Lysin CF-301 Exhibits a Low Propensity for Decreased Susceptibility and Prevents Daptomycin (DAP) Resistance in a Rabbit Model of *S. aureus* Infective Endocarditis (IE)

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Introduction

CF-301 is a novel, recombinantly-produced, bacteriophage-derived lysin (cell wall hydrolase) which is in Phase 2 of clinical development for the treatment of *S. aureus* bacteremia including IE used in addition to standard of care antibiotics.

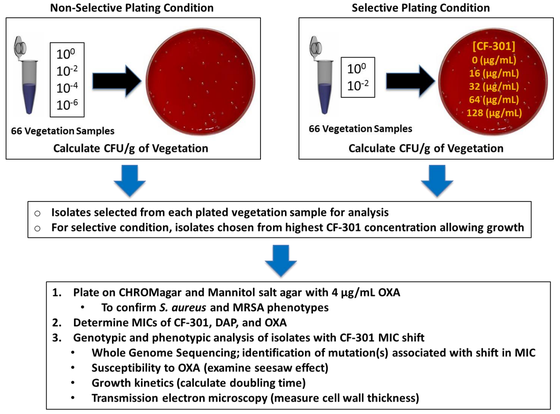
We previously used in vitro serial passage assays to examine the propensity for CF-301 resistance (1,2). While resistance was not detected, we identified a stable 2-fold increase in MIC; this reduced susceptibility did not proceed beyond 2-fold. Furthermore, we demonstrated the ability of sub-MIC CF-301 to suppress resistance to conventional antibiotics when used in combination. In accordance with FDA guidance (3), the phenotypic and genotypic changes associated with 2-fold MIC shifts were studied and unique mutations were identified in *murA*, *oatA* and *lyrR*. Each mutation was (separately) associated with a reduced growth rate, a "seesaw effect" on oxacillin (OXA) resistance, and a 50% increase in cell wall thickness. Isogenic mutants were constructed to recapitulate each mutation and confirm the impact on CF-301 susceptibility.

As a complement to the in vitro resistance analysis, we now report use of the MRSA rabbit IE model to assess changes in the MIC of CF-301 and DAP in animals treated with DAP alone, CF-301 alone or CF-301 in addition to DAP. We collected *S. aureus* isolates from IE vegetations and used an ex vivo format to evaluate the effect of treatment on MIC values. Similar to our in vitro study findings, we observed a low propensity for resistance to CF-301 (defined by MIC shifts of no more than 2-fold) and the ability of CF-301 to suppress the appearance of DAP resistance. We furthermore identified a series of mutations associated the 2-fold shift in CF-301 MIC, which were distinct from the mutations previously identified in serial passage resistance experiments. Additional phenotypes associated with the 2-fold shift in CF-301 MIC include an increase in cell wall thickness and a reduction in OXA MIC values.

Methods

The standard indwelling catheter-induced model of aortic valve IE in rabbits utilizing MRSA strain MW2 was employed (4). Animals were given DAP (4 mg/kg, IV QD x 4 d) alone or in addition to a single-dose of CF-301 (0.09 mg/kg to 1.4 mg/kg; IV). Vehicle controls and animals treated with a CF-301 alone were included. At 24 h after the last dose of DAP, valvular vegetations were sterilely removed from all treatment groups and quantitatively cultured. To assess potential emergence of CF-301 resistance, CF-301 treatment groups (alone and with DAP) were plated on TSAB (non-selective condition) and TSAB supplemented with CF-301 over a range of concentrations (selective conditions). To study the potential emergence of DAP resistance, DAP treatment groups (alone and with CF-301), were parallel plated on TSAB ± DAP. Multiple colonies from each condition were subcultured and both CF-301 and DAP MICs for each colony was determined. To screen for the seesaw effect, we also determined OXA MICs for all isolates. Additional phenotypic changes associated with altered susceptibility to CF-301 were examined using growth curves and transmission electron microscopy. Mutant genotypes were identified by whole genome sequencing.

Study Outline



Non-Selective Plating Condition

❑ ***Analysis of CF-301, DAP, and OXA MIC of isolates recovered from non-selective plating on TSAB***

Treatment Groups[a]	Log_{10} CFU/g of Vegetation[b]	n[c]	CF-301 MIC (µg/mL)[d] 0.25	0.5	1	2	4	DAP MIC (µg/mL)[e] 0.125	0.25	0.5	1	2	4	8	OXA MIC (µg/mL)[f] <2	4	8	16	32	64
Pre-Treatment Control	7.02±1.47	24			24					24									24	
Buffer treatment Control	7.26±1.54	32			31	1				30	2						2		30	
DAP (4)	4.03±2.58	24			24							2	22					24		
CF-301 (1.4)	8.24±0.02	24			24				2	20	2				7				17	
CF-301 (0.7)	7.93±0.12	24		1	23				2	20	2							1	23	
CF-301 (0.35)	8.17±0.58	24		3	21					24					6				18	
CF-301 (0.18)	8.65±0.58	16			16				1	15									16	
CF-301 (1.4) DAP (4)	<2.17±0.09	0																		
CF-301 (0.7) DAP (4)	<2.32±0.17	0																		
CF-301 (0.35) DAP (4)	4.02±0.17	47		16	30	1				47									47	
CF-301 (0.18) DAP (4)	3.16±0.33	48			48					48									48	
CF-301 (0.09) DAP (4)	6.27±0.19	60		5	55						3	57							60	
Sterilization																				

[a]Number in parenthesis represents dose in mg/kg
[b]Limit of detection is indicated by the less than sign for each group
[c]Number of isolates analyzed in MIC assays from each group
[d]The CF-301 MIC of *S. aureus* strain MW2 is 1 µg/mL
[e]The DAP MIC of *S. aureus* strain MW2 is 0.5 µg/mL
[f]The OXA MIC of *S. aureus* strain MW2 is 64 µg/mL

No decreased susceptibility to CF-301

CF-301 suppressed DAP^R

CF-301 exposure resulted in increased OXA susceptibility

- **No decreased susceptibility to CF-301 was observed**
- **Up to 8-fold shift in DAP MIC observed in DAP alone treatment**
- **CF-301 suppressed emergence of DAP resistance in combinations down to 0.18 mg/kg**
- **CF-301 exposure appears to increase OXA susceptibility (≥8-fold shift)**

Selective Plating Condition

❑ ***Analysis of CF-301, DAP, and OXA MIC of isolates recovered from selective plating on TSAB supplemented with a range of CF-301 concentrations***

Treatment Groups[a]	Log10 CFU/g of Vegetation[b] 0	16	32	64	128	n[c]	CF-301 MIC (µg/mL)[d] 0.25	0.5	1	2	4	DAP MIC (µg/mL)[e] 0.125	0.25	0.5	1	2	4	8	OXA MIC (µg/mL)[f] <2	4	8	16	32	64
Pre-Treatment Control	7.02±1.47	4.3±2.0	<2.3±0.04	<2.3±0.04	<2.3±0.04	19		1	18					19									19	
Buffer treatment Control	7.26±1.54	3.3±1.1	4.9±1.8	3.1±1.6	<2.1±0.1	44			44					44									44	
DAP (4)	4.03±2.58	3.0±2.0	2.9±1.9	2.7±1.4	<2.1±0.1	8		1	7							1	7					8		
CF-301 (1.4)	8.24±0.02	7.1±0.06	5.9±0.03	4.2±1.7	2.8±1.2	24			20	4			3	20	1				3				21	
CF-301 (0.7)	7.93±0.12	6.7±0.1	5.7±1.1	3.8±1.8	<2.1±0.1	24		2	19	3			2	21	1							3	21	
CF-301 (0.35)	8.17±0.58	6.9±0.2	6.4±0.5	5.5±0.6	3.4±1.3	24			22	2			3	21					7				17	
CF-301 (0.18)	8.65±0.58	7.1±0.1	7.1±0.1	6.6±0.9	4.6±0.7	16			14	2				16									16	
CF-301 (1.4) DAP (4)	<2.17±0.09	<2.1±0.1	<2.1±0.1	<2.1±0.1	<2.1±0.1	0																		
CF-301 (0.7) DAP (4)	<2.32±0.17	<2.3±0.1	<2.3±0.1	<2.3±0.1	<2.3±0.1	0																		
CF-301 (0.35) DAP (4)	4.02±0.17	<2.2±0.1	<2.2±0.1	<2.2±0.1	<2.2±0.1	0																		
CF-301 (0.18) DAP (4)	3.16±0.33	<2.2±0.2	<2.2±0.2	<2.2±0.2	<2.2±0.2	0																		
CF-301 (0.09) DAP (4)	6.27±0.19	<2.2±0.1	<2.2±0.1	<2.2±0.1	<2.2±0.1	0																		
Sterilization																								

[a]Number in parenthesis represents dose in mg/kg
[b]Limit of detection is indicated by the less than sign for each group
[c]Number of isolates analyzed in MIC assays from each treatment group (chosen from the highest CF-301 concentration supporting growth)
[d]The CF-301 MIC of *S. aureus* strain MW2 is 1 µg/mL
[e]The DAP MIC of *S. aureus* strain MW2 is 0.5 µg/mL
[f]The OXA MIC of *S. aureus* strain MW2 is 64 µg/mL

≤2-fold shift increase

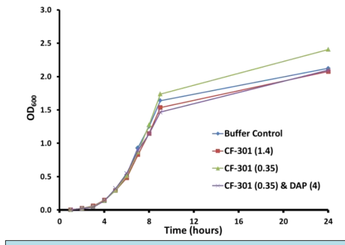
Cannot assess suppression of DAP^r

CF-301 exposure resulted in increased OXA susceptibility

- **A modest ≤2-fold increase in CF-301 MIC observed**
- **The ability of CF-301 to suppress DAP^R could not be assessed**
- **CF-301 exposure appears to increase OXA susceptibility (≥8-fold shift)**

Growth Kinetics

❑ ***We examined the growth kinetics of 2-fold shifted mutants generated in the rabbit IE model***
❑ ***Doubling time (Td) of CF-301 MIC-shifted mutants were analyzed in 0.25x LB at 37°C over 24 hours***

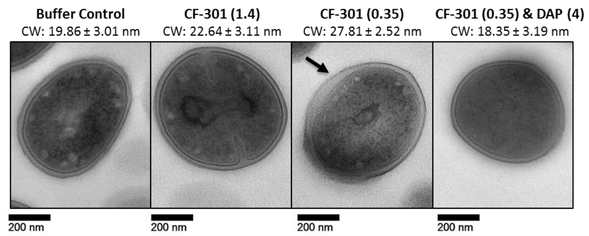


	MIC (µg/mL) CF-301	DAP	OXA	Growth Curve Td (mins)
Buffer Control	1	0.5	64	56
CF-301 (1.4)	2	1	1	60
CF-301 (0.35)	2	0.5	64	57
CF-301 (0.35) & DAP (4)	2	1	32	53

- **No significant change in growth kinetics was observed**

Cell Wall Ultrastructure

❑ ***Based on the findings from in vitro resistance studies (increased cell wall thickness), we examined the cell wall of 2-fold shifted mutants generated in the rabbit IE model***
❑ ***Average cell wall thickness (CW) was based on 50 individual cells at x13,000 mag***



- **With the exception of mutants generated in the 0.35mg/kg treatment group, the 2-fold shifted MIC variants did not demonstrate a change in cell wall thickness**
- **In the 0.35 mg/kg group, the 2-fold shifted variants demonstrated a slight increase in cell wall thickness (see arrow)**

Whole Genome Sequencing and SNP/INDEL Analysis

❑ ***CF-301 MIC-shifted mutants were analyzed by WGS to identify mutations associated with decreased susceptibility***
❑ ***Comparisons were made to isolate recovered from Buffer Control treatment group and to MW2 reference genome***

Whole Genome Sequencing: Reference Position(s)[a]	Overlapping Annotation[b]	Ref	Allele	Amino Acid Change	Treatment Groups[c]: Buffer Control	CF-301 (1.4)	CF-301 (0.35)	CF-301 (0.35) DAP (4)
2492859	*HlgCB* (near)	T	C	43bp upstream *HlgCB*	-	+	-	-
1366472	*mprF*	T	A	L291I	-	+	-	+
661605	MW_RS03160	G	A	G192D	-	-	+	-
34167	*RlmH*	G	A	K159R	-	+	-	-
34171	*RlmH* (near)	C	A	3bp downstream *RlmH*	-	+	-	-
37034	IS*431 mec* (transposase)	T	C	T25A	-	+	-	-

[a]Position in the reference genome of Staphylococcus aureus MW2 (Genbank accession: NC_003923.1)
[b]Annotated open reading frames overlapping computationally predicted polymorphisms
[c]Number in parenthesis represents dose in mg/kg; +/- indicate detection of mutation when compared to that of the Buffer Control (reference genome)

- **Unique mutation sets were identified in loci encoding enzymes involved in membrane synthesis (*mprF*), hemolysin production (*hlgAB*), and methicillin resistance (IS431 *mec*)**
- **Isogenic mutants are now under construction**

Conclusions

- **Emergent resistance to CF-301 (+/- DAP) was not observed in the rabbit IE model**
- **Modest shifts in CF-301 MICs of only up to 2-fold were observed; we do expect that 2-fold shifted MIC variants will remain susceptible to the clinical CF-301 dose of 0.25 mg/kg, based on previously presented exposure target attainment studies and PK modeling (5)**
- **CF-301 has a low propensity for resistance in this model, consistent with the findings from previous in vitro serial passage experiments**
- **CF-301 suppresses the appearance of DAP resistance when used in combination**

References

1. Schuch et al., 2014. J Infect. Dis. 209:1469-78
2. Oh et al., 2017 Low Propensity of Resistance Development *in vitro* in *Staphylococcus aureus* with Lysin CF-301 (ASM 2017 Poster #Friday-330)
3. Microbiology Data for Systemic Antibacterial Drugs – Development, Analysis, and Presentation, Guidance for Industry (FDA-CDER, 2016)
4. Xiong et al., 2011. AAC. 55:5325-5330
5. Rotolo et al., 2016 (ASM 2016 Poster)