Evaluation Strategies for Triple-Drug Combinations against Carbapenemase-Producing *Klebsiella Pneumoniae* in an *In Vitro* Hollow-Fiber Infection Model

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Mounting antimicrobial resistance to carbapenemase-producing *Klebsiella pneumoniae* (CPKP) highlights the need to optimize currently available treatment options. The objective of this study was to explore alternative dosing strategies that limit the emergence of resistance to preserve the utility of last-line antibiotics by: (i) evaluating the pharmacodynamic (PD) killing activity of simulated humanized exposures to monotherapy and two-drug and three-drug combinations against CPKP bacterial isolates with different resistance mechanisms; and (ii) optimizing polymyxin B (PMB) exposure simulated in the three-drug combination regimens to maximize the killing activity. Two CPKP clinical isolates (BAA2146 (NDM-1) and BRKP76 (KPC-2)) were evaluated over 168 hours using a hollow-fiber infection model simulating clinically relevant PMB, fosfomycin, and meropenem dosing regimens. PMB-based three-drug combinations were further optimized by varying the initial exposure (0–24 hours) or maintenance dose received over the duration of treatment. The area under the bacterial load-versus-time curve (AUCFU) was used to determine PD activity. Overall reductions in PMB exposure ranged from 2 to 84%. BAA2146 and BRKP76 had median (range) AUCFUs of 11.0 (10.6–11.6) $\log_{10}$ CFU hour/mL and 7.08 (7.04–11.9) $\log_{10}$ CFU hour/mL, respectively. The PMB "front loaded" 2.5 mg/kg/day + 0.5 mg/kg maintenance dose in combination with meropenem and fosfomycin was a promising regimen against BRKP76, with an overall reduction in PMB exposure of 56% while still eradicating the bacteria. Tailored triple-combination therapy allows for the optimization of dose and treatment duration of last-line agents like PMB to achieve adequate drug exposure and appropriate PD activity while minimizing the emergence of resistance.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Knowledge regarding the design of optimal dosing strategies against carbapenemase-producing *Klebsiella pneumoniae* that achieve both adequate exposure at the site of infection and prevent resistant subpopulation growth is minimal.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Using the hollow-fiber infection model, we explored alternative dosing strategies of polymyxin B (PMB), fosfomycin, and meropenem that limited the emergence of resistance to preserve the utility of these last-line antibiotics.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ PMB-based triple-drug combinations can be further optimized to reduce the overall PMB exposure. PMB "front

loaded" 2.5 mg/kg/day + 0.5 mg/kg maintenance dose was a promising regimen against BRKP76 (KPC-2 producer) with a 56% reduction in PMB exposure while still eradicating the bacteria.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Dose and treatment duration of last-line agents like PMB can be optimized to achieve adequate drug exposure and appropriate pharmacodynamic activity while minimizing the emergence of resistance. The design and optimization strategy proposed here will be useful for prolonging the life span of existing antibiotics.

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The increasing prevalence and lack of effective treatment options for carbapenemase-producing *Klebsiella pneumoniae* (CPKP) are a significant threat to human health on a global scale.[1] The SENTRY Antimicrobial Surveillance Program reported a significant increase in carbapenem-resistant *Enterobacteriaceae* (CRE) from 0.6% to 2.95% over 20 years (1997–2016) across 42 countries, with *Klebsiella pneumoniae* (KP) comprising 71.1% of CRE isolates.[2] A change in the epidemiology of carbapenemase genes was also reported, with increased dissemination of isolates carrying genes encoding NDM (2007–2009: 4.3% vs. 2014–2016: 12.7%) and a constant number of KPC-encoding genes (2007–2009: 49.7% vs. 2014–2016: 54.2%).[2,3] Ceftazidime-avibactam was the first drug approved under the Generating Antibiotic Incentives Now (GAIN) Act, delivering a new agent to combat resistant bacteria. Nevertheless, the clinical use of this drug was soon followed by reports of resistance.[4–9] Although not unexpected, this "arms race" against bacteria and their capacity to subvert therapy raises the issue of the best clinical approach to combat multidrug resistant (MDR) pathogens.

Serine β-lactamases and metallo-β-lactamases (MBLs) are two classes of carbapenemase that differ in their hydrolytic mechanisms and hydrolysis rates.[10] The serine β-lactamases (e.g., KPC and OXA enzymes) can be inhibited by novel beta-lactamase inhibitors, whereas MBLs (e.g., NDM, VIM, and IMI enzymes) are inhibited by agents that can chelate divalent cations.[11] Colistin, tigecycline, fosfomycin, carbapenems, and gentamicin are "old" antibiotics that have retained their activity and remain viable components of two-drug and three-drug treatment regimens.[12,13] Polymyxins (i.e., polymyxin B (PMB) and colistin) are an important lipopeptide antibiotic class against these pathogens due to their potent bactericidal activity, particularly when the epidemiology warrants agents active against MBL-carrying KP. Over the last 2 decades, PMB has re-emerged and gained clinical utility against MDR Gram-negative pathogens and it is commonly used as a backbone antibiotic in two-drug and three-drug combinations for CPKP.[14] Additionally, carbapenem-containing combinations are often favored due to lower mortality rates being observed in several retrospective studies.[15,16] Fosfomycin represents an attractive option given its activity against KPC-producing and NDM-1 producing *Enterobacteriaceae*, despite the additional benefit of a third drug being unclear.[17,18]

Knowledge regarding the design of optimal dosing strategies that achieve both adequate exposure and prevent resistant subpopulation growth is minimal. Understanding the relationship between drug exposure achieved at the site of infection and bacterial killing dynamics is critical for limiting the selective pressure that drives the emergence of resistance. In particular, the development of polymyxin resistance is concerning and highlights the need to optimize polymyxin-based therapy to conserve its efficacy.

The *in vitro* hollow-fiber infection model (HFIM) can be used to simulate the full pharmacokinetic (PK) time course and associated pharmacodynamics (PD) by simulating clinically relevant dosing regimens over extended durations. The objective of this study was to explore alternative dosing strategies that limit the emergence of resistance to preserve the utility of these last-line antibiotics by: (i) evaluating the PD killing activity of simulated humanized exposures to monotherapy and two-drug and three-drug combinations against CPKP bacterial isolates with different resistance

mechanisms; and (ii) optimizing PMB exposure simulated in the three-drug combination regimens to maximize the killing activity.

METHODS

Bacterial isolates
Two KP clinical isolates were employed for all studies: BAA2146 was obtained from the American Type Culture Collection (ATCC) and BRKP76 was obtained from a patient treated at the Instituto Dante Pazzanese de Cardiologia, Sao Paulo, Brazil. The minimum inhibitory concentrations (MICs) were determined in triplicate by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[19] Both isolates are susceptible to PMB (MIC 0.5 mg/L) and fosfomycin (BAA2146 MIC, 16 mg/L; BRKP76 MIC, 32 mg/L), and both are resistant to meropenem (MIC 64 mg/L). BAA2146 is an NDM-1 producer and BRKP76 is a KPC-2 producer. Polymerase chain reaction was performed using primer sets for β-lactamase Ambler class A (GES and KPC), B (NDM, VIM, and IMP), and D (OXA-48 and OXA-40), as previously described.[20]

Hollow-fiber infection model
The HFIM was used as previously described[21] to simulate monotherapy and two-drug and three-drug combination regimens using PMB, meropenem, and fosfomycin against an initial inoculum of ~ 10^7 colony forming unit (CFU)/mL of BAA2146 or BRKP76 over a duration of 168 hours in triplicate (see **Supplementary Materials**).

Simulated antimicrobial PK
The simulated PK profiles for PMB, meropenem, and fosfomycin were based on published clinical PK studies.[22–25] Peristaltic pumps (Masterflex L/S; Cole-Parmer, Vernon Hills, IL) were used to provide continuous CAMHB flow through the central compartment to simulate a terminal half-life of 2 hours (meropenem) and 4 hours (PMB and fosfomycin), which correspond to an elimination rate constant of 0.35 and 0.17 hours^{-1}, respectively. A supplement compartment was used to simulate the appropriate drug concentrations. The PK parameters ($fC_{max,avg(0-24)}$, $fC_{max,avg(0-168)}$, $fAUC_{0-24}$, and $fAUC_{0-168}$) used to simulate each of the polymyxin-based three-drug regimens in combination with meropenem (2 g every 8 hours as a 3-hour extended infusion) and fosfomycin (8 g every 8 hours as a 0.5-hour infusion) are provided in **Table 1**. Simulated PK profiles in the HFIM reflect the unbound concentration in plasma and represent relevant concentrations for the treatment of bloodstream infections.

Pharmacodynamic analysis
Serial samples obtained at 0, 2, 4, 8, 24, 28, 32, 48, 56, 72, 96, 120, 144, and 168 hours were used for bacterial quantification (see **Supplementary Materials**). To assess bacterial killing, the area under the bacterial load-versus-time curve (AUCFU) from 0 to 168 hours was calculated for each regimen evaluated using Phoenix WinNonlin software (Certara, Princeton, NJ).

Emergence of resistance
To assess for the emergence of resistance, population analysis profiles were determined by plating samples collected at 0 (baseline), 24, 48, 72, 96, 120, 144, and 168 hours on PMB-containing MHA (2, 16, and 32 mg/L) and on fosfomycin-containing MHA (16, 32, abd 128 mg/L) for all the regimens evaluated.

RESULTS

PK analysis: PMB dose optimization
The PK profile of each of the PMB-based three-drug combinations was compared with the clinical standard, PMB "front

Table 1 PK Exposure Parameters of PMB-based three drug combinations

Description of regimen	$f\mathrm{C}_{\text{max,avg(0–24)}}$, mg/L	$f\mathrm{AUC}_{0–24}$, mg hour/L	$f\mathrm{C}_{\text{max,avg(0–168)}}$, mg/L	$f\mathrm{AUC}_{0–168}$, mg hour/L	% Change in $f\mathrm{AUC}$ 0–24	% Change in $f\mathrm{AUC}$ 0–168
PMB FL with maintenance[a] (PMB FL 2.5 + Maint 1.5)	3.67	40.1	3.49	268.2	**	**
PMB FL with maintenance[a,d] (PMB FL 2.5 + Maint 0.5)	3.67	40.1	1.54	118.4	0%	−56%
PMB maintenance 0.5[b,d] (PMB Maint 0.5)	1.07	11.7	1.14	87.6	−71%	−67%
PMB maintenance 1.5[b,d] (PMB Maint 1.5)	3.21	35.1	3.42	262.8	−13%	−2%
PMB FL 2.5[c,d] (PMB FL 2.5)	3.67	40.1	0.56	43.4	0%	−84%
PMB FL 5[c,d] (PMB FL 5.0)	7.34	80.1	1.54	86.8	+200%	−68%

The free steady-state area under the concentration-time curve over 24 hours ($f\mathrm{AUC}_{24}$) and the average peak free drug concentration ($f\mathrm{C}_{\text{max,avg}}$) were determined for each of the PMB-based three-drug combinations. The reduction in PMB exposure was calculated by using PMB FL 2.5 + Maint 1.5 (**) as the reference. FL, front loaded; PK, pharmacokinetic; PMB, polymyxin B.
[a]Loading dose administered as a 1-hour infusion every 12 hours for the first 24 hours followed by a maintenance dose as a 1-hour infusion for the duration of treatment. [b]Maintenance dose administered as a 1-hour infusion every 12 hours over the duration of treatment (0 to 168 hours). [c]Loading dose administered as a 1 hour infusion every 12 hours for the first 24 hours and then discontinued. [d]Administered in combination with MEM (2 g every 8 hours as a 3 hours extended infusion ($f\mathrm{AUC}_{24}$: 913.5 mg hour/L; $f\mathrm{C}_{\text{max,ss}}$: 70.0 mg/L; $f\mathrm{C}_{\text{min,ss}}$: 12.4 mg/L)) and FOF (8 g every 8 hours as a 0.5 hours infusion ($f\mathrm{AUC}_{24}$: 4,000 mg hour/L; $f\mathrm{C}_{\text{max,ss}}$: 295.1 mg/L; $f\mathrm{C}_{\text{min,ss}}$: 80.5 mg/L)).

loaded" (FL) 2.5 + Maintenance (Maint) 1.5, to determine the percentage PMB exposure reduction (**Table 1**).

PMB Maint 0.5 resulted in the greatest reduction (71%) within the first 24 hours. Whereas, PMB Maint 1.5 was associated with a 13% reduction in PMB exposure (**Table 1**). PMB FL 2.5 + Maint 0.5 and PMB FL 2.5 regimens resulted in no change, whereas PMB FL 5.0 doubled the exposure within the first 24 hours ($f\mathrm{C}_{\text{max,avg(0–24)}}$ and $f\mathrm{AUC}_{0–24}$).

All PMB-based three-drug combinations, with the exception of PMB Maint 1.5, were associated with a > 50% reduction in the total PMB exposure ($f\mathrm{C}_{\text{max,avg(0-168)}}$ and $f\mathrm{AUC}_{0–168}$) (**Table 1**). The greatest reduction over the duration of treatment (i.e., 7 days) was seen with PMB FL 2.5 followed by PMB FL 5.0, PMB Maint 0.5 and PMB FL 2.5 + Maint 0.5 (84%, 68%, 67%, and 56%, respectively). PMB Maint 1.5 resulted in a minimal reduction of 2% (**Table 1**).

PD analysis

The time course of changes in bacterial density in response to mono-, two-, and three-drug combinations against BAA2146 and BRKP76 are shown in **Figure 1**. AUCFUs, a measure of bacterial reduction in response to drug treatment, are reported in **Table S1** for each regimen. AUCFUs associated with growth control for BAA2146 and BRKP76 were 12.9 $\log_{10}$ CFU hour/mL and 12.8 $\log_{10}$ CFU hour/mL, respectively.

Monotherapy

Monotherapy with PMB, meropenem, and fosfomycin evaluated against BAA2146 and BRKP7 resulted in no discernible early activity, with minimal bacterial reductions ranging between ~ 2 and 4 $\log_{10}$ CFU/mL within the first 4 hours. Complete regrowth was

observed for each regimen against both strains by 24 hours with AUCFUs > 12.3 $\log_{10}$ CFU hour/mL (**Figure 1**). The absence of any sustained activity is represented by AUCFUs similar to the growth control.
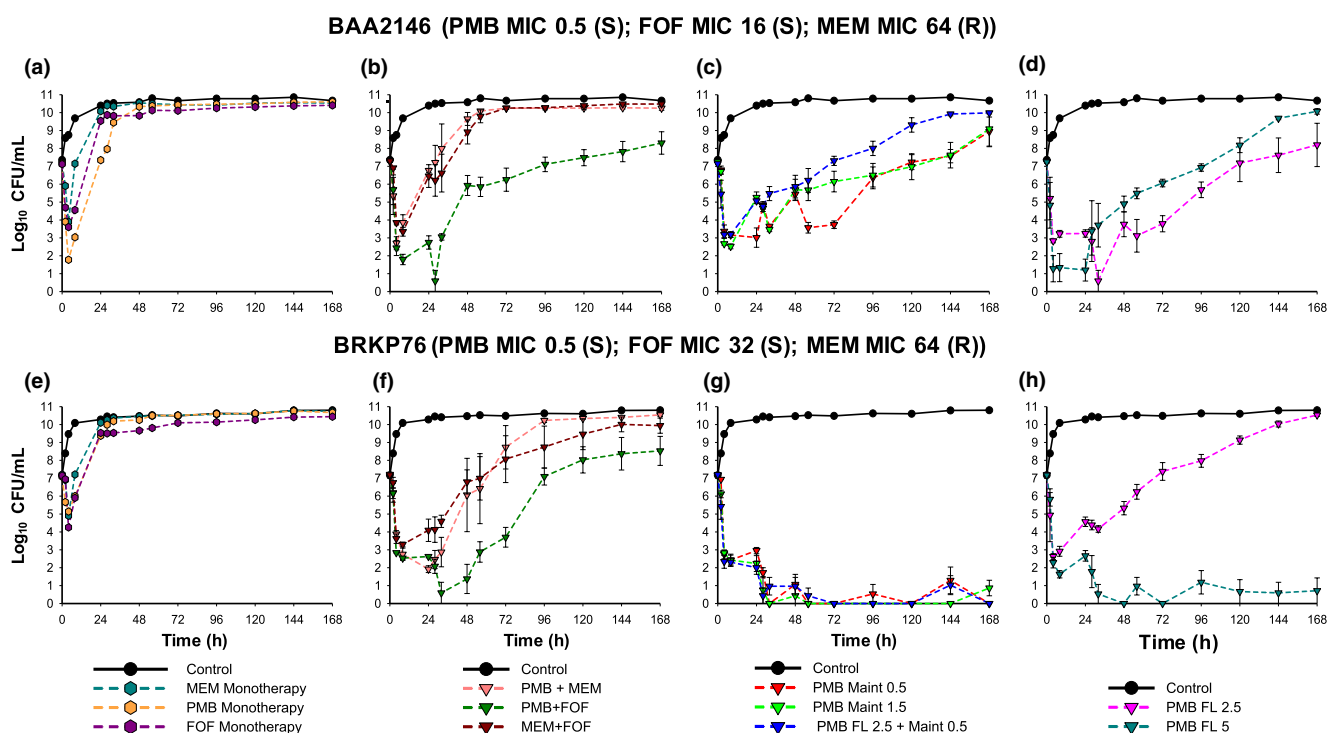
Two-drug combinations

PMB and meropenem, PMB and fosfomycin, and meropenem and fosfomycin all resulted in greater bactericidal activity compared with monotherapy, with bacterial reductions between ~ 3 and 5 $\log_{10}$ CFU/mL within the first 4 hours and delayed regrowth (**Figure 1**). PMB and fosfomycin resulted in the greatest bacterial reductions (BAA2146 AUCFU: 10.3 $\log_{10}$ CFU hour/mL; BRKP76 AUCFU: 11.0 $\log_{10}$ CFU hour/mL) compared with either of the other two-drug combinations. However, there was no sustained activity for any regimen, with regrowth similar to growth control observed by 48 hours for BAA2146 and by 96 hours for BRKP76.

Three-drug combinations

The three-drug regimens demonstrated substantial early bactericidal activity within the first 4 hours against both strains, with bacterial reductions between ~ 4 and 5 $\log_{10}$ CFU/mL (**Figure 1**). Against BAA2146, none of the three-drug regimens showed sustained killing activity, and therefore the AUCFUs associated with these regimens were much higher compared with BRKP76, ranging from 10.6 to 11.6 $\log_{10}$ CFU hour/mL.

Conversely, against BRKP76, PMB Maint 0.5, PMB Maint 1.5, PMB FL 2.5 + Maint 0.5, and PMB FL 5.0 eradicated bacteria by 48 hours and sustained this activity over 168 hours. The increased killing activity resulted in smaller AUCFUs for these four regimens (7.04–7.39 $\log_{10}$ CFU hour/mL). PMB FL 2.5

Figure 1 Time course of bacterial density in response to monotherapy (**a, e**), two-drug combinations (**b, f**), and three-drug combinations (**c–d, g–h**) against an inoculum of ~ 10^7 CFU/mL of BAA2146 (**a–d**) and BRKP76 (**e–h**) in the hollow-fiber infection model (HFIM). Each time point represents the mean ± standard error of three independent HFIM experiments performed. CFU, colony forming unit; MIC, minimum inhibitory concentration; PMB, polymyxin B. [Colour figure can be viewed at wileyonlinelibrary.com]

had no sustained activity, resulting in a higher AUCFU of 11.9 $\log_{10}$ CFU hour/mL.

PK/PD analysis
Further analyses were conducted to understand the impact of optimization of PMB exposure on PD killing activity (**Figure 2**). PMB FL 2.5 showed the greatest reduction in PMB exposure of 84% over the duration of the experiment, with minimal killing activity against both BAA2146 and BRKP76, resulting in AUCFUs of 10.6 and 11.9 $\log_{10}$ CFU hour/mL, respectively.

PMB FL 5.0 doubled the PMB exposure within the first 24 hours but reduced the overall PMB exposure over 168 hours by 68%. This regimen resulted in complete regrowth of BAA2146 but eradicated BRKP76 (BAA2146: 11.5 $\log_{10}$ CFU hour/mL; BRKP76: 7.07 $\log_{10}$ CFU hour/mL).

PMB Maint 1.5 was associated with a 2% reduction in PMB exposure over 168 hours, whereas PMB Maint 0.5 and PMB FL 2.5 + Maint 0.5 achieved reductions of 67% and 56%, respectively. PMB Maint 0.5 and PMB FL 5.0 had similar reductions in PMB exposure over 168 hours but significantly differed in the $fC_{max,avg(0-24)}$ achieved: 1.07 mg/L compared with 7.34 mg/L. All three regimens resulted in complete regrowth of BAA2146 but eradicated BRKP76 (BAA2146: 10.7–11.6 $\log_{10}$ CFU hour/mL; BRKP76: 7.04–7.39 $\log_{10}$ CFU hour/mL).

Emergence of resistance
With the BAA2146 strain, fosfomycin resistance emerged with PMB FL 2.5, PMB FL 5.0, PMB FL 2.5 + Maint 0.5, and PMB Maint 0.5

regimens. Subpopulation growth on MHA containing 16, 32, and 128 mg/L of fosfomycin was similar to that of the total population by 144 hours (**Figure S1b–e**). Subpopulation growth on PMB-containing plates remained similar to the control for PMB FL 2.5 and PMB FL 5.0 (**Figure S1b,c**). PMB Maint 0.5 resulted in amplification of PMB resistance by 168 hours. With PMB FL 2.5 + Maint 0.5 and PMB Maint 1.5, a higher proportion of subpopulations grew on MHA containing 2 mg/L of PMB but remained similar to control on 16 and 32 mg/L PMB-containing plates (**Figure S1d,f**).

With the BRKP76 strain, PMB Maint 0.5, PMB Maint 1.5, PMB FL 2.5 + Maint 0.5, PMB FL 2.5, and PMB FL 5.0 suppressed the emergence of resistance (**Figure S2c–f**). Resistance emerged to fosfomycin with the PMB FL 2.5 regimen. Subpopulation growth on MHA containing 16, 32, and 128 mg/L fosfomycin was similar to that of the total population beyond 120 hours (**Figure S2b**).

DISCUSSION
The present HFIM studies highlight the bacterial killing kinetics and suppression of emergence of resistance using PMB-based triple-drug combinations against two clinical KP isolates, BRKP76 and BAA2146. Alternative PMB dosing strategies in combination with meropenem (2 g every 8 hours as a 3-hour extended infusion) and fosfomycin (8 g every 8 hours as a 0.5-hour infusion), presented here, show that a reduction in overall PMB drug exposure is possible as part of a three-drug combination while maintaining the same PD activity, specifically against BRKP76. The evaluation of different dosing strategies in a KPC-producing
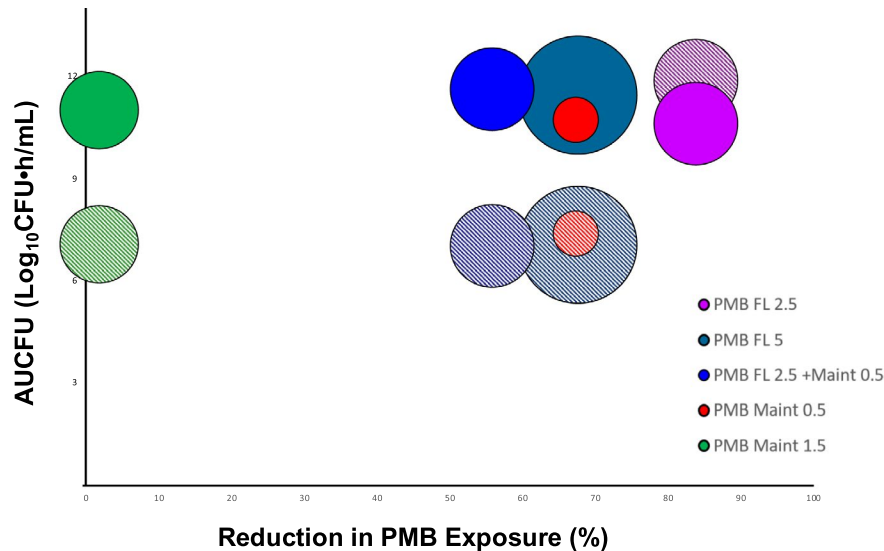
Figure 2 Reduction in PMB exposure from 0 to 168 hours and the corresponding pharmacodynamic activity of each three-drug combination for the BAA2146 strain (solid) and BRKP76 strain (shaded). In the BRKP76 isolate, PMB maintenance (Maint) 0.5 and PMB FL 2.5 + Maint 0.5 resulted in eradication with bacterial counts below the limit of quantification for PMB Maint 1.5 and PMB FL 5. The size of the dot corresponds to the pharmacokinetic exposure received within the first 24 hours. AUC, area under the time curve; CFU, colony forming unit; FL, front loaded; PMB, polymyxin B. [Colour figure can be viewed at wileyonlinelibrary.com]

and NDM-producing isolate exposed the underlying complexity of drug-microbe interactions and the need to better understand the various factors that determine clinical outcomes.

Combination treatments using more than two antibiotics are gaining wider acceptance as an approach to treating highly resistant pathogens, given the ability of these multiple agents to increase PD activity by affecting multiple targets (i.e., produce mechanistic synergy).[14,26] Mechanistic synergy has been adopted in other therapeutic areas, such as in HIV, tuberculosis, and oncology, where combination chemotherapy is standard of care.[27–29] The increased PD activity associated with our use of combination therapy may result from PMB binding to lipid A present in the lipopolysaccharide located on the outer membrane of Gram-negative bacteria,[30] thus disrupting the integrity of the outer membrane and consequently enabling fosfomycin and meropenem entry to enhance target-site drug concentrations inside bacteria.

Over the last 2 decades, last-line agents like polymyxin have played a pivotal role in the management of CRE. However, mounting reports of polymyxin resistance associated with treatment failure and increased mortality are concerning. Macesic *et al.* conducted a comprehensive genomic survey of clinical KP isolates and found suboptimal polymyxin exposure to be a major contributor to the emergence of polymyxin resistance rather than in-hospital transmission.[31] Given the increasing MICs for PMB among CPKP strains, optimization of PMB exposure is essential to increase its efficacy while minimizing its toxicity profile. In our study, PMB FL 2.5 and PMB FL 5.0 represented aggressive polymyxin dosing strategies with a PMB FL dose consisting of two doses administered within the first 24 hours and then discontinued for the duration of treatment. A previous *in vitro* study of *Acinetobacter baumannii* isolates found the combination of PMB "burst 5" and doripenem resulted in rapid and extensive killing (> 8 $\log_{10}$ CFU/mL), with a 3.49 $\log_{10}$ reduction in

AUC_{cfu} compared with growth control by 240 hours.[32] The cumulative exposure to PMB was reduced by 75% over 240 hours when compared with the combination of PMB "traditional" with doripenem (120 vs. 480 mg/mL hour). Early use of PMB-based combination therapy is associated with significant increases in bacterial clearance rates when compared with delayed administration (65.2% vs. 29.4%, $P = 0.025$; odds ratio (OR) = 0.533) as well as a decrease in 30-day mortality (39.1% vs. 70.6%, $P = 0.045$; OR = 0.461) and overall mortality (43.5% vs. 82.4%, $P = 0.022$; OR = 0.321).[33] Clinically, the benefit of achieving early, adequate bacterial load reduction with PMB FL 5.0 should be weighed against the risk of toxicity, given that the likelihood of nephrotoxicity substantially increases when average steady-state plasma concentrations are greater than ~ 2 mg/L.[34] Of note, the nephrotoxicity associated with PMB has been shown to be dose-dependent and reversible upon discontinuation of treatment.[35] Identifying the appropriate dosing strategy in critically ill patients with significant comorbidities and high bacterial burden is important given the risk of sepsis arising from inadequate treatment, which can also lead to kidney injury.[36] In patients where higher concentrations are not feasible or warranted, PMB Maint 0.5, PMB Maint 1.5, and PMB FL 2.5 + Maint 0.5 should be considered as part of a three-drug combination. Clinical guidelines currently recommend a loading dose of 2.0–2.5 mg/kg based on total body weight and a daily maintenance dose of 1.25–1.5 mg/kg every 12 hours infused over 1 hour. These recommendations are based on achieving a PK/PD therapeutic target $AUC_{ss,24 hours}$ of ~ 50 mg hour/L, which equates to a target C_{ss} of 2 mg/L.[37] Given that a loading dose is often recommended with the use of PMB, PMB FL 2.5 + Maint 0.5 might be the most promising alternative dosing strategy as it allows for early bactericidal activity while considering a lower maintenance dose in combination with fosfomycin and meropenem.

Understanding how to minimize the selection of antimicrobial resistance is fundamental for the design of antibiotic treatment regimens. Antimicrobial stewardship programs have identified the reduction in duration of antibiotic therapy as a key element in reducing the likelihood of resistance emerging.[38] Colistin front-loaded regimens have been previously found to be a highly beneficial strategy in achieving high initial peak concentrations followed by de-escalation of therapy resulting in lower exposure and a reduction in bacterial burden of at least 3 $\log_{10}$.[39] Our study found PMB FL 2.5 and PMB FL 5.0 to be associated with promising initial bactericidal killing activity and viable treatment options in preventing the emergence of PMB resistance. Minimizing antibiotic exposure, however, is not always without a cost. Suboptimal exposure with the PMB Maint 0.5 regimen against BAA2146 resulted in the emergence of PMB resistance due to the selection of antimicrobial-resistant subpopulations. Although both dosing strategies discussed here resulted in extensive regrowth beyond 72 hours in the case of BAA2146, there were clear differences in the emergence rate of PMB resistance, suggesting that optimization of antimicrobial therapy requires a clear understanding of the relationship among dose, duration, and the antibiotic PDs. PMB-based triple-drug combinations have previously been found to provide the flexibility needed to reduce the PMB footprint and consequently the likelihood of resistance emerging. A recent HFIM study evaluated the use of a PMB single-dose ("burst") regimen of 5.53 mg/kg in combination with meropenem and rifampicin, which resulted in bacterial eradication of a KPC-2-producing strain.[40] Similar PD activity was observed against the BRKP76 isolate in our study but not against BAA2146 highlighting the need to evaluate similar dosing regimens in additional isolates. Differences in the PD outcome could be attributable to differences in the resistance mechanisms of the two isolates (i.e., KPC-2 vs. NDM-1). Genetic analysis of NDM-producing *Enterobacteriaceae* has revealed this MBL to be located in a very mobile genetic element encoding numerous additional resistance determinants, including β-lactamase genes, quinolone resistance genes, and 16S RNA methylase genes.[41] NDM-1 producers have been associated with unpredictable and complex patterns of MDR compared with those harboring KPC-2, often resulting in treatment failure.[42] Future studies should focus on understanding the impact of PMB exposure on bacterial gene expression and the intricacies responsible for the development/suppression of resistance in order to further refine our approach.

Although the HFIM does not consider the potential impact of the host immune response on bacterial eradication, it is a valuable tool for optimizing exposure to individual components of combination therapy. Our study shows that there is value in time-course data and understanding the complexities of PD activity, including the emergence of resistance, in response to PK over time. PK/PD indices have traditionally been used to predict clinical efficacy. However, these indices represent the PD activity of the drug at a particular time point corresponding to a reductionist approach when optimizing antimicrobial therapy. Mechanism-based PD modeling can be used to further explore the antimicrobial efficacy of different drug regimens and dosing strategies. Additionally, clinical trials evaluating these triple-drug combinations should be

prioritized to truly understand the implications of dose optimization in terms of safety and efficacy.

With treatment options becoming extremely limited, an understanding of the interplay between drug exposure at the site of infection, its killing activity against the pathogen, and bacterial pathogen characteristics (e.g., resistance mechanisms and virulence genes expressed) are taking center stage with regard to optimization and individualization of combination therapy. Optimization of new and old antibiotics deserves greater attention. The design and optimization strategy proposed here will help prolong the lifespan of existing approved antibiotics. Although clearly a complex task, administering the right drugs, at the right dose, at the right time has clinical value worth pursuing.

SUPPORTING INFORMATION
Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

CONFLICTS OF INTEREST
All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
E.G. and G.G.R. wrote the manuscript. G.G.R. designed the research. E.G., J.K.D., R.S., and P.O.H. performed the research. E.G., C.S.A., K.S.K., J.L., T.V., and G.G.R analyzed the data.

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