DEAR COLLEAGUE,

We are pleased to welcome you at the 8th International Workshop on Clinical Pharmacology of Tuberculosis Drugs at the Declan Suites Hotel in San Diego, CA, USA.

The treatment of tuberculosis is increasingly becoming a challenge worldwide. All around the world experts in the field are involved in intensive research to be able to cope with the various concerns with regard to treatment.

The aim of this abstract-driven workshop is to make a significant contribution to the optimization of TB treatment, by bringing experts together and having them present and discuss the latest important scientific findings in the TB clinical pharmacology field. Additionally, scientific, regulatory or strategy issues that are highly relevant to the optimization of TB treatment will be exchanged and discussed.

We hope this workshop will surpass your expectations and you will find it an inspiring event.

On behalf of the Organizing Committee,

ROB AARNOUTSE, PHARM.D., PH.D
Radboudumc, The Netherlands
Chair 2015
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GENERAL INFORMATION

BADGE POLICY
All registered delegates are provided with an identity badge. Please wear it at all times to ensure admission to the meeting.

CERTIFICATE OF ATTENDANCE
Certificates of attendance will be sent by email in the week following the workshop.

CONFERENCE MATERIALS
Presentations and abstracts will be posted on the workshop website shortly after the meeting.

EVALUATION FORMS
Your feedback is very valuable to us and enables us to further improve this workshop. An evaluation form will be distributed after the PM break. Please complete the evaluation form and return it to the meeting secretariat.

MEETING SECRETARIAT
The meeting secretariat is situated in the Rhapsody Foyer (lobby level). The conference organizers can be addressed for all questions concerning the logistics of the meeting.

MOBILE PHONES
As a courtesy to speakers and other delegates, we request that all mobile phones and pagers are turned off before entering the meeting room.

POSTER PRESENTERS
Poster presenters are requested to stand near their poster during the poster sessions. Please hang your poster as soon as possible. All posters must be displayed from the opening until the closure of the workshop.

Directly after the lunch break we will have a guided poster session in which the poster presenters will be asked to present their poster orally in 2-3 minutes. Members of the faculty will guide/moderate this session.

SPEAKERS
Speakers are requested to submit their presentation as early as possible, preferably in the break prior to their session.

WORKSHOP DINNER
The workshop dinner is scheduled on Friday evening 17 September for those delegates who are pre-registered. Onsite registrations are accepted upon availability. Please gather in the hotel lobby at 6.15 PM.

Disclaimer

This conference is intended for educational purposes only and aims to offer participants the opportunity to share information. The Organizing Secretariat of this conference, Expert Medical Events, cannot accept any liability for the scientific content of the sessions or for any claims which may result from the use of information or publications from this conference. Expert Medical Events disclaims all liability for injuries or losses of whatever nature incurred by individuals attending the conference.
PROGRAM
## PROGRAM

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Refined Pharmacokinetics for Tuberculosis Drug Discovery: Determining Drug Concentration at the Site of Infection
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O. Clewe #24

Initial development of assays for MDR-TB drugs using LC/MS/MS in small hair samples: Implications for pharmacologic monitoring of outcomes
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Rada Savic, PharmD, PhD - University of California, San Francisco, USA
N. Zhang #09

02.30 PM  Concentration-response effects of combination therapy in a murine model of tuberculosis: optimizing the dosing regimen for short-course therapy
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03.00 PM  Dose optimization for combinations of three or more drugs: an application to dose selection in regimen-based tuberculosis drug development
P. Abel zur Wiesch #12

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Rovina Ruslami, MD, PhD - Padjadjaran University, Faculty of Medicine, Indonesia
S. Browne #13

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C. Chen #14

05.00 PM  Prediction of drug partitioning into tubercular pulmonary lesions
S. Irwin #15

05.15 PM  Drug Partitioning and Local Microenvironmental Conditions affect Bedaquiline, Clofazimine and Pyrazinamide Treatment Responses in Mycobacterium tuberculosis Infected C3HeB/FeJ Mice
J.F. Marier #16

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05.45 PM  Discussion

06.00 PM  Closure and adjournment
INVITED SPEAKERS
CLOFAZIMINE: CURRENT STATUS AND FUTURE PROSPECTS

ERIC NUERMBERGER, MD is Associate Professor of Medicine and International Health at Johns Hopkins University, where he completed a residency in Internal Medicine and a fellowship in Infectious Diseases.

Since 2001, he has been primarily engaged in pre-clinical TB drug development research using in vitro and murine models. This work has informed the clinical development of moxifloxacin, rifapentine, PA-824 and PNU-100480 for TB and the pre-clinical development of numerous other compounds. This work has been funded by the National Institutes of Health, the Food and Drug Administration, the Bill and Melinda Gates Foundation, the Global Alliance for TB Drug Development, and Pfizer.

THE PHARMACOKINETICS/PHARMACODYNAMICS OF DELAMANID

SURESH MALLIKARJUN, PHD, FCP is currently a Senior Director in Clinical Pharmacology in the Otsuka Novel Products group, OPDC in Rockville, Maryland. He obtained his B.Pharm degree from the University of Bombay and then joined the M.Pharm program at the Government College of Pharmacy, Bangalore. He obtained a PhD from Virginia Commonwealth University, Richmond, Virginia in the area of Pharmacokinetics.

He has over 27 years of drug development experience in several therapeutic areas, i.e., central nervous system, cardiovascular, respiratory, anti-infective and oncology. He started his career at the Food and Drug Administration followed by positions at Procter and Gamble Pharmaceuticals and Otsuka America Pharmaceuticals. He is a diplomate of the American Board of Clinical Pharmacology and is a Fellow of the American Association of Clinical Pharmacology. He holds an adjunct faculty position at Virginia Commonwealth University and is an author of over 50 publications and abstracts.
INVITED SPEAKERS

THE SYSTEMS PHARMACOLOGY APPROACH TO IMPROVE TB TREATMENT

RADA SAVIC, PHARMD, PHD is an Assistant Professor at the Department of Bioengineering and Therapeutic Sciences at the University of California San Francisco since 2011. Rada holds a PharmD from University of Belgrade, Serbia and Ph.D. in Pharmacometrics from Uppsala University in Sweden. She completed two postdoctoral fellowships in biostatistics (INSERM, Paris) and HIV Clinical Pharmacology (Stanford University).

Her current research laboratory has 11 full time modelers, focusing on drug development and treatment optimization questions in TB and HIV. More specifically, her UCSF laboratory is applying quantitative model-based principles to study drug, biomarker and disease dynamics and response, in order to determine the optimum dose, schedule and treatment duration of various therapies, potentially bringing novel therapies to patients with unmet need more quickly.

TB AND DIABETES MELLITUS: PHARMACOLOGICAL ASPECTS OF THE CONVERGENCE OF TWO EPIDEMICS

ROVINA RUSLAMI, MD, PHD is the head of Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. She completed a residency in the Internal Medicine at the same institution in 2001, and obtained her PhD in Clinical Pharmacology of anti tuberculosis treatment at the Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands in 2009. She completed postdoctoral fellowship form KNAW, the Netherlands, and continue working on the area of optimizing the treatment of TB Meningitis. Her current research activity is as the PI of ReDEFINe study - (Rifampicin Dose Finding Study), a double blind randomized control trial phase IIb Clinical Trial, funded by USAID & NIH through PEER Health program (NCT02169882).

Besides working on TB Meningitis, she is also interested at the concurrent TB and Diabetes Mellitus, focusing on improving care for both TB and Diabetes Mellitus. She is the site co-PI of TANDEM project (www.tandem-fp7.eu) funded by the EU.
ABSTRACTS
Abstract: 1

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Penetration of rifampin and rifapentine into diseased lung in the rabbit cavity pulmonary disease model of TB

D. Rifat1, B. Prideaux2, M. Urbanowski1, B. Luna3, M. Marzinke4, V. Dartois2, R. Savic5, W. Bishai3, K. Dooley4

1Johns Hopkins University School of Medicine, The Center for Tuberculosis Research, Baltimore, USA; 2Rutgers New Jersey Medical School, Public Health Research Institute, Newark, USA; 3Johns Hopkins University School of Medicine, The center for Tuberculosis Research, Baltimore, USA; 4Johns Hopkins University School of Medicine, Clinical Pharmacology Analytical Laboratory, Baltimore, USA; 5UCSF School of Pharmacy, Department of Bioengineering, San Francisco, USA; 6Johns Hopkins University School of Medicine, Division of Clinical Pharmacology and Infectious Disease, Baltimore, USA

Introduction: Rifampin (RIF) and rifapentine (RPT) are potent sterilizing drugs. In mouse models daily RPT can cure tuberculosis (TB) in 3 months. In clinical trials, substitution of 10 mg/kg of daily RIF with 10 mg/kg of daily RPT is not more efficacious. Evidence suggests that higher doses of daily RPT may improve microbiologic outcomes, but there is little information about the penetration of rifamycins into infected areas in humans.

Materials and methods: In this study we used the rabbit cavitary pulmonary TB model that has human-like TB pathology (necrotic granulomas and cavities) to study compartmental pharmacokinetics (PK) of rifamycins. Diseased rabbits were given 10mg/kg of RIF and 30mg/kg of RPT by direct intravenous (IV) injection within 10-15 min, respectively and then necropsies were performed 2, 3 and 6 hours post-dose. Drugs were quantified in plasma samples that were collected at different time points before necropsies and in the lung tissues as well using liquid chromatography tandem-mass spectrometry (LC-MS/MS). In addition, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging was conducted to demonstrate the spatial distribution of RIF and RPT within pulmonary lesions.

Results: The half-life of RPT is much shorter in rabbits than in humans. After single doses of IV RIF or RPT at doses intended to mimic human PK, penetration of RIF into caseum in necrotic lesions was poor. RPT may penetrate even more poorly into caseum in cavitary lesions than RIF. Penetration into small granulomatous lesions was excellent for both RIF and RPT, and the two drugs remained in small lesions longer than in uninvolved lung tissue.

Conclusions: PK studies using rabbits with pulmonary TB that have human-like lung pathology may provide valuable insights into anti-TB drug distribution and penetration in humans.

No conflict of interest
Abstract: 2
Pharmacokinetics and Pharmacodynamics of Approved Drugs

Pharmacokinetics of first-line antitubercular drugs in plasma and PBMCs and two week antimicrobial response.
I. Motta1, A. Calcagno1, L. Baietto2, P. Bigliano1, C. Costa1, K. Baruffi1, G. Fattiguso2, R. Milano3, A. D’Avolio2, G. Di Perri1, V. Ghisetti3, S. Bonora1
1Ospedale Amedeo di Savoia, Unit of Infectious Diseases, Torino, Italy; 2Ospedale Amedeo di Savoia, Laboratory of Clinical Pharmacology and Pharmacogenetics, Torino, Italy; 3Ospedale Amedeo di Savoia, Laboratory of Microbiology and Virology, Torino, Italy

Introduction: Several studies have reported low plasmatic concentrations of antitubercular drugs in tuberculosis (TB) patients. Aim of this research was to describe plasma and intra PBMCs concentrations (that might be useful to estimate drug distribution inside alveolar macrophages and better predict compartmental efficacy) of first-line drugs and two week antimicrobial response.

Material & Methods: A prospective observational pilot study was conducted between 2013 and 2015 in adult patients with Mycobacterium tuberculosis infection. Enrolled patients received weight-adjusted dose intravenous treatment for two weeks and then orally. Maximal, trough plasma and intra PBMCs concentrations (validated HPLC/MS-MS methods) and TTP were measured at week 2 and 4. Data are expressed as medians (IQR).

Results: 24 patients were included: 19 with pulmonary TB and 5 subjects treated ex juvantibus. At week 2 median (IQR) plasma and intra PBMCs RIF Cmax were 6652 ng/ml (4474-7873) and 7104 ng/ml (4362-11124). INH and ETA were 3782 ng/ml (2259-5837), 0 ng/ml (0-129) and 5627 ng/ml (3325-10785), 133830 ng/ml (59739-256971), respectively. Plasma and intraPBMCs PZA Cmax were 26955 ng/ml (22522-35191) and 947 ng/ml (0-2563), respectively. Significant correlations were found between plasma and intra PBMCs Cmax of RIF at week 2 and 4 (r=0.65, p=0.001 and r=0.5, p=0.03) and of INH (r=0.45, p=0.04) and ETA (r=0.642, p=0.002) at week 4. Oral administration was associated with lower maximum plasma concentrations of INH (p=0.03). TTP (available in 11 patients) was 289 (169-415) hours at baseline and 543 (325-1032) at week 2; a significant higher increase in TTP was observed in patients with RIF plasma maximum concentrations over 8000 ng/ml at week 2 (617 vs. 90.5 hours, p=0.023).

Conclusions: The observed robust correlation between intraPBMCs and plasmatic concentrations supports the use of plasma drug monitoring; however the novel finding of high intracellular ethambutol accumulation warrants further studies. Given recent data on higher dosage and the here observed better microbiological outcome in patients with higher rifampicin Cmax, the use of tailored rifampicin doses is warranted.

Abbreviations: PBMCs peripheral blood mononuclear cells, TTP time to liquid culture positivity, IQR interquartile range, Cmax maximum concentration, RIF rifampicin, INH isoniazid, ETA ethambutol, PZA pyrazinamide.

No conflict of interest

Due to a poster presentation at the ICAAC this abstract cannot be presented at the 8th International Workshop on Clinical Pharmacology of TB Drugs
Abstract: 3

Population PK/PD modeling

Population pharmacokinetics and penetration of first and second line anti-TB drugs in pulmonary lesions from adults with active tuberculosis

S. Gupta¹, V. Dartois², R. Savic¹

¹University of California San Francisco, Bioengineering and Therapeutics, San Francisco, USA; ²Public Health Research Institute and New Jersey Medical School, Public Health, New Brunswick, USA

Introduction: Treatment of patients with tuberculosis (TB) is challenging due to the ineffective penetration of drugs to the sites-of-action of the pathogen. Most anti-TB medications have been developed taking into account pharmacokinetics (PK) and pharmacodynamics (PD) of the drugs in plasma, not in pulmonary tissues. We hypothesize that incomplete distribution of drugs into lesions impacts treatment duration and failure. To test this, we examined the lesion-specific penetration pharmacokinetic properties of 5 standard anti-TB drugs at active site-of-action.

Materials & Methods: Fifteen Korean adults with difficult-to-treat TB consented to surgical removal of lung after a single dose of each of the five common anti-TB drugs: isoniazid (300mg), rifampicin(600mg), pyrazinamide (1500mg), kanamycin (1000mg) and moxifloxacin (400mg). Absolute drug concentrations of standard drugs as well as background regimen of linezolid and clofazamine were measured in plasma, and pulmonary lesions using HPLC-MS over a 24 hour dosing interval. Nonlinear mixed-effects analysis was performed by modeling all available plasma, lesion and uninvolved-lung drug concentrations. In total, 295 plasma, 369 uninvolved lung and 892 (range 4-55/drug) samples from each of 8 discrete lesions (necrotic nodule, caseum from closed nodule, caseous fibrotic nodule, caseum from cavity, cavity wall, fibrotic issue, small cellular nodule and fungal ball) were used to estimate penetration ratios (R) and rates (k) from plasma to lesions and uninvolved lung. The model-building process was performed in a step-wise fashion, developing first the structural plasma PK model, including variability. In a second step, a full model describing penetration into uninvolved lung or individual discrete lesions was developed, keeping the parameters of the plasma PK model fixed. In the final step, all estimates were opened to confirm stability of model. The likelihood test (LRT) was used to evaluate statistical significance for inclusion of additional parameters in the nested models, assuming that the objective function value (OFV) is X² distributed.

Results: All the drugs were best described by 1-compartment plasma disposition model linked to the first-order absorption model following oral dosing. The distribution into lung and lesion were described using effect compartment models where drug penetrates from central plasma compartment to lung or lesion. All drugs except for kanamycin showed an improved penetration into cellular lesions compared to acellular lesions. Moxifloxacin and clofazamine showed the highest penetration into all lesions irrespective of cellularity. We observed a large inter-individual variability in the distribution and penetration of the standard-drugs for each of the standard drugs. Penetration ratios were between 0.263-0.762 for rifampin, 0.381– 0.658 for pyrazinamide, 0.0321 – 3.05 for isoniazid, 1.23-4.42 for moxifloxacin, 0.137-1.19 for kanamycin, 1.55-16.5 for clofazamine and 0.0945-0.71 for linezolid.

Conclusion: Our study is the first of its kind that quantifies the penetration and distribution of standard as well as novel anti-TB drugs at the site-of-action. The large variation in spatial distribution of the drugs, explains well the large variation observed in treatment duration and outcome of TB. The penetration rates and ratios from this study will help to guide development of agents with optimal penetration to treat drug-sensitive and resistant TB.

No conflict of interest
Abstract: 4

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Association of anti-tuberculosis drug concentrations with drug-related adverse events in TB/HIV co-infected patients in Uganda


1Infectious Diseases Institute, Makerere University department of medicine, Kampala, Uganda; 2Infectious diseases, University Hospital Zurich University of Zurich, Zurich, Switzerland; 3Infectious Disease Institute, Makerere University department of medicine, Kampala, Uganda; 4Infectious Disease, University Hospital Zurich University of Zurich, Zurich, Switzerland; Clinical Chemistry, University Hospital Zurich University of Zurich, Zurich, Switzerland

Introduction: Anti-tuberculosis (TB) drugs are generally well tolerated; however mild, severe or life threatening adverse events may occur. Pyrazinamide has been associated with arthralgia and isoniazid with peripheral neuropathy which can be debilitating. All four first line anti-TB drugs are potentially hepatotoxic. There is not much data on the association between anti-TB drug levels and drug-related adverse events in sub-Saharan Africa. We aimed to assess the correlation between anti-TB drug levels and the incidence of anti-TB drug-related adverse events in TB/HIV co-infected adults.

Material & Methods: The SOUTH study is an ongoing study conducted at the TB/HIV integrated clinic at the Infectious Diseases Institute in Kampala, Uganda. A cohort of TB/HIV co-infected patients were evaluated for adverse events between May 2013 and May 2014. Patients were evaluated every two weeks during the first two months and monthly subsequently. Patients were initiated on antiretroviral therapy (ART) 2 weeks after starting anti-TB medication. Pharmacokinetic blood sampling of isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z) was done 1 hour, 2 hours and 4 hours post dose at 2 weeks, 8 weeks and 24 weeks after initiation of anti-TB treatment using ultra violet high - performance liquid chromatography (UV-HPLC). Potential toxicities were assessed at each visit by patient interview for arthralgia and peripheral neuropathy; clinical examination for vibration sensation; and serum alanine transferase (ALT) measurements. Adverse events were graded according to the National Institute of Health Division of AIDS toxicity tables. Logistic regression accounting for repeated measures was used to determine associations between tertiles of serum anti-TB drug serum concentrations and adverse events. We further compared continuous TB drug levels of patients with and without adverse events using Wilcoxon rank-sum and Kruskal-Wallis tests.

Results: We evaluated 149 subjects in this study of whom 103 (69%) experienced at least one adverse event. Patients with/without adverse events did not differ with regards to gender (51% vs. 57% male), median age (33 vs. 34 years), median BMI (19.2 vs. 19.2 kg/m2) and median CD4 cell count (160 vs. 188 cells/µL) (all P>0.6). Contrary to clinical assumptions, there was no evidence of an association between the serum levels of anti-TB drugs and the prevalence of their most common adverse events (all P>0.05, Figure). We did, however, observe a reduction over time of arthralgia and peripheral neuropathy but not liver toxicity. At weeks 2, 8 and 24 the prevalence of arthralgia was 57%, 53%, 15%, (P<0.001); peripheral neuropathy: 63%, 43% and 47% (P<0.015); and elevated ALT levels: 21%, 24%, 15% (P=0.82).

Conclusion: We observed lower concentrations of isoniazid and rifampicin in our study population of HIV/TB co-infected patients. The implications of these findings are not yet clear. We therefore need to correlate our findings with the response to TB treatment.

No conflict of interest

ENCORE ABSTRACT (ECCMID 2015)
Abstract: 5

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Pharmacokinetics and pharmacodynamics of pyrazinoic acid in murine models of tuberculosis

J.-P. Lanoix1, R. Tasneen1, M. Pin1, V. Dartois2, E. Nuernberger2

1Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2Public Health Research Institute and New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ, USA

Background: Control of tuberculosis (TB) is jeopardized by the increasing prevalence of multidrug-resistant TB (MDR-TB), which is accompanied by additional resistance to pyrazinamide (PZA). PZA is a prodrug that requires conversion to pyrazinoic acid (POA) for its antimicrobial effect. Mutation of the gene encoding the mycobacterial PncA amidase (pncA) that catalyzes this conversion is the most common cause of PZA resistance. One way to circumvent this resistance could be to administer POA directly to patients. To determine whether systemic delivery of POA or host-mediated conversion of PZA or other prodrugs to POA could be efficacious, we studied the systemic and intrapulmonary PK and efficacy of orally administered POA in murine TB models.

Material & Methods: Dose-ranging plasma, epithelial lining fluid (ELF) and lung lesion concentration-time profiles of POA were determined in infected and uninfected BALB/c and C3HeB/FeJ mice after single doses of PZA or POA and after 4 weeks of PZA in infected C3HeB/FeJ mice. Dose-ranging efficacy of POA administered orally was determined in C3HeB/FeJ and BALB/c mice receiving up to 450 mg/kg twice daily for up to 8 weeks.

Results: Dose-proportional POA exposures were observed in plasma after administration of PZA or POA. Median plasma POA AUC0-∞ values were 139-222 µg-h/mL and 178-287 µg-h/mL when administered as 150 mg/kg doses of PZA and POA, respectively. Concentrations in lesions of C3HeB/FeJ mice mirrored those in plasma. However, unlike PZA exposures which increased in dose-proportional fashion in ELF, POA concentrations in ELF plateaued at low doses of either PZA or POA. Nevertheless, POA concentrations in ELF of infected mice administered POA directly were at least as high as those in mice administered the same dose of PZA. In a chronic model of TB in BALB/c mice, PZA 150 mg/kg reduced the lung CFU count by over 2 log10 after 4 weeks of treatment, whereas POA was ineffective until the dose reached 450mg/kg administered once or twice daily, which reduced the lung CFU count by ~0.7 log10 compared to no treatment (p<0.001). No additional killing was observed between 4 and 8 weeks. Even POA doses as high as 450 mg/kg BID had no demonstrable bactericidal activity in C3HeB/FeJ mice.

Conclusions: Oral administration of POA at doses producing plasma exposures higher than those produced by PZA 150 mg/kg was significantly less effective than PZA in two chronic murine infection models, indicating that systemic administration of POA must attain higher plasma POA exposures than what are observed with PZA to have activity comparable to PZA. These result suggest that intrabacillary conversion of prodrugs to POA is likely to be a more efficient strategy for maximizing intrabacillary POA concentrations. Poor activity of directly administered POA relative to PZA may also be related to poor penetration of POA into ELF and/or intracellular compartments harboring bacilli, suggesting that alternative delivery strategies that overcome these limitations, including aerosol administration, may also improve potency.

No conflict of interest
Abstract: 6

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Pharmacodynamics of sulfamethoxazole in in vitro and in vivo models of tuberculosis

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Background: There is increasing interest in repurposing sulfamethoxazole (SMX) for use against multidrug-resistant tuberculosis (MDR-TB), based on its MIC90 of ≤38 µg/mL against clinical isolates, a concentration readily achievable in blood with oral dosing. However, because of the unique pathology of TB and the wide range of SMX doses employed to treat various infections, optimal dose selection for TB treatment will require a better understanding of the time course of SMX activity against M. tuberculosis and the relationships between the time-course of drug exposure and its bacteriostatic and bactericidal effects. We are exploring the pharmacodynamics of SMX in in vitro models, including an intracellular infection model and a hollow fiber model (HFM), and in 2 murine models to inform dose selection.

Materials & Methods: All experiments used M. tuberculosis H37Rv. Time-kill studies were performed in against extracellular bacilli in complete 7H9 broth and against intracellular bacilli engulfed by murine J774 cells, using SMX concentrations up to 1280 µg/mL. Dose ranging and dose fractionation experiments were performed in the HFM, using doses producing AUC0-24h values between 240 and 3000 µg-h/mL, and in acute and chronic BALB/c infection models, producing plasma AUC0-24h values ranging to over 25,000 µg-h/mL.

Results: In vitro, SMX exhibited concentration-dependent bactericidal activity against extracellular bacilli in terms of both the time to onset and the magnitude of the bactericidal effect. The concentrations limiting growth to 10% and 1% of drug-free controls after 14 days were 5-10 and 20 µg/mL, respectively, consistent with reported MICs. However, these concentrations had virtually no effect on growth for the first 7 days before exerting a bactericidal effect over the next 7 days to produce the net effect. The lowest concentration to reduce the starting inoculum by 99% (MBC) was 160 µg/mL. Similar time-kill kinetics were observed in the HFM. When regimens producing an AUC0-24h of ~3000 µg-h/mL were administered as a single bolus, a twice daily bolus or a continuous infusion, only the latter 2 regimens, which maintained concentrations above 120 µg/mL for at least 80% of the dosing interval reduced the CFU count by >1 log10 over 14 days. On the other hand, SMX had no measurable effect against intracellular bacilli, even at 640 µg/mL, and in the acute murine infection model, where bacilli reside intracellularly, plasma AUC0-24 values of approximately 6,000 and 12,000 µg-h/mL were required to delay and prevent death, respectively, but still enabled a 2 log10 increase in lung CFU counts over 4 weeks of treatment, with a benefit favoring twice daily administration. Results from the chronic infection model will be available at the meeting.

Conclusions: SMX exhibits substantial bactericidal activity against extracellular bacilli at concentrations achievable in serum, but is much less effective against intracellular bacilli. The poor results in an acute murine infection model likely relate to poor intracellular penetration and, possibly, a delayed onset of antibacterial effect.

No conflict of interest
Abstract: 7

**Pharmacokinetics and Pharmacodynamics of Approved Drugs**

**Pharmacokinetics of rifampicin and moxifloxacin in the PanACEA-MAMS-TB-01 trial**

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**Background:** The PanACEA-MAMS-TB-01 trial evaluated whether combinations of high-dose rifampin (R), moxifloxacin (M) and SQ109 (Q) combined with isoniazid (H), pyrazinamide (Z) and ethambutol (E) reduced time to culture conversion sufficiently to select for a phase III treatment-shortening trial. Here we describe the pharmacokinetics (PK) of rifampicin and moxifloxacin in this trial.

**Materials and methods:** Adult South-African and Tanzanian patients with pulmonary TB were randomized to 12 weeks of 1. R10HZQ; 2. HR20ZQ with 20 mg/kg R; 3. HR20ZM with moxifloxacin 400 mg QD; 4. HR35ZE with 35 mg/kg R; 5. a control arm with standard R10HZE for 8 weeks. All patients received RH to complete 26 weeks of treatment. Intensive PK sampling took place in 20 patients in each arm after 4 weeks of treatment. Samples were collected prior to and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after intake of TB drugs with a light meal. AUC0-24 and Cmax values were described with a geometric mean (GM), range and geometric coefficient of variation (GCV) and were compared to recent data assessed with the same analytical methods.

**Results:** GM rifampicin AUC0-24 and Cmax values after 10 mg/kg rifampicin were 24 h*mg/L (min-max 12-53 h*mg/L) and 5.8 mg/L (min-max 2.1-12 mg/L) in the HRZE arm (n=19) and 17 h*mg/L (5.0-45 h*mg/L) and 3.3 mg/L (0.7-8.9 mg/L) in the HRZQ arm (n=18). Doubling the dose of rifampicin to 20 mg/kg resulted in a more than proportional increase in rifampicin GM AUC0-24 to 70 h*mg/L (39-149 h*mg/L) and a GM Cmax of 12 mg/L (6.6-24 mg/L) in the HR20ZQ arm (n=20), whereas GM AUC0-24 was 55 h*mg/L (15-121 mg/L) and GM Cmax was 11 mg/L (6.6-24 mg/L) in the HR20ZM arm (n=20). The highest dose of rifampicin in the HR35ZE arm resulted in a GM AUC0-24 of 170 h*mg/L (103-266 h*mg/L) and a GM Cmax of 27 mg/L (15-39 mg/L). ANOVA on log-transformed AUC0-24 values showed significant differences in rifampicin AUC0-24 between the arms on 10, 20 and 35 mg/kg, but not between arms with the same rifampicin doses. GCV for rifampicin AUC0-24 was 26-66% dependent on arm. Exposure to 10 mg/kg rifampicin in the HRZE arm was comparable to data from the PanACEA-HIGHRIF1 and HIGHRIF2 studies, but was much lower than observed in other populations. Exposures at 20 and 35 mg/kg were lower than in PanACEA-HIGHRIF1. GM moxifloxacin AUC0-24 in the HR20ZM arm (n=20) was 24 h*mg/L (min-max 13-33 h*mg/L). This is lower than exposures to moxifloxacin without rifampicin, but comparable to moxifloxacin with standard dose rifampicin. GCV for moxifloxacin AUC0-24 was 27%.

**Conclusions:** Increasing the dose of rifampicin increased exposures effectively. Co-administration of moxifloxacin with rifampicin resulted in lower than previously observed exposures to moxifloxacin and may warrant for an increase in moxifloxacin dose. Higher exposures to rifampicin and addition of moxifloxacin may explain the favourable reduction in time to culture conversion in the HR20ZM and HR35ZE arms as presented previously. PK results of SQ109 and HZE, assessment of PK determinants and PK-PD analyses are pending.

No conflict of interest
Abstract: 8

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Contribution of different oxazolidinones to the efficacy of novel regimens containing bedaquiline and pretomanid in murine models of tuberculosis

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Background: New regimens based on two or more novel agents are sought to shorten or simplify treatment of tuberculosis (TB). Pretomanid (PMD) is a nitroimidazole in phase 3 trials with significant bactericidal activity alone and in combination with bedaquiline (BDQ). We previously showed the novel combination of BDQ+PMD plus the oxazolidinone sutezolid (SZD) to have sterilizing activity superior to that of the first-line regimen in a BALB/c mouse model of TB. The present experiments compared the activity of several candidate oxazolidinones in combination with BDQ+PMD in C3HeB/FeJ mice which, unlike BALB/c mice, develop caseous lesions.

Materials & Methods: C3HeB/FeJ mice were aerosol-infected with ~100 CFU of M. tuberculosis H37Rv 6-8 weeks prior to treatment. Control mice received (i) rifampin 10mg/kg, isoniazid 10 mg/kg, pyrazinamide 150mg/kg and ethambutol 100mg/kg for 8 weeks followed by rifampin and isoniazid alone (RHZE), or (ii) BDQ 25mg/kg plus PMD 100mg/kg. Test mice received BDQ+PMD plus one of the following: SZD 50mg/kg; linezolid (LZD) 25, 50 or 100mg/kg; tedizolid phosphate (TZD) 10 or 20mg/kg; or AZD5847 phosphate 125mg/kg (AZD). Single-dose PK in uninfected mice and multi-dose PK in infected mice were determined in plasma for SZD, LZD and TZD (only single-dose PK for AZD). Efficacy was determined on the basis of (i) lung CFU counts after 4 and 8 weeks of treatment, and (ii) the proportion of mice with culture-positive relapse after 12, 18 and 24 weeks of treatment.

Results: In two experiments, mean lung CFU counts ranged from 0-1 log10 lower among mice treated with 4-8 wks of BDQ+PMD compared to RHZE. Addition of SZD and LZD 100mg/kg significantly increased the bactericidal activity of BDQ+PMD after 4 and 8 weeks. Addition of TZD 20mg/kg did so at 8 weeks only. These doses produce plasma AUCs comparable to 1200 mg daily doses of SZD or LZD and TZD doses 400 mg. LZD 25 mg/kg and TZD 10mg/kg did not add to the bactericidal activity of BDQ+PMD. LZD 50mg/kg appeared additive but the difference was not statistically significant after controlling for multiple comparisons. Relapse rates after 12 and 18 weeks of treatment will be presented at the meeting.

Conclusions: Combination of BDQ+PMD with either SZD, LZD or TZD produced regimens with superior bactericidal activity compared to RHZE, although the additive activity of the oxazolidinones was dose-dependent. If pending relapse results follow suit, these results would complement prior results in BALB/c mice indicating that novel BDQ+PMD+oxazolidinone combinations may provide effective short-course regimens for multidrug- and extensively drug-resistant forms of TB.

No conflict of interest
Abstract: 9

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Concentration-response effects of combination therapy in a murine model of tuberculosis: optimizing the dosing regimen for short-course therapy

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Introduction: The importance of rifampin (RIF) and pyrazinamide (PZA) to the modern short-course regimen for tuberculosis (TB) is well demonstrated. When administered for the first 2 months in combination with RIF and isoniazid (INH), PZA contributes important sterilizing activity which shortens the treatment duration from 9 months to 6 months without increasing the risk of relapse. Remarkably, continuation of PZA beyond the first 2 months lends no further benefit. Furthermore, the addition of INH to the combination of RIF and PZA reduces the activity of the regimen in an exposure-dependent way in some murine models at least one clinical study. Therefore, a quantitative analysis of the dose-response relationship of these three drugs individually and in combinations is critical in order to optimize the drug regimen for the short-course therapy.

Methods: Lung colony-forming unit (CFU) count data were collected from published and unpublished studies of RIF, PZA and INH evaluated as single agents and their combinations (RIF-PZA, RIF-INH and RIF-PZA-INH) BALB/c mice for a dose-response analysis. Among these studies, the dose ranges tested for RIF, PZA and INH were from 3 to 640 mg/kg, 4.2 mg/kg to 900 mg/kg, and 1.56 mg/kg to 50 mg/kg, respectively. The dosing frequency varied between once to twice daily, two to seven times a week dosing, for up to 6 months. In addition, previously published and unpublished plasma concentration-time data of the three drugs in mice were studied. Pharmacokinetic-pharmacodynamic analyses were performed using non-linear mixed effects modeling. Linear and Emax models were used to relate dose and concentration to CFU count, with a proportional factor to capture antagonistic, additive or synergistic effects of drug combinations.

Results: A 2-compartment model with saturated rate of absorption best described the PK characteristics of RIF in mice. PZA showed nonlinear pharmacokinetic properties with saturated absorption and decreased clearance as the dose decreases. In contrast, INH has increased absorption and decreased clearance at high dose levels. The concentration-response relationship between the individual drugs and outcome were non-linear. EC50s for these three drugs used individually and in their combinations were obtained. A synergistic effect of RIF and PZA was observed whereas INH showed an antagonistic effect when added to the RIF-PZA combination.

Conclusions: The EC50 values of the individual drugs and concentration-dependent antagonism between INH and the RIF-PZA combination suggests that drug doses and combination regimens can be optimized further. Increased dosing and optimal combination treatment may improve treatment outcomes.

No conflict of interest
Abstract: 10

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Model based evaluation of higher doses of rifampicin using a semi-mechanistic model incorporating auto-induction and saturation of hepatic extraction

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Background: Rifampicin is a key drug for the treatment of tuberculosis. It is hepatically cleared and undergoes extensive first pass metabolism, whose saturation with larger doses has been reported since early pharmacokinetic (PK) studies. Rifampicin also induces its own metabolism via Pregnane X Receptor (PXR), WHO currently recommends 8-12 mg/kg, which is believed to be sub optimal and higher doses may potentially improve treatment outcomes. We sought to describe rifampicin PK among tuberculosis patients accounting for the auto induction of clearance and saturation of hepatic extraction, and explore exposures associated with doses higher than the currently recommended.

Materials and Methods: Blood samples were collected from 61 HIV/TB co infected patients in South Africa who had a median (range) age of 32 (18 47) years and weight of 55 (34 99) kg. Samples were collected just prior to the dose and at 1, 2, 4, 6, 8, and 12 h post-dose on the 1st, 8th, 15th and 29th days of treatment. Data were analysed using nonlinear mixed effects modelling in NONMEM VII. A one compartment model with first-order elimination and a well stirred hepatic model were explored to describe rifampicin disposition. Change in clearance over time was described using an exponential maturation model. Allometric scaling was applied to all clearance and volume parameters. The M6 method was applied to handle concentrations below the limit of quantification The final model was employed to simulate steady-state rifampicin exposures (AUC0-24) using the demographic data of 870 tuberculosis patients from South Africa and West Africa (200 repetitions). Based on the current weight bands, dose exposure relationship was assessed using doses equivalent to 1.5, 2, 2.5, 3 and 3.5 times the current dose.

Results: Rifampicin PK was best described using a transit compartment absorption and a well-stirred liver model with saturation of hepatic extraction. For a typical individual, volume of the liver compartment and liver plasma flow were assumed to be 1 L and 50 L/h respectively. Free fraction of unbound rifampicin was fixed to 20%. Fat free mass was the best descriptor of body size for all clearance and volume parameters. The model estimated a Michaelis constant (Km) of 3.2 mg/L. Maximum intrinsic clearance almost doubled from the first day of treatment to steady state: for a typical individual, it increased from 93 to 176 L/h with an induction half-life of 4.5 days. As expected, the current model predicts that increases in dose of rifampicin result in more than linear increases in drug exposures. Our simulations show that giving patients 20 mg/kg rather than 10 mg/kg results in 3.2 times higher AUC and further increasing the dose to 35 mg/kg (3.5 times), the AUC becomes 10.8 times higher.

Conclusion: We developed a model for rifampicin PK that characterises auto-induction of clearance and saturation of metabolism, which is evident on first pass extraction even at the current doses. The model correctly predicts that increasing the dose of rifampicin results in a more than proportional increase in drug exposure and results are closely in line with those from recent clinical trials.

Document not received
Abstract: 11

**Drug Development and optimization: Approaches & Tools**

**Dose optimization for combinations of three or more drugs: an application to dose selection in regimen-based tuberculosis drug development**

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**Background:** Regimen-based tuberculosis (TB) drug development is now in place with several novel three- and four-drug combinations advancing through clinical testing as a single unit of development. However, dose selection for individual drug components that optimizes the safety and efficacy of the regimen as a whole has not been addressed due to lack of efficient methods capable of optimizing more than two-drug combinations. Consideration of multiple treatment phases and variable drug and dose combinations further complicates the problem of rational dose selection in these multicomponent drug regimens. Advances in computational algorithms from engineering and statistics provide approaches and tools that can be adapted to address these aspects of dosage regimen design.

**Materials & Methods:** We developed a novel and versatile computational method for dose optimization that has the capability to optimize dosage regimens for combinations of three or more drugs, including regimens with multiple treatment phases and variable drug and dose combinations. This method combines pharmacokinetic/pharmacodynamic (PK/PD) modeling with a biologically inspired genetic algorithm to identify optimized dosage regimens in a process analogous to natural selection. Bayesian parameter estimation together with hierarchical population statistical modeling is used to facilitate in vitro-in vivo and preclinical-to-clinical translation of experimental data. Optimized dosage regimens are identified as all feasible regimens that optimally balance safety, efficacy, and suppression of drug resistance, from which individual regimens that meet a desired therapeutic performance can be chosen.

**Results:** We illustrate the capability of this method for a three-drug anti-TB combination based on typical, but hypothetical, preclinical and clinical experimental data. The PK/PD model includes drug-susceptible, drug-resistant, and persistent Mycobacterium tuberculosis subpopulations, together with the adverse effects of drug-induced QT prolongation and hepatotoxicity. For specified daily and intermittent schedules of administration we identify individual component doses (for a fixed dose combination) that simultaneously maximize the safety, efficacy, and suppression of drug resistant mutants for the regimen as a whole. We also demonstrate the capability of this computational framework to identify the optimal timing and dose combinations for two- and three-phase treatment regimens.

**Conclusions:** This computational framework addresses a fundamental and unresolved problem of dose optimization in combinations of three or more drugs. While this problem is associated with multiple therapeutic areas, our application is to the specific needs of TB. Central to this method is the replacement of current clinical utility-based approaches that aim for a unique solution for an optimal dosage regimen with the more general concept of trade-off (or Pareto) optimization. This removes the need for a priori decisions on conflicting therapeutic objectives, and provides solutions to complex dose optimization problems that are inaccessible to conventional methods. Importantly, this approach provides for an iterative preclinical-to-clinical development of novel anti-TB therapies to lower the risk of Phase 3 clinical trial failures for complex combination regimens.

No conflict of interest
Abstract: 12

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Mathematical model of drug-target binding predicts optimal antibiotic treatment

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Background: The rise of antibiotic resistance underlines the need for employing existing antibiotics prudently. However, we do not have a good understanding how bacterial killing depends on how antibiotic concentration fluctuates over time within a patient. Some antibiotics are most effective when they are given in single, large doses, for others it is important to minimize fluctuations of concentration. Three general pharmacokinetic descriptions of drug exposure are widely used: (i) the total concentration of the substance integrated over a time interval (area under the curve), (ii) the peak concentration, or (iii) the time during which the concentration exceeds a specific threshold. Determining optimal treatment regimens requires costly and time-intensive experiments.

Materials & Methods: To predict optimal dosing and thereby accelerate the design of treatment strategies, we developed a modeling framework that integrates bacterial population biology with the intracellular reaction kinetics of antibiotic-target binding (Abel zur Wiesch et al., Science Translational Medicine 2015). Here, we employ this model to investigate how the kinetics of drug-target binding affect bacterial response to fluctuating antibiotic concentrations.

Results: We find that biochemical parameters of drug-target interaction, for example transmembrane diffusion and the half-life of the antibiotic-target complex, can largely explain which pharmacokinetic measure best predicts antibiotic efficacy for different antibiotics. If the drug-target complex dissociates rapidly, the antibiotic must be kept constantly above MIC to prevent bacterial replication. If antibiotics cross bacterial cell envelopes slowly to reach their target, there is a delay in the onset of action that may be reduced by high initial antibiotic concentrations. Finally, slow drug-target dissociation and slow diffusion out of cells prolong antibiotic efficacy. In this case, the area under the curve is the best predictor of antibiotic efficacy.

Conclusions: Our model explains how biochemical characteristics of drug-target binding determine optimal pharmacokinetics and can be used as tool in the rational design of treatment regimens.

No conflict of interest
Abstract: 13

**Pharmacokinetics and Pharmacodynamics of Approved Drugs**

**Digitizing MTB Medicines: Using compounding methods to enable mobile capture of MTB medication adherence**

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**Introduction:** Directly Observed Therapy (DOT) is universally recommended to ensure Mycobacterium Tuberculosis Complex (MTB) treatment adherence; however DOT is resource intensive, intrusive and expensive. High costs prohibit use of DOT in resource-limited settings contributing to increased rates of multi-drug resistant MTB. A CE marked/FDA cleared digital device (Proteus Digital Heath, Redwood City, CA) can for the first time detect actual medication ingestion events, via an ingestible sensor (IS), wearable patch and paired mobile device. Medication ingestion data is automatically uploaded from the patch to a secure Internet server where the healthcare worker can login to confirm ingestions remotely. A simple, accessible method to combine the IS with oral MTB medications is co-encapsulation of the medication and IS within a gelatin capsule. This can be performed by compounding pharmacists. Herein we investigate the bio-availability and sensor emitter profile of co-encapsulated IS and fixed dose combinations of INH and Rifampin for use in the continuation phase treatment of MTB.

**Materials and Methods:** The IS was combined with fixed dose combinations of Isoniazid (INH) and Rifampin (Rifamate™ and Rifinah™ Sanofi Aventis) via co-encapsulation within certified gelatin capsules, with or without micro-cellulose back-fill. Co-encapsulated (CoE) products underwent in-vitro dissolution testing, and Rifamate underwent in-vivo bioequivalence evaluation in patients with active MTB. The dissolution testing utilized standard USP apparatus and liquid chromatography methods and compared the amount of INH and Rifampin drug substance appearing in solution per unit time in the co-encapsulated and native formulations. Dissolution profile comparisons took place at 6 time points. A randomized bioequivalence (BE) study of co-encapsulated IS Rifamate versus native Rifamate was conducted in 12 patients with active MTB during the continuation phase of treatment. Plasma INH and rifampin were assayed using validated HPLC methods, and the pharmacokinetic parameters were analyzed using non-compartmental methods (Phoenix/WinNonlin software). Sensor emitter profile testing was performed.

**Results:** Dissolution testing for CoE Rifinah with IS, (containing INH and Rifampin 100mg/300mg) was 100% at 43-45 mins, meeting USP requirements. In the randomized BE study the patients mean age was 41 yrs and 71% were male. PK analysis of CoE Rifamate with IS versus native Rifamate, showed INH and RIF plasma concentrations were bioequivalent using the population method ratio test (95% confidence level). Median INH Cmax were 3.85 and 4.27 mcg/ml; median AUC0-12h were 13.34 and 12.50 mcg/ml for native and CoE Rifamate with IS, respectively. Median rifampin Cmax were 12.12 and 11.79 mcg/ml; median AUC0-12h were 45.19 and 43.76 mcg/ml for native and CoE Rifamate with IS, respectively. Sensor emitter profile testing showed the activation time was slightly delayed when co-encapsulated, as expected. The amplitude and lifetime activation profile were similar for the CoE product and native sensor.

**Conclusions:** Co–encapsulation of MTB medication with the IS to produce digitized medications for TB treatment did not affect bio-equivalence or the IS activation amplitude and lifetime profiles. Co-encapsulation of MTB medications with the IS is a feasible option for digitization of these medications and provides a model for the digitization of all MTB drug compounds.

*No conflict of interest*
Abstract: 14

Drug Development and optimization: Approaches & Tools

Prediction of drug partitioning into tubercular pulmonary lesions

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Mycobacterium tuberculosis generates structurally and biochemically diverse pulmonary lesions in humans, ranging from cellular granulomas containing intracellular M. tuberculosis to granulomas with a firm necrotic caseous core harboring anaerobic persistent bacilli and fully fibrotic cavities containing intracellular and extracellular bacilli. An ideal drug or drug combination should possess capabilities of reaching all bacterial populations and penetrating the full spectrum of lesions in order to exert antitubercular activities and sterilize the infection. However, treatment failure and our recent studies by MALDI-MSI have shown that existing drugs have different abilities to reach M. tuberculosis residing in various lesions.

In order to facilitate drug discovery and predict in vivo drug penetration and distribution, we developed two in vitro assays addressing drug accumulation in both cellular granulomas and necrotic caseum.

We measured intracellular concentrations in THP-1 macrophages and calculated the intracellular/extracellular [I/E] ratios of a list of anti-TB drugs. Using a rapid equilibrium dialysis (RED) device, we also determined the unbound drug fractions in M. tuberculosis infected rabbit caseum and in caseum surrogate.

The results showed that the two in vitro assay inversely correlated: as intracellular accumulation in THP-1 macrophages decreases, the free/unbound fraction in caseum increases, with a concomitant gradual decrease in cLogP. Distinct drug accumulation patterns predicted by these two in vitro assays were also confirmed and supported by lesion PK studies using MALDI-MSI.

In conclusion, our two newly developed in vitro assays are useful pre-clinical tools to prioritize compounds from hits to leads with favorable partitioning properties. The results can also be used to rationally design combinational therapies containing anti-TB agents that reach all compartments of pulmonary lesions.

MALDI-MSI: Matrix-assisted laser desorption ionization coupled to mass spectrometry imaging

No conflict of interest
Abstract: 15

Drug Development and optimization: Approaches & Tools

Drug Partitioning and Local Microenvironmental Conditions affect Bedaquiline, Clofazimine and Pyrazinamide Treatment Responses in Mycobacterium tuberculosis Infected C3HeB/FeJ Mice

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BALB/c and Swiss mice are routinely used to validate the effectiveness of tuberculosis drug regimens; although these mouse strains fail to develop human-like pulmonary granulomas exhibiting caseous necrosis. Both drug partitioning and the microenvironmental conditions within human granulomas may negatively impact drug efficacy, and this may not be accurately reflected in non-necrotizing lesions found within conventional mouse models.

The C3HeB/FeJ mouse model has been increasingly utilized as it develops hypoxic, caseous necrotic granulomas which may more closely mimic the lesion heterogeneity found within human pulmonary granulomas.

Of all single agents evaluated in the BALB/c and C3HeB/FeJ mice, the efficacy of 3 drugs (bedaquiline, clofazimine and pyrazinamide) was found significantly different between the two mouse strains. BALB/c mice consistently displayed a highly uniform treatment response to all three drugs, while C3HeB/FeJ mice displayed a dichotomous response composed of responsive and less-responsive mice.

Plasma pharmacokinetic analysis of dissected lesions from BALB/c and C3HeB/FeJ mice revealed that PZA penetrated lesion types from both mouse strains with similar efficiency, however the pH of the necrotic caseum of C3HeB/FeJ granulomas was determined to be 7.5 which is in the range where pyrazinamide is essentially ineffective. Both clofazimine and bedaquiline preferentially accumulated within the highly cellular regions in the lungs of both mouse strains. Bedaquiline was present at reduced but still biologically relevant concentrations within the central caseum, whereas clofazimine showed highly diminished efficacy under these hypoxic, elevated pH conditions. Interestingly, combination therapy of BDQ with PZA had the greatest effect against the less-responsive mouse subpopulation in C3HeB/FeJ mice, thereby reducing the bacterial load in all mice.

In conclusion, the differential treatment response which resulted from the heterogeneous pulmonary pathology in the C3HeB/FeJ mouse model revealed several factors which may impact treatment efficacy. Besides drug partitioning across the necrotic lesions, drugs have to show activity in these caseous lesions which show to be hypoxic and are at neutral, slightly elevated pH.

No conflict of interest
Abstract: 16

Drug Development and optimization: Approaches & Tools

Quantitative modeling of time to positivity following dosing of bedaquiline and rifafour in patients with pulmonary tuberculosis infection

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Introduction: The Critical Path to TB Drug Regimens (CPTR) is a collaborative initiative to help address the scientific, clinical, regulatory and legal challenges posed by focusing on development of novel drug combinations rather than single agents. The goal of this project was to develop a quantitative model to assess longitudinal changes in time to positivity (TTP) as a continuous measure in patients with TB and its relevant sources of variability following administration of various combination products.

Materials & Methods: Quantitative models were developed to understand longitudinal changes in TTP based on data collected in 313 patients with TB enrolled in Phase II trials. As part of these trials, patients were treated with bedaquiline or Rifafour (rifampicin, isoniazid, pyrazinamide and ethambutol). The effect of HIV infection, multidrug resistance, treatment with second-line therapy, and cavitory disease on the rate of TTP was assessed. Analysis was performed using nonlinear mixed-effect modeling.

Results: A Gompertz model adequately characterized the longitudinal behavior of TTP. The shape of TTP was characterized by a rapid prolongation of TTP, followed by a saturable/plateau effect observed at 42 days (a value that corresponded to negative growth). HIV infection was an important covariate describing variability in rate of TTP progression. Following treatment of bedaquiline or rifafour, the rate of TTP progression to negative growth in patients with HIV was slower than that in patients without HIV (0.033 vs. 0.0495 day⁻¹, respectively). These values corresponded to TTP half-life of 21 and 14 days, respectively, suggesting that time to negative growth would be observed after approximately 100 and 70 days of treatment, respectively.

Conclusions: A quantitative model was developed to assess the longitudinal behavior of TTP in HIV and non-HIV patients and investigate sources of variability. HIV patients displayed a 50% slower rate to negative growth than that observed in subjects without HIV following treatment of either bedaquiline or Rifafour. The longitudinal behavior of TTP derived with the current model will be correlated to the probability of durable cure in a next step to optimize combination products in Phase III studies.

Conflict of interest: Consultant for the Critical Path Institute
Abstract: 17

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Determination of levofloxacin plasma concentrations by high performance liquid chromatography and UV detection for use in a multidrug-resistant tuberculosis hospital

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Background: Individual pharmacokinetic variability for fluoroquinolones may contribute to poor treatment outcomes in patients with multidrug-resistant tuberculosis (MDR-TB). Therapeutic drug monitoring is underutilized in MDR-TB endemic settings. Kibong’toto National TB Hospital and the affiliated Kilimanjaro Clinical Research Institute in Tanzania now have capacity for therapeutic drug monitoring with HPLC.

Methods: We developed a method for determination of levofloxacin concentrations from human plasma by HPLC/UV detection on the Dionex UltiMate 3000 (Thermo Scientific). Levofloxacin and phenacetin (internal standard) were extracted from plasma by SPE using Oasis HLB Extraction Cartridges (30 µm). Cartridges were conditioned with methanol and water prior to the addition of 500 µL of serum sample followed by two washes with 10% methanol and 5% NH4OH respectively and subsequently eluted with 2% formic acid in 90% methanol. The eluents were evaporated and the residue reconstituted in 150 µL of 5% acetonitrile. Separation by HPLC was achieved with injecting 100 µL onto a C18 column (Acclaim 120) at 25°C with mobile phase of acetonitrile gradient of 5-55% with 10mM-monobasic potassium phosphate of pH 4.5 (0.5 mL/min). Chromatograms were analyzed with Chromeleon 7.2 software. Calibration curves were constructed using spiked plasma samples over the concentration ranges of 0.625–40 µg/mL. The method was repeated in triplicate to test for intraday and interday variability. For further validation, identical samples were assayed at a commercial referral laboratory.

Results: Optimum detection for levofloxacin was at 295 nm. Recovery of the internal standard was 75-76%. The assay was linear over the concentration range of 2.5-40.0 µg/ml (R2=0.98). In separate patient samples (N=4) with previously determined concentrations from another laboratory, patient sample accuracy range was 95.6%-112%. Both intraday (c.v. 0.2-2.2%) and interday precision (c.v 0.8-2.5%) showed excellent repeatability and reproducibility.

Conclusions: The proposed methodology for determination of levofloxacin concentrations achieved acceptable accuracy and reproducibility along a clinically meaningful range. Further testing will take place onsite from stored plasma of Tanzanian patients being treated for MDR-TB at different intervals of therapy. Protocols could then be further developed to study prospectively the application of therapeutic drug monitoring and dose adjustment for levofloxacin (or other fluoroquinolones).

No conflict of interest
Abstract: 18

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Determining protein binding of levofloxacin and moxifloxacin in vitro using ultrafiltration

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Introduction: Fluoroquinolones are commonly used for the treatment of tuberculosis and other bacterial infections. Knowledge of protein binding is important when developing dosing regimens as only the free fraction is active at the site of action. Limited information exists with regards to protein binding of levofloxacin (LEVO) and moxifloxacin (MOXI). Our objective is to determine the protein binding of LEVO and MOXI in vitro in different plasma matrices using ultrafiltration.

Methods: Protein binding of both LEVO and MOXI was determined in two different plasma matrices as follows: freshly donated plasma and freshly donated plasma after stripping with charcoal. Both plasma matrices were spiked with LEVO and MOXI at two different concentrations 1 and 10 mcg/ml. Later samples were warmed in a water bath at 37 °C for one hour. Then we transferred 1 ml from each of the four LEVO and MOXI solutions into ultrafiltration units. Ultrafiltration units were centrifuged at 1000 g and 37 °C for 16 minutes. 60 ul of each ultra-filtrate was collected and transferred to a 1.5 ml microcentrifuge tube. 60 ul of internal standard was added to all samples. Drug concentrations were determined using a validated high-performance liquid chromatography (HPLC) with a fluorescence detector assay.

Results: Protein binding of both LEVO and MOXI was relatively low. Median protein binding of LEVO and MOXI in both matrices were 41 % and 38 % respectively. No concentration dependent binding was noticed for either LEVO or MOXI. Protein binding was noticeably lower in fresh plasma. Median protein binding of LEVO in fresh plasma and stripped plasma was 38% and 44.25%. Median protein binding of MOXI in fresh plasma and stripped plasma was 29.25% and 46.6%.

Conclusion: Protein binding for both LEVO and MOXI was relatively low and consistent with previous reports. Protein binding in non-stripped plasma was lower for both MOXI and LEVO. Next we will determine the protein binding of LEVO and MOXI in clinical samples for patients with TB.

No conflict of interest
Abstract: 19

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Stability of second-line tuberculosis drugs mixed with readily available, nutritious foods

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Background: Pediatric dosage forms are lacking for most tuberculosis (TB) drugs, making them difficult to give and unpalatable to children. Commonly, drugs are admixed with various foods to make them easier to give to children, but the stability of these food and drug combinations remains unknown.

Methods: We prepared dosage forms of cycloserine (250 mg), levofloxacin (500 mg), and 2 dose sizes of ethionamide (150 mg and 250 mg) in foods that are available to parents: milk, yogurt, peanut paste, banana, and vitamin drink. Samples then were extracted and assayed using assays previously validated for plasma samples.

Results: Milk: Cycloserine and levofloxacin were fully recovered from milk. Ethionamide (125 mg) was partly recovered from milk (median 51%), and the 250 mg dose was 76% recovered. Yogurt: Cycloserine and levofloxacin were fully recovered from yogurt. Ethionamide (125 mg) in yogurt was inconclusive, with concentrations increasing with time, but the 250 mg dose was fully recovered. Peanut paste: Cycloserine was 40-60% recovered from peanut paste. Levofloxacin was not recovered from the peanut paste due to an extracted interference with the assay. Ethionamide (125 mg) was poorly recovered from peanut paste (2-26%), and the 250 mg dose was not recovered. Banana: Cycloserine showed degradation over time in banana, down to 46% by 24 hours. Levofloxacin was not recovered from banana, possibly due to binding in the matrix. Ethionamide (125 mg) was variably recovered from banana (24-94%, median 39%), and the 250 mg dose was not recovered. Vitamin drink: Cycloserine was not measurable from the vitamin drink due to matrix effects. Levofloxacin was fully recovered. Ethionamide (125 mg) showed recovery >100% from the vitamin drink, due to an assay interference, while the 250 mg dose was not quantifiable due to an assay interference.

Conclusion: All drugs and doses could be recovered from milk and yogurt, although ethionamide showed a decrease of roughly 25-50% in milk. Drug recovery from peanut paste was variable. It was best for cycloserine, poor for ethionamide, and inconclusive for levofloxacin. Drug recovery from banana was poor. Levofloxacin was fully recovered from the vitamin drink, but assay interferences prevented conclusive findings for the other drugs. Milk and yogurt appear to be the preferred vehicles for short-term stability when mixed with these second-line drugs. Overall, yogurt performed the best.

No conflict of interest
Abstract: 20

Pharmacokinetics and Pharmacodynamics of Approved Drugs

Low isoniazid and rifampicin concentrations in TB/HIV co-infected patients in Ugandan


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Background: There is limited data on exposure to anti-tuberculosis (TB) drugs in this region. Charles Peloquin et al (2002) has described reference ranges however some studies have demonstrated that patients actually achieve concentrations below these ranges. There is limited data about exposure to anti-TB drugs in the HIV/TB co-infected population in Sub-Saharan Africa. Our objective is to describe the concentration of anti-TB drug levels in a well characterized prospective cohort of adult patients starting treatment for pulmonary TB.

Methods: This study is an ongoing study being carried out in the TB/HIV integrated clinic at the Infectious Diseases Institute in Kampala, Uganda. Sputum culture and microscopy was done for all patients. We performed pharmacokinetic blood sampling of anti-TB drugs was done 1 hour, 2 hours and 4 hours post dose at 2 weeks, 8 weeks and 24 weeks after initiation of anti-TB treatment using ultra violet high-performance liquid chromatography (UV-HPLC) . We describe the maximum concentration (Cmax) of isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) and compare them with the values observed by Peloquin et al referenced in other studies.

Results: We started 113 HIV infected adults on a fixed dose combination of HREZ. The median age of our population was 33 years. 52% were male with a median BMI of 19kg/m2 and a median CD4 cell count of 142cells/µL. In 90% of the participants, the diagnosis of TB was based on microscopy and or cultures. The boxplot graph shows the median Cmax and IQR of H and R. Levels of H were found to be below the reference ranges (3-6µg/mL) in 54/77(70.1%), 38/59(64.4%) and 15/24(62.5%) participants at weeks 2, 8 and 24. Rif levels were also found to be below the reference ranges (8-24ug/mL) in 41/66(62.1%), 26/48(54.2%) and 8/10(6%) participants at weeks 2, 8 and 24 respectively. The mean Cmax of E and Z were within the reference range at week 2 and 8; mean Cmax of 3.2±SD2.1ug/mL and 4.0±SD3.1ug/mL for E and 41.6±SD13.1ug/mL and 42.6±SD16.4ug/mL for Z.

Conclusion: We observed lower concentrations of isoniazid and rifampicin in our study population of HIV/TB co-infected patients. The implications of these findings are not yet clear. We therefore need to correlate our findings with the response to TB treatment.

No conflict of interest

ENCORE ABSTRACT (HIV DRUG THERAPY GLASGOW 2014)
Abstract: 21

Pharmacokinetics and Pharmacodynamics of Approved Drugs

**Therapeutic drug monitoring of ethambutol in patients with tuberculosis**

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**Background:** Ethambutol is an essential first-line drug used in the treatment of tuberculosis (TB). However, absorption of the drug varies and is not always complete. Low drug concentrations may contribute to failure of TB treatment. The objective was to determine the prevalence of low drug concentrations for ethambutol, and explore possible correlations between drug concentration and age, sex or dose.

**Methods:** We retrospectively extracted data from patients who received therapeutic drug monitoring for ethambutol between April 2012 and June 2014. Patients that did not have both 2 and 6 hour samples were excluded. Cmax was determined as the higher concentration between the 2 and 6 hour samples, and Tmax was the time of Cmax. Data were divided as daily (7/7 or 5/7, normal Cmax 2-6 mcg/mL) or as intermittent (2-3 times weekly, normal Cmax 4-12 mcg/mL). Patients’ absorption was classified as normal absorption (2 hour Cmax in the normal range), delayed absorption (6 hour Cmax in the normal range), or malabsorption (both 2 and 6 hour samples below the normal range). Statistical analyses were performed using JMP software (V.10.0).

**Results:** There were data for 242 patients (80 females, 160 males and 2 patients of unknown sex). Patient age spanned from 0.5 year to 97.6 years, with a mean age of 51 years. Of the 242 patients, 50.8% had normal absorption, 11.2% had delayed absorption and 38.0% had malabsorption. In the daily dosing patients (n=205), doses were between 200 and 2000 mg (mean 1106 mg) produced a Cmax range from 0 and 7.26 mcg/mL (mean of 2.55 mcg/mL), and a mean Tmax of 2.88 hours. There was no significant relationship between Cmax and sex. Weak but statistically significant positive associations were observed between Cmax and age (R² = 0.04, p < 0.05) and Cmax and dose (R² = 0.07, p <0.05). In the intermittent dosing patients (n=37), doses were between 800 and 3600 mg (mean 2254 mg) produced a Cmax range from 0.60 and 11.51 mcg/mL (mean Cmax of 4.39 mcg/mL), and a mean Tmax of 3.87 hours. There were no significant relationships between Cmax and sex, age or dose.

**Conclusion:** The available data set was restricted to the information provided on the sample requisition, and did not include complete patient data. Cmax was not well predicted based on sex, age or dose in patients receiving intermittent dosing. Cmax showed a modest positive association with age and dose (but not sex) in patients receiving daily dosing.Nearly 40% of patients displayed malabsorption, showing the importance of monitoring for low drug concentrations in patients taking ethambutol.

*No conflict of interest*
Abstract: 22

Drug Development and optimization: Approaches & Tools

Model-Based Design and Analysis of Phase IIa Trials within Tuberculosis Drug Development

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Introduction: The demand for new drugs to treat pulmonary tuberculosis (TB) is high due to high prevalence of multi-drug resistant TB. Phase IIa within TB drug development is the first study in patients and is crucial for decision-making for further development. Current design and analysis of Phase IIa trials within TB are not always informative. We explore the advantages of model-based design and analysis using a pharmacokinetic-pharmacodynamic (PKPD) approach within Phase IIa TB drug development, aiming to produce more informative and less costly trials.

Material & Methods: Phase IIa studies were simulated for three hypothetical anti-TB drugs with high (Drug A), modest (Drug B), and low (Drug C) bactericidal efficacy using the Multistate Tuberculosis Disease Model. Longitudinal colony forming unit (CFU) data was simulated for 4 doses of study drug and rifampicin as reference for 14 days following daily administration of drug. Analysis was performed using traditional (t-test), empirical (mono- and bi-exponential regression models) and PKPD approaches to investigate power to find a drug effect at different sample sizes. All dose groups were included in the PKPD approach whilst the other approaches only included the highest dose group.

Results: For Drug A the required total sample size to reach 90% power followed the order: PKPD (n=10); mono-exponential model (n=20); t-test (n=30); bi-exponential model (n=955), from lowest to highest. The required sample sizes for Drug B were: PKPD (n=40); mono-exponential model (n=85); t-test (n=90); bi-exponential model (n>1250). For Drug C the required sample sizes were 200 for the PKPD approach and >1250 for the other approaches.

Conclusions: A model-based design and analysis using a PKPD approach can provide supportive evidence for trial designs that reduce number of patients required to determine a drug effect, reducing costs. The PKPD approach achieves this because it provides exposure-response information in contrast to the other analyses.

No conflict of interest
Abstract: 23

Population PK/PD modeling

Refined Pharmacokinetics for Tuberculosis Drug Discovery: Determining Drug Concentration at the Site of Infection

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Introduction: Fusidic acid is a well characterised antibacterial drug that has been shown to possess good antymycobacterial activity. It has however failed to deliver positive results in the tuberculosis mouse model; a model shown not to extrapolate well to clinical outcomes due to the complex pathology of tuberculosis. Organ sampling and analysis to evaluate compound exposure closer to the site of infection was used to evaluate the murine pharmacokinetic properties of a novel prodrug of fusidic acid, GKFA17.

Material and methods:
1. LC/MS/MS methods to analyse GKFA17 and its three metabolites in mouse whole blood and organs were developed.
2. Pharmacokinetic evaluation of GKFA17 and its active metabolites in blood was performed in C57/Bl6 mice, n = 3 for oral and intravenous groups, followed by organ distribution, n = 3 at 4 time points.
3. Analysis of blood, spleen, liver, kidney, brain, heart and lung data to compare exposure.

Results: An inactive metabolite was identified and retrospectively quantified and added to the existing analysis. The results showed GKFA17 at a dose of 10 mg/kg was rapidly metabolised to fusidic acid, which partitioned greatly into the liver and did not reach its proposed target site. The inactive metabolite had highest exposure in all tissues and explain the lack of fusidic acid efficacy in the tuberculosis mouse model.

Conclusions: A preclinical method for determining drug pharmacokinetics at the proposed site of tuberculosis infection was successfully developed. This model can be used in the drug discovery process to better identify promising anti-tuberculosis compounds.
Abstract: 24

Population PK/PD modeling

Evaluation of rifampicin effect on mycobacterium tuberculosis using a multistate pharmacometric model

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Introduction: Mycobacterium tuberculosis (M. tuberculosis) can exist in different states in vitro, which can be denoted fast-, slow- and non-multiplying. A multistate pharmacometric model describing the natural growth of M. tuberculosis H37Rv, providing predictions of the bacterial number in a fast, slow and non-multiplying state over time, has previously been developed. The model could be used to characterize the effect of antitubercular drugs on bacteria from different types of cultures and different bacterial states over time and hence provide further insight into the kill kinetics of the studied drug.

Methods: The data consisted of M. tuberculosis H37Rv pre-grown to log phase (4 days) and stationary phase (100 days) in a hypoxia driven in vitro system. The log and stationary phase bacteria were incubated with different concentrations of rifampicin (RIF) and viability determined by colony forming unit (cfu) counting at different time points. The data was modeled using a nonlinear mixed-effects approach (NONMEM 7.3, I.D. Solutions, Ellicott City, Maryland, USA). The effect of RIF on the different bacterial states was evaluated using different linear and nonlinear relationships to exposure.

Results: The effect of RIF on the log and stationary phase bacteria was described both individually and simultaneously. Effects of RIF were evaluated, as applicable, on both the fast, slow and non-multiplying bacterial states as kill-rate or inhibition of growth. The model that best described both the log and stationary phase bacteria consisted of kill-rates of the fast, slow and non-multiplying states and inhibition of growth of the fast multiplying state.

Conclusions: By taking RIF specific in vitro pharmacokinetics into account the multistate model was successfully used to describe the in vitro effect of RIF on both log and stationary phase M. tuberculosis H37Rv simultaneously.

No conflict of interest
Abstract: 25

Initial development of assays for MDR-TB drugs using LC/MS/MS in small hair samples: Implications for pharmacologic monitoring of outcomes

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Background: Pharmacokinetic (PK) variability may be an important determinant of multidrug resistant tuberculosis (MDR-TB) therapeutic outcomes and may explain differences in response among trial participants and in real-world settings. Plasma PK assessments require phlebotomy and a cold chain, and are generally not repeated frequently enough to characterize drug exposure over time. Noninvasive, readily available PK assessments in biomatrices without need for biohazard precautions or cold chain in collection and shipment could have a major impact on MDR-TB drug development and monitoring. Our group has expertise in the development and validation of antiretroviral concentrations in small hair samples and we sought to extend these methods to MDR-TB.

Materials and Methods: We developed a multi-analyte assay for measuring second-line anti-TB drug concentrations (levofloxacin, moxifloxacin, linezolid, pyrazinamide) in small hair samples. Sample preparation consisted of hair pulverization using an Omni Bead Ruptor® tissue homogenizer, extraction of drug with an aqueous methanol solution, liquid-liquid extraction, and then evaporation of the combined organic layer extract prior to reconstitution to the mobile phase. After injection of sample extract into the LC/MS-MS system (Agilent LC 1260-AB Sciex API 5500 equipped with a Synergi Polar-RP column), mass spectrometric detection was achieved with positive ionization by electrospray ionization (ESI) and mass scanning was performed via multiple reaction monitoring (MRM). Quantitation of each drug was performed by isotope dilution method using deuterium- or 13C-labeled standard of each drug as internal standards.

Results: We extracted and quantified levofloxacin (LFX, 15.1 ng/mg), moxifloxacin (MFX, 16.9 ng/mg), linezolid (LZD, 7.2 and 12.8 ng/mg), and pyrazinamide (PZA, 15.1 ng/mg) from hair specimens in two patients on directly observed MDR-TB treatment; additional co-administered drugs included cycloserine, ethambutol, and PAS. Utilizing a 6-point calibration curve, each assay showed high sensitivity (LLQ 0.1-1 ng/mg) and wide linear dynamic range (0.1-40 ng/mg) using 20-30 strands of human hair (~1-3 mg). The drug concentrations observed for each extracted drug were well within their linear ranges, and the within-run precision (5 intra-subject iterations) of our method was within 15% coefficient of variation for each drug (PZA 13.2%; LZD 6.6-12.9%; LFX 7.8%; MFX 6.5%). In addition, we have successfully incorporated bedaquiline, delamanid, and isoniazid into this multi-analyte assay panel, and extraction procedures from hair are currently in progress.

Conclusions: We demonstrate proof of concept for the development of assays to accurately and noninvasively determine long-term exposure to key second-line anti-TB drug concentrations in hair. Such tools could aid in optimizing MDR-TB treatment regimens through improved understanding of the individual contribution of each drug within a multidrug regimen, exposure-response relationships for each drug, and monitoring of treatment response. In addition, given that MDR-TB treatment is increasingly provided in community settings, such tools could improve assessment of adherence. Further validation of these hair assays and testing their utility in clinical settings is needed.

No conflict of interest
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<td>Model-Based Design and Analysis of Phase Ila Trials within Tuberculosis Drug Development</td>
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<td>Tasneen, R.</td>
<td>Contribution of different oxazolidinones to the efficacy of novel regimens</td>
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<td>containing bedaquiline and pretomanid in murine models of tuberculosis</td>
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<td>Wiltshire, C.</td>
<td>Association of anti-tuberculosis drug concentrations with drug-related adverse events in TB/HIV co-infected patients in Uganda</td>
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<td>Wiltshire, C.</td>
<td>Low isoniazid and rifampicin concentrations in TB/HIV co-infected patients in Ugandan</td>
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<td>Zhang, N.</td>
<td>Concentration-response effects of combination therapy in a murine model of tuberculosis: optimizing the dosing regimen for short-course therapy</td>
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