

## Introduction

**Background:** Lysins are bacteriophage-derived enzymes that degrade bacterial cell wall peptidoglycan. The use of lysin in addition to cytoplasmic membrane targeting antibiotics such as daptomycin (DAP) is attractive alternative to antibiotic monotherapy currently used to treat *S. aureus* related infections (1).

**Motivation:** Daptomycin (DAP) has proven to be a viable alternative amid vancomycin resistance, nevertheless the use of DAP post vancomycin treatment has led to the development of DAP non-susceptible (DNS) strains (2, 3, 4). The cell wall hydrolytic activity of exebacase (CF-301) is rapidly bactericidal resulting in the osmotic lysis of the targeted cell. Resistance to lysins is rare due to the lysin's ability to bind and cleave highly conserved targets within the cell walls (5).

**Objective:** The objective of this study was to evaluate the impact of DAP and exebacase co-administration against MRSA MW2 compared to DAP alone in a two compartment PK/PD in-vitro model.

**Significance:** Previous experience with exebacase demonstrated that it has a low propensity for bacterial resistance. Exebacase can ultimately lead to a more efficient treatment of serious *S. aureus* infections and potentially lead to optimizing patient outcomes and preserving antibiotic therapy for serious MRSA infections.

## Methods

**Bacterial strains:** MRSA MW2 and 494 strains from the Anti-Infective Research library (ARL) were used for susceptibility testing experiments and MW2 strain was further evaluated in PK/PD models vs. exebacase and DAP alone and exebacase in addition to DAP.

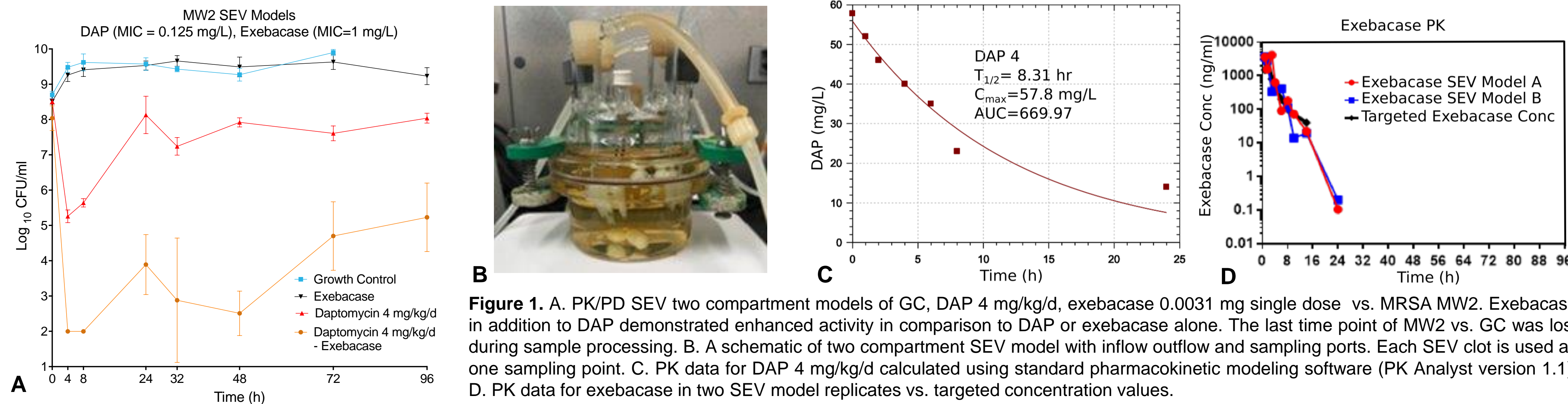
**Media/ Antibiotics:** All susceptibility tests and models were performed in MHB broth containing 25% horse serum and equivalent of 50 mg/L calcium. Exebacase was supplied by the company (Contrafact Corporation) and DAP was purchased commercially Sigma Chemical Company (St. Louis, MO).

**Susceptibility Testing:** MIC tests were performed using broth micro-dilution method. Bactericidal activity and synergy were defined as  $>3 \log_{10}$  CFU/ml and  $>2 \log_{10}$  CFU/ml reduction from baseline (compared to the most active agent alone) respectively.

**PK/PD Models:** MW2 strain was also tested in *in-vitro* PK/PD simulated endocardial vegetation (SEV) PK/PD models. In the SEV PK/PD models, the regimens tested consisted of: exebacase 0.0031 mg/ml single dose on Day 1 injected at a rate simulating human elimination, DAP at a human equivalent dose of 4 mg/kg/day  $fC_{max}=57.8 \mu\text{g/ml}$  during 4 days, DAP 4 mg/kg/day in addition to 0.0031 mg/ml single dose of exebacase and growth control. All the model experiments were performed in duplicate over 96 h. Model samples were plated and counted using an automated colony counter and differences in  $\log_{10}$  CFU/g of vegetation between regimens was evaluated. Development of resistance was checked for all regimens.

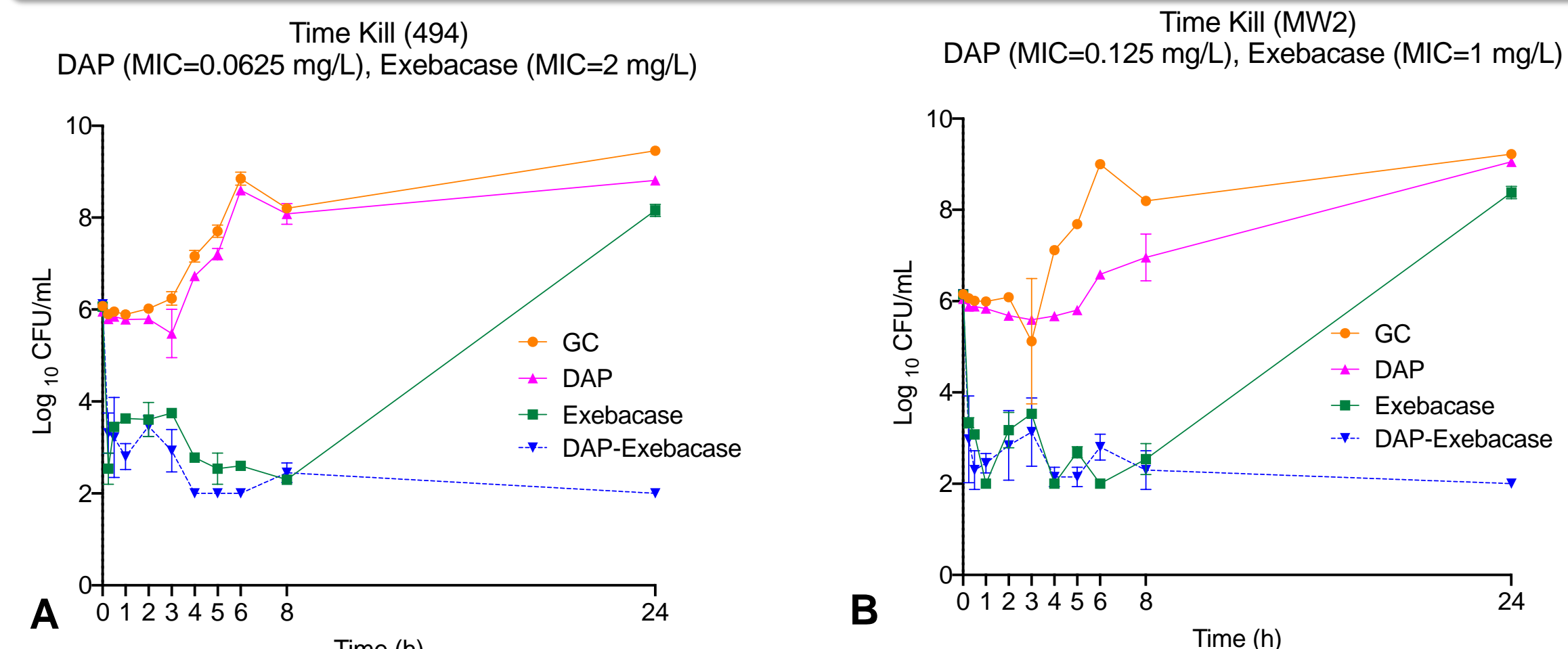
## Results

### PK/PD Models



**Figure 1.** A. PK/PD SEV two compartment models of GC, DAP 4 mg/kg/d, exebacase 0.0031 mg single dose vs. MRSA MW2. Exebacase in addition to DAP demonstrated enhanced activity in comparison to DAP or exebacase alone. The last time point of MW2 vs. GC was lost during sample processing. B. A schematic of two compartment SEV model with inflow outflow and sampling ports. Each SEV clot is used as one sampling point. C. PK data for DAP 4 mg/kg/d calculated using standard pharmacokinetic modeling software (PK Analyst version 1.1). D. PK data for exebacase in two SEV model replicates vs. targeted concentration values.

### Time Kill Experiments



**Figure 2.** A (MRSA 494) and B (MRSA MW2) tested in a 24h time kill experiments for GC, DAP, exebacase alone or in addition to DAP - at 1xMIC against MRSA strains. Synergy was observed as early as 15 minutes exposure to the DAP+exebacase and stayed at detection limit for the duration of 24 h time kill experiment.

## Conclusions

- Co-administration of DAP and exebacase in this model offers encouraging results for the clearance of MRSA strains 494 and MW2 compared to DAP alone.
- The use of exebacase in addition to antibiotic therapy can potentially lead to optimizing patient outcomes and preserving antibiotic therapy for serious MRSA infections.

## References

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## Disclosures

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