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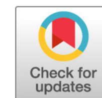

Efficacy of Human-Simulated Epithelial Lining Fluid Exposure of Meropenem-Nacubactam Combination against Class A Serine β-Lactamase-Producing *Enterobacteriaceae* in the Neutropenic Murine Lung Infection Model

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ABSTRACT Nacubactam is a novel, broad-spectrum, β-lactamase inhibitor that is currently under development as combination therapy with meropenem. This study evaluated the efficacy of human-simulated epithelial lining fluid (ELF) exposures of meropenem, nacubactam, and the combination of meropenem and nacubactam against class A serine carbapenemase-producing *Enterobacteriaceae* isolates in the neutropenic murine lung infection model. Twelve clinical meropenem-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* isolates, all harboring KPC or IMI-type β-lactamases, were utilized in the study. Meropenem, nacubactam, and meropenem-nacubactam (1:1) combination MICs were determined in triplicate via broth microdilution. At 2 h after intranasal inoculation, neutropenic mice were dosed with regimens that provided ELF profiles mimicking those observed in humans given meropenem at 2 g every 8 h and/or nacubactam at 2 g every 8 h (1.5-h infusions), alone or in combination. Efficacy was assessed as the change in bacterial growth at 24 h, compared with 0-h controls. Meropenem, nacubactam, and meropenem-nacubactam MICs were 8 to >64 μg/ml, 2 to >256 μg/ml, and 0.5 to 4 μg/ml, respectively. The average bacterial density at 0 h across all isolates was 6.31 ± 0.26 $\log_{10}$ CFU/lung. Relative to the 0-h control, the mean values of bacterial growth at 24 h in the untreated control, meropenem human-simulated regimen treatment, and nacubactam human-simulated regimen treatment groups were 2.91 ± 0.27, 2.68 ± 0.42, and 1.73 ± 0.75 $\log_{10}$ CFU/lung, respectively. The meropenem-nacubactam combination human-simulated regimen resulted in reductions of -1.50 ± 0.59 $\log_{10}$ CFU/lung. Meropenem-nacubactam human-simulated ELF exposure produced enhanced efficacy against all class A serine carbapenemase-producing *Enterobacteriaceae* isolates tested in the neutropenic murine lung infection model.

KEYWORDS β-lactam, β-lactamase inhibitor, Gram negative, OP0595, RG6080, carbapenemase, lung epithelial lining fluid, nacubactam

Enterobacteriaceae is a family of Gram-negative bacteria that is implicated in various infections, including nosocomial pneumonia. Respiratory tract infections caused by drug-resistant *Enterobacteriaceae* strains are difficult to treat and a major public health burden (1–3). Resistance to β-lactams among *Enterobacteriaceae* strains occurs primarily through production of β-lactamases, with carbapenemases representing the most challenging β-lactamase family owing to their ability to hydrolyze almost all β-lactams (4). Furthermore, the worldwide emergence of Ambler class A serine carbapenemases,

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TABLE 1 Phenotypic and β-lactamase profiles of the *K. pneumoniae*, *E. coli*, and *E. cloacae* isolates utilized in *in vivo* efficacy studies

Isolate			MIC (μg/ml) for:		
Identification	**Bacterial species**	**β-Lactamase(s) encoded**	**Meropenem**	**Nacubactam**	**Meropenem-nacubactam[a]**
EC 548[b]	*E. coli*	KPC-3, TEM-1	8	2	0.5
KP 652[b]	*K. pneumoniae*	KPC-3	64	>256	1
ECL 72	*E. cloacae*	AmpC, KPC-3, TEM-1	32	>256	1
KP 651[b]	*K. pneumoniae*	KPC-2	64	>256	2
KP 599[c]	*K. pneumoniae*	KPC-2, SHV-11	>64	64	2
KP 604[c]	*K. pneumoniae*	KPC-3, TEM-1, SHV-11	>64	2	2
KP C4-10	*K. pneumoniae*	CTX-M-15, SHV-11, TEM-1, OXA-9, KPC-3	>64	>256	2
KP C8-9	*K. pneumoniae*	SHV-12, TEM-1, KPC-2	>64	>256	2
ECL 119[b]	*E. cloacae*	NMC-A	32	128	2
KP C30-27	*K. pneumoniae*	SHV-11, TEM-1, KPC-2	>64	>256	4
ECL 118[b]	*E. cloacae*	NMC-A	64	>256	4
KP 648[b]	*K. pneumoniae*	KPC-3	>64	>256	4

[a]Meropenem/nacubactam concentration ratio of 1:1.
[b]Clinical isolates obtained from FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA).
[c]Clinical isolates obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

particularly *Klebsiella pneumoniae* carbapenemases (KPCs), is a cause of concern, given the limited selection of treatment options for carbapenemase-producing *Enterobacteriaceae* infections (5–8).

Nacubactam is a novel, non-β-lactam, diazabicyclooctane, β-lactamase inhibitor with *in vitro* activity against class A β-lactamases such as KPC, class C, and some class D β-lactamases, and it can thus restore the activity of β-lactam antibiotics against β-lactamase-producing organisms. In addition to β-lactamase inhibition, nacubactam possesses the following mechanisms: (i) intrinsic antimicrobial activity against *Enterobacteriaceae* via penicillin-binding protein 2 (PBP2) inhibition and (ii) synergy with various β-lactam agents (enhancer effect) (9–13). Nacubactam is being developed as a combination therapy with meropenem for the treatment of serious Gram-negative bacterial infections, including lung infections.

The purpose of this study was to evaluate the efficacy of human-simulated epithelial lining fluid (ELF) exposures of meropenem, nacubactam, and a meropenem-nacubactam combination against class A serine carbapenemase-producing *Enterobacteriaceae* strains in a murine neutropenic lung infection model. Assessing drug concentrations at the site of action is advisable for investigational agents (14, 15), as it allows the simulation of the observed human drug profiles in animal models and thus yields insights into antimicrobial efficacy at clinically relevant exposures. (This study was presented in part at IDWeek 2018, San Francisco, CA, 3 to 6 October 2018 [16].)

RESULTS

***In vitro* susceptibility studies.** All 12 *Enterobacteriaceae* isolates utilized in the study demonstrated *in vitro* resistance to meropenem using Clinical and Laboratory Standards Institute (CLSI) breakpoints (17). Meropenem and nacubactam MICs ranged from 8 to >64 μg/ml and from 2 to >256 μg/ml, respectively. The MICs of meropenem-nacubactam (concentration ratio of 1:1) varied from 0.5 to 4 μg/ml. The MICs of meropenem, nacubactam, and meropenem-nacubactam, as well as the β-lactamase profiles of the 12 isolates examined, are shown in Table 1.

Lung ELF drug exposure studies. Meropenem and nacubactam were detected in mouse bronchoalveolar lavage (BAL) fluid after subcutaneous administration of two different single doses of each agent (meropenem, 50 mg/kg and 100 mg/kg; nacubactam, 10 mg/kg and 20 mg/kg). The ELF pharmacokinetics of meropenem and nacubactam single doses were well characterized using a one-compartment model; the best-fit pharmacokinetic parameters for nacubactam in the neutropenic lung infection model were as follows: volume of distribution (V) (conditioned on the unknown bioavailability in ELF), 0.42 liters/kg; rate constant for input into ELF (k_{01}), 40.64 h^{-1}; rate constant for elimination from ELF (k_{10}), 0.50 h^{-1}. The ELF profile of meropenem was utilized to develop a murine meropenem monotherapy regimen consisting of 6 doses during each

TABLE 2 Meropenem and nacubactam %$T >$ ELF concentration values estimated in humans and in mice

Drug and species	%$T >$ ELF concentration of:								AUC_{0-24} (μg·h/ml)
	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml	8 μg/ml	16 μg/ml	32 μg/ml	64 μg/ml	
Meropenem									
Human (2 g every 8 h, 1.5-h infusion)	94.58	78.33	60.83	42.08	20.83	0.00	0.00	0.00	104
Mouse[a]	92.50	81.25	61.25	40.00	21.25	5.83	0.00	0.00	121
Mouse[b]	88.75	75.83	55.00	33.75	18.83	0.00	0.00	0.00	94
Nacubactam									
Human (2 g every 8 h, 1.5-h infusion)	100.00	100.00	100.00	80.42	55.42	26.67	0.00	0.00	265
Mouse[c]	100.00	100.00	88.75	72.08	53.33	16.67	0.00	0.00	220
Mouse[d]	100.00	99.58	86.25	73.75	55.00	19.58	0.00	0.00	240

[a]Meropenem human-simulated regimen administered alone.
[b]Meropenem human-simulated regimen coadministered with nacubactam human-simulated regimen.
[c]Nacubactam human-simulated regimen administered alone.
[d]Nacubactam human-simulated regimen coadministered with meropenem human-simulated regimen.

8-h dosing interval (i.e., a total of 18 doses for the 24-h study duration), as follows: 0 h, 15 mg/kg; 1.5 h, 19 mg/kg; 2.75 h, 19 mg/kg; 4 h, 17 mg/kg; 5.5 h, 9 mg/kg; 7.25 h, 5 mg/kg (repeated every 8 h); this provided ELF exposure similar to that achieved in humans following a dose of meropenem of 2 g every 8 h, as a 1.5-h infusion. Likewise, a murine nacubactam monotherapy regimen that provided ELF exposure similar to that achieved in humans following a dose of nacubactam of 2 g every 8 h, as a 1.5-h infusion, consisted of 3 doses during each 8-h dosing interval, as follows: 0 h, 9 mg/kg; 1.5 h, 9 mg/kg; 4 h, 1.25 mg/kg (repeated every 8 h). Confirmatory pharmacokinetic studies showed that the selected murine regimens simulated the exposures in humans on the basis of the percentage of the dosing interval during which the ELF drug concentration exceeded the ELF concentration threshold (%$T >$ ELF) for a concentration range of 0.5 to 64 μg/ml, as well as the ELF area under the concentration-time curve (AUC) profile (Table 2). Figure 1 depicts the confirmatory murine ELF pharmacokinetic profiles of the nacubactam human-simulated regimen and the meropenem human-simulated regimen, compared with the respective human ELF profiles.

When the meropenem and nacubactam murine human-simulated regimens were administered concomitantly, the meropenem exposure achieved in mice was unaltered, as shown in Fig. 1. However, the nacubactam elimination from ELF was enhanced and the ELF exposure was observed to be slightly reduced, which necessitated an increase in the nacubactam dose for animals receiving the combination in order to attain the target human ELF exposure. As a result, the nacubactam human-simulated regimen for combination treatment with meropenem was as follows: 0 h, 9 mg/kg; 1.5 h, 9 mg/kg; 4 h, 3 mg/kg (during each 8-h dosing interval). Following dose adjustment, the target nacubactam exposure upon meropenem coadministration was confirmed, as shown in Fig. 1. The mechanism of the pharmacokinetic interaction between meropenem and nacubactam was not studied in this investigation.

***In vivo* efficacy studies.** In this lung infection model, 0-h control mice displayed an overall growth value of 6.31 $\pm$ 0.26 $\log_{10}$ CFU/lung (mean $\pm$ standard deviation) across all isolates examined, which increased by an average of 2.91 $\pm$ 0.27 $\log_{10}$ CFU/lung in untreated mice after 24 h. Relative to the 0-h control, the mean values for bacterial growth at 24 h in the meropenem monotherapy and nacubactam monotherapy treatment groups were 2.68 $\pm$ 0.42 and 1.73 $\pm$ 0.75 $\log_{10}$ CFU/lung, respectively. In comparison to meropenem monotherapy, nacubactam monotherapy resulted in a lower bacterial burden ($P < 0.001$) for all isolates studied. The combination of meropenem and nacubactam resulted in bacterial reductions ranging from -0.73 ± 0.26 to -2.16 ± 0.21 $\log_{10}$ CFU/lung, with 10 of the 12 isolates studied achieving >1-$\log_{10}$ CFU/lung reduction at 24 h. The results of the bacterial density studies for each isolate are depicted in Fig. 2.

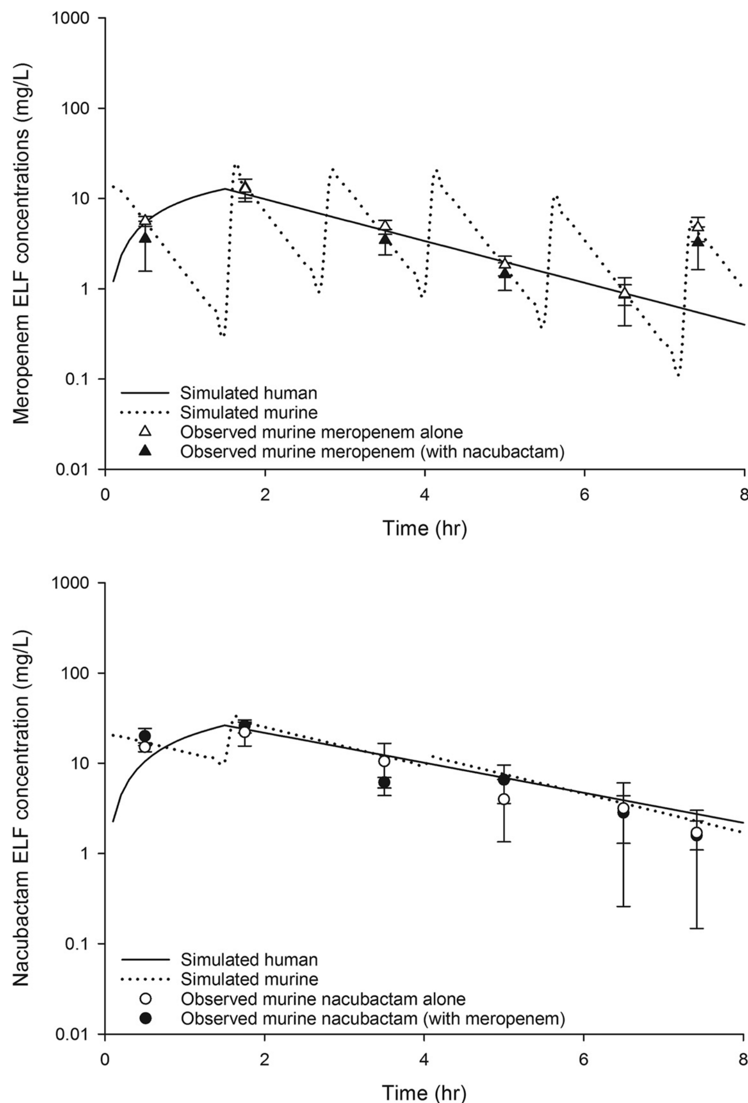

FIG 1 Observed meropenem (top) and nacubactam (bottom) ELF concentrations in the neutropenic lung infection model, compared with human ELF profiles of meropenem (2 g every 8 h, as 1.5-h infusion) and nacubactam (2 g every 8 h, as 1.5-h infusion). Data are presented as mean ± standard deviation.

DISCUSSION

The Centers for Disease Control and Prevention estimates that more than 9,000 health care-associated infections each year are caused by carbapenemase-resistant *Enterobacteriaceae* (CRE), resulting in approximately 600 deaths (18). Currently, there are limited therapeutic options available for patients with CRE infections. Given that the predominant antimicrobial resistance mechanism among *Enterobacteriaceae* strains is β-lactamase production, the combination of a β-lactamase inhibitor with activity against carbapenemases and an existing broad-spectrum β-lactam agent is an attractive therapeutic strategy. Nacubactam, a novel β-lactamase inhibitor under clinical development, demonstrates a potent spectrum of *in vitro* activity against CRE in combination with meropenem (9, 11, 12). *In vivo*, antimicrobial activity requires the achievement of sufficient drug levels at the site of infection. For pulmonary infections, assessment of ELF drug concentrations enables more robust predictions of the exposure-response relationships, compared with plasma concentrations (19–24), but oftentimes data on the drug exposures at these critical sites are limited. In the present study, the availability of data on the meropenem-nacubactam bronchopulmonary

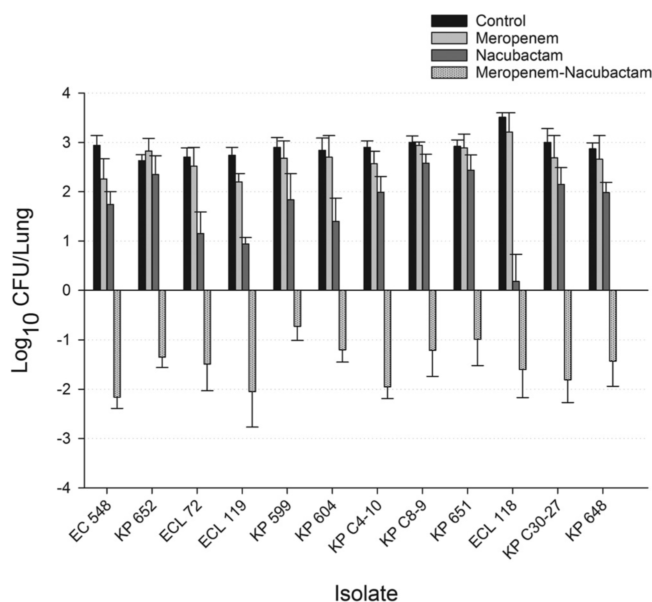

FIG 2 Changes in bacterial growth (mean ± SD) at 24 h, relative to 0-h controls, with meropenem, nacubactam, and meropenem-nacubactam against *Enterobacteriaceae* strains harboring class A serine carbapenemases.

pharmacokinetics in healthy adults provided the ability to evaluate the efficacy of the combination using the human-simulated ELF exposures in a murine lung infection model, which improves the translation application of the outcomes from this study to the clinic.

Meropenem-nacubactam MICs, determined using a 1:1 methodology, were 16- to 256-fold lower than meropenem MICs against CRE isolates in this study. These findings are supported by a similar result from an *in vitro* assessment by Morrisey et al., demonstrating that nacubactam in combination with meropenem had better *in vitro* activity against extended-spectrum β-lactamase (ESBL)-, class B-, class C-, class D-, and KPC-producing *Enterobacteriaceae* strains, compared with meropenem alone (11).

Focusing on class A carbapenemases, particularly KPC, the current study demonstrated enhanced *in vivo* activity, i.e., >1-log-unit bacterial density reductions, in 10 of 12 isolates with human-simulated ELF exposures of the meropenem-nacubactam combination, compared with either agent alone, in a lung infection model. The observation of nacubactam monotherapy resulting in a significantly lower bacterial burden, relative to meropenem monotherapy, among the *Enterobacteriaceae* isolates studied is attributed to the intrinsic antimicrobial activity of nacubactam mediated via PBP2 inhibition, as observed in a PBP-binding assay study (12).

Previously reported *in vitro* experiments demonstrated the activity of nacubactam in combination with either piperacillin, cefepime, or meropenem against a variety of β-lactamases, including CTX-M-15- and KPC-expressing *Enterobacteriaceae* strains (24, 25). Additionally, the activity of nacubactam (previously OP0595) in combination with cefepime, as the β-lactam backbone, against CTX-M-15-positive *E. coli* ($n = 2$) and KPC-positive *K. pneumonia* ($n = 2$) isolates was evaluated by Morinaka et al. in a thigh infection model (25). Treatment with either cefepime alone or nacubactam alone did not decrease the bacterial density in the murine thigh; however, combination treatment with cefepime and nacubactam decreased the bacterial density by 3 to 4 $\log_{10}$ CFU/thigh, relative to the untreated 24-h control (25). In comparison, human-simulated ELF exposures of meropenem-nacubactam in the current study resulted in reductions in the bacterial burden of 4 to 5 $\log_{10}$ CFU/lung, relative to the untreated 24-h control. Against contemporary clinical *Enterobacteriaceae* isolates ($n = 317$), meropenem and meropenem-nacubactam MIC_{90} values were 8 mg/liter and 0.25 mg/liter, respectively,

while cefepime and cefepime-nacubactam MIC_{90} values were >64 mg/liter and 0.5 mg/liter, respectively (26). In addition, our observations are in agreement with the recent findings of Monogue and colleagues (27). Utilizing a murine urinary tract infection model, the authors demonstrated that human-simulated meropenem-nacubactam plasma exposure had potent activity against meropenem- and ceftazidime-avibactam-resistant *Enterobacteriaceae* strains. Isolates in that study harbored a range of β-lactamases, including ESBL, KPC, OXA, and NDM enzymes (27).

The need for new antimicrobial agents has contributed to renewed interest in β-lactam/β-lactamase inhibitor combinations. The recently approved agents ceftazidime-avibactam and meropenem-vaborbactam both have activity against class A (i.e., ESBL and KPC) and class C β-lactamases (10, 13, 28). However, recent real-world reports of resistance developing with ceftazidime-avibactam therapy are concerning (29). In contrast to avibactam and vaborbactam, nacubactam possesses a dual mechanism of action in addition to a synergistic effect in combination with β-lactams (12, 13). Future studies are needed to compare the activity of meropenem-nacubactam to those of ceftazidime-avibactam and meropenem-vaborbactam and to examine whether meropenem-nacubactam offers a potential alternative to ceftazidime-avibactam for resistant strains.

In conclusion, this study demonstrated that, in lung-infected mice administered a meropenem-nacubactam regimen that achieved exposures in pulmonary ELF comparable to those observed in humans following the currently examined clinical doses of the combination, the regimen resulted in enhanced efficacy against a variety of clinical isolates harboring KPC-2, KPC-3, or IMI-type β-lactamases. These translational data support the potential role of nacubactam in combination with meropenem for treatment of human lung infections due to class A carbapenemase-producing *Enterobacteriaceae* strains, and further studies are warranted.

MATERIALS AND METHODS

Antimicrobial test agent. Analytical grade nacubactam (batch no. R07079901-001-009; Roche Laboratories, Basel, Switzerland) was used for all *in vitro* and *in vivo* testing. Analytical grade meropenem (lot no. M0608A; Tecoland Corp., Irvine, CA) and commercially available meropenem in 1-g vials (lot no. 0017D61; Fresenius Kabi USA) were utilized for *in vitro* and *in vivo* testing, respectively. Commercial vials of meropenem were reconstituted as described in the prescribing information, with dilution in sterile normal saline (Hospira, Inc., Lake Forest, IL) as appropriate to achieve the desired concentrations.

Bacterial isolates. Eight *Klebsiella pneumoniae*, 1 *Escherichia coli*, and 3 *Enterobacter cloacae* clinical isolates were utilized in the studies. Of these 12 *Enterobacteriaceae* isolates, 6 isolates were obtained from the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA), 2 isolates were obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland), and the remaining 4 isolates were from the Center for Anti-Infective Research and Development isolate repository. All isolates were maintained in skim milk (BD Biosciences, Sparks, MD) at −80°C. Each isolate was subcultured twice on Trypticase soy agar with 5% sheep blood (BD Biosciences) and grown for 18 to 20 h at 37°C under 5% CO_2 prior to use in the experiments.

Susceptibility testing. The MICs of meropenem, nacubactam, and meropenem-nacubactam were determined for all isolates using the broth microdilution methodology outlined by the CLSI (17). For meropenem-nacubactam MICs, doubling dilutions of meropenem and nacubactam were utilized at a 1:1 concentration ratio. MIC values were determined in triplicate, and the modal MIC was reported.

Neutropenic lung infection model. Pathogen-free, female ICR mice (20 to 22 g) were obtained from Envigo RMS, Inc. (Indianapolis, IN). Animals were provided food and water *ad libitum* and were maintained and used in accordance with National Research Council recommendations. Mice were rendered transiently neutropenic with intraperitoneal injections of cyclophosphamide (250 mg/kg on day −4 and 100 mg/kg on day −1). Uranyl nitrate (5 mg/kg on day −3) was administered to produce a controlled degree of renal impairment, to assist with the development of human-simulated drug exposures. After 18 to 20 h of incubation of the isolate second transfer, a bacterial suspension of approximately 10^7 CFU/ml in 3% hog gastric mucin was made for inoculation. The mice were anesthetized using vaporized isoflurane (2 to 3% [vol/vol] in an oxygen carrier), and lung infection was produced by intranasal inoculation of 50 μl of inoculum 2 h prior to therapy initiation. This study was approved by the Hartford Hospital Institutional Animal Care and Use Committee.

Bronchopulmonary pharmacokinetics and human-simulated ELF exposures. Human-simulated dosing regimens in mice that provided a percentage of the dosing interval above the ELF concentration and an ELF AUC similar to those achieved in a nonrandomized, open-label, one-treatment, one-group study to investigate the intrapulmonary lung penetration of RO7079901 in 21 healthy volunteers, conducted by F. Hoffmann-La Roche (ClinicalTrials registration no. NCT03182504), were developed. In the clinical trial, study participants received a single dose of nacubactam (2-g intravenous infusion of

nacubactam over 1.5 h) coadministered with meropenem (2-g intravenous infusion of meropenem over 1.5 h).

Initially, single-dose ELF pharmacokinetic studies of meropenem (50 mg/kg and 100 mg/kg) and nacubactam (10 mg/kg and 20 mg/kg) were performed in the murine infection model. Using the pharmacokinetic parameter estimates derived from the single-dose pharmacokinetic studies, human-simulated ELF regimens of meropenem and nacubactam were developed. Confirmatory pharmacokinetic studies in which mice received meropenem or nacubactam human-simulated regimens alone or in combination were undertaken to ascertain whether the meropenem and nacubactam regimens resulted in the expected ELF exposures. All single-dose and confirmatory pharmacokinetic studies were conducted in lung-infected mice ($n = 36$ mice) to examine meropenem and nacubactam ELF profiles. Following intracardiac blood collection, BAL fluid was collected at 6 sampling time points, with 6 mice contributing to each time point; a catheter was inserted into the trachea of the mice, and the lungs were lavaged with 4 aliquots of 0.4 ml of normal saline. Plasma and BAL fluid were analyzed for drug and urea concentrations by F. Hoffmann-La Roche Ltd. (Basel, Switzerland) via high-performance liquid chromatography-mass spectrometry. The ELF concentrations (C_{ELF}) of nacubactam or meropenem were determined using the equation: $C_{ELF} = C_{BAL} \times Urea_{plasma}/Urea_{BAL}$, where $C_{BAL,}$ $Urea_{BAL,}$ and $Urea_{plasma}$ are the concentration of drug in BAL fluid, the concentration of urea in BAL fluid, and the concentration of urea in plasma, respectively. Pharmacokinetic parameters for single-doses studies were calculated using Phoenix 64 (WinNonlin 6.4, NLME 1.3).

***In vivo* efficacy studies.** The 12 *Enterobacteriaceae* strains were used to infect cohorts of 30 mice each. Treatment was initiated 2 h following bacterial inoculation. Treated mice (6 mice per group) received subcutaneous injections (0.1 ml/agent) of either human-simulated meropenem alone, human-simulated nacubactam alone, a meropenem-nacubactam combination, or saline (24-h controls) at each treatment time point. The lung tissue harvesting procedure for all study mice began with euthanization by CO_2 exposure, followed by cervical dislocation. Lungs from all animals were harvested 24 h after the initiation of therapy. After sacrifice, the lungs were removed aseptically and individually homogenized in normal saline. Tenfold serial dilutions of the lung homogenates were plated on Trypticase soy agar with 5% sheep blood for CFU determination. Untreated control mice (6 mice per group) were sacrificed 2 h postinoculation, to serve as the 0-h control animals. Efficacy was quantified by the change in bacterial density in the mice after 24 h, relative to the 0-h untreated controls.

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