrovirals within 2 to 8 weeks of starting TB therapy (World Health Organization 2013a). It is therefore essential for us to understand potential drug-drug interactions between antiretrovirals and bedaquiline.

The pharmacodynamics of bedaquiline

Bedaquiline has the novel mechanism of action of inhibiting mycobacterial ATP synthase. In vitro testing has shown it to have bactericidal and sterilizing activity against drug-sensitive and drug-resistant *Mycobacterium tuberculosis* (Chahine et al. 2014).

Area under the concentration time curve (AUC) refers to the plot of concentration of a drug in the blood plasma over a period of time (Brunton et al. 2008). It is a measure of the amount of drug the patient is exposed to. The pharmacokinetic parameter that correlates best with bedaquiline pharmacodynamics effect is thought to be bedaquiline AUC (Brunton et al. 2008). Drug interactions which decrease the AUC of bedaquiline may therefore result in decreased efficacy.

As part of a phase 2 randomized, placebo controlled trial in 47 patients with newly diagnosed MDR-TB, 23 patients received bedaquiline (400mg daily for 2 weeks, followed by 200mg 3 times a week for 6 weeks) (Diacon et al. 2009). The primary efficacy end point was the conversion of sputum cultures at 8 weeks, but the investigators also collected sputum at weeks 1, 2, 4, and 6 weeks. The rate of bactericidal activity improved between week 1 and 4, suggesting a time-dependent killing (Chahine et al. 2014).

The pharmacokinetics of bedaquiline

Pharmacokinetics refers to the movement of a drug into, through, and out of the body. The pharmacokinetics of a drug is described by measurements of its absorption, distribution, metabolism, and excretion (Brunton et al. 2008).

Bedaquiline is well absorbed after oral administration with food (food increases the bioavailability of bedaquiline by 95%) (Andries et al. 2005). After ingestion, bedaquiline reaches a maximum concentration in the blood at a median of 4 to 5 hours (Andries et al. 2005).

Bedaquiline has a large volume of distribution, approximately 164L. Bedaquiline has a very long half-life of approximately 5.5 months, which is thought to be due to its slow release following uptake in peripheral tissues (Chahine et al. 2014).

Bedaquiline is metabolized in the liver by the enzyme cytochrome P450 (CYP) 3A to a less active metabolite called N-monodesmethyl (M2) (Andries et al. 2005).

A drug which induces the CYP3A enzyme will result in increased metabolism of bedaquiline. This will result in lower plasma concentrations of bedaquiline, and consequently may result in treatment failure or the development of drug resistance.

A drug which inhibits the CYP3A enzyme will decrease the metabolism of bedaquiline. This will result in higher plasma concentrations of bedaquiline, and consequently may result in toxicity.

The dosing schedule of treatment of bedaquiline

When a patient starts on bedaquiline, they initially receive a loading dose of 400mg once daily for 2 weeks. This is followed by a maintenance dose of 200mg 3 times per week for a further 22 weeks (World Health Organization 2013b).

In a double-blind randomized placebo-controlled which included 47 newly diagnosed multi-drug resistant TB (MDR-TB), 23 patients received bedaquiline using the above dosing schedule (Diacon et al. 2009). Serial bedaquiline plasma concentrations were measured during the loading dose period at week 24 over 24 hours, and during the maintenance dose period at week 8 over 48 hours.

The majority of patients achieved steady-state plasma concentrations above the target of 600ng/ml. By week 8, steady-state plasma trough concentrations were reached 36 to 48 hours post-dose.

The efficacy of bedaquiline

In a randomized placebo-controlled trial conducted by Diacon et al., 160 patients with newly diagnosed MDR-TB were assigned to receive either bedaquiline or placebo in combination with a standard MDR-TB regimen (Diacon et al. 2014).

Patients on bedaquiline had a significantly faster median time to sputum culture conversion of 83 days (95%CI 56 to 97) compared to 125 days (95%CI 98 to 168) in the placebo group (hazard ratio 2.44 in the bedaquiline group; 95%CI 1.57 to 3.80; P<0.01). At 24 weeks, the bedaquiline group had a significantly higher rate of culture conversion of 79% compared to 58% in the placebo group (P=0.008). At 120 weeks, the bedaquiline group still had a significantly higher rate of culture conversion (62% vs 44%; P=0.04).

In France, a retrospective cohort study was conducted in patients with MDT-TB who received bedaquiline for compassionate use in combination with a median of 4 other

drugs (Guglielmetti et al. 2015). Of the 35 patients included in the study, 54% had XDR-TB, and the remainder pre-XDR. 1 death occurred, and was reported as unrelated to TB or anti-TB treatment. Bedaquiline treatment was stopped early in 2 patients who developed QT prolongation and 2 patients who developed raised liver enzymes. After 6 months of bedaquiline treatment, 28 of 29 cases achieved culture conversion.

Small cohort studies of 5 patients from India also reported excellent clinical outcomes, and had no significant adverse effects with the use of bedaquiline in patients with drug-resistant TB (Udwadia et al. 2014).

Bedaquiline toxicity

Bedaquiline is known to cause the following toxicities: QT prolongation (which can result in a potentially fatal cardiac arrhythmia), hyperuricaemia, phospholipidosis, elevation of liver enzymes, nausea, joint pain, headache, chest pain, and haemoptysis (Janssen Therapeutics 2012).

In the randomized placebo-controlled conducted by Diacon et al., 12 deaths were reported (Diacon et al. 2014). There were 10 deaths in the 79 patients who received bedaquiline, and 2 deaths in the 81 patients in the placebo group. TB was reported as the cause of death in 5 of the 10 bedaquiline group deaths. The reason for the higher number of deaths in the bedaquiline group remains unclear, and careful monitoring of patients with specific emphasis on QT prolongation is mandatory (World Health Organization 2013a). In addition, this highlights the importance of identifying drug interactions which may increase bedaquiline concentrations, and thus result in increased toxicity.

Drug-drug interactions with bedaquiline

Bedaquiline is metabolized by the liver enzyme CYP3A. Drugs that induce or inhibit this enzyme have the potential to affect the pharmacokinetics of bedaquiline.

HIV-TB co-infection is common, and it is therefore essential that we explore the potential drug interactions between bedaquiline and antiretrovirals. Antiretroviral regimens consist of 3 antiretroviral drugs. Typically, these regimens are made up of a backbone of 2 nucleotide reverse transcriptase inhibitors (NRTIs) and a third drug, either a non-nucleotide reverse transcriptase inhibitor or a protease inhibitor. The NRTIs do not affect the CYP3A enzyme, and are therefore unlikely to affect bedaquiline pharmacokinetics. The NNRTIs and protease inhibitors do, however, affect the CYP3A enzyme, and may therefore have an effect on bedaquiline pharmacokinetics.

Important drug-drug interaction studies relevant to HIV-TB co-infection

Rifampicin

Rifampicin is an anti-TB drug, and a potent inducer of CYP3A.

In a study looking at the effect of rifampicin on bedaquiline pharmacokinetics, 32 participants received 2 doses of bedaquiline, one with and one without rifampicin co-administration (Svensson et al. 2015). Blood sampling was done over 14 days after each bedaquiline dose. Rifampicin reduced the AUC of bedaquiline by approximately 58%. Therefore bedaquiline cannot be administered in combination with rifampicin in an anti-TB regimen.

Efavirenz

Efavirenz is a NNRTI which induces CYP3A (Rathbun & Liedtke 2011).

In a phase one 2-period sequential design pharmacokinetic drug interaction study conducted by Dooley et al., investigators evaluated the potential drug interaction between efavirenz and bedaquiline in 37 healthy volunteers (Dooley et al. 2012).

On study day 1, subjects received a single 400mg dose of bedaquiline orally. Serial blood samples for measurement of bedaquiline plasma concentrations were collected pre-dose and up to 14 days after the dose. For days 15 to 28, participants receive a night time 600mg dose of efavirenz to achieve efavirenz steady-state. A second 400mg dose of bedaquiline was then administered, and serial blood samples collected pre-dose and for 14 days after the dose. The efavirenz dose was continued until the end of the pharmacokinetic sampling.

Bedaquiline concentrations were then measured using high-performance liquid chromatography with tandem mass spectrometry. Concentration profiles when subjects received bedaquiline alone, and after co-administration of efavirenz were then compared.

Co-administration of efavirenz decreased the AUC of bedaquiline by 18% (95%CI 11% to 25%). Initially, this modest change in concentration was thought unlikely to have clinical significance. However, subsequent population pharmacokinetic nonlinear mixed effects modelling of the data by the same group of investigators estimated that the steady state bedaquiline concentrations could be reduced by as much as 52% (relative standard error 3.7%) when co-administered with efavirenz . Efavirenz can therefore not be co-administered with bedaquiline.

Nevirapine

Nevirapine is a NNRTI and inducer of CYP3A (Rathbun & Liedtke 2011).

In a phase one 2-period sequential design pharmacokinetic drug interaction study conducted by van Heeswick et al, investigators evaluated the potential drug-interaction between nevirapine and bedaquiline in 16 HIV-1 infected participants (van Heeswijk et al. 2011).

Participants first received a single dose of bedaquiline followed by 14 days of serial plasma sampling. Subsequently, participants were initiated on a nevirapine-based antiretroviral regimen. After 14 days on nevirapine, a second dose of bedaquiline was administered, followed by 14 days of serial plasma sampling.

Co-administration of nevirapine did not have an effect on the AUC of bedaquiline. Subsequent population pharmacokinetic modelling of this data by Svensson et al. to estimate concentrations at steady-state also showed that nevirapine was unlikely to have a significant effect on bedaquiline concentrations (Svensson et al. 2014). Nevirapine is therefore considered safe for co-administration with bedaquiline.

Lopinavir/ritonavir (LPV/r)

Ritonavir is an inhibitor of CYP3A, and is used in combination with lopinavir, also a protease inhibitor (Rathbun & Liedtke 2011).

In a randomized open-label cross-over drug interaction study conducted in 16 healthy volunteers by van Heeswick et al., a single-dose of bedaquiline was administered alone and in combination with LPV/r, followed by serial plasma sampling (van Heeswijk et al. 2010).

The bedaquiline AUC increased by 22% (95%CI 11% to 34%) when co-administered with LPV/r. The authors felt the clinical significance of this increase was unclear. Subsequent population pharmacokinetic modelling of this bedaquiline single-dosing data by

Svensson et al. to estimate bedaquiline concentrations at steady-state (when patients have reached the maintenance dosing phase of treatment) estimated a 35% (relative standard error 9.2%) decrease in bedaquiline clearance, which equates to an almost 3-fold increase in bedaquiline AUC (Svensson et al. 2014). The authors recommend careful monitoring of patients who are receiving co-administered LPV/r, as these patients are at high risk for toxicity with such high bedaquiline concentrations.

Identification of gaps and needs for further research

- There is no data on drug-drug interactions with bedaquiline at steady-state during the maintenance dosing phase of treatment.
    - The phase 1 studies upon which current recommendations have been made used single dose bedaquiline. There is no bedaquiline pharmacokinetic data at steady-state during the maintenance dose phase of treatment. Because bedaquiline accumulates in tissues and consequently has a very long half-life, it is likely to behave very differently with chronic dosing compared to single-dosing.
    - Population pharmacokinetic modelling provides limited insight into the possible pharmacokinetics of bedaquiline at steady-state.
    - Further research is required and should include drug-drug interaction studies at steady-state during the maintenance dosing phase of bedaquiline treatment.

- There is limited data in patients infected with TB, and in patients with TB-HIV co-infection.
    - Most of the currently available data is from studies done in healthy volunteers. The pharmacokinetics of bedaquiline may be affected by the disease states of tuberculosis and HIV.

    - Further research is required and should include the pharmacokinetic evaluation of bedaquiline in patients infected with TB, and patients with TB-HIV co-infection.

- There is limited data on the pharmacokinetics of bedaquiline in the general population.
    - The phase 1 drug-drug interaction studies had small sample sizes, and therefore provide poor estimates of bedaquiline pharmacokinetics because of the wide inter-individual variability of drug pharmacokinetics.
    - Further research is required and should include pharmacokinetic evaluation of large numbers of patients on bedaquiline and should identify co-factors which may affect bedaquiline pharmacokinetics (e.g. age, sex, weight, race).

- There is no data on the effect of drug-drug interactions on the clinical outcomes for patients co-infected with TB and HIV.
    - No studies have looked at clinical outcomes such as sputum conversion, TB cure rates, and toxicity when bedaquiline is co-administered with antiretrovirals.
    - Further research is required including cohort studies of patients with TB and TB-HIV co-infection looking at clinical outcomes.

References

Andries, K., Verhasselt, P., Guillemont, J., Göhlmann, H.W.H., Neefs, J.M., Winkler, H., Van Gestel, J., Timmerman, P. et al. 2005. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. *Science*. 307(5707):223–227.

Brunton, L. and Hilal-Dandan, R. 2008. *Goodman and Gillman's Manual of Pharmacology and Therapeutics.* 11th Edition. San Diego: McGraw-Hill Companies, Inc.

Calligaro, G.L., Moodley, L., Symons, G., Dheda, K. 2014. The medical and surgical treatment of drug-resistant tuberculosis. *Journal of Thoracic Disease*, 6(3):186–195.

Chahine, E.B., Karaoui, L.R. & Mansour, H. 2014. Bedaquiline : A Novel Diarylquinoline for Multidrug-Resistant Tuberculosis. *Annals of Pharmacotherapy*, 48(1):107–115.

Diacon, A.H., Pym, A., Grobusch, M., Patientia, R., Rustomjee, R., Page-Shipp, L., Pistorius, C., Krause, R. et al. 2009. The Diarylqunoline TMC207 for Multidrug-Resistant Tuberculosis. *The New England Journal of Medicine*, 360(23): 2397–2405.

Diacon, A.H., Pym, A., Grobusch, M.P., de los Rios, J.M., Vasilyeva, I., Leimane, V., Andries, K., Bakare, N. 2014. Multidrug-Resistant Tuberculosis and Culture Conversion with Bedaquiline. *New England Journal of Medicine*, 371(8):723–732.

Dooley, K.E., Park, J.G., Swindells, S., Allen, R., Haas, D.W., Cramer, Y., Aweeka, F., Wiggins, I. et al. 2012. Safety, tolerability, and pharmacokinetic interactions of the antituberculous agent TMC207 (bedaquiline) with efavirenz in healthy volunteers: AIDS Clinical Trials Group Study A5267. *Journal of acquired immune deficiency syndromes*. 59(5):455–462.

Guglielmetti, L., Le Du, D., Jachym, M., Henry, B., Martin, D., Caumes, E., Veziris, N., Metivier, N., Robert, J. 2015. Compassionate Use of Bedaquiline for the Treatment of Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis : Interim Analysis of a French Cohort. *Clinical Infectious Diseases*, 60(2):188–194.

Janssen Therapeutics. 2012. Sirturo information leaflet. Ittusville, NJ. Page 1–9.

van Heeswijk, R., Vandevoorde, A., Meyvisch, P., De Marez, T., De Beule, K., Mc Neeley, D, Hoetelman, R. 2010. The effect of lopinavir/ritonavir on the pharmacokinetics of TMC207, an investigational antimycobacterial agent. Proceeding of the *18th*

International AIDS Conference. 18-23 July 2010. Vienna, Austria.

van Heeswijk, R., Vandevoorde, A., Meyvisch, P., De Marez, T., McNeeley, D., Hoetelman, R. 2011. The effect of nevirapine on the pharmacokinetics of TMC207, an investigational antimycobacterial agent, in HIV-1-infected subjects. Proceeding of the *6th International AIDS Conference*. 17-20 July 2011. Rome, Italy.

Rathbun, R.C. & Liedtke, M.D. 2011. Antiretroviral Drug Interactions: Overview of Interactions Involving New and Investigational Agents and the Role of Therapeutic Drug Monitoring for Management. *Pharmaceutics*, 3(4):745–781.

Singh, H., Kaur Natt, N., Garewal, N., Pugazhenthan, T. 2013. Bedaquiline : a new weapon against MDR and XDR-TB. *International Journal of Basic & Clinical Pharmacology*. 2(2):96–102.

Svensson, E.M., Murray, S., Karlsson, M.O., Dooley, K. 2015. Rifampicin and rifapentine significantly reduce concentrations of bedaquiline, a new anti-TB drug. Journal of Antimicrobial Chemotherapy. 70(4):1106–1114.

Svensson, E.M., Dooley, K.E. & Karlsson, M.O., 2014. Impact of Lopinavir-Ritonavir or Nevirapine on Bedaquiline Exposures and Potential Implications for Patients with Tuberculosis. *Antimicrobial Agents and Chemotherapy*. 58(11):6406–6412.

Udwadia, Z., Amale, R. & Mullerpattan, J., 2014. Initial experience of bedaquiline use in a series of drug-resistant tuberculosis patients from India. *The International Journal of Tuberculosis and Lung Disease*, 18(11):1315–1318.

World Health Organization, 2013a. Consolidated guidelines for the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. Geneva, Switzerland.

World Health Organization, 2013b. *The use of bedaquiline in the treatment of multidrug-resistant tuberculosis: Interim policy guidance*. Geneva, Switzerland.

World Health Organization, 2015. *Global Tuberculosis Report 2015*. Geneva, Switzerland

World Health Organization, 2010. Treatment of tuberculosis guidelines. Geneva, Switzerland.

Part C: JOURNAL READY MANUSCRIPT

The following manuscript was submitted to the Journal of Antimicrobial Chemotherapy on the 25th September 2015 in accordance with their requirements.

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The article is available from: http://www.ncbi.nlm.nih.gov/pubmed/26747099 or http://jac.oxfordjournals.org/cgi/content/full/dkv447?ijkey=1PRbownPuVDTzkn&keytype=ref

Drug-drug interactions between bedaquiline and the antiretrovirals lopinavir-ritonavir and nevirapine in HIV-infected patients with drug resistant tuberculosis.

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Abstract

Objectives: Bedaquiline is a new antituberculosis drug, which is metabolised by cytochrome P450 (CYP) 3A4. Concomitant antiretroviral therapy (ART) is important for all HIV-infected patients treated for tuberculosis, but several antiretrovirals inhibit or induce CYP3A4. Single dose drug-drug interaction studies found no significant interactions with nevirapine or lopinavir-ritonavir (LPV/r), but these findings could be misleading, especially because of bedaquiline's long terminal half-life. We evaluated the effect of nevirapine and LPV/r on bedaquiline exposure.

Methods: We conducted a parallel group pharmacokinetic study of three groups of participants who were on bedaquiline as part of therapy for drug-resistant tuberculosis: no ART (HIV seronegative), nevirapine-based ART, and LPV/r-based ART. Non-compartmental analyses were done and exposure of bedaquiline and its M2 metabolite compared between the no ART and the two ART groups.

Results: We enrolled 48 participants: 17 in the no ART, 17 in the nevirapine, and 14 in the LPV/r groups. The following median bedaquiline pharmacokinetic parameters were significantly higher in the LPV/r group than in the no ART group: $AUC_{0\text{-}48}$ (67002 versus 34730 $ng.hr.mL^{-1}$; P=0.003); T_{max} (6 versus 4 hours; P=0.003); and half-life (55 versus 31 hours; P=0.004). On multivariate analysis bedaquiline exposure was increased by LPV/r, male sex and time on bedaquiline. Bedaquiline exposure was not significantly different between the nevirapine and the no ART groups. M2 metabolite exposure was not significantly different in either of the antiretroviral groups compared with the no ART group.

Conclusions: LPV/r significantly increased bedaquiline exposure. The clinical significance of this interaction remains to be determined.

Introduction

Bedaquiline, which has recently been approved for the treatment of drug-resistant tuberculosis, is a diarylquinoline antimycobacterial with the novel mechanism of action of selectively inhibiting mycobacterial ATP synthase.[1] Bedaquiline is primarily metabolized in the liver by the cytochrome P450 (CYP) isoenzyme 3A4 to a less active N-monodesmethyl metabolite (M2).[1] Co-administered drugs that induce CYP3A4 could result in reduced bedaquiline concentrations, which could reduce efficacy and select for resistant mutants, while drugs that inhibit CYP3A4 could result in increased bedaquiline concentrations, which could increase the risk of toxicity, notably QT prolongation.[2] Tuberculosis and HIV co-infection is common, and their concurrent treatment has become the standard of care following studies showing reduced mortality when antiretroviral therapy (ART) is initiated soon after starting tuberculosis therapy.[3] The World Health Organization's recommended first- and second-line ART regimens include drugs which may affect bedaquiline exposure by inducing and/or inhibiting CYP3A4.[4]

The non-nucleoside reverse transcriptase inhibitor nevirapine is a moderate inducer of CYP3A4,[5] while the protease inhibitor lopinavir/ritonavir (LPV/r) is a potent inhibitor of CYP3A4.[6] Drug-drug interaction (DDI) studies found only modest effects of nevirapine and LPV/r on bedaquiline exposure.[1] However, both DDI studies used single doses of bedaquiline in participants at steady state on LPV/r or nevirapine, which may under-estimate the magnitude of the DDIs when bedaquiline reaches steady-state following chronic administration. Non-linear mixed effects modelling estimated 3- and 2-fold increases in average concentrations at steady-state for bedaquiline and M2 respectively when bedaquiline is co-administered with LPV/r, and no significant effect when co-administered with nevirapine.[7] There are currently no data on bedaquiline pharmacokinetics when co-administered long term in patients on nevirapine- or LPV/r-based ART.

We conducted an observational pharmacokinetic study in patients with drug-resistant tuberculosis during the maintenance dose phase of their bedaquiline therapy, and compared bedaquiline pharmacokinetic parameters in HIV-infected patients on nevirapine or LPV/r with HIV-uninfected patients not on ART.

Methods

Adults (≥18 years) with drug-resistant tuberculosis receiving bedaquiline from the South African national access program[8] were approached for enrolment into the study. We enrolled consenting participants into three groups: no ART (HIV-uninfected), on nevirapine-based ART and on LPV/r-based ART. Participants on ART must have been on nevirapine for at least 2 weeks and on LPV/r and for at least 3 days. Exclusion criteria were other drugs known to inhibit or induce CYP3A4, pregnancy, or gastrointestinal diseases that may interfere with pharmacokinetics.

Intensive pharmacokinetic sampling was performed once participants had completed the 2 week loading dose phase and at least one week of the maintenance dose phase of bedaquiline treatment. Pharmacokinetic samples were collected before and 1, 3, 4, 5, 6, 8, 24, and 48 hours after a 200mg dose of bedaquiline (administered after a meal). Plasma was separated by centrifugation within 1 hour of sampling, and then stored at -70^{o}C.

Bedaquiline and M2 concentrations were determined using a LC-MS/MS assay validated according to FDA and EMA guidelines. Plasma samples (20 µl) were processed by precipitation with acetonitrile containing the isotope labelled internal standards, di-methyl-d3 amine bedaquiline and 4-methyl(^{13}C)-d3 amino M2. The supernatant was concentrated and reconstituted in injection solution consisting of equal parts acetonitrile and 0.1% formic acid in water. Chromatographic separation was achieved on a Phenomenex Gemini-NX C18, 5µ (50 x 2.0 mm) analytical column using acetonitrile and 0.1% formic acid (37:63, v/v) as the mobile phase, delivered at a constant flow rate of 500 µl/min. An AB Sciex API 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode, monitoring the transition of the protonated molecular ion at m/z 555.2 to the product ion at m/z 58.1 for bedaquiline, and the protonated molecular ion at m/z 541.1 to the product ion at m/z 480.1 for M2. The internal standard transitions monitored were the protonated molecular ion m/z 561.1 to the product ion at m/z 64.3 for di-methyl-d3 amine bedaquiline and the protonated molecular ion m/z 545.3 to the product ion m/z 480.1 for 4-methyl-(^{13}C)-d3 amino M2. Electro spray ionisation was used for ion production. The assay

was validated over the concentration range of 0.00977 – 5 µg/ml for bedaquiline and 0.00313 – 0.2 µg/ml for M2. During inter-day sample analysis, the accuracies (%Nom) for bedaquiline were 97.2%, 96.9% and 104.1% at the high (3.75 µg/ml), medium (1.75 µg/ml) and low (0.0293 µg/ml) QC levels respectively with precision less than 10% across all three levels. The accuracies for M2 were 97.5%, 101.4% and 99.9% at the high (0.150 µg/ml), medium (0.075 µg/ml) and low (0.00938 µg/ml) QC levels respectively with precision less than 7%.

A sample size of 14 participants in each group was required to detect a 50% difference in bedaquiline steady-state AUC between the no ART and on ART groups, with 80% power and 5% significance based on the variance calculated from the steady state concentrations at week 8 reported in the phase 2 study.[9] As we would likely be using non-parametric statistical testing, we increased the sample size by 15% to 16 participants in each of three groups. Pharmacokinetic parameters were derived from non-compartmental analysis. The Mann-Whitney U-test was used to compare pharmacokinetic parameters in parallel groups of participants on nevirapine or LPV/r versus participants not on ART. Multivariate linear regression was used to estimate the impact of covariates on bedaquiline $AUC_{0\text{-}48}$.

Results

The participants' characteristics and the pharmacokinetic parameters of the three groups are shown in Table 1. There were no significant differences in participant characteristics between either the nevirapine or the LPV/r groups and the no ART group, but duration on bedaquiline at the time of sampling was longer in participants on LPV/r (P=0.06). There were no significant differences in the bedaquiline and M2 pharmacokinetic parameters between the nevirapine and the no ART groups. By contrast, the bedaquiline AUC, T_{max} and half-life were significantly higher in the LPV/r than in the no ART group. There were no significant differences in M2 pharmacokinetic parameters between the LPV/r and no ART groups.

Multivariable linear regression modelling controlling for covariate effects (Table 2) showed LPV/r and male sex were associated with a significant increase in bedaquiline $AUC_{0\text{-}48}$ of 62%

and 47%, respectively. Time on bedaquiline was also associated with increased bedaquiline AUC_{0-48}, but this just failed to reach statistical significance.

Discussion

We found that bedaquiline exposure was almost twice as high in the LPV/r group as in the no ART group. Variables associated with higher plasma bedaquiline exposure on multivariate analysis were LPV/r co-administration, men (presumably due to less body fat than women), and time on bedaquiline (of borderline significance). Nevirapine did not significantly affect bedaquiline exposure, and neither antiretroviral affected M2 exposure.

Our findings are similar to the estimates of a non-linear mixed effects model derived from single dose interaction studies.[7] Svennson et al estimated no significant effect of nevirapine on bedaquiline exposure, but approximately a threefold increase in bedaquiline and a twofold increase in M2 average steady state concentrations, reached at 6 months, when co-administered with LPV/r.[7] However, the magnitude of the interaction at about 3 months, which is when we sampled our participants, was simulated to be no effect on M2 and approximately a twofold increase in bedaquiline exposure.[7]

The clinical implications of the interaction between LPV/r and bedaquiline that we found are unclear, but the toxicity of bedaquiline could be enhanced. Notably QT prolongation, which correlates with M2 concentrations,[10] could occur at 6 months when the increase in M2 concentrations is maximal.[7] We concur with the World Health Organizations' view that co-administration of LPV/r with bedaquiline "should be used with extreme caution and only in a closely monitored setting when other options are not available".[11]

Limitations of our study include the failure to achieve the desired sample size in the LPV/r group (14 instead of 16 participants) due to the small proportion of patients on second-line ART in the bedaquiline access programme, and that pharmacokinetic sampling was not done

when participants were at steady state. A strength of our study is that it was conducted in the diseased populations of interest.

In conclusion, we found that concomitant LPV/r increased almost doubled the plasma bedaquiline exposure. Future studies should explore the effect of LPV/r and other ritonavir-boosted protease inhibitors on M2 and bedaquiline pharmacokinetic parameters, and on toxicity, at steady state. The clinical implications of this drug-drug interaction remain to be determined.

Acknowledgements

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Transparency declaration

GM served on the data safety and monitoring board for Janssen for the TMC207 (bedaquiline) C208 and C207 phase 2 trials in patients with multidrug-resistant tuberculosis, 2007–2012.

References

1. Van Heeswijk RP, Dannemann B, Hoetelmans RM. Bedaquiline: a review of human pharmacokinetics and drug-drug interactions. *J Antimicrob Chemother* 2014; 69: 2310–8.
2. Diacon AH, Pym A, Grobusch MP *et al*. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. *N Engl J Med* 2014; 371: 723–32.
3. Uthman OA, Okwundu C, Gbenga K *et al*. Optimal Timing of Antiretroviral Therapy Initiation for HIV-Infected Adults With Newly Diagnosed Pulmonary Tuberculosis: A Systematic Review and Meta-analysis. *Ann Intern Med* 2015; 163: 32-9.
4. World Health Organization. *Consolidated guidelines for the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. June 2013*. http://www.who.int/hiv/pub/guidelines/arv2013
5. Back D, Gibbons S, Khoo S. Pharmacokinetic drug interactions with nevirapine. *J Acquir Immune Defic Syndr* 2003; 34 Suppl 1:S8-14.
6. Wyen C, Fuhr U, Frank D *et al*. Effect of an antiretroviral regimen containing ritonavir boosted lopinavir on intestinal and hepatic CYP3A, CYP2D6 and P-glycoprotein in HIV-infected patients. *Clin Pharmacol Ther* 2008; 84: 75-82.
7. Svensson EM, Dooley KE, Karlsson MO. Impact of lopinavir/ritonavir or nevirapine on bedaquiline exposures: potential implications for patients with TB/HIV co-infection. *Antimicrob Agents Chemother* 2014; 58: 6406–6412.
8. Ndjeka N, Conradie F, Schnippel K *et al*. Treatment of drug-resistant tuberculosis with bedaquiline in a high HIV prevalence setting: an interim cohort analysis. *Int J Tuberc Lung Dis* 2015; 19: 979-85.
9. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L. The Diarylquinoline TMC207 for Multidrug-Resistant Tuberculosis. *N Engl J Med* 2009; 360: 2397–405.
10. Sirturo™ prescribing information. http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000lbl.pdf
11. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis, 2014. http://www.who.int/tb/publications/pmdt_companionhandbook

Table 1. Participant characteristics and plasma pharmacokinetic parameters of bedaquiline and M2 metabolite. Unless otherwise stated, figures are medians (interquartile range).

	No ART (N = 17)	**Nevirapine (N = 17)**	**LPV/r (N = 14)**
Participant characteristics			
Male sex (%)	13 (77%)	10 (59%)	9 (64%)
Age, years	25 (22-38)	37 (21-46)	32 (26–40)
Weight, kg	56.7 (52.5–64.7)	58.7 (54.6–69.2)	53.8 (48–60)
Days on bedaquiline at PK sampling	43 (34-79)	70 (35-79)	95 (43–114)
Bedaquiline			
AUC_{0-48}, $ng.hr.mL^{-1}$	34730 (27466-52862)	35174 (28372-64158)	67002 (51862-88255)
P value*	Referent	P=0.502	P=0.003
C_{max}, ng/mL	1970 (1100-2640)	2000 (1420-2450)	2235 (1670-2850)
P value*	Referent	P=0.642	P=0.234
T_{max}, hours	4 (3-5)	5 (4-6)	6 (5-8)
P value*	Referent	P=0.197	P=0.003
Half-life, hours**	31 (24-37)	33 (26-44)	55 (44-93)
P value*	Referent	P=0.564	P=0.004
M2 metabolite			
AUC_{0-48}, $ng.hr.mL^{-1}$	7449 (6064-9060)	8358 (4943-9537)	6561 (3846-10626)
P value*	Referent	P=0.617	P=0.606
C_{max}, ng/mL	169 (140-244)	185 (128-281)	172 (103-258)
P value*	Referent	P=0.705	P=0.921

T_{max}, hours	8 (5-8)	6 (0-8)	8 (3-24)
P value*	Referent	P=0.391	P=0.587
Half-life, hours***	208 (120-447)	240 (124-477)	243 (72-1233)
P value*	Referent	P=0.5056	P=0.9379

* P-values are the difference between the no ART and either nevirapine or LPV/r groups

** The half-life of bedaquiline for one patient in the nevirapine group could not be estimated

*** The half-life of M2 could not be estimated in 2, 7, and 8 of the participants from the no ART, nevirapine, and LPV/r groups, respectively.

Table 2. Multivariable linear regression on covariate effects on bedaquiline AUC_{0-48} (no ART is the reference group).

	Change in geometric mean AUC_{0-48}	**95% confidence interval**	**P value**
Nevirapine	17% increase	-17% to 65%	0.340
LPV/r	62% increase	13% to 132%	0.010
Male sex	47% increase	8% to 98%	0.014
Time on bedaquiline (per week increase)	2% increase	-1% to 5%	0.060
Weight (per 10 kg increase)	11% decrease	-32% to 0.2%	0.107
Age (per year increase)	0.9% increase	-1% to 24%	0.186

Part D: APPENDICES

CONTENTS

Appendix 1: UCT Human Research Ethics Committee (letter of approval)

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

24 July 2013

HREC REF: 444/2013

Prof G Maartens
Clinical Pharmacology
K46, OMB

Dear Prof Maartens

PROJECT TITLE: DRUG-DRUG INTERACTIONS BETWEEN BEDAQUILINE AND ANTIRETROVIRALS: A POPULATION PHARMACOKINETIC APPROACH

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study subject to the following:

Please clarify the time commitments required in the Informed Consent Forms.

Approval is granted for one year till the 30th July 2014

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s.thomas

Appendix 2: WITS Human Research Ethics Committee (letter of approval)

R14/49 Drs F Conradie & JF Ferreira

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130855

NAME: (Principal Investigator)	Drs F Conradie & JF Ferreira
DEPARTMENT:	Department of Medicine/Clinical HIV Research Unit Sizwe Tropical Diseases Hospital Klerksdorp Tshepong Hospital
PROJECT TITLE:	Drug-drug Interactions between Bedaquiline and Antiretrovirals: A Population Pharmacokinetic Approach
DATE CONSIDERED:	30/08/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Signed Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/09/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES