# Doing more with less: Application of microsampling, LC/MS/MS and MS imaging for the measurement of drug, metabolites and lipid biomarkers in biofluids and tissues following the IV & PO administration of gefitinib to the mouse

**Ian Wilson**

**Imperial College, London, UK**

16th EBF Open Symposium

# Aiding Decision Making in Discovery

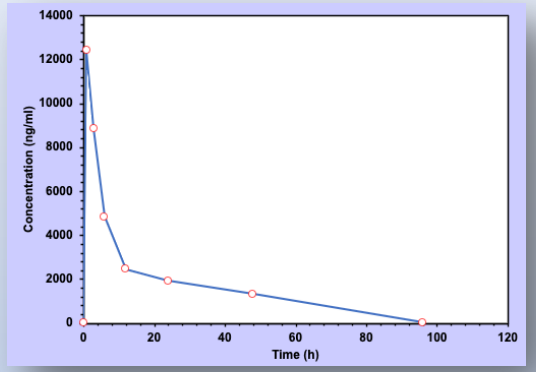
- **Despite advances in compound evaluation in drug discovery many compounds still fail in development/clinical and fail to get to market.**

- **Issues in drug discovery development.**
  - **Compound attrition due to unexpected toxicity, lack of efficacy and off target pharmacology**

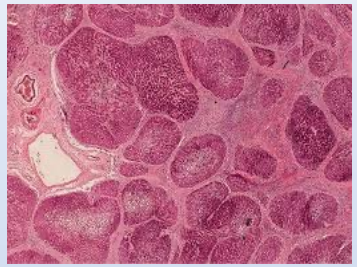
- **We need more information, but with no additional animal studies (3R's).**

- **Aim is to use microsampling and modern analytical technologies to maximize information recovery and knowledge generation from lean animal studies.**

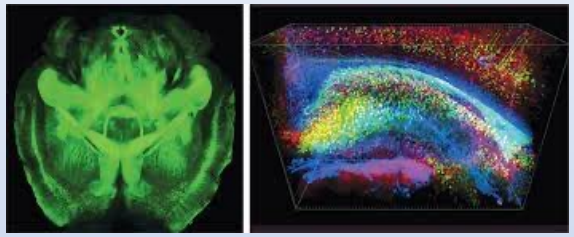
# What Do We Need To Know to Progress Drugs?



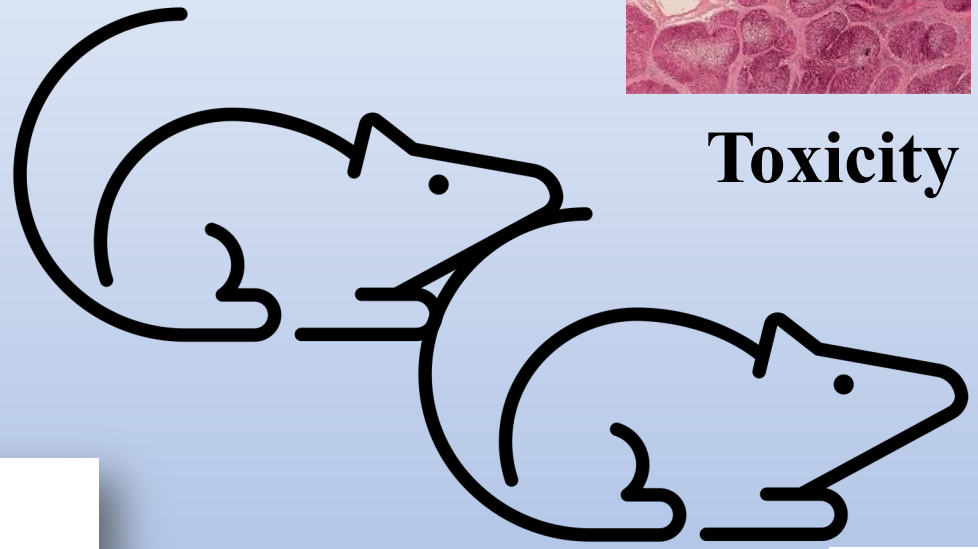

Pharmacokinetics



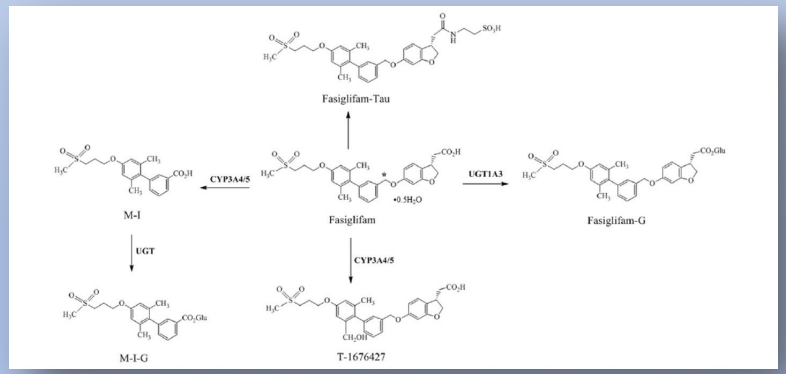
Toxicity



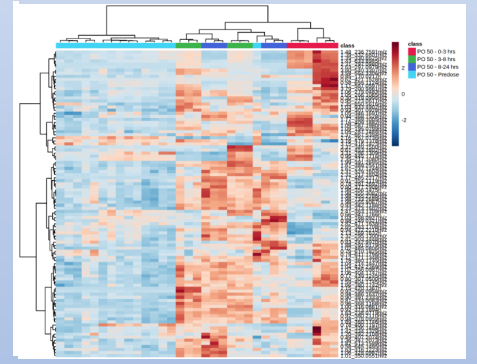
Drug Distribution



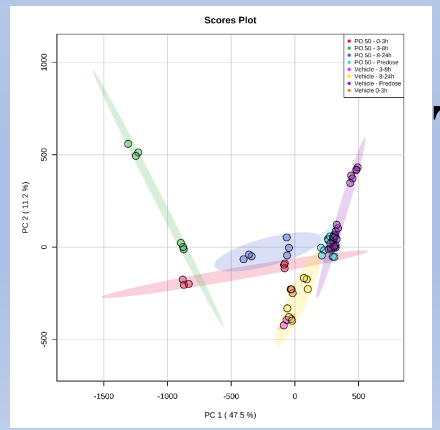
Inter Animal Variation




Drug Metabolism



Target engagement




Off target pharmacology

# Study Design (Late stage discovery)

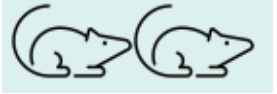
3 Dose Groups (males)

Plasma collection

Urine collection

Tissue collection

*Vehicle*



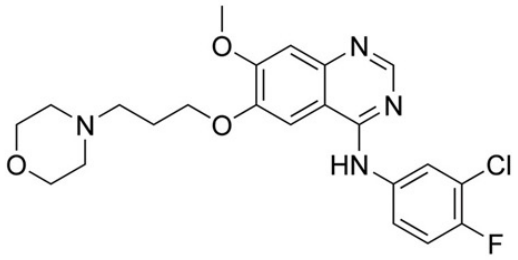
Oral Dose 50mg/Kg

IV Dose 10mg/Kg

Pre dose, 15, 30, 45min, 1hr, 2hr, 3hr, 6hr, 8hr & 24hrs

Pre dose, 0-3hr, 3-8hr, 8-24hr

1, 3, 6, 8 and 24hrs

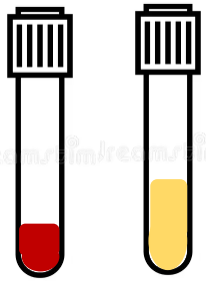



**Gefitinib**

- **Pharmacokinetics**
- **Metabolite ID**
- **Metabolomics**
- **Proteomics**
- **Imaging**
- **Transcriptomics**

# Sample Analysis

**50µL Blood**
**Urine**



**10uL Plasma**

**PK Analysis**
UPLC-MS/MS (QqQ)

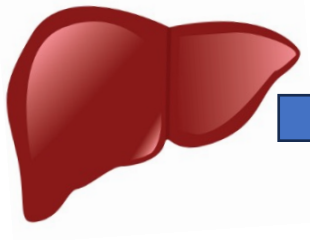
**2 µl Plasma and 20 µl Urine**

**Metabolite ID, Metabolomics,**
UPLC-cIM-MS

**20 µl Plasma**

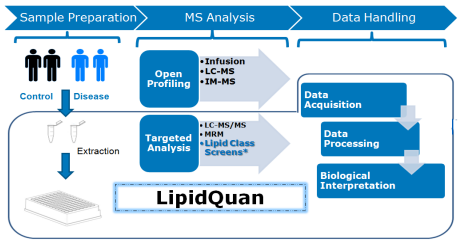
**Plasma Lipidomics**
UPLC-cIM-MS, UPLC-MS/MS (QqQ)

**Liver/ Tissue**



**50mg Liver**

**Tissue Lipidomics**
UPLC-MS/MS (QqQ)

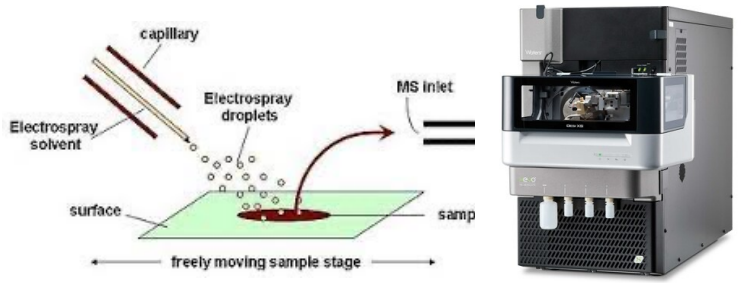



**Tissue Extract**

**Proteomics**
UPLC-cIM-MS

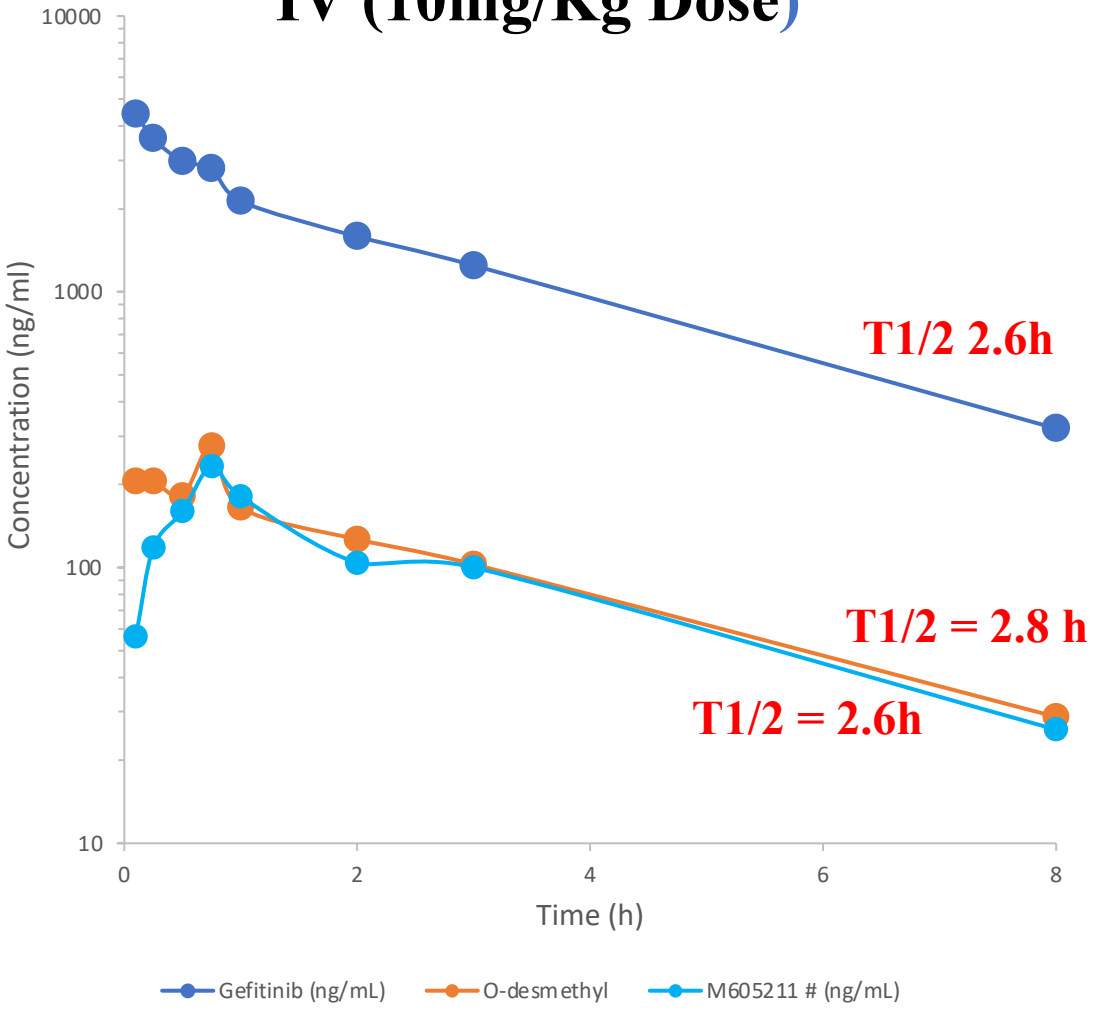
**Liver Slice**

**Tissue Imaging**
MS/MS (QqQ)

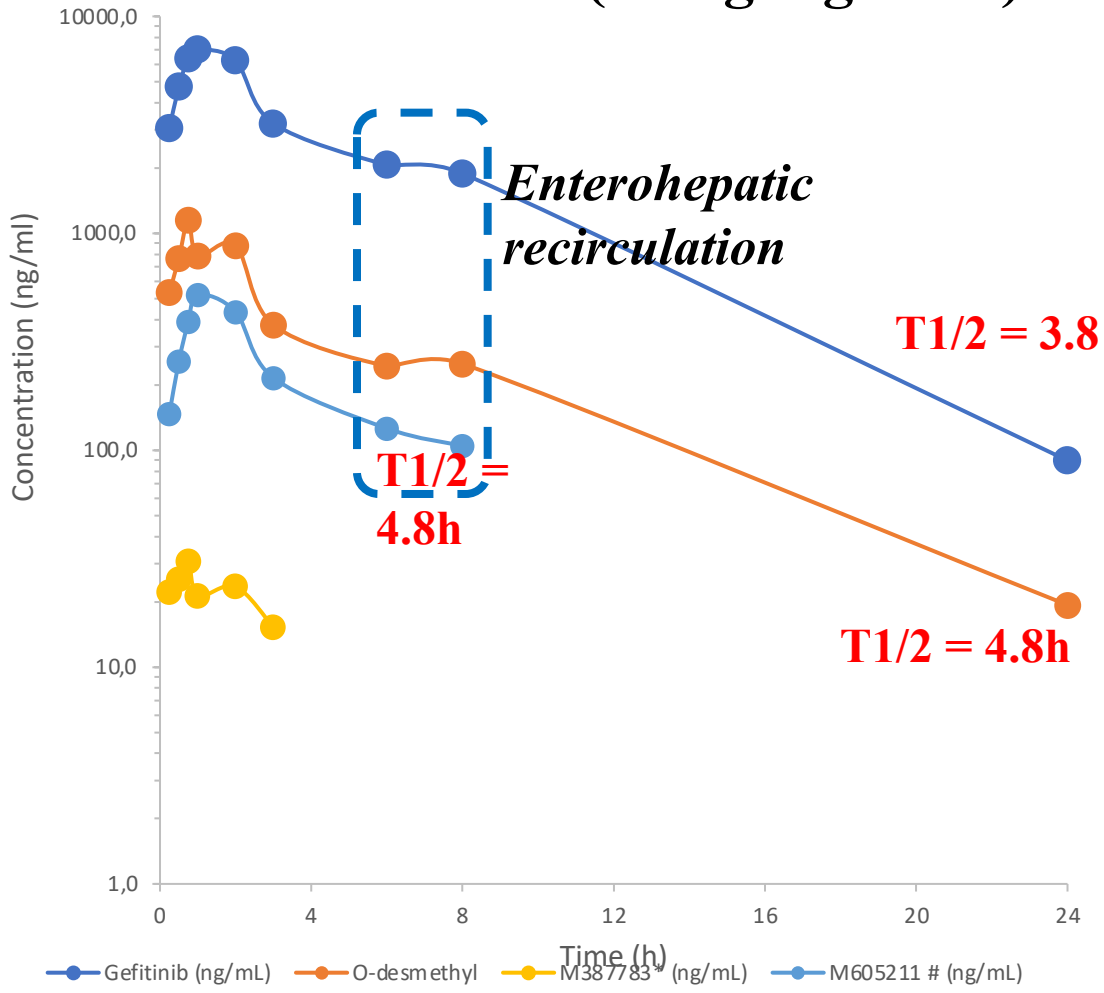


# Pharmacokinetics

## IV (10mg/Kg Dose)



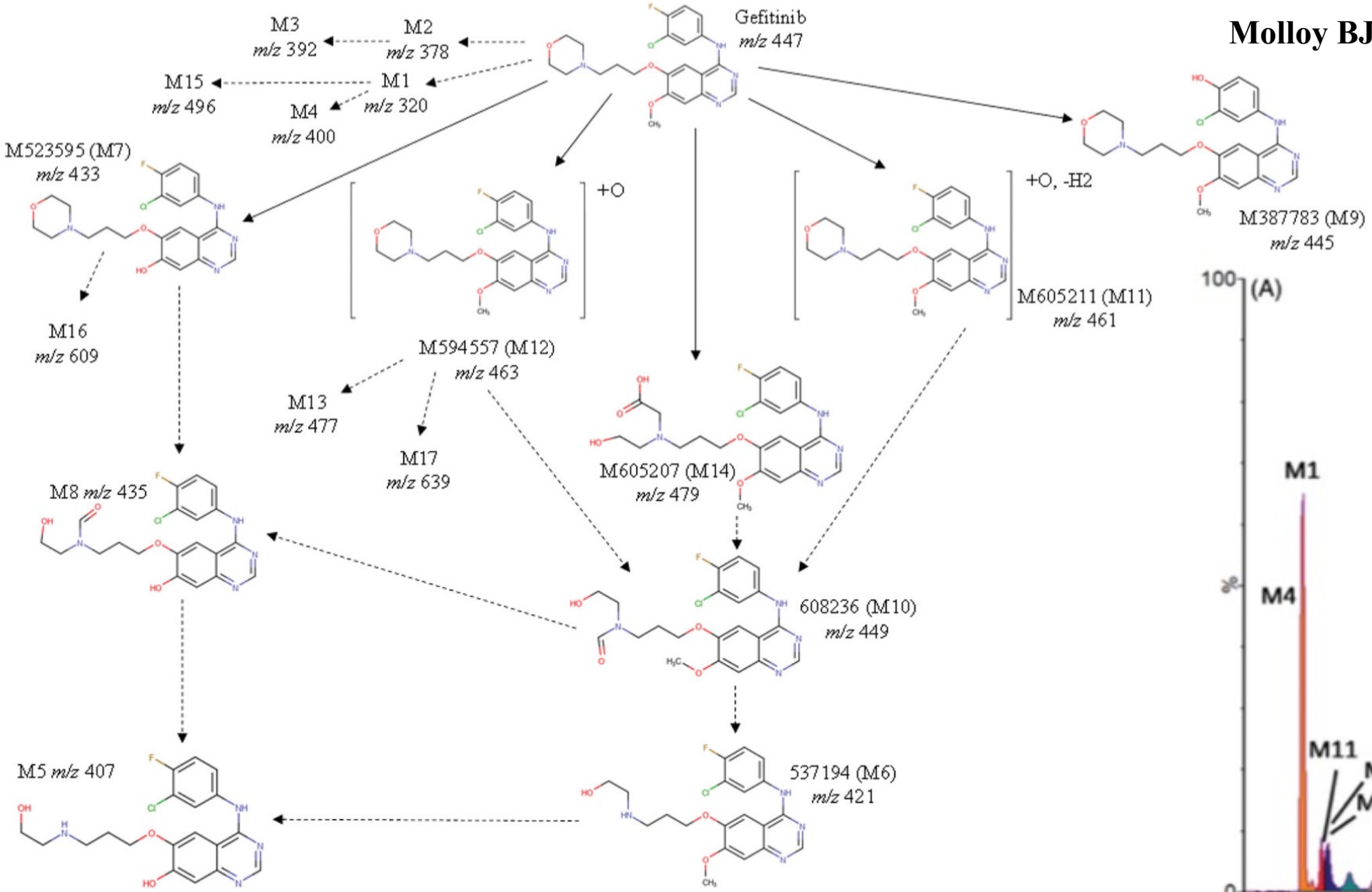

## PO (50mg/Kg Dose)



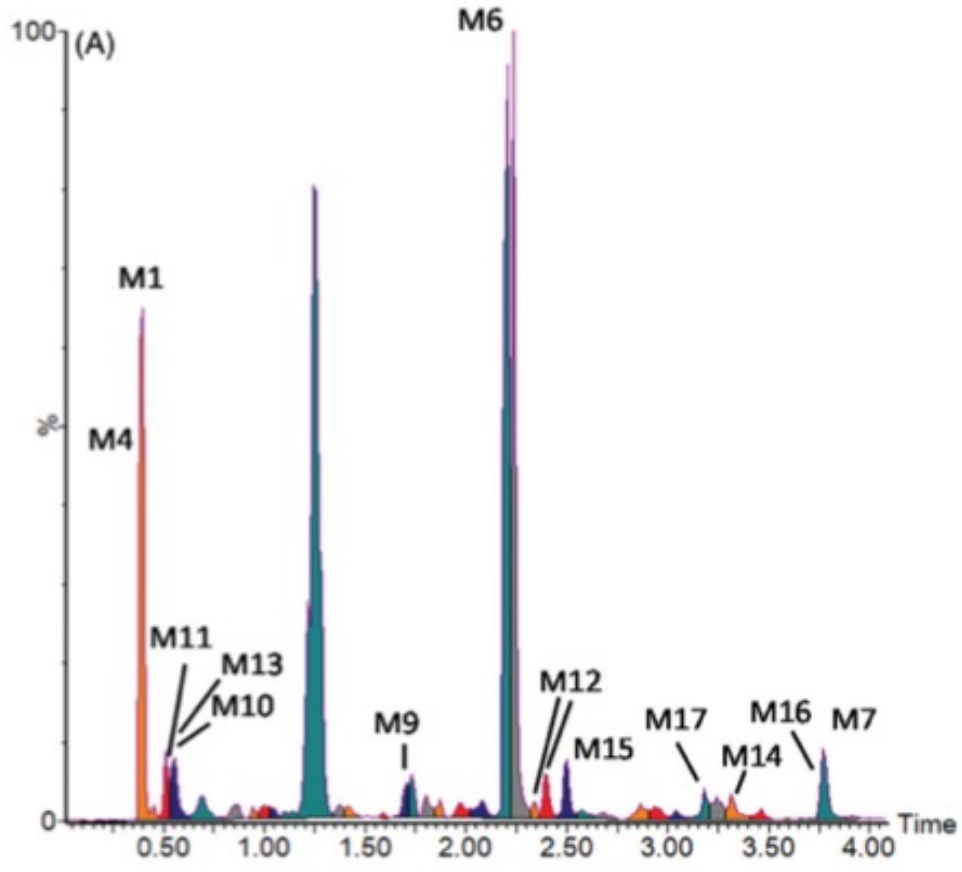

**Molloy et al, Xenobiotica 2021;51(4):434-446**

Tandem Quadrupole LC-MS/MS

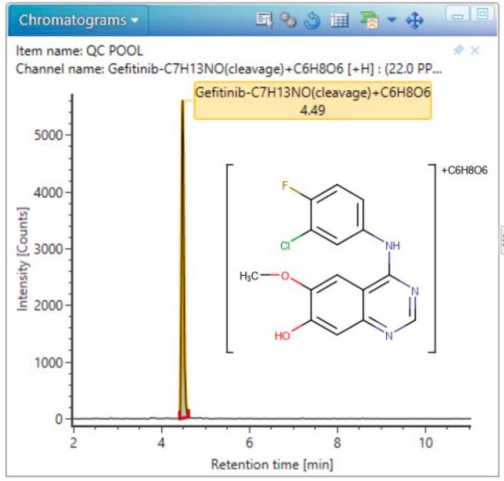
# Gefitinib - Metabolism

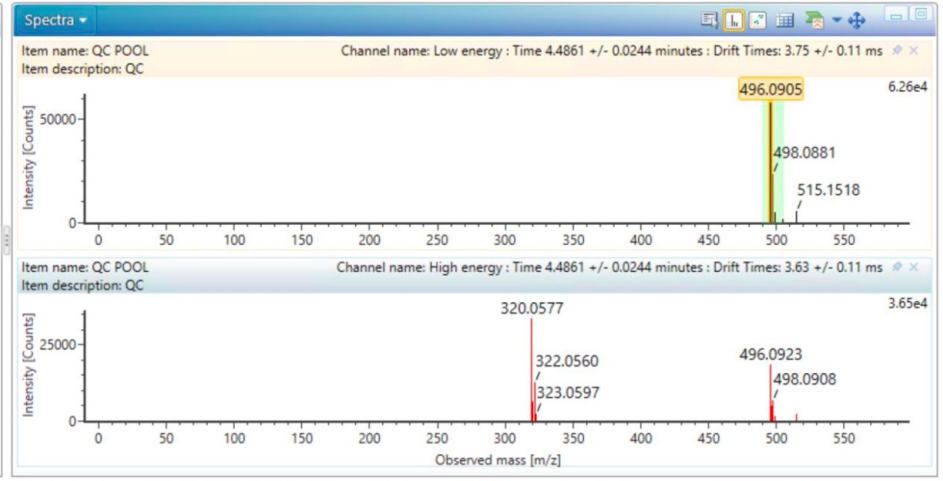



Molloy BJ, et- al Xenobiotica. 2021;51(4):434-446.

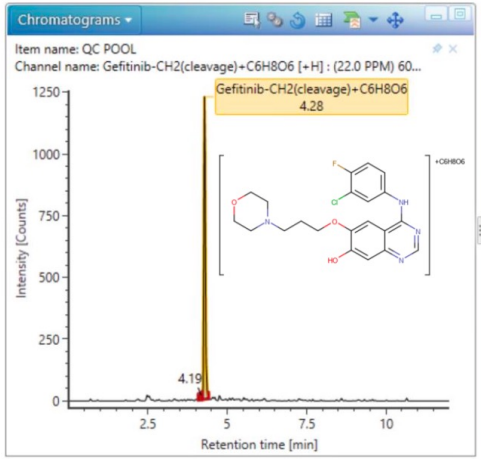


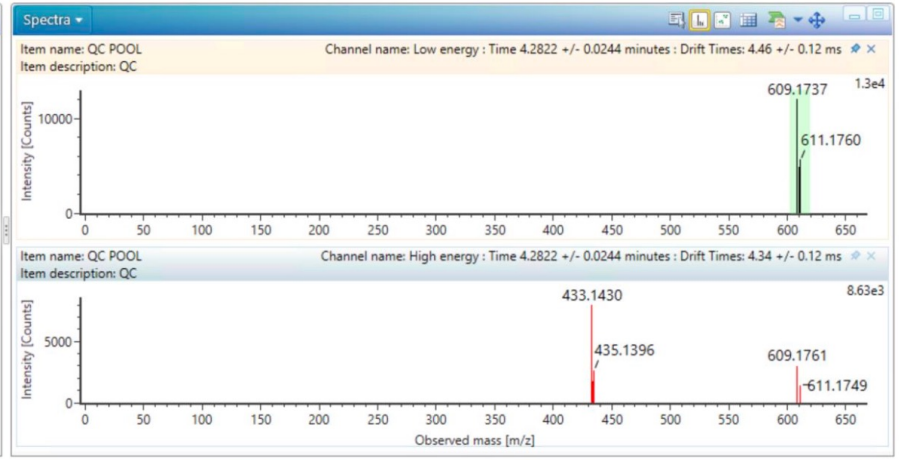

Reversed – Phase -IM-MS

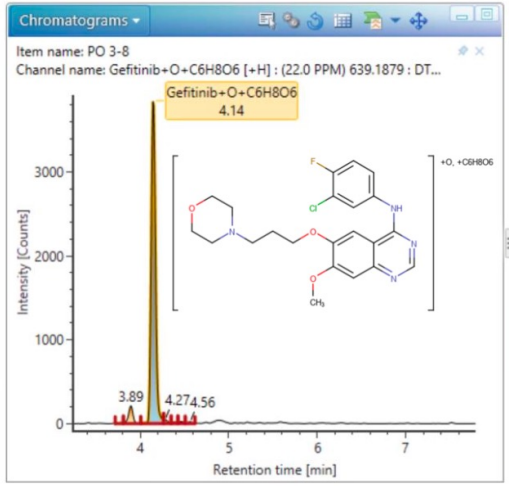


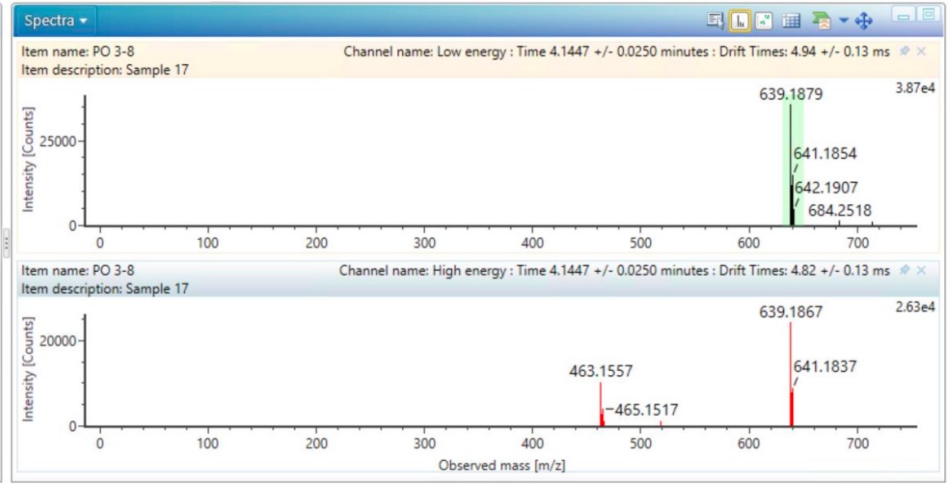




# 3 New Glucuronides Identified (+ 1 novel sulphate)



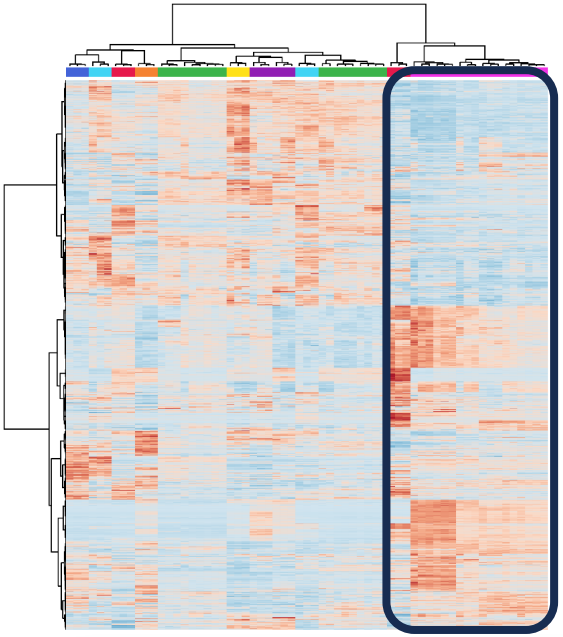



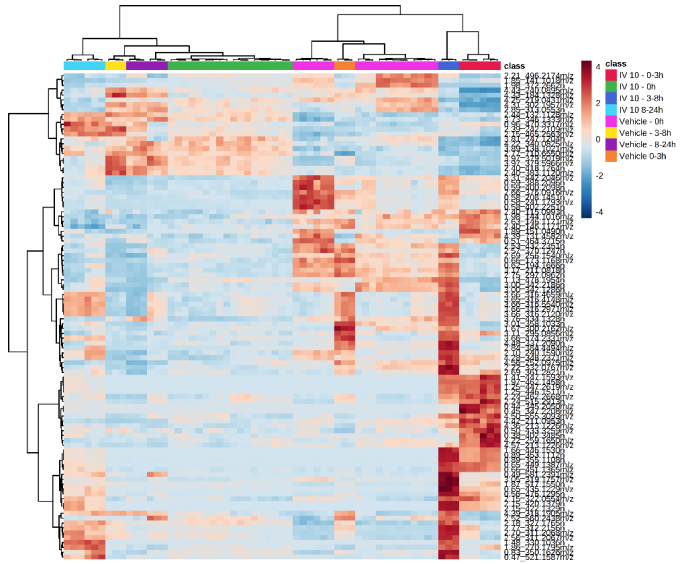



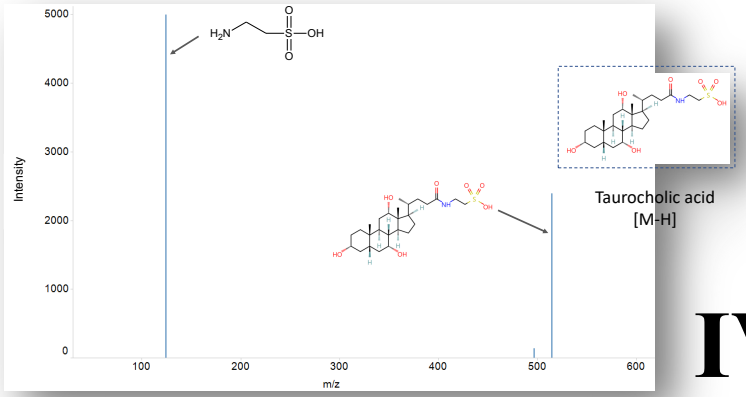

**LC-IM-MS Using MassMetaSite Data Analysis**

# Target Engagement & Off-Target Pharmacology via Metabolic Phenotyping

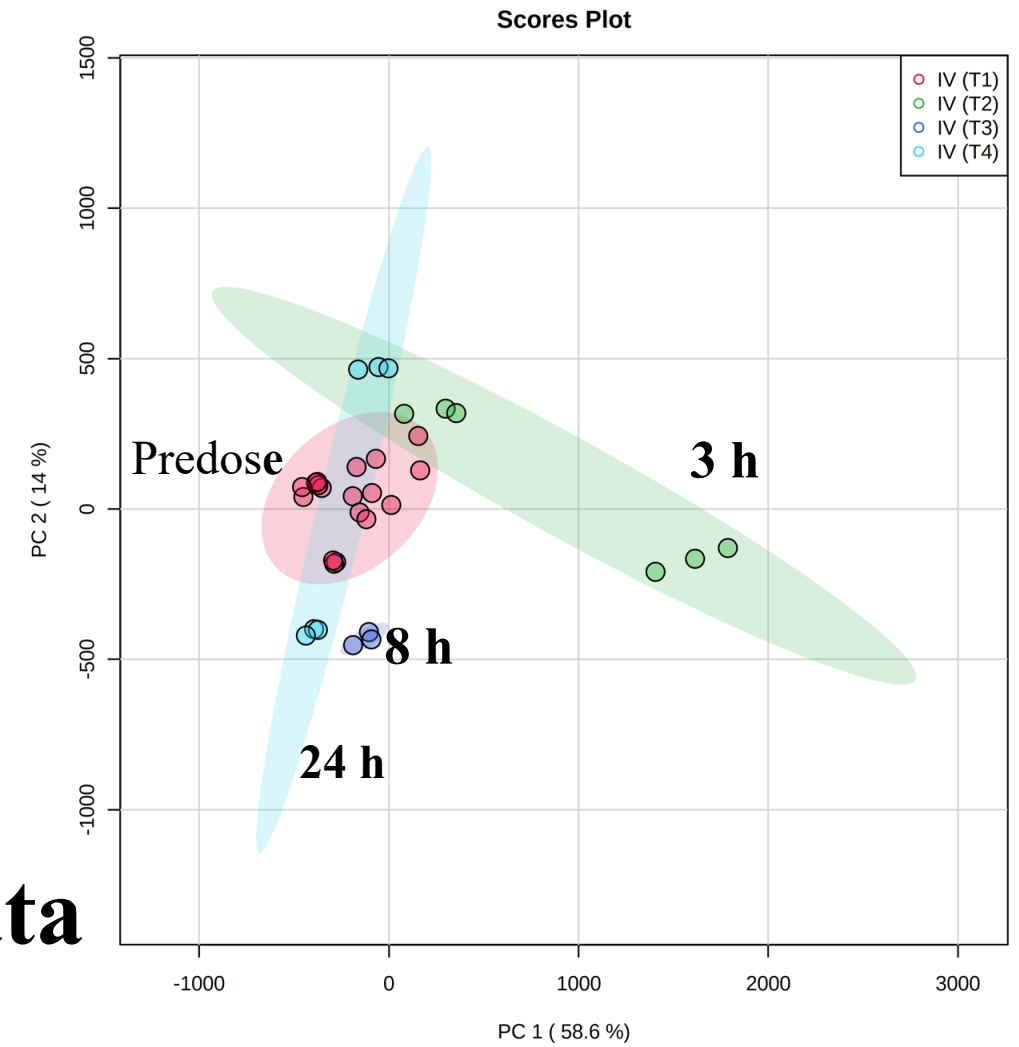





*Top 100 (t-test) features*




Excluding Gefitinib metabolites

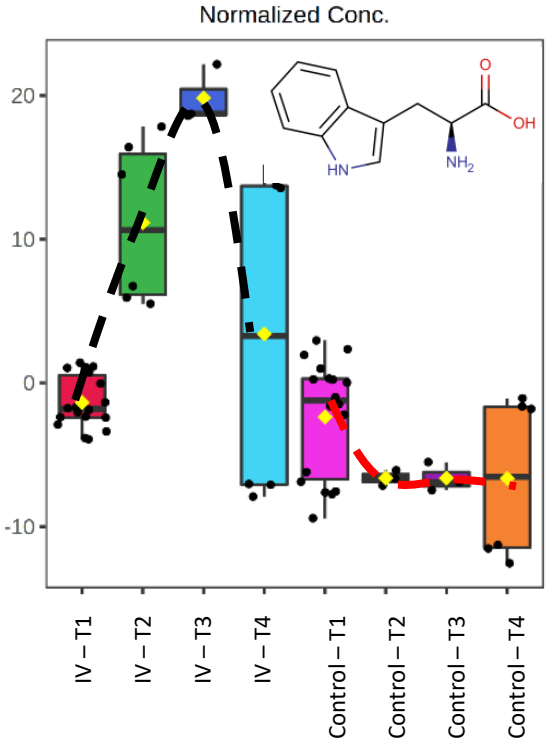



# IV Dose, Urine Data

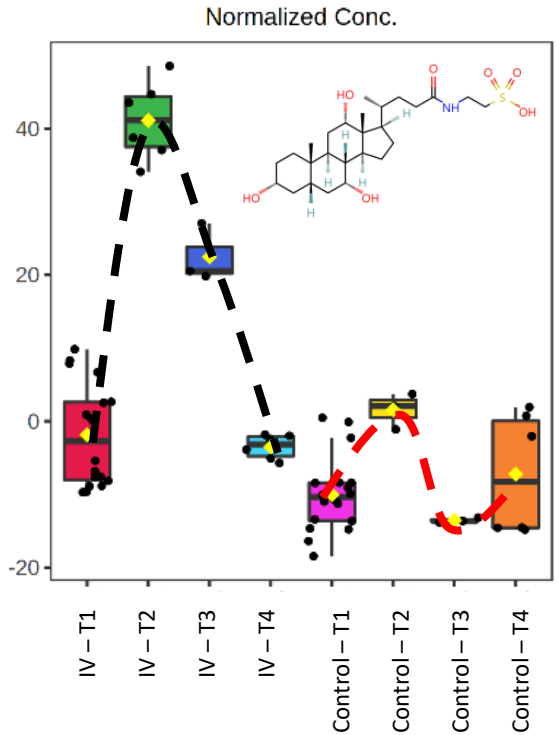
Wilson *et-al*, Metabolites 2021, 11, 379.

# +ve PK Correlated IV Urinary “Biomarkers”

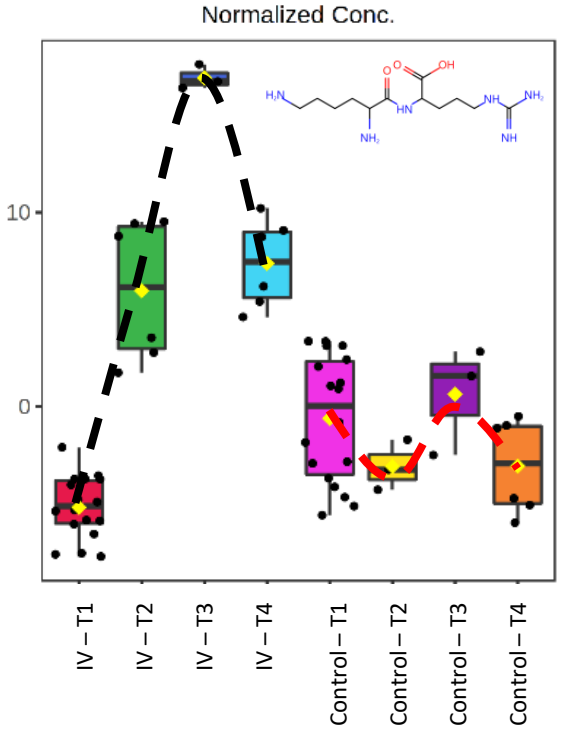
Tryptophan

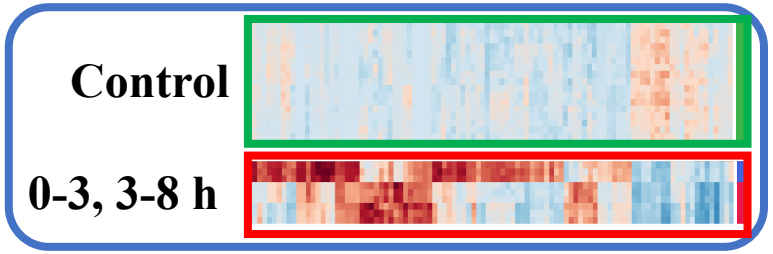



Taurocholic acid



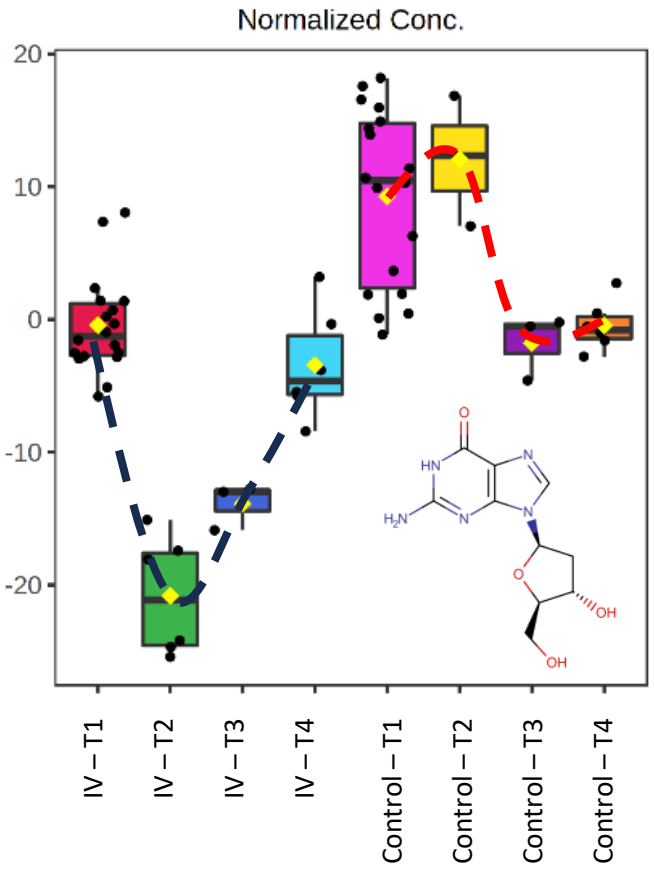

Arginyl-lysine



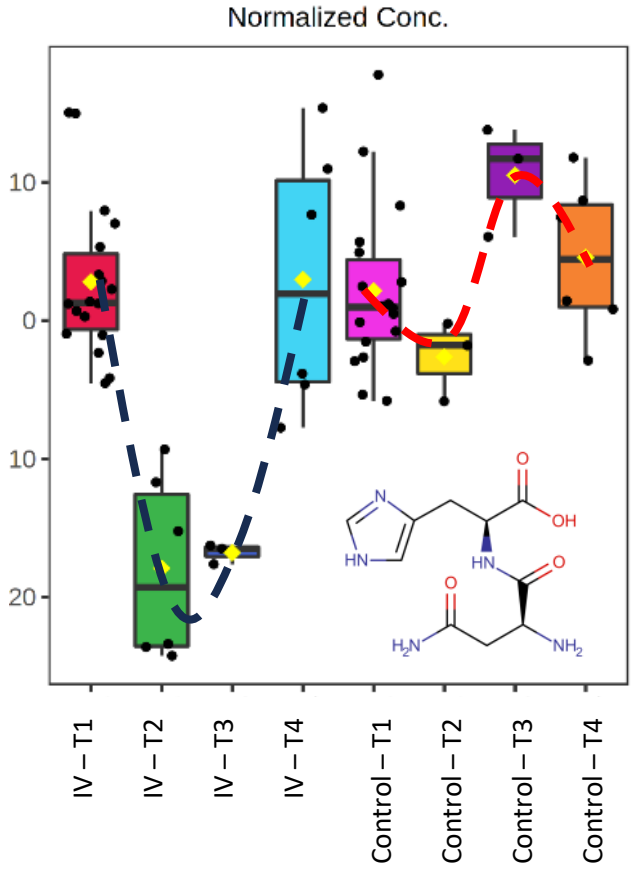



# -ve PK Correlated Urinary IV “Biomarkers”

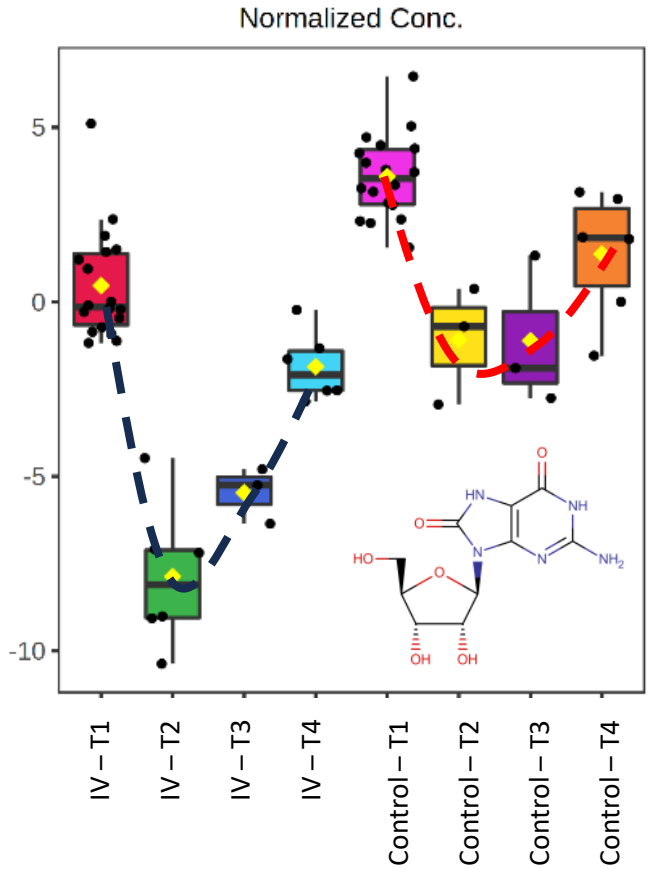
Deoxyguanosine




Asparaginyl-histidine



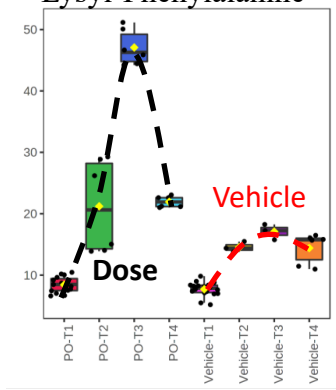

8-hydroxy-deoxyguanosine



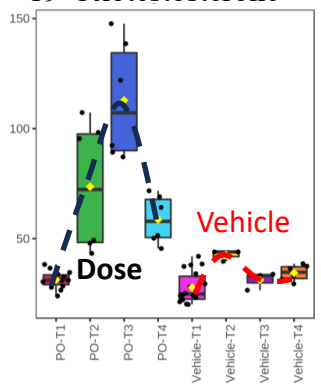
# PO Data Off Target Pharmacology

## (+ve PK Correlation)

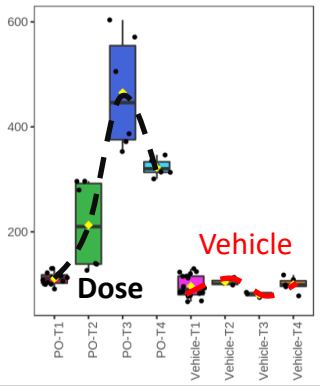
Lysyl-Phenylalanine



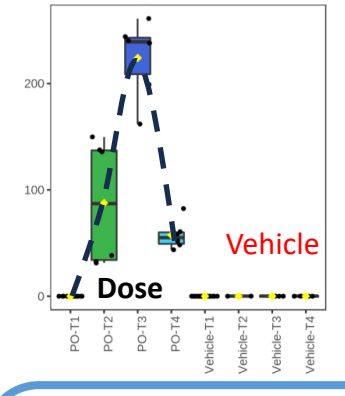

19-Oxotestosterone

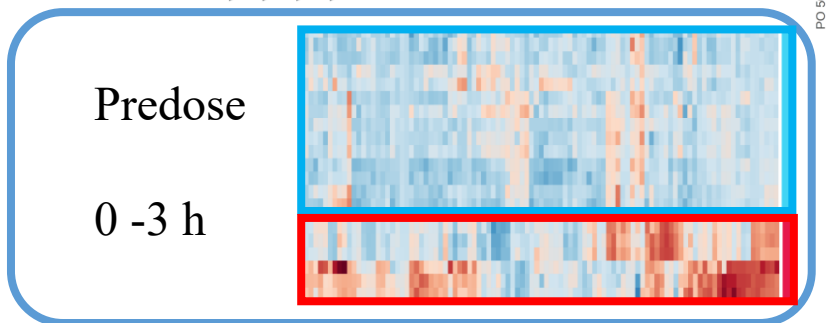



8-Hydroxy-deoxyguanosine



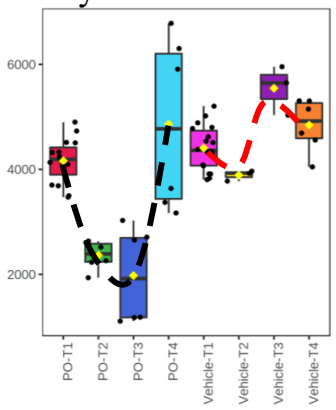

3'-L-asparaginyl-AMP



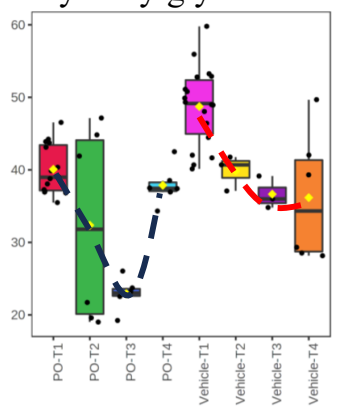




## (-ve PK Correlation)

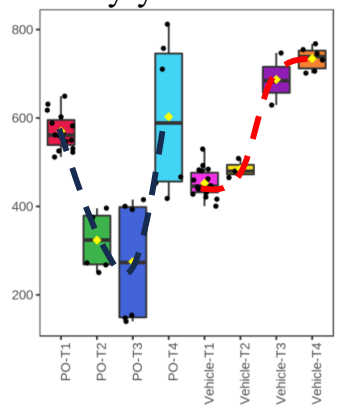
acylcarnitine



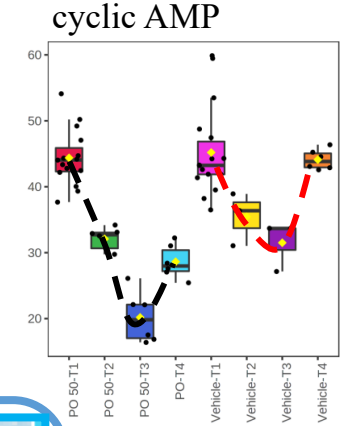

myristoylglycine



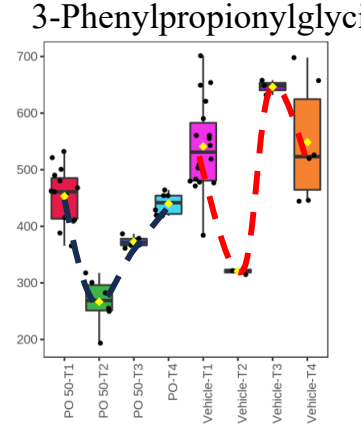

isobutyryl-carnitine



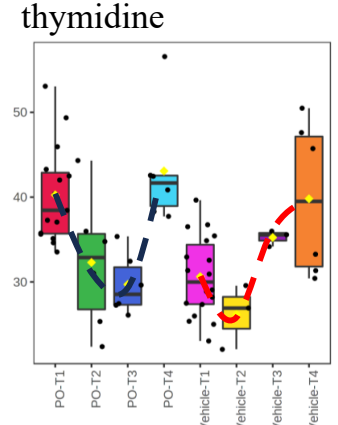

cyclic AMP



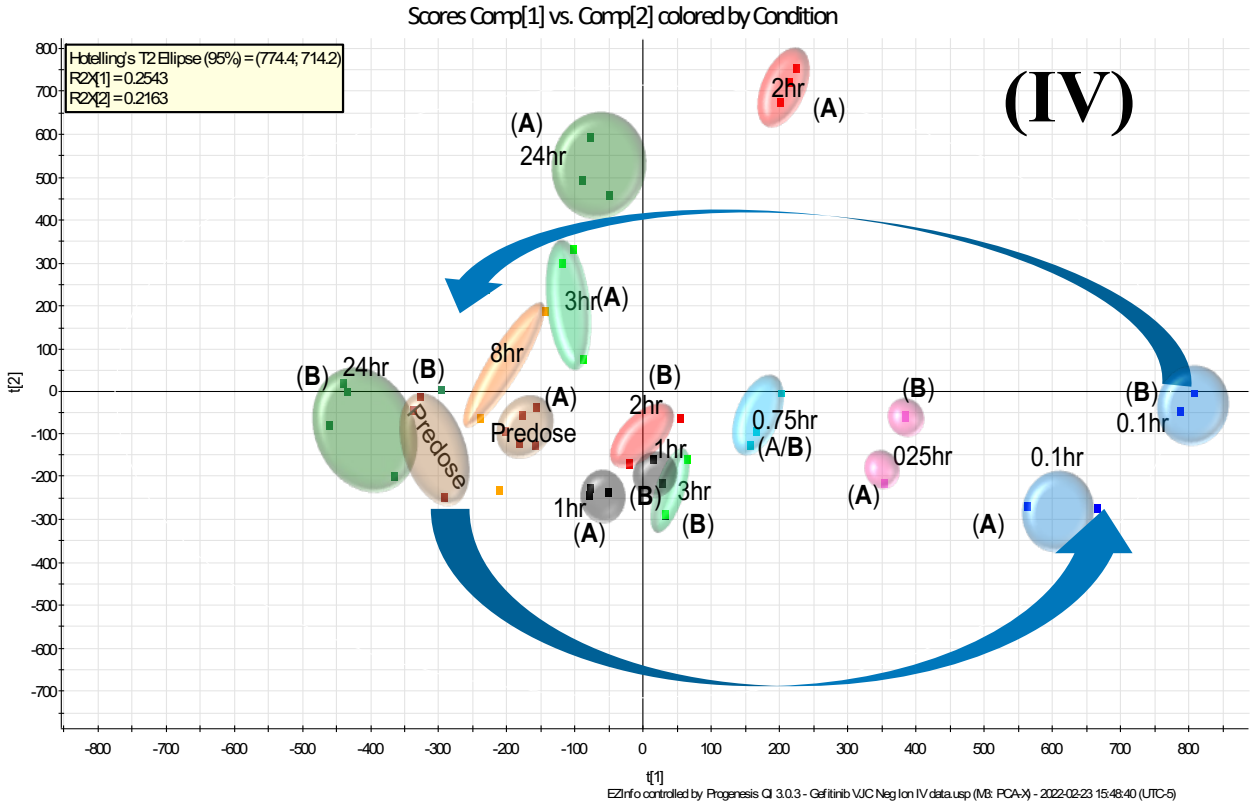

3-Phenylpropionylglycine

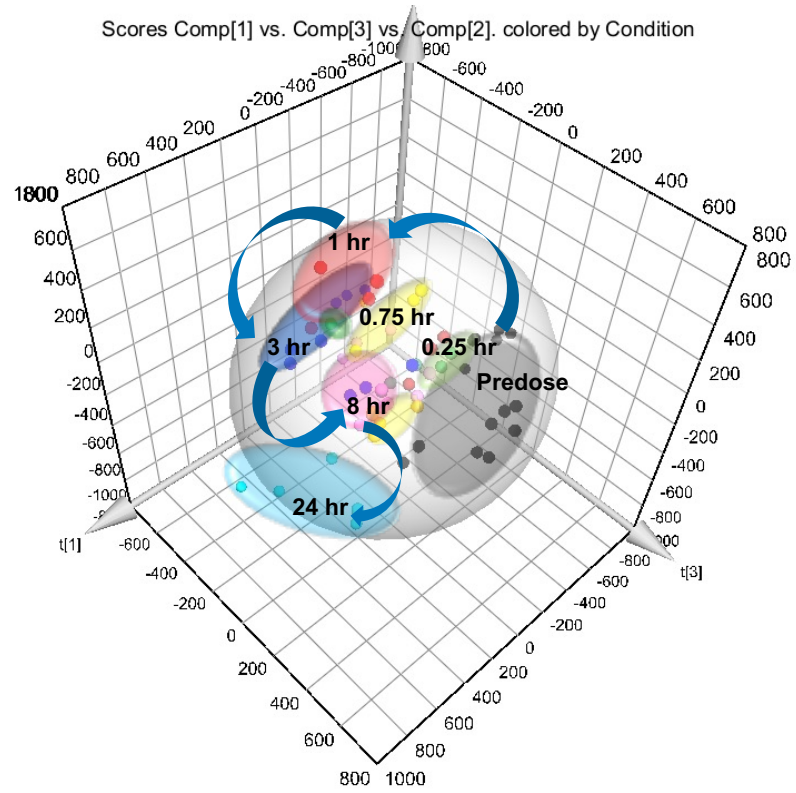



thymidine



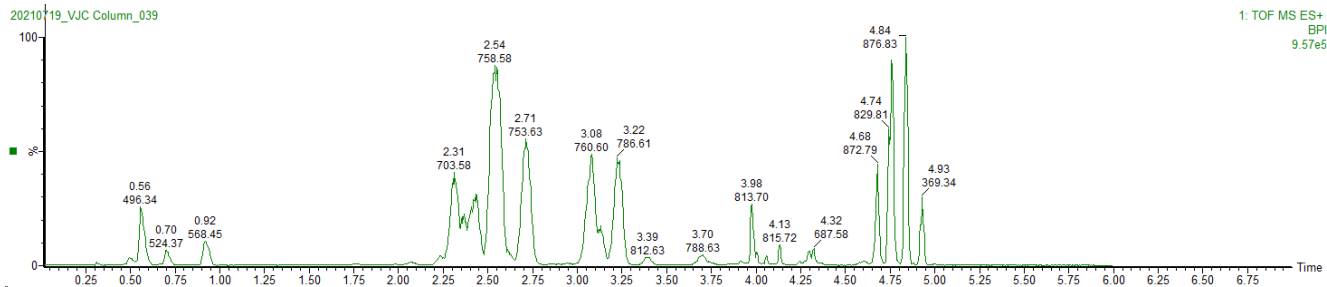
# Plasma IV and PO Lipidomics



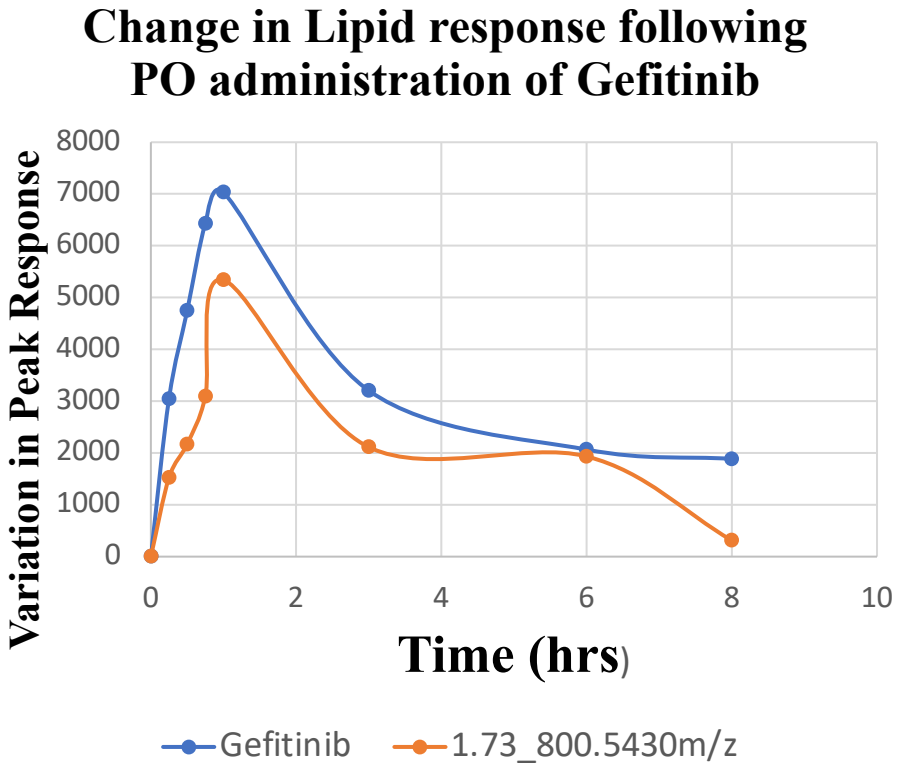



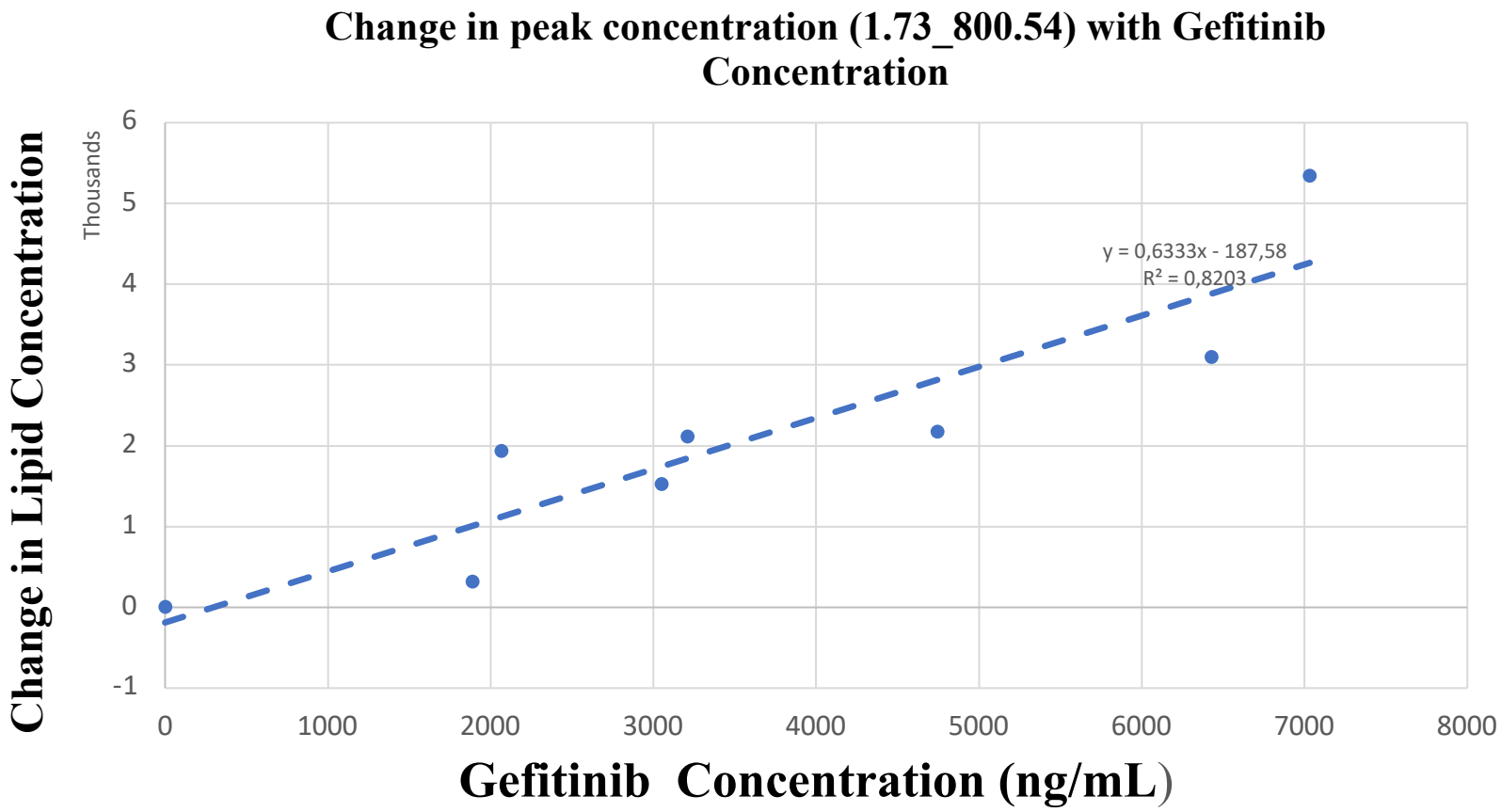

(PO)


## Discovery & Targeted LC-MS/MS



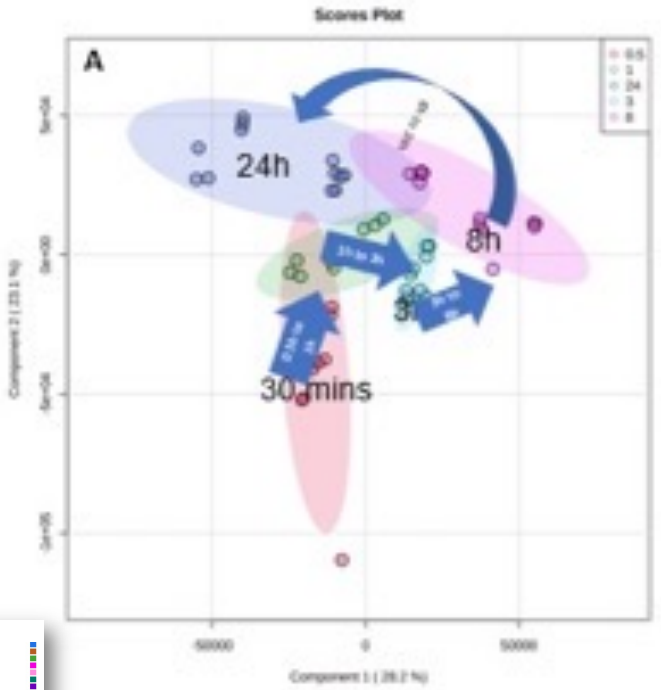
# Drug concentration related response (”Biomarker?”)

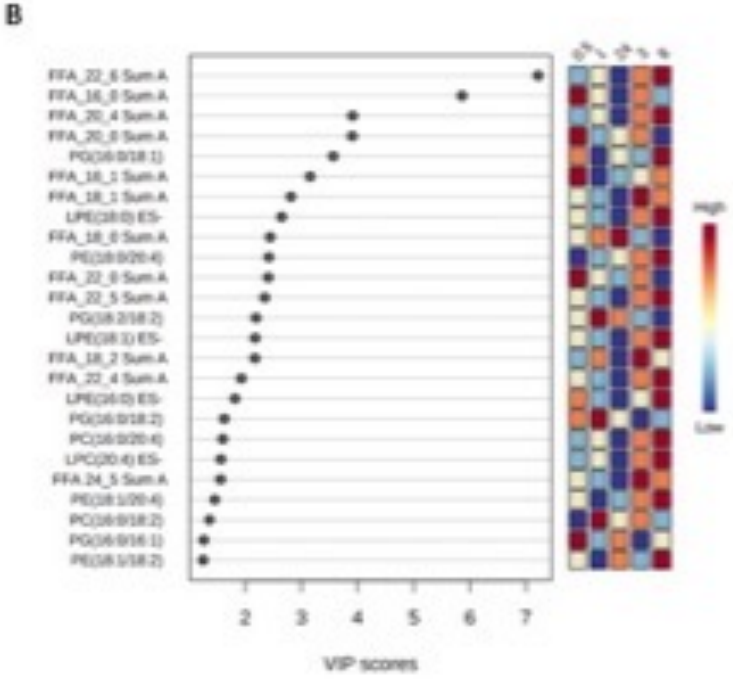


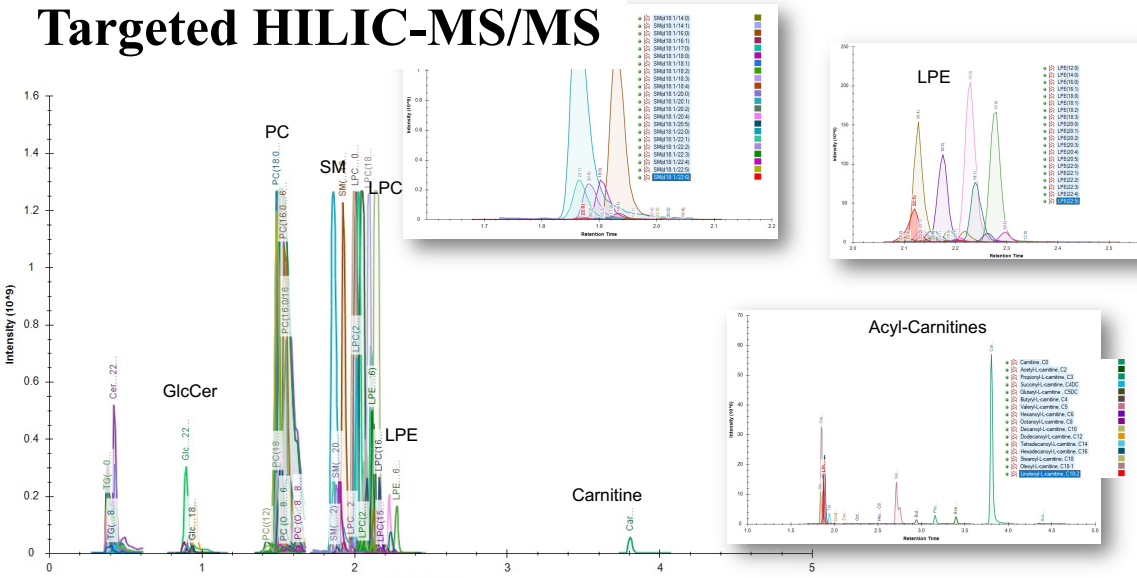
# Tissue Lipidomics

- **Tissue extraction using organic solvent**
- **400+ lipid measured using HILIC-MS/MS (QqQ)**
- **Time related trajectory observed**

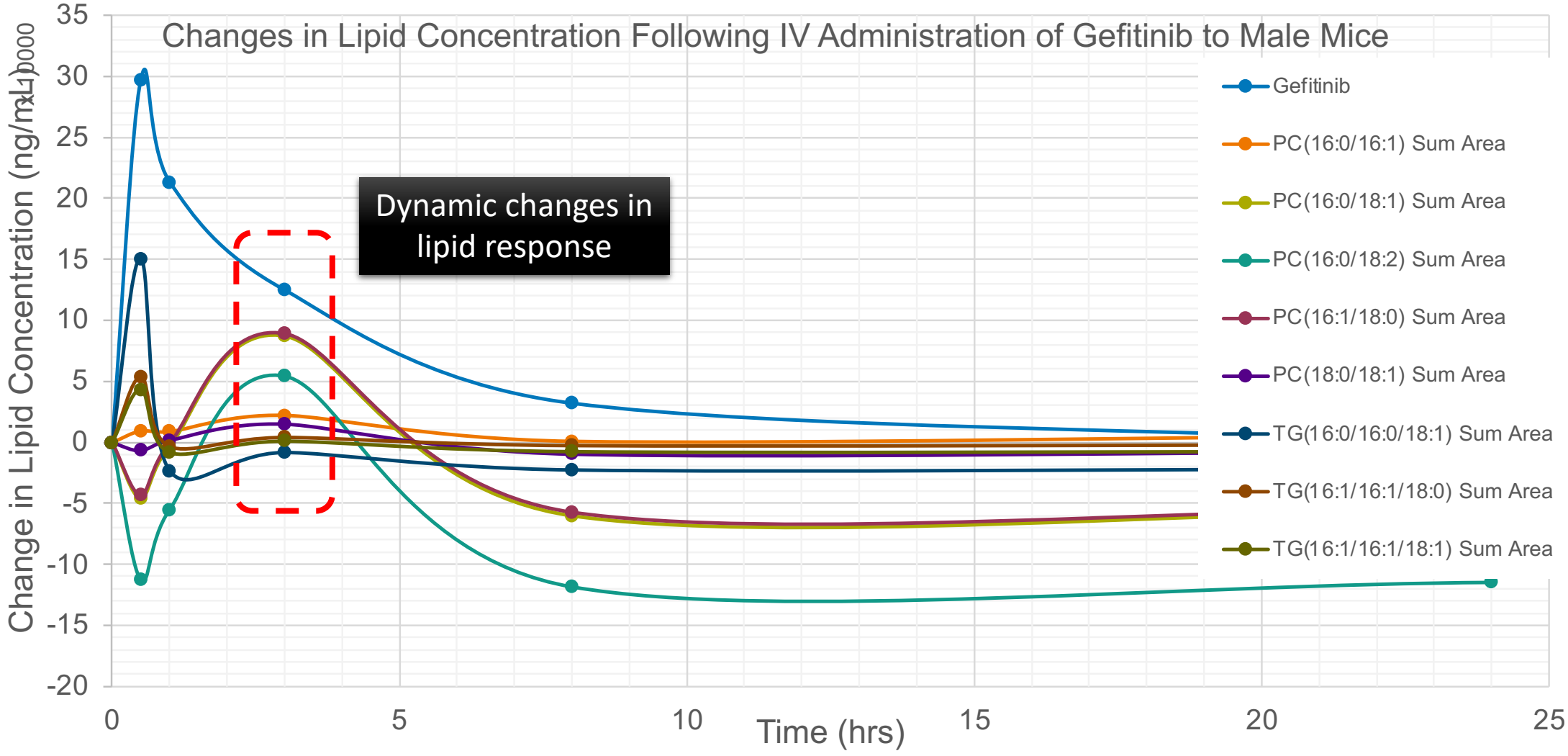


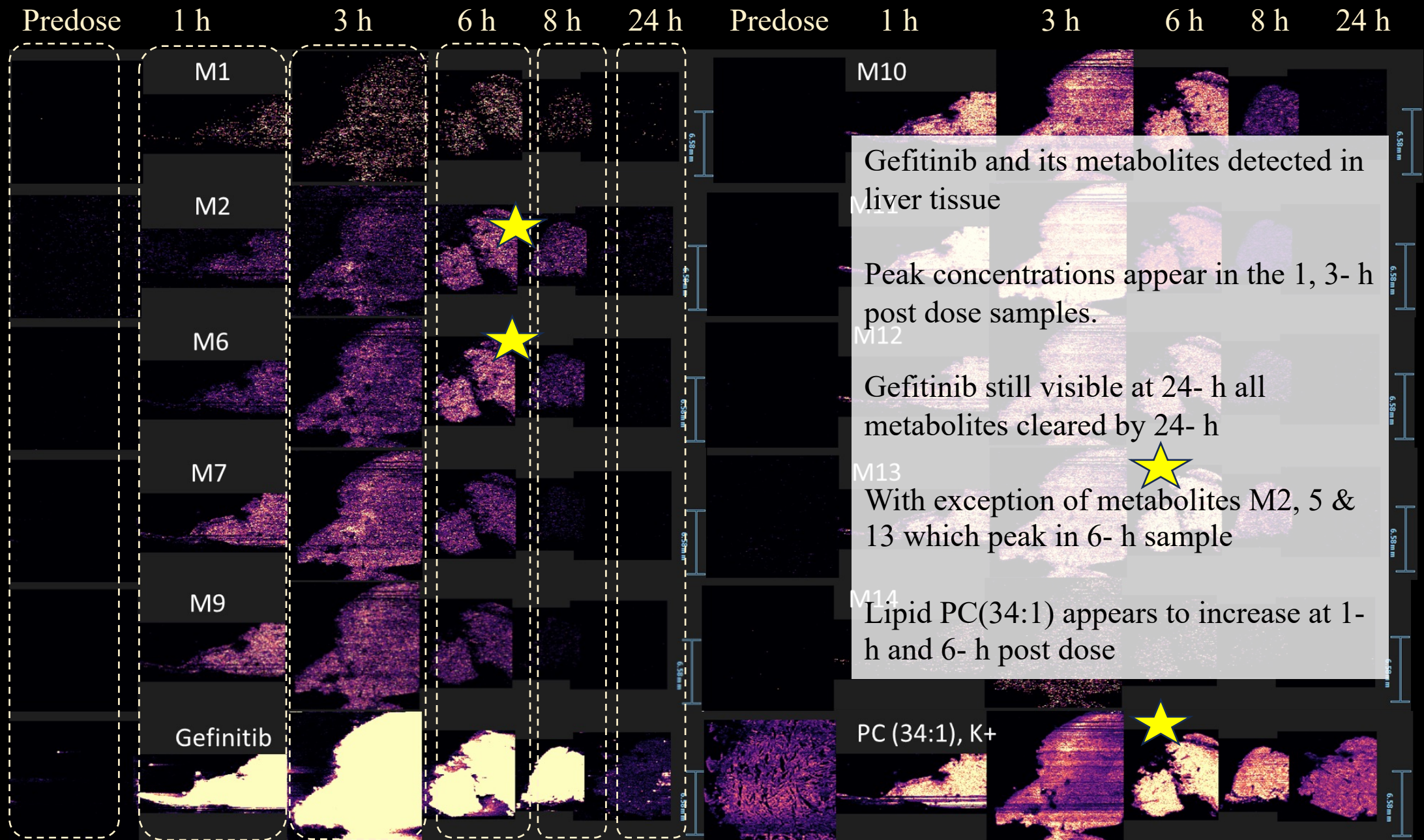

**Targeted HILIC-MS/MS**



# Tissue Lipid – PK Correlation



# Tissue Imaging (Targeted QqQ MS)




Gefitinib and its metabolites detected in liver tissue

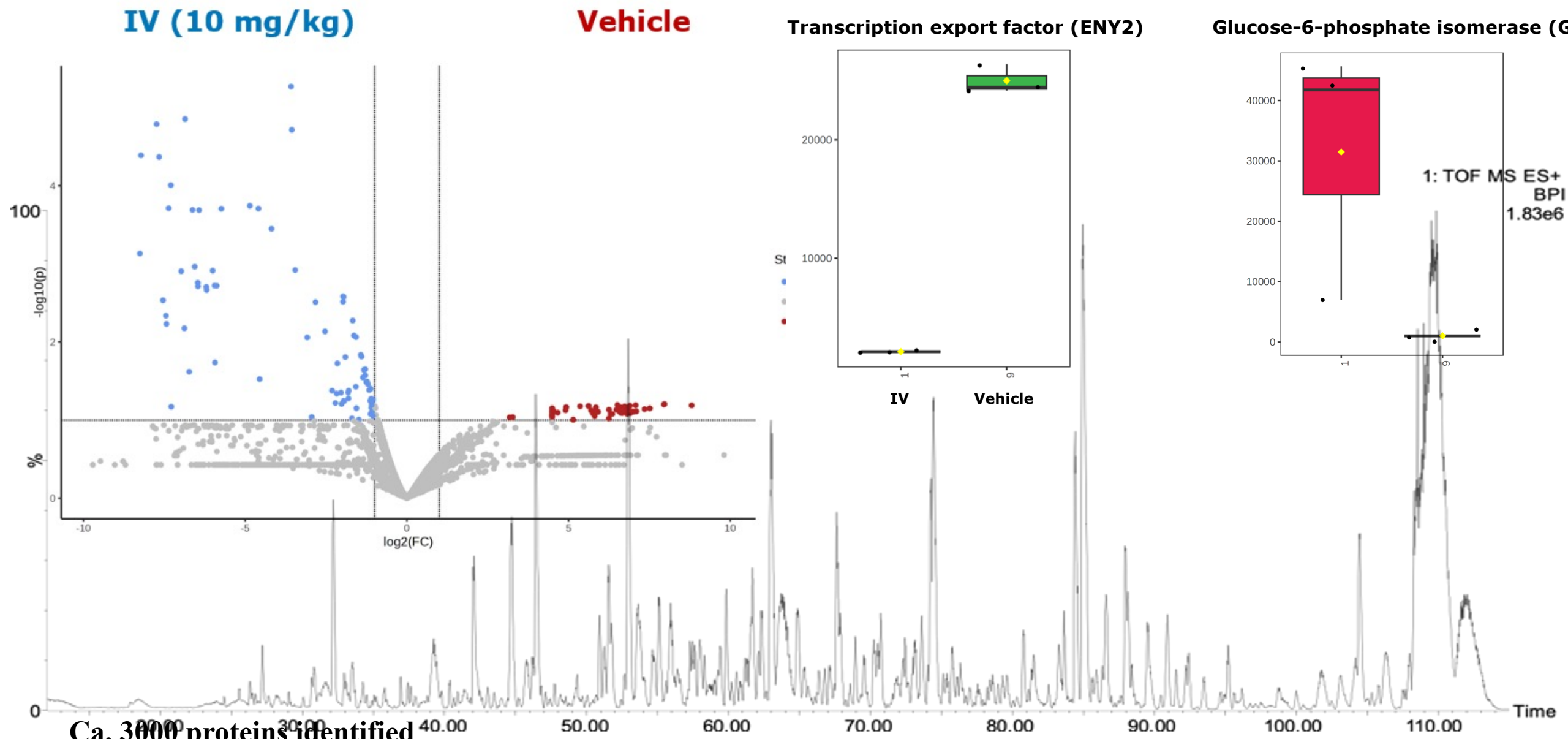
Peak concentrations appear in the 1, 3- h post dose samples.

Gefitinib still visible at 24- h all metabolites cleared by 24- h

With exception of metabolites M2, 5 & 13 which peak in 6- h sample

Lipid PC(34:1) appears to increase at 1- h and 6- h post dose

# And Finally - Liver Proteomics




Ca. 3000 proteins identified

# Conclusions (what we have learned)

- **Using modern LC-MS/MS & accurate mass IM/MS a huge amount of information can be obtained from a small late – stage discovery animal study.**
- **Gefitinib specific results:-**
  - **PK consistent with previous publications**
  - **New Metabolites, 3 glucuronides 1 sulphate conjugate detected**
  - **Dysregulated endogenous metabolites and lipids correlate with PK indicating pharmacometabodynamic effects**
  - **Targeted imaging showed presence of drug and metabolites in liver tissue whilst untargeted imaging shows e.g., PC 34:1 changes**
- **However, samples are small and precious, so planning is key to maximizing data recovery.**

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