Direct Lytic Agents (DLAs), a Novel Family of Antimicrobial Agents, Exert Potent *in vitro* Bactericidal Activity Against Gram-negative (GN) Pathogens Which Cause Pulmonary Infections in CF Patients, Including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*

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BACKGROUND

Gram-negative (GN) bacteria including *Pseudomonas aeruginosa* are common and problematic pathogens for persons with cystic fibrosis (pwCF) and are associated with pulmonary exacerbations (PEx) and decline in lung function and mortality. Traditional antibiotics are largely ineffective in eradicating such bacteria from the lungs of CF patients. Although recent data indicate that triple combination CFTR modifier therapy (elexacaftor-tezacaftor-ivacaftor) affords significant improvements in lung function and reductions in PEx, the long-term impact of this treatment on airway infection in pwCF is not known. Given the deleterious effects of chronic airway infection (in particular by GN pathogens) on the clinical course of CF and that infection persists despite CFTR modifier therapy, there is a continued need to evaluate new, novel modalities to treat bacterial infections for pwCF.

ContraFect is developing novel direct lytic agents (DLAs) as potential treatment modalities for multi-drug resistant (MDR) bacterial infections. DLAs are comprised of two distinct classes of biologics: lysins (cell wall hydrolases) and amurins (outer membrane-disrupting peptides). The anti-staphylococcal lysin exebacase is the first DLA to enter human clinical trials in the US and is now in Phase 3 for the treatment of *Staphylococcus aureus* bacteremia including right sided endocarditis. ContraFect also has GN-targeting DLAs in preclinical development, including lysin CF-370 and amurin peptides. Here, we report *in vitro* profiling of CF-370 and amurins against a heterogenous array of CF patient isolates obtained from each of nine GN species most commonly associated with CF. We determined MIC values, antibiofilm activity and assessed the impact of CF patient sputum on DLA activity. These studies were conducted to inform the potential for development of these agents to improve clinical outcomes for CF patients with pulmonary infections caused by GN pathogens.

METHODS

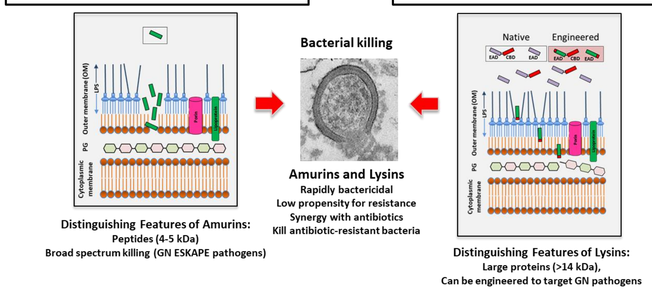
Isolates of *P. aeruginosa*, *Stenotrophomonas maltophilia, Achromobacter xylosoxidans, Achromobacter ruhlandii, Achromobacter dolens, Burkholderia cenocepacia, Burkholderia multivorans, Burkholderia gladioli* and *Pandorea apista* were obtained from the strain collection of the CFF *Burkholderia cepacia* Research Lab and Repository at the University of Michigan (BcRLR). MICs were determined by broth microdilution. The Minimal Biofilm Eradication Concentration (MBEC) assay was performed using the Calgary Biofilm Device in a manner similar to that described (Jones and Wozniak; doi: 10.1128/mBio.00864-17). Time-kill assays were performed in the presence and absence of pooled sputum from CF donors in a manner similar to that described (Zhang et a., 2005; DOI:10.1128/AAC.49.7.2921–2927.2005).

DLAs Targeting Gram-Negative Bacteria

Lysins are recombinant, purified cell wall hydrolase enzymes that elicit rapid bacteriolytic effects vs. target bacteria. As a class, lysins demonstrate key microbiological attributes: 1) potent bactericidal activity, 2) eradication of biofilms (including persister cells), 3) potent synergy with conventional antibiotics, 4) extended post-antibiotic effect and 5) low propensity for resistance development, no cross resistance with antibiotics, suppression of antibiotic resistance and the potential to re-sensitize antibiotic-resistant strains to antibiotics. CF-370 is a recently described lysin, engineered for high-level activity against *P. aeruginosa* and related GN organisms. The amurins are distinct from lysins, and represent a novel class of small peptides with potent, broad spectrum antimicrobial activity against GNs. While the mechanism of action (MOA) of amurins is different from lysins, they share many of the key hallmark microbiological attributes described above.

Amurins are non-enzymatic, bacteriolytic peptides that disrupt the outer membrane (OM)

Lysins are enzymes that cleave and degrade the cell wall peptidoglycan to facilitate osmotic lysis



DLAs Demonstrate Potent Activity Against CF Isolates

- Minimal inhibitory concentrations (MICs) for CF-370 and each of 3 amurin peptides (AM1, AM2, and AM3) were determined by broth microdilution
- Up to 30 contemporary clinical CF isolates from each of 9 of the most common Gram-negative species afflicting CF patients (Salsgiver et al., 2016, Chest, Feb;149(2):390-400) were tested
- Heterogeneous isolate sets (including MDR and XDR forms, as well as mucoid, non-mucoid, and small colony variant (SCV) types) were obtained from the BcRLR for testing

Lysin CF-370

Organism	n	MIC_{50}	MIC_{90}	MIC Range
Pseudomonas aeruginosa	20	0.25	0.5	0.06-1
Stenotrophomonas maltophilia	23	0.5	2	0.03-2
Achromobacter xylosoxidans	30	8	>32	0.06->32
Achromobacter ruhlandii	30	2	>32	0.06->32
Achromobacter dolens	29	32	>32	0.25->32
Bulkholderia cenocepacia	29	>32	>32	0.5->32
Bulkholderia gladioli	29	>32	>32	0.5->32
Bulkholderia multivorans	30	16	>32	0.06->32
Pandoraea apista	30	>32	>32	0.5->32

CF-370 MIC of QC strains: *P. aeruginosa* ATCC 27853 = 0.5 µg/mL, *E. coli* ATCC 29212 = 0.125 µg/mL

	AM1			AM2			AM3		
Organism	n	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Pseudomonas aeruginosa	30	0.25	0.5	0.016-1	0.06	0.25	0.016-0.5	0.06	0.25	0.016-1
Stenotrophomonas maltophilia	25	0.5	1	0.016-1	0.5	1	0.03-2	0.5	1	0.016-2
Achromobacter xylosoxidans	30	0.5	2	0.016-4	2	64	0.016->128	2	16	0.016->128
Achromobacter ruhlandii	30	1	4	0.125-8	2	16	0.016->128	1	128	0.06-128
Achromobacter dolens	30	1	2	0.06-4	4	8	0.016-16	2	8	0.016-16
Bulkholderia cenocepacia	30	>128	>128	0.06->128	>128	>128	0.06->128	>128	>128	0.016->128
Bulkholderia gladioli	30	8	64	0.03-128	4	8	0.016-16	32	128	0.016->128
Bulkholderia multivorans	30	128	>128	0.016->128	>128	>128	0.016->128	>128	>128	0.016->128
Pandoraea apista	30	64	>128	4->128	>128	>128	4->128	>128	>128	32->128

MICs of QC strains: *P. aeruginosa* ATCC 27853 - 1, 0.5, and 0.25 µg/mL for AM1, AM2, and AM3, respectively; *E. coli* ATCC 29212 - 1 , 0.25, and 0.125 µg/mL for AM1, AM2, and AM3, respectively.

- Uniformly potent activity (green) was observed against *P. aeruginosa* and *S. maltophilia*
- AM1 was active against *Achromobacter* spp., while CF-370, AM2 and AM3 exhibited more variability, with a broader range of MICs (yellow)
- Poor activity was observed against *Burkholderia* spp. and *P. apista*

- No differences in DLA activity were observed for MDR (**Table A**, see below) or carbapenem-resistant (**Table B**, see below) isolate subsets compared to the overall susceptibility of each species
- Similarly, no differences in susceptibility were observed for mucoid forms or SCVs (data not shown)

A

		CF-370 MIC (µg/mL)		
Organism type	MDR (n)	MIC_{50}	MIC_{90}	Range
P. aeruginosa	8	0.25	0.5	0.0625-0.5
S. maltophilia	5	0.25	0.5	0.0625-0.5

		AM1 MIC (µg/mL)		
Organism type	MDR (n)	MIC_{50}	MIC_{90}	Range
P. aeruginosa	13	0.25	0.5	0.016-0.5
S. maltophilia	8	0.5	1	0.5-1
A. xylosoxidans	14	1	4	0.5-4

B

		CF-370 MIC (µg/mL)		
Organism type	Carbapenems^{R} (n)	MIC_{50}	MIC_{90}	Range
P. aeruginosa	12	0.25	0.5	0.0625-0.5
S. maltophilia	21	0.25	0.5	0.0625-0.5

		AM1 MIC (µg/mL)		
Organism type	Carbapenems^{R} (n)	MIC_{50}	MIC_{90}	Range
P. aeruginosa	17	0.25	0.5	0.016-0.5
S. maltophilia	23	0.5	1	0.5-1
A. xylosoxidans	7	1	4	0.5-4

DLAs are Active Against Biofilms

- The capacity to form biofilms can provide bacteria an enormous advantage to establish infections, including VAP and CF lung infections, in susceptible hosts
- For this reason, we determined minimal biofilm eliminating concentrations (MBECs) using isolate sets from each GN pathogen
- Biofilms were treated for 2 hours with CF-370, AM1, AM2, or AM3

	CF-370				AM1				AM2				AM3			
Organism	n	$MBEC_{50}$	$MBEC_{90}$	Range	n	$MBEC_{50}$	$MBEC_{90}$	Range	n	$MBEC_{50}$	$MBEC_{90}$	Range	n	$MBEC_{50}$	$MBEC_{90}$	Range
Pseudomonas aeruginosa	23	1	2	0.06 - 4	24	0.5	1	0.06 - 2	23	0.25	1	0.016 - 2	23	0.125	1	0.016 - 1
Stenotrophomonas maltophilia	24	1	4	0.06 - 4	26	1	2	0.25 - 2	26	1	2	0.12 - 4	26	1	2	0.12 - 16
Achromobacter xylosoxidans	9	8	>256	1 - >256	10	0.5	1	0.12 - 2	10	2	8	0.12 - >32	10	1	2	0.12 - 8
Achromobacter ruhlandii	10	4	64	2 - 64	10	2	4	2 - 8	10	2	16	1 - 16	10	2	4	0.5 - 8
Achromobacter dolens	10	32	128	4 - 128	10	2	4	1 - 8	10	4	8	1 - 8	10	2	16	1 - 32
Bulkholderia cenocepacia	10	>256	>256	1- >256	10	16	32	2 - 32	10	16	32	2 - 32	10	16	32	1 - 32
Bulkholderia gladioli	10	256	>256	4 - >256	10	4	16	0.5 - 32	10	4	8	1 - 32	10	4	16	1 - 32
Bulkholderia multivorans	10	8	64	1 - >256	10	16	16	2 - 16	10	8	32	0.06 - 32	10	16	16	1 - 32
Pandoraea apista	10	32	64	8 - 64	10	8	16	4 - >32	10	8	16	8 - >32	10	8	8	4 - 16

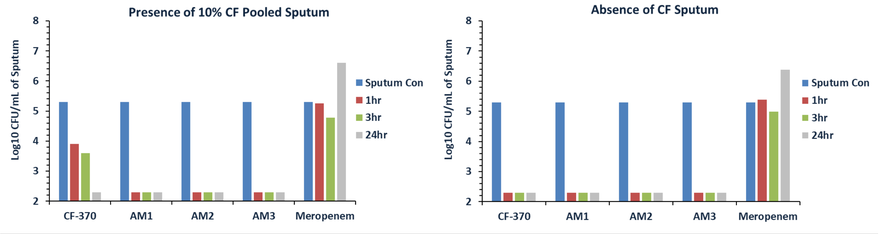
- MBEC values for *P. aeruginosa* and *S. maltophilia* were similar to MIC values, consistent with potent antibiofilm activity (green) for CF-370, AM1 and AM2 (AM3 was primarily active vs. *P. aeruginosa*)
- Variable activity was observed against isolates of some of the *Achromobacter* spp. tested (yellow), with AM1 demonstrating the most potent effect
- DLAs are poorly against biofilms produced by *Burkholderia* spp. and *P. apista*

DLAs are Active in CF Patient Sputum

- To examine the potential for CF sputum interference of DLA activity, each agent was examined using the time-kill assay format (Zhang et al., 2005; DOI:10.1128/AAC.49.7.2921–2927.2005) in the presence and absence of 10% CF patient sputum (obtained from the BcRLR)
- Three organisms (*P. aeruginosa*, *A. xylosoxidans*, and *S. maltophilia*) were tested using 4x MIC levels of each DLA (or a meropenem comparator) in media with/without sputum; cultures were incubated at 37°C for 24 hrs for quantitative plating.

P. aeruginosa

MIC (µg/mL)				
CF-370	AM1	AM2	AM3	MER
0.5	1	0.25	0.125	0.125



- DLA activity against *P. aeruginosa* was not inhibited by the presence of sputum and maintained a bactericidal effect over the 24 hour timecourse
- The DLAs were similarly active against *A. xylosoxidans* and *S. maltophilia* (data not shown)
- Meropenem was poorly active in the presence and absence of sputum

Findings and Conclusions

- The DLAs, including lysin CF-370 and amurin peptide AM1, are active against some of the most prevalent GN pathogens associated with chronic lung infections in CF patients
- The activity range includes *P. aeruginosa*, *S. maltophilia*, and *Achromobacter* spp.
- These findings suggest a therapeutic potential for the treatment of infections caused by MDR GN pathogens for which there are limited treatment options and for CF patients in particular

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