A workflow for application of the general pharmacodynamic interaction model on high-throughput combinatorial data in order to identify, quantify and characterise drug combinations that can overcome multi-drug-resistance

R. Michelet (1), A.R. Brochado (2,3), A. Typas (2,4), S.G. Wicha (5), C. Kloft (1)

(1) Dept. of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, Freie Universitaet Berlin, Germany,
(2) European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany
(3) Wuerzburg University, Chair of Microbiology, Wuerzburg, Germany
(4) European Molecular Biology Laboratory, Structural & Computational Biology Unit, Heidelberg, Germany
(5) University of Hamburg, Institute of Pharmacy, Dept. of Clinical Pharmacy, Hamburg, Germany

Background and Objectives

Antimicrobial resistance is one of the key challenges in the current global healthcare system [1]. As new antibiotics are lacking, combinations of existing drugs can help to treat multi-drug-resistant (MDR) bacterial infections. In order to detect synergistic combinations between antibiotics, human-use (non-antibiotic) drugs and other compounds (e.g. food additives), we previously combined ~3000 compound pairs and assessed their interaction in three gram-negative species [2]. In the current work, a robust workflow to quantitatively characterise these interactions using the General PharmacoDynamic Interaction (GPDI) model [3] is presented. Using this approach, not only the magnitude, but also directionality of an interaction between two or more compounds can be elucidated, possibly identifying interesting combinations for further non-/clinical development.

Methods

- A model selection & evaluation workflow was established using R (v. 3.4.4) and RStudio (v. 1.1.447) and is depicted in Figure 1.

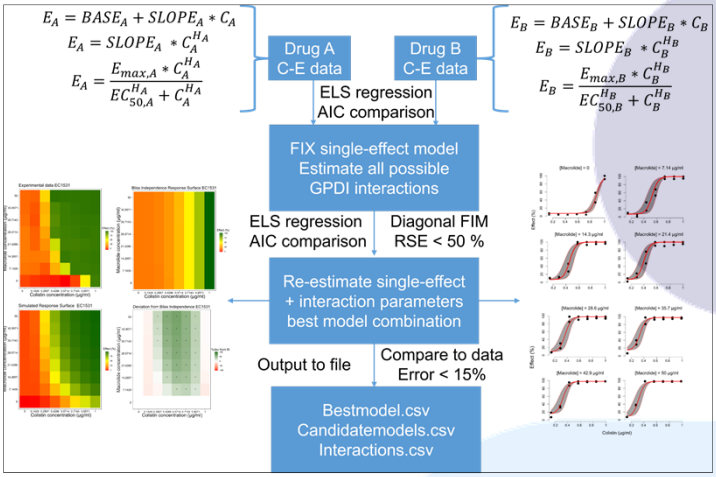

Figure 1. Developed GPDI model selection and evaluation workflow for one example combination in one strain. In step 1, linear, power and E_{MAX}-type models are fitted to single concentration-effect (C-E) data by extended least squares (ELS) regression using the Nelder-Mead and BFGS algorithm. In step 2 the parameters of the best single-effect model are fixed and all possible GPDI interactions are estimated and the best model is selected based on parameter precision (assessed using the diagonal of the Fisher Information Matrix (FIM), calculated from the Hessian outputted by the last successful algorithm) and the Akaike Information Criterion (AIC) (penalty of 2 points per parameter). In step 3, the full set of parameters of the best model is re-estimated and model evaluation is performed by comparing the model to the experimental C-E data, for which >15% deviation from observed effect, or no overlap with the 95% confidence interval of the t-distribution estimated from the data, were considered significant deviations. Finally, the best model is used to simulate a response surface, which is compared to the Bliss Independence surface [4], in order to visualise the interaction and the C-E curves are simulated and overlaid with the data for different combinations of concentrations. This procedure is then repeated over the all unique combinations of drugs and strains in the input dataset.

- The GPDI model framework [3] was used for the quantification of interactions. Possible interactions that were incorporated in the workflow are monodirectional and bidirectional interactions on the slope for linear and power models and on E_{max} and EC_{50} for E_{max}-type models, both with and without estimation of an interaction EC_{50}.

$$GPDI_{A\ on\ B} = 1 + \left(\frac{INT_{max,A\ on\ B} * C_A}{EC_{50,A\ on\ B} + C_A}\right)$$

$$GPDI_{B\ on\ A} = 1 + \left(\frac{INT_{max,B\ on\ A} * C_B}{EC_{50,B\ on\ A} + C_B}\right)$$

- The parameters of the GPDI model were then used to assess the magnitude and direction of the interaction in order to inform hypotheses about the interaction mechanism and select interesting candidates for further development.

Results

- A validation dataset [2] consisting of extended-dose data (8x8 checkerboard experiments, 242 drug combinations in susceptible gram negative strains and 7 synergistic combinations in a set of 6 *E.coli* and *K. pneumoniae* MDR clinical isolates) was first analysed using the developed workflow.
- The experimental data was described well for most combinations and similar synergies and antagonisms as conventional response-surface analyses were identified.
- The well-estimated interaction parameters allowed for, apart from quantitative description of the interaction, identification of the nature of the interactions and putative perpetrator and victim drugs could be identified. The observed interaction categories are presented in Figure 2.

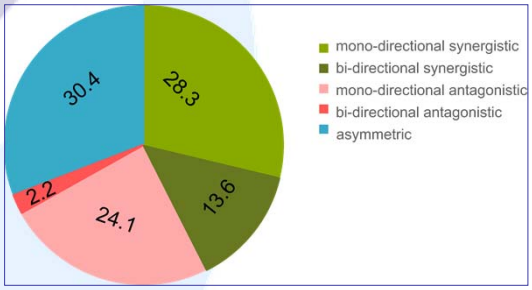

Figure 2. Distribution of the quantified interactions in the validation dataset. 41.9% of the interactions were purely synergistic, 26.3% purely antagonistic and 30.4% of the interactions were asymmetric, meaning that e.g. drug A was synergistic for drug B but drug B antagonistic for drug A.

- In the clinical isolates, strong synergies between colistin and macrolide drugs [5] and between colistin and loperamide were characterised. Weaker synergies were quantified between doxycycline and procaine (bi-directional effect), and vanillin and spectinomycin (only for *E. coli*). These interactions were in line with the earlier described interactions using conventional methods [2].

Discussion and Conclusions

- In order to interrogate a high-throughput dataset consisting of combinations of antibiotics, human-use (non-antibiotic) drugs and other compounds, a quantitative framework needed to be set up.
- For this, a robust model selection and estimation workflow was set up to apply different versions of the GPDI model and to select the most fitting model structure per combination.
- The workflow was first applied to two smaller datasets for validation purposes and to further quantify earlier detected synergies.
- This workflow can now be applied on the larger dataset consisting of 3000 combinations to identify the complete set of promising candidates.
- Furthermore, clustering approaches should be applied to the generated model repository in order to group interactions according to their intensity and directionality to inform mechanistic hypothesis generation.
- Finally, the most promising interactions will be pushed towards further pre-clinical testing and eventual clinical application.

References:

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For additional information, please contact
Dr. ir. Robin Michelet
Email: Robin.michelet@fu-berlin.de

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