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In vivo pharmacokinetic/pharmacodynamic evaluation of cefepime/taniborbactam combination against cefepime-non-susceptible Enterobacterales and *Pseudomonas aeruginosa* in a murine pneumonia model

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Background: Cefepime/taniborbactam is a cephalosporin/bicyclic boronate β-lactamase inhibitor combination in clinical development for nosocomial pneumonia due to MDR Gram-negative bacteria. A murine pneumonia model was used to characterize cefepime/taniborbactam *in vivo* pharmacodynamics against Enterobacterales and *Pseudomonas aeruginosa* strains.

Methods: Clinical cefepime-non-susceptible Enterobacterales and *P. aeruginosa* strains expressing serine carbapenemases and/or other cefepime-hydrolysing β-lactamases with cefepime/taniborbactam combination MICs of 0.12–16 mg/L were used. Cefepime and taniborbactam human-simulated regimens equivalent to clinical doses (i.e. 2/0.5 g q8h) were established in the pneumonia model. The *in vivo* activity of the cefepime human-simulated regimen given alone or concomitantly with escalating taniborbactam exposures against eight Enterobacterales and four *P. aeruginosa* strains was assessed. Taniborbactam pharmacokinetics were evaluated to determine systemic exposures of regimens used; taniborbactam $f\mathrm{AUC}_{0-24}$/MIC values required for efficacy were estimated using the Hill equation. In addition, the *in vivo* activity of the cefepime/taniborbactam combination human-simulated regimen was assessed against 18 strains.

Results: Among Enterobacterales, median taniborbactam $f\mathrm{AUC}_{0-24}$/MIC values associated with stasis and 1 log kill were 0.96 and 4.03, respectively, while for *P. aeruginosa*, requirements were 1.35 and 3.02 for stasis and 1 log kill, respectively. The cefepime/taniborbactam human-simulated regimen produced >2 log kill in 14/18 strains and >1 log kill in 18/18 strains.

Conclusions: Cefepime/taniborbactam produced marked *in vivo* bactericidal activity against cefepime-non-susceptible Enterobacterales and *P. aeruginosa* isolates with cefepime/taniborbactam MICs up to and including 16 mg/L in the pneumonia model. Assessments of the probability of clinical attainment of the identified targets should be undertaken to support the selected cefepime/taniborbactam dose for treatment of nosocomial pneumonia.

Introduction

Hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP) are serious nosocomial infections prevalent among patients requiring critical care. HABP/VABP due to carbapenem-resistant and MDR Gam-negative strains are particularly challenging to treat and associated with high morbidity and mortality.[1] Toxicity to some of the antibiotics of last resort, notably polymyxins, in addition to poor therapeutic outcomes, creates further challenges when utilizing these agents for management of HABP/VABP in critically ill patients.

New β-lactam/β-lactamase inhibitor (BLBLi) combinations have played pivotal roles in enriching the antibiotic armamentarium as they are generally well tolerated and offer coverage against MDR Gram-negative strains, minimizing the need for less-tolerable last-line agents. Several BLBLi combinations have recently been

approved for HABP/VABP caused by Enterobacterales and *Pseudomonas aeruginosa*[2] and are active against carbapenem-resistant subsets, including ceftazidime/avibactam, ceftolozane/tazobactam and imipenem/cilastatin/relebactam. Other BLBLis in the pipeline include cefepime/taniborbactam, a combination of cefepime, a fourth-generation cephalosporin, and taniborbactam, a novel bicyclic boronic acid-containing β-lactamase inhibitor. Taniborbactam inhibits most clinically relevant serine β-lactamases belonging to Ambler classes A, C and D, and MBLs (class B) of VIM, NDM, SPM-1 and GIM-1 types.[3]

The objective of this study was to investigate cefepime/taniborbactam pharmacodynamics against clinical cefepime-non-susceptible Enterobacterales and *P. aeruginosa* isolates expressing serine β-lactamases (such as CTX-M, KPC and OXA-48-like) as well as inducible AmpC-expressing *P. aeruginosa* in a translational murine pneumonia model[4] to support clinical dose selection for HABP/VABP treatment with cefepime/taniborbactam (2/0.5 g every 8 h as 4 h infusion) in Phase III studies.

Methods

Antimicrobial test agents

Taniborbactam (batch numbers CA19-1355 and CA20-0265; Venatorx Pharmaceuticals Inc., Malvern, PA, USA) was used for *in vivo* and *in vitro* testing. For *in vitro* testing, taniborbactam master stock (10 mg/mL) was prepared in DMSO. For *in vivo* testing, taniborbactam was reconstituted to 10 mg/mL using sterile water for injection (Hospira, Inc., Lake Forest, IL, USA). Subsequent dilutions in sterile 0.9% normal saline (NS) solution (B. Braun Medical Inc., Irvine, CA, USA) were made to attain final concentrations that would deliver the required doses based on study mice population's mean weight. Cefepime HCl (batch number LRAB8503, Sigma–Aldrich, Inc., St. Louis, MO, USA) was used for *in vitro* testing. Cefepime 1 g vials (lots 108307C and 108725C, WG Critical Care, LLC, Paramus, NJ, USA) were reconstituted and diluted with 0.9% NS for *in vivo* testing. Taniborbactam and cefepime were administered via subcutaneous injections of 0.1 mL volumes.

Bacterial isolates and susceptibility testing

Thirteen Enterobacterales and five *P. aeruginosa* were provided by Venatorx Pharmaceuticals, Inc.[5] or selected from the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA, USA) or the Hartford Hospital Center for Anti-Infective Research and Development isolate repository. Isolates harboured genes for serine carbapenemases, ESBLs and/or inducible AmpC cephalosporinases. Cefepime and cefepime/taniborbactam combination MICs at a fixed taniborbactam concentration of 4 mg/L were determined in triplicate using reference broth microdilution methodology with concurrent quality control as outlined by CLSI.[6,7] Modal MICs were utilized to characterize the isolates for final analyses.

Neutropenic murine pneumonia model

Specific pathogen-free, female ICR mice weighing 20–22 g were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA) and housed in HEPA-filtered cages in groups of six at controlled room temperature, provided with food and water *ad libitum* and allowed to acclimatize for a minimum of 48 h before experimentation commencement. A 12 h light/12 h dark cycle was maintained. The protocol was approved by the Institutional Animal Care and Use Committee at Hartford Hospital (Assurance #A3185-01).

Mice were rendered transiently neutropenic by injecting cyclophosphamide intraperitoneally at 250 and 100 mg/kg 4 days and 1 day before inoculation, respectively. Uranyl nitrate 5 mg/kg was administered intraperitoneally 3 days before inoculation to produce a controlled degree of renal impairment reducing the dosing requirement for humanizing the regimens of the test agents.

Prior to mice lung inoculation, two transfers of the organisms (previously frozen at −80°C in skimmed milk) were performed onto Trypticase soy agar plates with 5% sheep blood (TSA II™; Becton, Dickinson & Co.; Sparks, MD, USA) and incubated at 37°C for approximately 24 h. After 18–24 h incubation of the second bacterial transfer, a bacterial suspension of approximately 10^7 cfu/mL in NS with 3% mucin was made for inoculation. Inoculum bacterial densities were confirmed by serial dilution and culture of an aliquot for quality control. Mice were anaesthetized using vaporized isoflurane (2%–3% v/v in an oxygen carrier) until respiratory rate visually decreased. Pneumonia was induced via intranasal inoculation with 50 μL of the bacterial suspension 2 h prior to the initiation of antimicrobial therapy. Mice were allowed to fully recover from anaesthesia in an oxygen-enriched chamber prior to randomization into treatment and control groups. All experiments were conducted in a non-blinded design, i.e. the handling staff were aware of group allocation at the different experimental stages.

Plasma and bronchopulmonary pharmacokinetics

The purpose of these studies was to establish human-simulated regimens of cefepime and taniborbactam in the murine pneumonia model equivalent to clinical doses of 2 and 0.5 g, respectively, administered every 8 h as 4 h infusions based on the unbound (free) plasma exposures. Following the selection and the confirmation of the cefepime and taniborbactam human-simulated regimens, additional studies were conducted to quantify the plasma and bronchopulmonary exposures achieved following the administration of the taniborbactam dosages utilized in the dose-ranging studies in combination with the cefepime human-simulated regimen.

Cefepime pharmacokinetic data in healthy adult volunteers collected in Phase I studies upon taniborbactam co-administration[8] and cefepime and taniborbactam pharmacokinetic parameters in the murine model were utilized for simulation. Cefepime protein-binding percentages utilized in the simulations were 20% and 0% in humans and mice, respectively,[9] while taniborbactam protein-binding percentages were 0% and 19.4% in humans[10] and mice,[11] respectively.

Infected mice (6–10 groups of six mice each) received the estimated human-simulated regimen of cefepime as monotherapy or in combination with that of taniborbactam or fractions of the established taniborbactam human-simulated regimen: 1.56% or 12.5% of the taniborbactam human-simulated regimen doses (equivalent to 7.8 or 62.5 mg every 8 h as 4 h infusion, respectively).

At 6–10 different timepoints, groups of six mice were euthanized by CO_2 asphyxiation followed by blood collection via intracardiac puncture and cervical dislocation. Following blood collection, but prior to cervical dislocation, bronchoalveolar lavage (BAL) fluid was collected from the mice at the same timepoints using methods previously described.[12] Formic acid in water (2% v/v) was added to BAL samples (equal parts) prior to freezing. All sample tubes were stored at −80°C until drug and urea concentration determination. Cefepime, taniborbactam and urea concentrations in plasma and BAL fluid were assayed by either Keystone Bioanalytical, Inc. (North Wales, PA, USA) or by Venatorx Pharmaceuticals, Inc. using qualified LC-MS/MS methods.

Cefepime and taniborbactam concentrations in the epithelial lining fluid (ELF) were estimated by correcting the drug concentration in BAL fluid for the dilution with NS during lavage using the following formula[13]:

$Compound_{ELF} = Compound_{BAL} \times (Urea_{plasma}/Urea_{BAL})$, where $Compound_{BAL}$ is the measured concentration of either cefepime or taniborbactam in the BAL fluid sample and $Urea_{plasma}$ and $Urea_{BAL}$ are the concentrations of urea in paired plasma and BAL fluid samples from each mouse, respectively.

Statistical outliers for each respective analyte were removed by the IQR method. A pharmacokinetic model was fitted to the plasma and ELF concentrations of each of cefepime and taniborbactam and the best-fit estimate parameters were determined by the non-linear least-squares techniques (WinNonlin, Version 8.3, Pharsight Corp., Mountain View, CA, USA). Compartment model selection was based on visual inspection of the fit and comparison of model diagnostics.

These parameters were utilized to estimate the plasma and ELF exposures and the ELF penetration ratios as the ratio of the ELF AUC_{0-24} to unbound (free) plasma AUC_{0-24}.

Taniborbactam dose-ranging studies

The purpose of these studies was to assess the *in vivo* bactericidal activity of the human-simulated exposure of cefepime monotherapy (2 g every 8 h as 4 h infusion) and of cefepime in combination with escalating taniborbactam exposures against cefepime-non-susceptible β-lactamase-producing Enterobacterales and *P. aeruginosa*.

Eight Enterobacterales and four *P. aeruginosa* isolates were selected with a range of cefepime/taniborbactam MICs (0.12 to 16 mg/L), expressing various types of Class A, C and D β-lactamases.

Mice (six per group) were prepared and inoculated, and treatment commenced 2 h later with cefepime monotherapy human-simulated regimen or cefepime human-simulated regimen in combination with taniborbactam as 1.56%, 6.25%, 12.5% or 100% of the taniborbactam human-simulated regimen.

For each isolate tested, six untreated mice were used as 0 h controls to determine the pre-treatment inoculum density, and six additional mice as 24 h controls. The 24 h control animals received NS in the same volume and schedule as the most frequently dosed drug regimen. Dosing order of different treatments and control groups was kept consistent to minimize confounding. After 24 h, all animals were euthanized by CO_2 asphyxiation followed by cervical dislocation then lungs were removed aseptically and individually homogenized in NS. Serial dilutions were plated onto TSA plates with 5% sheep blood for cfu enumeration. Bacterial densities at 24 h were evaluated to determine the change in log_{10} cfu/lungs achieved with each control/treatment. Statistical outliers were excluded systematically after application of the IQR rule.

We had previously reported that $fAUC_{0-24}$/MIC was the pharmacokinetic/pharmacodynamic (PK/PD) driver of taniborbactam activity.[11] Thus, for taniborbactam pharmacodynamic analyses, $fAUC_{0-24}$/MIC values were estimated for the different taniborbactam exposures for each isolate using the bioactive, free-drug exposures and isolate-specific cefepime/taniborbactam combination MIC value. A sigmoidal inhibitory E_{max} model was fitted to the data using WinNonlin and the effective taniborbactam indices ($fAUC_{0-24}$/MIC) required to achieve net stasis, 1 log and 2 log bacterial cell killing from the starting bacterial burden (0 h groups) for each isolate were estimated. The goodness of fit for each of these relationships was characterized using the coefficient of determination (R^2).

In vivo activities of cefepime and cefepime/taniborbactam human-simulated exposures

The purpose of these studies was to assess the *in vivo* bactericidal activity of cefepime human-simulated exposure as monotherapy (2 g every 8 h as 4 h infusion) and cefepime in combination with taniborbactam human-simulated exposure (cefepime/taniborbactam, 2/0.5 g every 8 h as 4 h infusion) against cefepime-non-susceptible β-lactamase-producing Enterobacterales ($n=13$) and *P. aeruginosa* ($n=5$).

Mice (six per group) were inoculated then treatment was commenced 2 h later. For each isolate tested, six untreated mice were used as 0 h controls, and six mice (receiving NS) as 24 h controls. Dosing order of different treatments and control groups was kept consistent to minimize

confounding. After 24 h, animals were euthanized, and lung homogenates were processed as described before for cfu enumeration. Bacterial densities at 24 h were evaluated to define the changes in log_{10} cfu/lungs. Statistical outliers were excluded systematically after application of the IQR rule.

Results

In vitro susceptibility

Cefepime and cefepime/taniborbactam combination MICs at a fixed taniborbactam concentration of 4 mg/L for the tested isolates are shown in Table 1. One isolate of *P. aeruginosa* (PSA 1715) was intermediate to cefepime and all other isolates were cefepime-resistant based on the MIC breakpoints defined by the CLSI.[6] Extent of cefepime MIC potentiation by taniborbactam was 32- to >4096-fold for Enterobacterales and 4- to >256-fold for *P. aeruginosa*.

Cefepime and taniborbactam plasma and bronchopulmonary pharmacokinetics

Cefepime and taniborbactam were detected in mouse plasma and BAL fluid following subcutaneous administration in all pharmacokinetic studies. Cefepime and taniborbactam plasma and ELF concentration–time profiles are shown in Figures 1 and 2. Cefepime and taniborbactam plasma and ELF pharmacokinetics were described satisfactorily using one-compartment models with first-order elimination. Best-fit pharmacokinetic parameters in the neutropenic pneumonia model are shown in Tables S1 to S4 (available as Supplementary data at *JAC* Online). Best-fit parameters in plasma were utilized to develop a cefepime human-simulated regimen of 2 g every 8 h as 4 h infusion as monotherapy and in combination with a taniborbactam human-simulated regimen of 0.5 g every 8 h as 4 h infusion or deescalating proportions of the latter established regimen. The murine dosing regimens are summarized in Table S5.

Comparisons of cefepime and taniborbactam $fAUC_{0-24}$, fC_{max} and $\%fT_{>MIC}$ values estimated in plasma and ELF at each MIC doubling dilution from 2 to 128 mg/L in humans and mice receiving the examined regimens are presented in Tables 2 and 3. The established murine cefepime and taniborbactam human-simulated regimens reasonably approximated the targeted human plasma exposures. Across the doses examined, taniborbactam plasma and ELF AUC_{0-24} values were dose-proportional across the range of dose studies ($R^2=1$).

Cefepime penetration ratio into the ELF was estimated to be 0.65. Penetration of cefepime into the ELF was unaltered by taniborbactam co-administration at any dosing level. Taniborbactam penetration ratio into the ELF was mostly consistent between different taniborbactam dosages and ranged between 0.85 and 1.11.

Taniborbactam dose-ranging studies

Enterobacterales

At 0 h, average ($\pm$SD) bacterial burdens were 6.78 ± 0.23 log_{10} cfu/lungs. For all the tested isolates, adequate growth in the lungs was achieved; the bacterial burdens increased over 24 h by an average magnitude of 2.57 ± 0.32 log_{10} cfu/lungs in the

Table 1. A summary of all the Enterobacterales and *P. aeruginosa* isolates selected for the *in vivo* efficacy studies. All isolates were examined in the *in vivo* efficacy of cefepime/taniborbactam human-simulated exposures studies. Isolates designated by bolded CAIRD IDs were also examined in taniborbactam dose-ranging studies

Isolate			MIC (mg/L)		
CAIRD ID	Source	Source ID	Cefepime	Cefepime/taniborbactam	β-Lactamase encoded
EC 747	ATCC	BAA 2340	512	2	KPC
EC 753[a]	IHMA	1904038	>512	2	CTX-M-15
EC 755[a],b	IHMA	1886025	>512	16	CTX-M-15, EC-TYPE, OXA-1, TEM-1
ECL 108	CDC[e]	0163	>512	2	KPC-2, ACT-7, CTX-M-15, OXA-1, TEM-1B
KP 647	CDC[e]	0112	>512	0.12	KPC-3
KP 679	CDC[e]	0066	>512	4	OXA-232, OXA-9, TEM-1A, CTX-M-15, OXA-1
KP 681[c]	CDC[e]	0075	>512	1	CTX-M-15, OXA-1, OXA-232, SHV-1
KP 731[d]	CAIRD		>512	1	SHV-11, TEM-1, KPC-3
PSA 1711	CDC[e]	0518	128	16	PDC-103, KPC-2
PSA 1714	CDC[e]	0528	32	4	PDC-1
PSA 1715	CDC[e]	0529	16	4	PDC-5
PSA 1844	CDC[e]	0356	>512	2	KPC-2, PDC-42
EC 720	CDC[e]	0437	>512	16	CTX-M-15, OXA-1
EC 748	CDC[e]	0001	512	2	ESC-35, OXA-1, KPC-3
ECL 124[d]	CAIRD		>512	8	TEM-OSBL, CTX-M-15, ACT-7
KP 579[d]	CAIRD		>512	16	SHV-11, TEM-1, CTX-M-15, OXA-48
KP 744[d]	CAIRD		>512	4	SHV-32, TEM-1, CTX-M-15, OXA-48
PSA 1681[d]	CAIRD		>512	8	OXA-486, PDC-8, OXA-10, CTX-M-2

CAIRD, Center for Anti-Infective Research and Development; EC, *E. coli*; ECL, *Enterobacter cloacae*; KP, *K. pneumoniae*; PSA, *P. aeruginosa*; OSBL, original-spectrum β-lactamase.

[a]PBP3 characterized by a 4-amino-acid (YRIK) insertion after position 333 and an A413V substitution.

[b]G137D substitution in OmpC known to be present.

[c]Mutation in OmpK35 porin known to be present.

[d]MICs were previously reported.[11]

[e]Molecular mechanisms of β-lactam resistance listed as posted on the FDA-CDC Antimicrobial Resistance Isolate Bank website at the time of study execution.

untreated controls. Consistent with the isolates' non-susceptibility to cefepime, robust increase in the bacterial burden was observed in the lungs of the mice receiving the cefepime monotherapy human-simulated regimen, indicating lack of *in vivo* activity; the bacterial burdens increased over 24 h by an average magnitude of $2.12 \pm 0.46 \log_{10}$ cfu/lungs compared with the 0 h controls. Compared with the cefepime monotherapy human-simulated regimen, taniborbactam co-administration at all tested ratios enhanced the degree of bacterial killing. Average maximal reduction of burden (I_{max}) at 24 h achieved due to taniborbactam co-administration was $5.59 \pm 1.09 \log_{10}$ cfu/lungs, estimated relative to the bacterial densities in the lungs of the mice treated with the cefepime monotherapy human-simulated regimen (Figure 3). For the composite of eight Enterobacterales isolates, the plasma $f\text{AUC}_{0-24}$/MIC ratios associated with static, 1 log kill and 2 log kill endpoints were 0.22, 1.45 and 36.46, respectively (Figure 4; $R^2 = 0.72$). As for the individual isolates, exposure–response relationships were strong based upon the coefficient of determination (median $R^2 = 0.94$). The median $f\text{AUC}_{0-24}$/MIC associated with static, 1 log kill and 2 log kill endpoints were 0.96, 4.03, and 49.1, respectively (Figure 5).

P. aeruginosa

Across the four isolates, average initial bacterial burdens at 0 h were $6.66 \pm 0.25 \log_{10}$ cfu/lungs and increased over 24 h by 3.29 ± 0.41 and $2.36 \pm 0.49 \log_{10}$ cfu/lungs in the untreated controls and those that received the cefepime monotherapy human-simulated regimen, respectively.

Compared with cefepime monotherapy, taniborbactam co-administration enhanced the bacterial killing, resulting in average I_{max} of $5.07 \pm 0.62 \log_{10}$ cfu/lungs (Figure 3). For the composite of four *P. aeruginosa* isolates, plasma $f\text{AUC}_{0-24}$/MIC associated with stasis, 1 log kill, and 2 log kill were 0.03, 0.93 and 18.22, respectively (Figure 4; $R^2 = 0.70$). Across the fits for the individual isolates, median $f\text{AUC}_{0-24}$/MIC associated with stasis, 1 log kill, and 2 log kill were 1.35, 3.02, and 6.29, respectively (median $R^2 = 0.89$; Figure 5).

In vivo activities of cefepime and cefepime/taniborbactam human-simulated exposures

At 0 h, average bacterial burdens were $6.73 \pm 0.18 \log_{10}$ cfu/lungs and increased over 24 h by 2.49 ± 0.47 and $1.92 \pm 1.17 \log_{10}$ cfu/
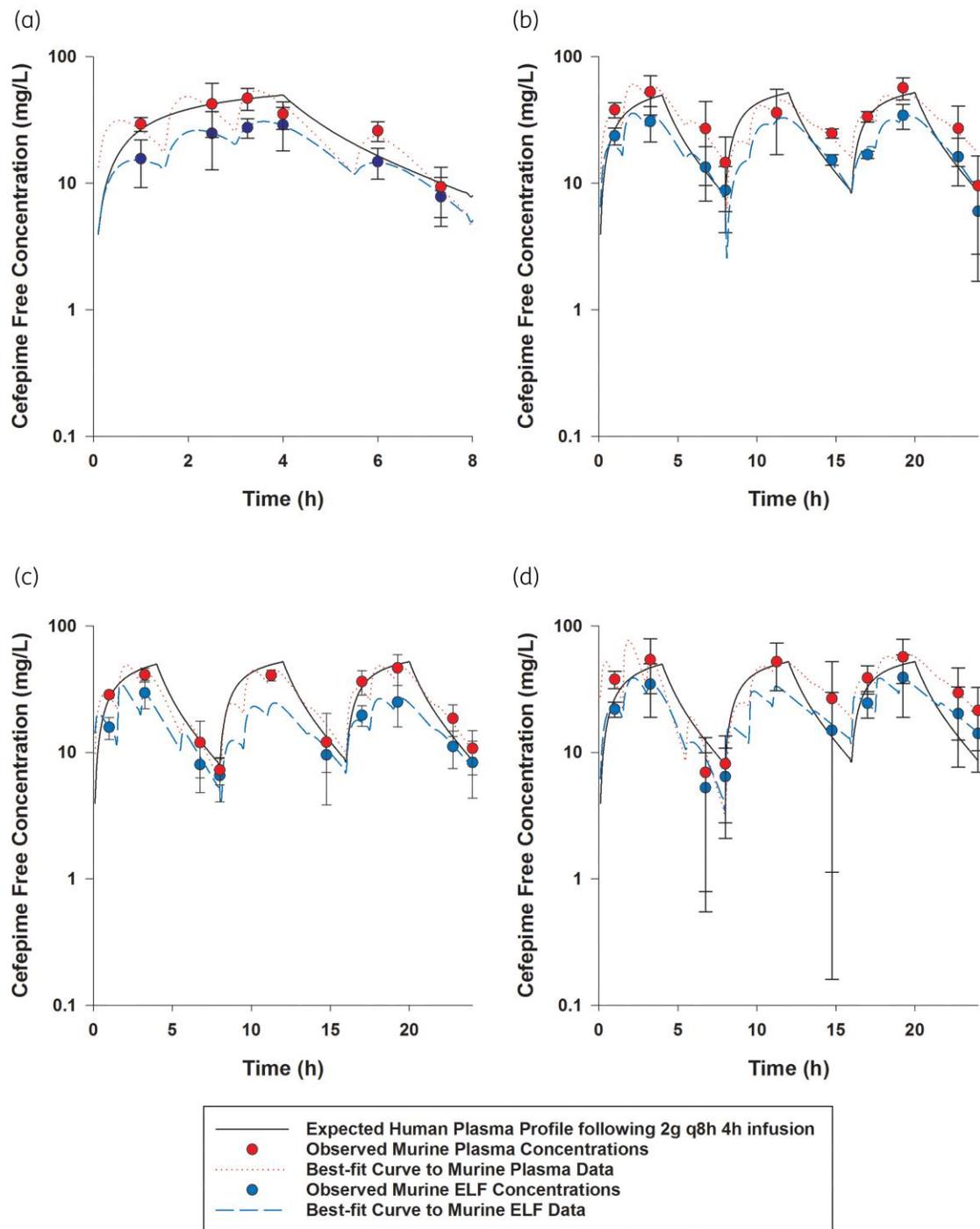
Figure 1. Cefepime free plasma and ELF concentrations achieved in mice receiving human-simulated regimen of cefepime (2 g every 8 h as 4 h infusion): (a) as monotherapy; (b) in combination with 1.56% of the doses of the human-simulated regimen of taniborbactam; (c) in combination with 12.5% of the doses of the human-simulated regimen of taniborbactam; and (d) in combination with 100% of the doses of the human-simulated regimen of taniborbactam. Data are means ± SDs. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

lungs in the untreated controls and those that received the cefepime monotherapy human-simulated regimen, respectively. Cefepime and taniborbactam human-simulated exposures co-administration resulted in ≥1 log reduction at 24 h compared

with the initial bacterial burdens against all 18 isolates and >2 log reduction against 14 of 18 isolates (Figure 6). Using a Student's *t*-test, cefepime and taniborbactam combination human-simulated exposures' bactericidal activities against
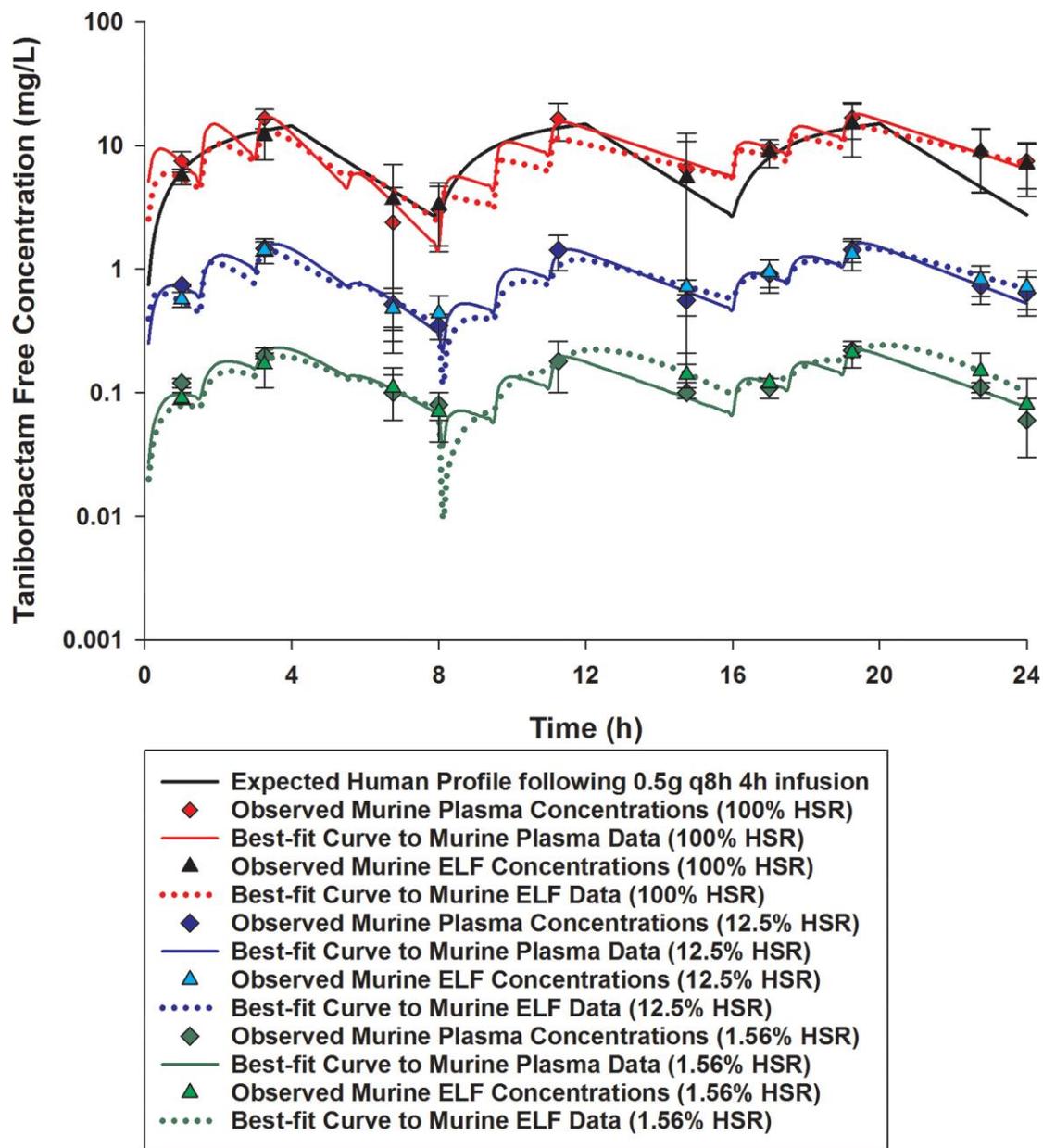
Figure 2. Taniborbactam free plasma and ELF concentrations achieved in mice receiving 1.56%, 12.5% or 100% of the doses of the human-simulated regimen of taniborbactam (0.5 g every 8 h as 4 h infusion) in combination with cefepime human-simulated regimen (2 g every 8 h as 4 h infusion). Data are means ± SDs. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Enterobacterales and *P. aeruginosa* isolates were compared and found to be not significantly different between the two species ($P=0.70$). Cefepime and taniborbactam combination human-simulated exposures' magnitude of killing against the four isolates with cefepime/taniborbactam MICs = 16 mg/L was comparable to that achieved against isolates of lower MICs ($P=0.98$).

Discussion

Taniborbactam ability to restore cefepime activity *in vitro* against clinical cephalosporin- and carbapenem-resistant

Enterobacterales and *P. aeruginosa* was illustrated as part of a recent global surveillance study.[5] A total of 99.5% of Enterobacterales ($n=13\,731$) and 94.2% of *P. aeruginosa* ($n=4619$) isolates were inhibited at a cefepime/taniborbactam concentration of ≤ 8 mg/L, with MIC_{90} values of 0.25 and 8 mg/L, respectively. Importantly, cefepime/taniborbactam was active *in vitro* against a variety of resistant subsets including piperacillin/tazobactam, ceftazidime/avibactam, ceftolozane/tazobactam, meropenem/vaborbactam and MDR phenotypes.[5] Cefepime/taniborbactam combination thus has the potential to fulfil the unmet need of combating infections due to carbapenem-resistant

Table 2. Comparison of cefepime plasma and ELF exposures achieved in humans receiving 2 g every 8 h as 4 h infusion versus infected mice receiving cefepime human-simulated regimen subcutaneously

Matrix/regimen	Species	%$fT_{>MIC}$ for an MIC (mg/L) of:							fAUC$_{0-24}$ (mg·h/L)	fC_{max} (mg/L)	ELF:plasma penetration
		2	4	8	16	32	64	128			
Plasma											
	Human	100	100	99	73	45	0	0	700.9	52.0	
Monotherapy	Mouse	100	100	93	77	35	0	0	656.8	54.0	
In combination with taniborbactam 1.56% HSR	Mouse	100	100	100	97	52	0	0	821.0	60.6	
In combination with taniborbactam 12.5% HSR	Mouse	100	100	98	80	37	0	0	668.2	48.9	
In combination with taniborbactam 100% HSR	Mouse	100	99	95	88	59	3	0	858.4	76.9	
ELF											
Monotherapy	Mouse	100	100	90	50	0	0	0	428.4	31.0	0.65
In combination with taniborbactam 1.56% HSR	Mouse	100	100	98	70	14	0	0	516.7	35.8	0.63
In combination with taniborbactam 12.5% HSR	Mouse	100	100	93	51	2	0	0	406.1	34.2	0.61
In combination with taniborbactam 100% HSR	Mouse	100	100	95	74	17	0	0	545.2	39.0	0.64

HSR, human-simulated regimen.

Table 3. Comparison of taniborbactam plasma and ELF exposures achieved in humans receiving 0.5 g every 8 h as 4 h infusion versus infected mice receiving taniborbactam in combination with cefepime human-simulated regimen subcutaneously

Matrix/regimen	Species	%$fT_{>MIC}$ for an MIC (mg/L) of:							fAUC$_{0-24}$ (mg·h/L)	fC_{max} (mg/L)	ELF:plasma penetration
		2	4	8	16	32	64	128			
Plasma											
	Human	99	83	53	0	0	0	0	205.2	15.0	
Taniborbactam 1.56% HSR	Mouse	0	0	0	0	0	0	0	3.2	0.2	
Taniborbactam 12.5% HSR	Mouse	0	0	0	0	0	0	0	22.1	1.6	
Taniborbactam 100% HSR	Mouse	98	93	65	5	0	0	0	229.9	18.1	
ELF											
Taniborbactam 1.56% HSR	Mouse	0	0	0	0	0	0	0	3.5	0.2	1.11
Taniborbactam 12.5% HSR	Mouse	0	0	0	0	0	0	0	21.0	1.5	0.95
Taniborbactam 100% HSR	Mouse	100	89	51	0	0	0	0	195.2	15.1	0.85

HSR, human-simulated regimen.

and MDR Gram-negative pathogens with a coverage that surpasses currently approved comparators.

We have previously demonstrated cefepime/taniborbactam combination's potent *in vivo* activity against Enterobacterales and *P. aeruginosa* clinical isolates including carbapenem-resistant isolates in the murine thigh and complicated urinary tract infection models.[11,14] Using a taniborbactam dose-fractionation design in the thigh infection model, we established that the PK/PD index that best correlated with taniborbactam inhibitory activity (i.e. protection of cefepime from β-lactamase hydrolysis) was fAUC$_{0-24}$/MIC.[11] The current study in the murine pneumonia model adds to the understanding of cefepime/taniborbactam *in vivo* pharmacodynamics, and the data have important implications for the clinical dose selection for the HABP/VABP indication.

A cefepime human-simulated regimen was included in all *in vivo* pharmacodynamics assessments as a negative control to demonstrate that the efficacy of the cefepime/taniborbactam combination was not due to unexpected *in vivo* killing with the β-lactam monotherapy. Model validation using a different antibiotic was not performed concomitantly in this investigation. Nevertheless, the neutropenic murine pneumonia model utilized is well validated for antibacterial PK/PD investigations as supported by literature,[15,16] as the efficacies of various agents are generally predictable based on the MICs demonstrating the translational application of the model. An example from one of our recent investigations where the *in vivo* activity of a levofloxacin human-simulated regimen equivalent to 750 mg q24h was concordant with the *in vitro* MICs of Enterobacterales strains; bacteriostatic activity (-0.46 ± 0.42 log$_{10}$ cfu/lung) and bacterial killing (-3.02 ± 0.31 log$_{10}$ cfu/lung) were observed in lung-infected mice with isolates of susceptible MICs (1 and 0.12 mg/L, respectively), while >2 log$_{10}$ cfu/lung bacterial growth was observed with seven levofloxacin-resistant isolates.[4] While clinically relevant *in vivo* activity in pneumonia due to the target organisms studied
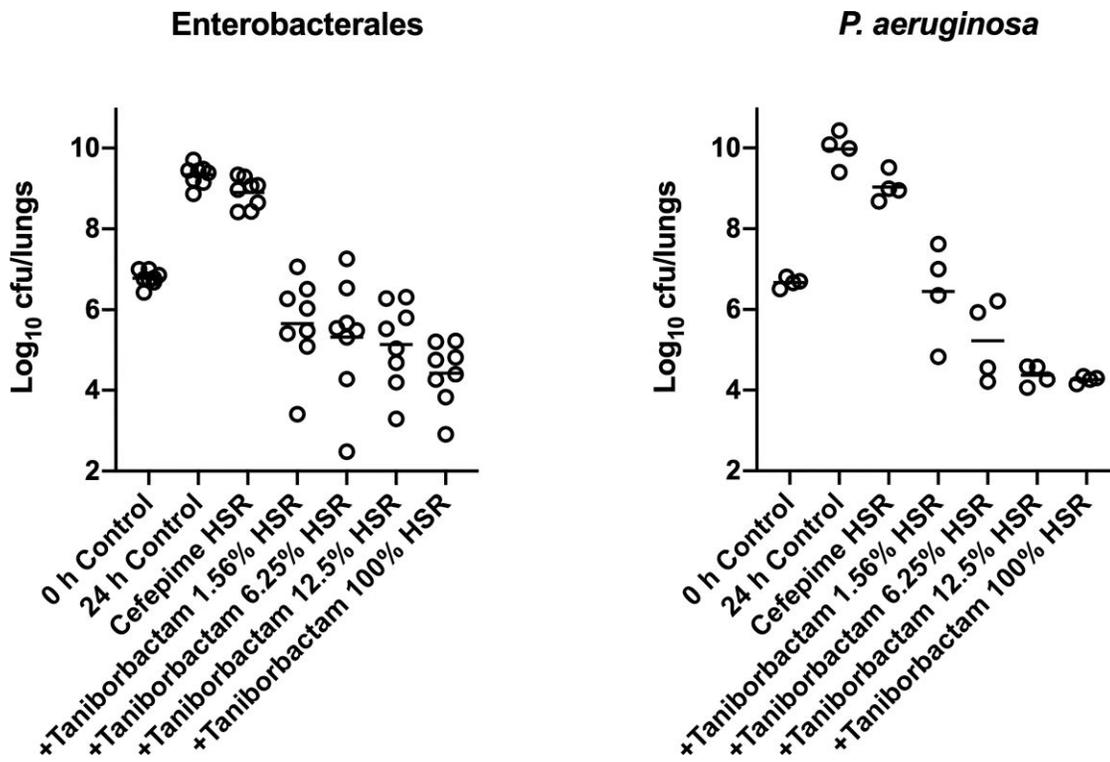
Enterobacterales

P. aeruginosa

Figure 3. Taniborbactam dose-ranging efficacy in combination with cefepime human-simulated exposure against Enterobacterales ($n=8$, left) and *P. aeruginosa* isolates ($n=4$, right) in the neutropenic pneumonia model. Each dot represents the mean $\log_{10}$ cfu/lungs for one bacterial strain per regimen.
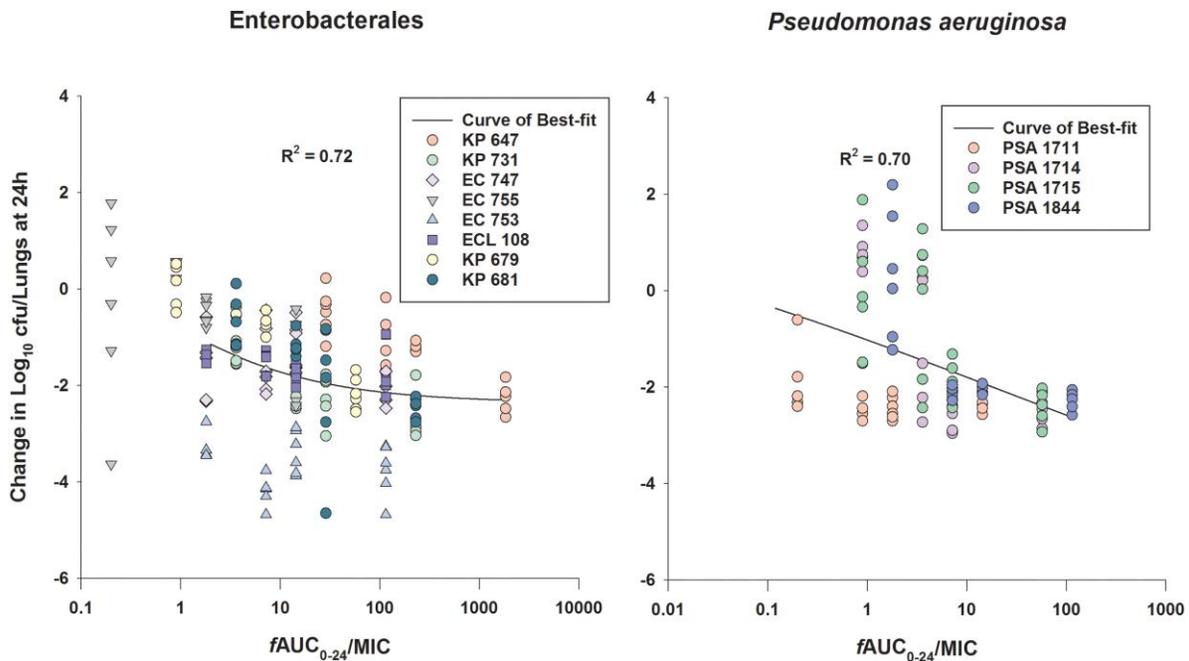
Enterobacterales

Pseudomonas aeruginosa

Figure 4. The curves of best fit to the taniborbactam $f\text{AUC}_{0-24}$/MIC and the changes in $\log_{10}$ cfu/lungs at 24 h relative to 0 h groups for the composites of examined Enterobacterales ($n=8$) and *P. aeruginosa* ($n=4$) isolates in the neutropenic pneumonia model. The solid lines represent the curves of best fit, while the circles represent the actual changes in bacterial burdens observed in the individual mice. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.
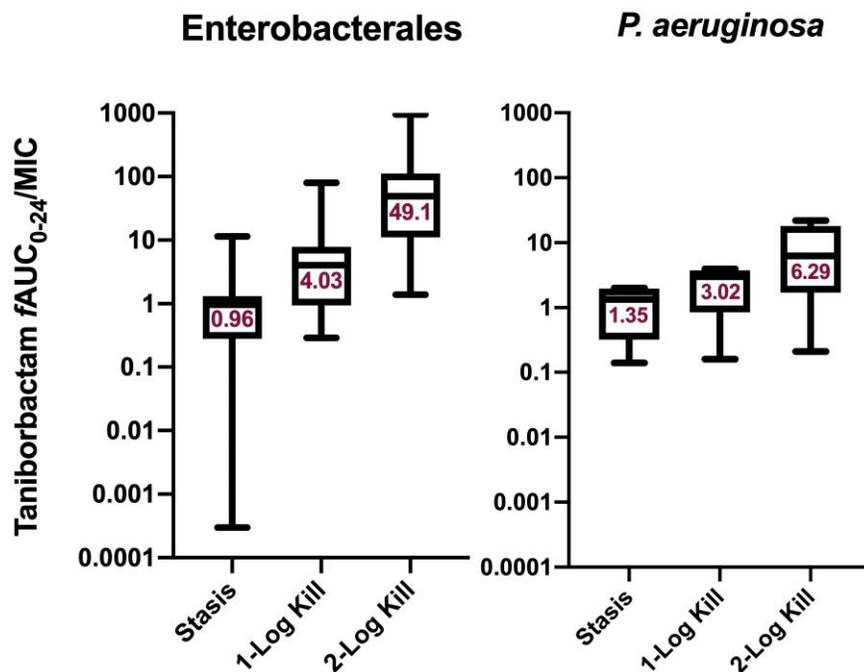
Enterobacterales

P. aeruginosa

Figure 5. Taniborbactam fAUC$_{0-24}$/MIC required to achieve efficacy endpoints against Enterobacterales ($n=8$) and *P. aeruginosa* ($n=4$) when co-administered with the cefepime human-simulated regimen. Median values are displayed on the plots. Median 2 log kill target for Enterobacterales calculated based on seven isolates (target not achieved against one isolate). Hinges represent 25th and 75th percentiles. Whiskers represent 10th and 90th percentiles. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

in the current investigation is inferred by the use of this translational model, which incorporates humanized drug exposures, HABP/VABP trial data in man are required to fully assess the clinical utility of the compound for this pulmonary indication.

In the current investigation, we observed activity of cefepime/taniborbactam against a diverse group of cefepime-non-susceptible, serine-β-lactamase-producing Enterobacterales and *P. aeruginosa* isolates including carbapenemase producers. In order to identify the magnitude of taniborbactam fAUC$_{0-24}$/MIC required to achieve efficacy endpoints that correlate with clinical outcome,[16] a dose-ranging study design was implemented, in which a fixed cefepime clinical exposure was administered while varying taniborbactam exposure. This design allows the assessment of taniborbactam target threshold required to restore the *in vivo* efficacy of the pharmacodynamically optimized clinical cefepime exposure without the potential confounding effect of varying cefepime activity.

Against Enterobacterales, median taniborbactam plasma targets required for stasis and 1 log kill in the neutropenic pneumonia model were comparable to those previously reported in the neutropenic thigh infection model (0.96 and 4.03 versus 1.18 and 2.62, respectively).[11] However, against *P. aeruginosa*, plasma target exposures in the pneumonia model were several folds higher than those observed in the thigh infection model (1.35 and 3.02 versus 0.29 and 0.46, respectively).[11] Given the high extent of taniborbactam ELF penetration in the murine model, the difference in threshold plasma targets is unlikely the function of major discrepancies in taniborbactam exposures at infection sites between models. The higher threshold targets against *P. aeruginosa* in the

pneumonia model might be attributed to differences in infection pathogenesis as the formation of biofilm on airway epithelial cells serves as a barrier to limit antibiotic access to bacterial cells.[17] Other possible explanations are differences in starting inoculum between models (~1 log higher in the pneumonia model), thus the higher exposure requirements for achieving the efficacy endpoints, and inter-isolate variabilities as the isolates examined were different between the two models. Nevertheless, given that the estimated average taniborbactam fAUC$_{0-24}$ associated with a 0.5 g dose every 8 h in healthy volunteers is ~205 mg·h/L, the targets derived in the pneumonia model predict that this taniborbactam dose, in combination with cefepime 2 g every 8 h as 4 h infusion, would provide sufficient exposure to achieve bactericidal activity against cefepime-resistant Enterobacterales and *P. aeruginosa* isolates with cefepime/taniborbactam MICs up to and including 16 mg/L in patients. Nonetheless, the impact of variable pharmacokinetics among critically ill patients on the probability of pharmacodynamic target attainment should be assessed to support clinical breakpoint determination.

Non-clinical PK/PD assessments, utilizing mean or median human concentration–time profiles from healthy subjects for establishing the murine human-simulated regimens, is a practical approach that provides reasonable approximation to the exposures observed among the majority of target patients. The assumption herein is that inter-subject variability in pharmacokinetic exposures exists among patients as well as among mice on a comparable scale and the mean murine profile typically falls within the reported coefficient of variation of the concentrations for the majority of the dosing interval.[18] Efficacy assessments
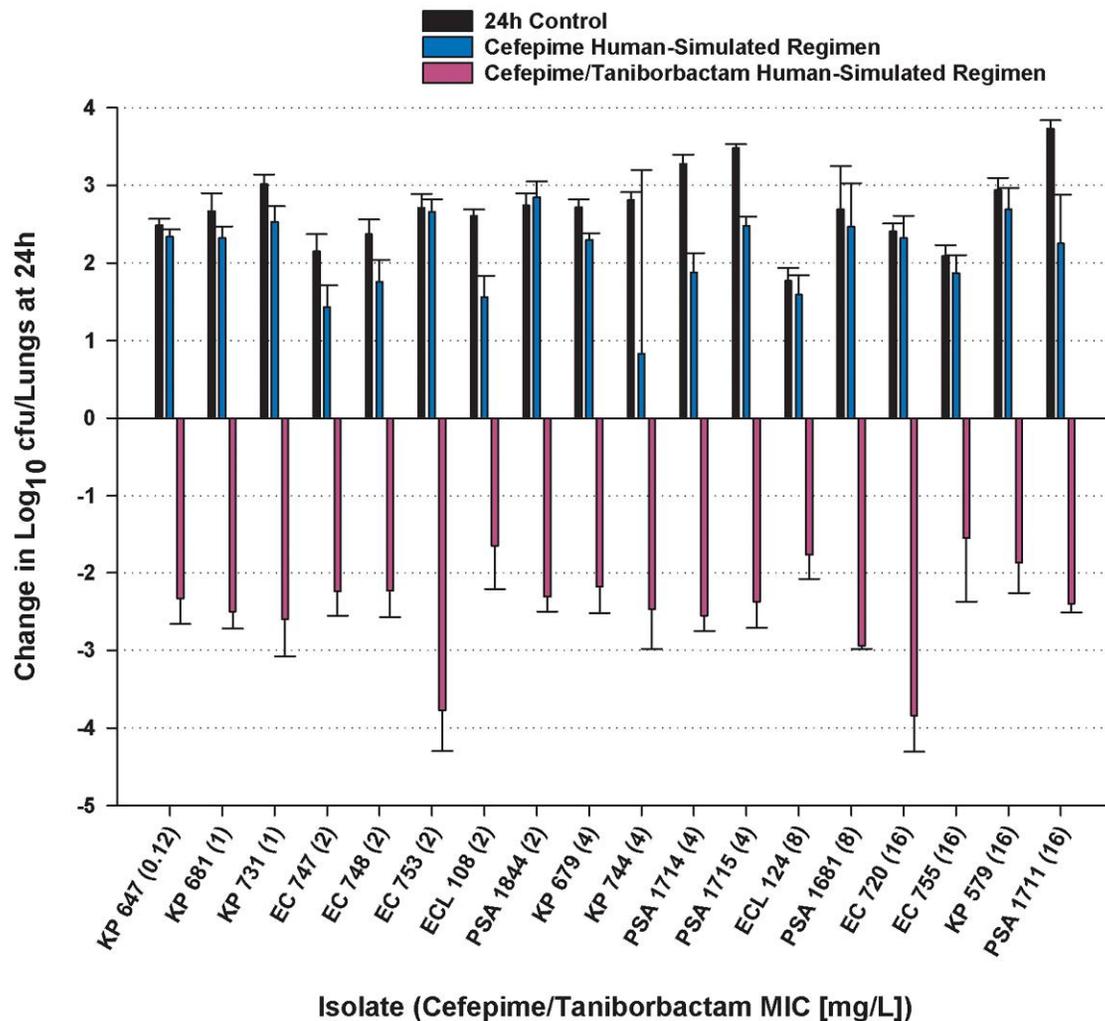
Figure 6. Comparative efficacy of the human-simulated exposure of cefepime monotherapy and in combination with taniborbactam human-simulated exposure against 13 Enterobacterales and 5 *P. aeruginosa* clinical isolates expressing serine β-lactamases. Data are means ± SDs. Value between parentheses following strain designation represents cefepime/taniborbactam MIC in mg/L. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

utilizing human-simulated regimens allow for screening antibiotics such as preclinical and clinical candidates against phenotypically and genotypically diverse isolates including those at the upper end of the MIC distribution not frequently isolated from patients during clinical trials. In the current investigation, administration of cefepime/taniborbactam human-simulated exposures resulted in ~2 log kill against the majority of the examined Enterobacterales and *P. aeruginosa* isolates including isolates with combination MICs exceeding the MIC$_{90}$ from recent surveillance.[5] The high degree of killing observed was consistent with predictions from the dose-ranging studies and further supported cefepime/taniborbactam clinical dose selection where a stringent threshold (>1 log kill) is predictive of clinical efficacy for severe infections such as pneumonia.[16] It should be noted that by humanizing cefepime/taniborbactam exposures according to plasma concentrations, the corresponding concentration–time profile in mouse ELF, the infection site, may be considerably different compared with the human ELF profile in pneumonia patients.

Differences in ELF pharmacokinetic profile estimates and the inter-subject variability observed among infected mice, healthy humans and patients with pneumonia are to be expected, which should be taken into account when considering the translation applicability of the preclinical data. Nevertheless, the robustness of the dose-ranging study design through the use of a wide range of taniborbactam exposures may allow for informative PK/PD analyses when ELF data from cefepime/taniborbactam Phase I bronchopulmonary pharmacokinetic studies are considered.[10,19]

In summary, co-administration of taniborbactam with a cefepime plasma human-simulated regimen markedly enhanced the latter's *in vivo* efficacy against clinical cefepime-non-susceptible Enterobacterales and *P. aeruginosa* isolates expressing a broad range of serine β-lactamases in the neutropenic pneumonia model with cefepime/taniborbactam MICs up to and including 16 mg/L. Taniborbactam fAUC$_{0-24}$/MIC targets required for various efficacy endpoints were identified. Assessments of the probability of the clinical attainment of these PK/PD targets should be undertaken to

support the proposed cefepime/taniborbactam dose (2/0.5 g every 8 h as 4 h infusion) for treatment of HABP/VABP due to Enterobacterales and *P. aeruginosa* with these resistant phenotypes.

Supplementary data

Tables S1 to S5 are available as Supplementary data at *JAC* Online.

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