

Large multi-scale modeling of long term toxic effects in organotypic cultures

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Project Aims and Benefits

The aim of our project is to establish model-driven *in silico* approaches capable of predicting long term toxic events and assessing individual or stratified risks in a fast and reliable way. Thus, the benefit will be the reduction or even replacement of usually tedious preclinical studies (Fig.1). The liver plays a central role in metabolic activation of compounds and liver toxicity is one of the major toxic manifestations. Therefore, NOTOX concentrates on the holistic description of both cellular and organ-level liver toxicity.

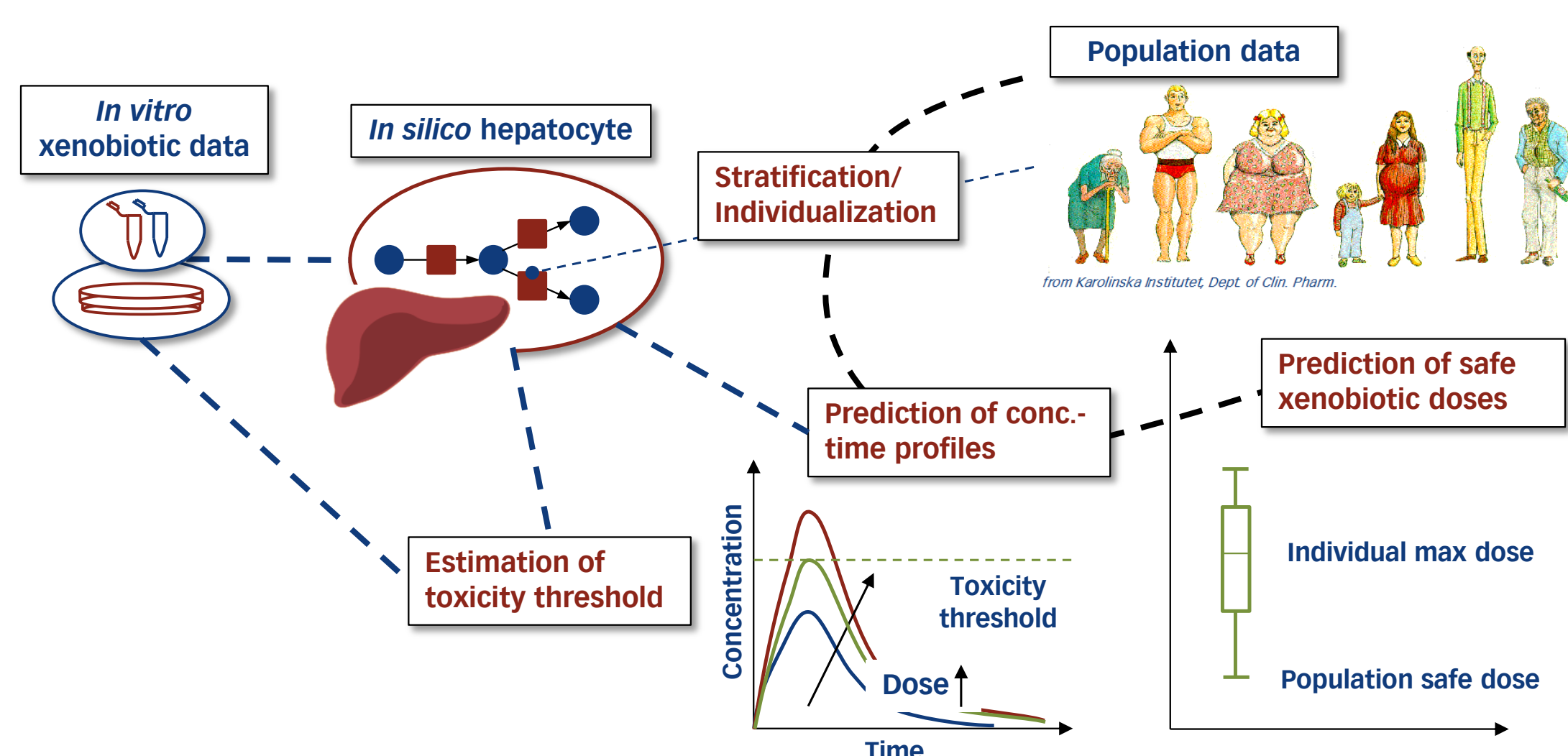


Fig. 1. Replacement of cost- and time-intensive preclinical studies by fast computational approaches. Detailed *in silico* hepatocyte models which are validated by experiments permit the prediction of dose-dependent concentration-time profiles of xenobiotics. The knowledge of appropriate toxicity thresholds and the implementation of population data into predictive models enable individualized or stratified estimations of maximum serum concentrations of drugs and drug-metabolites as well as individualized risk assessment.

Modeling of Short Term Cellular Toxicity

- Modeling of acute toxicity related to Acetaminophen (APAP) overdose, comprising APAP degradation, glutathione metabolism and ROS synthesis in hepatocytes (Fig. 2)
- Parameterization by literature values of kinetic parameters and initial concentrations
- Model validation will be done in the first step on HepaRG monolayer cultures (Fig. 3) in 2012

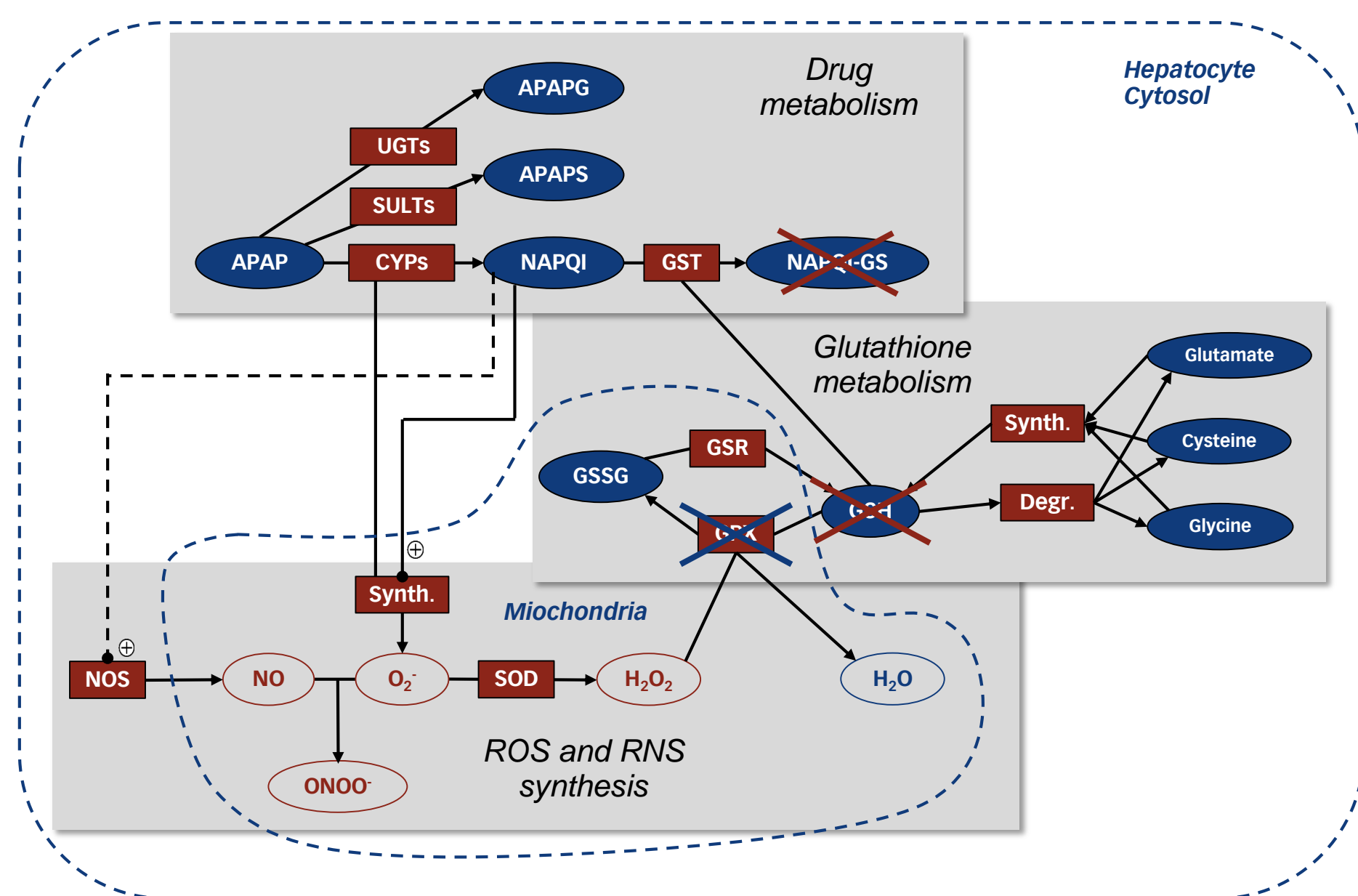


Fig. 2. Model of acetaminophen (APAP) metabolism, glutathione metabolism, and ROS- and RNS-synthesis in hepatocytes. APAP is degraded by phase II conjugation enzymes, UGTs and SULTs, and by phase I CYP catalyzed oxidation to NAPQI. NAPQI is detoxified by GST-enzymes to NAPQI-GS. Therefore, glutathione has to be regenerated from the amino acids glutamate, cysteine, and glycine. NAPQI stimulates NO-synthesis and binds to the mitochondrial membrane causing oxidative stress resulting in an elevated synthesis of reactive oxygen species, O_2^- and H_2O_2 , and reactive nitrogen species, $ONOO^-$.

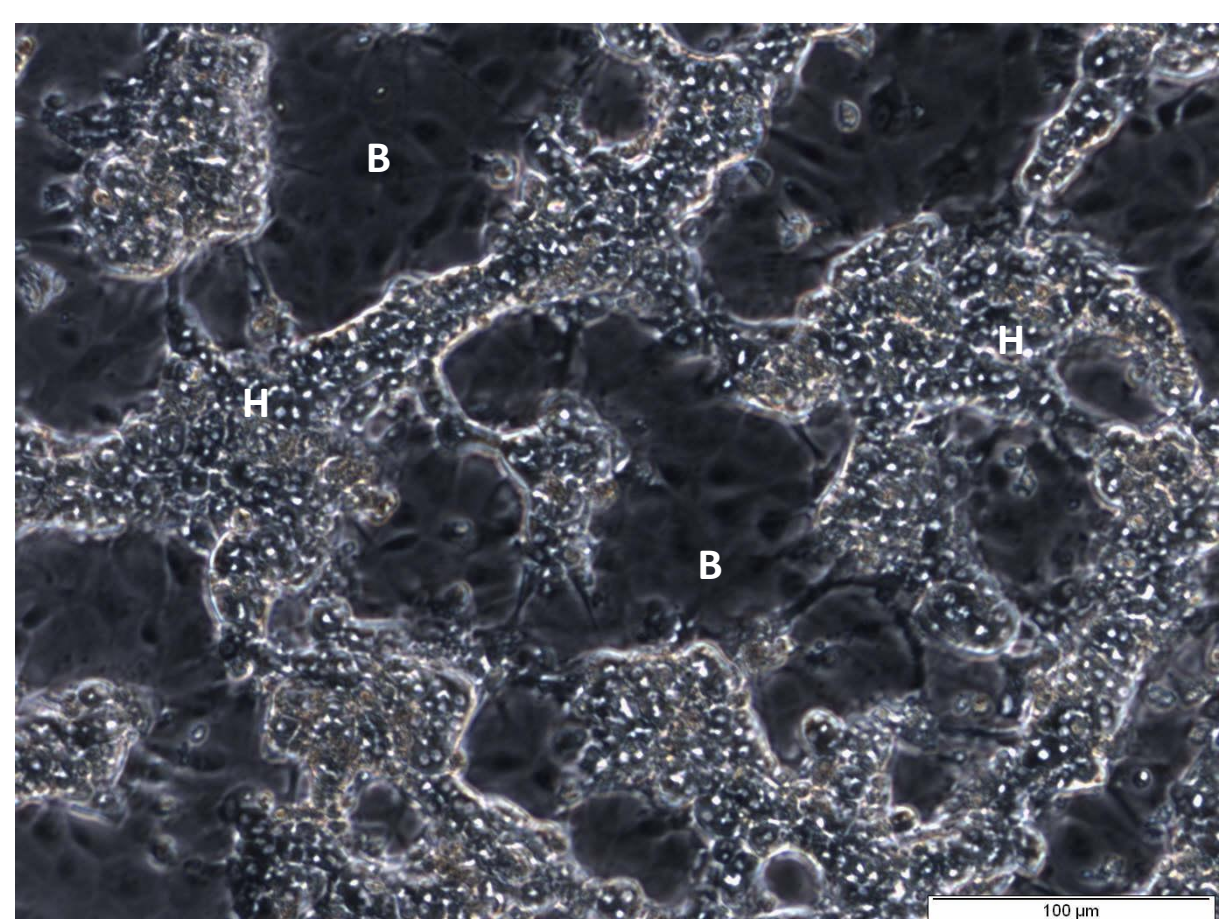


Fig. 3. 2D HepaRG cultures upon 2 weeks maintenance. This cell line consists of two cell types, namely hepatocyte-like cells (H) and biliary-like cells (B). Such heterogeneity can also be observed in real liver tissue. In addition, HepaRG cells show metabolic competence comparable to primary human hepatocytes, making it a very interesting cell line to study hepatotoxicity.

Modeling of Long Term Toxicity

- Necessitates the implementation of toxicity models into large-scale models of metabolic and regulatory pathways (currently under construction)
- This allows combined simulations of xenobiotic and central hepatic metabolism, signaling, enzyme modification and gene regulation
- First flux analyses on HepaRG culture indicate strong influence of APAP on central carbon metabolism (Fig. 4)

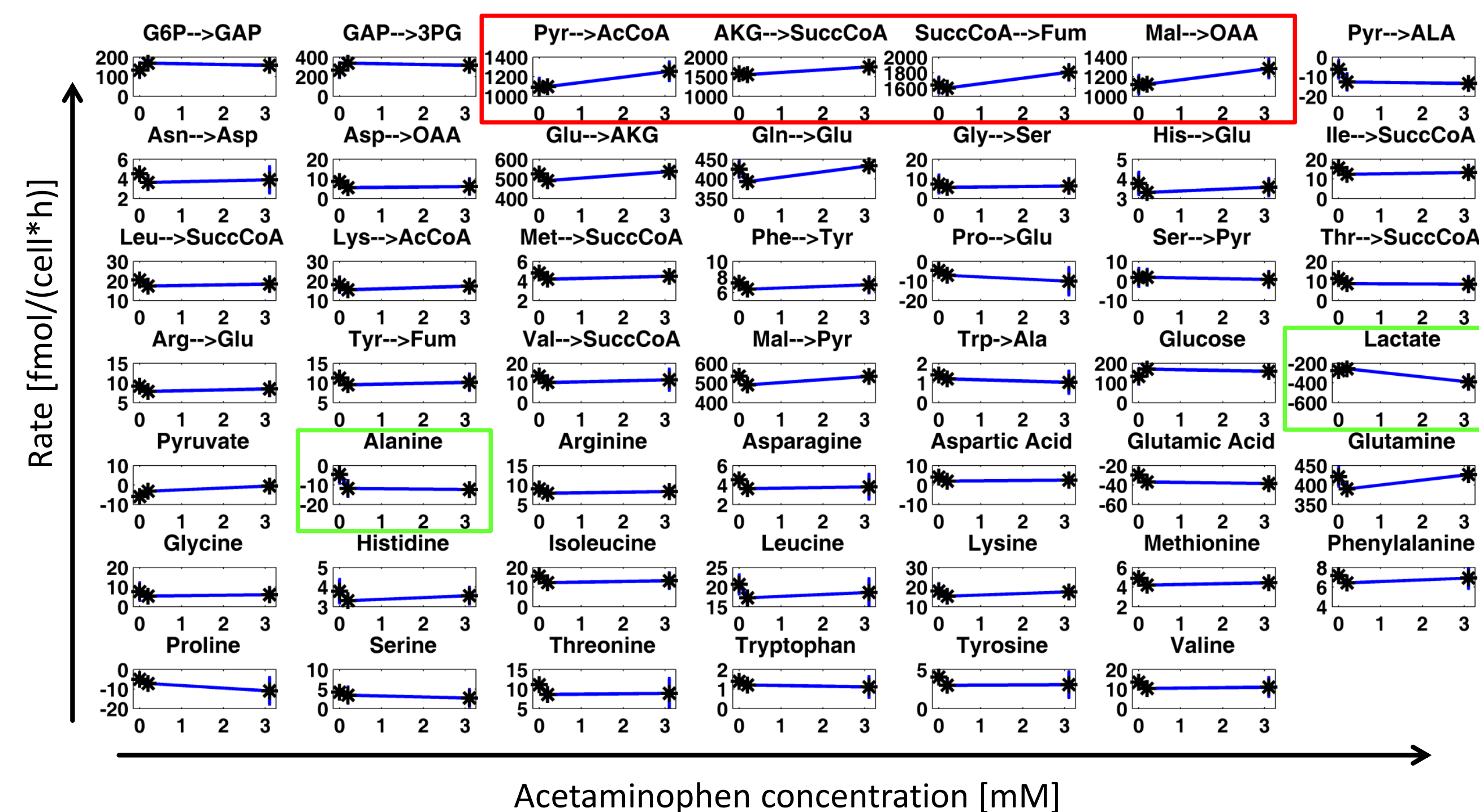


Fig. 4. Intra- and extracellular fluxes in HepaRG cells treated with different concentrations (subtoxic and slightly toxic) of acetaminophen. Untreated control was included. Cells were incubated with the drug for 24 h, after which supernatants were analyzed using HPLC. Fluxes were calculated with metabolic flux analysis (MFA) using a published HepG2 network model. We can observe a low concentration dependent increase in the Krebs cycle (s. red box), indicating a higher demand for energy, most likely due to oxidative stress caused by acetaminophen metabolites. In some cases the effects of acetaminophen were already observed at subtoxic to slightly toxic concentrations (e.g. Alanine, green box). In other cases effects did not occur until exposure to slightly toxic concentrations (e.g. Lactate, green box).

Modeling of Organotypic Cultures

- For the modeling and prediction of organ-level toxicity, inflammation, as well as organ recovery, organotypic culture models have to be established
- The organotypic culture models include the single cell fate, death or proliferation, cell-cell-interactions, cell aggregation and the morphological structure of the organ
- Currently, *in silico* 3D-models and modeling tools are under development, based on spheroid investigations, describing spatial organization as the superimposed mechanism of cell-death and proliferation (Fig. 5)

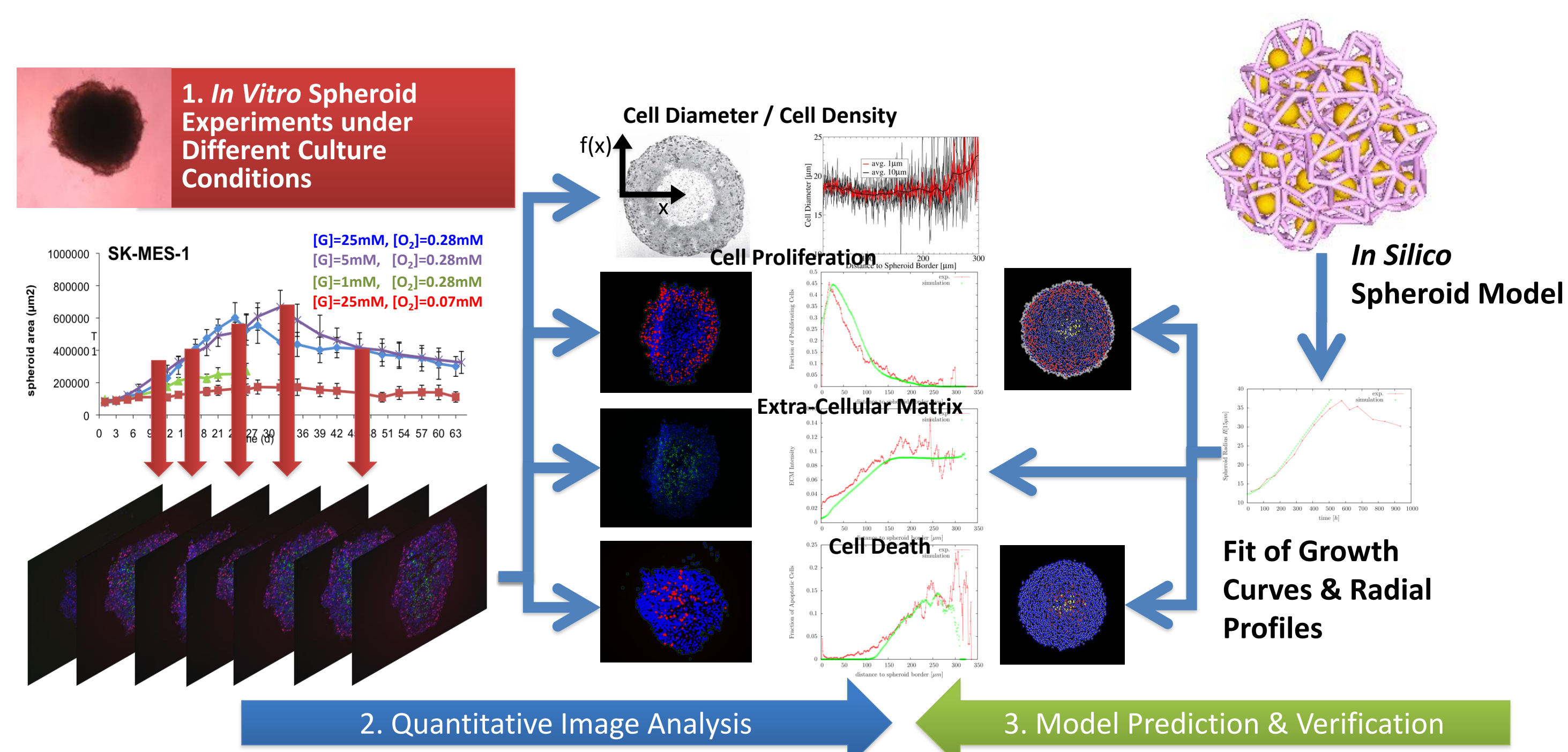


Fig. 5. Individual-based models can help to identify the correct mechanisms on the cellular and molecular cell leading to phenomena on the tissue scale. As a proof of principle the sketch shows how one has to parameterize a predictive model in such a way that not only the quantitative behavior in time (growth curves, left & right) are captured but also the spatial organization as well (central column). Part of this work has been funded by BMBF-LUNGSSYS.

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