New preclinical data on adjunctive antibacterial therapies

SASP: in vitro activity against Pseudomonas aeruginosa in combination with conventional antibiotics

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Objectives: 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. The in vitro bactericidal activity of SASPject has been assessed in MDR P. aeruginosa clinical and non-clinical strains. Furthermore, single dosing of SASPject in combination with conventional antibiotics against P. aeruginosa has been assessed using time-kill analysis.

Methods: MIC values for conventional antibiotics were calculated using standard CLSI guidelines. In vitro time kill analysis using multi drug resistant (MDR) clinical P. aeruginosa strains and ATCC27853 were carried out in (Cation adjusted Mueller Hinton broth) CAMHB. 1 ml of cells at 10⁵ cfu/ml was added to 1 ml of SASPject at 10⁹ Units (U)/ml. Combination treatments included meropenem at the sensitive threshold (2 µg/ml) and 0.5 to 3 x MIC and reactions were incubated statically at 37 °C for 48 h. Total viable cell counts were taken at 0, 1, 6, 24 and 48 h using MHB agar.

Results: For the majority of P. aeruginosa strains tested a single dose of SASPject was rapidly bactericidal with cell numbers reduced to below the detection limit of 10 cfu/ml within 1 hour. Following single dosing with a combination of SASPject and meropenem at 0.5 up to 3 x MIC cell numbers remained below the limit of detection during the 48 h monitoring period for both the meropenem resistant strain and ATCC7853. In comparison strains exposed to a single dose of conventional antibiotics showed regrowth after 6 h. A strain with intermediate sensitivity to meropenem showed increased bactericidal sensitivity within 6 h to single dose of SASPject and meropenem at 2 µg/ml. Cell numbers declined to approximately the detection limit and were sustained at this level for 48 h. In comparison regrowth had occurred within 24 h following a single dose of meropenem at 2 µg/ml.

Conclusions: Time-kill analyses show the highly bactericidal nature of SASPject compared to conventional antibiotics. Single dosing of SASPject in combination with antibiotics may increase the effectiveness of conventional antibiotics over a 24-48 h time period. Furthermore, the potential additive effect seen in SASPject plus antibiotic combinations indicates a potential role in addressing the unmet clinical need for novel antibacterial approaches with activity against Gram negative pathogens while potentially extending the life-span of current antibiotics.