F—1550 ICAAC | WASHINGTON

# SASPject: A Novel Antibacterial Technology Targeting MDR *Pseudomonas aeruginosa* Demonstrating a Low Propensity for Resistance Development

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**5-9 September 2014**

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## ABSTRACT

**Background:** 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. For novel antibacterials to be successful it is critical that they demonstrate a low propensity for the development of resistance. The unique mode of action of SASPject, including the ability of SASP to prevent DNA replication and gene transcription in a DNA sequence-independent manner, coupled with rapid bactericidal activity suggests SASPject has a low propensity to induce resistance compared to other antibiotics. Here the potential for resistance of *Pseudomonas aeruginosa* (*Pa*) to develop in response to *Pa* SASPject therapy was measured in a 52-day passaging study

**Methods:** Passaging studies: *Pa* 3176 cultures at ~$10^5$ cfu/ml in Luria Bertani broth supplemented with magnesium, calcium, and glucose (LB+) were passaged daily after overnight growth at 37 °C in the presence of *Pa* SASPject at 1.25 x $10^6$, 6.25 x $10^5$ and 3.13 x $10^5$ U/ml. A control without added *Pa* SASPject was also passaged in parallel. Susceptibility of the cultures to SASPject was assessed daily by 3 h kill assays, with ~$10^5$ cfu/ml *P. aeruginosa* and ~1.5 x $10^9$ U/ml SASPject incubated statically at 37 °C.

**Results:** Bacterial kill to below the detection limit of 10 cfu/ml was observed in each 3 h kill assay against *Pa*. No decrease in sensitivity was observed under any of the three *Pa* SASPject concentrations measured during 52 days of monitoring, after which the experiment was stopped.

**Conclusion:** The unique mode of action of SASP means that mutations in bacterial DNA cannot lead to resistance as SASP inactivates bacterial DNA in a sequence-independent manner. This is supported by the data presented here, where resistance did not develop within 52 days of passaging *Pa* with *Pa* SASPject. In conclusion the SASPject technology platform displays a low propensity for resistance development in the treatment of *Pa.*

## 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 (1), 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).

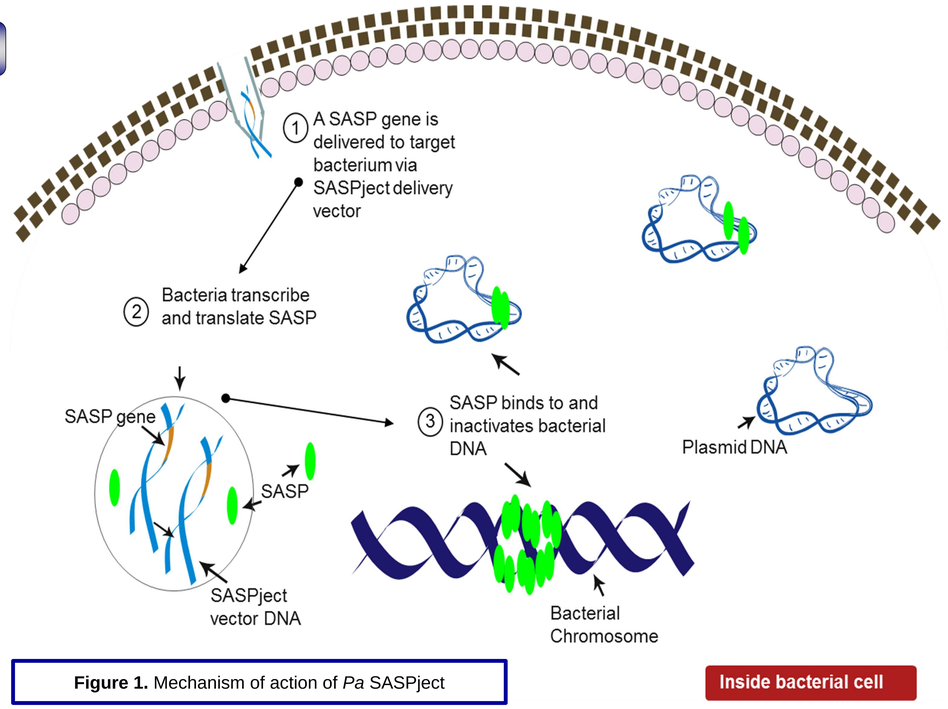


**Figure 1.** Mechanism of action of *Pa* SASPject

*Pa* SASPject has demonstrated a rapid bactericidal activity against a geographically diverse range of MDR *Pa* strains (4) supporting the previously published effective SASPject therapeutic targeting *S. aureus* , PT1.2 (2,3). Due to SASPject's unique mode of action the technology has demonstrated a low propensity for resistance development to *S. aureus* (5). In this study, *P. aeruginosa* has been serially passaged in the presence of three concentrations of *Pa* SASPject representing inhibitory to sub-inhibitory concentrations of *Pa* SASPject. The susceptibility of serially passaged *Pa* was measured at regular intervals.

## METHODS

**Passaging studies**

*P. aeruginosa* Asia strain 3176 was streaked onto a fresh Luria-Bertani agar plate containing $MgSO_4$ (5 mM), $CaCl_2$ (5 mM $CaCl_2$) and Glucose (0.1% w/v) (LB+) and grown overnight at 37 °C. Four individual colonies were subsequently re-suspended into 5 ml LB+ broth and grown overnight at 37 °C with shaking (350 rpm). The following day the $OD_{600}$ of the overnight culture of *Pa* strain 3176 was measured and diluted to $10^5$ cfu/ml in fresh LB+ broth. *Pa* SASPject was added under three different exposure regimes 1.25 x $10^6$, 6.25 x $10^5$ and 3.13 x $10^5$ U/ml.

Triplicate cultures were used for each of the exposure regimes while a no exposure control culture was inoculated with *Pa* strain 3176. Cultures were incubated overnight, static at 37 °C. After overnight culture the $OD_{600}$ was measured and cultures were diluted back to $10^5$ cfu/ml and *Pa* SASPject was added at the same exposure concentrations. This procedure was repeatedly daily.

The susceptibility of the passaged cultures exposed to *Pa* SASPject were assessed on a daily basis by a 3 h kill assay. Briefly, an aliquot of the overnight culture diluted for serial passaging studies ($10^5$ cfu/ml) was incubated with *Pa* SASPject (1 x $10^9$ U/ml) and incubated for 3 h statically at 37 °C. Samples were then diluted and plated onto LB+ agar plates. Following overnight incubation at 32 °C viable cells were enumerated.

## RESULTS

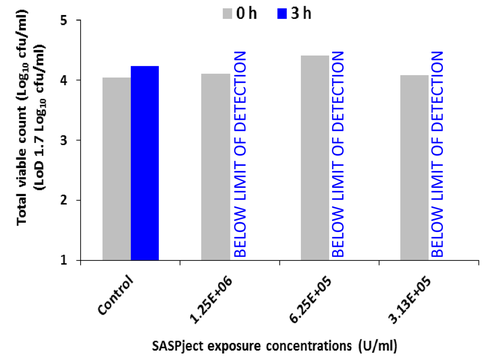


**Figure 2. 3 h kill assay of *Pa* Asian strain 3176 (1 x $10^5$ cfu/ml) by *Pa* SASPject (1 x$10^9$ U/ml) after 20 days exposure to *Pa* SASPject therapeutic**




**Figure 3. 3 h kill assay of *Pa* Asia strain 3176 (1 x $10^5$ cfu/ml) by *Pa* SASPject (1x $10^9$ U/ml) after 40 days exposure to *Pa* SASPject therapeutic**




**Figure 4. 3 h kill assay of *Pa* Asia strain 3176 (1 x $10^5$ cfu/ml) by *Pa* SASPject (1x $10^9$ U/ml) after 52 days exposure to *Pa* SASPject therapeutic**

- After 52 days of serial passaging the susceptibility of *P. aeruginosa* Asia strain 3176 to *Pa* SASPject was unaffected, with complete removal of the bacterial load to below the detection limit within 3 hours of exposure (Figures 2-4)
- Differing exposure concentrations of SASPject had no effect on the potential for resistance to develop in *P. aeruginosa* with removal to below the detection limit within 3 h (Figures 2-4)

## CONCLUSIONS

- *Pseudomonas aeruginosa* remains susceptible to *Pa* SASPject following 52 days of exposure
- Sub-inhibitory concentrations of SASPject do not increase the potential for the development of resistance of kill of *P. aeruginosa.*
- Observations reported here reflect those seen with PT1.2 against MRSA (5) and highlight that the unique mode of action of SASP means both Gram-positive and Gram-negative bacteria are extremely unlikely to develop resistance
- Further studies are required to characterise *Pa* SASPject binding and gene delivery *in vivo*

## REFERENCES

1. Fairhead H. 2009. SASP gene delivery: a novel antibacterial approach. *Drug News Perspect.* **22 (4)**, 197-203
2. Hatzixanthis K., Wilkinson A., Fairhead H. Double-blind, placebo-controlled Phase I study of PT1.2, a novel anti-bacterial protein (SASP delivery vector). 50th ICAAC Boston 2010. Abstract F1-2086b
3. Mushtaq S., Livermore, D., Wilkinson A., Fairhead H. A novel antibacterial protein which shows rapid bactericidal activity against MRSA in presence of other antibiotics. 19th ECCMID Helsinki. Abstract P1081
4. Cass J., Cullen S., Castillo A.L., Wang H., Wilkinson A., Fairhead H. SASP: A novel antibacterial technology targeting MDR *Pseudomonas aeruginosa* demonstrating a low propensity for resistance development . ICAAC Washington 2014. Abstract F-1550
5. Holme S., Math V., Pitts K., Bernard A., Wang H., Wilkinson A., Fairhead., Low propensity for *S. aureus* to develop resistance to SASP or its delivery vector ICAAC Washington 2008. Abstract F1-4001

## ACKNOWLEDGEMENTS

- Phico Therapeutics Ltd is grateful to Professor Mark Wilcox (University of Leeds & the General Infirmary, Leeds, UK).