Lysin CF-301 (Exebacase) Activates Latent Host Factors in Human Blood to Potentiate Bacteriolysis

ContraFect
MOLECULAR TREATMENTS FOR INFECTIOUS DISEASE

2ND International Symposium on Antimicrobial Hydrolytic Enzymes
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SESSION ONE: GRAM-POSITIVE LYSINS AS THERAPEUTICS

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Background on ContraFect

Lysin platform: novel alternatives to conventional antibiotics

- Bacteriophage-derived therapeutic enzymes; potent, targeted and rapidly bactericidal
- Proprietary research and collaborative discovery program with The Rockefeller University
- Portfolio of lysins targeting Gram-positive (and Gram-negative) organisms

Lead program: CF-301 for treatment of *Staphylococcus aureus* bacteremia

- First and only lysin to enter Ph2 clinical trials in the U.S.
- Fast track status granted by FDA
- Phase 1: Complete – No clinical adverse safety signals observed
- Phase 2: Enrollment completed in 3Q2018

Nature
Aug 2002
Schuch et al.

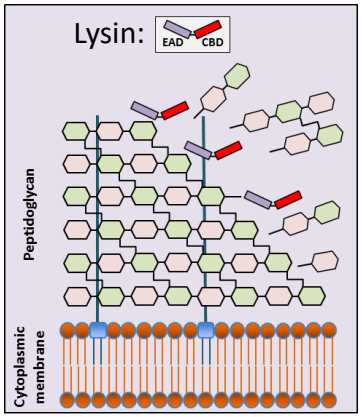
JID
May 2014
Schuch et al.

CF-301 studies at ContraFect

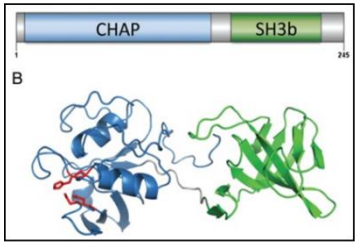
- In vitro antimicrobial activity (and anti-biofilm activity) of lysin CF-301
- Efficacy of lysin CF-301 in addition to daptomycin (DAP) or vancomycin (VAN) in a rabbit model of infective endocarditis (IE) due to MRSA
- CF-301 may improve clinical outcomes in patients with biofilm-associated infections, such as infective endocarditis

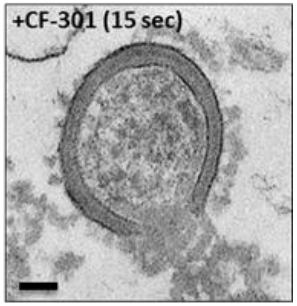
CF-301 Overview: Hallmark Properties

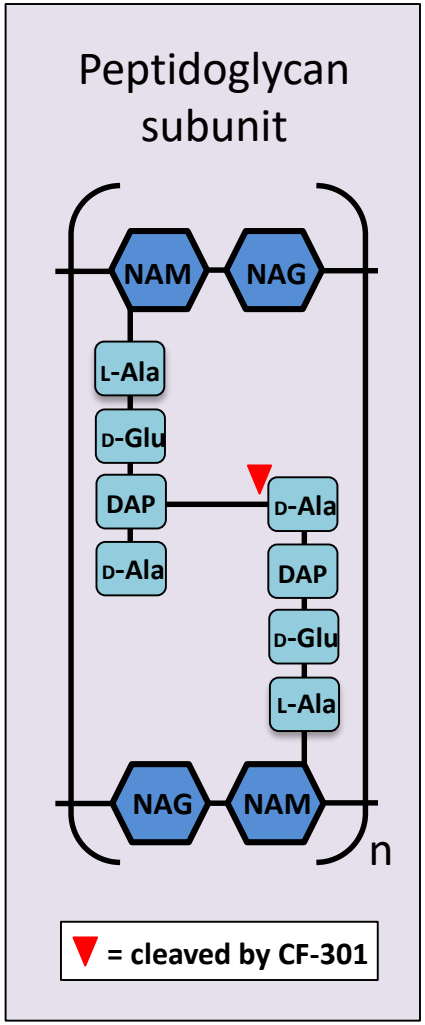
- Novel MOA: peptidoglycan hydrolysis and osmotic lysis
- Modular protein design
- Potent and rapidly bactericidal activity
- Targeted killing (species specificity)
- Eradicates biofilms
  - Kills persisters and SCVs
- Synergy with antibiotics
- Extended postantibiotic effect
- Low propensity for resistance
- Suppresses antibiotic resistance
- **High-level activity in human blood matrices**



CF-301 Structure



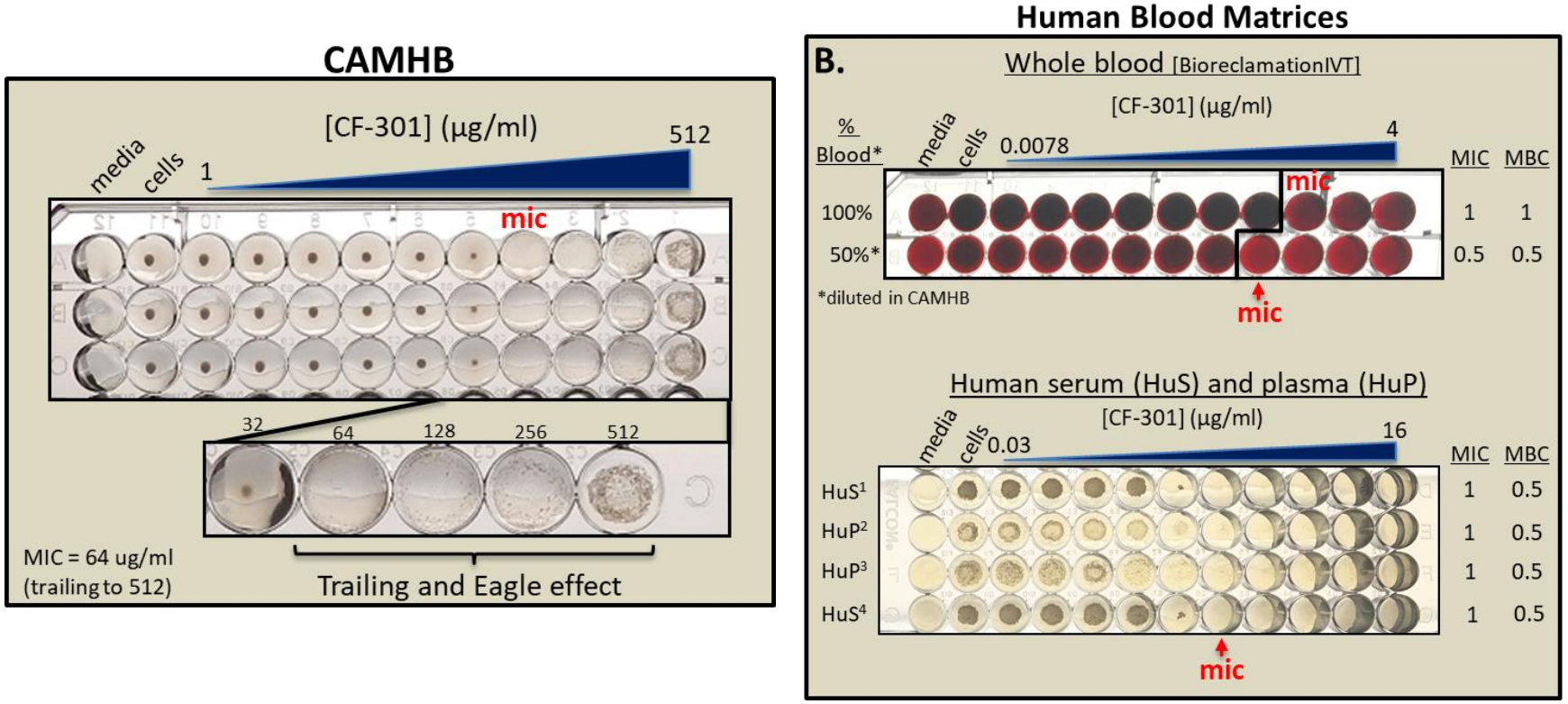




Fischetti et al., 2006 (Nat Biotechnol 24:1508-11)
Gilmer et al., 2013 (AAC, 57:2743-50)
Schuch et al., 2014 (JID, 209:1469-78)
Wittekind and Schuch, 2016 (COM, 33:18-24)
Schuch et al., 2017 (AAC, e02666-16)
ASM (Microbe), ECCMID posters (2014-2017)

CF-301: Potentiation of Activity in Blood

During development of an AST method to support clinical trials, we observed far more potent activity in human blood matrices compared to standard AST media (i.e., caMHB):



- The activity of CF-301 in caMHB does not reflect potency observed in human blood, plasma, and serum

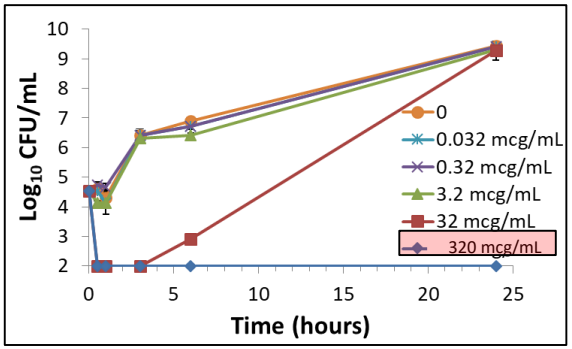
CF-301: Potentiation of High-Level Activity

A comparison of CF-301 activity in caMHB and blood matrices was performed in the time kill and MIC formats

Time kills, *S. aureus* MW2:

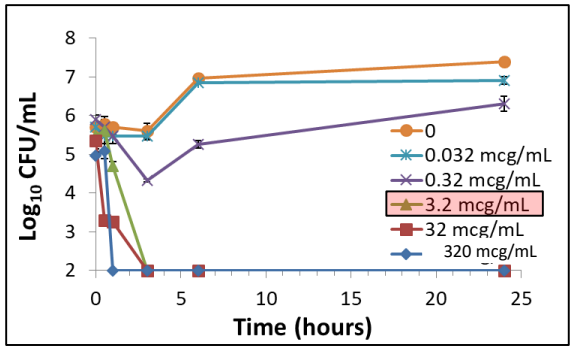
= minimum sterilizing concentration

caMHB



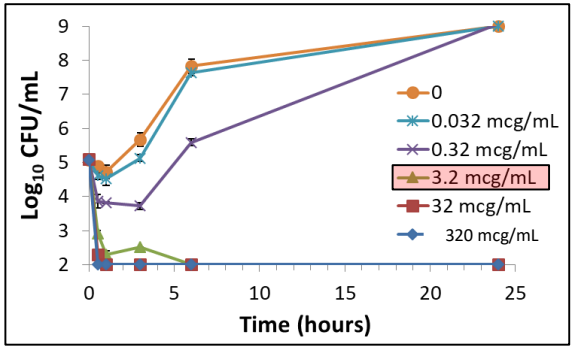
Mean, 10 replicates

whole blood



Mean, 10 individuals, citrate

serum



Mean, 10 individuals, 4 pooled samples

- 100-fold difference also observed in 15 additional strains (no difference for VAN)

MIC range analysis (*clinical and lab strains*):

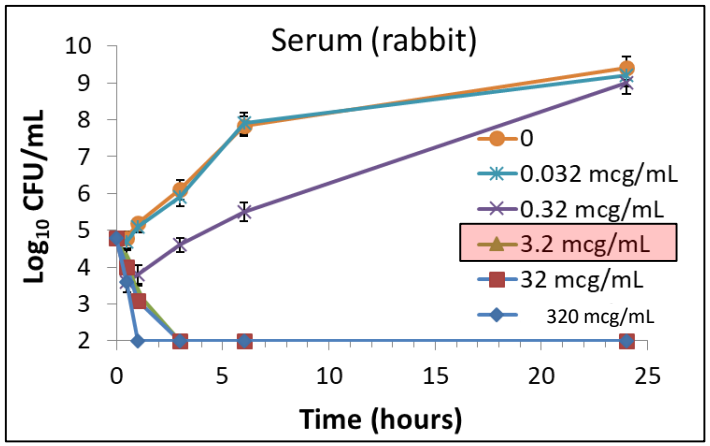
S. aureus type	N	MIC in caMHB (µg/mL)			MIC in Human serum (µg/mL)		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
MSSA (clinical, 2011)	74	16	32	8-32	0.5	1	0.25-1
MRSA (clinical, 2011)	75	32	32	2-128	0.5	1	0.25-2
Other* (lab strains)	22	4	32	0.5-32	0.5	1	0.25-2

Human pooled serum, broth microdilution method

- MICs were 32- to 64-fold lower in human serum (including data from 61 additional sources)

CF-301: Hierarchy of Activity in Animal Blood

Time-kill and MIC studies extended to matrices of different animals:

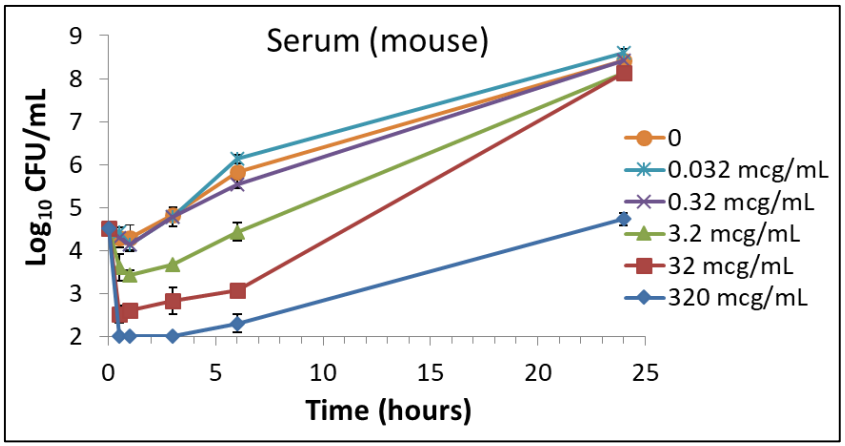


Rabbit=Human (Dog, Horse)

MIC_{90}=1 µg/mL

Rat

MIC=16 µg/mL



Mouse < caMHB

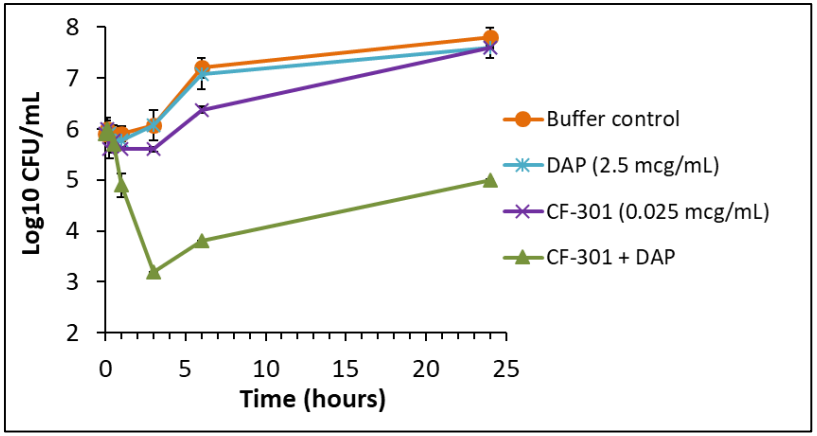
Mouse MIC_{90}= 64 µg/mL

- Horse serum is the basis for our CLSI-approved AST format
- Complement inactivated serum supports potentiation
- Anticoagulants do not affect activity
- Delipidized serum does not support potentiation effect*

CF-301: Synergy with Antibiotics in Serum

Time-kill and checkerboard studies used to examined synergy with DAP or VAN

Time-kill curves (CF-301+DAP)



Checkerboard assays (CF-301+DAP)

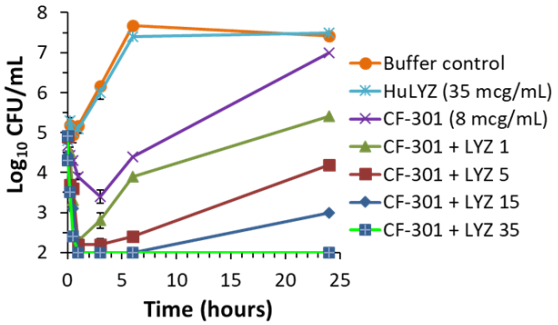
Strain number	Human Serum		caMHB	
	ΣFIC_{min}	ΣFIC_{avg}	ΣFIC_{min}	ΣFIC_{avg}
NRS 271	0.25	0.39	0.38	0.5
NRS 100	0.25	0.29	0.5	0.75
ATCC 43300	0.25	0.29	0.5	0.87
HPV 107	0.38	0.44	0.5	0.64
CAIRD 456	0.38	0.44	0.5	0.75
JMI 227	0.25	0.29	0.5	0.64
JMI 1280	0.25	0.29	0.5	0.57
JMI 4789	0.25	0.29	0.5	0.64
MW2	0.25	0.5	0.5	0.63

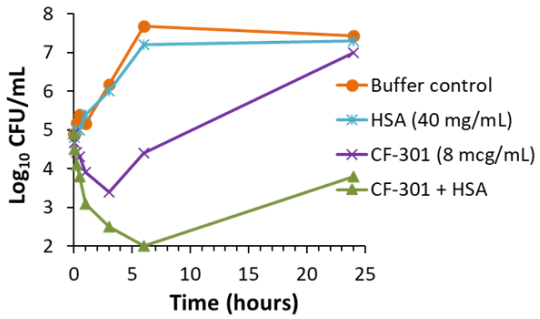
- Synergy in both formats with DAP and (not shown) VAN
- Minimum synergistic concentration in serum (0.025 ug/mL) was 160× lower than in caMHB (Schuch et al., 2014, JID)
- Findings extend to range of strains in both formats

CF-301: Synergy with Human Blood Components

Hypothesis: CF-301 synergizes with and/or activates host factors in human blood

- Features of components: 1) protease-sensitive; 2) inactivated at temperatures >80°C; 3) abundant
- Used a checkerboard assay screen for synergy of blood components with CF-301
- Native and recombinant forms of human serum albumin and human neutrophil-derived lysozyme
- Confirmed in time-kill (below) and lytic assay:





Agent	Description	ΣFIC_{min}
β-Defensin 3	Human AMP (hBD-3)	1
LEAP-1	Human AMP	0.75
LEAP-2	Human AMP	1
LL-37	Human AMP	1
LL-18-37	Derivative of LL-37	≥1.16
Lactoferrin	Human milk	≥1.16
Lactoferrin	Bovine colostrum	≥1.16
Lactoferricin H	Human AMP	≥1.16
Histatin-5	Human AMP	≥1.16
HNP-1	Human AMP	1
HNP-2	Human AMP	≥1.16
factor IV 18	Human AMP	≥1.16
Lysozyme	Human, recombinant	≤0.05
Lysozyme	Hen egg-white	≤0.563
Lysozyme	Human neutrophil	≤0.056
Serum albumin	Human, fraction V	≤0.086
Serum albumin	Human, recombinant	≤0.1
Serum albumin	Rabbit, from serum	≤0.1

CF-301: Recapitulation of the Blood Effect

Supplement media with blood factors (in presence of CF-301) to recapitulate the 32-fold MIC reduction in serum compared to non-supplemented media

- HSA + HuLYZ support full potentiation (Green)
- Rabbit SA is equivalent to HSA
- Rodent SA does not support potentiation at physiological level (20 mg/mL), but can at 40 mg/mL
- HSA or MSA (to 40 mg/mL) added to murine serum causes 16-fold MIC decrease (not shown)

Supplementation	Fold decrease in CF-301 MIC compared to base media with no supplementation	
	caMHB	caMHB/HiHuS
HuLYZ (10 µg/mL)	2	2
HSA (40 mg/mL)	16	8
HuLYZ (10 µg/mL) + HSA (40 mg/mL)	32	32
Rabbit SA (40 mg/mL)	16	8
Rat SA (20 mg/mL)	2	1
Rat SA (40 mg/mL)	8	16
Mouse SA (20 mg/mL)	1	2
Mouse SA (40 mg/mL)	8	16

CF-301: Potential Interaction with HSA

Western blot analyses (anti-CF-301 antibodies) of MIC well samples from different incubation conditions

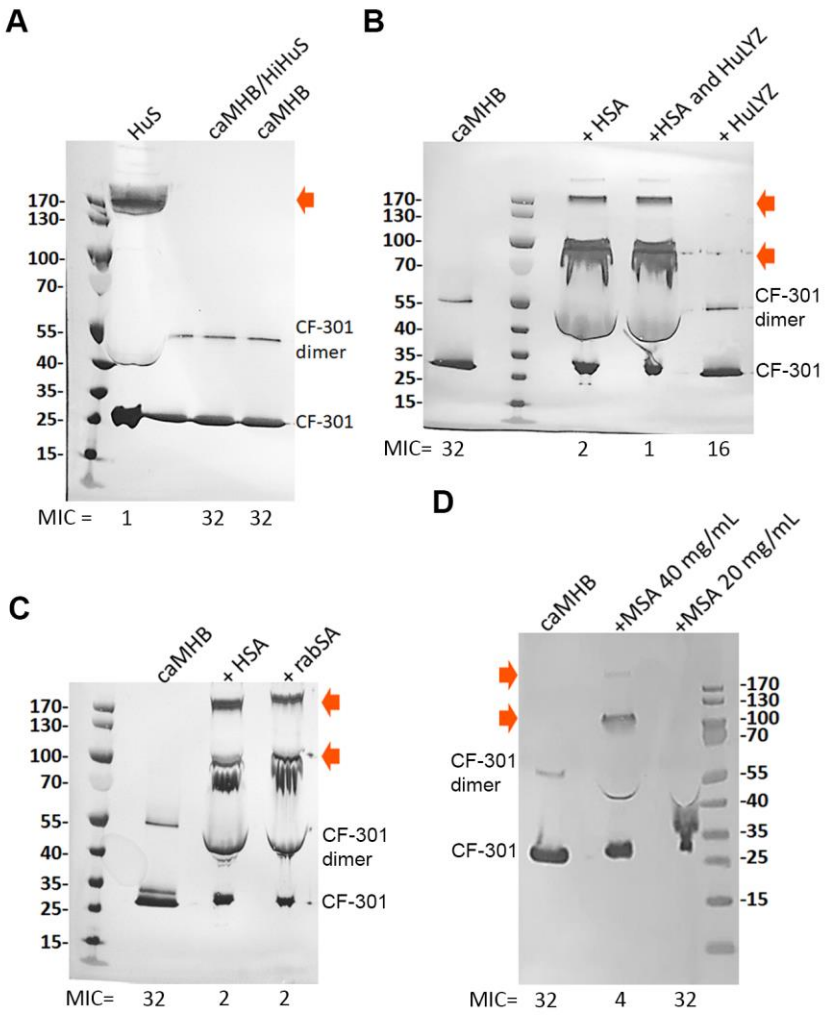
(A) In HuS, we see CF-301 monomer, dimer + 170 kDa band (mass spec indicates that HSA + CF-301 are the most abundant proteins)

(B) Supplementation of caMHB with HSA lowers the MIC and high M_r bands appear

(C) Supplementation with Rabbit Sa (40 mg/mL) result in low MICs and high M_r bands

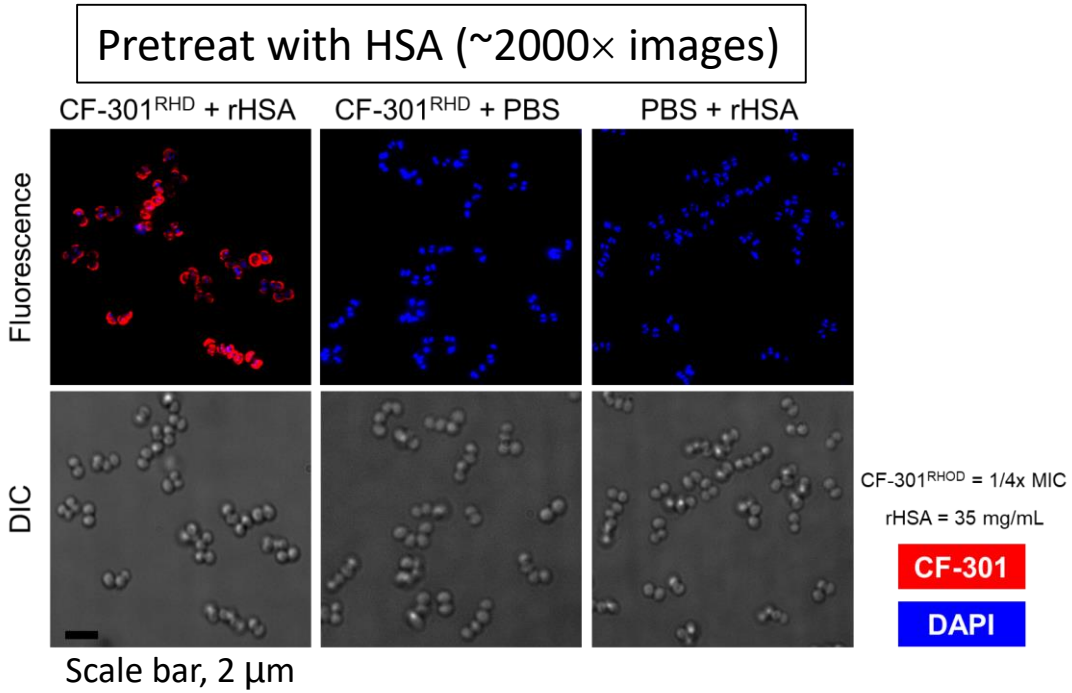
(D) Supplementation with Mouse SA produces low MICs and high Mr bands only at 40 mg/mL (supraphysiological level)

- Potential interaction with HSA (high M_r bands) associated with low MIC values



CF-301: CF-301 Leverages HSA Activity

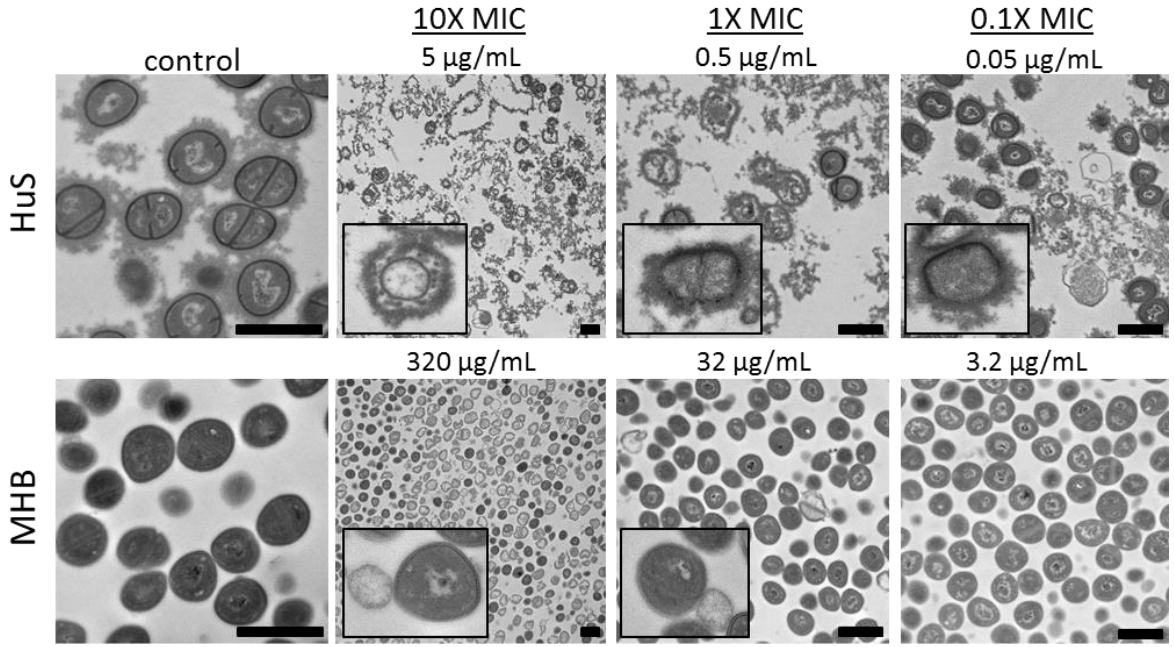
Fluorescence microscopy used to test the effect of 30 min pretreatment of *S. aureus* MW2 with rHSA on subsequent labeling with CF-301^{RHODAMINE}



- Pretreatment with HSA promotes high-level CF-301 surface labeling
- Similar result for pretreatments using human or rabbit serum, but not with rodent

CF-301: Bacteriolysis in Human Serum

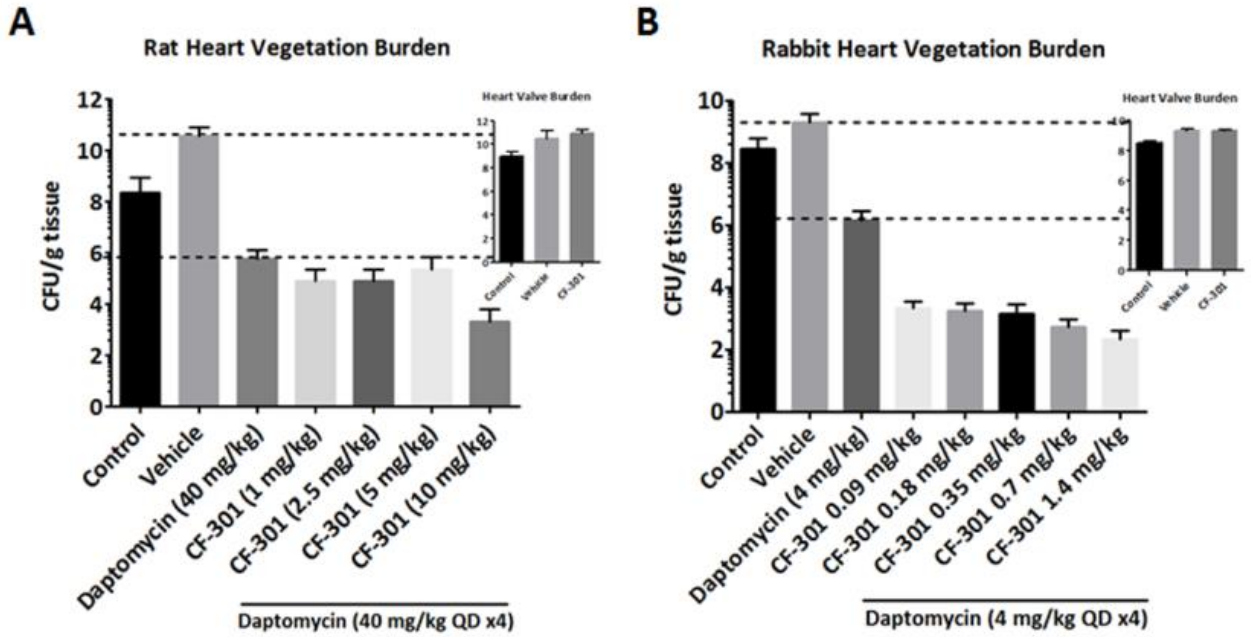
TEM after 15 min treatments with either buffer or CF-301 (10×, 1×, 0.1× MIC) in serum or MHB:



- Classic images of “membrane bubbling” at 10× and 1× MIC in MHB
- In HuS, we see circumferential dissolution of the cell wall, with staphylococci (and debris) encased in a proteinaceous sheath (“capsule-like shield”), possibly including HAS
- HSA may act to concentrate CF-301 at the staphylococcal cell surface

CF-301: In Vivo Validation of Ex Vivo Findings

The MRSA infective endocarditis (IE) model was used to compare efficacy profiles of CF-301 in animals for which ex vivo studies predicted synergy with host factors (i.e., rabbits) vs. in animals for which synergy was not expected (i.e., rats)



- In rat IE model, single CF-301 dose of 10 mg/kg administered with DAP HTD equivalent (40 mg/kg) was required for ≥6-$\log_{10}$ decrease compared to vehicle control
- In rabbit model the same 6-log10 decrease was achieved at total dose of 0.09 mg/kg in combination with DAP
- Comparable synergistic efficacy was observed at >50 fold lower doses in the rabbit vs the rat model

Summary and Conclusions

- We describe the unique ability of CF-301 to leverage/activate latent antimicrobial host factors in human blood to potentiate MRSA bacteriolysis
- Our working hypothesis holds that the mechanism of synergy is based on improved accumulation kinetics for CF-301 at the bacterial cell surface mediated by binding to HSA, resulting in more rapid and efficient killing; the activity of CF-301, in turn, facilitates access of HuLYZ to nascent peptidoglycan
- Our findings distinguish CF-301 from small molecule antibiotics which typically exhibit diminished systemic activity because of serum protein binding
- The influence of blood matrices (including human) on CF-301 activity is particularly important considering the intended clinical plan to develop CF-301 for systemic use
- The unique properties of CF-301 which enable bactericidal potentiation of antimicrobial activity via activation of 'latent' host factors in human blood may have important therapeutic implications for the durable improvements in clinical outcomes of serious, antibiotic-resistant staphylococcal infections

Acknowledgements

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