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Lysin CF-301 Demonstrates In Vitro Synergy with Conventional Antibiotics against *Staphylococcus aureus*

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ABSTRACT

Background: CF-301 is a novel, recombinantly-produced bacteriophage-derived lysin (cell wall hydrolase) and is the first agent of this class to enter Phase 2 of clinical development in the US for the treatment of bacteremia including endocarditis due to *S.aureus*. While the intended clinical use for CF-301 is in combination with standard of care antibiotics, including daptomycin, vancomycin, and semi-synthetic penicillins, additional antistaphylococcal antibiotics may be considered. In the current study, we evaluated the in vitro activity of CF-301 combined with up to 12 antibiotics including those currently used to manage serious staphylococcal infections and others that have important therapeutic roles in less serious infections.

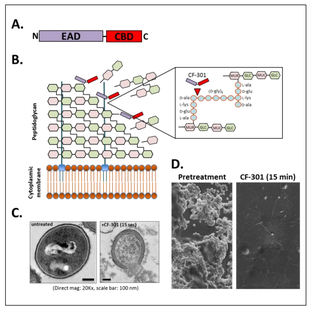
Methods: MICs were determined by broth microdilution (BMD) in human serum (HuS) and aa AST medium, consisting of cation-adjusted MHB with 25% horse serum and 0.5 mM DTT (caMHB-HSD), recently endorsed by the CLSI for use with CF-301 (1). Synergy was assessed by checkerboard microdilution using the fractional inhibitory concentration index (FICI) for each combination. An FICI mean was derived from each checkerboard based on two consecutive FIC values along the growth/no growth interface. Synergy was defined as an FICI of ≤0.5; strongly additive was >0.5-<1; indifference was 1 to <2; and antagonism was ≥2.

Results: CF-301 synergized with daptomycin , vancomycin, semi-synthetic penicillins (oxacillin and nafcillin) , a first generation cephalosporin (cefazolin) and both clindamycin and azithromycin against the majority of MSSA and MRSA strains tested in both AST media and HuS (in >95% of assayed combinations). Synergy was observed at a lower frequency with either linezolid (13/20 strains) or televancin (13/20 strains). For levofloxacin, gentamicin, and sulfamethoxazole-trimethaprim the majority of interactions were either strongly additive or indifferent (45% and 46% of combinations, respectively).

Conclusion: The broadly synergistic activity of CF-301 with many conventional antistaphylococcal antibiotics against MSSA and MRSA suggests that CF-301 may afford therapeutic benefit by potentiating the activity antibiotics to treat serious infections for which there is an unmet medical need to improve outcomes.

INTRODUCTION

Hallmark features of CF-301 include a rapid bacteriolytic effect against a range of *S. aureus* isolates, potent anti-biofilm activity, a low propensity for resistance, and synergy with antibiotics (2). To support the intended clinical use of CF-301 in addition to antistaphylococcal antibiotics (3), we sought to extend our previous synergy studies to include a broader range of antibiotics from different classes tested in both the CF-301 AST medium (caMHB-HSD) and in human serum.



Characteristics of lysins including CF-301. **(A)** Lysins have an N-terminal enzymatically active domain (EAD) fused to a C-terminal cell wall binding domain (CBD) (4). **(B)** Lysins rapidly degrade cell wall peptidoglycan to trigger lysis. The CF-301-sensitive bond is indicated. **(C)** Bacteriolytic effect of CF-301. **(D)** Removal of catheter biofilm by CF-301.

METHODS and RESULTS

Checkerboard assays were used to determine the interaction and potency of CF-301 in combination with a range of antibiotics . To establish checkerboards, each of the 2 antimicrobial agents to be tested were serially diluted in a 2-dimensional fashion (one agent across the x-axis, and one across the y-axis) to include all combinations over a specified clinically relevant range based on single-agent MIC values (5). Each combination was tested against 10 MSSA and 10 MRSA strains in AST medium; a subset of strains were also tested in 100% human serum. The MIC for each strain is presented below in either the new AST medium for CF-301 or human serum.

Table 1. Single Agent MIC Values (μg/mL) in caMHB-HSD (CF-301 AST Medium) and Human Serum[1]

Resistance	Strain #	Antibiotics[2] CF-301	DAP	VAN	OXA	LZD	TLV	NAF	CFZ	CLN	AZN	LEV	GM	SXT
MRSA	NRS 271	0.25	0.5	1	64	16	2	16	32	32	0.031	64	0.5	4
	NRS 100	0.5	0.5	1	256	2	4	64	256	0.25	0.062	0.5	0.125	32
	ATCC 43300	1	0.5/16	1/1	8/128	1	4	2/8	16	>512/>1024	>512/>512	0.25/0.5	0.5/0.25	4/16
	HPV 107	0.5	0.5	1	8	2	2	2	16	2	0.062	0.25	0.5	8
	CAIRD 426	0.5	1	1	4	2	4	16	8	4	0.031	0.25	32	16
	JMI 227	0.25	0.5/16	1/1	8/32	2	2	4/16	2	2/16	2/2	16/64	0.25/0.5	8/16
	JMI 1280	0.5	1/16	1/1	256/1024	2	4	256/1024	16	>512/>1024	>512/>512	32/64	0.5/0.5	8/16
	JMI 4789	0.5	1/16	1/1	64/128	2	4	4/32	4	2/32	0.031/0.031	0.25/0.5	0.5/0.5	8/32
	MW2	0.5	1/16	1/1	64/128	2	2	4/128	4	2/16	0.062/0.062	0.25/0.25	0.5/0.125	8/16
	ATCC 33591	0.5	1	2	256	2	2	64	128	>512	>512	0.25	1	32
MSSA	ATCC BAA-1718	0.5	0.25	0.5	1	2	2	0.5	0.5	0.5	0.031	0.25	1	8
	NRS 107	0.5	0.25	1	1	1	4	0.5	1	0.5	0.015	0.25	0.5	16
	NRS 143	0.5	0.25	1	0.5	2	4	0.5	1	2	0.062	0.25	1	4
	NRS 112	0.5	0.125	1	0.5	2	4	0.25	1	2	0.062	0.25	1	8
	NRS 161	0.5	0.25	1	0.5	2	2	0.25	1	2	0.062	0.125	0.5	4
	NRS 111	0.5	0.25/16	1/1	0.5/4	2	4	0.5/4	0.5	2/32	0.062/0.031	0.25/0.25	1/0.062	8/16
	ATCC 29213	0.5	0.25/16	0.5/1	0.5/4	2	4	0.5/4	1	1/32	0.062/0.032	0.125/0.25	0.5/0.062	8/16
	ATCC 49521	0.5	0.5/16	1/1	1/64	2	2	0.25/8	1	2/32	0.031/0.031	0.125/0.25	0.5/0.125	8/32
	JMI 2559	0.5	0.25/16	1/1	0.5/4	2	2	0.5/4	0.5	2/32	0.062/0.062	0.125/0.25	1/0.25	8/32
	JMI 3126	0.5	0.25/16	1/1	0.5/4	2	4	0.5/4	1	1/32	0.062/0.062	0.25/0.25	1/0.25	8/32

[1]The MIC value for each agent determined in caMHB-HSD (CF-301 AST medium) is shown. For a subset of 5 MSSA and 5 MRSA strains a second MIC value, determined in 100% human serum, is indicated after the slash mark (i.e., MIC determined in caMHB-HSD/MIC determined in human serum).
[2]Agent abbreviations are as follows: DAP (daptomycin), VAN (vancomycin), OXA (oxacillin), VAN (vancomycin), LZD (linezolid), TLV (televancin), NAF (nafcillin), CFZ (cefazolin), CLN (clindamycin), AZN (azithromycin), LEV (levofloxacin), GM (gentamicin), and SXT (trimethoprim-sulfamethoxazole)

Using the single-agent MIC values determined above in caMHB-HSD, the following series of checkerboard assays were performed. FICI values are reported.

Table 2. FICI values in caMHB-HSD

Resistance	Strain #	Antibiotics DAP	VAN	OXA	LZD	TLV	NAF	CFZ	CLN	AZN	LEV	GM	SXT
MRSA	NRS 271	0.254	0.5	0.563	0.531	0.5	1.063	0.5	0.375	0.312	0.625	1.063	1.031
	NRS 100	0.5	0.5	0.5	0.5	0.5	0.313	0.5	0.375	0.312	1.031	0.375	0.75
	ATCC 43300	0.375	0.5	0.313	0.75	0.5	0.375	0.5	n.d.	n.d.	1.031	1.031	0.531
	HPV 107	0.375	0.5	0.375	0.75	1.031	0.375	0.37	0.5	0.375	0.5	1.031	0.75
	CAIRD 426	0.375	0.313	0.313	0.5	0.5	0.375	0.313	0.25	0.5	0.563	0.5	0.75
	JMI 227	0.5	0.281	0.375	0.625	0.5	0.375	0.75	0.375	0.5	1	0.508	1
	JMI 1280	0.5	0.5	0.156	0.5	0.75	0.5	0.188	n.d.	n.d.	1	1	0.75
	JMI 4789	0.375	0.5	0.375	0.5	0.5	0.375	0.5	0.375	0.375	0.75	0.508	0.504
	MW2	0.375	0.5	0.375	0.625	1.031	0.25	0.5	0.5	0.375	1	0.75	1.031
	ATCC 33591	0.5	0.5	0.5	0.375	0.504	0.5	0.25	n.d.	n.d.	1	1	0.508
MSSA	ATCC BAA-1718	0.5	0.75	0.5	0.5	0.5	0.375	0.75	0.5	0.375	1	1.031	0.508
	NRS 107	0.5	0.5	0.375	0.75	0.5	0.5	0.5	0.375	0.312	0.516	0.508	1.031
	NRS 143	0.5	0.5	0.5	0.503	0.5	0.5	0.75	0.5	0.312	1	1.031	1.031
	NRS 112	0.75	0.75	0.312	0.625	0.75	0.5	0.5	0.375	0.5	1.031	1.031	0.625
	NRS 161	0.5	0. 5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.625	0.75	0.75
	NRS 111	0.5	0.625	0.375	0.375	0.5	0.25	0.5	0.5	0.375	1	0.625	0.75
	ATCC 29213	0.375	0.5	0.312	0.625	0.75	0.312	0.5	0.375	0.5	0.75	1.031	0.516
	ATCC 49521	0.5	0.5	0.5	0.5	0.5	0.375	0.5	0.375	0.5	0.75	0.563	1.031
	JMI 2559	0.375	0.5	0.315	0.625	0.5	0.25	0.5	0.5	0.5	0.563	0.5	1.031
	JMI 3126	0.5	0.625	0.375	0.625	0.625	0.25	0.5	0.5	0.375	1.031	0.5	1.031

- DAP, VAN, OXA, LZD, TLV, NAF, CFZ, CLN and AZN were predominantly synergistic (>50% with FICI values ≤0.5); remainder were strongly additive; indifference was rare
- LEV, GM and SXT were predominantly strongly additive or indifferent

= synergy
= strongly additive
= indifferent

Using single-agent MIC values determined in human serum, the following series of checkerboard assays were performed. FICI values are reported.

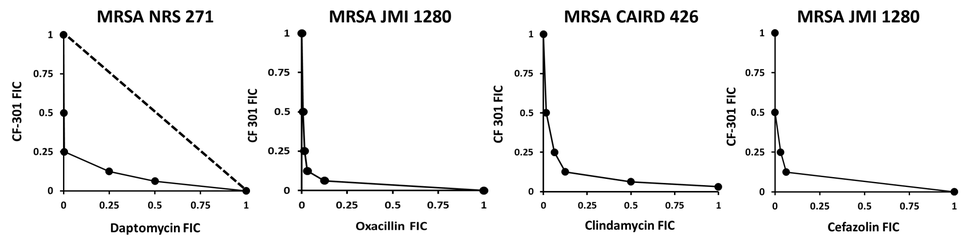
Table 3. FICI Values in 100% Human Serum

Resistance	Strain	Antibiotics DAP	VAN	OXA	NAF	CLN	AZN	LEV	GM	SXT
MRSA	ATCC 43300	0.25	0.5	0.5	0.625	n.d.	n.d.	0.5	0.75	1.031
	JMI 227	0.375	0.5	0.25	0.562	0.375	0.5	0.5	1	1
	JMI 1280	0.313	0.375	n.d.	n.d.	n.d.	n.d.	0.5	0.75	0.625
	JMI 4789	0.25	0.375	0.5	0.375	0.312	0.375	0.562	0.625	1
	MW2	0.375	0.5	0.25	0.312	0.5	0.375	0.562	1	1
MSSA	NRS 111	0.375	0.5	0.625	0.5	0.5	0.375	0.5	0.625	1.031
	ATCC 29213	0.375	0.5	0.312	0.5	0.25	0.375	0.375	1	1
	ATCC 49521	0.375	0.375	0.507	0.5	0.25	0.375	0.5	0.508	0.75
	JMI 2559	0.375	0.375	1	0.562	0.25	0.375	0.5	1	1.03
	JMI 3126	0.5	0.375	1	0.562	0.312	0.375	0.5	1	1.03

The human serum was obtained from Sigma-Aldrich and consists of pooled type AB serum from 50-70 male donors.

- Results were very similar to that obtained using the CF-301 AST medium, with the exception that LEV synergized with CF-301 against the majority of strains tested

Isobolograms are often used to represent the results of the checkerboard assay and FICI values. Isobolograms are shown for a subset of combinations tested. The dashed line is the theoretical curve expected for an additive effect.



Conclusions

- CF-301 acts synergistically in vitro with the majority of antibiotics of different classes, that are used commonly used to treat invasive staphylococcal infections
- These classes of antibiotics include: lipopeptide (daptomycin), glycopeptide (vancomycin and televancin), semi-synthetic pencillin (oxacillin and nafcillin), cephalosporin (cefazolin), macrolide (azithromycin), oxazolidinone (linezolid) and lincosamide (clindamycin)
- No antagonism (FICI ≥2) was observed with any antibiotic
- CF-301 may provide therapeutic benefit by augmenting the efficacy of antistaphylococcal antibiotics to address unmet medical needs and improve clinical outcomes

REFERENCES

1. https://clsi.org/education/microbiology/ast/ast-meeting-files-resources/
2. Schuch, R. *et al.,* 2014. The Journal of Infectious Diseases. 209:1469-78.
3. ClinicalTrials.gov (2017) Safety, Efficacy and Pharacokinetics of CF-301 vs. Placebo in Addition to Antibacterial Therapy for Treatment of *S. aureus* Bacteremia.
4. Wittekind , M., and R. Schuch, 2016. Current Opinion in Microbiology. 33:18-24.
5. Pillai, S., R. Moellering, and G. Eliopoulos. 2005. Antimicrobial combinations, p. 365-440. In V. Lorian (ed.), Antibiotics in laboratory medicine, 5th ed. Lippincott Williams & Wilkins, Baltimore, MD.