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SASPject: Microbiological Characterisation of a Novel Therapeutic Targeting MDR *Pseudomonas aeruginosa*

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ABSTRACT

Background: SASPs are a class of unique antibacterial proteins that prevent DNA replication and gene transcription through binding to bacterial DNA. The mode of SASP binding is DNA sequence-independent and thus mutations have no impact upon SASP activity. Phico's SASPject technology uses a synthetic biology approach to create nano-delivery vehicles (NDV), capable of delivering a SASP gene expression system to target bacteria. SASP production *in situ* in target cells is rapidly bactericidal. NDV gene delivery activity has been assessed against a large panel of recent, geographically diverse clinical isolates – an essential requirement for novel antibacterial technologies. Furthermore, the rapidly bactericidal activity of *Pseudomonas aeruginosa* (*Pa*) SASPject against Pa has been assessed.

Methods: Spectrum activity: 539 clinical *Pa* strains from multiple sites across Asia, EU, North and South America were grown in Luria Bertani broth supplemented with magnesium, calcium and glucose (LB+); ~10^7 cfu was used to inoculate LB+ soft agar and poured onto LB+ plates. 10 µl spots of a preparation of 10^9 *Pa* NDV U were inoculated onto the soft agar overlay. Kill efficiency: Diluted overnight suspensions of MDR *Pa* 2046 were inoculated (final inoculum 10^6 cfu/ml) into LB+ broth, ~10^9 *Pa* SASPject U/ml were added and incubated statically at 37 °C with total Viable Counts every hour (h) for 6 h.

Results: *Pa* NDV gene delivery activity was achieved in 92% of *Pa* strains, spanning carbapenem, aminoglycoside, fluroquinolone and polymixin resistance profiles. *Pa* SASPject was rapidly bactericidal with *Pa* cells reduced to below the detection limit of 50 cfu/ml within 1 h. Continued monitoring over 6 h showed no bacterial regrowth.

Conclusion: *Pa* NDV screening demonstrated a broad spectrum against an extensive range of recent, geographically diverse clinical *P. aeruginosa* strains enabling utility in *Pa*-targeted SASPject. Furthermore, SASPject's unique mode of action, together with its rapid bactericidal activity indicates a potential role in addressing the unmet clinical need for novel antibacterial approaches with activity against Gram negative pathogens.

INTRODUCTION

The emergence of multidrug resistant Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli, Acinetobacter baumannii,* and *Klebsiella spp.* amongst others is a global public health issue and has highlighted the urgent need for new therapeutic options, ideally with novel mechanisms of action..

SASPject™ is a new class of antibacterial currently under development (1). The SASPject platform technology comprises NDV's designed to deliver a gene encoding an antibacterial protein (SASP) into target bacteria (Figure 1). SASPs are small proteins expressed by sporulating bacteria which, when delivered into bacteria non-specifically, inactivate bacterial DNA and lead to rapid cell death. Due to the specific nature of the NDV-bacterium interaction, single-species or multi-species or genera can be selectively targeted. Consequently, non-selective inhibition of commensal bacteria, which is a significant drawback of some conventional antibiotic therapies, can be avoided.

Previously, a SASPject antibacterial (PT1.2) against *Staphylococcus aureus*, including a diverse range of antibiotic-resistant strains including MRSA and VISA/hVISA (2), has been developed. PT1.2 successfully passed through a Phase I clinical trial for nasal decolonisation of *S. aureus* in humans and demonstrated excellent *in vitro* activity (2, 3). *Pa* NDV is in development for treating *P. aeruginosa*, including MDR strains. Here we report the first data on the binding and gene transfer efficacy of *Pa* NDV against a diverse range of clinical *P. aeruginosa* isolates from around the globe and demonstrate the rapid mode of action *Pa* SASPject.

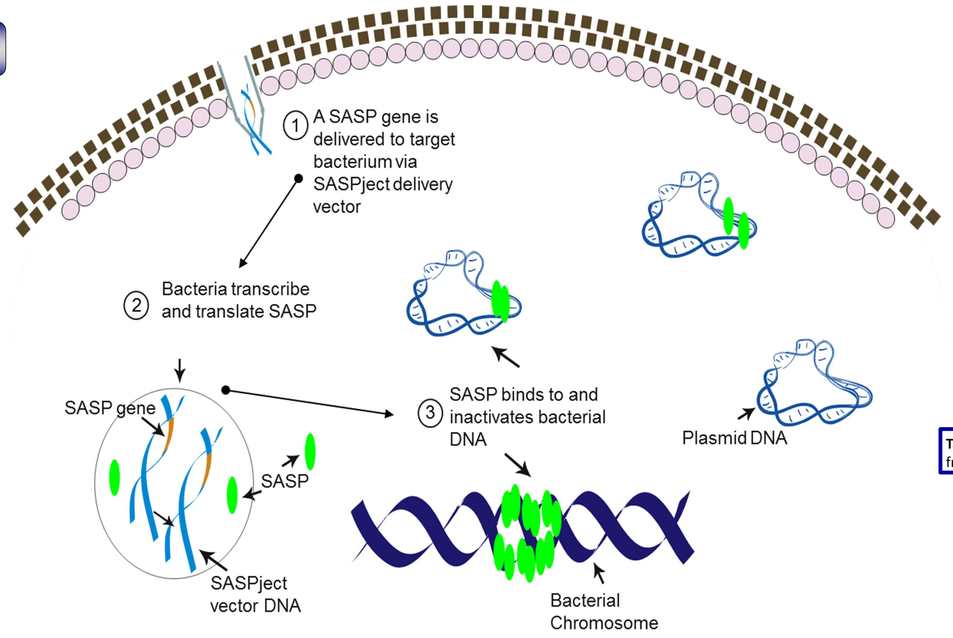

Figure 1. Mechanism of action of *Pa* SASPject

METHODS

Bacterial isolates

- Five hundred and fifty three *P. aeruginosa* isolates of clinical specimens were tested for spectrum of activity studies and included (but not limited to) sputum, tissue, blood, peritoneal dialysis fluid, cough swabs, urine and abscess pus. Testing was carried out both at Phico Therapeutics Ltd and with external partners Micromyx and IHMA Europe Sárl . Clinical isolates were of recent clinical interest with >90 % collected between 2010-2013 and were collected from multiple sites across the following geographical regions :
  - Europe: 232
  - Asia: 148
  - Americas: 159

Spectrum activity – agar medium

- 10^7 cfu of each *P. aeruginosa* culture was suspended in Luria-Bertani broth supplemented with $MgSO_4$ (5 mM), $CaCl_2$ (5 mM) and glucose (0.1 % w/v) (LB+ broth) and supplemented with 0.4 % agar bacteriological No. 1 (Oxoid), mixed and poured onto the surface of an LB+ agar (1 % w/v) plate and allowed to solidify
- *Pa* NDV stocks were diluted to 10^{-7} in LB+ broth
- 10^9 U of *Pa* NDV and subsequent dilution series were inoculated onto *P. aeruginosa* lawns and inocula were absorbed into the agar prior to incubation (overnight, 32 °C); *P. aeruginosa* lawns were assessed for killing by *Pa* NDV

6 Hour (h) kill curve – liquid medium

- *Pa* SASPject cultures were prepared as above and diluted in LB+ broth to ~10^5 cfu/ml and exposed to *Pa* SASPject (10^9 U/ml) of LB+ broth only in a 6 h kill curve
- *P. aeruginosa* viable counts were determined at hourly intervals

RESULTS

		No. of strains	% Coverage
EU	Germany	20	100
	Greece	74	89
	Italy	110	93
	UK	28	93
	Total	**232**	**92**
Asia	East Asia	50	84
	India	51	90
	Japan	30	93
	South Korea	8	88
	Taiwan	9	100
	Total	**148**	**88**
Americas	Argentina	22	91
	Brazil	35	97
	Canada	13	77
	USA	89	97
	Total	**159**	**94**
Global	**Overall Total**	**539**	**92**

Table 1. Summary table of *Pa* NDV host range against clinical *P. aeruginosa* strains from diverse geographical regions

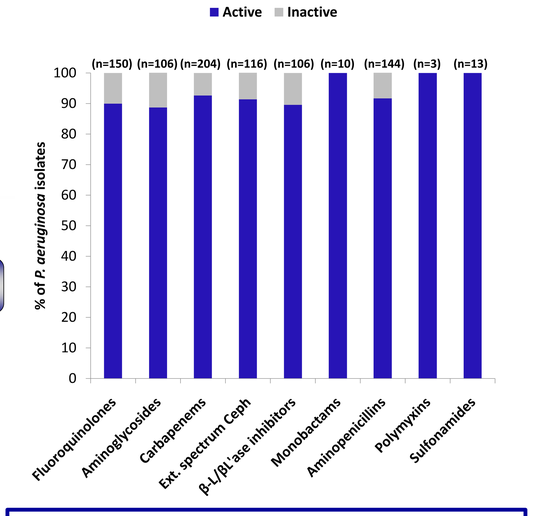

Figure 2. Effect of conventional antibiotic resistance on host range of *Pa* NDV

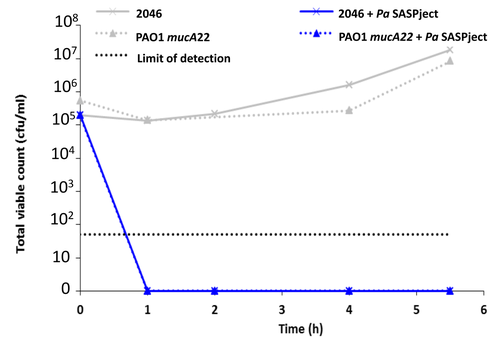

Figure 3. 6 h kill curve of MDR *P. aeruginosa* strain 2046 and a *mucA22* mutant of PAO1 (1 x10^5 cfu/ml) hyper expressing alginate by *Pa* SASPject (1 x 10^9 U/ml)

Pa NDV host range

- *Pa* NDV demonstrated binding and gene delivery against 92 % of *P. aeruginosa* isolates across the EU, Asia and Americas (Table 1)
- Activity was demonstrated against clinical isolates of *P. aeruginosa* resistant to a wide array of antimicrobial agents including aminoglycosides and carbapenems (Figure 2)

SASPject kill curves

- *Pa* SASPject was bactericidal against MDR *P. aeruginosa* strain 2046 and an alginate hyper-expressing *mucA22* mutant of PAO1 within 1h (Figure 3)
- Continued monitoring demonstrated no re-growth of either *P. aeruginosa* strains within 6 h

CONCLUSIONS

- The binding and gene delivery spectrum of *Pa* NDV was impressive against a geographically diverse range of clinically significant *P. aeruginosa* isolates, including MDR strains
- Kill of *P. aeruginosa* PAO1 by *Pa* SASPject was rapid and bactericidal within 1 h and reflected observations with PT1.2 against MRSA
- As a new class of antibiotics, SASPject represents an attractive and novel therapeutic option against MDR *P. aeruginosa,* given the rapid bactericidal killing and also ability of SASP to limit horizontal transfer of antibiotic resistance genes *in vitro* (4)
- Furthermore, the low propensity for resistance to develop to SASPject's unique mode of action (5) demonstrates the advantages of this new class of antibacterial
- Further studies are required to characterise *Pa* SASPject binding and gene delivery *in vivo*

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