In Vivo Synergy and Efficacy of P128 in Combination with Standard of Care Antibiotics in Methicillin Resistant *Staphylococcus aureus* (MRSA) Bacteremia

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Aim: To demonstrate the potential of chimeric ectolysin, P128 as a candidate for antimicrobial development for the treatment of systemic MRSA infections.

Background

- P128 is a chimeric recombinant ectolysin with rapid bactericidal activity on *S. aureus* and coagulase negative Staphylococci (CoNS).
- P128 has demonstrated potent antibiofilm activity on MRSA and CoNS strains.
- These properties make it an attractive candidate for antibacterial development.
- Combination regimens are now favoured for treatment of bacterial infections due to rapid development of resistance to single-drug therapy.
- P128 is highly synergistic with standard-of-care (SoC) antibiotics in inhibiting *S. aureus* and CoNS *in vitro*.
- **This study investigates the *in vivo* efficacy of P128 alone and in combination with SoC antibiotics.**

Methods

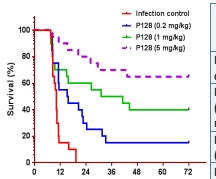
Efficacy in immunocompetant mice: BALB/c mice were challenged by intraperitoneal (IP) route with 10^9 CFU of MRSA strain COL; or with 10^9 CFU of MRSA strain USA300 in 5% mucin. P128 dose-response was tested for single doses of P128 (IP) in the range of 0.2 - 5 mg/kg, 3 or 2 h after bacterial challenge. In MRSA COL bacteremia, synergy with SoC antibiotics was tested using a single dose of 0.2 mg/kg of P128 combined with sub-therapeutic doses of vancomycin (55.0 mg/kg, SC, BID) or daptomycin (12.5 mg/kg, SC, BID). In MRSA USA300 bacteremia, synergy was tested with a single dose of 2.5 mg/kg of P128 combined with vancomycin (27.5 mg/kg, SC, BID) or daptomycin (12.5 mg/kg, SC, BID). The therapeutic dose equivalents of vancomycin and daptomycin in mice are 110 mg/kg and 50 mg/kg, respectively. Survival was monitored over 72 hours after infection.

***In vivo* bactericidal effect in neutropenic mice:** Neutropenia was induced using 2 doses of cyclophosphamide (150 mg/kg, 4 days prior and 100 mg/kg, 1 day prior to infection). Mice were infected (IP) with MRSA USA300, 5 x 10^8 CFU with 5% mucin and treated with single doses of 0.2, 1, 5 or 10 mg/kg of P128 (IP or IV) 2h after bacterial challenge. CFUs in blood were determined at 30 mins and 1 hr post-treatment.

Efficacy in bacteremia in rats: Female Wistar albino rats of 8-9 weeks age were infected with 10^9 CFU of MRSA USA300. Infection progressed slowly, simulating bacteremic infection in humans and mortality occurred over 14 days. Single doses of 0.5, 2.5 or 10.0 mg/kg of P128 were administered as a 60 minute-infusion, 2h after intravenous bacterial challenge and survival was monitored over 14 days.

Results: P128 is efficacious as monotherapy and synergises with SoC antibiotics Vancomycin and Daptomycin

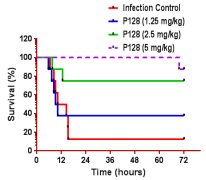
Dose response against MRSA COL



Group (n= 20)	Mortality 24 h	48 h	72 h	Mortality (%)
Infection control	20/20	20/20	20/20	100
P128 (0.2 mg/kg)	14/20	17/20	17/20	85
P128 (1 mg/kg)	8/20	12/20	12/20	60
P128 (5 mg/kg)	4/20	7/20	7/20	35

P128 shows dose-dependent efficacy and rescues mice from fatal bacteremia

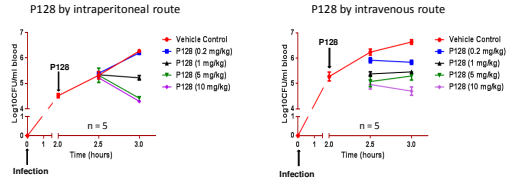
Dose response against MRSA USA300



Group (n=8)	Mortality 24 h	48 h	72 h	Mortality (%)
Infection control	7/8	7/8	7/8	88
P128 (1.25 mg/kg)	5/8	5/8	5/8	63
P128 (2.5 mg/kg)	2/8	2/8	2/8	25
P128 (5 mg/kg)	-	-	1/8	13

P128 shows dose-dependent efficacy in the mucin-enhanced virulence model of bacteremia

Treatment of bacteremia in neutropenic mice (MRSA USA300)



Time post infection (hours)	Mean±Log_{10} CFU/ml blood-IP dosing Vehicle	0.2 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
2	4.5±0.2	NE	NE	NE	NE
2.5	5.3±0.3	5.4±0.15	5.3±0.14	5.3±0.62	5.2±0.14
3	6.0±0.0	6.2±0.14	5.2±0.18*	4.4±0.15*	4.3±0.07*

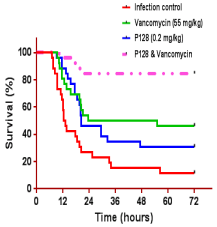
NE=Not Estimated, *P <0.001 compared to vehicle control

Time post infection (hours)	Mean±Log_{10} CFU/ml blood-IV dosing Vehicle	0.2 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
2	5.3±0.39	NE	NE	NE	NE
2.5	6.2±0.25	5.9±0.22	5.4±0.18	5.1±0.45	4.9±0.39
3	6.6±0.2	5.8±0.2	5.5±0.08*	5.3±0.34*	4.7±0.39*

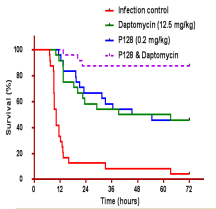
NE=Not Estimated, *P <0.001 compared to vehicle control

P128 dosing by both intraperitoneal and intravenous routes is effective in CFU reduction and controlling bacteremia in neutropenic mice

Combination with vancomycin and daptomycin against MRSA COL



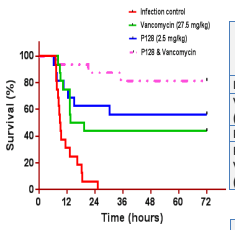
Group (n= 26)	Mortality 24 h	48 h	72 h	Mortality (%)
Infection control	19/26	22/26	23/26	88
Vancomycin (55 mg/kg)	13/26	13/26	14/26	54
P128 (0.2 mg/kg)	14/26	18/26	18/26	69
P128 (0.2 mg/kg) Vancomycin (55 mg/kg)	4/26	4/26	4/26	15



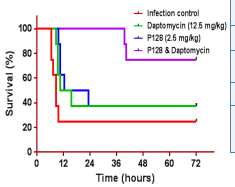
Group (n= 24)	Mortality 24 h	48 h	72 h	Mortality (%)
Infection control	21/24	22/24	23/24	92
Daptomycin (12.5 mg/kg)	10/24	12/24	13/24	46
P128 (0.2 mg/kg)	8/24	11/24	13/24	46
P128 (0.2 mg/kg) Daptomycin (12.5 mg/kg)	3/24	3/24	3/24	12

P128 shows *in vivo* synergy with SoC antibiotics: drug combinations are effective at sub-therapeutic dose levels

Combination with vancomycin and daptomycin against MRSA USA300



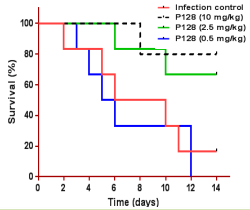
Group (n= 16)	Mortality 24 h	48 h	72 h	Mortality (%)
Infection control	15/16	16/16	16/16	100
Vancomycin (27.5 mg/kg)	9/16	9/16	9/16	56
P128 (2.5 mg/kg)	6/16	7/16	7/16	44
P128 (2.5 mg/kg) Vancomycin (27.5 mg/kg)	2/16	3/16	3/16	19



Group (n= 8)	Mortality 24 h	48 h	72h	Mortality (%)
Infection control	6/8	6/8	6/8	75
Daptomycin (12.5 mg/kg)	5/8	5/8	5/8	63
P128 (2.5 mg/kg)	5/8	5/8	5/8	63
P128 (2.5 mg/kg) Daptomycin (12.5 mg/kg)	0/8	2/8	2/8	25

P128 shows synergistic efficacy with SoC antibiotics: combinations are superior to either drug alone

Efficacy of P128 in rat model of bacteremia



Groups (n=6)	Mortality (%)
Infection control	83
P128 (0.5 mg/kg	100
P128 (2.5 mg/kg)	33
P128 (10 mg/kg)	20

A single dose of P128 by intravenous infusion rescues rats from mortality

Conclusions

- **P128 treatment resulted in dose-dependent survival in both mouse and rat models of bacteremia caused by MRSA strains of clinical significance.**
- **P128 given by both intraperitoneal and intravenous routes had a rapid, dose-dependant bactericidal effect.**
- **P128 synergized with vancomycin and daptomycin, resulting in efficacy at sub-therapeutic concentrations of both drugs.**
- **P128 is a potent, rapid and efficacious anti-microbial for Staphylococcal infections, including MRSA bacteremia, as stand alone treatment and in combination with SoC antibiotics.**

References

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All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee, GangaGen Biotechnologies Pvt Ltd, Bangalore, India.