Approaches to assess human metabolites in early clinical trials through MIST

人体药物代谢产物安全评估在早期临床试验中的应用研究

文波

Bo Wen

GlaxoSmithKline R&D, King of Prussia, PA 19406, USA

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2018 Nanjing International DMPK Symposium, June 29th – July 1th, Nanjing, China

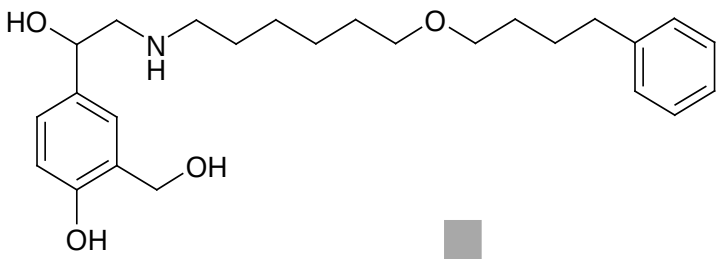
Outline

- Background
  - Why human metabolism?

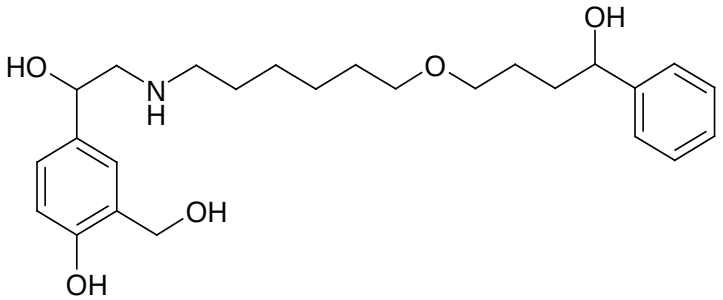
- Stable drug metabolites (“MIST”)
  - FDA and ICH guidances

- ‘Human first’ metabolism strategy
  - Can we MIST human first?

Human metabolism

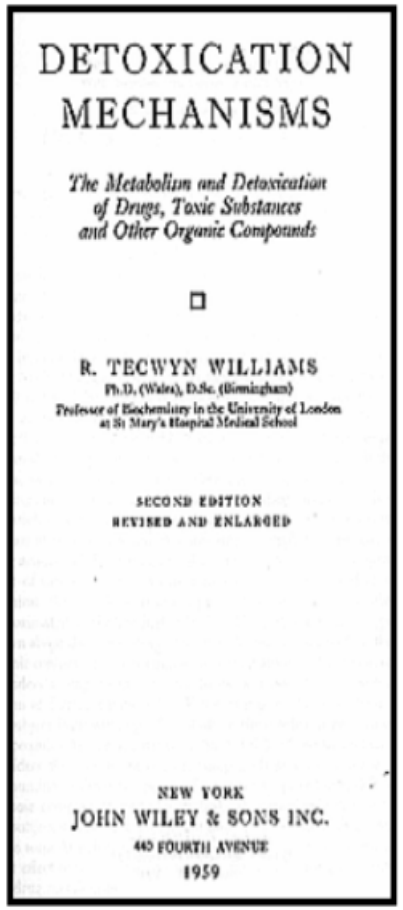


60% dose (faeces)



- ❑What it is? – **Structure**
- ❑Where it is? - **Distribution**
- ❑How much there is? – **Quantification**
- ❑How it happened? - **Mechanism**
- ❑Where it goes? – **Excretion**
- ❑What it did? – **Safety vs efficacy vs inert**

Human metabolism - why


DETOXICATION
MECHANISMS

The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds

R. TECWYN WILLIAMS
Ph.D. (Wales), D.Sc. (Birmingham)
Professor of Biochemistry in the University of London at St Mary's Hospital Medical School

SECOND EDITION
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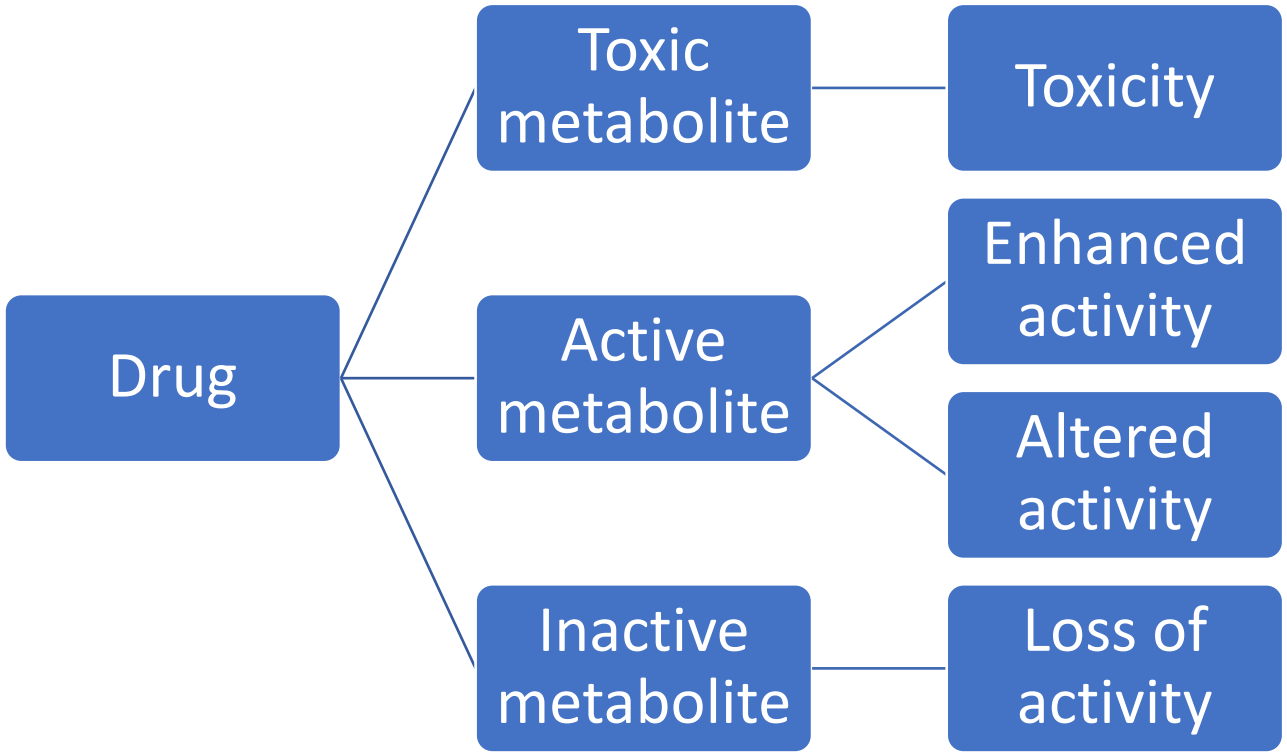
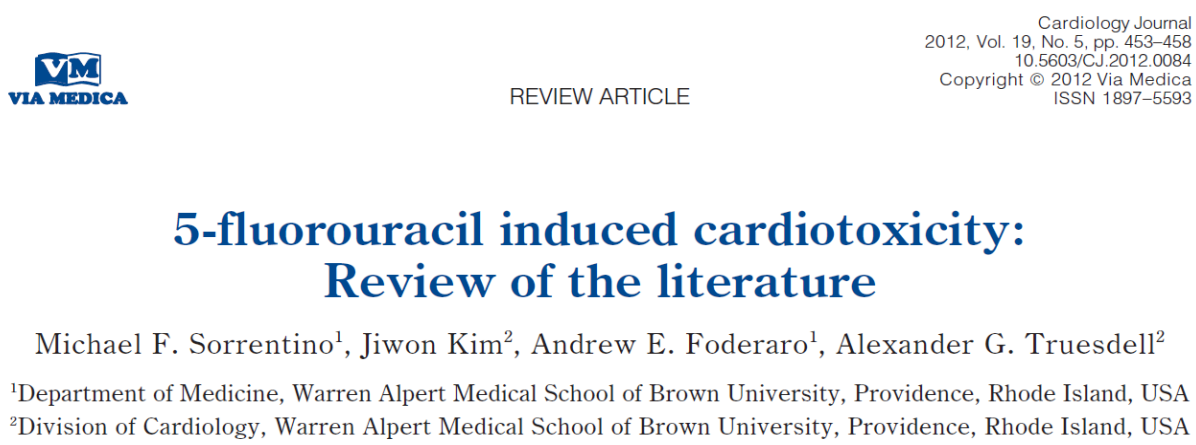
JOHN WILEY & SONS INC.
440 FOURTH AVENUE
1959



Human metabolism - Why
a case study of metabolism-related cardiac toxicity



VIA MEDICA

REVIEW ARTICLE

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2012, Vol. 19, No. 5, pp. 453–458
10.5603/CJ.2012.0084
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ISSN 1897–5593

5-fluorouracil induced cardiotoxicity: Review of the literature

Michael F. Sorrentino[1], Jiwon Kim[2], Andrew E. Foderaro[1], Alexander G. Truesdell[2]

[1]Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA
[2]Division of Cardiology, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Peng *et al. Cancer Commun (2018) 38:22*
https://doi.org/10.1186/s40880-018-0292-1

Cancer Communications

ORIGINAL ARTICLE Open Access

Cardiotoxicity of 5-fluorouracil and capecitabine in Chinese patients: a prospective study

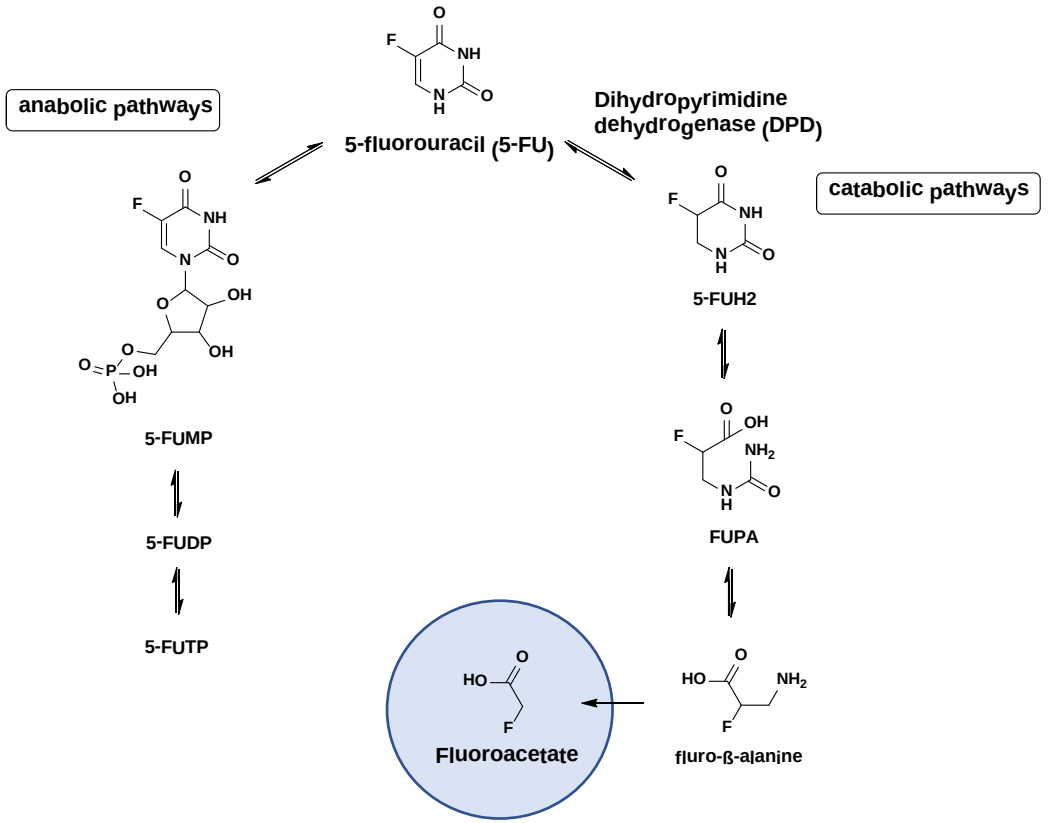
Jianjun Peng[1†], Chao Dong[2†], Chang Wang[3], Weihua Li[4], Hao Yu[5], Min Zhang[6], Qun Zhao[7], Bo Zhu[8], Jun Zhang[9], Wenliang Li[10], Fenghua Wang[11], Qiong Wu[12], Wenhao Zhou[13], Ying Yuan[14], Meng Qiu[15*] and Gong Chen[13*]

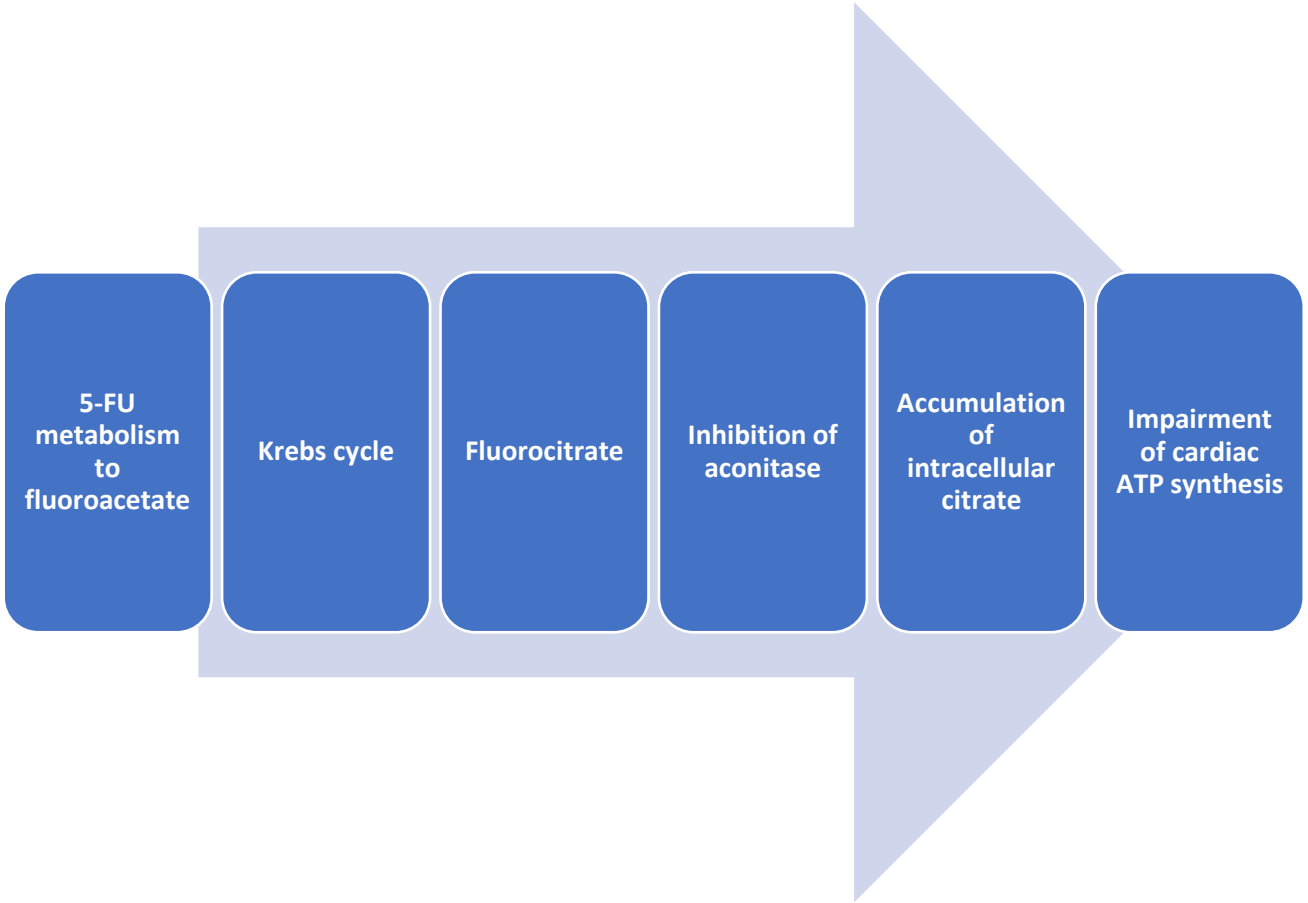
5-fluorouracil (5-FU): A case study of cardiotoxic anticancer drugs

- Analogue of uracil, widely used as an antitumor agent (incl. breast, gastric, pancreatic, prostate and bladder cancers)
- Considered as the second most frequent cause of cardiotoxicity by anticancer drugs, after anthracyclines
- High Vd, clearance primarily through hepatic metabolism

Arellano M, et al., *Br J Cancer.* 77:79-86 (1998)

Human metabolism – why: 5-fluorouracil (5-FU)





- ~ 90% of IV administered 5-FU dose is rapidly catabolized in liver by dihydropyrimidine dehydrogenase (DPD).
- Fluoroacetate, known as a highly cardiotoxic metabolite, enters Krebs cycle and is transformed into fluorocitrate, which inhibits aconitase leading to ↑intracellular citrate and ↓cardiac ATP synthesis.

Reis-Mendes AF, et al., *Curr Drug Metab.* 17:75-90 (2015)

Metabolites as the sole contributor to DDI: *Bupropion/Desipramine example*

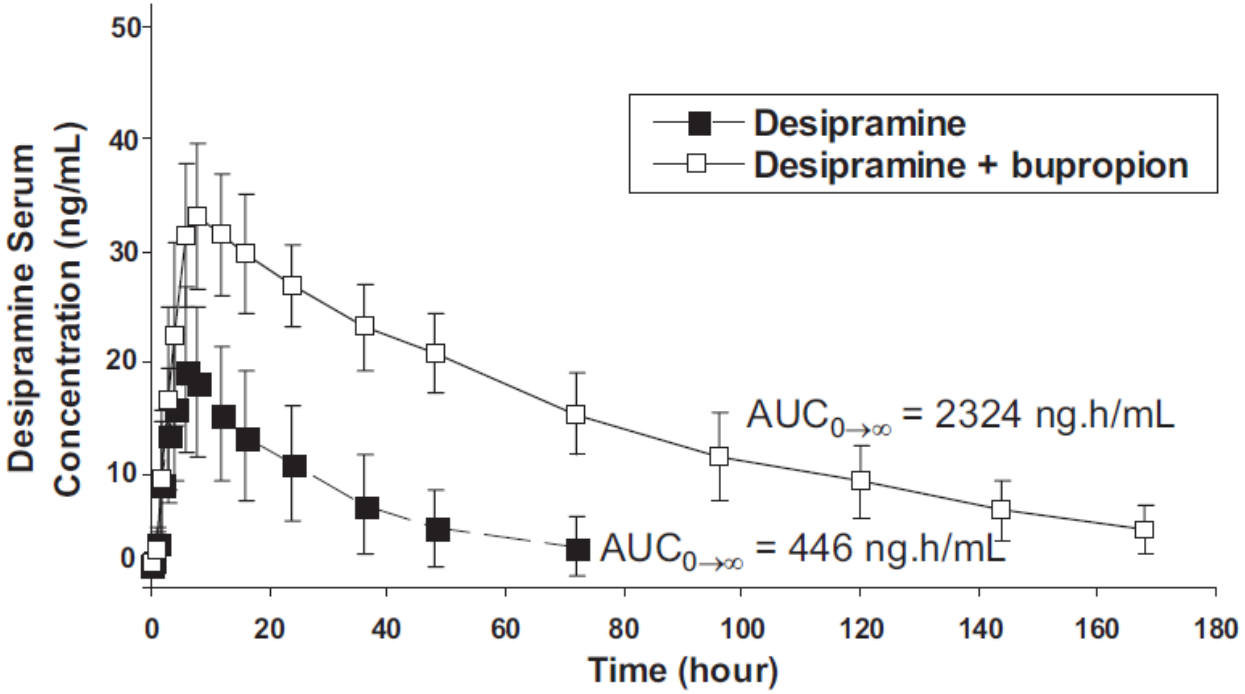


FIG. 2. Average serum concentrations of desipramine in extensive CYP2D6 metabolizers given a single p.o. dose of 50 mg of desipramine with (□) and without (■) 300 mg of bupropion codosing for 11 days.

1521-009X/43/4/620–630$25.00
DRUG METABOLISM AND DISPOSITION
http://dx.doi.org/10.1124/dmd.114.059345
Drug Metab Dispos 43:620–630, April 2015

Perspective

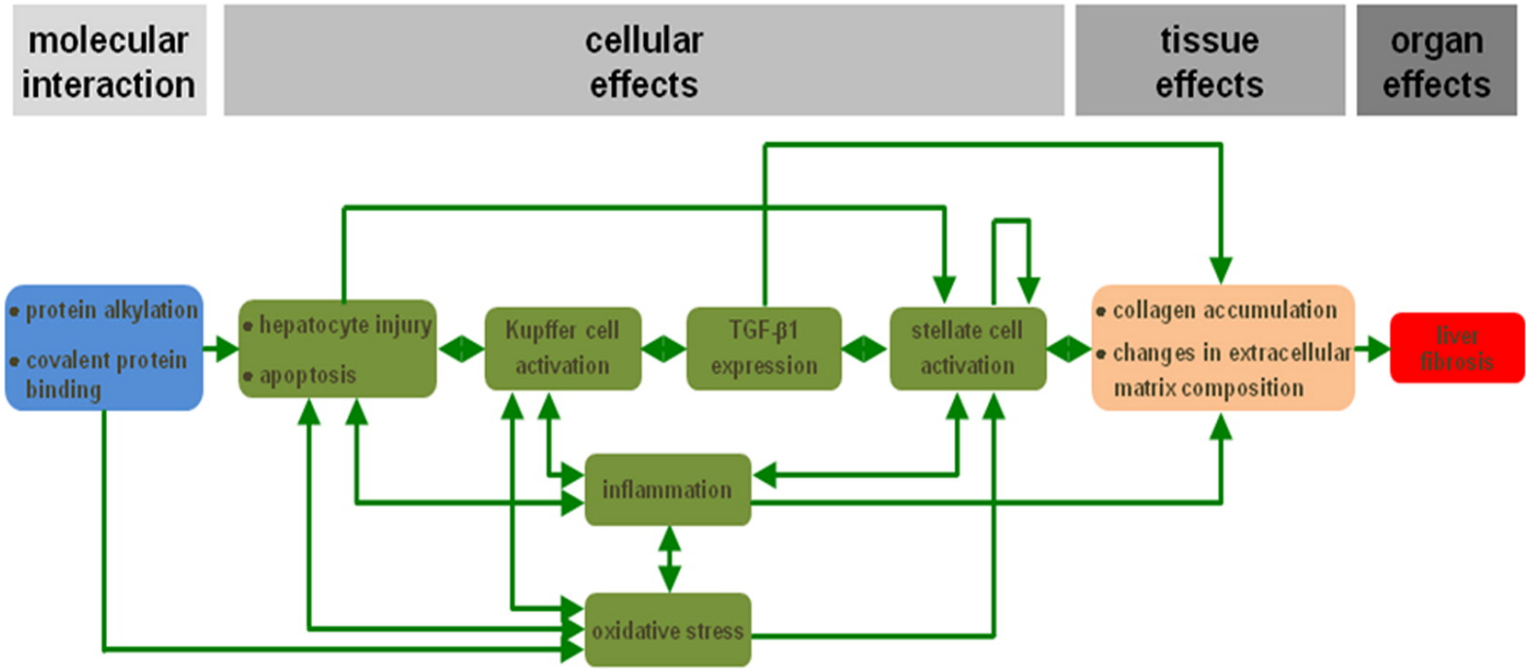
Contribution of Metabolites to P450 Inhibition–Based Drug–Drug Interactions: Scholarship from the Drug Metabolism Leadership Group of the Innovation and Quality Consortium Metabolite Group

Hongbin Yu, Suresh K. Balani, Weichao Chen, Donghui Cui, Ling He, W. Griffith Humphreys, Jialin Mao, W. George Lai, Anthony J. Lee, Heng-Keang Lim, Christopher MacLauchlin, Chandra Prakash, Sekhar Surapaneni, Susanna Tse, Alana Upthagrove, Robert L. Walsky,[1] Bo Wen,[2] and Zhaopie Zeng

- Bupropion not expected to inhibit CYP2D6 based on its in vitro CYP450 inhibition data.
- The bupropion metabolites contributed to the observed CYP2D6 inhibition.
  - high exposures
  - potent inhibitor (low K_i)

Reese MJ et al. *Drug Metab Dispos* 36:1198-1201 (2008)
Yu H, et al., *Drug Metab Dispos.* 43:620-30 (2015)

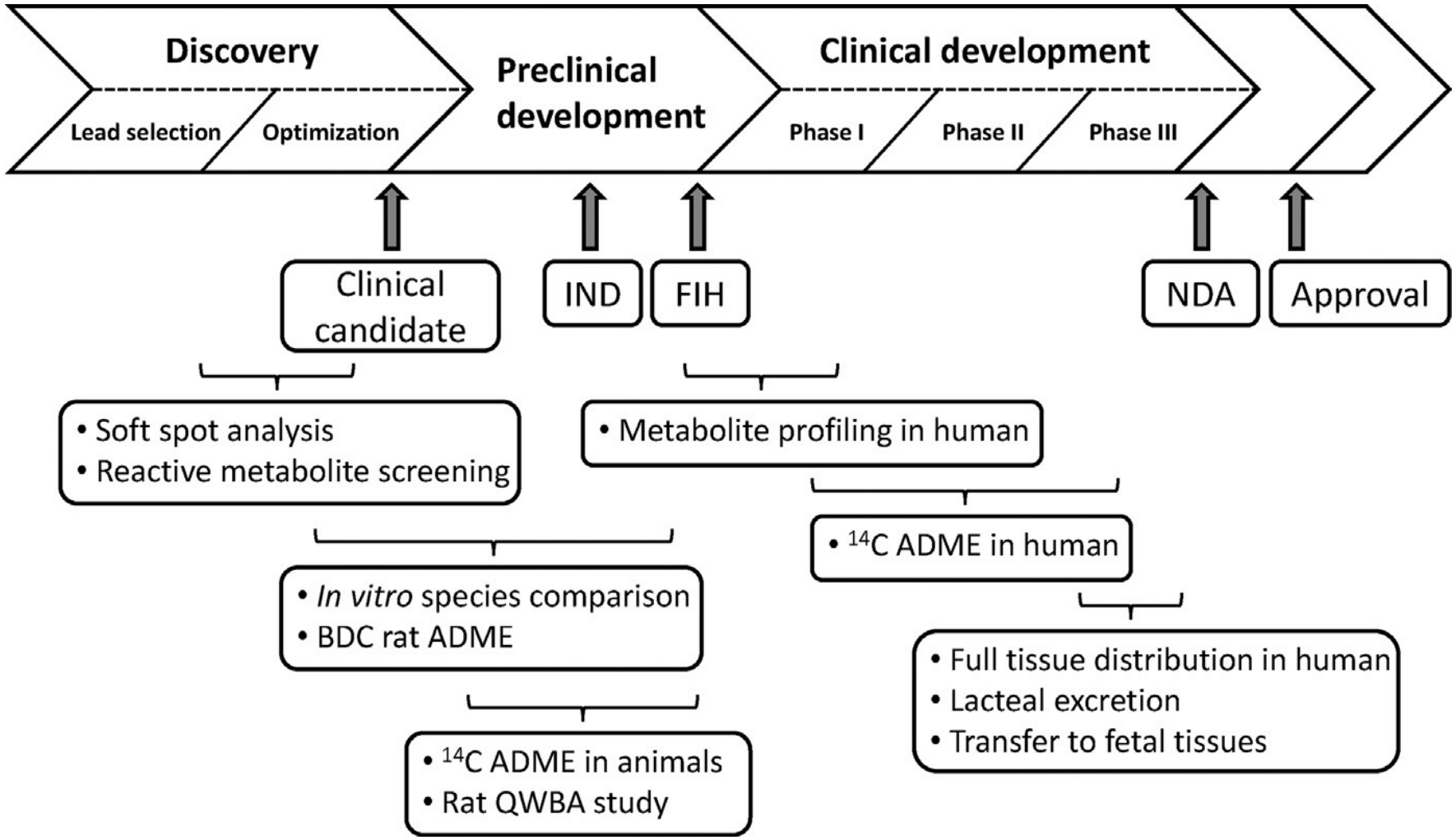
Reactive Metabolites in Adverse Outcome Pathways (AOPs)



- The MIE (blue) is considered protein alkylation and covalent protein binding in the liver. This serves as a trigger to provoke hepatocyte injury, including apoptosis, which in turn activates Kupffer cells.
- As a result, transforming growth factor β1 (TGF-β1) expression is induced, which is a key factor for stellate cell activation. The latter goes hand in hand with the occurrence of inflammation and oxidative stress.
- The overall end result is accumulation of collagen and changes in the extracellular matrix composition in the liver (orange), which becomes clinically manifested as the adverse outcome, namely, liver fibrosis (red).

Mathieu V, *Chem. Res. Toxicol.* 2015, 28:1391-1397.

Drug metabolism studies in drug discovery and development



Wen B and Zhu M, *Drug Metab Rev.,* 47:71-87 (2015)

Outline

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  - FDA and ICH guidances

- ‘Human first’ metabolism strategy
  - Can we MIST human first?

g (MIST)

2002

- Historical precedent for metabolite characterisation started to evolve into regulatory guidance
- Initiated by PhRMA paper (Baillie et al, 2002)
- Industry-wide view on metabolite safety

2003

- FDA response letter (Hastings et al, 2003)
- Challenged some of the key summaries of the PhRMA paper
- Especially around definition of major metabolite, e.g. Halothane

‘Are human metabolites of a drug candidate, as well as the parent compound, adequately evaluated for safety during nonclinical toxicology studies?’

Metabolites in

2008

- Finalised FDA MIST guide - Emphasis on circulating metabolites
- Increased focus on Steady State
- Recommendation that studies performed as early as possible
- All metabolites accounting for >10% drug exposure, defining unique and disproportionate

2010

- Harmonised ICH guidance (ICH M3 (R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials
- Broader scope, but did cover metabolites – recommendation 10% of the total drug-related material
- Helped focus the efforts around approaches to address early metabolism
- The nonclinical characterization of metabolites considered on a case-by-case basis

https://www.fda.gov/OHRMS/DOCKETS/98fr/FDA-2008-D-0065-GDL.pdf
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002941.pdf
https://www.fda.gov/downloads/Drugs/.../Guidances/ucm079266.pdf

Harmonised ICH M3(R2) guidance: Q&A 2012

...characterisation of metabolite toxicity generally considered adequate when animal exposure is at least 50% the exposure seen in humans

...important to have adequate exposure to metabolite in 1 species used in general tox, 1 species used in carc and 1 species used in embryofetal development

...a single dose radio study provides a reasonable estimate...but need to factor in changes at steady state

...exposure comparison conducted at MTD in animal compared to therapeutic dose in human

...10% threshold...when a metabolite comprises greater than 10% of the measured total exposure to drug and metabolites (usually group mean AUC)

...nonclinical studies for metabolites – consider design on case by case basis

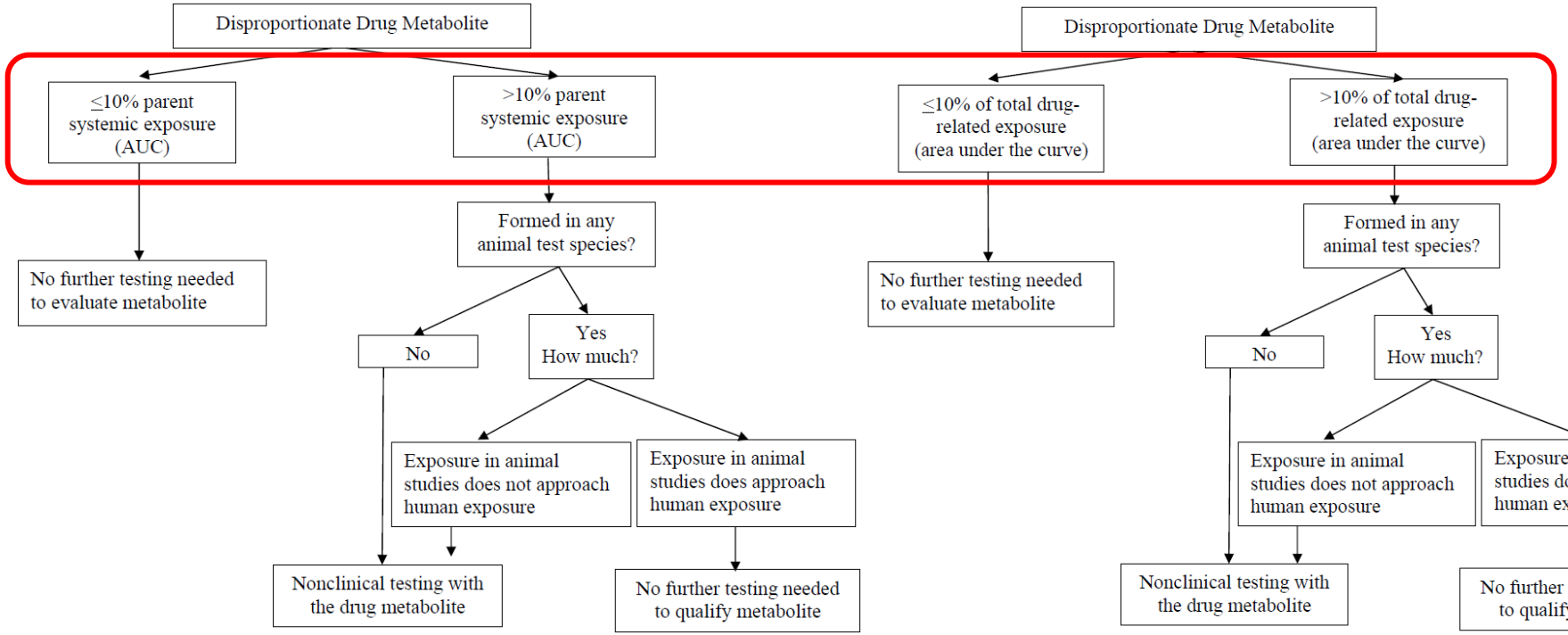
...or NOAEL if toxicity at MTD not mentionable or poses an unacceptable human risk

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

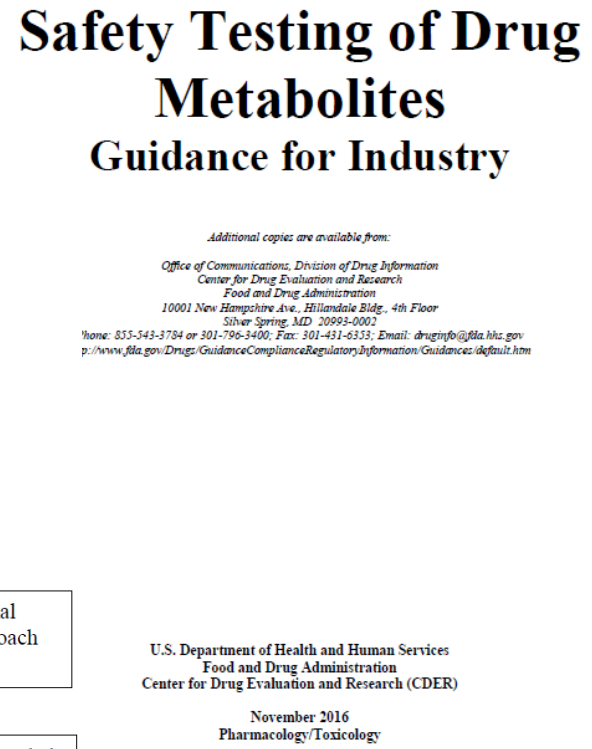
http://www.ema.europa.eu/docs/en_GB/document_library/Other/2011/07/WC500109298.pdf

Decision trees from the FDA MIST guidance
in alignment with the ICH M3(R2) guidance

MIST guidance Feb 2008



MIST guidance Nov 2016



Safety Testing of Drug Metabolites

Guidance for Industry

Additional copies are available from:

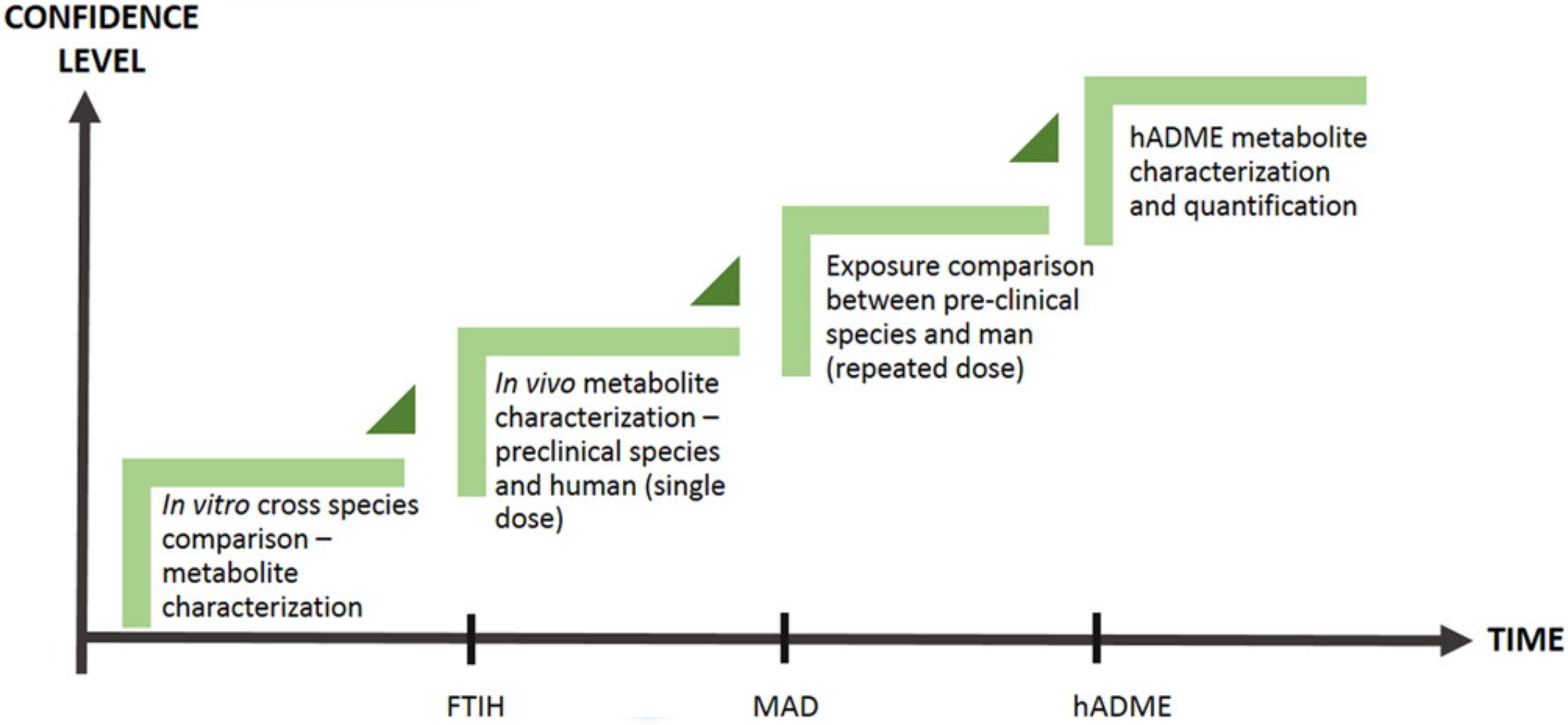
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353; Email: druginfo@fda.hhs.gov
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

November 2016
Pharmacology/Toxicology

https://www.fda.gov/OHRMS/DOCKETS/98fr/FDA-2008-D-0065-GDL.pdf
https://www.fda.gov/downloads/Drugs/.../Guidances/ucm079266.pdf

Standard ‘tiered’ approach in MIST



Haglund J, et al, *Chem Res Toxicol.* 27:601-610 (2014)

Building evidence and confidence for HRS

Where we are?

in silico

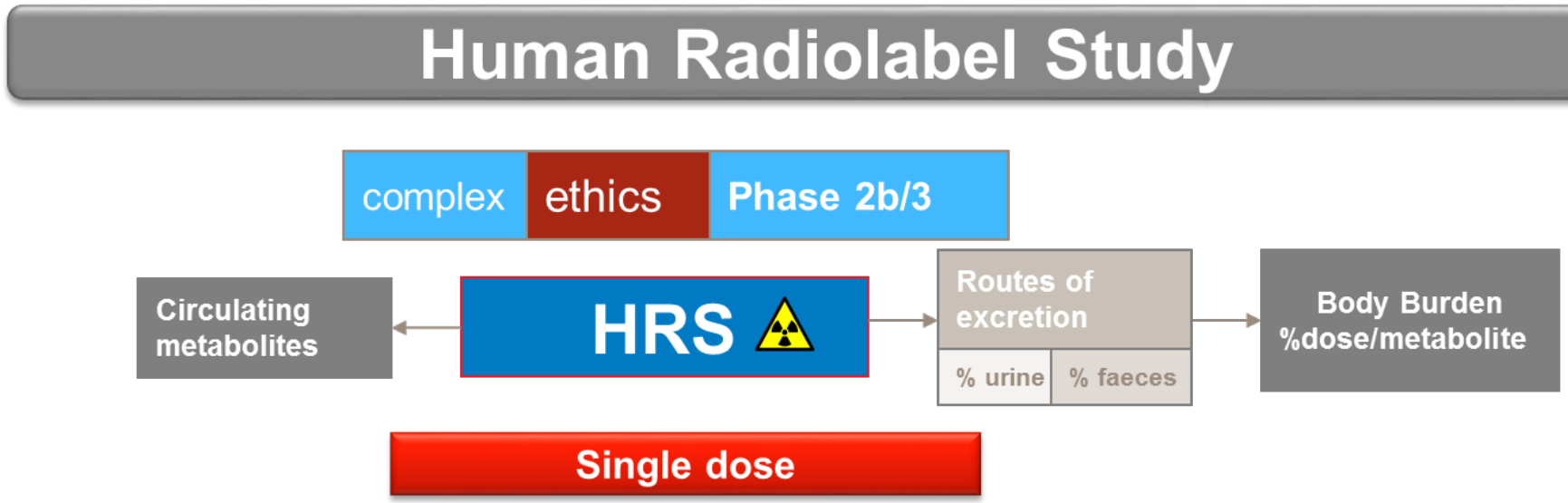
Too many options, how predictable for human especially circulation

in vitro

"artificial" system, good for routes about 70% predictable
Not routinely used to select toxicology species

Pre-clinical *in vivo*

Resource intensive
ID & quan on non-human metabolites
What are likely to be relevant?



Which and how much metabolites will circulate in human?

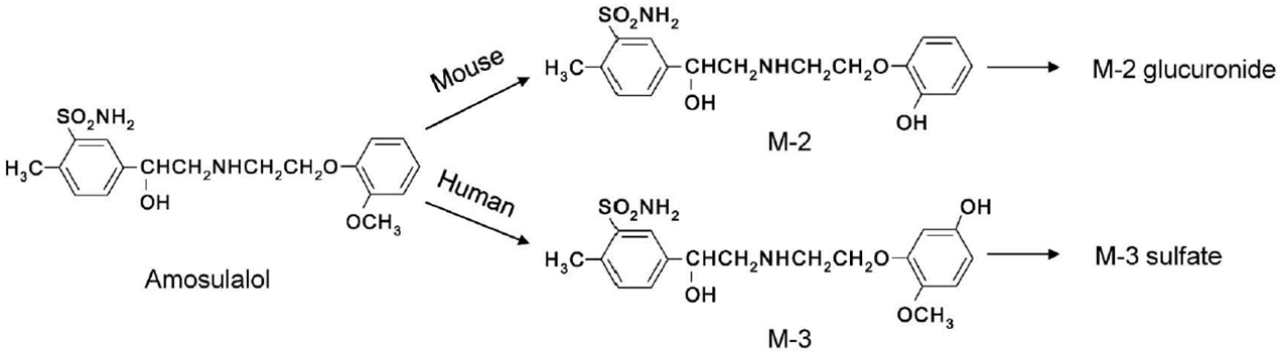


Fig. 9. Major metabolic pathways of amosulalol in mice and humans
M-3 sulfate, the major metabolite in humans, was found as one of the minor metabolites in mice.[45)]

Supplemental material to this article can be found at:
http://dmd.aspetjournals.org/content/suppl/2013/03/01/dmd.112.050278.DC1

1521-009X/41/5/933–951$25.00
DRUG METABOLISM AND DISPOSITION
http://dx.doi.org/10.1124/dmd.112.050278
Drug Metab Dispos 41:933–951, May 2013

Minireview

Which Metabolites Circulate?

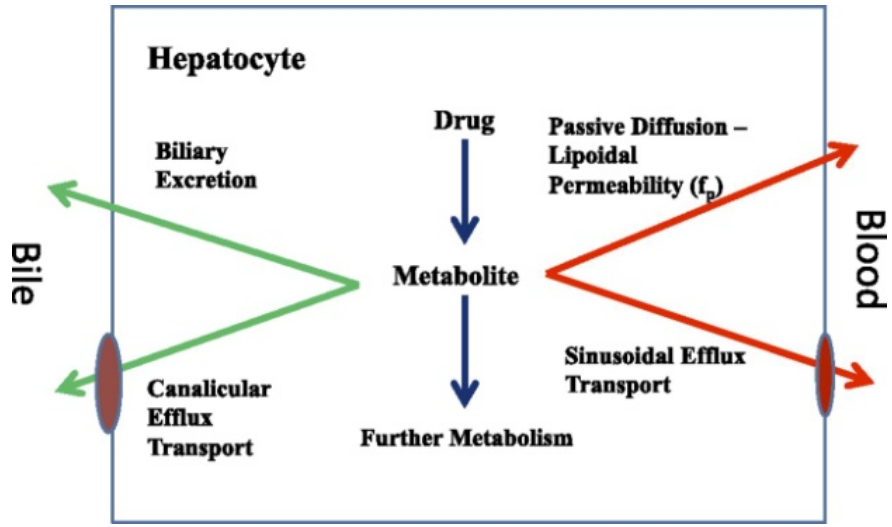
Cho-Ming Loi, Dennis A. Smith, and Deepak Dalvie

Pharmacokinetics, Dynamics, and Metabolism, Pfizer Worldwide Research and Development, San Diego, California (C.-M.L., D.D.); and Department of Chemistry, University of Capetown, South Africa and Institute of Translational Medicine, University of Liverpool, United Kingdom (D.A.S.)

Received November 20, 2012; accepted March 1, 2013

ABSTRACT

Characterization of the circulating metabolites for a new chemical entity in humans is essential for safety assessment, an understanding of their contributions to pharmacologic activities, and their potential involvement in drug-drug interactions. This review examines the abundance of metabolites relative to the total parent drug [metabolite-to-parent (M/P) ratio] from 125 drugs in relation to their structural and physicochemical characteristics, lipoidal permeability, protein binding, and fractional formation from parent (f_m). Our analysis suggests that f_m is the major determinant of total drug M/P ratio for amine, alcohol, *N*- and *S*-oxide, and carboxylic acid metabolites. Passage from the hepatocyte to systemic circulation does not appear to be limiting owing to the vast majority of metabolites formed being relatively lipid permeable. In some cases, active transport plays an important role in this process (e.g., carboxylic acid metabolites). Differences in total parent drug clearance and metabolite clearance are attenuated by the reduction in lipophilicity introduced by the metabolic step and resultant compensatory changes in unbound clearance and protein binding. A small subclass of these drugs (e.g., terfenadine) is unintentional prodrugs with very high parent drug clearance, resulting in very high M/P ratios. In contrast, arenol metabolites show a more complex relationship with f_m due largely to the new metabolic routes (conjugation) available to the metabolite compared with the parent drug molecule. For these metabolites, a more thorough understanding of the elimination clearance of the metabolite is critical to discern the likelihood of whether the phenol will constitute a major circulating metabolite.



For a given circulating metabolite with >25% parent

- f_m > 15%
- cLogD > -1
- Structural motifs

Smith DA and Dalvie D, *Xenobiotica.* 42:107-26 (2012)
Anderson S, et al, *Chem Res Toxicol.* 22:243-56 (2009)
Kamimura H, *Drug Metab Pharmacokinet.* 25:223-35 (2010)

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  - Can we MIST human first?

"Human First" Metabolism *in alignment with MIST guidance*

- Metabolites identified only in human plasma or metabolites presented at disproportionately higher levels in humans than animals should be considered for safety assessment
- The metabolite level that riggers an assessment for nonclinical safety testing has been defined as 10% of the total drug-related exposure - harmonized FDA and ICH guidances
- Traditionally human metabolites are identified and quantified in a single-dose 14C radiolabeled human ADME study during phase II or after POC is achieve; however, the information may not be adequate to assess metabolism at steady state, and may be too late for timely initiation of a large-scale clinical trial if any human metabolite requires safety evaluation.
- Identification of qualitative and quantitative differences in drug metabolism between humans and animals in non-clinical safety assessment **as early as possible** is critical to avoid a delay of drug development.

B. Identification of Metabolites

Metabolite concentrations cannot be inferred by measurement of parent drug concentrations. The metabolic profile of the drug should be identified during the drug development process. This identification can be accomplished at different stages of development using in vitro and in vivo methods. In vitro studies can use liver microsomes, liver slices, or hepatocytes from animals and humans and generally should be conducted before initiation of clinical trials. In vivo metabolism study results in nonclinical test species generally should be available early in drug development, and their results will either confirm the results obtained from the in vitro studies or reveal quantitative and/or qualitative differences in metabolism across species. It is the latter situation that may pose a safety concern. Human in vivo metabolism studies usually have been conducted relatively later in drug development, but we strongly recommend in vivo metabolic evaluation in humans be conducted as early as feasible.

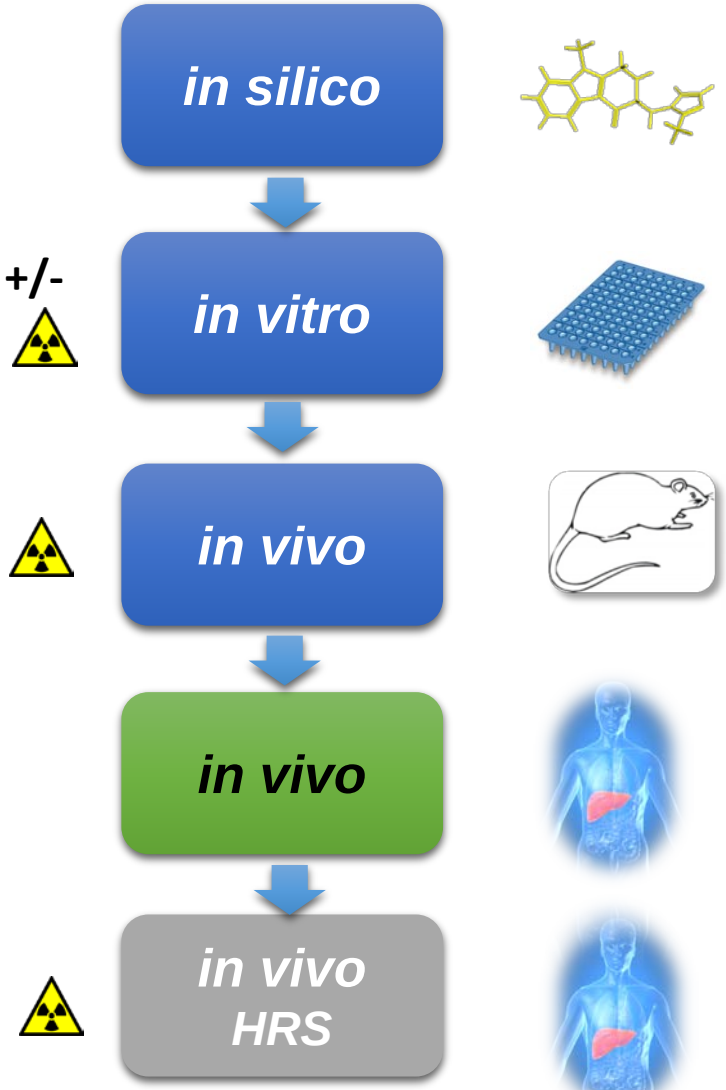
Contains Nonbinding Recommendations

V. TIMING OF SAFETY ASSESSMENTS

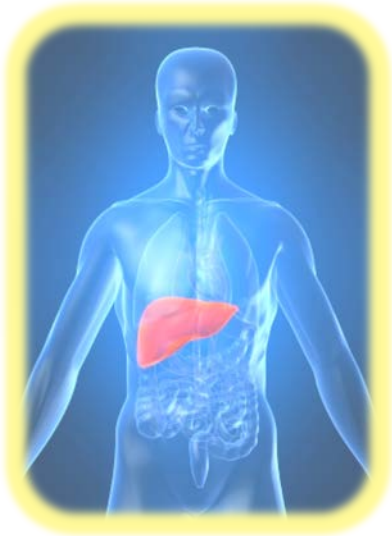
Early identification of disproportionate drug metabolites can provide clear justification for nonclinical testing in animals, assist in interpreting and planning clinical studies, and prevent delays in drug development. If toxicity studies of a drug metabolite are warranted, studies should be completed and study reports provided to the FDA before beginning large-scale clinical trials.

https://www.fda.gov/downloads/Drugs/.../Guidances/ucm079266.pdf

Can we get certain earlier?



Putting human first FTIH studies

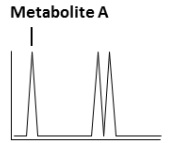


Obtain "certain" data then contextualise:

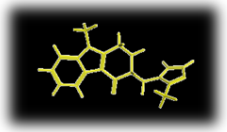
- non-clinical
- mechanisms

"cold metabolism"

- Need to detect



- Need to identify



- Need to quantify

PLASMA	%
DRUG	70
MET. A	30

MIST without radiolabel or reference standards?

	Conventional GLP bioanalysis	Radiometric calibration	Mixed matrix peak area comparison	NMR-based approach
Data output	Absolute concentration	Absolute concentration	Animal:human ratio	Absolute concentration
Reagent needs	Metabolite standards	Radiolabelled drug	None	None
Special equipment needs	None	None	None	NMR
Resource investment	Very high	Moderate	Low	High

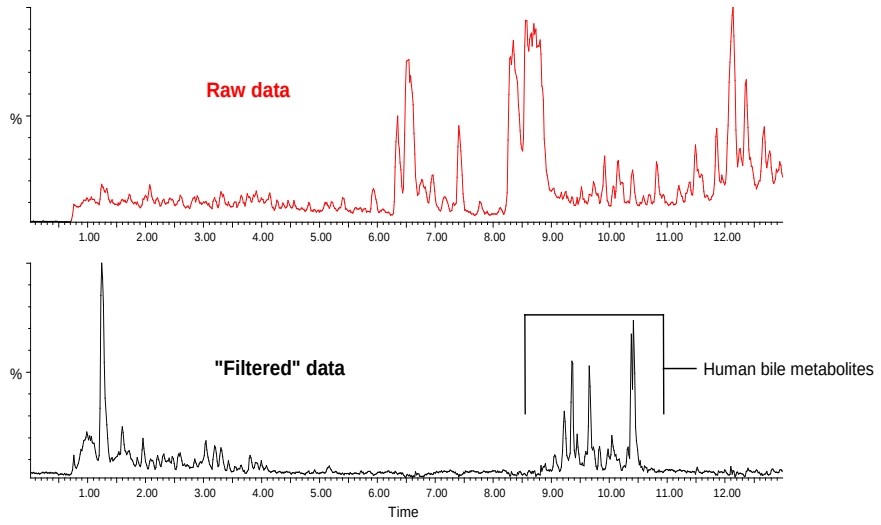
Zhang D, et al. *Drug Metab. Lett.* 1, 293-298, 2007
Ma S, et al. *Anal. Chem.* 83:5028-5036, 2011
Gao H, et al. *Drug Metab. Dispos.* 38:2147-2156, 2010
Espina R, et al. *Chem. Res. Toxicol.* 22:299-310, 2009

Metabolite ‘fishing’ with filters

What are our hooks? No radiotracer

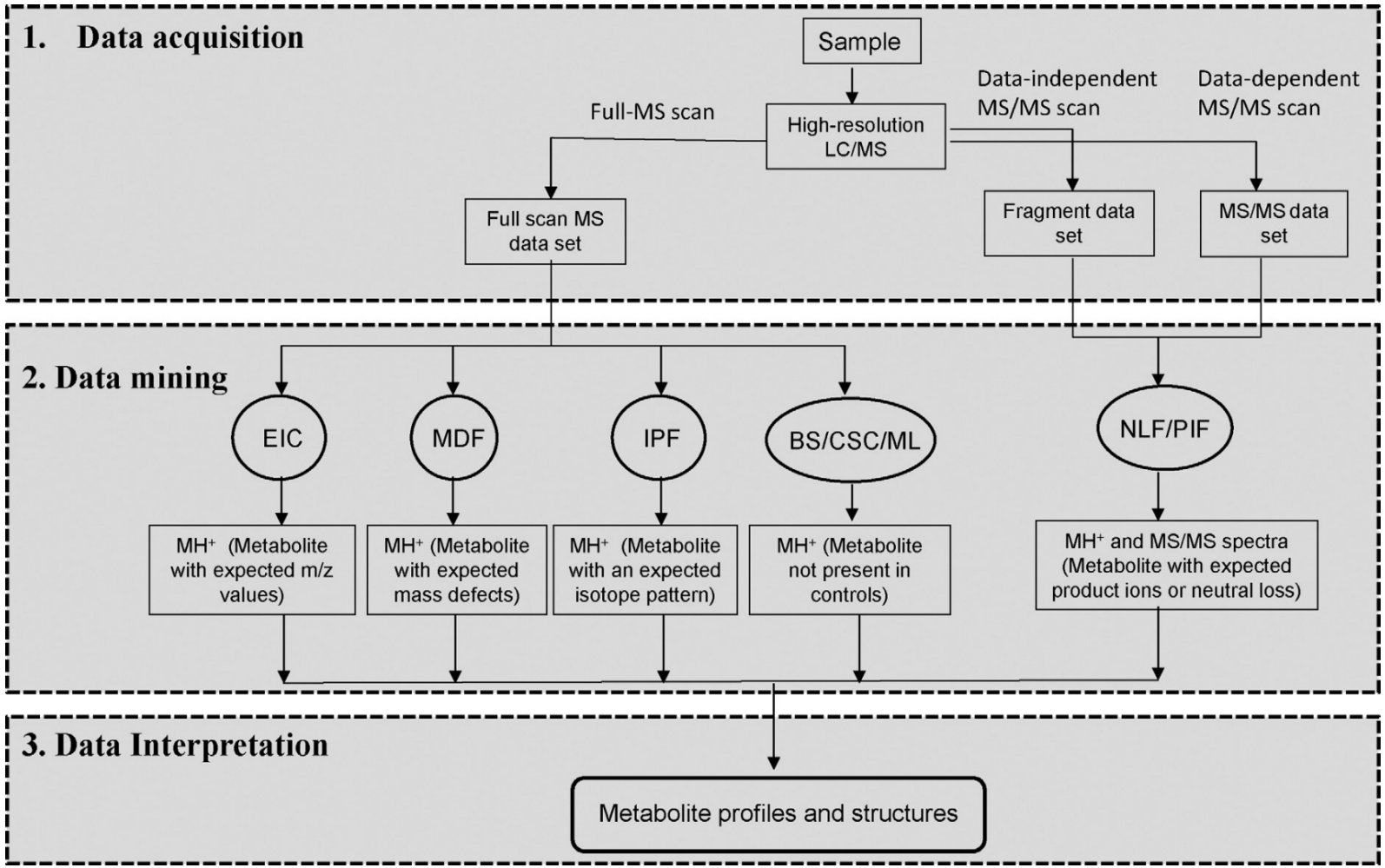
- MDF – fractional mass discriminator – taking advantage of nature’s imperfection
- The mass defect for parent is our hook (with a small window applied to account for metabolites, e.g. ± 50 mDa phase 1)

Element	Nuclide	Nominal mass	Exact mass	Mass defect	Isotopic abundance
Hydrogen	H	1	1.0078	0.00783	100.00%
	D	2	2.0141	0.0141	0.02%
Carbon	C^{12}	12	12.0000	0.0000	100.00%
	C^{13}	13	13.0034	0.00336	1.10%
Nitrogen	N^{14}	14	14.0031	0.003074	100.00%
	N^{15}	15	15.0001	0.0001	0.37%
Oxygen	O^{16}	16	15.9949	-0.0051	100.00%
	O^{17}	17	16.9991	-0.0009	0.04%
	O^{18}	18	17.9992	-0.0008	0.20%



Zhang H, et al., *J Mass Spectrom* 2003, 38:1110-1112.
Zhu M, et al., *Drug Metab Dispos* 2006, 34:1722-33.

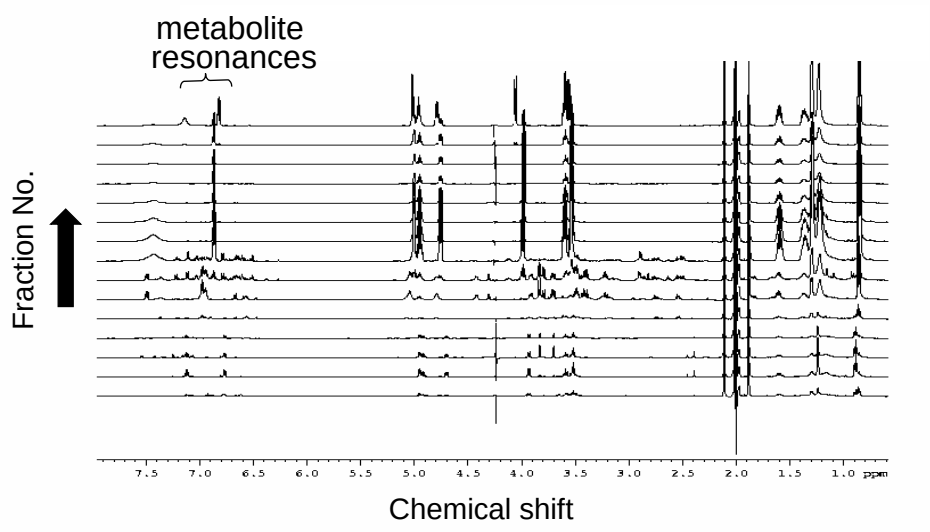
General HRMS workflow for metabolite detection and identification



Wen B and Zhu M, *Drug Metab Rev,* 47:71-87 (2015)

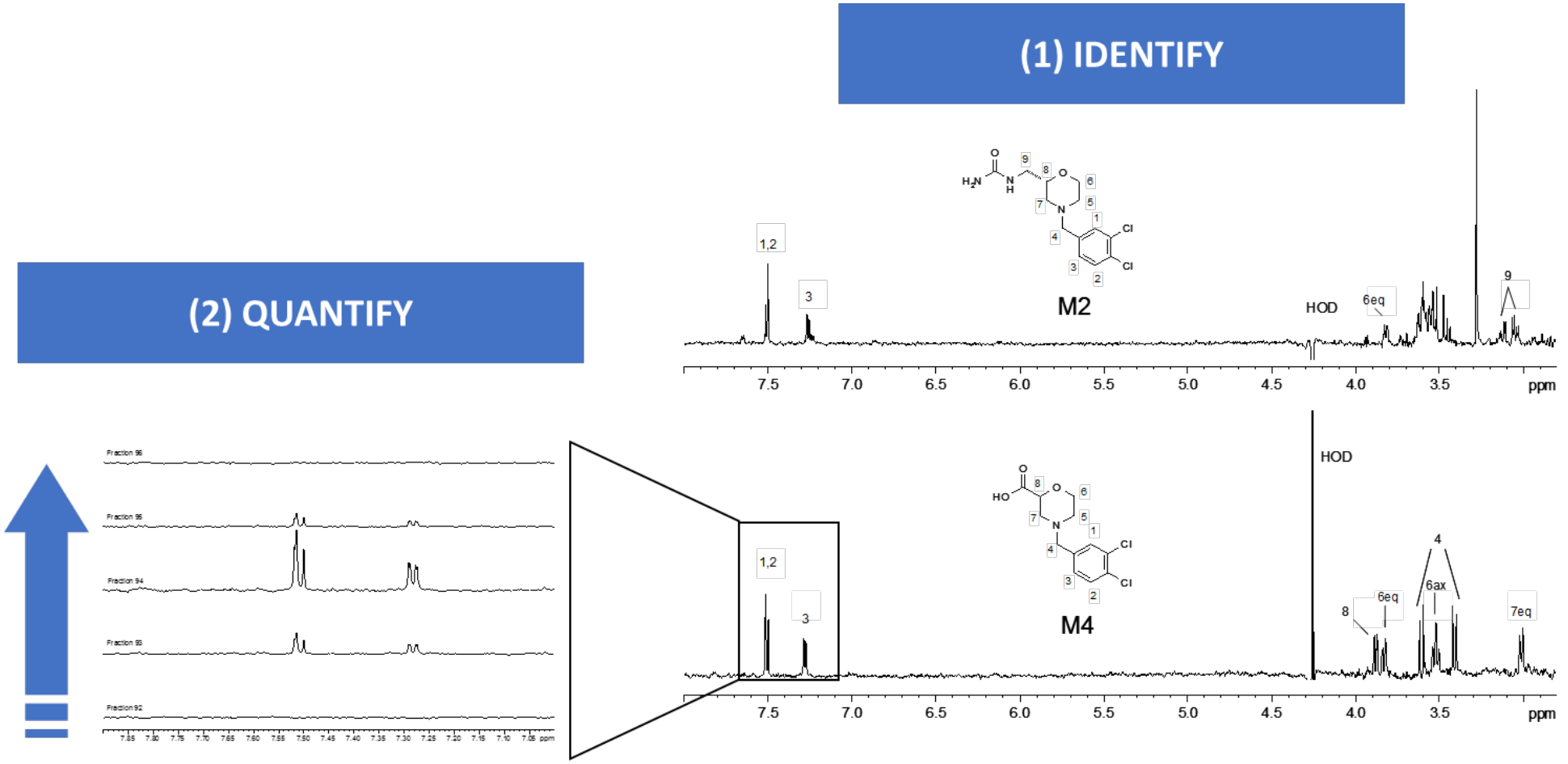
MIST with quantitative NMR

- NMR is a universal detector for organic compounds (C, H).
- Same NMR response (^{1}H, ^{19}F) for a nuclei independent of structural changes – making it the technique of choice for quantifying unknowns without radiolabel or reference standards
- Can provide both qualitative and quantitative data using the same sample, including "unweighable" metabolites isolated from biological sources
- A complimentary tool to LC/MS to aid structural elucidation and for further quantitative studies



Espina R, et al, *Chem Res Toxicol.* 22:299-310 (2009)
Vishwanathan K, et al, *Chem Res Toxicol.* 22:311-322 (2009)

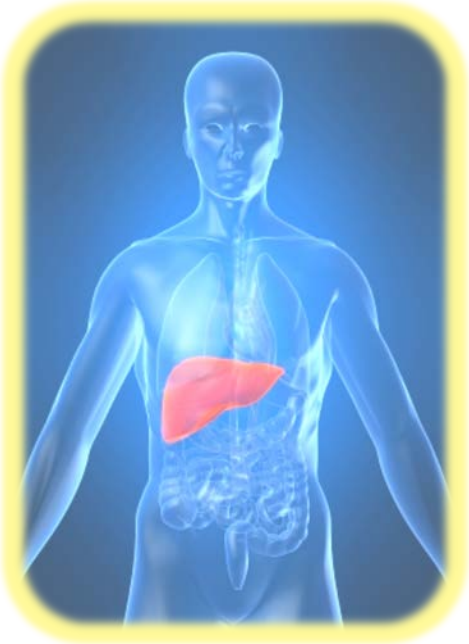
Structural elucidation and metabolite quantification in one NMR experiment



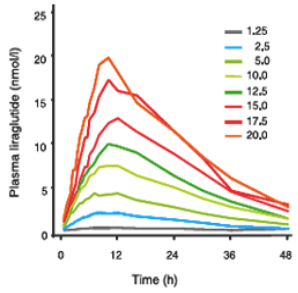
Dear GJ et al., *J Chromatogr B,* 2008, 876:182-190.

Putting human first – FTIH metabolism

- Technology evolved to allow more information with better quality
- **Can we take advantage of FTIH to get certain earlier** - generate a robust understanding of the metabolic fate of the drug

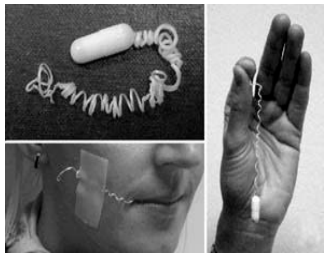


- Dose ascending design (SAD and MAD)
- Abundant sample
- Multiple dose - steady state metabolism
- **Free data** - blood samples collected for PK anyway



Plasma or Blood

Urine

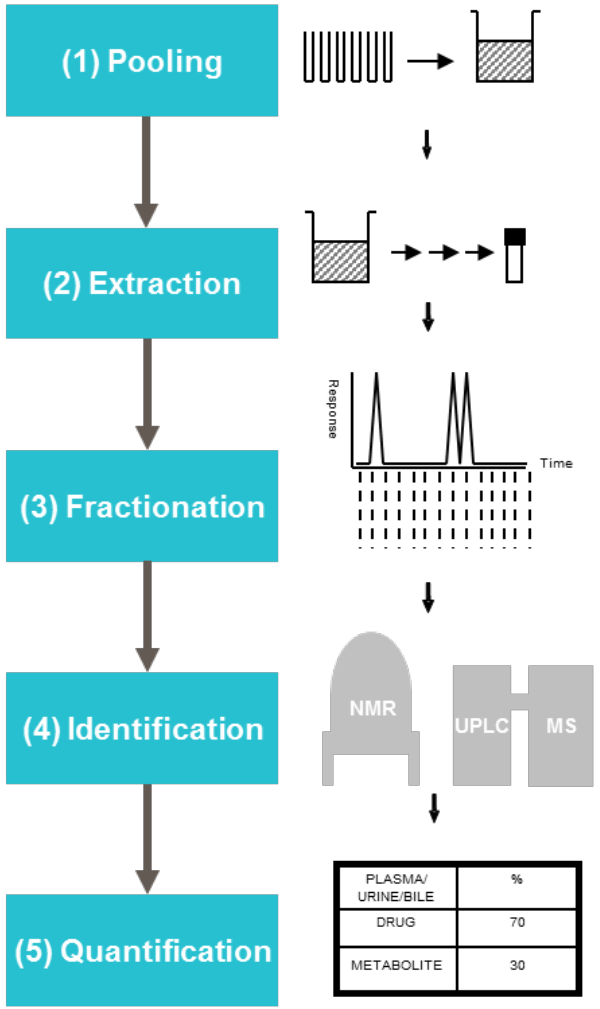


Bile string (Entero-test)

FTIH metabolism: advantages

- No reliance on radiolabel or synthetic standards or validated bioanalytical methods
- Robust data prior to a definitive human radiolabel study - what it is, where it is, how it happened, how much, where it goes
- Reduced animal experimentation – wait until more reason to believe – e.g. post-FTIH, post-POC
- Reduced up-front costs in support of differentiated development
- Help drive future clinical and safety studies based on reliable early assessment, prior to large clinical studies
- Steady state metabolism – more relevant in clinical chronic dosing

‘Human First’ Metabolism: Basic work flow



$$EM = \frac{(A_a^m / A_a^{IS})}{(A_h^m / A_h^{IS})}$$

‘Hamilton’ pooling of human plasma (MAD) and animal plasma (repeated dose tox. studies)

Matrix matching of human plasma with animal control plasma (50:50); IS added

Matrix matching of animal plasma with human control plasma (50:50); IS added

Metabolite profiling by LC-HRMS and/or NMR

Determine animal to human exposure multiples (EM) of metabolites

If EM > 1, the metabolite is covered in animal safety testing

If EM < 1, determine if the metabolite is >10% of DRM in human by NMR

If yes, determine EM with validated bioanalytical methods; prepare to find alternative animals or conduct safety assessment with the metabolites

If no, further testing is not required

Putting human first – FTIH metabolism

Once **structures** and **amounts** are known a metabolite risk assessment based on following criteria can be used to influence future clinical and safety programmes as appropriate

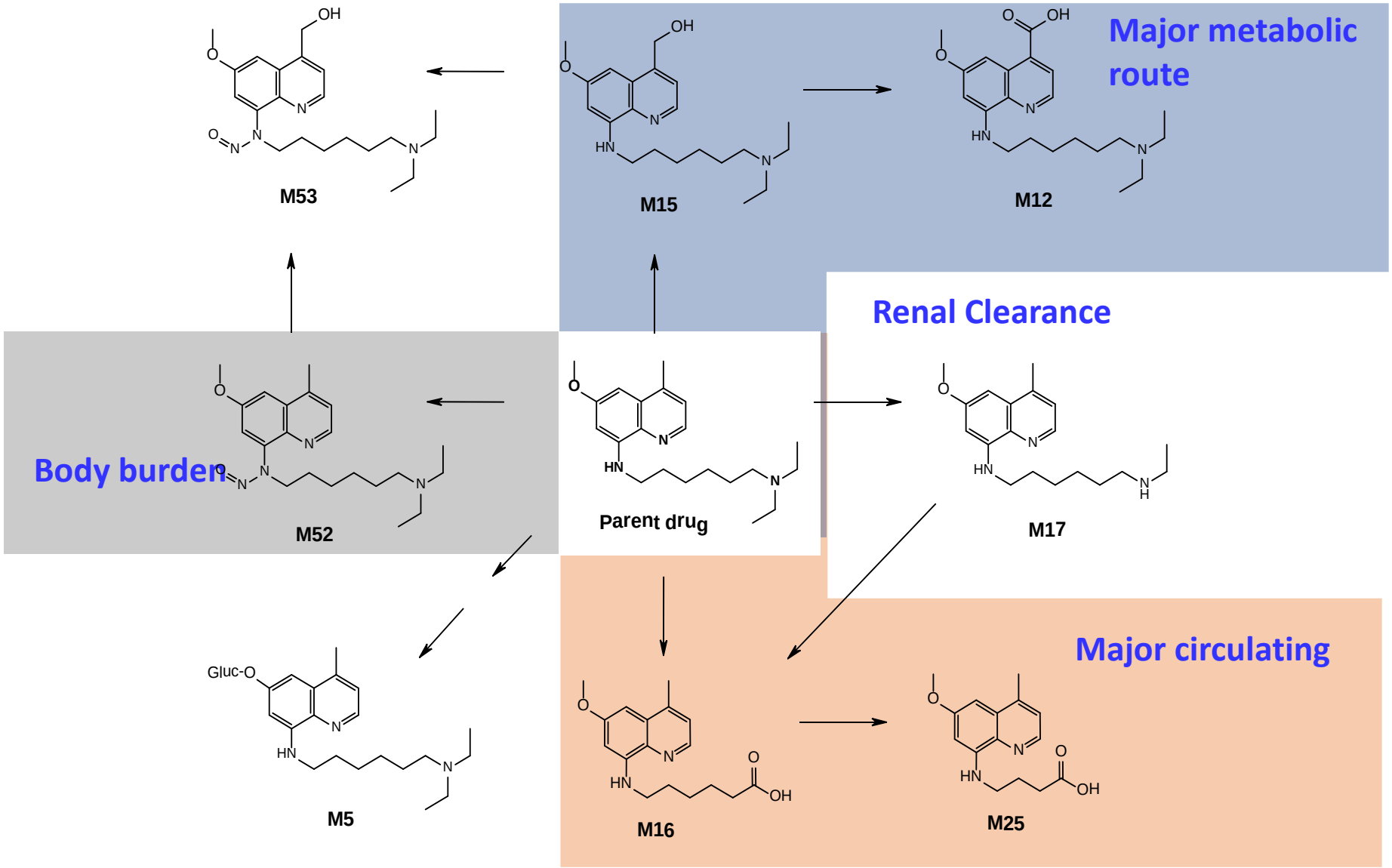
- Is it likely to be pharmacologically active (SAR)?
- Is it likely to be genotoxic (e.g. DEREK)?
- DDI implications?
- Is it human unique or disproportionate compared to animals?
- Is urine a major clearance route for parent or metabolites?

Case Study 1 – using ‘Human First’ approach

- Sitamaquine (SB-220) is an orally active 8-aminoquinoline which has shown encouraging efficacy against Visceral leishmaniasis
- Welfare/ethical issues precluded any further healthy subject studies and development was carefully continued in patient clinical studies
- The only option for generating in vivo human metabolism data, was via a ‘FTIH’ style study in patients
- Urine and plasma were examined by NMR and MS to determine the nature and amounts of metabolites present, and provided a good understanding of the metabolic fate in humans

Nedderman et al, *Xenobiotica* 41:605-622 (2011)

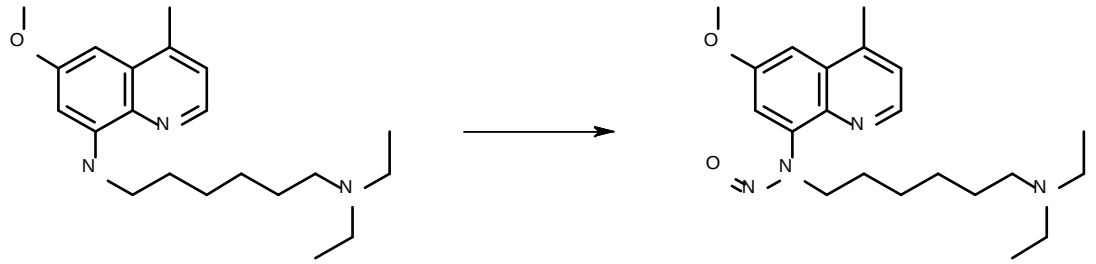
Case Study 1 – using 'Human First' approach



Nedderman et al, *Xenobiotica* 41:605-622 (2011)

Case Study 1 – using ‘Human First’ approach

- The major metabolites in human plasma were formed following N-dealkylation with subsequent oxidation - exposure coverage in toxicology species subsequently determined
- Approximately 20% dose eliminated in urine
- Of most concern was the identification of a notable, human specific circulating metabolite formed via. *N*-nitrosation
- The *N*-nitroso metabolite was a major component excreted in urine accounting for approximately 5 mg (25% administered dose)

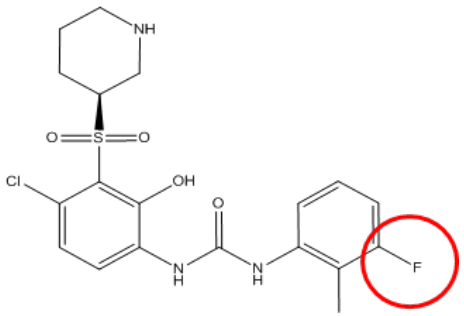


- Metabolite synthesised and a full assessment of its genotoxic potential made via a series of safety studies, including in vivo rat micronucleus test
- Concerns over genotoxic risk were decreased

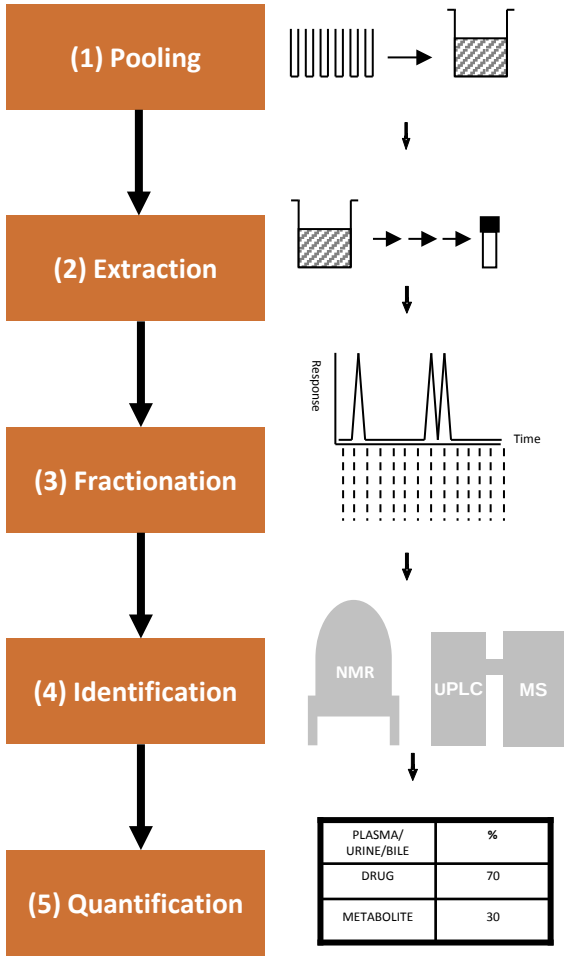
Nedderman et al, *Xenobiotica* 41:605-622 (2011)

Case Study 2 – using ‘Human First’ approach

- *O*-glucuronidation of GSK1325756 was predominant in rat hepatocytes but not human hepatocytes.
- Parent and low levels of oxidative metabolites detected in human blood, but not the O-glucuronide metabolite.
- Contribution of oxidative metabolism in human (i.e. CYP3A4-mediated) was unclear – key question
- Human bile was collected and analysed in early clinical development (non-invasive bile sampling using Entero-Test®); only 4% of administered dose recovered in urine.

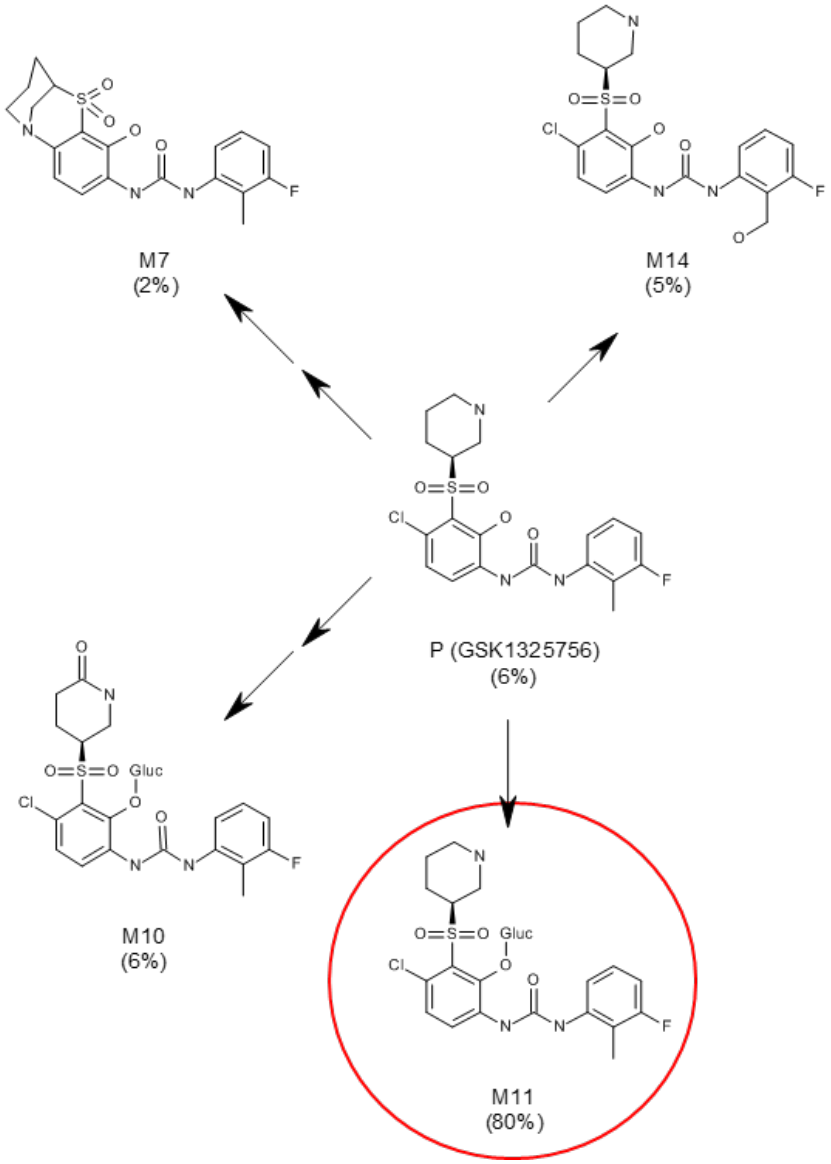


GSK1325756



Bloomer JC et al., *BJCP* 75:488-496 (2012)

Case Study 2 – using ‘Human First’ approach

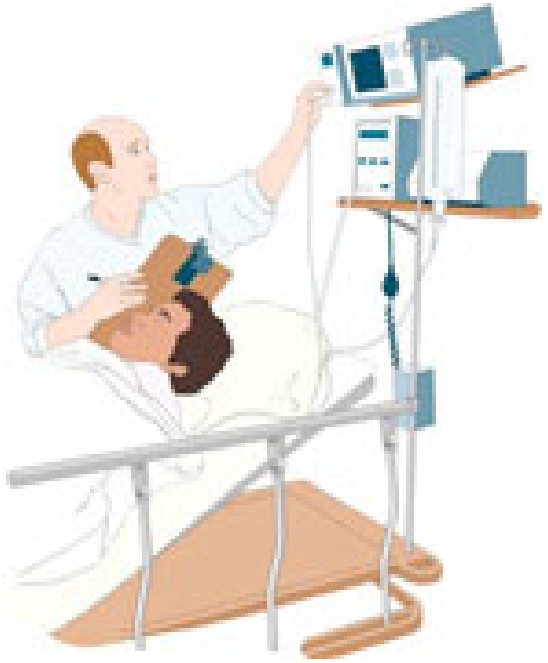


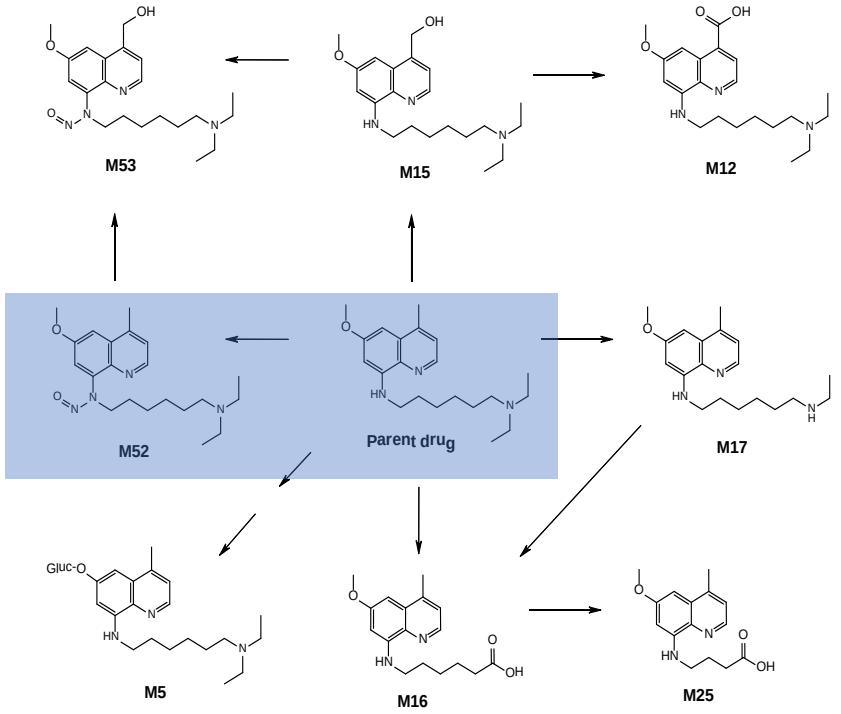
- O-glucuronidation confirmed as a major metabolic route (M11) in human, representing 80% of the DRM in bile
- CYP-mediated oxidative pathways responsible for a small fraction of clearance, and concerns of CYP-mediated victim drug interactions are reduced
- ‘Human first’ metabolism significantly contributed to assessment of the potential drug interaction risk

Bloomer JC et al., *BJCP* 75:488-496 (2012)

‘Human First’ metabolism in Patients

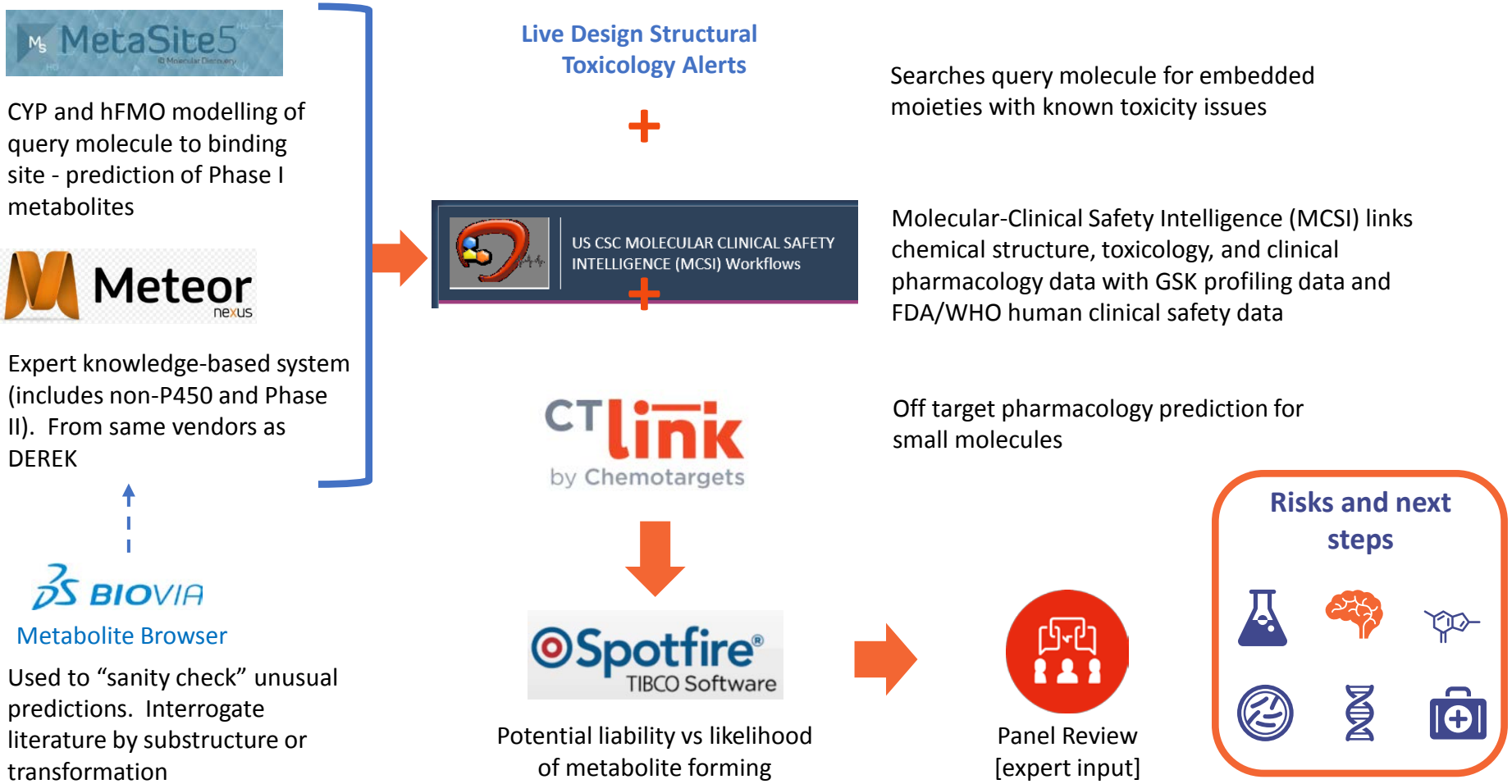
- Human first metabolism is a patient focused approach
- Unlike radiolabel, “cold metabolism” is more applicable to patients
- Increased relevance





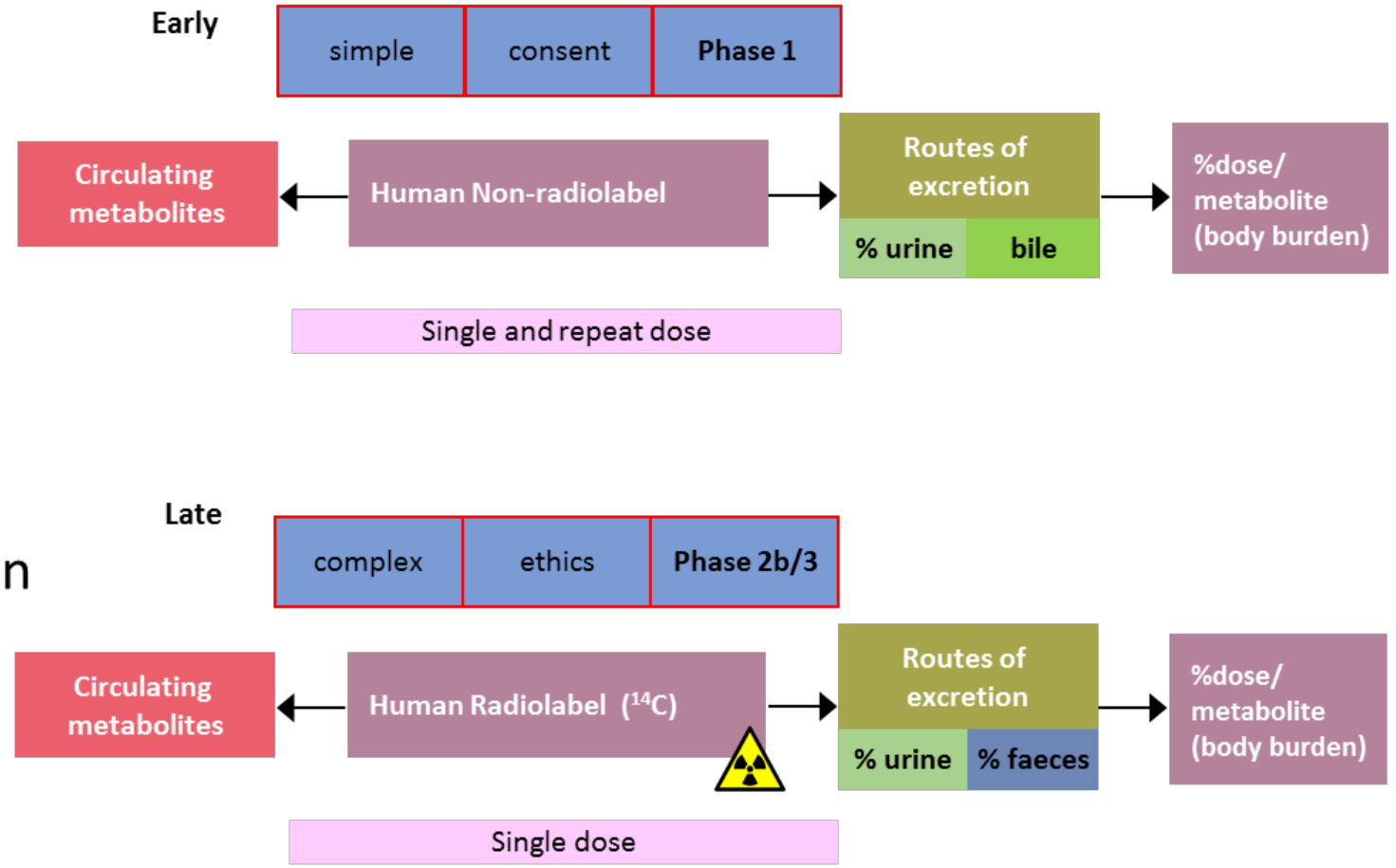
Metabolites relevant to patients (as opposed to healthy subjects)

In silico prediction



Take home message:

- Earlier (quality) data
- Less upfront cost
- 3Rs
- Better contextualisation
- Focus on the patient
- All this without compromising our ability to understand human ADME



Summary

- The implementation of regulatory guidances from the FDA and ICH requires that novel approaches be considered to adequately address the safety of drug metabolites as early as possible in development.

- With technological advances in recent years (namely LC/HRMS, cryoprobes for NMR), it is possible now to get certain about human metabolism in early development.

- 'Human first' metabolism strategy provides robust and quality data in metabolites safety assessment with no reliance on radiolabel or reference standards or validated bioanalytical method, helps drive clinical and safety studies both financially and regulatorily.

Acknowledgement

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- Stephanie North
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