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# Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: implications for the treatment of disseminated and meningeal tuberculosis in children

--Manuscript Draft--

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| **Abstract:** | Background: Ceftazidime-avibactam is an effective agent for the treatment of tuberculosis but requires frequent administration because of a short half-life. Due to a longer half-life, ceftriaxone could allow intermittent dosing.<br>Methods: First, we identified the MIC of ceftriaxone with 15 mg/L avibactam in 30 clinical Mycobacterium tuberculosis [ Mtb ] isolates. Next, two ceftriaxone exposure-effect studies in the intracellular hollow fiber model of tuberculosis (HFS-TB) that mimics disseminated disease in young children, were performed. Ceftriaxone was administered once or twice daily for 28 days to explore percentage of time that the concentration persisted above MIC [%T MIC ] ranging from 0 to 100%. In a third HFS-TB experiment, the “double cephalosporin” regimen of ceftazidime-ceftriaxone-avibactam was examined and analyzed using Bliss Independence.<br>Conclusion: The MIC 99 of the clinical strains was 32 mg/L, in the presence of 15 mg/L avibactam. Ceftriaxone %T MIC <42 had no microbial effect in the HFS-TB, %T MIC > 54% demonstrated a 4.1 log 10 CFU/mL Mtb kill, while %T MIC mediating E max was 68%. The “double cephalosporin” combination was highly synergistic. Monte Carlo experiments of 10,000 subjects identified the optimal ceftriaxone dose as 100 mg/kg twice a day.<br>Conclusion: The combination of ceftriaxone-avibactam at 100 mg/kg could achieve E |

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| | max in >90% of children. The ceftriaxone potent activity Mtb could potentially shorten therapy in children with disseminated tuberculosis. |
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| **Funding Information:** | Eunice Kennedy Shriver National Institute of Child Health and Human Development (1R01HD099756-01) — Dr. Shashikant Srivastava<br>UK Medical Research Council (MR/R007942/1) — Dr James Seddon |

Date: 06/29/2020

To,
Irja Lutsar, MD, PhD
Series Editor: Antimicrobial Reports
The Pediatric Infectious Disease Journal

Dear Dr. Lustar,

On behalf of all the authors, I would like to thank you and the reviewers for the comments and suggestions to improve the manuscript. We are submitting the revised manuscript entitled **"Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: implications for the treatment of disseminated and meningeal tuberculosis in children"** for your review and publication in the PDIJ.

All authors have read, reviewed and approved the revised manuscript, and declared the conflict of interest where applicable.

I am looking forward to hearing from you.

Best regards,



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**RE: PIDJ-220-406, Ceftriaxone plus avibactam for the treatment of disseminated and meningeal tuberculosis in children**

**Response to Editor**

**Query 1.** In addition, please revise the title as it is misleading. If my understanding is correct you present an *in vitro* data that may be relevant for clinical practice. Please assure that the title reflects that these are *in vitro* studies and cannot be directly translated into clinical practice.

**Response.** Thank you for the comment and the suggestion. We changed the manuscript title to “Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: implications for the treatment of disseminated and meningeal tuberculosis in children”.

**Reviewer's comments:**

**Reviewer #2:**

**Query 1.** This was a very interesting and novel study. I did not find any studies to compare with it. The methods and tables are clear to read. For young children, who more commonly have disseminated intracellular TB than older patients, there could be additional benefits due to the high concentrations achieved intracellularly. Since ceftriaxone-avibactam was effective against strains both drug-susceptible and MDR-TB clinical strains, this highlights an important potential role in the treatment of drug-resistant TB. It would be interesting to see the next steps in an RCT *in-vivo* study.

**Response.** We would like to thank the reviewer for the comments.

**Reviewer #3:**

**Query 1.** This is a well written manuscript. The information is critical to the clinical community. The only revisions are minor editing changes that will be made on final edit.

**Response.** We appreciate the reviewer’s comments and edited the manuscript for typographical errors and any other correction, where needed.

# Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: implications for the treatment of disseminated and meningeal tuberculosis in children

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**KEYWORDS:** Hollow fiber model, cephalosporin, optimal dose, children, tuberculosis

**ABBREVIATED TITLE:** Ceftriaxone-avibactam for children with tuberculosis

**RUNNING HEAD TITLE:** Ceftriaxone-avibactam and tuberculosis

## CONFLICT OF INTEREST

Tawanda Gumbo founded Praedicare, a drug development Contract Research Organization. All authors have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization and design, SS, TG, and TT; MIC and HFS-TB experiments, SS, DD, KNC, KM; PK/PD modeling, JvZ, SS and TG. Monte Carlo Experiments and Bliss Independence: TG; SS wrote the first draft of the manuscript; TT, JWA, JAS, and TG, reviewed the manuscript and provided clinically meaningful comments on the clinical dose selection and usages. All authors reviewed and approved the final version of the manuscript.

# Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: implications for the treatment of disseminated and meningeal tuberculosis in children~~Ceftriaxone plus avibactam for the treatment of disseminated and meningeal tuberculosis in children~~

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## ABSTRACT

**Background:** Ceftazidime-avibactam is an effective agent for the treatment of tuberculosis but requires frequent administration because of a short half-life. Due to a longer half-life, ceftriaxone could allow intermittent dosing.

**Methods:** First, we identified the MIC of ceftriaxone with 15 mg/L avibactam in 30 clinical *Mycobacterium tuberculosis* [*Mtb*] isolates. Next, two ceftriaxone exposure-effect studies in the intracellular hollow fiber model of tuberculosis (HFS-TB) that mimics disseminated disease in young children, were performed. Ceftriaxone was administered once or twice daily for 28 days to explore percentage of time that the concentration persisted above MIC [$\%T_{MIC}$] ranging from 0 to 100%. In a third HFS-TB experiment, the “double cephalosporin” regimen of ceftazidime-ceftriaxone-avibactam was examined and analyzed using Bliss Independence.

**Conclusion:** The $MIC_{99}$ of the clinical strains was 32 mg/L, in the presence of 15 mg/L avibactam. Ceftriaxone $\%T_{MIC}$ <42 had no microbial effect in the HFS-TB, $\%T_{MIC} \geq 54\%$ demonstrated a 4.1 $\log_{10}$ CFU/mL *Mtb* kill, while $\%T_{MIC}$ mediating $E_{max}$ was 68%. The “double cephalosporin” combination was highly synergistic. Monte Carlo experiments of 10,000 subjects identified the optimal ceftriaxone dose as 100 mg/kg twice a day.

**Conclusion:** The combination of ceftriaxone-avibactam at 100 mg/kg could achieve $E_{max}$ in >90% of children. The ceftriaxone potent activity *Mtb* could potentially shorten therapy in children with disseminated tuberculosis.

## INTRODUCTION

Drug-resistant *Mycobacterium tuberculosis* (*Mb*) is a major global health emergency; drug resistance has already been reported to the newly developed tuberculosis (TB) drugs delamanid and bedaquiline.(1-7) Given the limited number of candidates in the TB drug pipeline, “repurposing” drugs already approved for other indications could be an important strategy to achieve global TB control.(8, 9) Repurposed drugs have the advantage that post-licensure data on dosing and toxicities are already available. This makes them more “shovel ready” for use, especially in historically understudied populations, such as in children. Frequently, data on dosing and toxicity for children lag behind those in adults when new drugs are developed. Elsewhere we have shown that benzylpenicillin and ceftazidime, when used with avibactam, were highly effective against *Mtb*, despite the short half-life.(10, 11) In the present study, we wanted to optimize ceftriaxone, that has been used throughout the world for over fifty years for the treatment of Gram-positive and Gram-negative infections and has a long half-life (10-13) for the treatment of tuberculosis.

Ceftriaxone, an aminothiazolyl-oxyimino cephalosporin, is more β-lactamase stable, with a half-life of 6-8 hours that could allow once daily dosing in TB. Ceftriaxone has good cerebrospinal fluid [CSF] to serum penetration ratios., which makes it for the treatment of TB meningitis, commonly encountered in young children with disseminated disease.(14-16) Ceftriaxone is also safe during pregnancy as well as children and infants out of the neonatal period, suggesting

that if efficacy against *Mtb* is demonstrated, it would be useful drug in these neglected patient groups. In children <3 years, in whom there are higher rates of death and therapy failure, disseminated disease is predominantly intracellular.(17) Involvement of meninges, peritoneum and bone is more common that in older patients. Here, we examined the efficacy of ceftriaxone plus avibactam in the hollow fiber model of TB (HFS-TB).(10, 11, 18-20)

## MATERIALS ANDMETHODS

### Bacterial strain, cell lines, and supplies

The culture conditions and the growth medium for both *Mtb* ATCC #25177) and the THP-1 cells (ATCC TIB-202) were as reported previously.(10, 11, 20) We used our library of 30 clinical isolates [10 susceptible to rifampin and isoniazid and 20 multidrug-resistant (MDR; resistant to both rifampin and isoniazid) obtained from the Medical Research Council of South Africa. Hollow fiber cartridges were purchased from FiberCell (Frederick, MD), ceftriaxone from Baylor University Medical Center pharmacy, and avibactam from BOC Sciences (Shirley, NY). The BACTEC 960 system and Mycobacteria Growth Indicator Tubes (MGIT) were purchased from Becton Dickinson (Franklin Lakes, NJ).

### Ceftriaxone MIC distribution and concentration-response studies

First, we performed a concentration-response study to determine if avibactam monotherapy has any effect on the growth of *Mtb.* The experiment was performed with *Mtb* H37Ra using the avibactam concentrations of 0, 3.75, 7.5,

15, and 30 mg/L. Second, we used both MGIT and micro-broth dilution methods to identify ceftriaxone MICs for *Mtb* H37Ra laboratory strain, but only the micro-broth dilution method for the clinical isolates.(21) The ceftriaxone concentrations, in a two-fold dilution series, ranged from 0.25 to 128 mg/L. MICs were performed with and without the addition of 15 mg/L avibactam for the laboratory strain, but with avibactam for all clinical strains. Third, ceftriaxone concentrations-response studies (with 15 mg/L avibactam) were performed using the same concentrations as in the MIC experiments. The cultures were incubated at $37^0$C under shaking conditions for 7-days, followed by 10-fold serial dilutions and inoculation on Middlebrook 7H10 agar supplemented with 10% OADC agar. Colony forming units per milliliter (CFU/mL) were counted after 21 days of incubation at $37^0$C. All experiments were performed twice.

**Ceftriaxone hollow fiber system model of TB studies**

Using the intracellular HFS-TB model (11, 20, 22) we performed two different ceftriaxone experiments to identify [i] the optimal ceftriaxone exposure, and [ii] the best dosing schedule, based on the ceftriaxone serum half-life of $5.8 \pm 2.6$ hours encountered in infants and children <2 years.(13, 23) In the first HFS-TB experiment we used avibactam at a constant concentration of 1 mg/L, described as the pharmacokinetics/pharmacodynamics (PK/PD) target for avibactam for Gram negative bacilli in the ceftazidime-avibactam FDA approval document.(13) In the second HFS-TB experiment, avibactam was used at a concentration of 15 mg/L, which is the concentration achieved in children when given the standard

dose of the ceftazidime-avibactam, as well as the optimal avibactam concentration we identified in our *in vitro* experiments and elsewhere.(11, 24) The details of the THP-1 monocytes infection with *Mtb* and inoculation into the HFS-TB have been described in detail elsewhere.(20, 22) The HFS-TB units were treated with either a once daily or twice daily dosing schedule to achieve the percentage of time that the concentration persisted above MIC [$\%T_{MIC}$] ranging from 0 to 100%. Each HFS-TB unit was repetitively sampled to measure the ceftriaxone concentration as well as for *Mtb* CFU counts, using the methods described in detail elsewhere.(11, 20) A portion of the processed sample from the peripheral compartment of the HFS-TB was inoculated into MGIT tubes to determine the time-to-positivity (TTP) as a second readout for bacterial burden.

**Ceftazidime-avibactam as source of β-lactamase inhibitor**

Currently, avibactam is only commercially available as ceftazidime-avibactam,(24) and is thus the only available avibactam source for clinicians. In order to determine if there is synergy/antagonism/additivity between ceftriaxone-avibactam and ceftazidime-avibactam, ceftriaxone optimal exposure and ceftazidime-avibactam at optimal exposure of $\%T_{MIC}$ ≥63%(11) were administered to HFS-TB units as either [1] “monotherapy” [with avibactam] or [2] a combination as ceftriaxone-ceftazidime-avibactam. Samples for drug concentrations and CFU at various times were processed as described above.

**Drug concentration measurements**

The ceftazidime and avibactam concentration assays have been reported previously.(11) We used a stable-isotope dilution liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) to measure ceftriaxone concentrations. The calibrators, controls and internal standards (ceftazidime-d5) were included in each analytical run for quantification. The lower limit of detection of the method was 0.05 mg/L with a 6.2% and 7.4% inter- and intra-day variation.

**PK/PD analysis**

The observed drug concentrations were modeled using ADAPT 5 software.(25) Concentrations were analyzed using a one-compartmental model with first-order rate absorption and elimination. The primary pharmacokinetic parameters were estimated using the maximum likelihood expectation maximization [MLEM] algorithm and then used to calculate the secondary parameters such as half-life, peak concentration ($C_{max}$), and 24hr area under the concentration-time curve [$AUC_{0-24}$] for each HFS-TB. We used the inhibitory sigmoid $E_{max}$ model using $\%T_{MIC}$ versus *Mtb* burden, to estimate the PK/PD exposure associated with 50% [$EC_{50}$], or 80% [$EC_{80}$], or 90% [$EC_{90}$] of maximal bacterial kill [$E_{max}$].We also identified the PK/PD index linked to microbial kill using the same inhibitory sigmoid $E_{max}$ model for CFU/mL versus exposure [$\%T_{MIC}$ or $AUC_{0-24}$/MIC or $C_{max}$/MIC] based on corrected Akaike Information Criteria [AIC] scores.(26) GraphPad Prism (v7) was used for graphing.

## Bliss Independence versus Loewe Additivity

In our prior experiments evaluating the impact of ceftazidime-avibactam on *Mtb*, we found that ceftazidime has a different binding target [PonA1] from other beta-lactams and cephalosporins,(11) and the inhibitory sigmoid $E_{max}$ dose-response curves of ceftazidime-avibactam are not parallel with those of ceftriaxone-avibactam. Based on this, we assumed that ceftriaxone and ceftazidime work on independent targets, and fulfil the criteria for non-interaction, hence Bliss Independence.(27, 28) Thus, we utilized Bliss Independence for our modeling. Effect was measured as percentage change from day 0. Theoretical additivity [$X_T$] was calculated by adding the effect of ceftriaxone-avibactam "monotherapy" [A] to ceftazidime-avibactam "monotherapy" [B] on each sampling day. The observed effect [$X_o$] was the percentage change from day 0 in the ceftriaxone-ceftazidime-avibactam combination regimen. The combination index [$X_I$] is given by:

$$X_I=[X_T-A*B]/X_o \quad (1)$$

## Monte Carlo experiments

We utilized Monte Carlo experiments [MCE] to identify the optimal ceftriaxone doses for use in children.(18, 22, 29-31) The following pharmacokinetic parameter estimates were used in both subroutine PRIOR of ADAPT and the parameter files: plasma clearance of 0.051±0.024 L/hr/kg and a volume of distribution of 0.382±0.129 L/kg for 50 mg/kg dose, and clearance of 0.055±0.018 L/hr/kg and a volume of distribution of 0.387±0.056 L/kg for 75 and

100 mg/kg, based on Steele et al.(23) Each dose was infused intravenously over 10 minutes. The ratio for the ceftriaxone CSF-to-plasma AUC in neonates and infants with meningitis of 17%, as reported by Martin et al, was used for meningitis.(32) In children <5 years, at a dose of 50 mg/kg, ceftriaxone protein binding in serum is 82±8% while that in CSF during purulent meningitis is 9±6% protein bound, which is negligible.(33) Free drug ceftriaxone concentrations were used in the MCE; for CSF total ceftriaxone was assumed equivalent to free drug. The ceftriaxone MIC distribution used was from our 30 clinical isolates.

## RESULTS

### MIC and concentration-response in test tubes

Avibactam up to 30 mg/L concentration did not showed any inhibitory effect on *Mtb* growth. Therefore, avibactam on its own does not kill *Mtb*. The ceftriaxone MIC for *Mtb* H37Ra was 16 mg/L without avibactam but fell by two-tube dilutions to 4 mg/L in the presence of 15 mg/L avibactam. Therefore, we subsequently identified ceftriaxone MICs against 30 clinical isolates only in combination with 15 mg/L avibactam. The MIC range was 0.5-32 mg/L. The $MIC_{50}$ and $MIC_{90}$ were 4 mg/L and 32 mg/L, respectively. In the concentration-response studies, ceftriaxone $EC_{50}$ was calculated as 2xMIC, and effect was near maximal at 4xMIC, consistent with time driven effect ($r^2$=0.97).(34)

### HFS-TB experiment with 1mg/L avibactam

In the first ceftriaxone HFS-TB examined ceftriaxone, with an avibactam constant infusion concentration of 1 mg/L, the time-kill curves showed an exposure-dependent lowering of bacterial burden compared to non-treated control. After initial microbial kill below day 0 [stasis] on day 3, there was therapy failure due to the rebound growth by day 7. The Inhibitory sigmoid $E_{max}$ model showed highest $r^2$ on day 7, when the relationship was described by following equation:

$$\text{Effect}=5.41-1.07*(\%T_{MIC})^{1.0}/[19.90^{1.0}+(\%T_{MIC})^{1.0}] \quad (2),$$

so that the $EC_{50}$ was $\%T_{MIC}$=19.90, and $EC_{80}$ was calculated as $\%T_{MIC}$=79.60 [$r^2$=0.92]

**HFS-TB with 15 mg/L avibactam for exposure-effect and dose scheduling**

The ADAPT model predicted versus the observed ceftriaxone concentrations [$r^2$ of 0.99] with once daily or twice daily dosing schedule are shown in **Figure 1A** and **1B**. The ceftriaxone elimination rate constant [$K_{el}$] achieved in the HFS-TB extracellular compartments, reported as a "population" was 0.09±0.02 $hr^{-1}$ while volume of distribution was 1.39±0.21 L and half-life was 7.25±1.54 hr. The corresponding intracellular concentrations are shown in **Figure 1C**. The $K_{el}$ was 0.04±0.02 $hr^{-1}$ while volume of distribution was 0.62±0.17 L and half-life of 11.41±10.32 hr. Thus, clearance of ceftriaxone from intracellular compartment was slower.

**Figure 2A** shows the kill curves in the HFS-TB by these exposures, based on the TTP readout, while **Figure 2B** shows the kill curves based on the CFU/mL

readout. Lower the TTP, higher the bacterial burden. **Figure 2A** and **2B** show that there was microbial kill below day 0 [stasis] by several doses and dosing schedules, and a much greater depth of kill, which was sustained for up to 21 days in some doses, with 15 mg/l avibactam compared to what was observed in **Figure 2A** with 1 mg/L of avibactam.

**Figure 3A-C** shows the inhibitory sigmoid $E_{max}$ model output for the TTP readout for each PK/PD index: $C_{max}$/MIC or AUC/MIC or $\%T_{MIC}$. On day 28, the Akaike Information Criteria [AIC] scores for $C_{max}$/MIC was 15.58, for AUC/MIC was 13.75, while that for $\%T_{MIC}$ was 8.61. Based on this the PK/PD parameter linked to effect was $\%T_{MIC}$. **Figure 3D-F** show the model output for CFU/mL versus exposure. The same was apparent with the CFU/mL readout: on day 28, the AIC score for $C_{max}$/MIC was 18.12, for AUC/MIC was 14.34, while that for $\%T_{MIC}$ was 7.34. Based on this readout, the lowest AIC score was for $\%T_{MIC}$, which was chosen as the PK/PD index linked to *Mtb* kill. The relationship between $\%T_{MIC}$ and *Mtb* burden on day 28, at the end of the experiment, was described by the equation #3:

$$\text{Effect}=8.06-3.08*(\%T_{MIC})^{7.61}/[50.67^{7.61}+(\%T_{MIC})^{7.61}] \quad (3),$$

The $EC_{50}$ $\%T_{MIC}$ was 50.7%, while the $EC_{80}$ $\%T_{MIC}$ was 60% of dosing interval [$r^2$=0.91].

**HFS-TB to explore double β-lactam strategy**

**Figure 4A-C** shows that ceftriaxone-avibactam and ceftazidime-avibactam had "monotherapies" had identical kill curves by TTP **(Figure 4A)** and cfu/mL (**Figure 4B).** The figure also shows that the double β-lactam combination of ceftriaxone-ceftazidime-avibactam killed better than either cephalosporin "monotherapy". We calculated Bliss Independence for each day, and then an overall interaction factor [$X_I$] and 95% confidence intervals [CI] for all sampling days, and then an overall $X_I$, based on cfu/mL readout, with results shown in **Figure 4C**. When $X_I$ and its 95% CI were less than zero, as shown in **Figure 4C** for days 7 onwards, then there was synergy because the effect observed for the ceftriaxone-ceftazidime-avibactam combination regimen was greater than expected from adding the effect of ceftriaxone-avibactam "monotherapy" to that of ceftazidime-avibactam "monotherapy". On day 3, $X_I$ and its 95% CI crossed zero, which means the observed effect was exactly the same as the effect of the sum of the two monotherapies, so that on that day they were additive. Overall, when data for all the entire experiment was combined, the mean $X_I$ was -0.81 [95% CI: -1.01 to -0.63], indicating synergy [**Figure 6C**].

**Monte Carlo experiments [MCE]**

MCE of several different doses are shown in **Figures 5A** and **5B**. The pharmacokinetic parameter estimates and standard deviations in the MCE output were a clearance of 0.05±0.04 L/hr and volume of 0.38±0.22 L [half-life=5.21 hr] in 5,000 children, which are identical to the parameters in the domain of input, which is an internal validation step. For external validation we compared the

peak concentrations we identified in the MCE for children treated with 50 mg/kg of 232±134 mg/L to those of 220±64 mg/L observed in actual children in the clinic treated with the same dose, and 308±1106 mg/L versus 295±76 mg/L in children treated with 75/mg/kg.(13) This means our simulations represent the reality seen in clinical trials when it comes to drug concentrations. Target attainment probabilities [TAP] to achieve $\%T_{MIC}$ of 60% for disseminated disease without meningitis are shown in **Figure 5C**. The cumulative fraction of response [CFR] over the entire MIC distribution for 50 mg/kg once a day was 46.8% while that for twice a day was 76.0%. The CFR for 75 mg/kg once a day was 55.9% while that for twice a day was 83.4%. For meningeal TB, the TAPs are shown in **Figure 5D**. The CFR for 50 mg/kg once a day was 43.6% while that for twice a day was 72.7%. The CFR for 75 mg/kg once a day was 52.6% while that for twice a day was 80.3%. Thus, 75 mg/kg twice a day would be the best dose; the PK/PD derived susceptibility breakpoint for this dose is >16 mg/L. A dose of 100 mg/kg once-a-day achieved a CFR of 69.37%, while twice a day achieved a CFR of 90% in disseminated TB. It is unclear if this larger dose would be tolerated by the children.

## DISCUSSION

Here, first, we show that ceftriaxone has efficacy against *Mtb* even without avibactam, but the avibactam dramatically improved both potency and efficacy. We have found that *Mtb*'s natural resistance to cephalosporins is via degradation by BlaC [encoded by a lone gene] of the drug, and that otherwise *Mtb* has a

cephalosporin binding target and even a benzylpenicillin binding target.(11, 35, 36) Avibactam is a particularly effective inhibitor of *Mtb* BlaC.(11, 35, 37) Thus, ceftriaxone, when used with a β-lactamase inhibitor would be effective in children and adults, with particular relevance to neglected populations including young children and pregnant women. For young children, who more commonly have disseminated intracellular TB than older patients, there could be additional benefits due to the high concentrations achieved intracellularly. Since ceftriaxone-avibactam was effective against strains both drug-susceptible and MDR-TB clinical strains, this highlights an important potential role in the treatment of drug-resistant TB.

Second, we found that microbial kill of *Mtb* by ceftriaxone-avibactam was linked to $\%T_{MIC}$, with an optimal target of 60% of dosing interval. This exposure target is very similar to observations of 50-70% identified with cephalosporins and gram-negative bacteria. In the HFS-TB study that had suboptimal concentrations of avibactam, the $EC_{80}$ target of $\%T_{MIC}$=80% was higher than with avibactam, consistent with improved potency. We did not isolate any ceftriaxone resistant *Mtb* subpopulations, thus the PK/PD parameter linked to resistance suppression remains unknown.

Third, our clinical trial simulations show that a dose of 100 mg/kg twice a day could achieve a CFR of >90% in both disseminated and meningeal TB. However, there could be adverse events of this high dose such as nephrolithiasis,

cholestatic jaundice, neutropenia, hepatitis, eosinophilia etc.(13, 38) The once a day dose of 100 mg achieved a CFR of 69.4%; this rate could be acceptable in combination therapy in which the ceftriaxone gets help from companion drugs, and would have the virtue of more convenient dosing schedule in children and in resource limited settings. Otherwise, the dose of 100 mg/kg twice a day that could achieve CFR of 90% of the patients would be optimal, and for this dose the susceptibility breakpoint was an MIC >16 mg/L [32 mg/L].

Finally, we show that there is synergy between ceftazidime-avibactam and ceftriaxone; the commercially available preparation of ceftazidime-avibactam, therefore, can be used as source of avibactam. However, given that the high cost associated with the commercially available ceftazidime-avibactam combination, there is a need to develop a ceftriaxone-avibactam combination. The oral formulations of ceftriaxone (39) and avibactam (40) are in the developmental stage and in future this could potentially lead to a child friendly oral combination. Similarly, exploring ceftriaxone in combination with amoxicillin-clavulanic acid for potential synergy may also present a more cost-effective treatment option.

In summary, we show that ceftriaxone could be used for the treatment of TB, including drug-resistant forms, the 100 mg/kg dose for children and adults with TB could achieve exposure-target in a large proportion of patients.

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**Figure 1: Ceftriaxone concentration-time profile in the HFS-TB. (A)** Observed ceftriaxone concentrations versus ADAPT model-derived curves for the HFS-TB treated with a once daily dosing schedule. (**B)** Observed ceftriaxone concentrations achieved in the central compartment of the HFS-TB with a twice daily dosing schedule versus ADAPT model-derived curves. (**C)** Ceftriaxone concentration measured inside the THP-1 cells was multiple times higher than the concentration observed in the central compartment. The solid lines represent ADAPT PK model predicted ceftriaxone concentrations, and the symbols represent the observed concentration.

**Figure 2: Ceftriaxone kill curves in the HFS-TB. (A)** The TTP in the non-treated controls decreased from day 0 to day 28 indicating intracellular *Mtb* growth. In the ceftriaxone treated HFS-TB TTP was longer than the non-treated controls indicating inhibition of the intracellular *Mtb* growth. However, at days 21 and 28 the TTP was lower compared to day 14 showing regrowth. **(B)** Results of the cfu/mL readout confirmed the higher microbial kill with different ceftriaxone $\%T_{MIC}$ exposures with 15 mg/L avibactam, that sustained for longer durations of therapy.

**Figure 3: Ceftriaxone dose response and PK/PD parameter linked to the efficacy in the HFS-TB. (A)** TTP versus $C_{max}$/MIC, **(B)** TTP versus $AUC_{0-24}$/MIC, and **(C)** TTP versus $\%T_{MIC}$ on different timepoints. The model fit line for %TMIC on day 14 is missing as it did not converge. Inhibitory Sigmoid $E_{max}$ model results for **(D)** CFU/mL versus $C_{max}$/MIC, **(E)** $AUC_{0-24}$/MIC**,** and **(F)** percentage of time ceftriaxone concentration in the HFS-TB persisted above the MIC. The results show that on day 28, both by the TTP and cfu/mL readouts, the $\%T_{MIC}$ showed best Akaike Information Criteria score and therefore, identified as the PK/PD parameter linked to the ceftriaxone efficacy against *Mtb*.

**Figure 4. Ceftriaxone and synergy with ceftazidime-avibactam combination.** (**A)** TTP for the non-treated control was lowest showing high bacterial burden. Both ceftriaxone-avibactam and ceftazidime-avibactam as monotherapy showed higher TTP compared to the non-treated controls indicating microbial kill. The TTP for the combination of ceftriaxone-ceftazidime-avibactam was highest showing lowest bacterial burden in terms of TTP, (**B)** The bacterial burden in the HFS-TB with monotherapy or dual therapy shown as CFU/mL, where the direction of the curves is opposite to the TTP. Similar to the TTP results, the ceftriaxone-ceftazidime-avibactam combination showed best microbial kill, (**C)** Bliss Independence for each day was calculated based on the cfu/mL readouts. Shown in the figure are the overall interaction factor for each sampling day indicating that the dual β-lactam regimen was synergistic throughout the 28 days of the study.

**Figure 5. Monte Carlo experiments with different ceftriaxone doses.** (**A)** Simulated concentration-time profiles in serum with the 50 mg/kg once daily dose revealed peak concentrations 259±130 mg/L and $AUC_{0-24}$ of 1097±130 mg*h/L at steady state, similar to the peak of 220±64 mg/L observed in children clinically treated, and presented to the FDA.(13) (**B)** Simulated concentration-time profiles in serum with the 75 mg/kg once daily dose revealed peak concentrations 308±1106 mg/L and $AUC_{0-24}$ of 2531±158 mg*h/L at steady state, similar to the peak of 295±76 mg/L observed in children in the clinic. (**C**) Probability of target attainment for each of the two doses administered with either a once a day [QD] or twice a day [BID] schedule in children with disseminated disease. The once a day schedule achieves target in <90% of patients starting at an MIC=1 mg/L, while the twice a day dose extends the MIC range to a susceptibility breakpoint of ≥16 mg/L. **(D)** In the special case of disseminated TB that includes meningeal TB, the probability of target attainment is slightly worse at each dose, but the same MIC breakpoints are identified as in other disseminated disease.

Figure_1

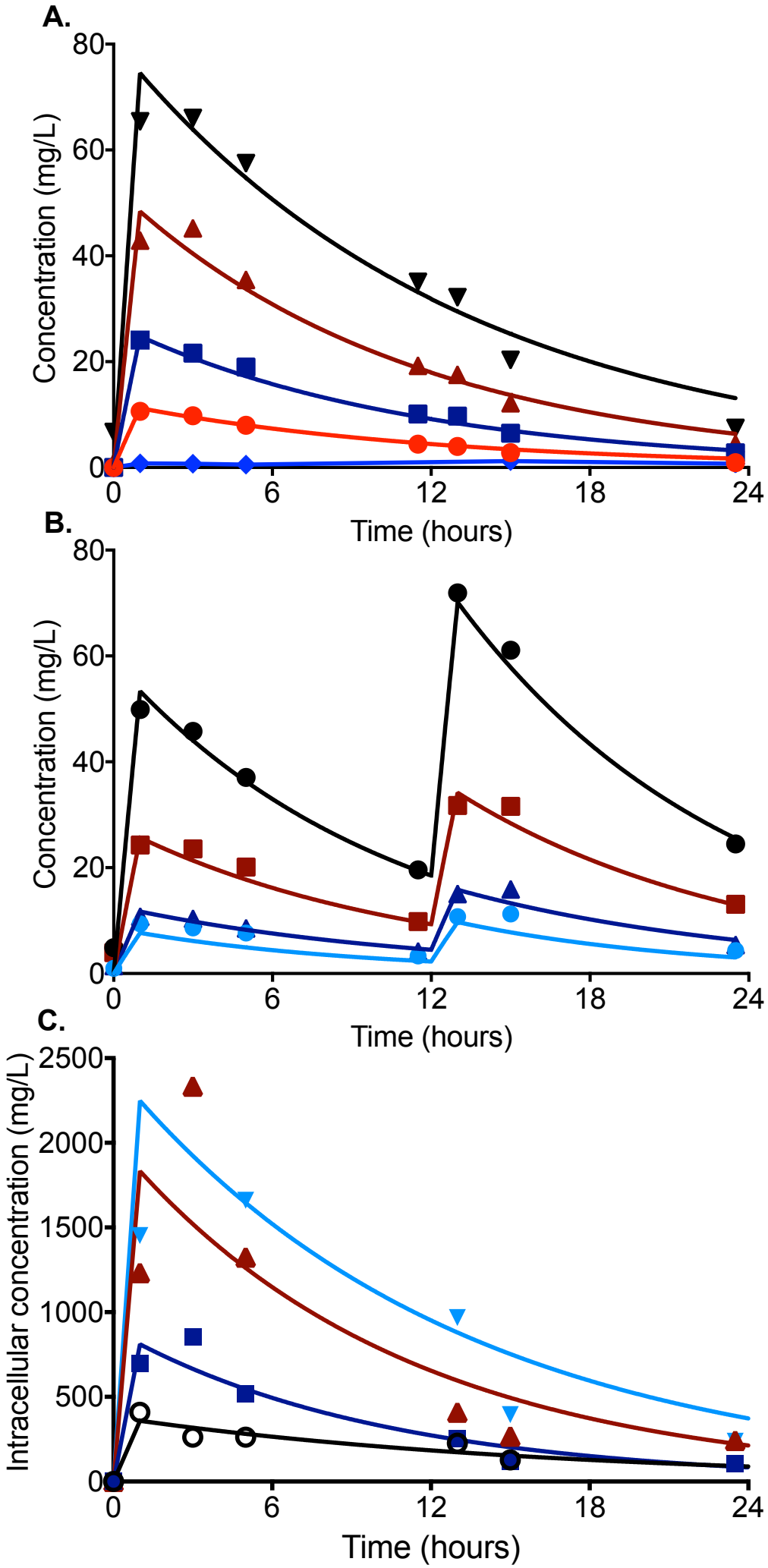

A.
Concentration (mg/L)
80
60
40
20
0
0
6
12
18
24
Time (hours)
B.
Concentration (mg/L)
80
60
40
20
0
0
6
12
18
24
Time (hours)
C.
Intracellular concentration (mg/L)
2500
2000
1500
1000
500
0
0
6
12
18
24
Time (hours)

Figure_2

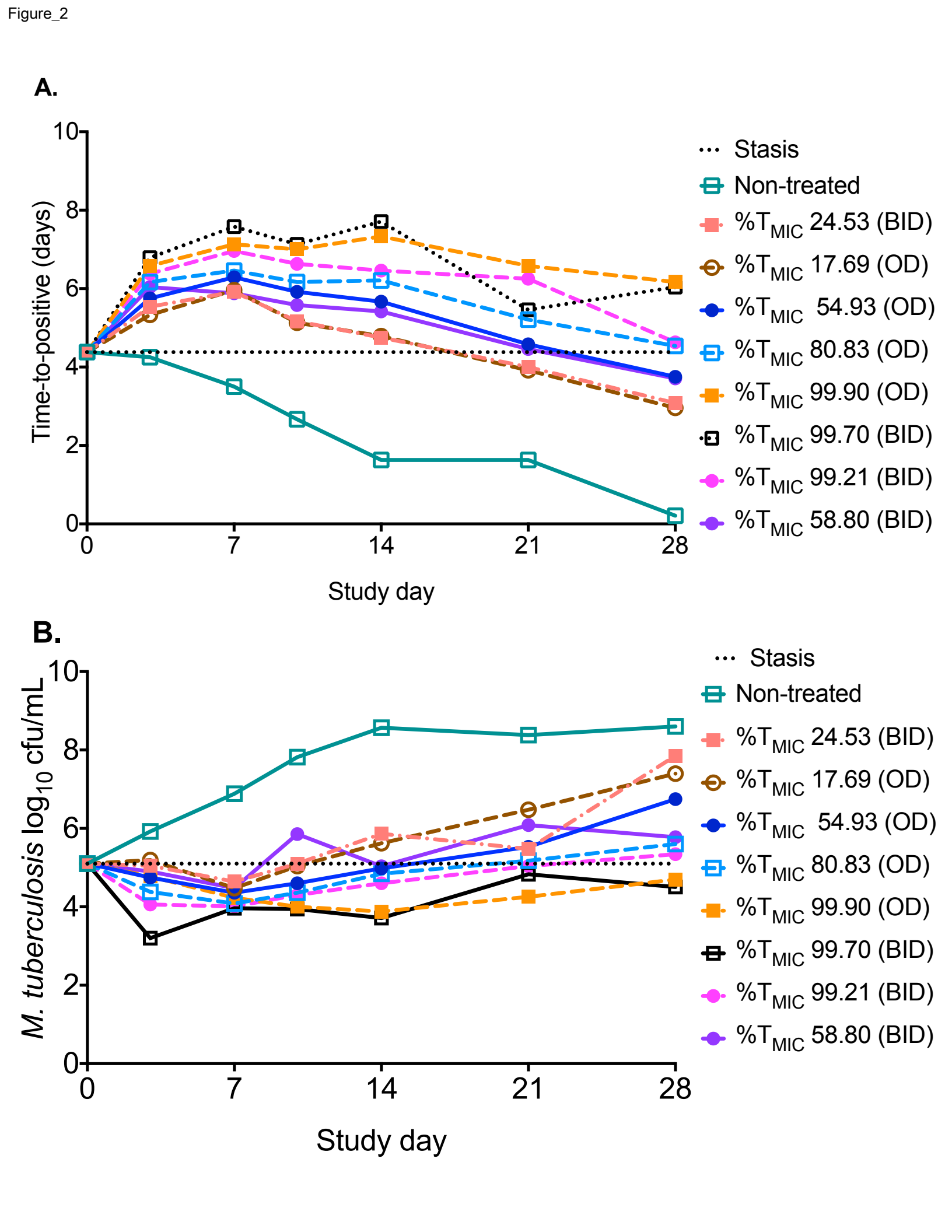

A.
Time-to-positive (days)
10
8
6
4
2
0
0
7
14
21
28
Study day
Stasis
Non-treated
%T MIC 24.53 (BID)
%T MIC 17.69 (OD)
%T MIC 54.93 (OD)
%T MIC 80.83 (OD)
%T MIC 99.90 (OD)
%T MIC 99.70 (BID)
%T MIC 99.21 (BID)
%T MIC 58.80 (BID)
B.
M. tuberculosis log10 cfu/mL
10
8
6
4
2
0
0
7
14
21
28
Study day
Stasis
Non-treated
%T MIC 24.53 (BID)
%T MIC 17.69 (OD)
%T MIC 54.93 (OD)
%T MIC 80.83 (OD)
%T MIC 99.90 (OD)
%T MIC 99.70 (BID)
%T MIC 99.21 (BID)
%T MIC 58.80 (BID)

Figure_3

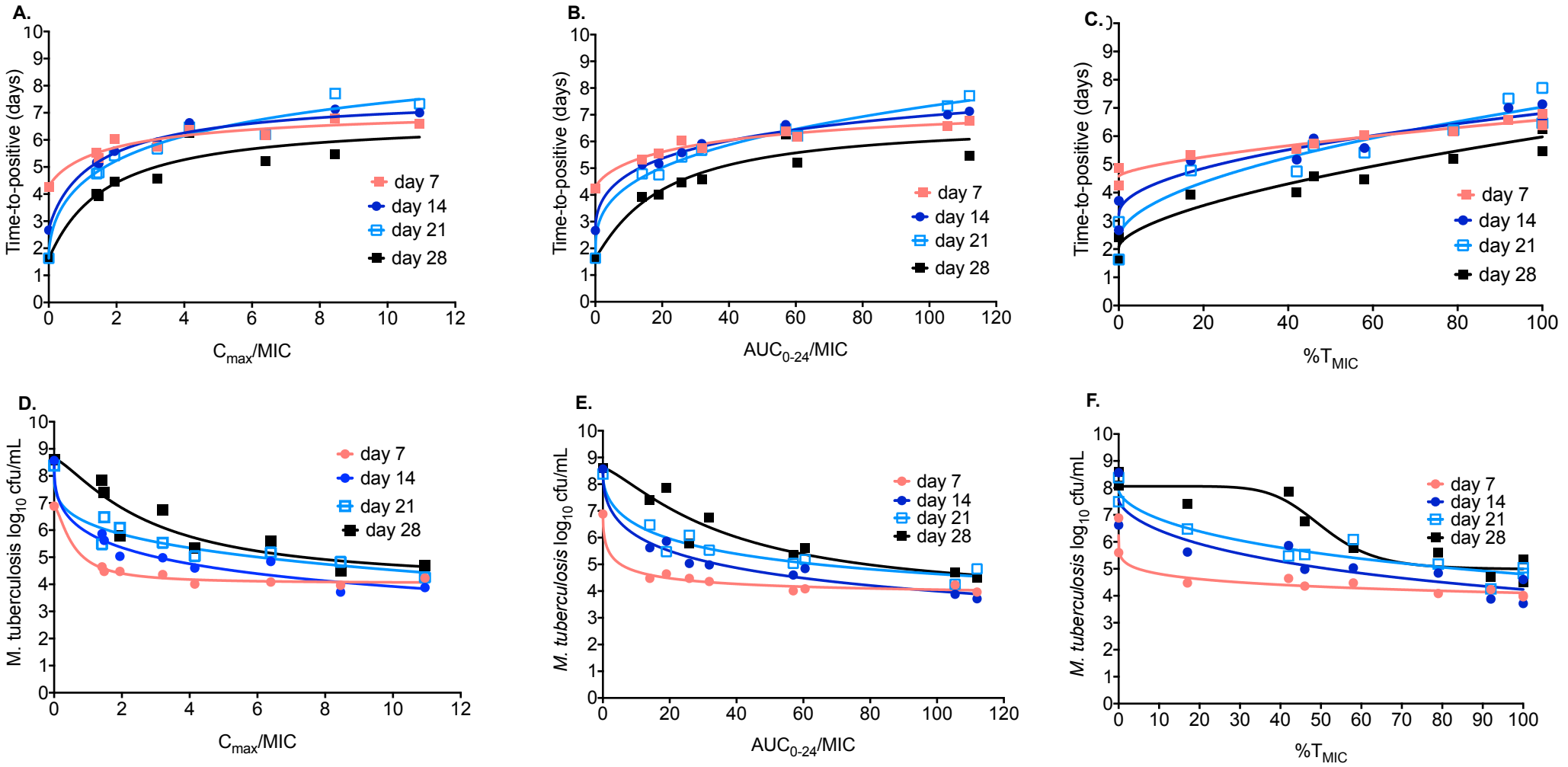
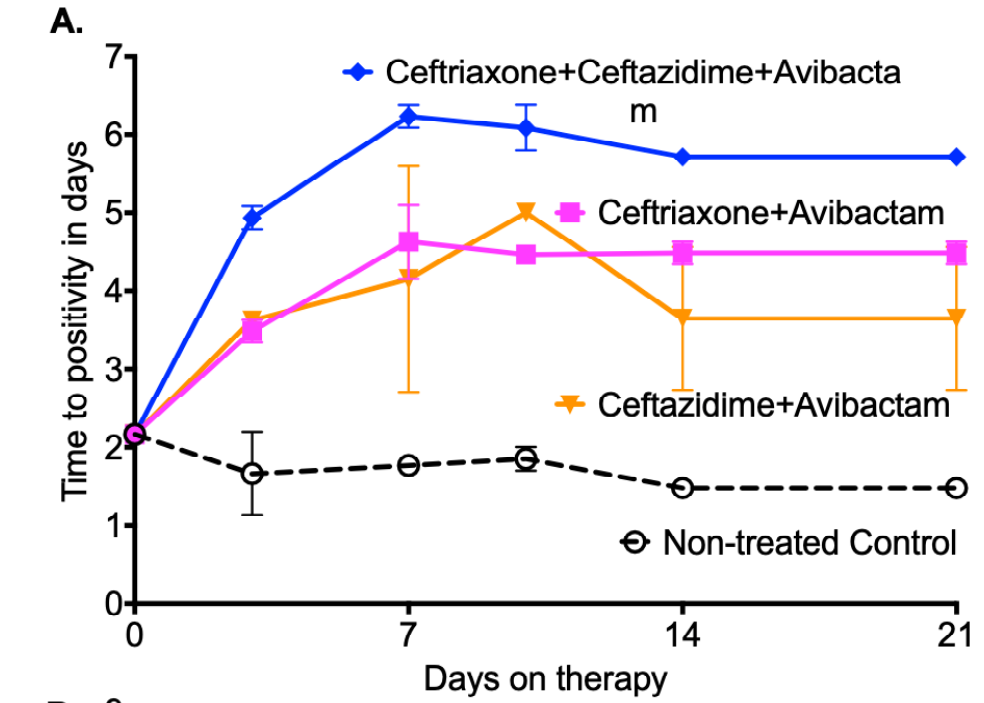
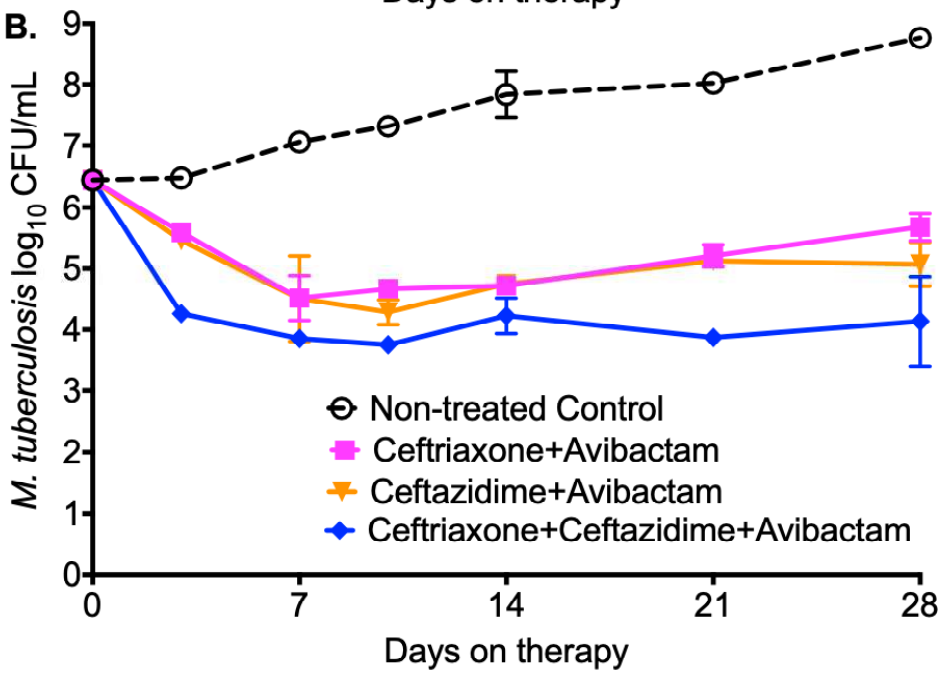

A.
Time-to-positive (days)
$C_{max}$/MIC
day 7
day 14
day 21
day 28
B.
Time-to-positive (days)
$AUC_{0-24}$/MIC
day 7
day 14
day 21
day 28
C.
Time-to-positive (days)
$\%T_{MIC}$
day 7
day 14
day 21
day 28
D.
M. tuberculosis $\log_{10}$ cfu/mL
$C_{max}$/MIC
day 7
day 14
day 21
day 28
E.
M. tuberculosis $\log_{10}$ cfu/mL
$AUC_{0-24}$/MIC
day 7
day 14
day 21
day 28
F.
M. tuberculosis $\log_{10}$ cfu/mL
$\%T_{MIC}$
day 7
day 14
day 21
day 28

Figure_4


A.
Ceftriaxone+Ceftazidime+Avibactam
Ceftriaxone+Avibactam
Ceftazidime+Avibactam
Non-treated Control
Time to positivity in days
Days on therapy
0
7
14
21


B.
M. tuberculosis log10 CFU/mL
Non-treated Control
Ceftriaxone+Avibactam
Ceftazidime+Avibactam
Ceftriaxone+Ceftazidime+Avibactam
Days on therapy
0
7
14
21
28

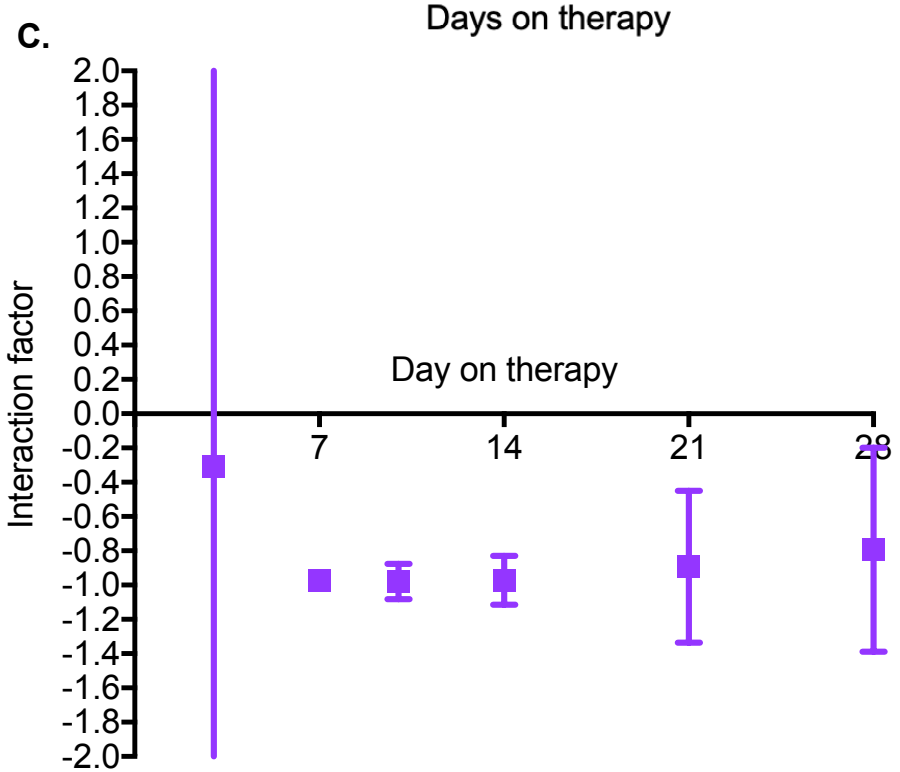
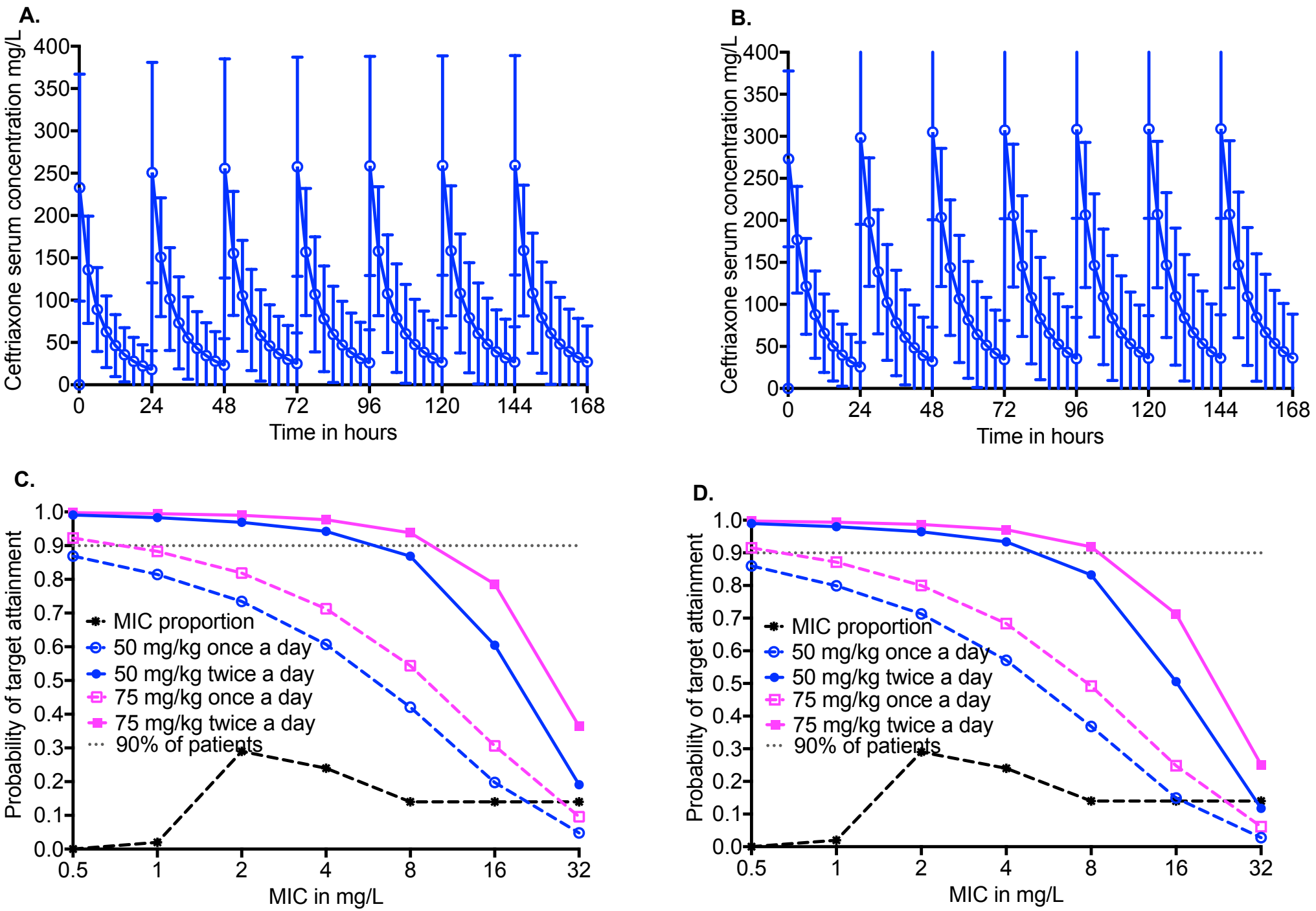

C.
Interaction factor
Day on therapy
7
14
21
28

Figure_5


A.
Ceftriaxone serum concentration mg/L
400
350
300
250
200
150
100
50
0
0
24
48
72
96
120
144
168
Time in hours
B.
Ceftriaxone serum concentration mg/L
400
350
300
250
200
150
100
50
0
0
24
48
72
96
120
144
168
Time in hours
C.
Probability of target attainment
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
MIC proportion
50 mg/kg once a day
50 mg/kg twice a day
75 mg/kg once a day
75 mg/kg twice a day
90% of patients
0.5
1
2
4
8
16
32
MIC in mg/L
D.
Probability of target attainment
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
MIC proportion
50 mg/kg once a day
50 mg/kg twice a day
75 mg/kg once a day
75 mg/kg twice a day
90% of patients
0.5
1
2
4
8
16
32
MIC in mg/L

## INTRODUCTION

Drug-resistant *Mycobacterium tuberculosis* (*Mb*) is a major global health emergency; drug resistance has already been reported to the newly developed tuberculosis (TB) drugs delamanid and bedaquiline.(1-7) Given the limited number of candidates in the TB drug pipeline, “repurposing” drugs already approved for other indications could be an important strategy to achieve global TB control.(8, 9) Repurposed drugs have the advantage that post-licensure data on dosing and toxicities are already available. This makes them more “shovel ready” for use, especially in historically understudied populations, such as in children. Frequently, data on dosing and toxicity for children lag behind those in adults when new drugs are developed. Elsewhere we have shown that benzylpenicillin and ceftazidime, when used with avibactam, were highly effective against *Mtb*, despite the short half-life.(10, 11) In the present study, we wanted to optimize ceftriaxone, that has been used throughout the world for over fifty years for the treatment of Gram-positive and Gram-negative infections and has a long half-life (10-13) for the treatment of tuberculosis.

Ceftriaxone, an aminothiazolyl-oxyimino cephalosporin, is more β-lactamase stable, with a half-life of 6-8 hours that could allow once daily dosing in TB. Ceftriaxone has good cerebrospinal fluid [CSF] to serum penetration ratios., which makes it for the treatment of TB meningitis, commonly encountered in young children with disseminated disease.(14-16) Ceftriaxone is also safe during pregnancy as well as children and infants out of the neonatal period, suggesting

that if efficacy against *Mtb* is demonstrated, it would be useful drug in these neglected patient groups. In children <3 years, in whom there are higher rates of death and therapy failure, disseminated disease is predominantly intracellular.(17) Involvement of meninges, peritoneum and bone is more common that in older patients. Here, we examined the efficacy of ceftriaxone plus avibactam in the hollow fiber model of TB (HFS-TB).(10, 11, 18-20)

## MATERIALS ANDMETHODS

### Bacterial strain, cell lines, and supplies

The culture conditions and the growth medium for both *Mtb* ATCC #25177) and the THP-1 cells (ATCC TIB-202) were as reported previously.(10, 11, 20) We used our library of 30 clinical isolates [10 susceptible to rifampin and isoniazid and 20 multidrug-resistant (MDR; resistant to both rifampin and isoniazid) obtained from the Medical Research Council of South Africa. Hollow fiber cartridges were purchased from FiberCell (Frederick, MD), ceftriaxone from Baylor University Medical Center pharmacy, and avibactam from BOC Sciences (Shirley, NY). The BACTEC 960 system and Mycobacteria Growth Indicator Tubes (MGIT) were purchased from Becton Dickinson (Franklin Lakes, NJ).

### Ceftriaxone MIC distribution and concentration-response studies

First, we performed a concentration-response study to determine if avibactam monotherapy has any effect on the growth of *Mtb.* The experiment was performed with *Mtb* H37Ra using the avibactam concentrations of 0, 3.75, 7.5,

15, and 30 mg/L. Second, we used both MGIT and micro-broth dilution methods to identify ceftriaxone MICs for *Mtb* H37Ra laboratory strain, but only the micro-broth dilution method for the clinical isolates.(21) The ceftriaxone concentrations, in a two-fold dilution series, ranged from 0.25 to 128 mg/L. MICs were performed with and without the addition of 15 mg/L avibactam for the laboratory strain, but with avibactam for all clinical strains. Third, ceftriaxone concentrations-response studies (with 15 mg/L avibactam) were performed using the same concentrations as in the MIC experiments. The cultures were incubated at $37^0$C under shaking conditions for 7-days, followed by 10-fold serial dilutions and inoculation on Middlebrook 7H10 agar supplemented with 10% OADC agar. Colony forming units per milliliter (~~cfu~~CFU/mL) were counted after 21 days of incubation at $37^0$C. All experiments were performed twice.

**Ceftriaxone hollow fiber system model of TB studies**

Using the intracellular HFS-TB model (11, 20, 22) we performed two different ceftriaxone experiments to identify [i] the optimal ceftriaxone exposure, and [ii] the best dosing schedule, based on the ceftriaxone serum half-life of 5.8 $\pm$ 2.6 hours encountered in infants and children <2 years.(13, 23) In the first HFS-TB experiment we used avibactam at a constant concentration of 1 mg/L, described as the pharmacokinetics/pharmacodynamics (PK/PD) target for avibactam for Gram negative bacilli in the ceftazidime-avibactam FDA approval document.(13) In the second HFS-TB experiment, avibactam was used at a concentration of 15 mg/L, which is the concentration achieved in children when given the standard

dose of the ceftazidime-avibactam, as well as the optimal avibactam concentration we identified in our *in vitro* experiments and elsewhere.(11, 24) The details of the THP-1 monocytes infection with *Mtb* and inoculation into the HFS-TB have been described in detail elsewhere.(20, 22) The HFS-TB units were treated with either a once daily or twice daily dosing schedule to achieve the percentage of time that the concentration persisted above MIC [$\%T_{MIC}$] ranging from 0 to 100%. Each HFS-TB unit was repetitively sampled to measure the ceftriaxone concentration as well as for *Mtb* ~~cfu~~ CFU counts, using the methods described in detail elsewhere.(11, 20) A portion of the processed sample from the peripheral compartment of the HFS-TB was inoculated into MGIT tubes to determine the time-to-positivity (TTP) as a second readout for bacterial burden.

**Ceftazidime-avibactam as source of β-lactamase inhibitor**

Currently, avibactam is only commercially available as ceftazidime-avibactam,(24) and is thus the only available avibactam source for clinicians. In order to determine if there is synergy/antagonism/additivity between ceftriaxone-avibactam and ceftazidime-avibactam, ceftriaxone optimal exposure and ceftazidime-avibactam at optimal exposure of $\%T_{MIC}$ ≥63%(11) were administered to HFS-TB units as either [1] “monotherapy” [with avibactam] or [2] a combination as ceftriaxone-ceftazidime-avibactam. Samples for drug concentrations and ~~cfu~~ CFU at various times were processed as described above.

**Drug concentration measurements**

The ceftazidime and avibactam concentration assays have been reported previously.(11) We used a stable-isotope dilution liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) to measure ceftriaxone concentrations. The calibrators, controls and internal standards (ceftazidime-d5) were included in each analytical run for quantification. The lower limit of detection of the method was 0.05 mg/L with a 6.2% and 7.4% inter- and intra-day variation.

**PK/PD analysis**

The observed drug concentrations were modeled using ADAPT 5 software.(25) Concentrations were analyzed using a one-compartmental model with first-order rate absorption and elimination. The primary pharmacokinetic parameters were estimated using the maximum likelihood expectation maximization [MLEM] algorithm and then used to calculate the secondary parameters such as half-life, peak concentration ($C_{max}$), and 24hr area under the concentration-time curve [$AUC_{0-24}$] for each HFS-TB. We used the inhibitory sigmoid $E_{max}$ model using $\%T_{MIC}$ versus *Mtb* burden, to estimate the PK/PD exposure associated with 50% [$EC_{50}$], or 80% [$EC_{80}$], or 90% [$EC_{90}$] of maximal bacterial kill [$E_{max}$].We also identified the PK/PD index linked to microbial kill using the same inhibitory sigmoid $E_{max}$ model for ~~cfu~~CFU/mL versus exposure [$\%T_{MIC}$ or $AUC_{0-24}$/MIC or

$C_{max}$/MIC] based on corrected Akaike Information Criteria [AIC] scores.(26) GraphPad Prism (v7) was used for graphing.

**Bliss Independence versus Loewe Additivity**

In our prior experiments evaluating the impact of ceftazidime-avibactam on *Mtb*, we found that ceftazidime has a different binding target [PonA1] from other beta-lactams and cephalosporins,(11) and the inhibitory sigmoid $E_{max}$ dose-response curves of ceftazidime-avibactam are not parallel with those of ceftriaxone-avibactam. Based on this, we assumed that ceftriaxone and ceftazidime work on independent targets, and fulfil the criteria for non-interaction, hence Bliss Independence.(27, 28) Thus, we utilized Bliss Independence for our modeling. Effect was measured as percentage change from day 0. Theoretical additivity [$X_T$] was calculated by adding the effect of ceftriaxone-avibactam "monotherapy" [A] to ceftazidime-avibactam "monotherapy" [B] on each sampling day. The observed effect [$X_o$] was the percentage change from day 0 in the ceftriaxone-ceftazidime-avibactam combination regimen. The combination index [$X_I$] is given by:

$$X_I=[X_T-A*B]/X_o \quad (1)$$

**Monte Carlo experiments**

We utilized Monte Carlo experiments [MCE] to identify the optimal ceftriaxone doses for use in children.(18, 22, 29-31) The following pharmacokinetic parameter estimates were used in both subroutine PRIOR of ADAPT and the

parameter files: plasma clearance of 0.051±0.024 L/hr/kg and a volume of distribution of 0.382±0.129 L/kg for 50 mg/kg dose, and clearance of 0.055±0.018 L/hr/kg and a volume of distribution of 0.387±0.056 L/kg for 75 and 100 mg/kg, based on Steele et al.(23) Each dose was infused intravenously over 10 minutes. The ratio for the ceftriaxone CSF-to-plasma AUC in neonates and infants with meningitis of 17%, as reported by Martin et al, was used for meningitis.(32) In children <5 years, at a dose of 50 mg/kg, ceftriaxone protein binding in serum is 82±8% while that in CSF during purulent meningitis is 9±6% protein bound, which is negligible.(33) Free drug ceftriaxone concentrations were used in the MCE; for CSF total ceftriaxone was assumed equivalent to free drug. The ceftriaxone MIC distribution used was from our 30 clinical isolates.

## RESULTS

### MIC~~s~~ and concentration-response in test tubes

Avibactam ~~of~~ up to 30 mg/L concentration did not showed any inhibitory effect on *Mtb* growth. Therefore, avibactam on its own does not kill *Mtb*. The ceftriaxone MIC for *Mtb* H37Ra was 16 mg/L without avibactam but fell by two-tube dilutions to 4 mg/L in the presence of 15 mg/L avibactam. Therefore, we subsequently identified ceftriaxone MICs against 30 clinical isolates only in combination with 15 mg/L avibactam. The MIC range was 0.5-32 mg/L. The $MIC_{50}$ and $MIC_{90}$ were 4 mg/L and 32 mg/L, respectively. In the concentration-response studies, ceftriaxone $EC_{50}$ was calculated as 2xMIC, and effect was near maximal at 4xMIC, consistent with time driven effect ($r^2$=0.97).(34)

**HFS-TB experiment with 1mg/L avibactam**

In the first ceftriaxone HFS-TB examined ceftriaxone, with an avibactam constant infusion concentration of 1 mg/L, the time-kill curves showed an exposure-dependent lowering of bacterial burden compared to non-treated control. After initial microbial kill below day 0 [stasis] on day 3, there was therapy failure due to the rebound growth by day 7. The Inhibitory sigmoid $E_{max}$ model showed highest $r^2$ on day 7, when the relationship was described by following equation:

$$\text{Effect}=5.41-1.07*(\%T_{MIC})^{1.0}/[19.90^{1.0}+(\%T_{MIC})^{1.0}] \quad (2),$$

so that the $EC_{50}$ was $\%T_{MIC}$=19.90, and $EC_{80}$ was calculated as $\%T_{MIC}$=79.60 [$r^2$=0.92]

**HFS-TB with 15 mg/L avibactam for exposure-effect and dose scheduling**

The ADAPT model predicted versus the observed ceftriaxone concentrations [$r^2$ of 0.99] with once daily or twice daily dosing schedule are shown in **Figure 1A** and **1B**. The ceftriaxone elimination rate constant [$K_{el}$] achieved in the HFS-TB extracellular compartments, reported as a "population" was 0.09±0.02 $hr^{-1}$ while volume of distribution was 1.39±0.21 L and half-life was 7.25±1.54 hr. The corresponding intracellular concentrations are shown in **Figure 1C**. The $K_{el}$ was 0.04±0.02 $hr^{-1}$ while volume of distribution was 0.62±0.17 L and half-life of 11.41±10.32 hr. Thus, clearance of ceftriaxone from intracellular compartment was slower.

**Figure 2A** shows the kill curves in the HFS-TB by these exposures, based on the TTP readout, while **Figure 2B** shows the kill curves based on the CFU/mL readout. Lower the TTP, higher the bacterial burden. **Figure 2A** and **2B** show that there was microbial kill below day 0 [stasis] by several doses and dosing schedules, and a much greater depth of kill, which was sustained for up to 21 days in some doses, with 15 mg/l avibactam compared to what was observed in **Figure 2A** with 1 mg/L of avibactam.

**Figure 3A-C** shows the inhibitory sigmoid $E_{max}$ model output for the TTP readout for each PK/PD index: $C_{max}$/MIC or AUC/MIC or $\%T_{MIC}$. On day 28, the Akaike Information Criteria [AIC] scores for $C_{max}$/MIC was 15.58, for AUC/MIC was 13.75, while that for $\%T_{MIC}$ was 8.61. Based on this the PK/PD parameter linked to effect was $\%T_{MIC}$. **Figure 3D-F** show the model output for ~~cfu~~CFU/mL versus exposure. The same was apparent with the ~~cfu~~CFU/mL readout: on day 28, the AIC score for $C_{max}$/MIC was 18.12, for AUC/MIC was 14.34, while that for $\%T_{MIC}$ was 7.34. Based on this readout, the lowest AIC score was for $\%T_{MIC}$, which was chosen as the PK/PD index linked to *Mtb* kill. The relationship between $\%T_{MIC}$ and *Mtb* burden on day 28, at the end of the experiment, was described by the equation #3:

$$\text{Effect}=8.06-3.08*(\%T_{MIC})^{7.61}/[50.67^{7.61}+(\%T_{MIC})^{7.61}] \quad (3),$$

The $EC_{50}$ $\%T_{MIC}$ was 50.7%, while the $EC_{80}$ $\%T_{MIC}$ was 60% of dosing interval [$r^2$=0.91].

**HFS-TB to explore double β-lactam strategy**

**Figure 4A-C** shows that ceftriaxone-avibactam and ceftazidime-avibactam had "monotherapies" had identical kill curves by TTP **(Figure 4A)** and cfu/mL (**Figure 4B).** The figure also shows that the double β-lactam combination of ceftriaxone-ceftazidime-avibactam killed better than either cephalosporin "monotherapy". We calculated Bliss Independence for each day, and then an overall interaction factor [$X_I$] and 95% confidence intervals [CI] for all sampling days, and then an overall $X_I$, based on cfu/mL readout, with results shown in **Figure 4C**. When $X_I$ and its 95% CI were less than zero, as shown in **Figure 4C** for days 7 onwards, then there was synergy because the effect observed for the ceftriaxone-ceftazidime-avibactam combination regimen was greater than expected from adding the effect of ceftriaxone-avibactam "monotherapy" to that of ceftazidime-avibactam "monotherapy". On day 3, $X_I$ and its 95% CI crossed zero, which means the observed effect was exactly the same as the effect of the sum of the two monotherapies, so that on that day they were additive. Overall, when data for all the entire experiment was combined, the mean $X_I$ was -0.81 [95% CI: -1.01 to -0.63], indicating synergy [**Figure 6C**].

**Monte Carlo experiments [MCE]**

MCE of several different doses are shown in **Figures 5A** and **5B**. The pharmacokinetic parameter estimates and standard deviations in the MCE output were a clearance of 0.05±0.04 L/hr and volume of 0.38±0.22 L [half-life=5.21 hr] in 5,000 children, which are identical to the parameters in the domain of input,

which is an internal validation step. For external validation we compared the peak concentrations we identified in the MCE for children treated with 50 mg/kg of 232±134 mg/L to those of 220±64 mg/L observed in actual children in the clinic treated with the same dose, and 308±1106 mg/L versus 295±76 mg/L in children treated with 75/mg/kg.(13) This means our simulations represent the reality seen in clinical trials when it comes to drug concentrations. Target attainment probabilities [TAP] to achieve $\%T_{MIC}$ of 60% for disseminated disease without meningitis are shown in **Figure 5C**. The cumulative fraction of response [CFR] over the entire MIC distribution for 50 mg/kg once a day was 46.8% while that for twice a day was 76.0%. The CFR for 75 mg/kg once a day was 55.9% while that for twice a day was 83.4%. For meningeal TB, the TAPs are shown in **Figure 5D**. The CFR for 50 mg/kg once a day was 43.6% while that for twice a day was 72.7%. The CFR for 75 mg/kg once a day was 52.6% while that for twice a day was 80.3%. Thus, 75 mg/kg twice a day would be the best dose; the PK/PD derived susceptibility breakpoint for this dose is >16 mg/L. A dose of 100 mg/kg once-a-day achieved a CFR of 69.37%, while twice a day achieved a CFR of 90% in disseminated TB. It is unclear if this larger dose would be tolerated by the children.

## DISCUSSION

Here, first, we show that ceftriaxone has efficacy against *Mtb* even without avibactam, but the avibactam dramatically improved both potency and efficacy. We have found that *Mtb*'s natural resistance to cephalosporins is via degradation

by BlaC [encoded by a lone gene] of the drug, and that otherwise *Mtb* has a cephalosporin binding target and even a benzylpenicillin binding target.(11, 35, 36) Avibactam is a particularly effective inhibitor of *Mtb* BlaC.(11, 35, 37) Thus, ceftriaxone, when used with a β-lactamase inhibitor would be effective in children and adults, with particular relevance to neglected populations including young children and pregnant women. For young children, who more commonly have disseminated intracellular TB than older patients, there could be additional benefits due to the high concentrations achieved intracellularly. Since ceftriaxone-avibactam was effective against strains both drug-susceptible and MDR-TB clinical strains, this highlights an important potential role in the treatment of drug-resistant TB.

Second, we found that microbial kill of *Mtb* by ceftriaxone-avibactam was linked to $\%T_{MIC}$, with an optimal target of 60% of dosing interval. This exposure target is very similar to observations of 50-70% identified with cephalosporins and gram-negative bacteria. In the HFS-TB study that had suboptimal concentrations of avibactam, the $EC_{80}$ target of $\%T_{MIC}$=80% was higher than with avibactam, consistent with improved potency. We did not isolate any ceftriaxone resistant *Mtb* subpopulations, thus the PK/PD parameter linked to resistance suppression remains unknown.

Third, our clinical trial simulations show that a dose of 100 mg/kg twice a day could achieve a CFR of >90% in both disseminated and meningeal TB. However,

there could be adverse events of this high dose such as nephrolithiasis, cholestatic jaundice, neutropenia, hepatitis, eosinophilia etc.(13, 38) The once a day dose of 100 mg achieved a CFR of 69.4%; this rate could be acceptable in combination therapy in which the ceftriaxone gets help from companion drugs, and would have the virtue of more convenient dosing schedule in children and in resource limited settings. Otherwise, the dose of 100 mg/kg twice a day that could achieve CFR of 90% of the patients would be optimal, and for this dose the susceptibility breakpoint was an MIC >16 mg/L [32 mg/L].

Finally, we show that there is synergy between ceftazidime-avibactam and ceftriaxone; the commercially available preparation of ceftazidime-avibactam, therefore, can be used as source of avibactam. However, given that the high cost associated with the commercially available ceftazidime-avibactam combination, there is a need to develop a ceftriaxone-avibactam combination. The oral formulations of ceftriaxone (39) and avibactam (40) are in the developmental stage and in future this could potentially lead to a child friendly oral combination. Similarly, exploring ceftriaxone in combination with amoxicillin-clavulanic acid for potential synergy may also present a more cost-effective treatment option.

In summary, we show that ceftriaxone could be used for the treatment of TB, including drug-resistant forms, the 100 mg/kg dose for children and adults with TB could achieve exposure-target in a large proportion of patients.

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**Figure 1: Ceftriaxone concentration-time profile in the HFS-TB. (A)** Observed ceftriaxone concentrations versus ADAPT model-derived curves for the HFS-TB treated with a once daily dosing schedule. (**B)** Observed ceftriaxone concentrations achieved in the central compartment of the HFS-TB with a twice daily dosing schedule versus ADAPT model-derived curves. (**C)** Ceftriaxone concentration measured inside the THP-1 cells was multiple times higher than the concentration observed in the central compartment. The solid lines represent ADAPT PK model predicted ceftriaxone concentrations, and the symbols represent the observed concentration.

**Figure 2: Ceftriaxone kill curves in the HFS-TB. (A)** The TTP in the non-treated controls decreased from day 0 to day 28 indicating intracellular *Mtb* growth. In the ceftriaxone treated HFS-TB TTP was longer than the non-treated controls indicating inhibition of the intracellular *Mtb* growth. However, at days 21 and 28 the TTP was lower compared to day 14 showing regrowth. **(B)** Results of the cfu/mL readout confirmed the higher microbial kill with different ceftriaxone $\%T_{MIC}$ exposures with 15 mg/L avibactam, that sustained for longer durations of therapy.

**Figure 3: Ceftriaxone dose response and PK/PD parameter linked to the efficacy in the HFS-TB. (A)** TTP versus $C_{max}$/MIC, **(B)** TTP versus $AUC_{0-24}$/MIC, and **(C)** TTP versus $\%T_{MIC}$ on different timepoints. The model fit line for %TMIC on day 14 is missing as it did not converge. Inhibitory Sigmoid $E_{max}$ model results for **(D)** ~~cfu~~CFU/mL versus $C_{max}$/MIC, **(E)** $AUC_{0-24}$/MIC**,** and **(F)** percentage of time ceftriaxone concentration in the HFS-TB persisted above the MIC. The results show that on day 28, both by the TTP and cfu/mL readouts, the $\%T_{MIC}$ showed best Akaike Information Criteria score and therefore, identified as the PK/PD parameter linked to the ceftriaxone efficacy against *Mtb*.

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**Figure 4. Ceftriaxone and synergy with ceftazidime-avibactam combination.** (**A)** TTP for the non-treated control was lowest showing high bacterial burden. Both ceftriaxone-avibactam and ceftazidime-avibactam as monotherapy showed higher TTP compared to the non-treated controls indicating microbial kill. The TTP for the combination of ceftriaxone-ceftazidime-avibactam was highest showing lowest bacterial burden in terms of TTP, (**B)** The bacterial burden in the HFS-TB with monotherapy or dual therapy shown as CFU/mL, where the direction of the curves is opposite to the TTP. Similar to the TTP results, the ceftriaxone-ceftazidime-avibactam combination showed best microbial kill, (**C)** Bliss Independence for each day was calculated based on the cfu/mL readouts. Shown in the figure are the overall interaction factor for each sampling day indicating that the dual β-lactam regimen was synergistic throughout the 28 days of the study.

**Figure 5. Monte Carlo experiments with different ceftriaxone doses.** (**A)** Simulated concentration-time profiles in serum with the 50 mg/kg once daily dose revealed peak concentrations 259±130 mg/L and $AUC_{0-24}$ of 1097±130 mg*h/L at steady state, similar to the peak of 220±64 mg/L observed in children clinically treated, and presented to the FDA.(13) (**B)** Simulated concentration-time profiles in serum with the 75 mg/kg once daily dose revealed peak concentrations 308±1106 mg/L and $AUC_{0-24}$ of 2531±158 mg*h/L at steady state, similar to the peak of 295±76 mg/L observed in children in the clinic. (**C**) Probability of target attainment for each of the two doses administered with either a once a day [QD] or twice a day [BID] schedule in children with disseminated disease. The once a day schedule achieves target in <90% of patients starting at an MIC=1 mg/L, while the twice a day dose extends the MIC range to a susceptibility breakpoint of ≥16 mg/L. **(D)** In the special case of disseminated TB that includes meningeal TB, the probability of target attainment is slightly worse at each dose, but the same MIC breakpoints are identified as in other disseminated disease.