ELRIG
Drug Discovery 2019

High-throughput metabolomics for liver safety and mechanisms of toxicity

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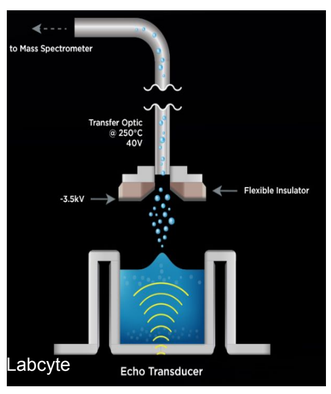
Aim: Improve sensitivity for cell-based liver toxicity predictions and gain mechanistic insight by measuring intracellular metabolites

I. The challenge of predicting Liver Injury

- Current cell-based Liver Toxicity assays mostly measure cell death
- Functional liver damage occurs much earlier than death
- Cytotoxicity endpoints peak at ~60% Sensitivity and 90% Specificity
- Measuring metabolites better reflects cell functioning and health
- Multiple metabolites can be linked in pathways to reveal mechanisms

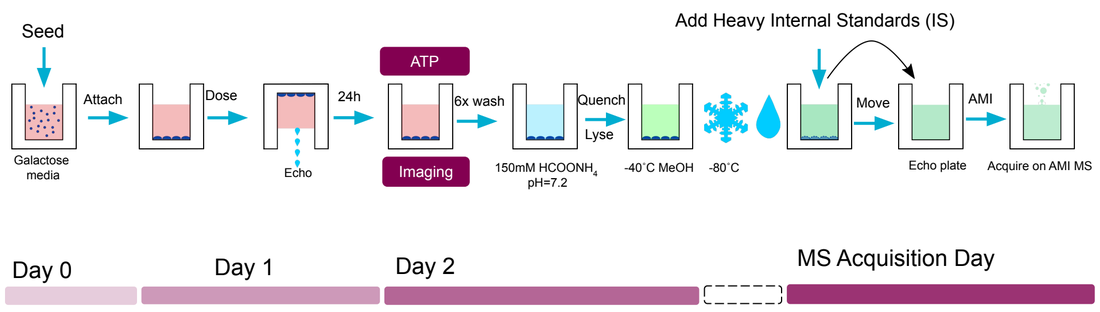
II. The opportunity of AMI MS



Acoustic Mist Ionisation Mass Spectrometry

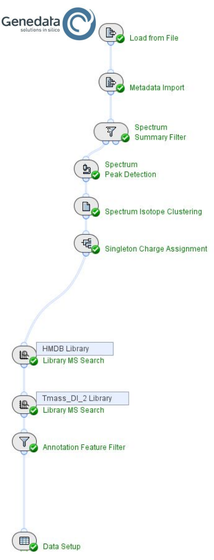
- Prototype technology conceived in DS
- Acoustic transducer ejects femtolitre droplets
- Tiny volumes - 150nL sample used
- High speed - 2 sec/sample; 20mins / 384 wells
- No chromatography selection
- ToF Detector for wide metabolite coverage

III. Experimental workflow



- 150 DILI (Drug Induced Liver Injury)-relevant compounds
- 9-point, half-log concentration-response
- 316µM top concentration

IV. Data processing



- Pre-processing performed in GeneData Expressionist
- Isotope cluster patterns used to detect metabolites
- Human Metabolite Database (HMDB) queried for best match
- Metabolite hits confirmed with manually curated library
- Detected >3000 peaks; 1000 have annotation
- Expressionist greatly reduced pre-processing times
- Before Expressionist – days to pre-process; now minutes

V. The data analysis challenge

- Highly multidimensional data with thousands of features spanning 6 orders of magnitude
- High proportion of raw features are noise
- Many features have high proportion of missing values
- Batch effects – within-plate, plate-to-plate and day-to-day variability
- Need to find “real” signals in variable background
- Non-monotonic concentration-responses – hormetic and biphasic

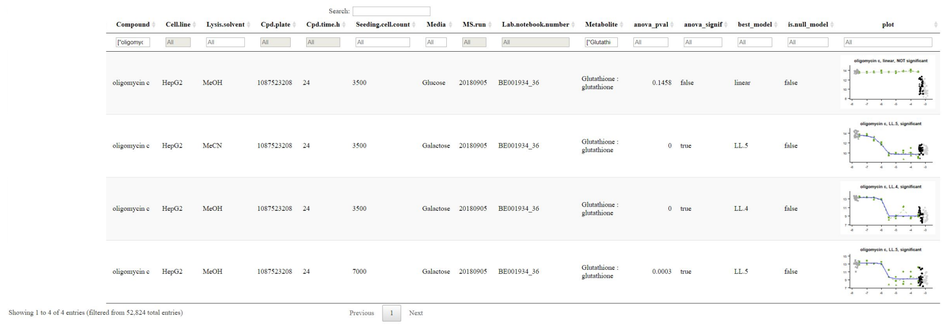
VI. The analysis pipeline

log-transformation | Missing values imputation | Plate-to-plate batch correction | Plate effect correction | Sample normalisation

- Custom pipeline in R
- Log-transformation to counter skewness
- k-NN (k-nearest neighbours) imputation of missing values
- Batch correction to reduce plate-to-plate and day-to-day variability
- Spatial plate effects normalisation to reduce within-plate variability
- Internal standard (IS) dependent and IS independent normalisation

VII. Automated concentration-response fitting

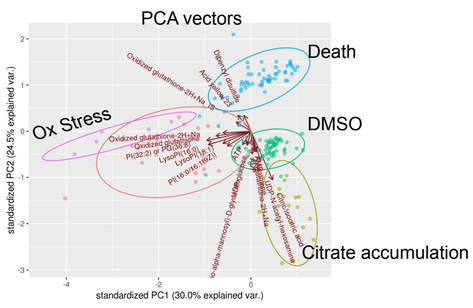
- GD Screener struggles to fit increasing and non-monotonic responses
- We built an automatic workflow to fit multiple dose-response models
- Fit straight line; sigmoidal; hormesis; multiphasic and bell-shaped models
- Used Bayesian information criterion (BIC) to penalise complex models
- Automated fit >60,000 metabolite-compound curves per experiment
- Interactive, searchable HTML table to quickly filter and examine curve fits



Left: Screenshot of the interactive report. **Right**: Examples of model fits to the response of two different metabolites to chlorpromazine treatment: LL4 (above) and gaussian (below).

VIII. Future application – towards a predictive assay

- Developed a pipeline to analyse challenging multiparametric dataset
- Working towards launching a metabolomics assay with improved sensitivity



Clustering of compounds to reveal mechanistic insight

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