SSX-2018

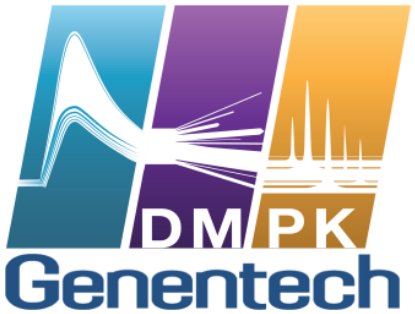
3rd Annual Conference

Organized by: SOCIETY FOR THE STUDY OF XENOBIOTICS (SSX)-INDIA

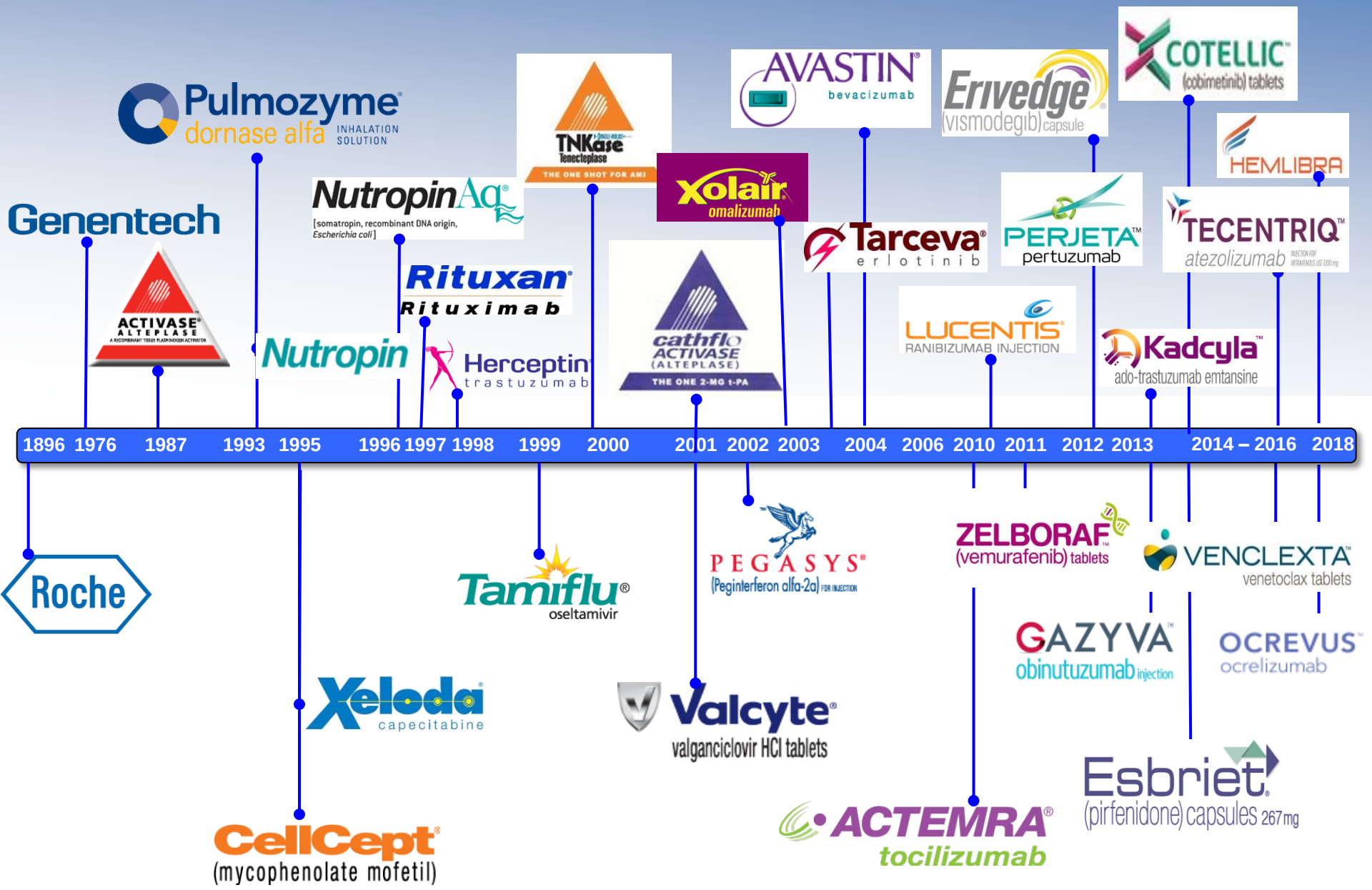
How to Handle MIST issues: The Use of the Mixed Matrix Method Illustrated with Clinical Examples

Marcel Hop

Genentech



We Make Medicines for People with Serious Diseases



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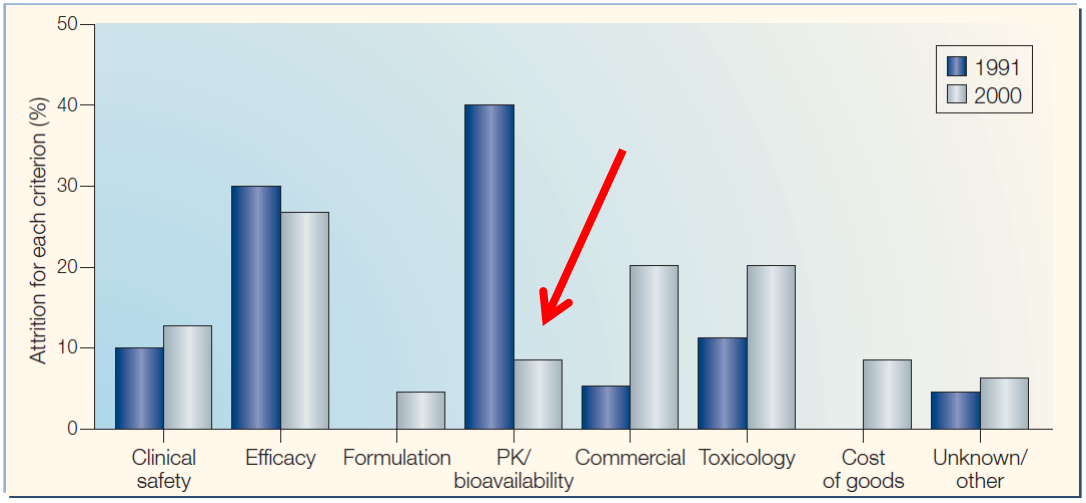
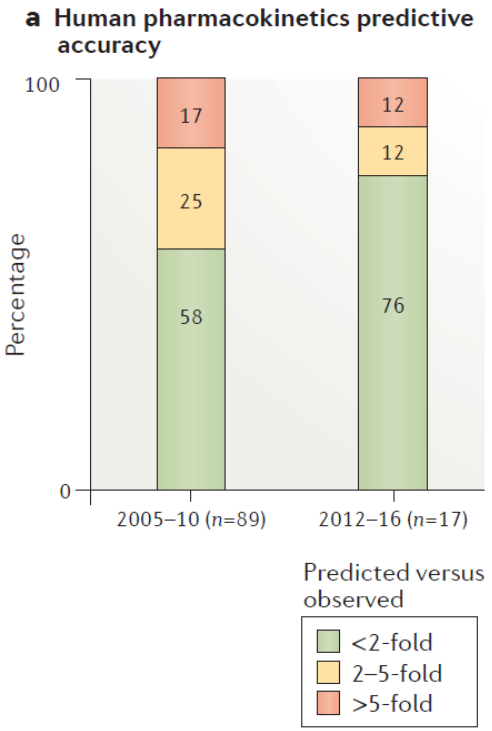
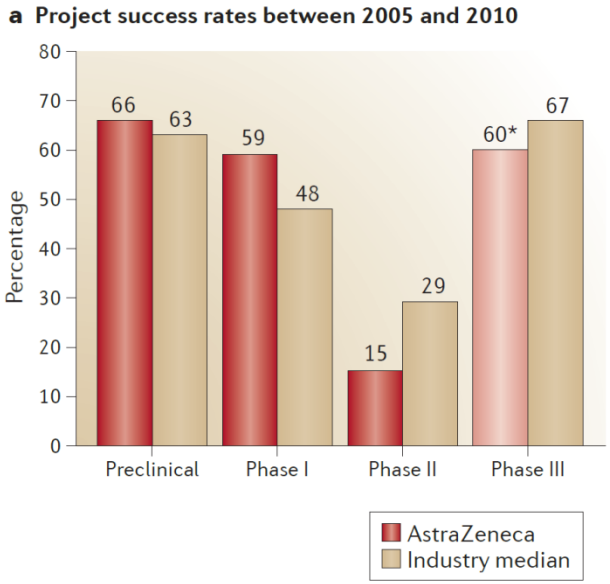
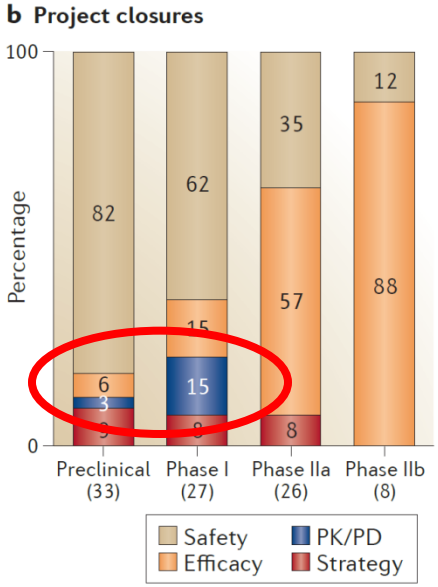
Attrition in Development Due to Poor PK is Limited









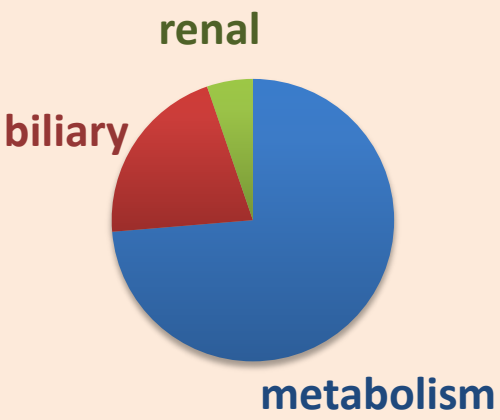
How about the impact of MIST?

Kola, Nature Rev. Drug Discov., 2004, 3, 711; Cook, Nature Rev. Drug Discov., 2014, 13, 419; Morgan, Nature Rev. Drug Discov., 2018, 17, 167.

Metabolism is a Major Drug Clearance Pathway

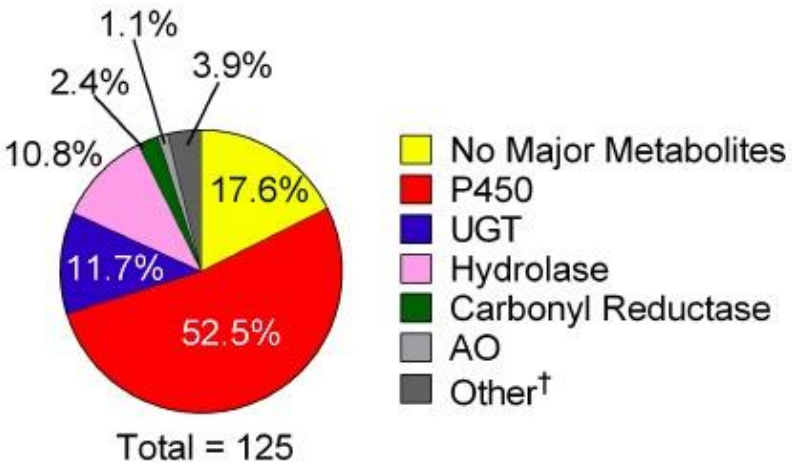
Top 200 Prescribed Drugs in 2002

Williams et al, 2004 DMD



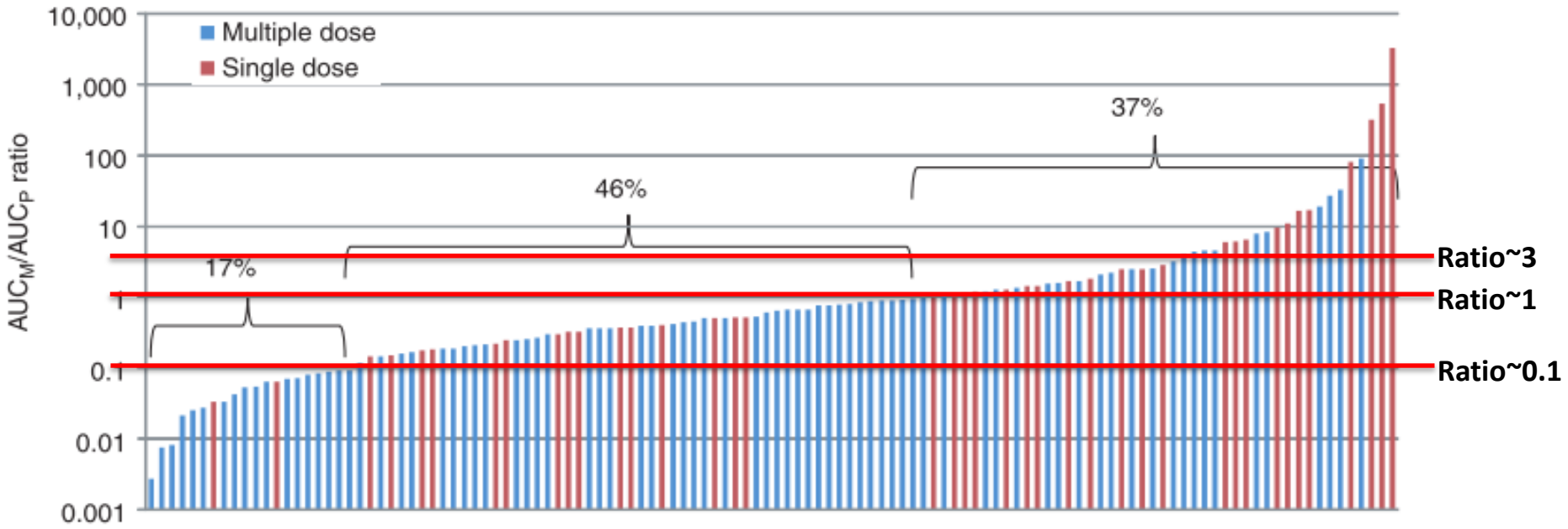
→P450 followed by UGT are major enzymes

Prevalence of Non–Cytochrome P450–Mediated Metabolism in Food and Drug Administration–Approved Oral and Intravenous Drugs: 2006–2015[R]



† - Other represents: sulfotransferases (0.8%), cytidine deaminase (0.8%), dehydropeptidase-I (0.8%), nucleotidases (0.8%), alcohol/aldehyde dehydrogenase (0.4%), flavin-containing monooxygenases (0.3%), glutathione conjugation (0.3%), gut microbes (0.3%), undefined/unknown (0.3%)

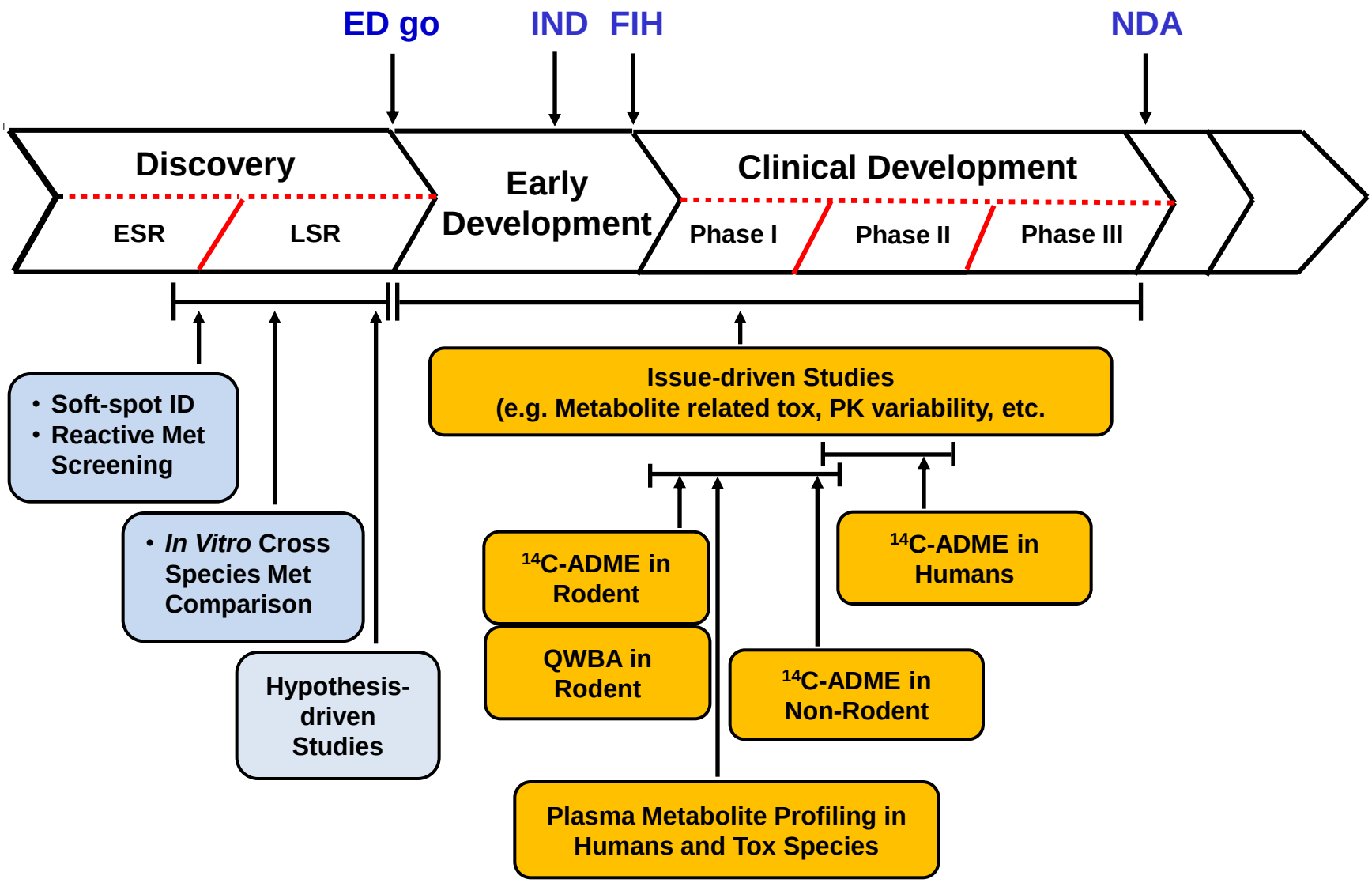
How Commonly is [AUC_m/AUC_p] >1 Observed for Drugs?



- Metabolite exposures > parent drug exposures have been observed for ~1/3 of drugs (retrospective analysis)
- A large number of drugs have metabolites that meet a >10% of parent criteria

Yeung, Clin. Pharmacol. Ther., 2011, 89, 105.

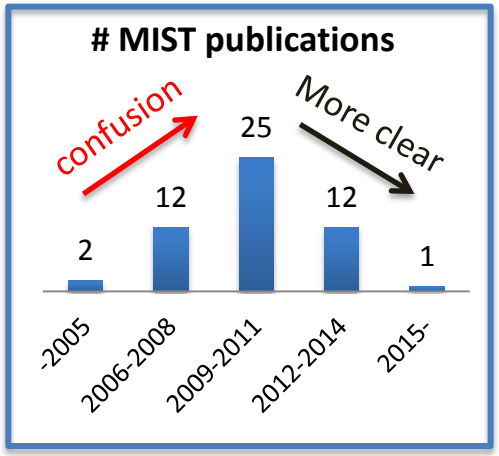
Metabolism Studies in Drug Discovery and Development



Brief History on Metabolite in Safety Testing (MIST)

- 2002, white paper on MIST published in *Toxicol. Appl. Pharmacol.*
  - >25% of the exposure of circulating drug-related material
- 2005, US FDA issued a draft guidance titled "Safety Testing of Drug Metabolites"
  - >10% of the administered dose or systemic exposure.
- 2008, US FDA issued a formal guidance on MIST.
  - >10% of systemic exposure of the parent drug at steady state
- 2009, ICH-M3 (R2)
  - >10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies
- 2016, FDA revised MIST guidance
  - >10% of total drug-related exposure

"The need for independent toxicity testing of major human metabolites is still infrequent."
Jeri El-Hage from FDA 2006



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Key Messages from MIST Guidance

- Addresses circulating human metabolites at steady state and their potential to elicit toxicities
- Studies to assess risks due to metabolites should be completed before large-scale clinical trials (Phase 3)
- MIST does not apply to oncology (S9) indications
- Most glucuronides are not of concern, except those that undergo chemical rearrangement (e.g., reactive acyl glucuronides)
- Low dose drugs (<10 mg daily) may warrant higher % of drug-related material
- The guidance does not specifically address prodrugs

Metabolism from FIH Studies - What is Essential?

There are four aspects/components to the metabolism data pertaining to MIST:

- Metabolite detection
- Metabolite identification
- Semi-quantitation of metabolite abundances (if any metabolites at greater than 10% of total exposure?)
- Quantitative assessment of metabolite coverage in preclinical safety species

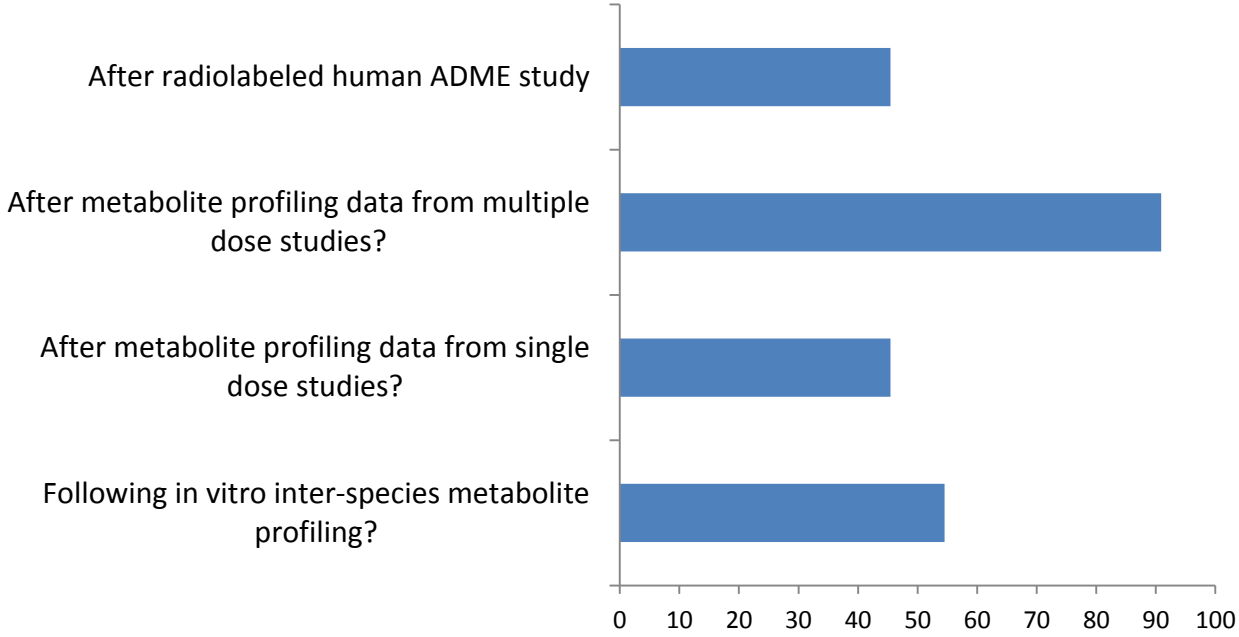
Comparison of four technologies applied in the context of MIST for metabolite profiling and structure elucidation

	Detection	Structure Elucidation	Quantitation
HRMS	Highly sensitive and effective	Partial information	Requires authentic standard for absolute quantitation. However, quantitative coverage assessment can be made without authentic standard.
NMR	Not sensitive enough	Highly effective (with biosynthesis)	Quantitative without an authentic standard
AMS	Highly effective (requires ^{14}C)	Not applicable	Quantitative, without an authentic standard
Biosynthesis	Not applicable	Highly effective (with NMR)	Can serve as standard for MS quantitation

Schadt, Drug Metab. Dispos., 2018, 46, 865.

When Do You Identify Potential MIST Issues?

Informal survey about MIST with responses from Agios, Boehringer Ingelheim, Bristol-Meyers Squibb, Chugai, Genentech, Merck, Novartis, Pfizer, Roche, Takeda, Unilabs

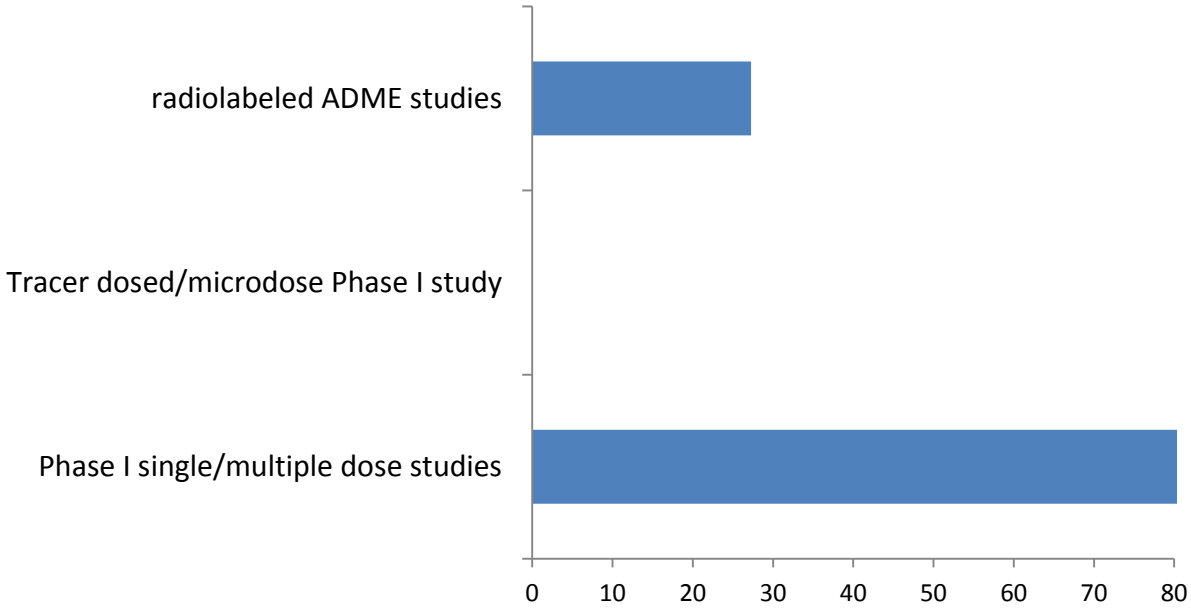


Following in vitro inter-species metabolite profiling?	55 %	6
After metabolite profiling data from single dose studies?	45 %	5
After metabolite profiling data from multiple dose studies?	91 %	10
After radiolabeled human ADME study	45 %	5

More than one answer could be provided

11 responses

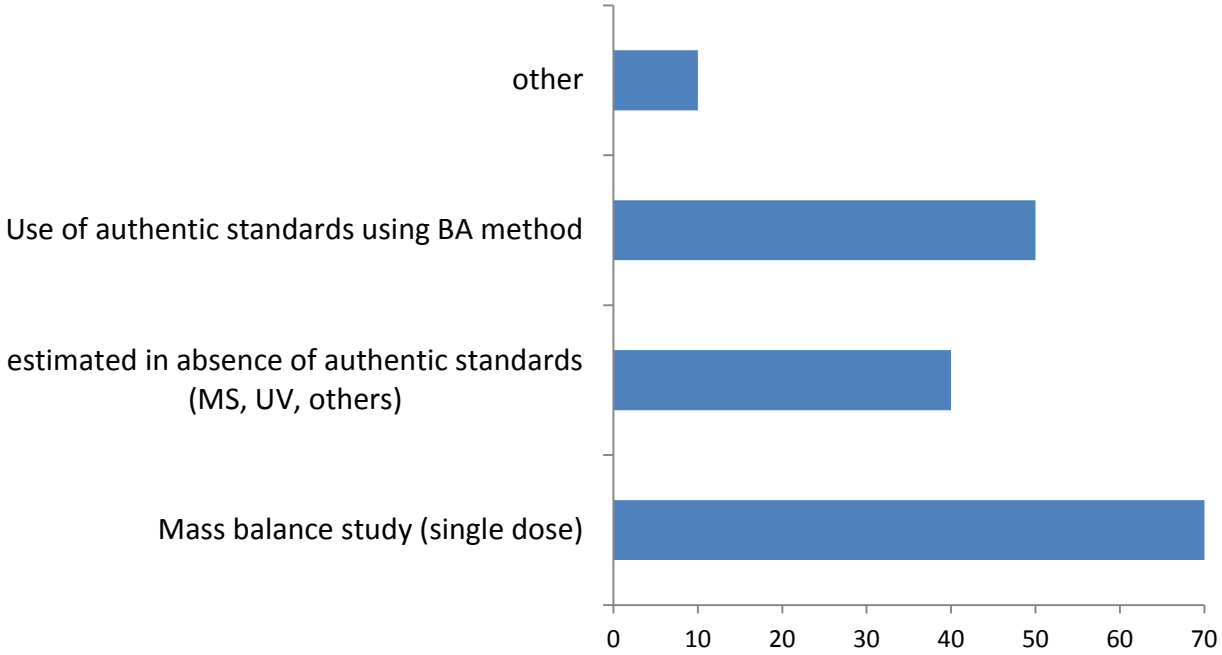
Which Earliest Clinical Study Data are Used to Decide if there is a Disproportionate Metabolite?



Phase I single/multiple dose studies	82 %	9
Tracer dosed/microdose Phase I study	0 %	0
radiolabeled ADME studies	27 %	3

More than one answer could be provided

How Do You Usually Determine Whether a Metabolite is >10 % or < 10 % of Total?



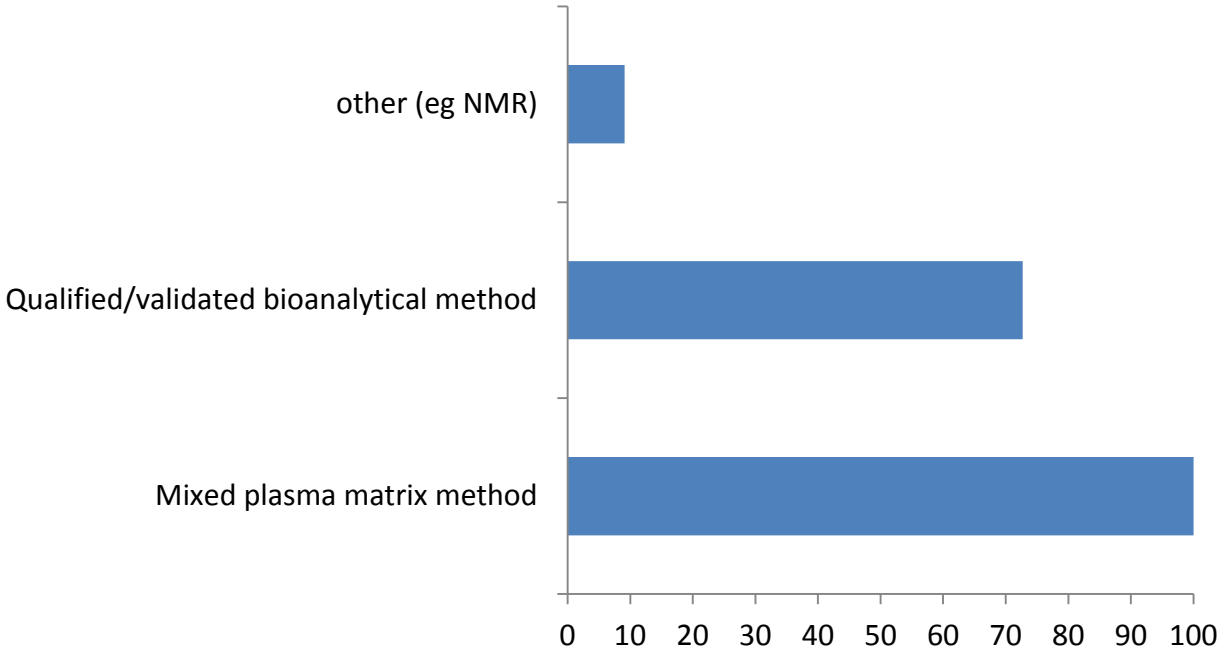
Mass balance study (single dose)	70 %	7
estimated in absence of authentic standards (MS, UV, others)	40 %	4
Use of authentic standards using BA method	50 %	5
other	10 %	1

More than one answer could be provided

Other: NMR - Sensitivity limitations have to be taken into account when using this.

10 responses

How Do You Usually Determine Metabolite Coverage in Clinical Studies and Assess Non-Clinical Coverage?



Mixed plasma matrix method	100 %	11
Qualified/validated bioanalytical method	73 %	8
other (eg NMR)	9 %	1

More than one answer could be provided

- Tiered approach: mixed plasma matrix method - first assessment; qualified/validated bioanalytical method - final assessment

Metabolism from FIH Studies - What is Essential?

There are four aspects/components to the metabolism data as it pertains to MIST:

- Metabolite detection
- Metabolite identification
- Semi-quantitation of metabolite abundances (if any metabolites at greater than 10% of total exposure?)
- Quantitative assessment of metabolite coverage in preclinical safety species

Mixed matrix methodology

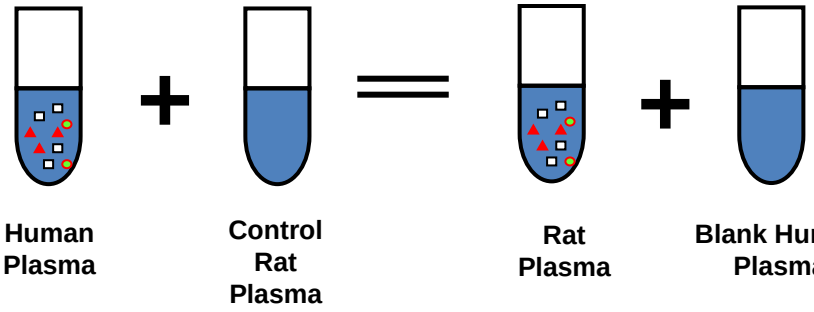
Mixed Matrix Method for Exposure Comparison

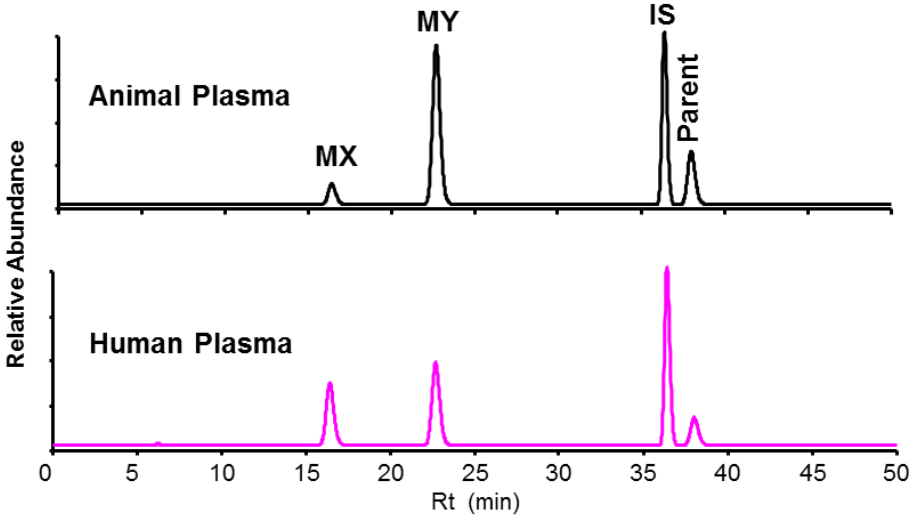
1. **Sample Pooling**
   AUC proportional pooling of plasma samples (pooled sample conc represents C_{avg}) + pooling across subjects/animals

2. **Matrix Equalization**
   Equalization of matrix by equal dilution with blank plasma from human/animal

3. **Sample Extraction**
   Internal standard (SIL-IS or analog) addition, protein precipitation

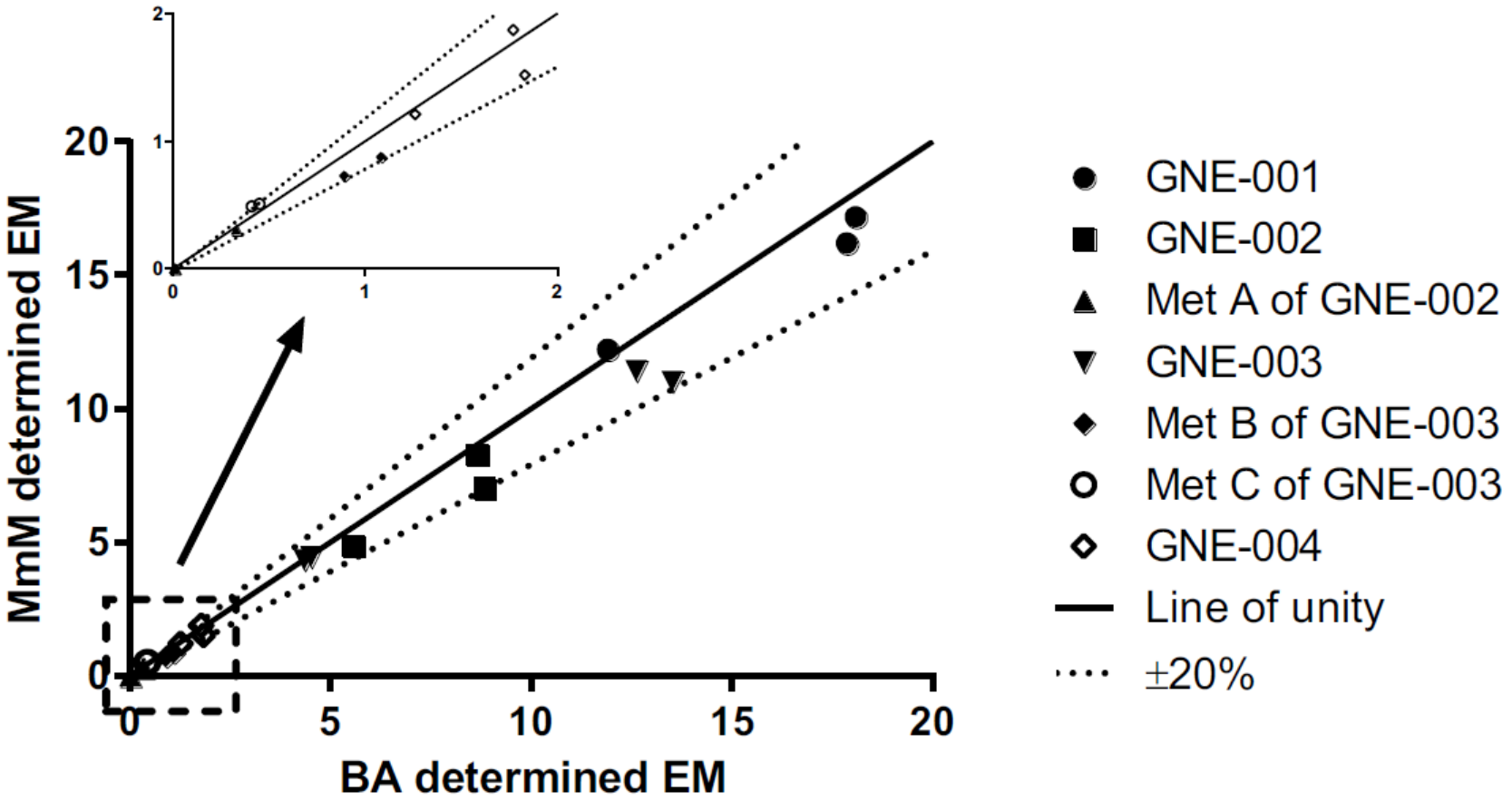
4. **MS Signal Comparison**
   LC-HRMS or LC-MRM analysis– direct comparison of MS response (IS normalized) between samples





Ma, Chem. Res. Toxicol., 2010, 23, 1871; Gao, Drug Metab. Dispos., 2010, 38, 2147.

Mixed Matrix Method Provides a Reliable Metabolite Exposure Comparison



The results are within ± 20% of those obtained from validated LC-MS/MS bioanalysis for multiple GNE development compounds and their metabolites.

Takahashi, Drug Metab. Lett., 2017, 11, 21.

Advantages and Disadvantages of Mixed Matrix Method

Advantages:

- No need for synthetic standards or radiolabeled compounds for mass spec. response correction for metabolites
- Simultaneous coverage determination of multiple metabolites
- The acquired LC-HRMS data set can be analyzed for quantitative assessment for any metabolite of interest, at any time during the development of a compound
- This approach provides accuracy close to that obtained from validated bioanalytical methods (~± 20%)

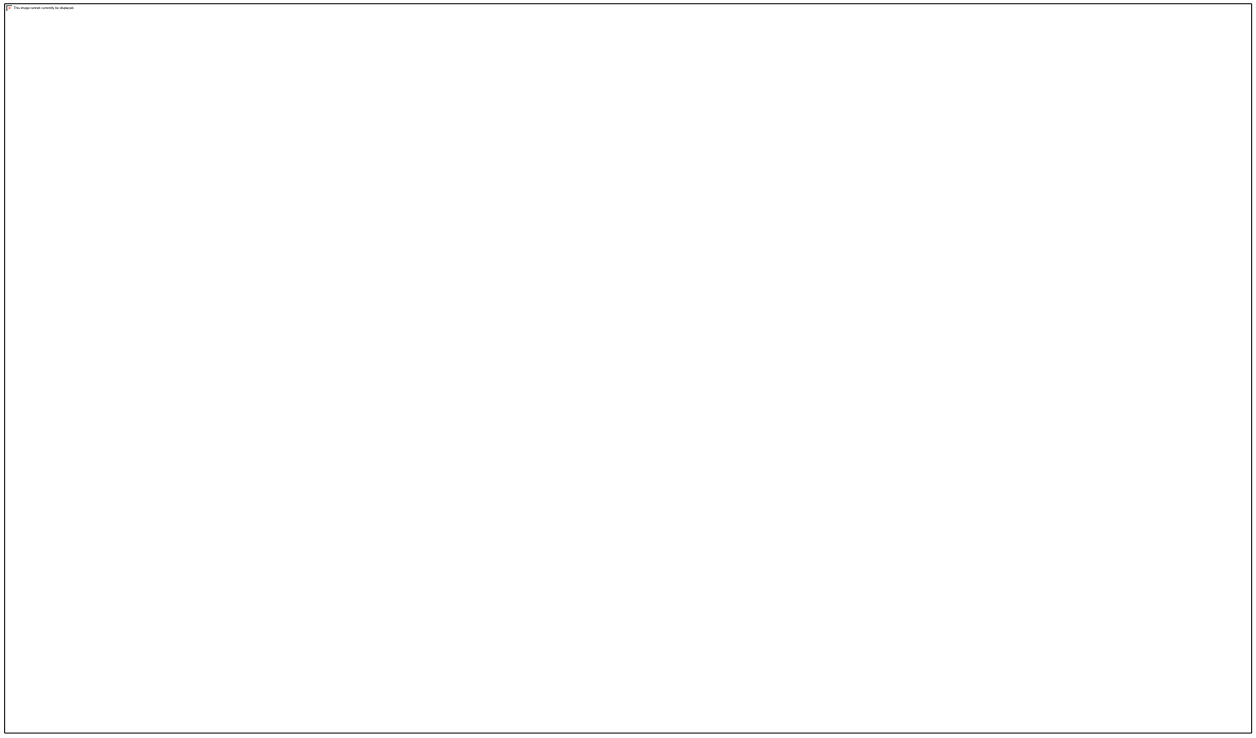
Disadvantage:

- Not absolute quantitation method. The metabolite concentration and exposure values can not be determined

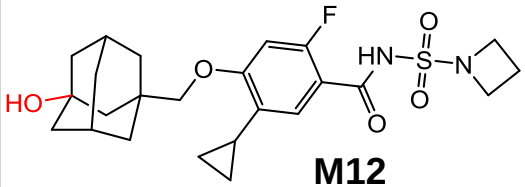
Case Study 1: GDC-0276

Indication: Moderate/severe pain; **Target:** Nav 1.7

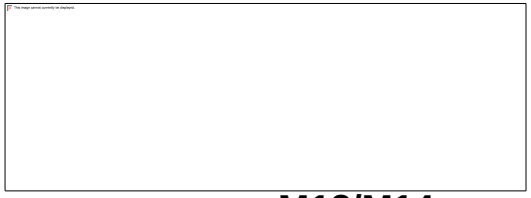
Background subtracted Plasma Metabolite Profile
GDC-0276 at steady state; 180 mg BID, Day 11, 0-12 h



CYP2C8,3A



M12



M13/M14

endo- and exo-conformation isomer

- M12 and M13 were estimated to account for >10% of total exposure.

Schadt, Drug Metab. Dispos., 2018, 46, 865.

M12 and M13 Exposure Coverage in Animals

Species	Exposure Ratios (Animal:Human)						
	GDC-0276			M12			**M13**
	BA data	Mixed Matrix	*Diff (%)*	BA data	Mixed Matrix	*Diff (%)*	**Mixed Matrix**
Dog (M)	8.8	7.0	*-21*	0.00828	0.00825	*-0.4*	**1.3**
Dog (F)	5.6	4.9	*-14*	0.00477	0.00515	*8.0*	**0.6**
Rabbit (F)	8.6	8.3	*-4.2*	0.327	0.308	*-5.8*	**15**

- Exposure estimates for parent and M12 based on validated BA method and mixed matrix experiment are consistent.
- M12 was clearly disproportionate in human and not covered in rat (data not shown, ~ 0.005x) and dog toxicology species.
- M13 exposures in male dogs and rabbit exceed human exposures at 270 mg BID

Studies Conducted with M12 and M13

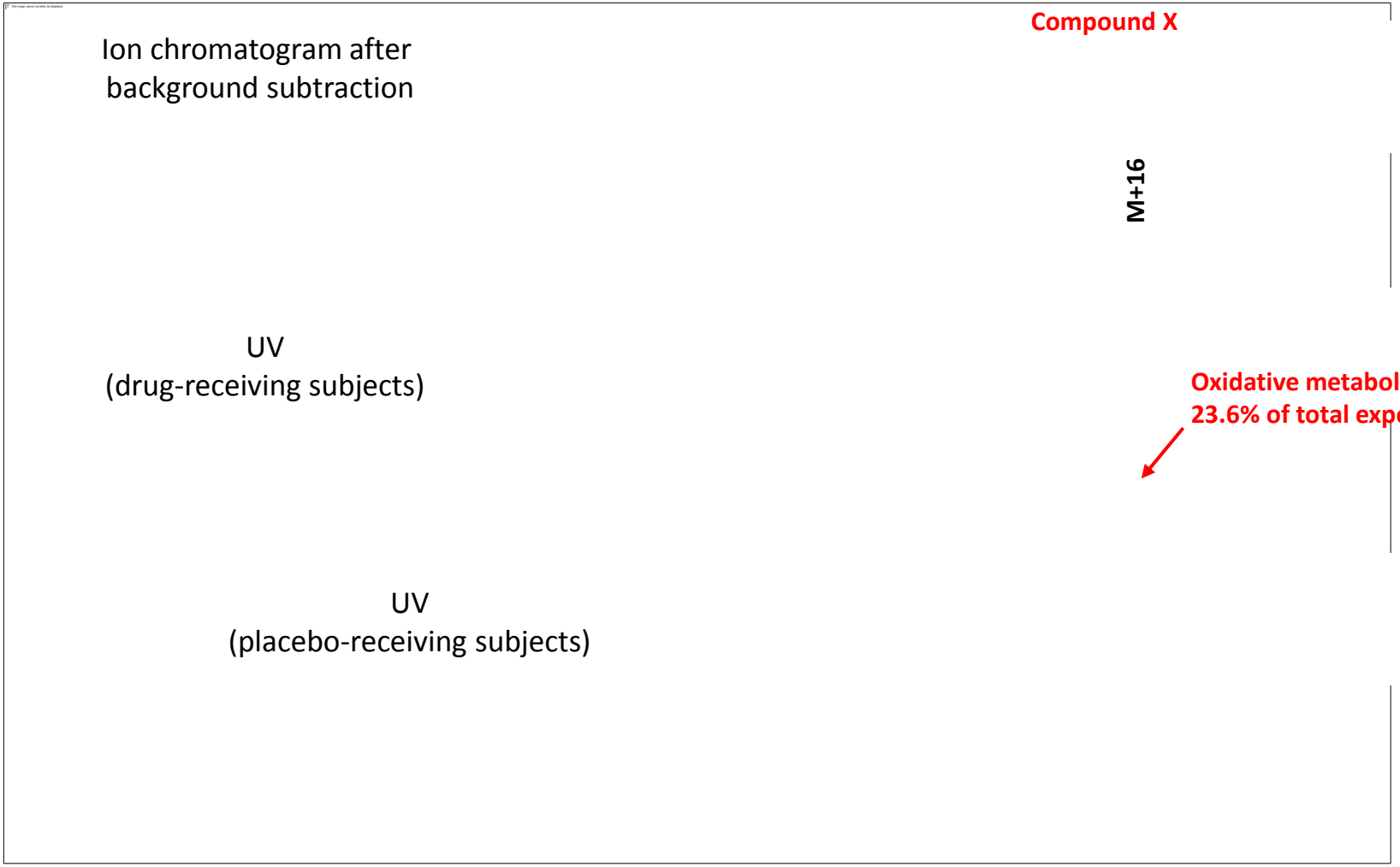
- M12 and M13 were synthesized and tested against the target (Nav1.7) to be inactive.
- M12 and M13 were tested in a secondary pharmacology panel and exhibited clean off-target profile and were not genotoxic.
- Due to its abundance with no coverage at tox species (< 0.01x), M12 was also tested in vivo toxicology study in rats (13-week GLP study).
- M13 was on the borderline for coverage in dog, but was covered in rabbit which provided coverage for the embryo fetal development study (seg II).
- In communication with EMA, the mixed matrix method was highlighted as appropriate to estimate the relative abundance of M13 in human compared to preclinical species.

Lessons Learned:

Exposure coverage is to compare to “marketed dose”. The efficacious clinical dose is not determined yet at early phases of clinical development. For GDC-0276 program, the recommended phase 2 dose decreased by a factor of 3 and this changed the coverage of M13 from a ratio of 0.9 in dog at 270 mg BID to 2.6 at 90 mg BID.

Case Study 2: Compound X

Phase: PhI SAD/MAD completed.

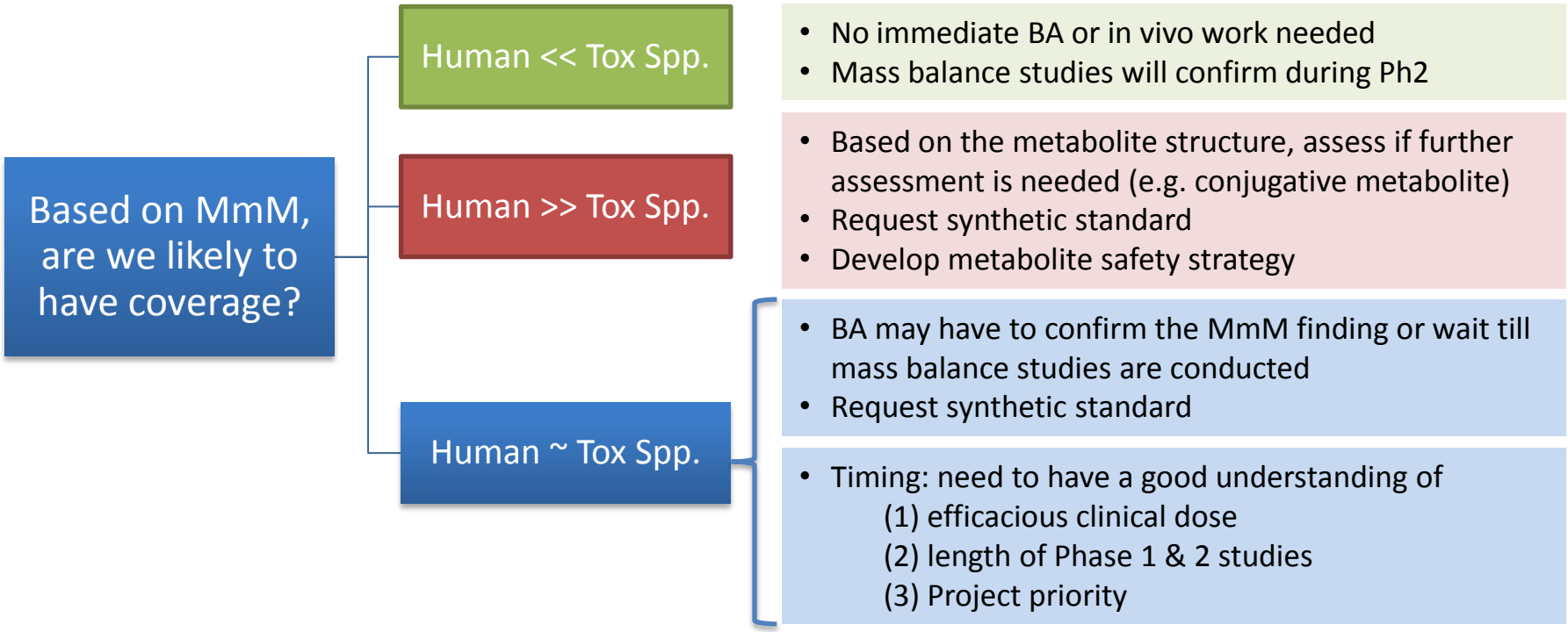


Metabolite Exposure Coverage in Rats and Monkeys

	Compound X from BA		LC-MS Mixed Matrix Method			LC-UV Method
	AUC (0-24hr) ng/mL*hr	EM (Cmpd X)	EM (Cmpd X)	% diff. from BA	EM (Metabolite)	EM (Metabolite)
Human (BID, 200mg, Day 7)	85120	---	---	---	---	---
Monkey (QD, 300 mpk, Day 7)	150706	1.77	1.87	5.6%	0.69	0.66
Monkey (QD, 30 mpk, Day 7)	107113	1.26	1.21	-4.0%	0.38	0.40
Rat (QD, 1000 mpk, Day 7)	155352	1.83	1.53	-16%	0.06	0.07

- The exposure of the oxidative metabolite in humans up to 200 mg BID was adequately covered in monkeys at 300 mpk (EM ~ 0.6).

Mixed Matrix Method Enables MIST Decision Making



Bioanalytical consideration to support comprehensive MIST strategy

- Does MmM trigger further metabolite assessment?
- Is BA method needed for in vivo tox studies (subchronic, chronic, repro, carc, etc.)?
- If relevant GLP tox studies have been completed, consider whether bridging PK or dedicated metabolite toxicity study is needed

Decision Tree for MIST Assessment Using Mixed Matrix Methodology

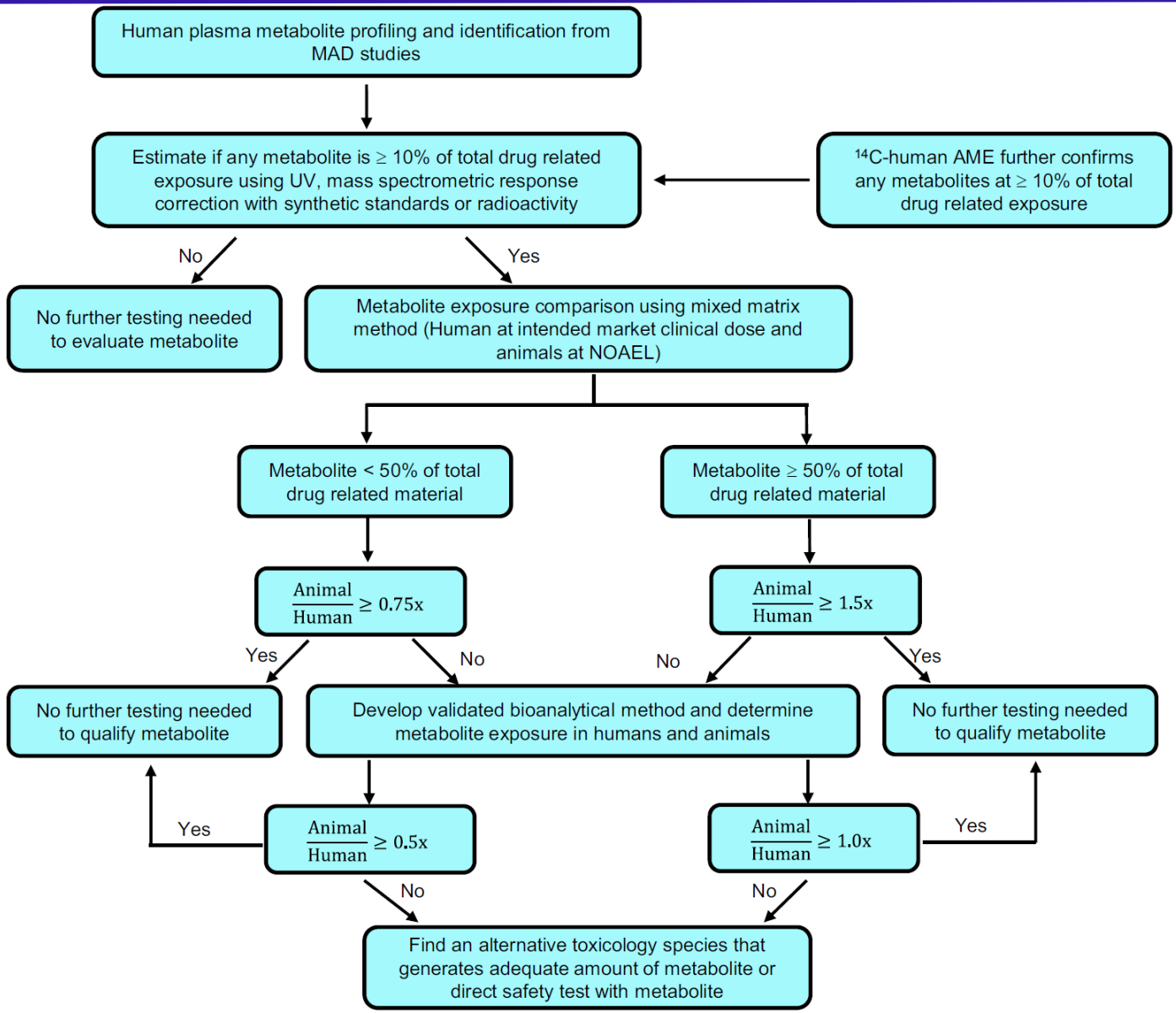


Fig. (2). A decision tree for using the mixed matrix approach for cross-species relative metabolite exposure comparison.

Takahashi, Drug Metab. Lett., 2017, 11, 21.

Implications of Species Coverage for MIST strategy

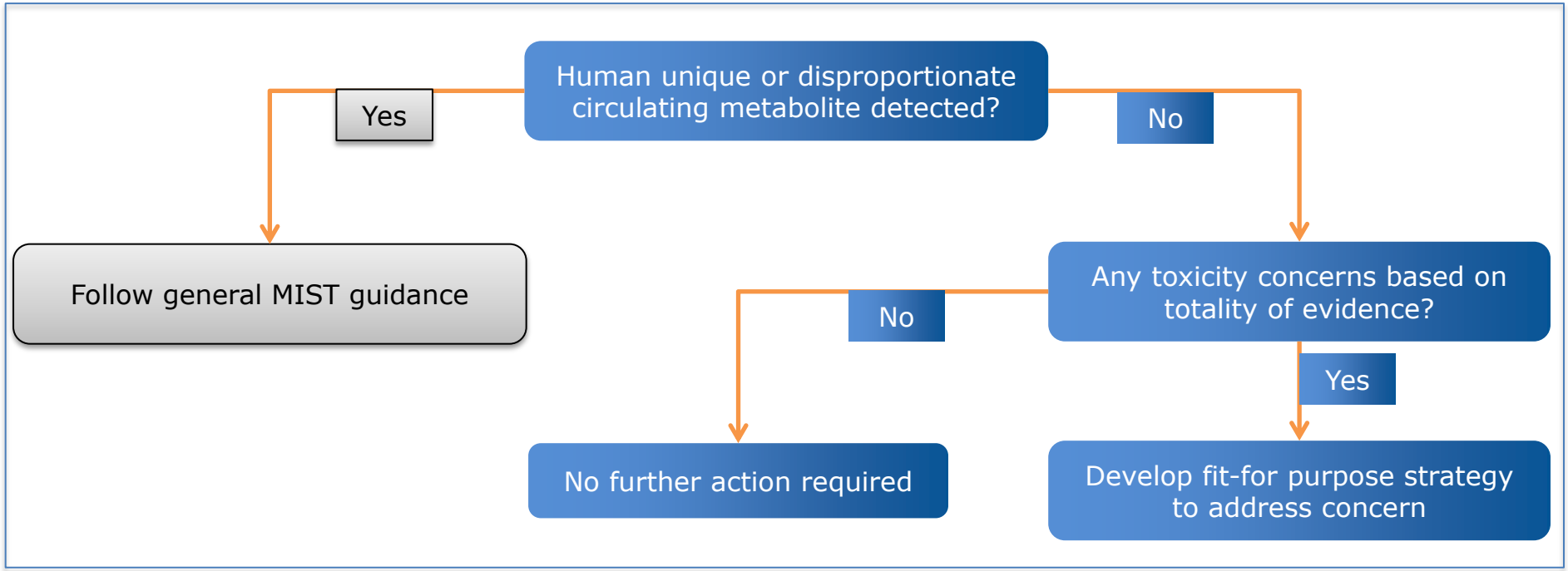
	Secondary Pharmacology	Geno-toxicity	Systemic Toxicity	Reproductive Toxicity(1)	Carcinogenicity Testing (2)
Rat induced S9 (in vitro)	☒	☑	☒	☒	☒
Rodent (in vivo)	☒	☑	☑	☑	☑
Non-Rodent (in vivo)	☒	☒	☑	☒	☒
Rabbit (in vivo)	☒	☒	☒	☑	☒

(1) When patient population include women of childbearing potential

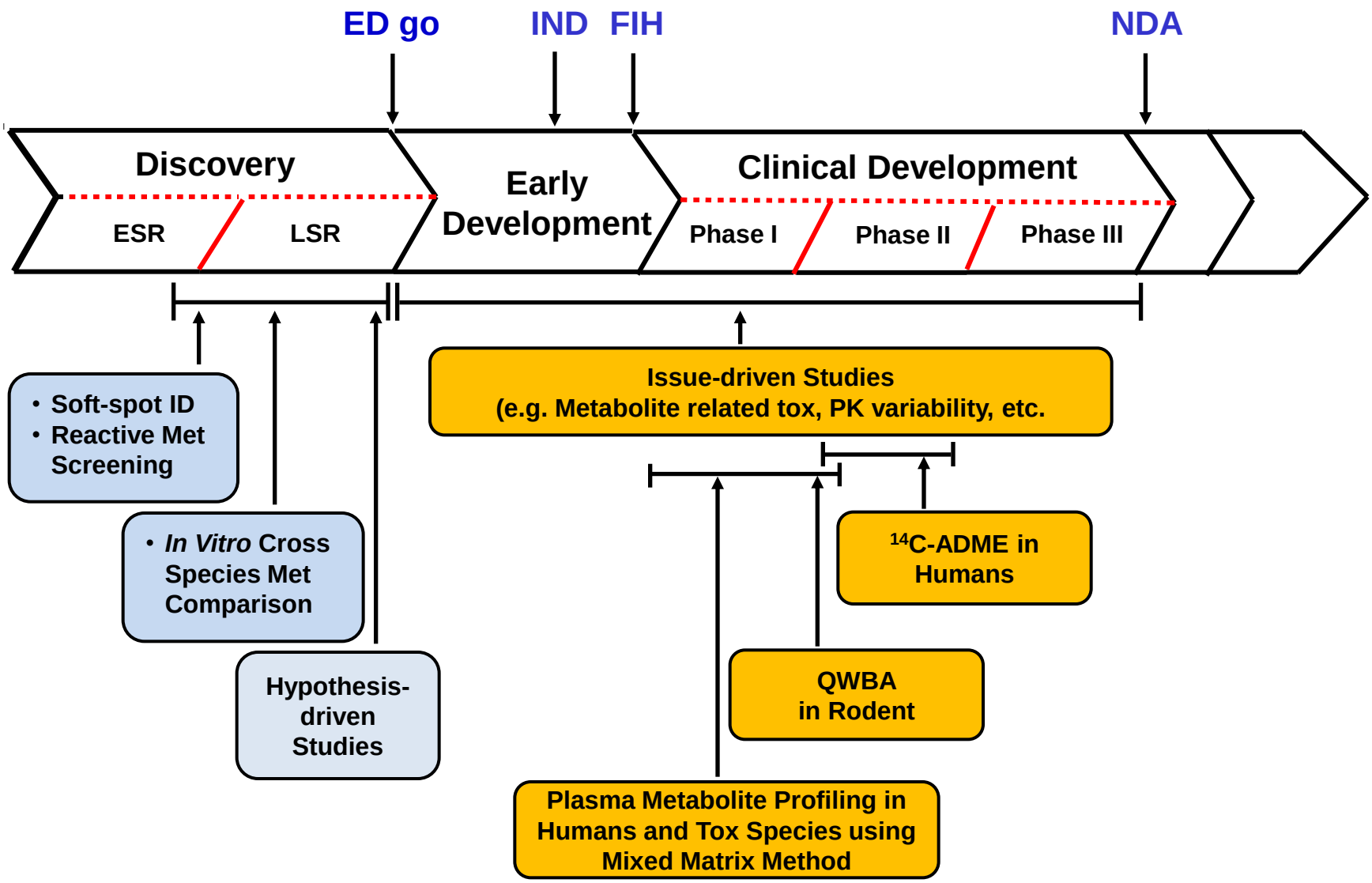
(2) When administered chronically (at least 6 month) or intermittent for chronic indication

Beyond MIST Assessment

There can be situations where (1) a circulating human metabolite may be less than 10% total in human or (2) where adequate coverage in nonclinical species can be demonstrated BUT there is still a concern based on metabolite structure or totality of safety evidence that require further nonclinical characterization on a case-by-case basis



Human First and Only Strategy?



Acknowledgements

- Genentech: Cyrus Khojasteh, Shuguang Ma, Ryan Takahashi, Jorg Blumel

Minireview

A Decade in the MIST: Learnings from Investigations of Drug Metabolites in Drug Development under the "Metabolites in Safety Testing" Regulatory Guidance

Simone Schadt, Bojan Bister, Swapan K. Chowdhury, Christoph Funk, Cornelis E. C. A. Hop, W. Griffith Humphreys, Fumihiko Igarashi, Alexander D. James, Mark Kagan, S. Cyrus Khojasteh, Angus N. R. Nedderman, Chandra Prakash, Frank Runge, Holger Scheible, Douglas K. Spracklin, Piet Swart, Susanna Tse, Josh Yuan, and R. Scott Obach

Schadt, Drug Metab. Dispos., 2018, 46, 865.