Lysin GN123 Resensitizes Carbapenem-Resistant *Pseudomonas aeruginosa* to Imipenem

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INTRODUCTION

Antibiotic-resistant Gram-negative (GN) pathogens pose a public health threat which necessitates the development of new antimicrobials with novel MOAs. Bacteriophage-derived lysins (cell wall hydrolases) are novel direct lytic agents (DLAs) currently in Phase 2 for the treatment of *S. aureus* bacteremia including endocarditis. Whereas the therapeutic use of lysins against GN pathogens has previously been precluded by the inability to efficiently penetrate the outer membrane (OM), we recently described proprietary lysins engineered to penetrate the OM and exert antimicrobial activity against *P. aeruginosa*. Here, we demonstrated the capacity of 12 GN lysins, including GN123, to synergize with imipenem and meropenem and resensitize carbapenem-resistant *P. aeruginosa* in vitro.

PURPOSE

Building upon ContraFect's broad experience with Gram-positive (GP) lysins, GN lysins are now under development for use in treating invasive *P. aeruginosa* infections. We focused here on the capacity of GN lysins to synergize with a range of antibiotics, including imipenem and meropenem. The ability to synergize with antibiotics, and in particular to drive the rensensitization of MDR organisms (including carbapenem-resistant *P. aeruginosa*), will be a key feature supporting the advancement of GN lysins for human clinical use.

METHODS

Synergy was examined in checkerboard assays in CAA media with human serum using carbapenem-resistant, -intermediate, and –susceptible clinical isolates of *P. aeruginosa*. Fractional inhibitory concentration index (FICI) values were determined for all combinations; values of ≤0.5 indicate synergy.

RESULTS: SYNERGY

Analysis of 12 lysins in combination with up to 12 antibiotics against the carbapenem-resistant clinical strain WC-452

Antibiotic	GN37	GN76	GN108	GN4	GN92	GN147
Amikacin	0.125	0.281	0.156	0.531	0.375	0.375
Azithromycin	0.188	0.156	0.060	0.094	0.063	0.250
Aztreonam	0.531	0.281	0.250	0.156	0.188	0.188
Ciprofloxacin	0.281	0.281	0.281	0.250	0.281	0.281
Colistin	0.156	0.250	0.133	0.156	0.094	0.188
Fosfomycin	0.313	0.125	0.188	0.250	0.5	0.250
Gentamicin	0.313	0.313	0.188	0.375	0.375	0.375
Imipenem	0.313	0.254	0.039	0.125	0.125	0.375
Meropenem	0.500	0.375	0.250	n.d.	0.375	0.375
Piperacillin	0.375	0.375	0.531	0.313	0.5	0.188
Rifampicin	0.281	0.281	0.125	0.156	0.094	0.281
Tobramycin	0.156	0.281	0.188	0.375	0.500	0.500

Antibiotic	GN121	GN123	GN150	GN351	GN370	GN428
Amikacin	0.375	0.25	0.313	0.250	0.125	0.281
Azithromycin	0.188	0.125	0.125	0.125	0.188	0.250
Aztreonam	0.625	0.375	0.188	0.125	0.188	0.156
Ciprofloxacin	0.313	0.375	0.250	0.375	0.281	0.125
Colistin	0.046	0.188	0.094	0.046	0.046	0.094
Fosfomycin	0.375	0.250	0.375	0.500	0.375	0.313
Gentamicin	0.375	0.375	0.375	0.125	0.250	0.250
Imipenem	0.375	0.188	0.375	0.156	0.094	0.188
Meropenem	0.313	0.125	n.d.	0.188	0.125	0.188
Piperacillin	0.375	0.500	0.500	0.281	0.125	0.375
Rifampicin	0.313	0.156	0.094	0.250	0.250	0.500
Tobramycin	0.188	0.188	0.500	0.153	0.188	0.188

Synergy

Highly additive

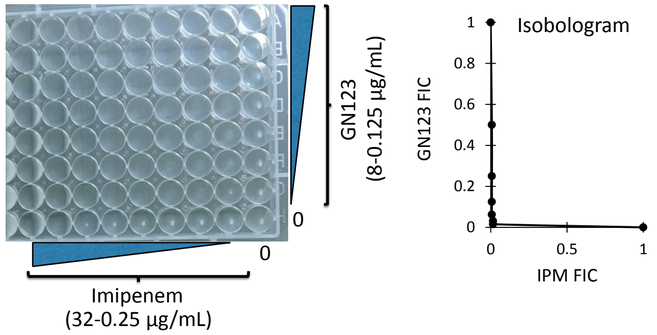
- GN lysins are synergistic across a broad range of antibiotics (a feature shared with GP lysins)
- For imipenem, the synergy is consistent with resensitization to the carbapenem antibiotic

RESULTS: RESENSITIZATION

To examine resensitization, 5 lysins (including GN123) were combined with 2 carbapenems (imipenem (IPM) and meropenem (MEM)) against up to 8 carbapenem^R and 2 carbapenem^S isolates. Resensitization occurs in synergistic combinations in which the carbapenem MIC values falls below established breakpoints. The interpretive categories (CLSI, M100 29th ed.) are, ≤2 (S), 4 (I), and ≥8 (R).

GN123 Resensitizes Carbapenem^R Isolates

Checkerboard Analysis of Clinical Isolate PA19 (IMP^R, MEM^R)



	Imipenem MIC (µg/mL)		GN123 (µg/mL)		
Isolate	Alone	Combination	Alone	Combination	FICI
PA19	32 (R)	0.5 (S)	8	0.125	0.03

Analysis of additional carbapenem^R isolates:

PA20	16 (R)	1 (S)	16	2	0.188
PA21	32 (R)	0.5 (S)	8	1	0.141
PA22	16 (R)	2 (S)	16	1	0.188
PA23	8 (R)	0.25 (S)	8	2	0.281
PA24	32 (R)	2 (S)	16	2	0.188
WC-452	16 (R)	1 (S)	16	2	0.188

Analysis of combinations using meropenem and GN123:

	Meropenem MIC (µg/mL)		GN123 (µg/mL)		
Isolate	Alone	Combination	Alone	Combination	FICI
PA19	32 (R)	0.5 (S)	8	0.25	0.046
PA20	16 (R)	0.5 (S)	16	1	0.094
PA21	32 (R)	1 (S)	8	1	0.156
PA22	16 (R)	1 (S)	16	1	0.125
PA23	16 (R)	0.5 (S)	8	1	0.156
PA24	32 (R)	2 (S)	16	0.5	0.094
WC-452	16 (R)	1 (S)	16	1	0.125

- Synergistic combinations with GN123 demonstrated reductions of imipenem and meropenem MICs to below breakpoint values for the carbapenems
- These observations are consistent with resensitization
- Resensitization observed with each of 7 carbapenem^R strains examined

Other Lysins Resensitize Carbapenem^R Isolates

Synergy Analysis Extended to 4 Additional Lysins (GN121, GN351, GN370, and GN428)

	Imipenem MIC (µg/mL)		GN121 (µg/mL)		
Isolate	Alone	Combo	Alone	Combo	FICI
PA19	32 (R)	1 (S)	1	0.125	0.155
PA20	16 (R)	0.5 (S)	1	0.25	0.265
PA21	32 (R)	1 (S)	1	0.125	0.155
PA22	32 (R)	2 (S)	2	0.25	0.188
PA23	16 (R)	0.125 (S)	1	0.25	0.257
PA24	32 (R)	1 (S)	1	0.125	0.155

	Meropenem MIC (µg/mL)		GN121 (µg/mL)		
Isolate	Alone	Combo	Alone	Combo	FICI
PA19	32 (R)	1	2	0.5	0.281
PA20	16 (R)	1	2	0.5	0.313
PA21	32 (R)	2	1	0.125	0.188
PA22	16 (R)	1	1	0.25	0.313
PA23	16 (R)	2	2	0.5	0.375
PA24	32 (R)	1	1	0.125	0.156
WC-452	16 (R)	1	1	0.06	0.123

Combinations	Antibiotic MIC		Lysin MIC		
vs. WC-452	Alone	Combo	Alone	Combo	FICI
IPM+GN351	16 (R)	0.5 (S)	1	0.125	0.156
IPM+GN370		0.5(S)	2	0.125	0.094
IPM+GN428		1 (S)	2	0.25	0.188
MEM+GN351	16 (R)	1 (S)	1	0.125	0.188
MEM+GN370		0.5 (S)	2	0.125	0.125
MEM+GN428		1 (S)	2	0.25	0.188

- All lysin combinations with IPM and MEM exhibit the resensitization phenotype

CONCLUSIONS

These findings indicate that GN lysins can resensitize *P. aeruginosa* to carbapenem antibiotics, driving MICs below breakpoint values in vitro. This novel ability of lysins to resensitize antibiotic resistant strains to conventional antibiotics may have important therapeutic implications and is a promising mechanism to combat and "reverse" antimicrobial resistance.

Disclosures: The authors are employees of ContraFect Corporation