Bacteriophage-Derived Lysins Exert a Potent Bactericidal Effect Against *Pseudomonas aeruginosa*

ContraFect
MOLECULAR TREATMENTS
FOR INFECTIOUS DISEASE

Antimicrobial Peptides

Gordon Research Conference

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VP of Research

ContraFect Corporation

Yonkers, NY

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Background on ContraFect

ContraFect is a clinical-stage biotech developing differentiated, first-in-class biologics for the treatment of life-threatening and drug-resistant infections

Lysin platform: novel class of anti-infectives

- Preclinical data supports multiple advantages as therapeutics
- Proprietary research at ContraFect and collaborative research at Rockefeller University
- Portfolio of lysins targeting Gram-positive and Gram-negative bacterial pathogens

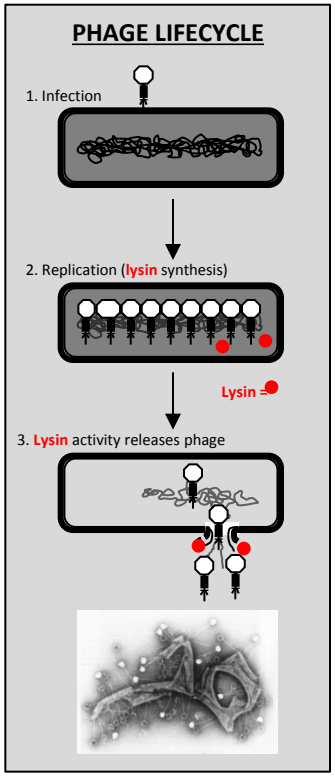
Lead program: Exebacase for treatment of *S. aureus* bacteremia and endocarditis

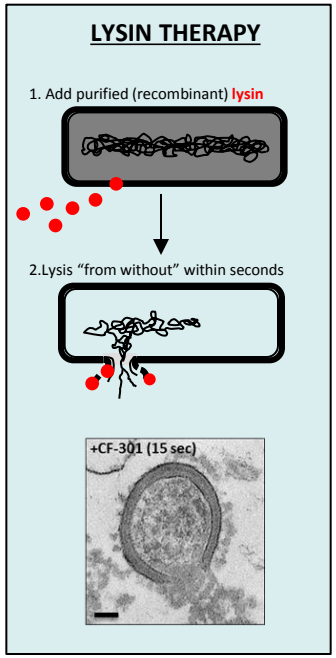
- Phase 1 complete – No clinical adverse safety signals observed
- Phase 2 ongoing – Positive Topline Phase 2 results were reported JAN2019, with higher clinical responder rates with CF-301 on top of SOC antibiotics vs SOC antibiotics alone
- Established Proof-of-Concept for lysins as human therapeutics

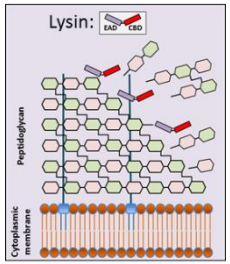
Broad pipeline of new agents

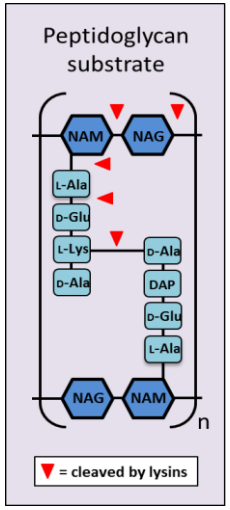
- Second generation antistaphylococcal lysin
- Novel lysins targeting the Gram-negative (GN) pathogen *Pseudomonas aeruginosa*
- Novel phage-derived lytic agents targeting a broad range of GN pathogens (ESKAPE)

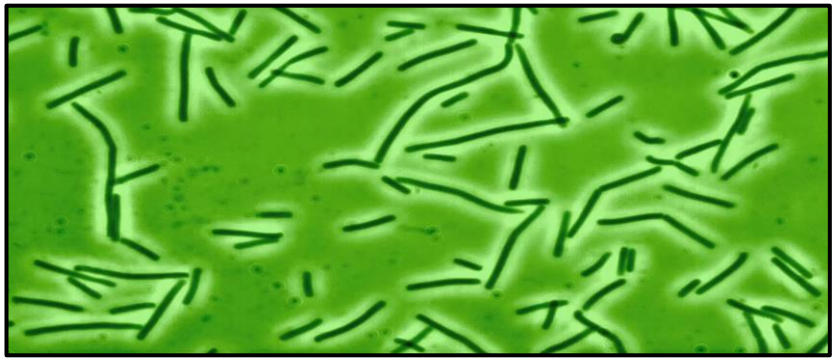
Lysins: Potential Alternatives to Conventional Antibiotics











Bacteriophage-derived, recombinantly-produced therapeutic proteins (cell wall hydrolase enzymes)

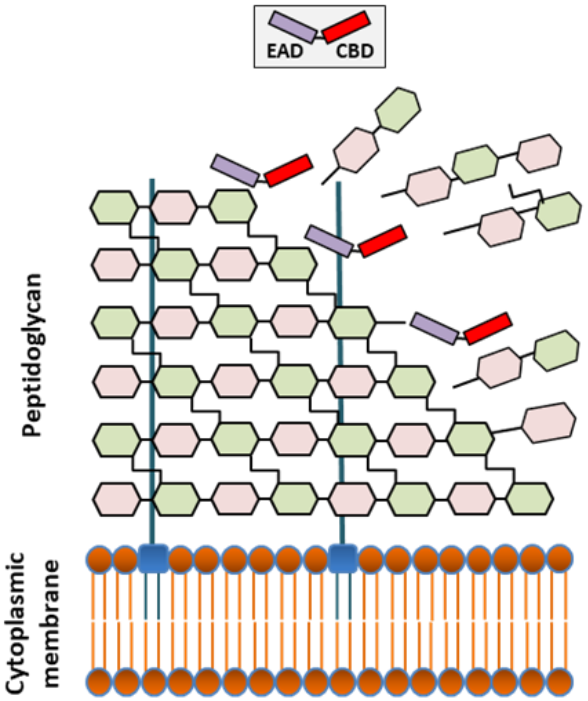
- Novel MOAs: peptidoglycan hydrolysis, osmotic lysis
- Rapidly bactericidal
- Potent ability to eradicate biofilms
- Targeted, species-specific killing
- Low propensity for resistance
- No antibiotic cross-resistance
- Synergy with standard-of-care (SOC) antibiotics
- Suppresses antibiotic resistance
- Extended post-antibiotic effect

Gram-negative (GN) lysin program (CARB-X funded)

- Discovery-stage program: engineering lysins to bypass the outer membrane and enable potent activity in human blood matrices
- Target indication: Cystic Fibrosis Pulmonary exacerbations; HABP/VABP caused by *P. aeruginosa*
- Potential to improve clinical cure rates for resistant GN infections
- Patents filed for all GN lysin candidates

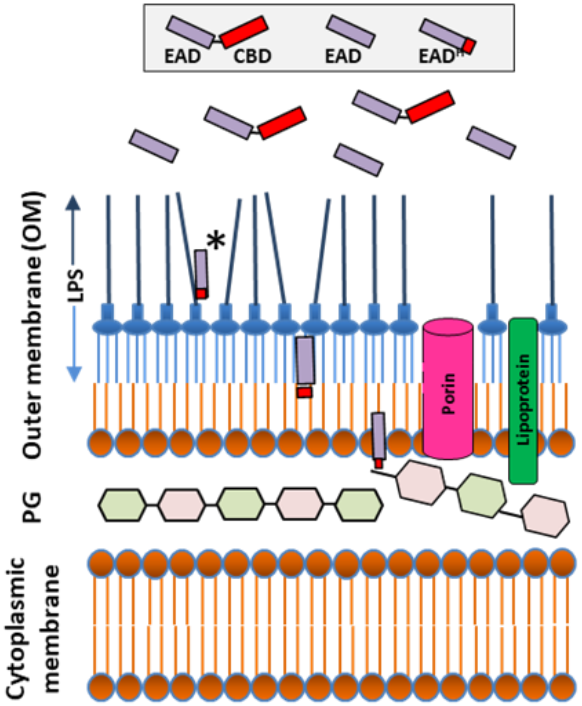
Challenges to developing Gram-negative (GN) lysins

Effective lysis by Gram-positive lysins



- Gram-positive lysins have an N-terminal enzymatically active domain (EAD) fused to a C-terminal cell wall binding domain (CBD)

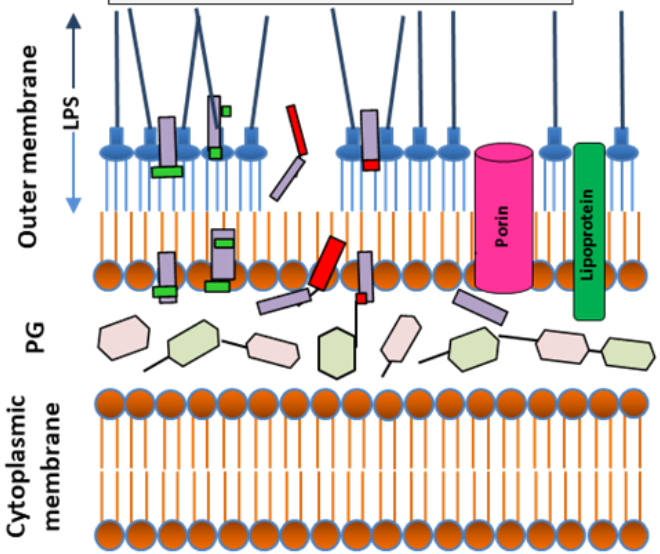
Gram-negative OM is a barrier to most* lysins



- Most purified Gram-negative lysins have no antimicrobial activity
- A subset (*) have "intrinsic" activity and are highly active in low ionic strength environments

Challenge: Activate GN lysins in human blood matrices

1. Combine with antibiotics or AMPs
2. Develop chimeras
3. Modify native sequences
4. Screen metagenome

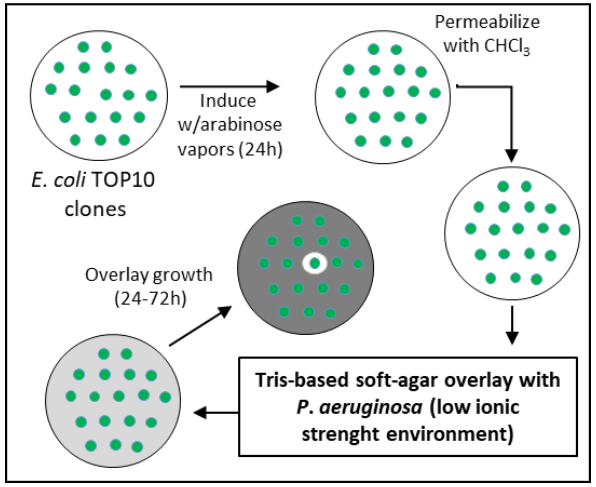


Identify and characterize native or engineered lysins active in human blood matrices and suitable for study in vitro activity and in vivo efficacy

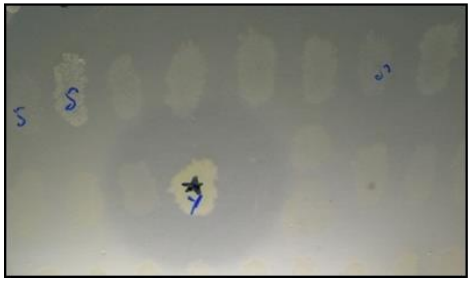
Lysin identification

Primary Screens

Over 500 phage lysins were identified in a bioinformatic analysis, cloned in *E. coli* and screened for bactericidal activity vs carbapenam-resistant *P. aeruginosa* isolate and lab strain



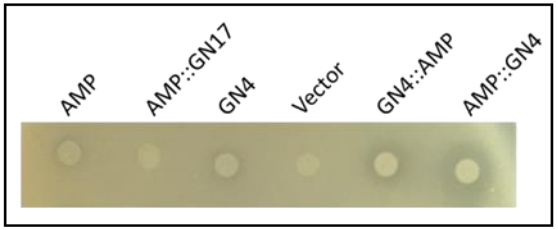
Identify lysins with intrinsic activity



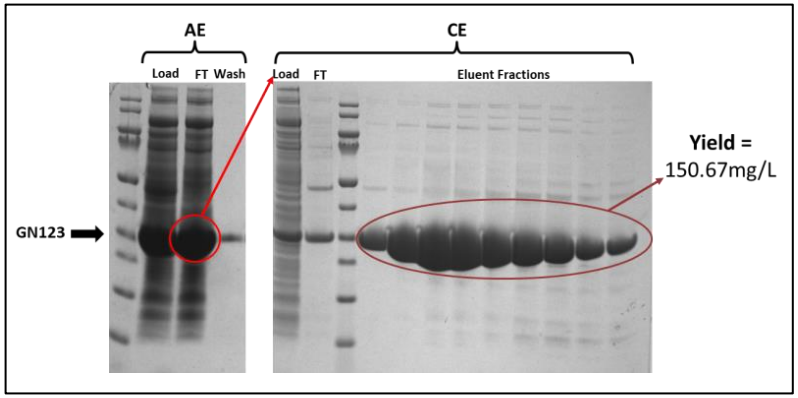
Secondary Screens

Engineer strategic protein sequence modifications and screen for activity using a modified agar overlay method in which the overlay is supplemented with human serum.

Identify highly active lysin derivatives



Purify by column chromatorgraphy



In vitro activity profile

GN Lysins Exhibit Potent In Vitro Activities

Activity in human serum (MIC assay)

	Lysin*	Type	CAA	CAA/HuS
GN4 family	GN3	Native	16	16
	GN4	Native	64	16
	GN147	Modified	16	4
	GN92	Modified	32	4
	GN146	Modified	2	2
	GN156	Modified	4	2
	GN178	Modified	8	1
	GN83	Modified	>128	>128
	GN122	Modified	2	2
	GN76	Native	64	8
	GN123	Native	8	4
	GN126	Modified	2	128
	T4LYZ	Native	>128	>128

	Lysin*	Type	CAA	CAA/HuS
GN37 family	GN37	Native	>128	16
	GN121	Modified	0.5	0.5
	GN94	Modified	16	2
	GN218	Modified	8	1
	GN11	Native	32	128
	GN13	Native	8	>128
	GN75	Modified	8	8
	GN14	Native	>128	32
	GN93	Modified	128	8
	GN328	Native	8	2
	GN7	Native	>128	128
	GN316	Native	16	<0.0625
	GN10	Native	16	8

Blue = native lysins serving as templates for purple shaded modified lysins

Purple = modified lysins derived from blue shaded lysins

- Engineered lysins have low MIC (0.5-4 µg/mL) values in serum

Potent antibiofilm activity (MBEC assay)

	Lysin	Type	MBEC (µg/mL)
GN4 family	GN3	Native	0.25
	GN4	Native	1
	GN147	Modified	0.25
	GN92	Modified	0.5
	GN146	Modified	2
	GN156	Modified	0.5
GN37 family	GN37	Native	0.25
	GN121	Modified	0.25
	GN150	Native	0.25
	GN13	Native	0.125
	GN9	Native	0.125
	GN10	Native	0.5

Lysin	Type	MBEC (µg/mL)
GN17	Native	0.125
GN76	Native	0.125
GN123	Native	4
GN126	Modified	0.125
GN83	Modified	1
GN94	Modified	2
GN80	Native	0.125
GN93	Modified	0.125
GN105	Native	4
GN108	Native	0.125
GN122	Native	1
T4LYZ	Native	>64

- MBEC values are below MICs for many GN lysins

Synergy with antibiotics (Checkerboard assay)

Antibiotic	GN37 LYSIN	GN76 LYSIN	GN108 LYSIN	GN4 LYSIN	GN92 Modified	GN121 Modified	GN147 Modified	GN150 LYSIN
Amikacin	0.125	0.281	0.156	0.531	0.375	0.375	0.375	0.313
Azithromycin	0.188	0.156	0.060	0.094	0.063	0.188	0.250	0.125
Aztreonam	0.531	0.281	0.250	0.156	0.188	0.625	0.188	0.188
Ciprofloxacin	0.281	0.281	0.281	0.250	0.281	0.313	0.281	0.250
Colistin	0.156	0.250	0.133	0.156	0.094	0.375	0.188	0.094
Fosfomycin	0.313	0.125	0.188	0.250	0.5	0.375	0.250	0.375
Gentamicin	0.313	0.313	0.188	0.375	0.375	0.375	0.375	0.375
Imipenem	0.313	0.254	0.039	0.125	0.125	0.500	0.375	0.375
Piperacillin	0.375	0.375	0.531	0.313	0.5	0.375	0.188	0.500
Rifampicin	0.281	0.281	0.125	0.156	0.094	0.313	0.281	0.094
Tobramycin	0.156	0.281	0.188	0.375	0.500	0.188	0.500	0.500

Synergy | Strongly Additive

- Broadly synergistic with many antibiotics

Non-hemolytic against hRBCs (Hemolysis assay)

	Lysin	Type	MHC (µg/mL)
GN4 family	GN3	Native	>128
	GN4	Native	>128
	GN147	Modified	>128
	GN92	Modified	>128
	GN146	Modified	>128
	GN156	Modified	>128
GN37 family	GN37	Native	>128
	GN121	Modified	>128
	GN150	Native	>128
	GN13	Native	>128
	GN9	Native	>128
	GN10	Native	>128

Lysin	Type	MHC (µg/mL)
GN17	Native	>128
GN76	Native	>128
GN123	Native	>128
GN126	Modified	>128
GN83	Modified	>128
GN94	Modified	>128
GN75	Modified	>128
GN7	Native	>128
GN11	Native	>128
GN14	Native	>128
GN54	Modified	>128

Control AMPs	MHC (µg/mL)
RR12	8
RR12polar	4
RR12hydro	32

- GN lysins exhibit no hemolytic activity

GN Lysins Exhibit Potent In Vitro Activities

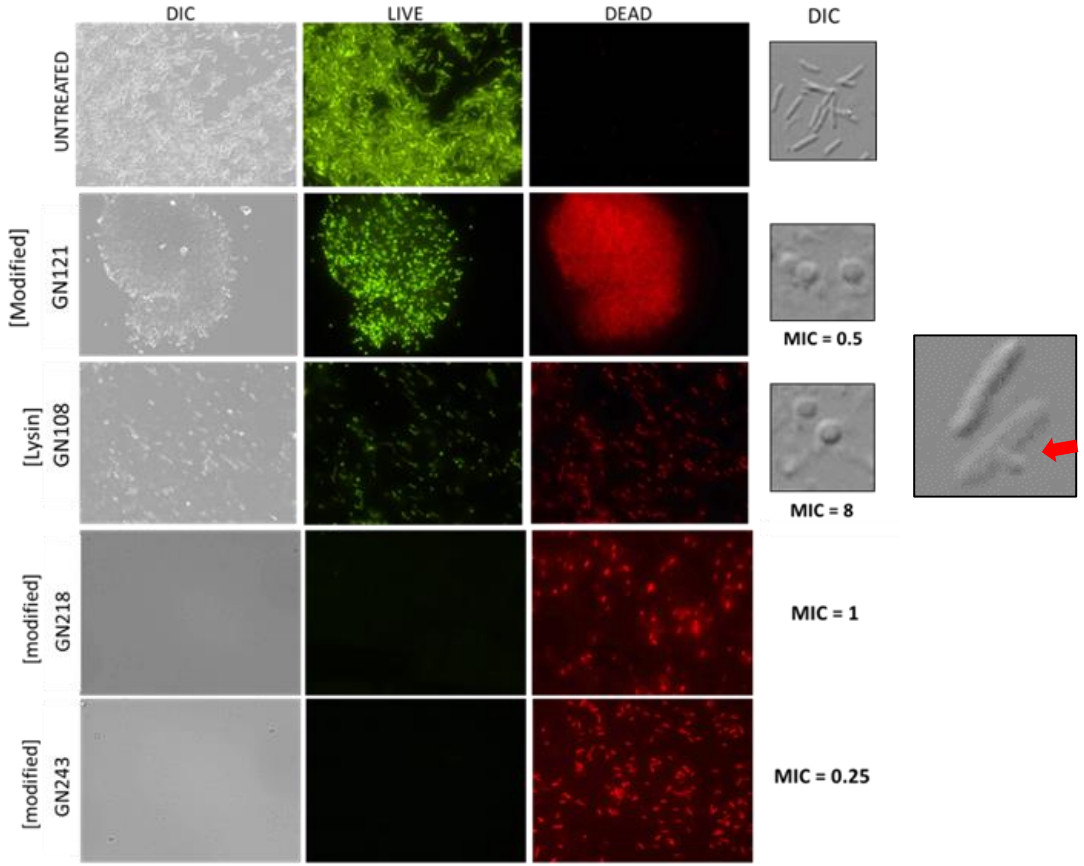
Bactericidal (Time-kill assay)

	HEPES (Log_{10} CFU/mL)		CAA/HuS (Log_{10} CFU/mL)		
GN	1hr	3hr	GN	1hr	3hr
3	<3.7	<3.7	3	5.8	<3.7
147	<3.7	<3.7	147	6.5	4.2
4	5.7	<3.7	4	6.0	<3.7
92	5.7	<3.7	92	6.2	<3.7
146	6.7	<3.7	146	5.9	4.0
156	5.7	<3.7	156	5.7	<3.7
83	<3.7	<3.7	83	5.7	<3.7
37	6.3	<3.7	37	6.2	<3.7
94	6.0	<3.7	94	6.4	<3.7
121	<3.7	<3.7	121	7.4	<3.7
150	5.7	<3.7	150	6.0	<3.7
13	6.7	<3.7	13	6.0	6.0
75	5.7	<3.7	75	5.9	<3.7
65	5.7	<3.7	65	6.0	<3.7
126	<3.7	<3.7	126	6.6	<3.7
7	7.5	6.2	7	7.0	7.0
9	6.7	5.7	9	5.6	<3.7
10	5.7	<3.7	10	6.1	<3.7
11	6.7	<3.7	11	6.7	7.0
14	<3.7	<3.7	14	5.7	<3.7
17	5.4	<3.7	17	6.4	4.2
40	7.0	5.7	40	6.6	6.7
43	6.4	<3.7	43	6.9	7.0
76	<3.7	<3.7	76	5.7	<3.7
80	7.7	6.7	80	6.7	7.0
93	6.0	<3.7	93	6.6	<3.7
105	6.6	<3.7	105	7.0	6.3
108	6.4	<3.7	108	5.7	<3.7
122	7.7	5.4	122	6.7	6.7
123	5.6	<3.7	123	6.7	<3.7
81	7.4	6.5	81	6.7	7.0
BLANK	7.7	7.2	BLANK	7.7	7.2

At a concentration of 10 µg/mL, many lysins exhibited decreases of ≥3-log10 CFU/mL. Buffer treated control ("blank") is indicated at the bottom (orange cells).

GN Lysins Kill Rapidly in 100% Human Serum

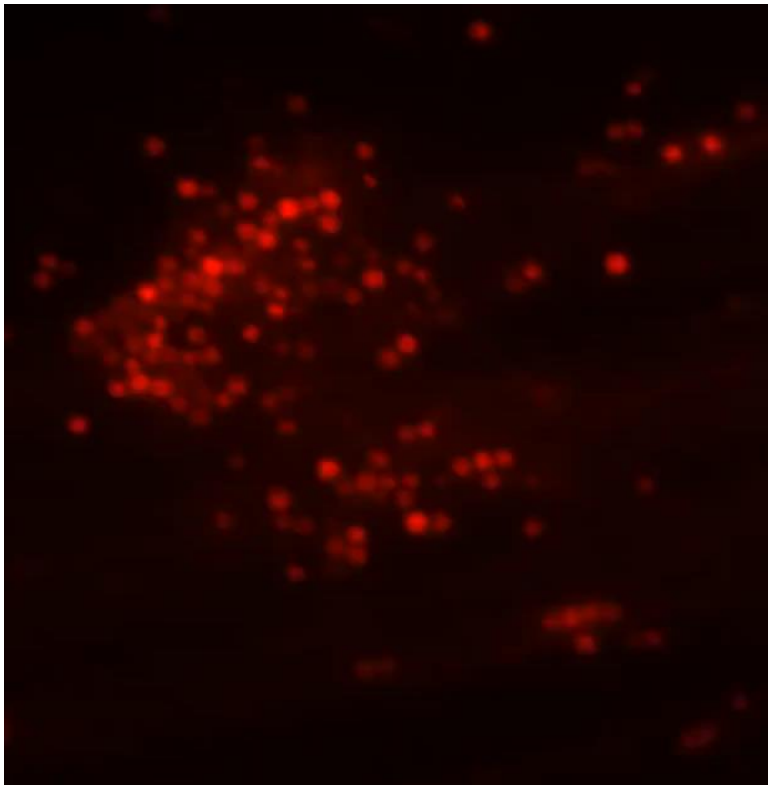
15 min treatment with buffer or lysin (10 µg/mL), LIVE/DEAD™ stained, and visualized by DIC and fluorescence microscopy (2000x mag)



- GN lysins are rapidly bacteriolytic activity in 100% human serum

Summary of GN lysin activities

Click on the picture below
View the rapid bacteriolytic effect in human serum on our website



- A screening and optimization strategy was developed to identify GN lysins with potent antimicrobial activity against *P. aeruginosa* in context of human serum
- The GN lysins identified here exhibit notable characteristics:
  - Rapid and potent bactericidal activity
  - Synergy with a range of antibiotics
  - Resensitization of antibiotic resistant strains
  - Ability to eradicate biofilm
  - Non-hemolytic
- The ability to engineer lysins to achieve activity in serum demonstrates that it is possible to develop lysins with potent activity against GN pathogens
- GN lysins represent a potential new therapeutic class of bactericidal agents to combat resistant GN pathogens through a unique mechanism of action

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