How discovery activities can influence metabolic profiling in the regulatory space ?

C. DELATOUR

EBF, 25th September 2015

Inspired by patients.
Driven by science.

Outline

- **Background**
  - Objectives
  - Shift in Workflow
  - Metabolite in safety testing (MIST)
  - Regulatory Guidance
- **Time line of metabolite profiling**
- **Tools**
- **Collaborations in Qual/Quan process**
- **Animal exposure challenging**
- **Study cases**
- **Metabolite profiling method**
- **Strategy and reporting**
- **Conclusion**

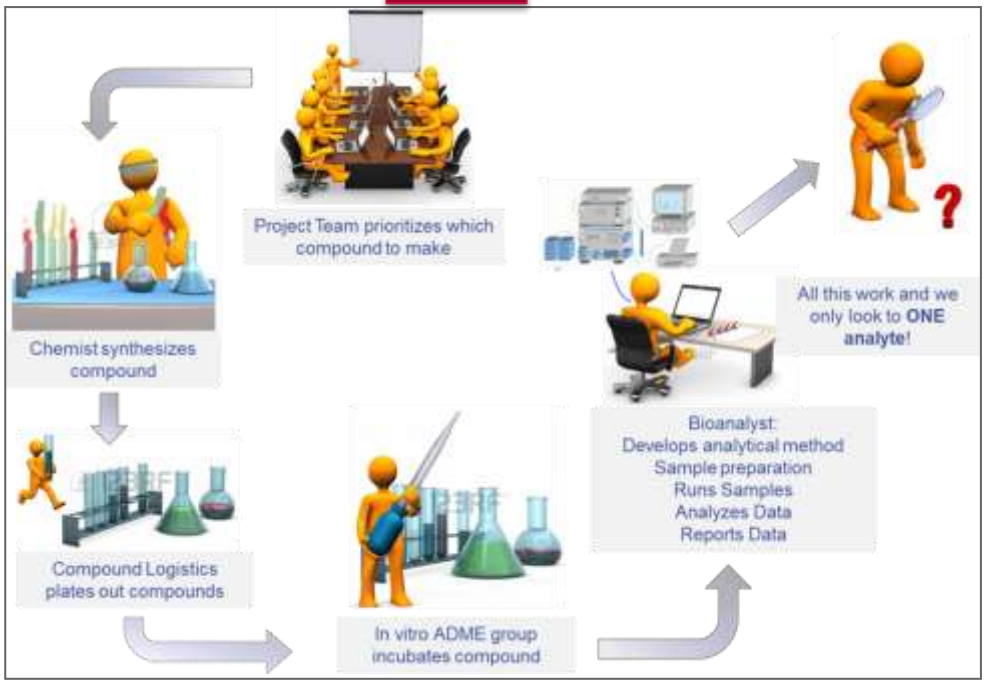
Metabolite Profiling Objectives

To ensure patient safety

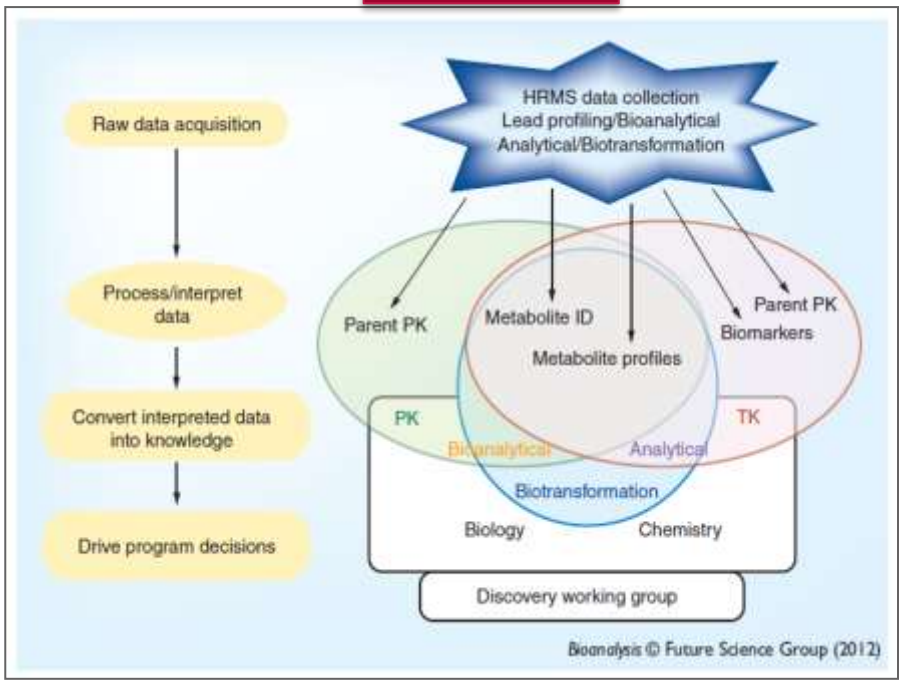
- Potential metabolite and contribution to the pharmacological response as well as side effects
- Identifying structural alerts and risk assessment
- Comparing metabolite exposure in preclinical toxicological species and the first study in healthy human volunteers

Shift from typical workflow to scientific understanding and knowledge

PAST



PRESENT



Shift

Knowledge on known compound(s)

Broad knowledge on (un)known compound(s)

Two Types of Toxic Mechanisms

Stable Metabolite with Reversible Interaction on Macromolecules

- Stable
- As circulating metabolite

- On-target pharmacology
- Off-target pharmacology

Reactive Metabolite with Irreversible Interaction on Macromolecules

- Unstable
- Find as “sign” metabolites in bile or excreta

- General tissue effects
- Immunoallergic
- Protein, nucleic alteration

Regulatory Guidelines

FDA

- **Metabolite(s) at 10% of parent drug in systemic exposure at Steady state (AUC)**
  - ✓ Circulating (excreted on case-by-case , drug has its own story)

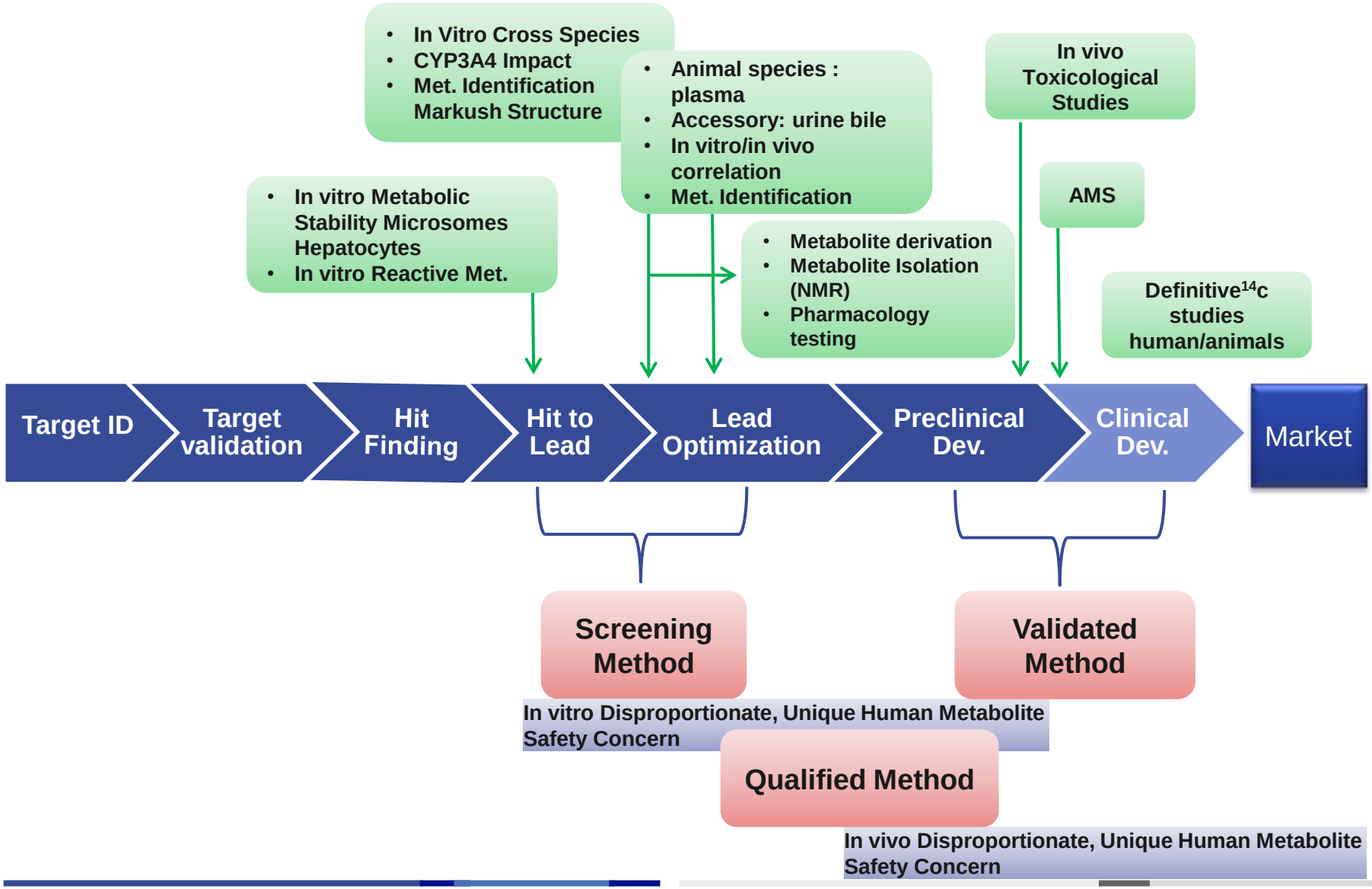
ICH

- **Metabolite(s) at 10% of total drug related exposure**
- **Animal exposure at least 50% of human metabolite exposure**
  - ✓ Circulating and excreted
  - ✓ Specific consideration on low dose drugs

DDI

- **Metabolite(s) at 25%**
  - ✓ Victim (Total Clearance)
  - ✓ Perpetrator (AUC)

Time line of Metabolite Profiling



Tools

Sample preparation: Ostro Plate

- Matrix effects
- Signal/ noise
- Metabolite detection
- Friendly
- Normalization
- Robustness

Ultra Performance Liquid Chromatography

- Matrix effects
- Signal/ noise
- Efficiency
- Normalization (Nano)
- Solvent (Nano)

Mass Spectrometry: HMRS

- Selectivity
- Sensitivity
- Minimal Optimization
- Full Scan Quantification
- Mass accuracy
- Data reprocessing
- Metabolite Profiling
- Biomarkers
- Fragmentation (MS^E)

Software: Metabolynx/ Mass MetaSite

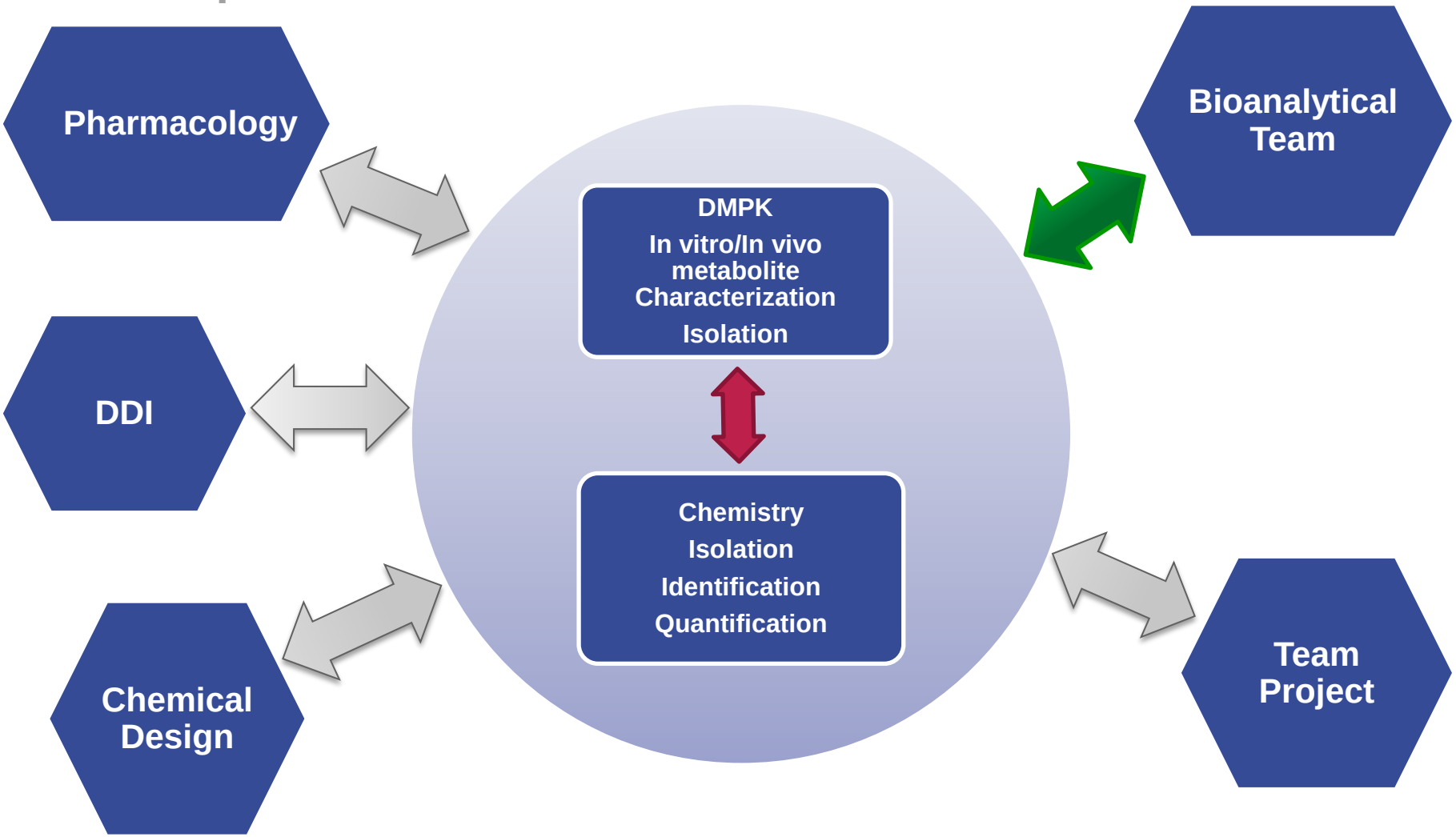
- Searches, identifies +integrates metabolites
- Assigns elemental composition
- Performs control sample comparison
- Automatic set up acquisition
- Displays kinetic metabolites formation
- Compatible MS equipment (Mass Metasite)

In silico prediction : MetaSite

- Metabolite Prediction
- Related to cytochrome-mediated Phase I reactions
- Thermodynamic (enzyme – substrate) and kinetic
- Ranking of metabolites

Collaborations in the Qual/Quan Process

Metabolite Profiling Screening / Qualified Method



Animal Exposure

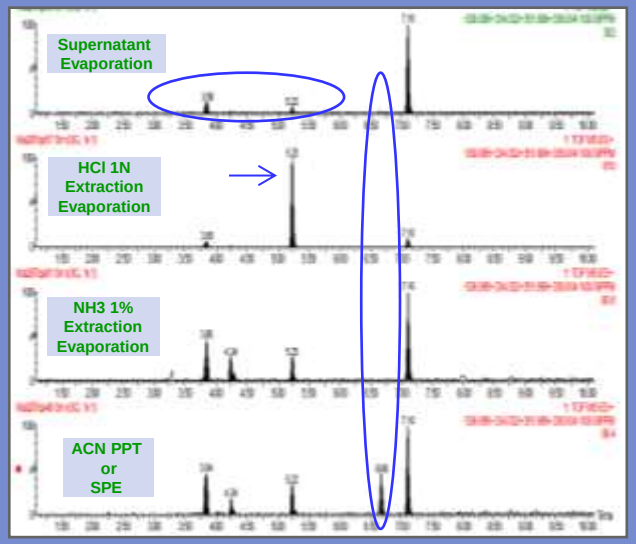
To ensure animal exposure for toxicological coverage

Challenges

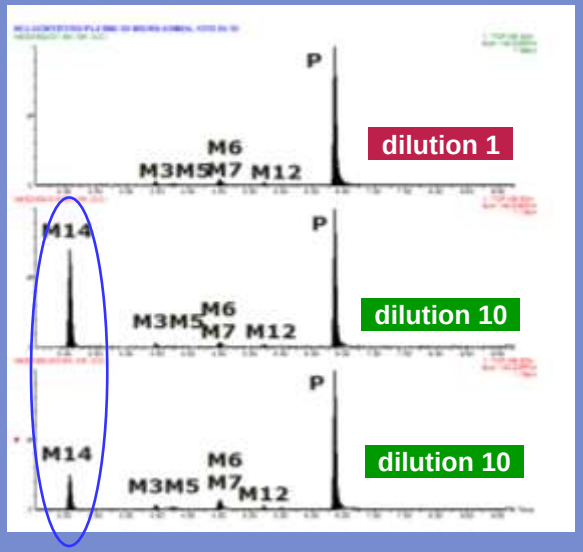
- Reproducible in vitro qualitative method (≤1µM)
- In vitro drug disappearance rate (<30%) should be close to the metabolite formation rate _ reflecting primary metabolism
- Ability to provide semi-quantitation data
- Response factor impact
- Matrix effect impact
- To characterize metabolic pathway (phenotype _ DDI _ Safety) in different species
- To give adequate information for validation

Study cases

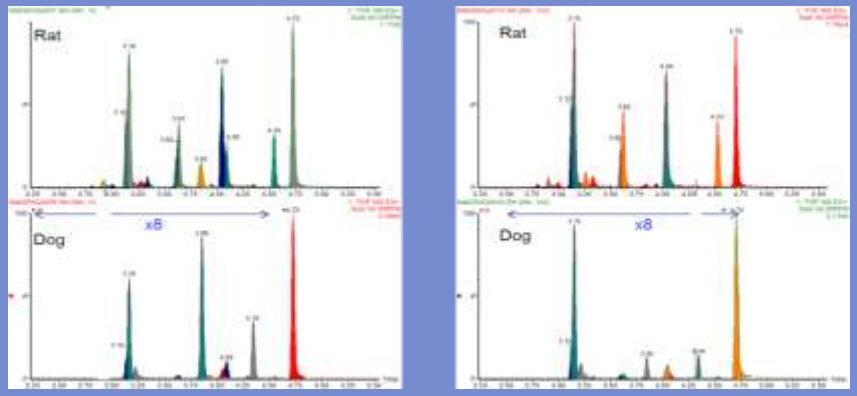
Sample preparation
Rat Plasma PO 1mg/kg



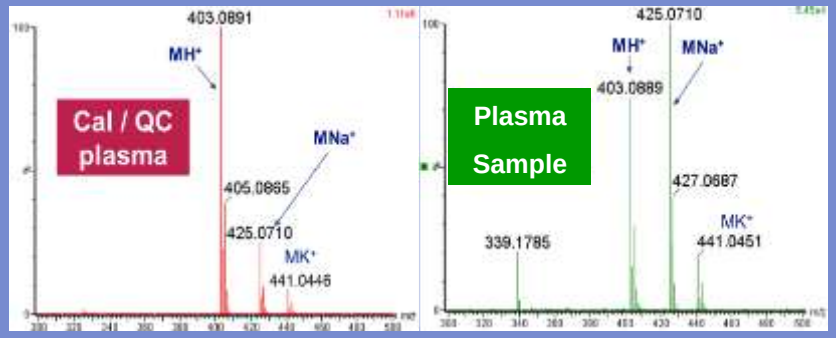
N-dealkylation
Monkey Plasma PO 3mg/kg



Isomerism
Rat/Dog Hepatocytes 1µM



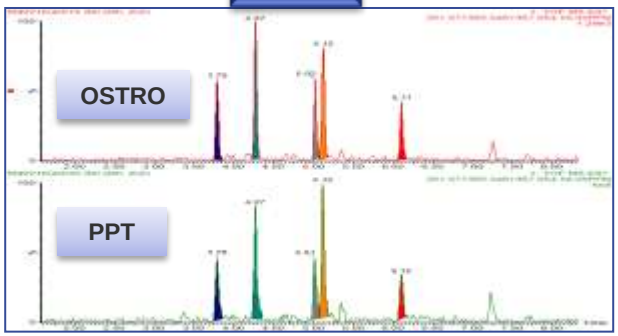
Ionization Process
Rat Plasma (PO 3mg/kg)



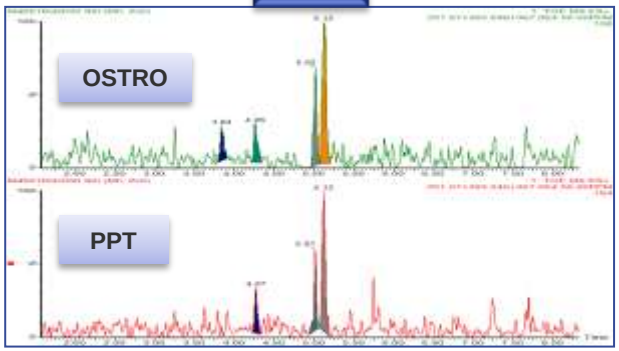
Metabolite Profiling Method

Rat PO 3mg/kg

PLASMA



BRAIN



Sample Clean-up

1V species plasma or brain homogenate + 4V human plasma

Ostro elution by Acetonitrile + 1% acetic acid

Dilution

Matrix Normalization
Robustness
Signal/Noise

Instrument: UPLC Acquity + PDA

Column :	C18 100mm, 1.8µM
Mobile Phase A :	Ammonium Acetate 10mM
Mobile Phase B :	Acetonitrile
Flow Rate:	4-400µL/min
Gradient	90 %A → 90%B

5min <RT $_{Drug}$>9 min

Strong Needle Wash:	5% Water/95% Acetonitrile
Weak Needle Wash:	95% Water/5% Acetonitrile
Wavelength:	210 →400nm

Efficiency
Reproducibility
Response Normalization
Spectra quality

Robustness

Instrument: Xevo G2S

Ionization Mode:	ESI+/-
TOF Optic Mode:	Resolution (35000 FWHM)
Acquisition Mode:	Centroid MS^E
Scan range:	100 - 850/1200 amu
Scan Time:	0.1s
Collision Energy:	Trap CE ramp from 15 to 35v
Software:	Metabolynx / MassMetaSite

1- Metabolite Profiling based on
- Structure
- Threshold
- Control Comparison
- Mass Data Filtering

2- Metabolite Quantification
- Selected Metabolite (exact mass / retention time)
- Sum in Excell Files

Accuracy
Full Mass Range
MS^E data
Signal Normalization

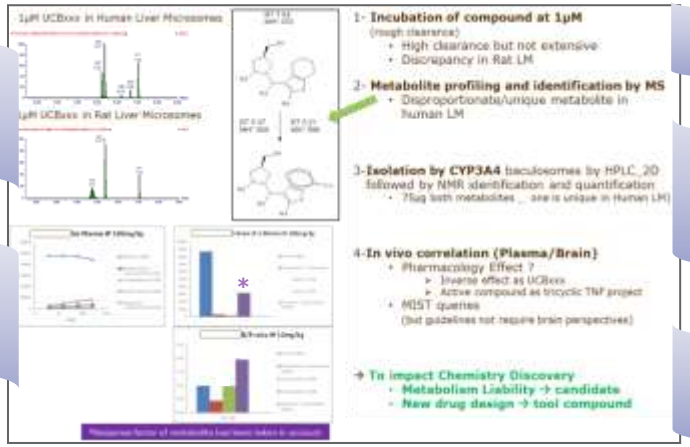
Identification
Quantification
Reporting

Strategy and Reporting

Biotransformation Team Implication

Target Validation | Hit Identification | Hit To Lead | Lead Optimization | Candidate Selection | Pre Clinical

Structure Reactivity | Identify Chemical Series wo safety issues | Metabolite Profiling Screening | Validation



Extensive/Intensive Disproportionate

Circulating Safety

Markush Structure

Cyp3A4 Implication <50µg Production

New Design Candidate

Strategy and Reporting

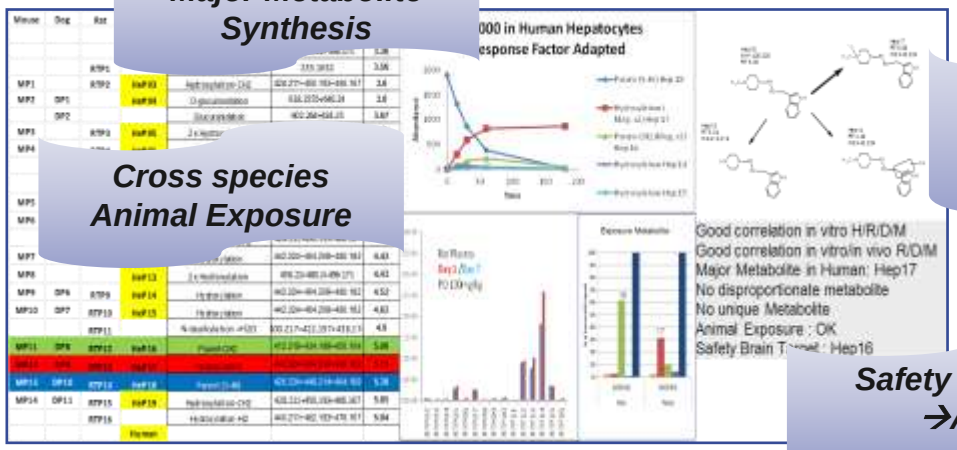
Biotransformation Team Implication

Target Validation	Hit Identification	Hit To Lead	Lead Optimization	Candidate Selection	Pre Clinical
	Structure Reactivity	Identify Chemical Series wo safety issues	Metabolite Profiling Qualified	Validation →BA Team	

AMS

Major Metabolite Synthesis

Cross species Animal Exposure



Metabolic Pathways

Safety concern →AMS

Conclusion

Tools

- Consider using high resolution mass spectrometry to quantify and identify drugs and its metabolites
- Ultra-performance of liquid chromatographic systems to correlate in vitro / in vivo cross species studies
- Better clean-up/sample preparation
- Metabolite Isolation 2D-LC / NMR and metabolite derivation
- Prediction software (Derek, Meteor, MetaSite,..)

Drivers

- To impact drug design
- To determine metabolite kinetic in human

  →To ensure animal exposure for toxicological coverage
- To improve prediction of unique metabolite

  →To predict risk assessment and at which concentration
- To translate these assessment to in vivo models
- To allow retrospective data reviewing to predict clinical situation