**Mechanism-based PK/PD modelling approach to optimise combination dosage regimens against multidrug-resistant clinical isolates of *Pseudomonas aeruginosa* and *in-vivo* evaluation in a murine thigh infection model**

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**Aims**: *Pseudomonas aeruginosa* (Pa) is an opportunistic Gram-negative pathogen that is prevalent in bloodstream, wound and respiratory infections and has a high propensity to become multidrug-resistant. Our primary aim was to systematically evaluate synergistic killing and suppression of resistance of Pa by combinations of carbapenem plus aminoglycoside (AGS) antibiotics. Secondly, we sought to propose optimised combination dosage regimens *via* Monte Carlo simulations (MCS) based on mechanism-based models (MBM) and human population pharmacokinetics (popPK). Thirdly, we aimed to prospectively evaluate the optimised combination regimens in a murine thigh infection model.

**Methods**: We studied the carbapenem imipenem (IPM) in monotherapy and combination with the AGS tobramycin (TOB) or amikacin (AMK) vs. 3 carbapenem- and AGS-resistant clinical Pa isolates. A MBM was developed to characterize the time-course of bacterial load reduction and resistance. The importance sampling algorithm (pmethod=4) was used for simultaneous estimation of all PD parameters using parallelized S-ADAPT (version 1.57) facilitated by SADAPT-TRAN. MCS were performed to optimise clinically relevant combination dosage regimens against a double-resistant clinical Pa isolate (FADDI-PA088) which represents the 98th percentile of the EUCAST (European Committee on Antimicrobial Susceptibility Testing) MIC distributions for IPM (MIC 16 mg/L) and TOB (MIC 32 mg/L). Inter-individual variability in PK and between curve variability of the PD parameters were incorporated in the MCS, which simulated 10,000 adult critically-ill patients with normal renal function. The optimised combination dosage regimens of IPM + TOB were tested in a murine thigh infection model *via* a humanized dosing scheme. Simulations were performed to identify IPM and TOB dosage regimens for mice that lead to similar plasma exposure profiles of both drugs as observed in humans. Thigh infections were established by injecting ~105 CFU of FADDI-PA088 intramuscularly into each posterior thigh muscle of neutropenic mice. The humanized dose of IPM at 60 or 77 mg/kg was administered subcutaneously (s.c.) every 2h and TOB at 73 mg/kg/day was fractionated in decreasing doses injected s.c. every 4h. Viable counts were determined in thigh homogenate at 0, 2, 6 and 24h. All animal experimentation was approved by the Monash Institute of Pharmaceutical Sciences animal ethics committee.

**Results**: Clinically relevant IPM + AGS concentrations achieved synergistic killing (>2 log10 vs. the most active monotherapy at 24h and 48h) and suppression of resistance for all isolates (MIC: IPM 16 to 32 mg/L & AGS 4 to 32 mg/L). MBM indicated that AGS significantly enhanced the imipenem (IPM) target site concentrations, characterized by an up to 2.9-fold decrease in IPM concentrations required to achieve half-maximal bacterial load reduction. The optimised combination regimens (IPM continuous infusion at 4g/day or 5g/day + tobramycin (TOB) 7 mg/kg q24h as 0.5h infusion) were predicted to achieve >2 log10 killing and prevent regrowth at 24 and 48h in 80.9 to 90.3% of simulated critically-ill patients. The bacterial load in the mice immediately before the start of treatment (2h after inoculation) was 4.79±0.08 log10 CFU/thigh (mean±standard deviation) and was increased up to 8.11±0.11 log10 CFU/thigh at 24h in untreated control mice. IPM+TOB combinations (*i.e*. IPM 4g/day or 5g/day continuous infusion + TOB 7 mg/kg q24h, 0.5h infusion) provided a clear benefit with ≥2.51 log10 and ≥1.50 log10 CFU/thigh of bacterial load reduction compared to the most active monotherapy at 24h. The MBM was translated well to describe the time-course of bacterial load reduction and resistance *in-vivo*.

**Conclusion**: The IPM plus TOB combination regimens, which were rationally optimised *via* a translational modelling approach, demonstrated a synergistic effect *in-vivo* against a double-resistant clinical Pa isolate and are therefore highly promising.