

## OVERVIEW OF THE SUPPLEMENTARY MATERIAL OF THE ARTICLE

### **In vitro discovery of promising anti-cancer drug combinations using maximization of a therapeutic index**

M. Kashif<sup>1</sup>, C. Andersson<sup>1</sup>, S. Hassan<sup>1</sup>, H. Karlsson<sup>1</sup>, W. Senkowski<sup>1</sup>, M. Fryknäs<sup>1</sup>,  
P. Nygren<sup>1</sup>, R. Larsson<sup>1</sup>, M.G. Gustafsson<sup>1,\*</sup>

<sup>1</sup>Uppsala University, Dept. Medical Sciences Cancer Pharmacology and Computational  
Medicine, Academic Hospital, 751 85 Uppsala, Sweden

<sup>2</sup>Uppsala University, Dept of Radiology, Oncology and Radiation Science, Academic  
Hospital, SE-751 85 Uppsala, Sweden.

\*Corresponding author: Mats.Gustafsson@medsci.uu.se

**The supplementary material is divided into 4 files and 3 movies, these are 1-SupplementaryData.pdf, that includes**

- a) Table S1: A set of 13 clinical/experimental drugs used in the iterative search using the MACS algorithm (pilot experiment).
- b) Table S2: Results of generation 0 of pilot experiment.
- c) Table S3: Results of generation 1 of pilot experiment.
- d) Table S4: Results of generation 2 of pilot experiment.
- e) Table S5: Results of generation 3 of pilot experiment.
- f) Table S6: Results of generation 0 of main experiment.
- g) Table S7: Results of generation 1 of main experiment.
- h) Table S8: Results of generation 2 of main experiment.
- i) Figure S1: Optimization overview of all iterations of pilot experiment.
- j) Figure S2: Factorial concentration-response and TS (therapeutic synergy) study of combination (Sunitinib, 17-AAG, Afungin, Trichostatin A).
- k) Figure S3: Factorial concentration-response study of combination (Sunitinib, 17-AAG, Afungin, Trichostatin A) in patient cells.
- l) Figure S4: Factorial concentration-response and TS study of combination (Rapamycin, 17-AAG, Trichostatin A).
- m) Figure S5: Factorial concentration-response study of combination (Rapamycin, 17-AAG, Trichostatin A) in patient cells.
- n) Figure S6: Factorial concentration-response and TS study of combination (Rapamycin, 17-AAG).
- o) Figure S7: Factorial concentration-response study of combination (Rapamycin, 17-AAG) in patient cells.
- p) Figure S8: Factorial concentration-response and TS study of combination (Sunitinib, 17-AAG, Afungin).
- q) Figure S9: Factorial concentration-response study of combination (Sunitinib, 17-AAG, Afungin) in patient cells.
- r) Figure S10: Analysis of patient data for the combination (17-AAG, Afungin, Trichostatin A).

**2- SupplementaryMethods-I-II-III.pdf, consists of  
Supplementary Methods I**

- a) Gene expression analysis of combination (17-AAG, Afungin, Trichostatin A).
- b) Statistical variability calculations.
- c) Performance monitoring of experimental/computational pipeline.
- d) Supplementary Figure S11: Performance monitoring of experimental/computational pipeline.

**Supplementary Methods II**

- a) Beckman Coulter® Biomek 2000 cherry picking program
- b) Matlab® code for prediction of IC10 and IC20 values from concentration response data.
- c) Supplementary Figure S12: Cell plate layout.

**Supplementary Methods III**

- a) Theory for determination for the number of degrees of freedom related to t-test for therapeutic synergy.

**3- Gene Expression Data.xls, includes**

- a) Contains differential gene expression signature of combination (17-AAG, Afungin, Trichostatin A).
- b) Compounds matching that signature when run CMap 02 (<https://www.broadinstitute.org/cmap/>).

**4- Movies**

- a) Supplementary Movie S1.mov, combination (17-AAG, Afungin, Trichostatin A) treatment of MelJuso<sup>YFP</sup>.
- b) Supplementary Movie S2.mov, MelJuso<sup>YFP</sup> treated with 17-AAG.
- c) Supplementary Movie S3.mov, MelJuso<sup>YFP</sup> treated with Bortezomib.