


Imipenem/funobactam (formerly XNW4107) *in vivo* pharmacodynamics against serine carbapenemase-producing Gram-negative bacteria: a novel modelling approach for time-dependent killing

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Background: Imipenem/funobactam (formerly XNW4107) is a novel β -lactam/ β -lactamase inhibitor with activity against MDR *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacterales strains. Using a neutropenic murine thigh infection model, we aimed to determine the pharmacokinetic/pharmacodynamic (PK/PD) index, relative to funobactam exposure, that correlated most closely with the *in vivo* efficacy of imipenem/funobactam combination and the magnitude of index required for efficacy against serine carbapenemase-producing clinical strains.

Methods: Dose-fractionation was conducted against three strains. Imipenem human-simulated regimen (HSR, 500 mg q6h 1 h infusion) efficacy in combination with escalating funobactam exposures against seven *A. baumannii*, four *P. aeruginosa* and four *Klebsiella pneumoniae* (imipenem/funobactam MICs 0.25–16 mg/L) was assessed as 24 h change in \log_{10} cfu/thigh.

Results: Increased funobactam fractionation enhanced efficacy, indicating time-dependent killing. Changes in \log_{10} cfu/thigh versus %fT > MIC were poorly predictive of efficacy; bactericidal activity was observed at %fT > MIC = 0%. Across different threshold plasma funobactam concentrations (C_T s), %fT > $C_{T(1 \text{ mg/L})}$ had the highest correlation with efficacy. Normalizing the %fT > $C_T = 1 \text{ mg/L}$ index to the respective isolate imipenem/funobactam MIC ($[\%fT > C_T]/\text{MIC}$) allowed integration of the isolate's susceptibility, which further enhanced the correlation. Median ($\%fT > C_{T(1 \text{ mg/L})}/\text{MIC}$) values associated with 1-log reductions were 9.82 and 9.90 for *A. baumannii* and *P. aeruginosa*, respectively. Median ($\%fT > C_{T(1 \text{ mg/L})}/\text{MIC}$) associated with stasis was 55.73 for *K. pneumoniae*. Imipenem/funobactam 500/250 mg q6h 1 h infusion HSR produced >1-log kill against 6/7 *A. baumannii*, 4/4 *P. aeruginosa* and stasis against 4/4 *K. pneumoniae*.

Conclusions: Imipenem/funobactam showed potent *in vivo* efficacy against serine carbapenemase-producers. The novel PK/PD index ($\%fT > C_T$)/MIC appeared to best describe *in vivo* activity.

Introduction

Infections due to carbapenem-resistant Gram-negative bacteria are an urgent public health threat.^{1–3} Specifically, in 2017 the WHO published a list of bacteria for which new antibiotics are urgently needed and named carbapenem-resistant Enterobacterales (CRE), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (CRAB) among the most critical group.⁴ Although multiple novel agents have been recently introduced

with activity against CRE and carbapenem-resistant *P. aeruginosa*,⁵ sparse therapeutic options exist for the treatment of CRAB infections.⁶ Additionally, current IDSA guidance recommends combination therapy for the treatment of moderate to severe CRAB infections.⁷ Among the limited armamentarium against CRAB, polymyxin and tetracycline derivatives have undesirable adverse effects and are often associated with poor clinical outcomes.^{8,9}

Imipenem/funobactam (formerly XNW4107) is a novel β -lactam/ β -lactamase inhibitor (BL/BLI) with *in vitro* activity against serine carbapenemase-producing *A. baumannii*, *P. aeruginosa* and Enterobacterales. Funobactam is a diazabicyclooctane BLI that confers protection against hydrolysis by Ambler Class A, C and D β -lactamases, including OXA-23 and OXA-24 found in *A. baumannii*.¹⁰ The investigational compound is advancing into Phase III clinical trials for complicated urinary tract infections and hospital acquired/ventilator-associated bacterial pneumonia (NCT05204368 and NCT05204563, respectively) as imipenem/cilastatin/funobactam 500/500/250 mg q6h. The objectives of this study were: (i) to determine and assess the magnitude of the pharmacokinetic/pharmacodynamic (PK/PD) index best predictive of efficacy when funobactam is co-administered with human-simulated plasma exposure of imipenem, and (ii) to assess the efficacy of a human-simulated regimen (HSR) of imipenem/funobactam 500/250 mg q6h against serine carbapenemase-producing *A. baumannii*, *P. aeruginosa* and *Klebsiella pneumoniae* in the neutropenic murine thigh infection model.

Methods

Antimicrobial test agents

For *in vivo* studies, commercially available imipenem-cilastatin vials (250/250 mg, Fresenius Kabi, Lake Zurich, IL, USA; lots 0001D95 and 0001E15) were utilized. Imipenem USP Reference-Standard was used for *in vitro* testing (Rockville, MD, USA; lot R038R0). Funobactam vials provided by WuXi Biologics Conjugation Co. Ltd (50 mg; Wuxi, Jiangsu, China; lots 20200907 and 20200504) were used for *in vitro* and *in vivo* studies. Placebo vials (lot 2216FFF1911010) provided by WuXi Biologics Conjugation Co. Ltd were diluted with 0.9% NaCl for dosing sham controls. Imipenem and funobactam were dissolved in 0.01 M phosphate buffer pH 7.2 and 0.9% NaCl, respectively, for *in vitro* studies.^{11,12} For *in vivo* testing, imipenem and funobactam were reconstituted and diluted with 0.9% NaCl to attain final concentrations that would deliver mean weight-based dosages.

Bacterial isolates and susceptibility testing

A total of 15 isolates (4 *P. aeruginosa*, 7 *A. baumannii* and 4 *K. pneumoniae*) were utilized (Table 1). Isolate selection for efficacy studies was based on phenotypic profiles, β -lactamases encoded and ability to establish infection in the model. Isolates were frozen in skim milk and stored at -80°C . Prior to experimentation, isolates were subcultured twice onto Trypticase Soy Agar with 5% sheep blood (Becton, Dickinson & Co., Sparks, MD, USA) and incubated for 18–24 h. Imipenem and imipenem/funobactam MICs (funobactam fixed at 8 mg/L) were determined by broth microdilution. *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC BAA 1705 were used as the quality controls for both imipenem^{11,12} and imipenem/funobactam.

Animals

Specific-pathogen-free female ICR mice weighing 20 to 22 g were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA) and allowed to acclimate for 48 h before study procedures. Mice were housed in groups of six at controlled room temperature in high-efficiency particulate air-filtered cages (Innovive, San Diego, CA, USA) with paper nesting material for enrichment. Food and water were provided *ad libitum* and a 12 h light/12 h dark cycle was maintained. Animals were utilized according to recommendations from the National Research Council of the National Academy of Sciences. The protocol was approved by the

Institutional Animal Care and Use Committee of Hartford Hospital (Assurance #A3185-01).

Neutropenic murine thigh infection model

Mice were rendered neutropenic by injecting cyclophosphamide via intraperitoneal (IP) injections at doses of 150 mg/kg and 100 mg/kg 4 days and 1 day prior to inoculation, respectively. In order to reduce the renal clearance of the study drugs and generate human-simulated exposures, uranyl nitrate at 5 mg/kg was given by IP injection 3 days before inoculation. One thigh per mouse was intramuscularly inoculated with 0.1 mL of $\sim 10^7$ cfu/mL bacterial suspension in normal saline (NS) 2 h prior to the initiation of antimicrobial or placebo therapy.

PK studies

Funobactam single-dose studies

Single-dose funobactam PK studies were carried out to determine murine PK parameters and assess if the co-administration of funobactam alters the disposition of imipenem. Escalating doses of funobactam (1, 10 and 20 mg/kg) were given subcutaneously with a previously established imipenem HSR¹³ to 108 infected mice (36 mice per funobactam dose). Groups of six were sacrificed at predefined timepoints. Blood was obtained via cardiac puncture and plasma was matched with an equal volume of stabilizing buffer (1:1 50% ethylene glycol/1 M pH 6.0 MES buffer) and stored at -80°C . Total imipenem and funobactam plasma concentrations were measured using a validated LC/MS-MS method by Keystone Bioanalytical, Inc. (North Wales, PA, USA). PK modeling and simulations were performed using Phoenix WinNonlin (Version 8.1, Pharsight Corp., Mountain View, CA, USA).

Ex vivo funobactam plasma protein-binding studies

Escalating doses of funobactam (1, 10 and 20 mg/kg) were administered subcutaneously in combination with the imipenem HSR to determine the funobactam plasma protein-binding using methods previously described.¹³ These doses covered the approximate exposure range seen in the dose-ranging and dose-fractionation studies. Triplicate plasma and ultrafiltrate samples were collected, treated with stabilizing buffer and stored at -80°C . Funobactam concentrations were determined by Keystone Bioanalytical, Inc.

Human-simulated exposure PK studies

PK studies were conducted to establish HSRs that approximated the target human exposures achieved after administration of imipenem/cilastatin 500/500 mg (imipenem HSR monotherapy) and imipenem/cilastatin/funobactam 500/500/250 mg (imipenem/funobactam HSR) q6h as 1 h infusions. To simulate the imipenem human profile in critical illness,¹⁴ previously reported murine PK parameters and percentage plasma protein-binding were utilized.¹³ The murine PK parameters generated during funobactam single doses, the estimated protein-binding percentage in mouse plasma, and the previously measured protein-binding percentage in humans (16.63%, unpublished data on file) were utilized to simulate a dosing regimen that approximated the target exposure in healthy volunteers.¹⁵

Following mathematical selection, confirmatory PK studies were undertaken following the same methodology as outlined above to establish that the murine HSRs approximated imipenem and funobactam target human exposures and confirm that the co-administration of funobactam did not alter imipenem exposures.

In vivo efficacy studies

All groups contained six mice. Controls were sacrificed just prior to antibiotic initiation (0 h controls) and 24 h later (24 h controls receiving placebo). Imipenem HSR monotherapy served as a negative treatment

Table 1. MICs of imipenem and imipenem/funobactam combination at a fixed funobactam concentration of 8 mg/L against a selection of *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates

CAIRD isolate ID	Source	Alternate isolate ID	Modal MIC (mg/L)		β -Lactamase(s) encoded ^a
			IPM	IPM/funobactam	
ACB 160	CDC	0277	>64 ^b	16	OXA-24, OXA-65, TEM-1B
ACB 179	CDC	0296	64 ^b	1	ADC-25, OXA-23, OXA-223
ACB 193	CDC	0310	>64 ^b	8	OXA-23, OXA-82
ACB 194	CDC	0311	64 ^b	4	ADC-25, OXA-23, OXA-82
ACB 209	CDC	0101	>64 ^b	8	OXA-65, OXA-24
ACB 246	JMI	990089	64	8	ADC-33, OXA-23, OXA-82
ACB 258	JMI	1043774	64	4	ADC-222, OXA-23, OXA-95
PSA 1844	CDC	0356	>64 ^b	1	KPC-2, PDC-42
PSA 1862	CDC	0763	64 ^b	4	GES-19, GES-20
PSA 1866	CDC	0767	64 ^b	8	GES-20
PSA 1869	CDC	0770	16 ^b	2	GES-19, GES-26
KP 648	CDC	0113	>64 ^b	1	KPC-3
KP 651	CDC	0120	16 ^b	0.5	KPC-2
KP 741	CAIRD	N/A	>32	0.5	SHV-11, CTX-M-55, OXA-48
KP 827	CDC	0848	>64 ^b	0.25	OXA-48, CTX-M-15, OXA-1, SHV-11, TEM-1B

ACB, *A. baumannii*; CAIRD, Center for Anti-Infective Research and Development; IPM, imipenem; KP, *K. pneumoniae*; N/A, not applicable; PSA, *P. aeruginosa*.

^a β -lactamase genes reported as posted on the FDA-CDC Antimicrobial Resistance Isolate Bank website at the time of study execution.

^bMIC reported as posted on the FDA-CDC Antimicrobial Resistance Isolate Bank website at the time of study execution.

control. Treatments were continued for 24 h, then animals were euthanized by CO₂ asphyxiation followed by cervical dislocation. Thighs were removed and individually homogenized in NS. Serial dilutions were plated on Trypticase soy agar with 5% sheep blood for cfu enumeration. Changes in log₁₀cfu/thigh at 24 h, relative to 0 h controls, were evaluated.

Funobactam dose-fractionation

Funobactam total daily doses of 3.6 and 7.2 mg/kg in combination with the imipenem HSR were administered as once daily and fractionated as q12h and q6h against three isolates. For a single isolate (ACB 258), the total daily dose of 7.2 mg/kg was further fractionated (q4h) to maximize the %fT > threshold funobactam plasma concentration parameter (%fT > C_T). Comparisons were made between the different regimens for bacterial densities after 24 h using one-way analysis of variance test followed by Tukey's test where the *P* value was ≤ 0.05 .

Funobactam dose-ranging and HSR studies

Fifteen isolates were tested against the imipenem HSR alone or in combination with 4–5 funobactam regimens [0.9, 1.8, 3.6, 7.2 (HSR of predicted clinical dose of 250 mg q6h) or 14.4 mg/kg, all administered q6h], representing escalating fractions of funobactam HSR. Additional PK studies were conducted to assess the funobactam exposures over the dose range for PK/PD analyses; funobactam 0.9 and 14.4 mg/kg (the lowest and highest doses utilized, respectively) were given with the finalized imipenem HSR of 500 mg q6h. Samples were obtained, processed and analysed, and exposures were estimated as described previously. A sigmoidal inhibitory maximum possible effect (*E*_{max}) model was fitted to the dose-ranging data using WinNonlin. The effective funobactam index required to achieve net stasis, 1-log and 2-log bacterial killing from the starting bacterial burden for each isolate as well as for the composite within each species was estimated and the goodness-of-fit for each relationship was characterized.

Results

In vitro susceptibility

Imipenem and imipenem/funobactam modal MICs for the 15 isolates are presented in Table 1. All isolates were resistant to imipenem per CLSI-established breakpoints.¹¹ The MICs in combination with funobactam decreased for all isolates with a range of reduction of >4-fold to >256-fold.

PK studies

Total funobactam plasma concentrations versus time profiles achieved following single-dose administration of 1, 10 and 20 mg/kg given concomitantly with imipenem HSR are presented in Figure S1 (available as [Supplementary data](#) at JAC Online). Dose-proportionality was confirmed across the examined dose range (Figure S2). PK was best described using a one-compartment model. The best-fit average PK parameters were: apparent volume of distribution, 4.62 L/kg; first-order absorption rate constant, 7.21 h⁻¹; and overall elimination rate constant, 0.41 h⁻¹.

The PK exposures of the lowest and highest doses of funobactam used in dose-ranging, 0.9 and 14.4 mg/kg q6h, respectively, co-administered with the imipenem HSR of 500 mg q6h were comparable to those achieved in humans following clinical doses of 31.25 and 500 mg q6h, respectively. Interpolation was applied to estimate the exposures achieved with regimens utilized in dose-ranging between 0.9 mg/kg q6h to 7.2 mg/kg q6h (Figure 1). Funobactam exposures achieved with the escalating regimens administered in the dose-ranging studies are

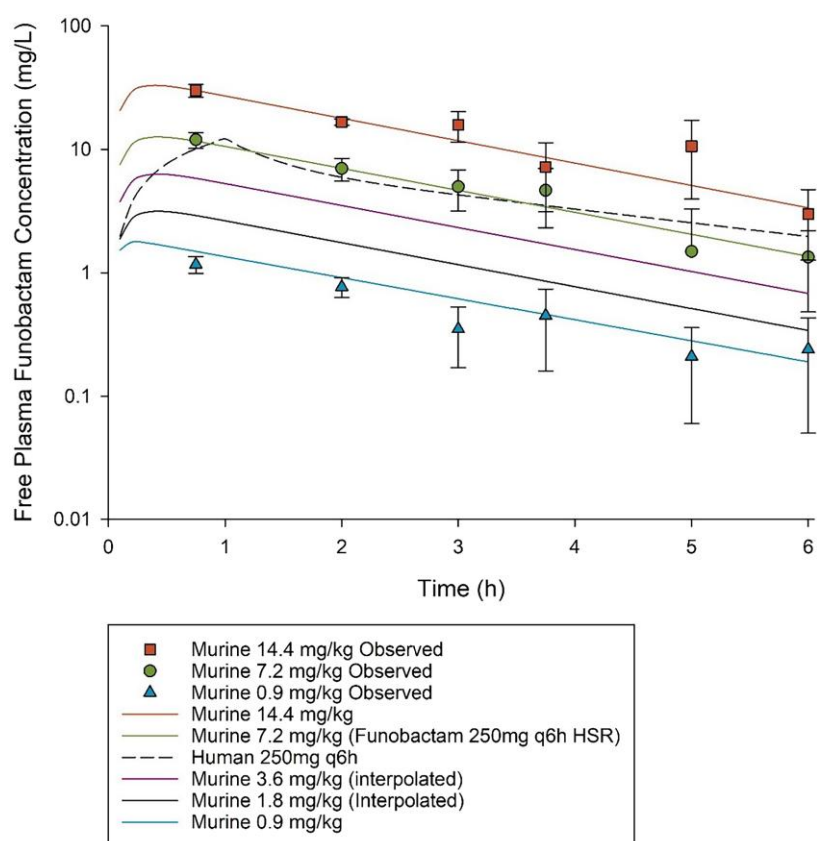


Figure 1. Funobactam free plasma concentration–time profiles achieved with the regimens administered in dose-ranging studies. Data are presented as means ± SD.

Table 2. Comparison of the funobactam pharmacokinetic exposures achieved with each regimen administered in the funobactam dose-ranging studies in the murine neutropenic thigh infection model

Funobactam dose	Clinical-equivalent dose q6h (1 h infusion)	%fT > MIC for MIC (mg/L):								fAUC ₀₋₂₄ (mg.h/L)	fC _{max} (mg/L)
		0.25	0.5	1	2	4	8	16	32		
0.9 mg/kg	31.25 mg	86%	57%	29%	0%	0%	0%	0%	0%	17.84 ^a	1.74 ^a
1.8 mg/kg	62.5 mg	100%	86%	57%	29%	0%	0%	0%	0%	35.68 ^b	3.48 ^b
3.6 mg/kg	125 mg	100%	100%	86%	57%	29%	0%	0%	0%	71.36 ^b	6.95 ^b
7.2 mg/kg	250 mg	100%	100%	100%	86%	57%	29%	0%	0%	142.71 ^a	13.90 ^a
14.4 mg/kg	500 mg	100%	100%	100%	100%	94%	67%	39%	10%	363.70 ^a	36.15 ^a

^aValues calculated from observed PK data.

^bLinear interpolation between 7.2 and 0.9 mg/kg.

summarized in Table 2. Funobactam co-administration did not alter imipenem HSR exposure profile (Figure 2).

Ex vivo plasma protein-binding

The average ± SD protein-binding percentages of funobactam after single-dose administration of 1, 10 and 20 mg/kg in the presence of imipenem HSR were 3.11% ± 3.73%, 5.44% ± 3.23%

and 2.03% ± 6.18%, respectively, with overall average of 3.53%. Thus, across the exposures tested, funobactam mouse plasma-bound fraction was low and not concentration-dependent.

Human-simulated exposure PK studies

The imipenem exposure attained following administration of 8 mg/kg at 0 h, 2.5 mg/kg at 1.25 h, 1.5 mg/kg at 2.5 h, and

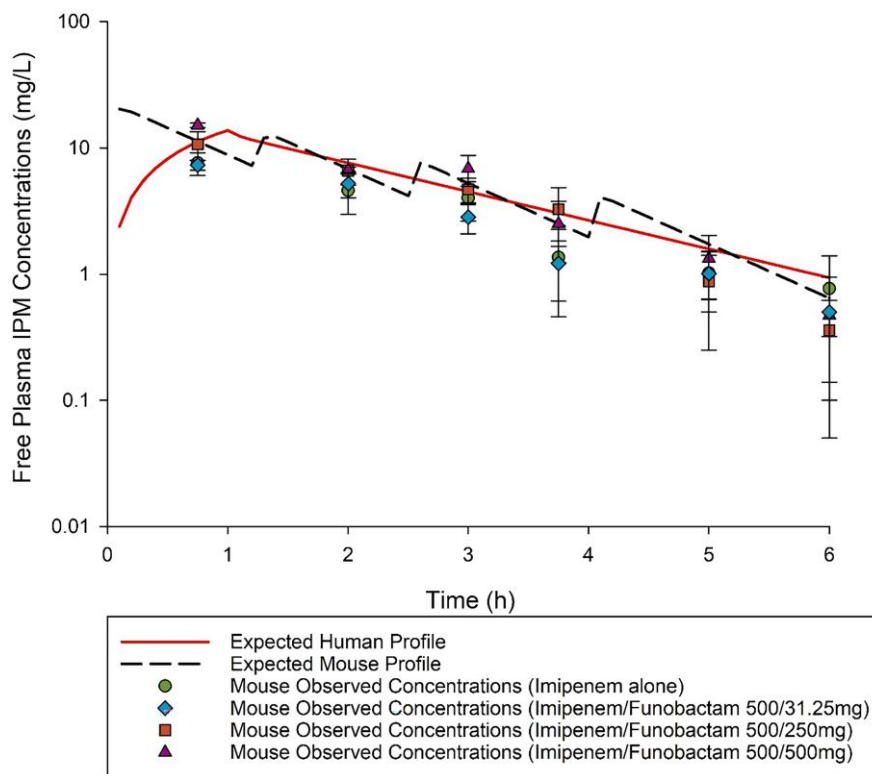


Figure 2. Imipenem (IPM) free plasma concentration–time profile after receiving imipenem human-simulated regimen alone and in combination with escalating doses of funobactam in a neutropenic thigh infection model compared with human receiving imipenem 500 mg q6h. Data are presented as means \pm SD.

0.9 mg/kg at 4 h repeated every 6 h, was similar to that expected in humans receiving 500 mg q6h 1 h infusion (Table 3). The imipenem/funobactam HSR (500/250 mg q6h 1 h infusion) reasonably approximated human exposures (Figures 1 and 2 and Table 3).

In vivo efficacy studies

Funobactam dose-fractionation

Funobactam exposures achieved with examined dosing regimens are available in Table S1. At 0 h, bacterial burden was $5.69 \pm 0.57 \log_{10}\text{cfu/thigh}$ across all three species. Adequate growth was observed in controls and imipenem HSR monotherapy groups: increase in burden of 3.51 ± 0.46 and $3.13 \pm 0.37 \log_{10}\text{cfu/thigh}$, respectively. Statistical differences in the 24 h change in $\log_{10}\text{cfu/thigh}$ were identified among some fractionations of the same total daily dose against ACB 258 and KP 827 ($P < 0.05$) (Figures S3–S5). The dosing frequency appeared to consistently impact the *in vivo* activity of funobactam, favouring the more fractionated administration. Importantly, statistical and visual trends suggested that the PD index best correlated with efficacy of funobactam was likely $\%fT > C_T$, as opposed to $\%fT > \text{MIC}$, as bactericidal activity was observed with regimens that achieved $\%fT > \text{MIC} = 0\%$ (Table S1).

Funobactam dose-ranging and HSR studies

A. baumannii. At 0 h, bacterial burden was $5.97 \pm 0.18 \log_{10}\text{cfu/thigh}$, and increased over 24 h by $2.32 \pm 0.84 \log_{10}\text{cfu/thigh}$ in untreated controls. Imipenem *in vitro* resistance was confirmed *in vivo*; bacterial burden of imipenem HSR monotherapy groups increased by $2.16 \pm 0.74 \log_{10}\text{cfu/thigh}$. All *A. baumannii* isolates reached 1-log kill upon administration of imipenem/funobactam 500/250 mg HSR, except for ACB 160, which had the highest imipenem/funobactam MIC of 16 mg/L, and five of seven isolates achieved ~ 2 -log kill (Figure 3a).

P. aeruginosa. 0 h bacterial burden was $5.64 \pm 0.41 \log_{10}\text{cfu/thigh}$, and increased at 24 h by 3.33 ± 0.62 and $2.64 \pm 1.18 \log_{10}\text{cfu/thigh}$ in sham controls and imipenem HSR monotherapy groups, respectively. All four *P. aeruginosa* isolates reached 2-log kill with imipenem/funobactam 500/250 mg HSR (Figure 3b).

K. pneumoniae. 0 h bacterial burden was $5.92 \pm 0.14 \log_{10}\text{cfu/thigh}$ and increased over 24 h by 3.15 ± 1.00 and $2.83 \pm 0.81 \log_{10}\text{cfu/thigh}$ in untreated controls and imipenem HSR monotherapy groups, respectively. All strains achieved stasis with the imipenem/funobactam 500/250 mg HSR; however, none reached 1-log kill (Figure 4). The figure also includes comparative efficacy of plasma HSRs of commercially available BL/BLIs

Table 3. Comparison of the pharmacokinetic profiles and %fT > MIC values achieved with imipenem and funobactam at each MIC in humans versus mice receiving human-simulated regimens

Regimen	Species	%fT > MIC for a MIC (mg/L) of:							fAUC ₀₋₂₄ (mg·h/L)	fC _{max} (mg/L)
		0.5	1	2	4	8	16	32		
IPM 500 mg q6h	Human ^a	100%	98%	76%	53%	25%	0%	0%	128.0	14.4
	Mouse	100%	92%	78%	55%	28%	6%	0%	146.8	20.9
Funobactam 250 mg q6h	Human ^b	100%	100%	99%	65%	25%	0%	0%	142.7	14.1
	Mouse	100%	100%	86%	57%	29%	0%	0%	142.7	13.9

IPM, imipenem.

^aHuman exposures estimated based on previously published population PK parameters in critically ill patients.¹⁴

^bHuman exposures estimated from Phase I PK study in healthy volunteers (NCT04482569).¹⁵

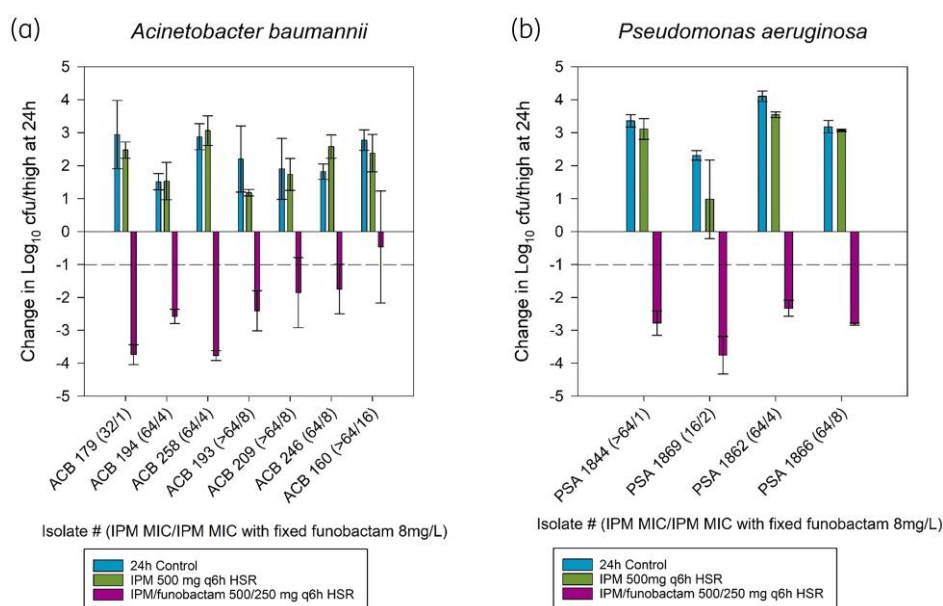


Figure 3. Comparative efficacy of the human-simulated exposure of imipenem (500 mg q6h) alone and in combination with human-simulated exposure of funobactam (250 mg q6h) against (a) seven *Acinetobacter baumannii* isolates and (b) four *Pseudomonas aeruginosa* isolates. Data are means \pm SDs. ACB, *Acinetobacter baumannii*; HSR, human-simulated regimen; IPM, imipenem; PSA, *Pseudomonas aeruginosa*.

(imipenem/relebactam, ceftazidime/avibactam and meropenem/vaborbactam) in a similar model during prior experiments.¹⁶

PK/PD analyses

Conventional PK/PD indices: fC_{max}/MIC , $fAUC/MIC$ and %fT > MIC

The relationships between funobactam fC_{max}/MIC , $fAUC/MIC$ and %fT > MIC (based on the imipenem/funobactam combination MIC) and change in bacterial burden seen in dose-ranging studies were explored for each isolate (Table 4). fC_{max}/MIC and $fAUC/MIC$ were both strong predictors of activity, whereas %fT > MIC was a poor predictor, as activity was observed at 0%fT > MIC against multiple isolates. The estimated funobactam %fT > MIC values for different efficacy endpoints were also equivalent to 0%, which disagreed with dose-ranging results demonstrating reasonable

funobactam dose-response against the majority of isolates. Given that neither fC_{max}/MIC nor %fT > MIC was a better predictor of the exposure-response relationship, $fAUC/MIC$ defaulted to the best predictive index of the conventional indices. However, and importantly, funobactam dose-fractionation studies provided evidence that imipenem/funobactam displayed time-dependent bactericidal activity *in vivo*. Whereas %fT > MIC was poorly predictive, several additional approaches were adopted to analyse alternative time-dependent funobactam exposure-response relationships.

Alternative time-dependent PK/PD indices

%fT > C_T. The %fT > C_T (at funobactam C_T ranging from 0.25 to 8 mg/L) were explored. For individual isolates, %fT > C_{T(1 mg/L)} was the best predictive threshold concentration as assessed by R² values, but was poorly predictive on the cumulative species level,

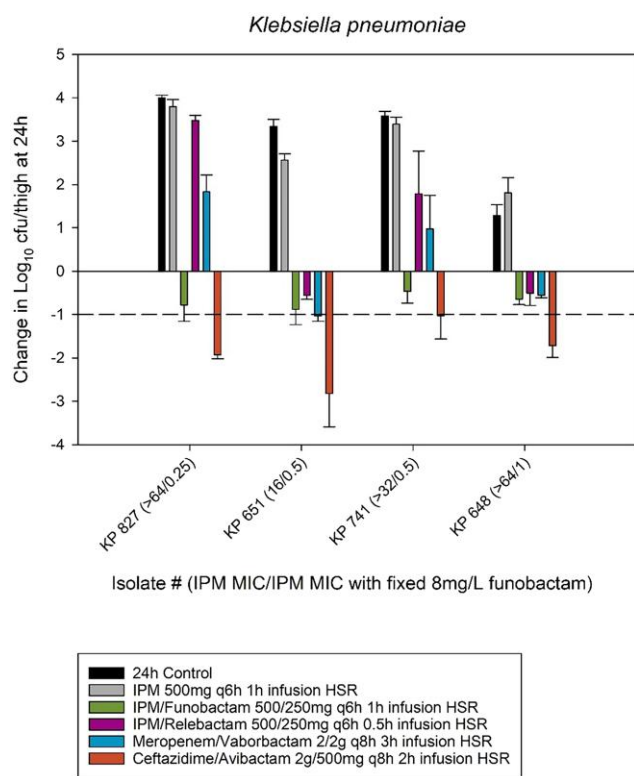


Figure 4. Comparative efficacy of the human-simulated exposure of imipenem (500 mg q6h) alone and in combination with human-simulated exposure of funobactam (250 mg q6h) against four *Klebsiella pneumoniae* isolates. The comparative efficacy of human-simulated exposures of three commercially available β -lactam/ β -lactamase inhibitors (imipenem/relebactam, ceftazidime/avibactam and meropenem/vaborbactam) against these isolates from previous unrelated experiments are also included. Data are means \pm SDs. Imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam MICs for: KP 827 (>32, >64, 1 mg/L), KP 651 (1, \leq 0.06, 1 mg/L), KP 741 (>32, 64, 1 mg/L) and KP 648 (4, 1, 2 mg/L). HSR, human-simulated regimen; IPM, imipenem; KP, *Klebsiella pneumoniae*.

particularly among the *A. baumannii* strains (Figure 5). Notably, estimated targets varied considerably within species and appeared to be higher for isolates with higher MICs compared with those with lower MICs (i.e. more resistant isolates required longer periods of time above the concentration threshold) (Table S2).

(%fT > C_T)/MIC. Given that efficacy studies collectively showed that imipenem/funobactam bactericidal activity varied by the combination MIC, with isolates of lower MIC values showing enhanced killing at lower funobactam exposures, and the fact that %fT > MIC as well as %fT > C_T offered poor predictions at the cumulative species level, a novel analysis was employed that normalizes the %fT > C_T (for C_T with the highest correlation to the efficacy data) to imipenem/funobactam combination MIC. In other words, the magnitude of time during which the inhibitor free plasma concentration remains above a threshold concentration required for *in vivo* killing would vary based on

the combination MIC. Thus, whereas %fT > C_T discounts the *in vitro* potency of the compound against the specific isolate, the novel index (%fT > C_T)/MIC allowed for a better comparison of PK/PD targets across isolates of the same species and was strongly predictive on both an individual isolate and cumulative species basis.

Across the different threshold plasma funobactam concentrations assessed, individual isolate analyses involving %fT > C_{T(1 mg/L)} had the highest correlation with efficacy. Normalizing the %fT > C_{T(1 mg/L)} index to imipenem/funobactam MIC [(%fT > C_{T(1 mg/L)})/MIC] allowed the integration of the isolate's susceptibility, which further enhanced the correlation and best described the bacterial killing (Figure 5). Funobactam PK/PD target thresholds for *in vivo* efficacy endpoints are summarized in Table 5.

The responses observed for each strain evaluated per each PK/PD index as detailed above are presented in Figures S6–S35.

Discussion

The isolates utilized in the current investigation are representative of imipenem/funobactam MIC distribution from recent surveillance of 261 clinical imipenem non-susceptible isolates from China, where imipenem/funobactam MIC₉₀ values for *A. baumannii* (n=106), *P. aeruginosa* (n=101) and *K. pneumoniae* (n=54) were 8, 32 and 2 mg/L, respectively.¹⁰ Through investigating the PD of funobactam in combination with imipenem HSR in the neutropenic thigh infection model, it was clear that funobactam co-administration markedly enhanced the *in vivo* efficacy of the latter against *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* isolates harbouring various serine carbapenemases. Dose-fractionation revealed time-dependent killing and a novel PK/PD index of (%fT > C_{T(1 mg/L)})/MIC best described the change in log₁₀cfu/thigh against clinical imipenem-non-susceptible isolates harbouring an array of serine BLs.

Traditional PK/PD indices of fAUC/MIC, fC_{max}/MIC and %fT > MIC have been used to predict antimicrobial activity and guide optimal dosing strategies for decades.¹⁷ In the case of BLIs, which often have little-to-no intrinsic antimicrobial activity, these classic indices may be insufficient to characterize activity when co-administered in a combination.¹⁸ It is well understood that there is high degree of co-linearity between these three conventional indices. Thus, well-designed dose-fractionation studies are required to deparameterize and pull apart the indices to appreciate a meaningful effect of one over the others, suggesting that it is more difficult to conclude that an index other than fAUC/MIC is best predictive of activity. Indeed, an additional fractionation of every 4 h was necessary against the *A. baumannii* isolate to empirically demonstrate time-dependent activity. Importantly, and often overlooked, fractionating the BLI ought to be done in the presence of humanized, or at least clinically relevant, fixed BL backbone exposures. Although funobactam fAUC/MIC was a strong predictor of change in cfu/thigh for this dataset, it could not be ignored that overall, dosing frequency appeared to consistently impact *in vivo* activity of funobactam across all three species, favouring the more fractionated administration. However, across numerous isolates and species, activity was observed at 0% fT > MIC, thus making this traditional index a poor descriptor. Alternatively, %fT > C_T was a more accepted model of describing time-dependent antibacterial killing and is the currently welcomed index of the non- β -lactam BLI avibactam, when

Table 4. Comparison of the funobactam stasis, 1-log kill, and 2-log kill exposure targets for the three traditional pharmacodynamic indices and goodness-of-fit (R^2) across all tested isolates in the presence of imipenem human-simulated plasma exposures in dose-ranging studies utilizing the murine neutropenic thigh infection model

Isolate (MIC, mg/L)	Enzyme(s)	Funobactam required to achieve:											
		%fT > MIC				fAUC ₀₋₂₄ /MIC				fC _{max} /MIC			
		Stasis	1-Log reduction	2-Log reduction	R ²	Stasis	1-Log reduction	2-Log reduction	R ²	Stasis	1-Log reduction	2-Log reduction	R ²
<i>Acinetobacter baumannii</i>													
ACB 179 (1)	ADC-25, OXA-23, OXA-223	12.60	14.05	15.81	0.99	8.65	9.51	10.54	0.99	0.91	0.99	1.09	0.99
ACB 194 (4)	ADC-25, OXA-23, OXA-82	0.00	1.03	12.64	0.24	2.77	3.75	5.84	0.79	0.25	0.35	0.58	0.79
ACB 258 (4)	ADC-222, OXA-23, OXA-95	3.71	7.75	15.20	0.87	8.75	11.89	16.41	0.91	0.85	1.16	1.60	0.91
ACB 193 (8)	OXA-23, OXA-82	0.02	0.97	2.13	0.21	0.47	0.93	2.77	0.93	0.05	0.09	0.27	0.93
ACB 209 (8)	OXA-65, OXA-24	1.60	7.88	32.73	0.66	6.24	11.71	24.07	0.79	0.62	1.14	2.36	0.79
ACB 246 (8)	ADC-33, OXA-23, OXA-82	0.00	0.00	N/A	0.27	5.24	5.93	N/A	0.81	0.51	0.58	N/A	0.81
ACB 160 (16)	OXA-24, OXA-65, TEM-1B	0.19	1.33	N/A	0.38	6.49	13.28	N/A	0.75	0.63	1.30	N/A	0.75
Mean		2.59	4.71	15.70	0.52	5.51	8.14	11.92	0.85	0.54	0.80	1.18	0.85
Median		0.19	1.33	15.20	0.38	6.24	9.51	10.54	0.81	0.62	0.99	1.09	0.81
Composite		0.31	4.14	20.04	0.54	4.38	8.15	16.22	0.73	0.43	0.79	1.58	0.73
<i>Pseudomonas aeruginosa</i>													
PSA 1844 (1)	KPC-2, PDC-42	31.55	37.16	46.16	0.88	19.42	22.91	28.62	0.88	1.89	2.24	2.79	0.88
PSA 1869 (2)	GES-19, GES-26	0.00	1.08	5.37	0.74	2.74	6.49	12.78	0.89	0.27	0.64	1.24	0.89
PSA 1862 (4)	GES-19, GES-20	0.00	0.00	0.00	0.25	3.72	4.08	4.89	0.97	0.36	0.39	0.47	0.97
PSA 1866 (8)	GES-20	0.00	0.00	0.00	0.53	4.54	5.63	7.68	0.97	0.45	0.55	0.75	0.97
Mean		7.89	9.56	12.88	0.60	7.60	9.78	13.49	0.93	0.74	0.95	1.31	0.93
Median		0.00	0.54	2.69	0.64	4.13	6.06	10.23	0.93	0.40	0.59	1.00	0.93
Composite		10.55	21.84	38.38	0.49	4.03	6.48	12.10	0.77	0.39	0.60	1.09	0.77
<i>Klebsiella pneumoniae</i>													
KP 827 (0.25)	OXA-48, CTX-M-15, OXA-1, SHV-11, TEM-1B	93.86	N/A	N/A	0.89	82.76	N/A	N/A	0.89	8.08	N/A	N/A	0.89
KP 651 (0.5)	KPC-2, CDC-120	17.38	N/A	N/A	0.93	19.90	N/A	N/A	0.95	1.94	N/A	N/A	0.95
KP 741 (0.5)	SHV-11, CTX-M-55, OXA-48	66.55	N/A	N/A	0.98	49.88	N/A	N/A	0.98	4.91	N/A	N/A	0.98
KP 648 (1)	KPC-3, CDC-113	13.20	N/A	N/A	0.96	8.26	N/A	N/A	0.96	0.84	N/A	N/A	0.96
Mean		47.75	N/A	N/A	0.94	40.20	N/A	N/A	0.95	3.94	N/A	N/A	0.95
Median		41.96	N/A	N/A	0.94	34.89	N/A	N/A	0.96	3.42	N/A	N/A	0.96
Composite		28.81	N/A	N/A	0.80	21.50	N/A	N/A	0.81	1.53	N/A	N/A	0.81

N/A, target not achieved based on the curve of best-fit.

co-administered with ceftazidime.¹⁹ Upon investigating %fT > C_T at thresholds from 0.25 to 8 mg/L in doubling dilutions, a free concentration of 1 mg/L funobactam with imipenem HSR was best predictive of change in log₁₀cfu/thigh on an individual isolate basis. Importantly, the estimated %fT > C_T targets varied considerably within species, with notable discrepancies by MIC values—they appeared to be greater for isolates with higher MICs compared with those with lower MICs. Previous investigations of another BL/BLI, ceftolozane/tazobactam, similarly found that in four BL-producing *Escherichia coli* strains, tazobactam %fT > C_T satisfactorily described the change in log₁₀cfu for individual isolates (R^2 range = 0.90–0.99), but did not co-model well.²⁰ However, a translational relationship that did allow for the co-modelling ($R^2 = 0.90$) was assigning an isolate-specific C_T equal

to the product of 0.5 and the MIC. Indeed, an inherent limitation of %fT > C_T is that, by definition, it does not consider the susceptibility (i.e. MIC) of the isolates. However, the application of this approach to our dataset yielded stasis or net bacterial killing at 0% fT > C_{T(0.5*MIC)} in multiple isolates (data not shown). Other novel PK/PD indices have been introduced recently—notably AUC: MIC * 1/τ.²¹ Reportedly, this index has utility for compounds with especially short half-lives in animals to gain better certainty around human dose predictions.²² Funobactam did not display a short half-life in our model with uranyl nitrate and therefore this PK/PD index was not investigated.

In this study, we explored an alternative approach using MIC as a correction factor applied to the best predictive %fT > C_T of 1 mg/L, producing the novel PK/PD index of (%fT > C_{T(1mg/L)l})/

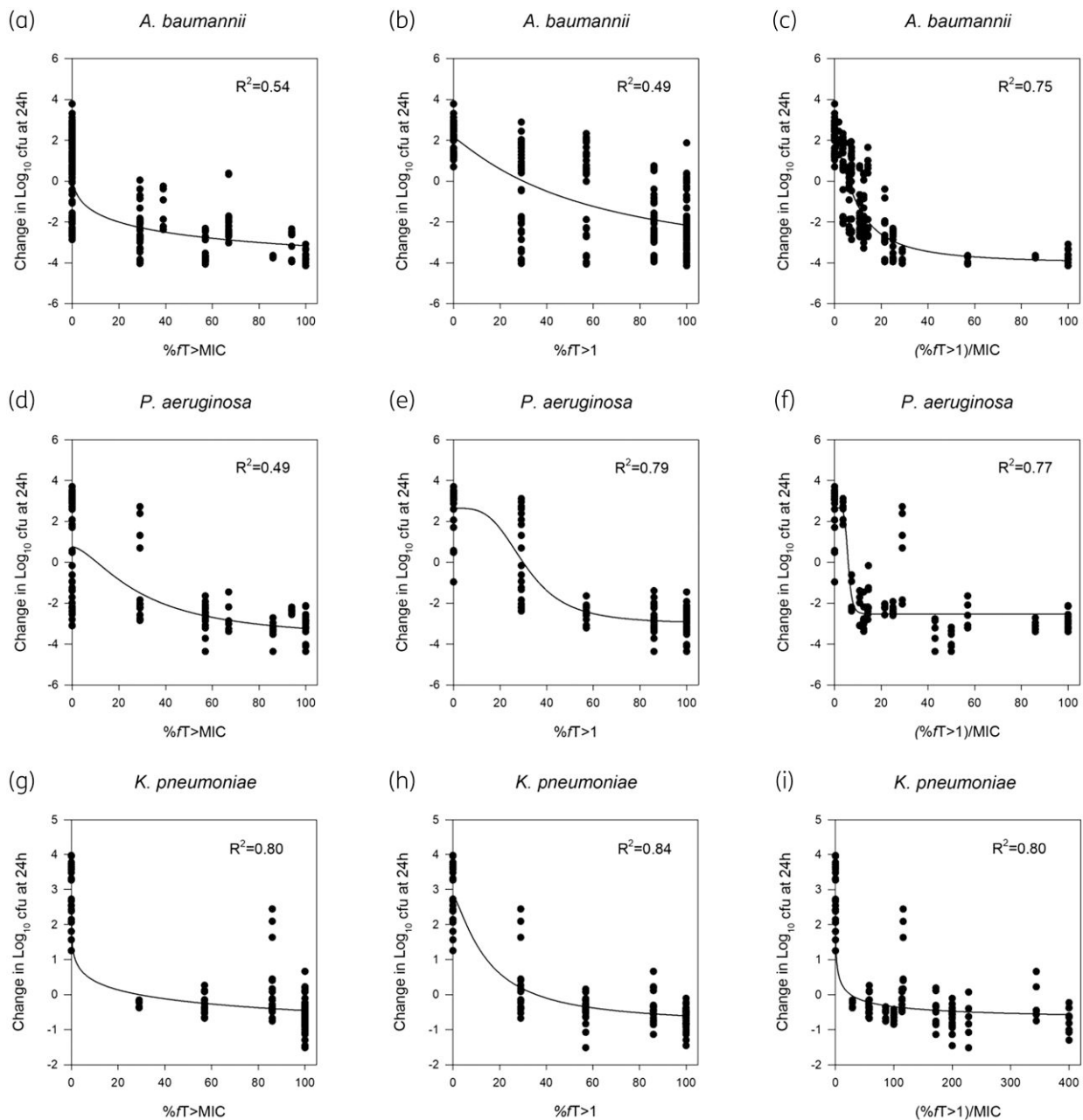


Figure 5. Comparison of the 24 h change in cfu/thigh versus funobactam exposure using different time-dependent PK/PD indices in serine carbapenemase-producing *Acinetobacter baumannii* ($n=7$) (a–c), *Pseudomonas aeruginosa* ($n=4$) (d–f) and *Klebsiella pneumoniae* ($n=4$) (g–i) isolates after receiving escalating doses of funobactam in combination with an imipenem human-simulated regimen of 500 mg q6h in the neutropenic murine thigh infection model.

MIC. Although we attempted to include isolates with sufficient diversity so that the finding from our study would better forecast for the target isolate populations, whether C_T of 1 mg/L will provide good correlation for strains and species beyond those assessed in our investigation cannot be ascertained. Accounting for the *in vitro* potency parameter, i.e. the MIC of the organism, yielded comparable goodness-of-fit for individual isolates, but markedly improved the predictive ability of the cumulative

analyses. This MIC correction factor makes intuitive sense and suggests that isolates with higher MICs require more time above the threshold concentration than those with lower MICs to achieve similar efficacy endpoints.

The magnitude of killing achieved with imipenem/funobactam 500/250 mg q6h HSR was profound against the non-fermenters: 24 h change in \log_{10} cfu/thigh ranged from -0.46 ± 1.69 to -3.77 ± 0.15 , and -2.33 ± 0.25 to -3.76 ± 0.57 among

Table 5. Comparison of the funobactam (%fT > C_T)/MIC (based on the MICs of imipenem in combination at a fixed funobactam concentration of 8 mg/L, and a C_T of 1 mg/L) stasis, 1-log kill and 2-log kill pharmacodynamic targets and goodness-of-fit (R²) across all tested isolates in the presence of imipenem HSR in dose-ranging studies utilizing the murine neutropenic thigh infection model

Isolate (MIC, mg/L)	Enzyme(s)	Funobactam (%fT > C _{T[1 mg/L])/MIC required to achieve:}			
		Stasis	1-Log reduction	2-Log reduction	R ²
<i>Acinetobacter baumannii</i>					
ACB 179 (1)	ADC-25, OXA-23, OXA-223	12.60	14.05	15.81	0.99
ACB 194 (4)	ADC-25, OXA-23, OXA-82	4.52	6.09	9.31	0.79
ACB 258 (4)	ADC-222, OXA-23, OXA-95	15.06	17.45	19.92	0.90
ACB 193 (8)	OXA-23, OXA-82	0.62	1.41	4.62	0.93
ACB 209 (8)	OXA-65, OXA-24	9.46	11.00	12.47	0.76
ACB 246 (8)	ADC-33, OXA-23, OXA-82	7.11	9.82	N/A	0.75
ACB 160 (16)	OXA-24, OXA-65, TEM-1B	6.14	6.38	N/A	0.65
Mean		7.93	9.46	12.42	0.82
Median		7.11	9.82	12.47	0.79
Composite		5.84	9.17	14.48	0.75
<i>Pseudomonas aeruginosa</i>					
PSA 1844 (1)	KPC-2, PDC-42	31.55	37.16	46.16	0.88
PSA 1869 (2)	GES-19, GES-26	5.54	11.51	19.40	0.88
PSA 1862 (4)	GES-19, GES-20	5.95	6.56	7.91	0.97
PSA 1866 (8)	GES-20	6.98	8.29	10.10	0.97
Mean		12.50	15.88	20.89	0.93
Median		6.46	9.90	14.75	0.92
Composite		5.81	6.55	7.90	0.77
<i>Klebsiella pneumoniae</i>					
KP 827 (0.25)	OXA-48, CTX-M-15, OXA-1, SHV-11, TEM-1B	134.52	N/A	N/A	0.89
KP 651 (0.5)	KPC-2, CDC-120	33.85	N/A	N/A	0.95
KP 741 (0.5)	SHV-11, CTX-M-55, OXA-48	77.60	N/A	N/A	0.98
KP 648 (1)	KPC-3, CDC-113	13.20	N/A	N/A	0.96
Mean		64.80	N/A	N/A	0.95
Median		55.73	N/A	N/A	0.96
Composite		26.07	N/A	N/A	0.80

N/A, target not achieved based on the curve of best-fit.

A. baumannii and *P. aeruginosa* isolates, respectively. In preclinical models, stasis has been linked to clinical efficacy for lower inoculum infection such as intra-abdominal or those involving the urinary tract, whereas a 1-log reduction has correlated similarly for higher inoculum infections including pneumonia, endocarditis or bacteraemia.²³ Going further, a 2-log reduction may be beneficial for optimized time-to-response or prevention of resistance development.²³ The bacterial killing against the four *K. pneumoniae* strains was lesser (-0.47 ± 0.28 to -0.88 ± 0.35), despite lower imipenem/funobactam MICs. However, these *K. pneumoniae* isolates were selected in part due to available comparative data with commercial BL/BLIs. For the KPC-producing *K. pneumoniae* isolates, imipenem/funobactam 500/250 mg q6h HSR achieved cfu reductions comparable to those seen previously with HSRs of imipenem/relebactam and meropenem/vaborbactam in the same model.¹⁶ The ceftazidime/avibactam HSR on the other hand provided greater cfu reductions compared with all the examined regimens. Imipenem/funobactam and ceftazidime/avibactam HSRs both led to net bacterial reduction against the OXA-48-harboring *K. pneumoniae* strains, whereas growth

was observed on imipenem/relebactam and meropenem/vaborbactam HSRs as expected due to lack of inhibition of enzyme-mediated hydrolysis with these latter compounds.¹⁶ Although the enzyme-mediated resistance mechanisms have been well characterized for these isolates, there may be additional underlying mechanisms of resistance at play, such as porin loss or alterations in efflux pump expression. Further experimentation with additional *K. pneumoniae* isolates and other Enterobacterales spp. is warranted.

In conclusion, the therapeutic armamentarium against CRAB is limited and represents a serious unmet medical need on the global scale.⁶ Imipenem/cilastatin/funobactam stands to bolster the sparse options available for treatment due to funobactam's broad inhibition of serine carbapenemases, including OXA-23 and OXA-24 enzymes, providing an advantage over commercially available BL/BLIs. Imipenem/funobactam combination (500/250 mg q6h as 1 h infusion) showed potent *in vivo* efficacy against serine carbapenemase-producing *A. baumannii* and *P. aeruginosa*, and bacterial reduction comparable to commercially available BL/BLIs against *K. pneumoniae* isolates, in the

neutropenic thigh infection model. These data support the consideration of this combination and the dosage for the treatment of serious infections due to these organisms in Phase III trials.

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Transparency declarations

X.L., X.C. and Y.W. are employed by Evopoint Biosciences Co. K.A. has received research funding from the study sponsor. A.J.F., A.V.B. and D.P.N. have none to declare.

Supplementary data

Figures [S1 to S35](#) and Tables [S1 and S2](#) are available as [Supplementary data](#) at JAC Online.

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