# Axcelead

## Drug Discovery Partners Inc.

創薬イノベーションアクセラレータを目指して
신약 개발의 혁신적인 서비스를 목표로
Solution providing for innovative drug discovery

**Strategies to determine the target human metabolites using *Radiolabeled compounds***
***- Example from nonclinical to clinical-***

**October 26, 2018**

**Drug Disposition & Analysis (DDA)**
**Fumihiro Jinno, Ph.D.**

KRCC KOREA RADIOCHEMICALS CENTER

SEKISUI





AXCELEAD Drug Discovery Partners

# Contents

- Evaluation of metabolite exposure in human: Why?
  - Toxicokinetics guideline, ICH S3A
  - ICH M3 (R2)、US-FDA MIST guidance
  - Metabolite issues making big impact for development schedule
- Examples for metabolite profiling studies using RI compounds
  - Case-1 Conventional human ADME study
  - Case-2 Microtracer human ADME study
  - ・Other information
  - Case-3 Nonclinical studies using RI compound for IND
- Strategy for metabolites exposure using RI compound
- Conclusion

ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
RI: radioisotope
RI compound: radiolabeled compound

# Evaluation of metabolite exposure in human: Why?

**Why?**

*Why is it necessary to evaluate drug exposure?*

3.2 Quantification of exposure
The quantification of systemic exposure provides an assessment of the burden on the test species and assists in the interpretation of similarities and differences in toxicity across species, dose groups and sexes. The exposure might be represented by plasma (serum or blood) concentrations or the AUCs of parent compound and/or metabolite(s).

**Ref: ICH S3A, NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES**

**Comparison of exposure between human and animals is important for assessment of the toxicity.**

# Evaluation of metabolite exposure in human: Why?

**Why?**

*Evaluation of metabolite exposure?*

**The important case: metabolite determination in toxicokinetics**

**(3.8 Determination of metabolites)**

- When the administered compound acts as a 'pro-drug' and the delivered metabolite is acknowledged to be the primary active entity.
- When the compound is metabolized to one or more pharmacologically or toxicologically active metabolites which could make a significant contribution to tissue/organ responses.
- When the administered compound is very extensively metabolized and the measurement of plasma or tissue concentrations of a major metabolite is the only practical means of estimating exposure following administration of the compound in toxicity studies

**Measurement of metabolite concentrations may be especially important when documentation of exposure to human metabolite(s) is needed in the non-clinical toxicity studies in order to demonstrate adequate toxicity testing of these metabolites. (Note 9)**

**Ref: ICH S3A, NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES**

*How about a guideline for assessment of human metabolite exposure?*

# ICH M3 (R2) Guideline

## GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS

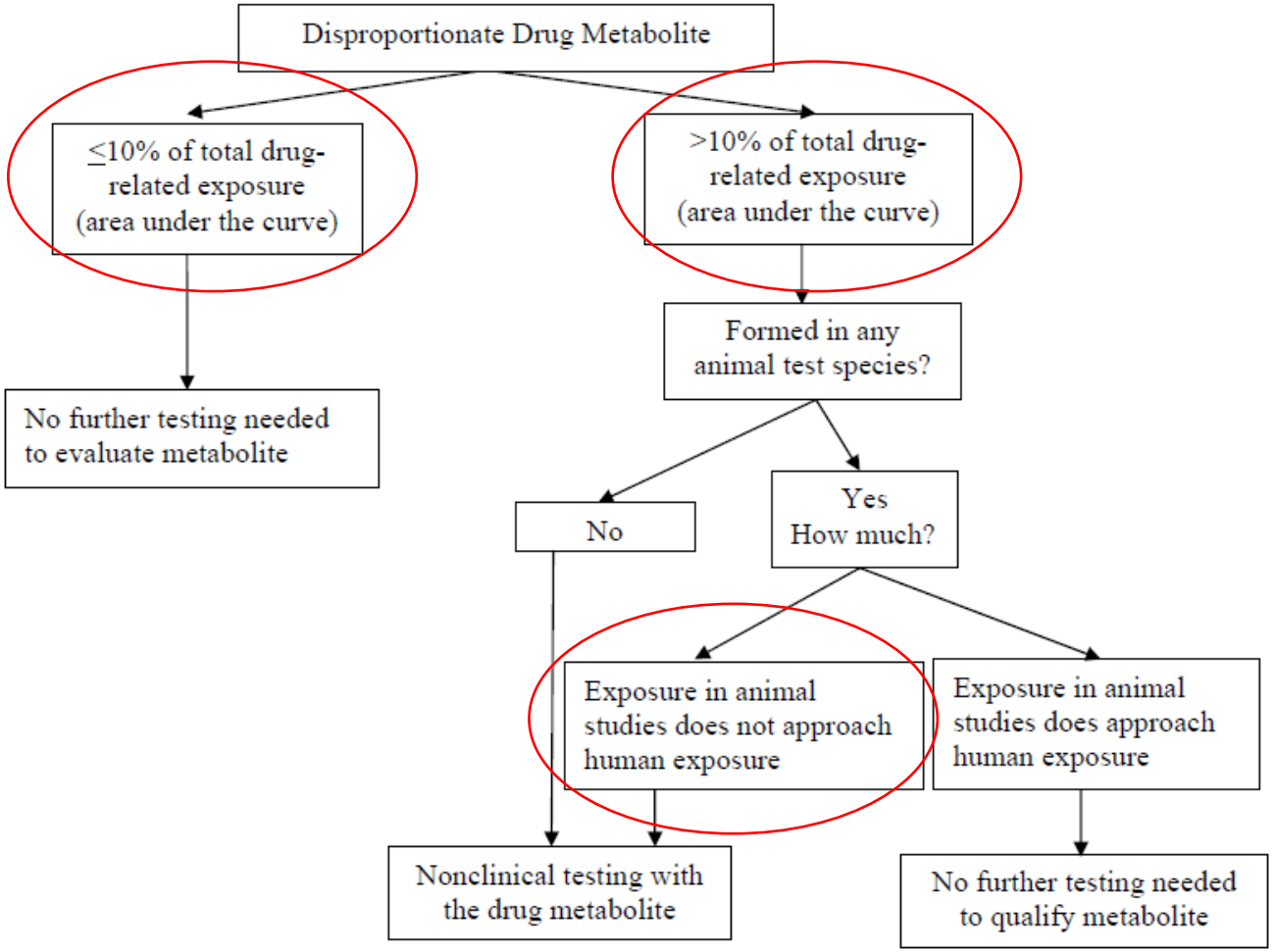
### 3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

.....

**Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Such studies should be conducted to support Phase III clinical trials. For drugs for which the daily administered dose is <10 mg, greater fractions of the drug related material might be more appropriate triggers for testing. Some metabolites are not of toxicological concern (e.g., most glutathione conjugates) and do not warrant testing. The nonclinical characterization of metabolites with an identified cause for concern (e.g., a unique human metabolite) should be considered on a case-by-case basis.**

# US-FDA MIST Guidance

## DECISION TREE FLOW DIAGRAM




(FDA Guidance for Industry :Safety Testing of Drug Metabolites, Revision 1, 2016)

# Metabolite issues making big impact for development schedule

**Cold (LC/MS etc)**

**SRD**
- Human unique or human disproportionate metabolite
- Active metabolite

**MRD**
- Higher level exposure in human plasma at steady state in therapeutic dose compared to NOAEL in tox species

***Hot*** ***(Radioisotope)*** → **human mass balance study**
- Reactive metabolite
- Newly detected human unique or human disproportionate metabolite

**GLP Tox studies for metabolites are required**

**API preparation**
**0.5-1 year**

**over 13 weeks**
**GLP Tox studies**

***moreover***

- ✓ **contribution to pharmacological activity**
- ✓ **possibility of DDI**

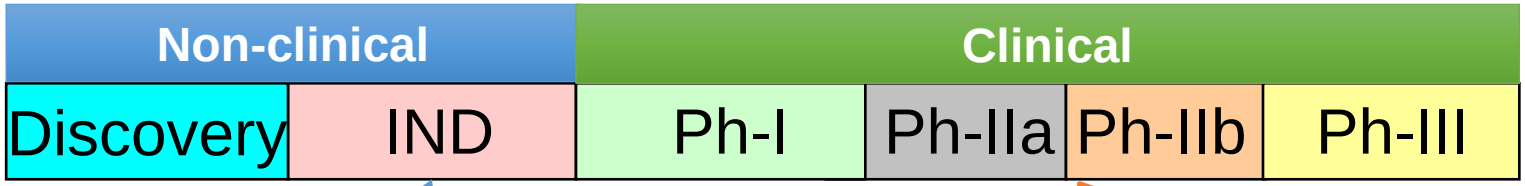
**Enormous delay of development schedule over 1.5 to 2 years**





AXCELEAD Drug Discovery Partners

# Examples for metabolite studies using RI compounds




① **Metabolite profiles in a conventional human ADME study**

② **Metabolite profiles in microtracer human ADME studies with tiny amount of RI compounds for exploratory early phase-I studies**

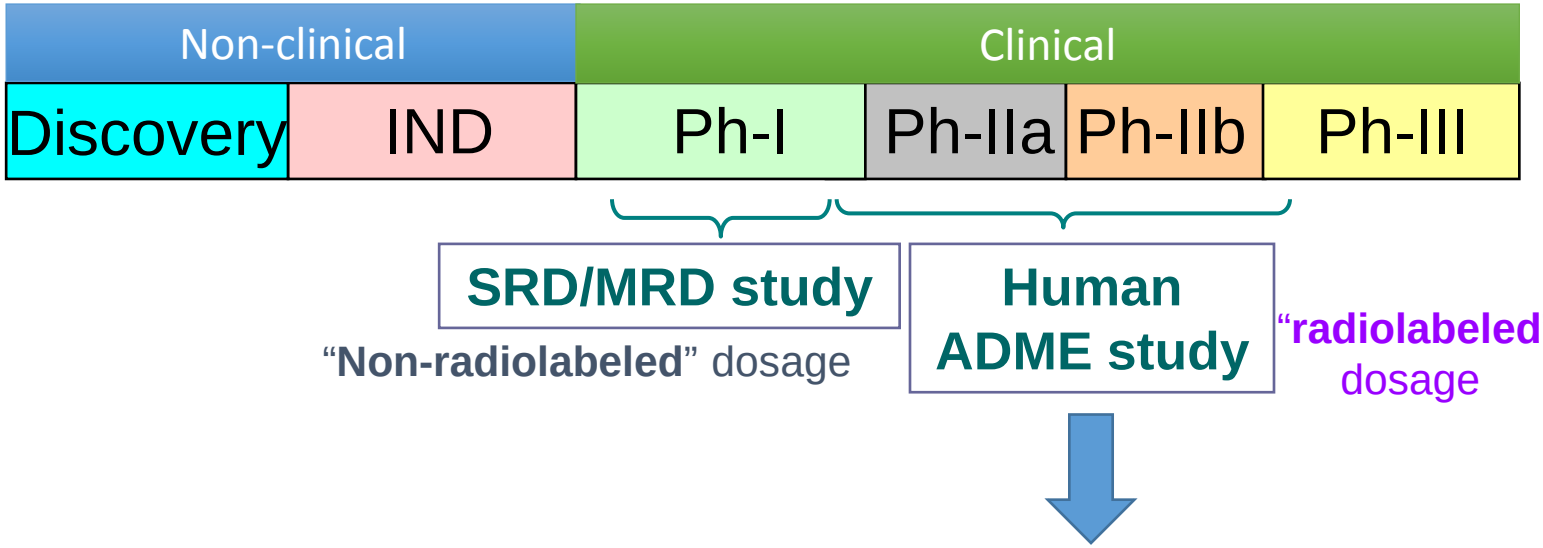
③ **Nonclinical studies using RI compound:**
**A case avoided delay of development schedule by evaluation of exposure using RI compound**





AXCELEAD Drug Discovery Partners

# Case-1: Human mass balance study using RI compound




**Information from a human mass balance study**

- ✓mass balance [adsorption,(distribution), metabolism, excretion] and route of elimination
- ✓metabolite identification (plasma, excreta)
- ✓clearance mechanisms
- ✓exposure of parent compound and its metabolites
- ✓detection of a covalent adduct and a reactive metabolites
- ✓support validated the animal species used for toxicological studies
- ✓metabolites contribution to the pharmacological / toxicological effects.

*possibility of DDI*

*significant caution for patients such as renal impaired*

**Benefit for NDA**

- ✓answer for inquiries from a regulatory authority with minimum time and resource



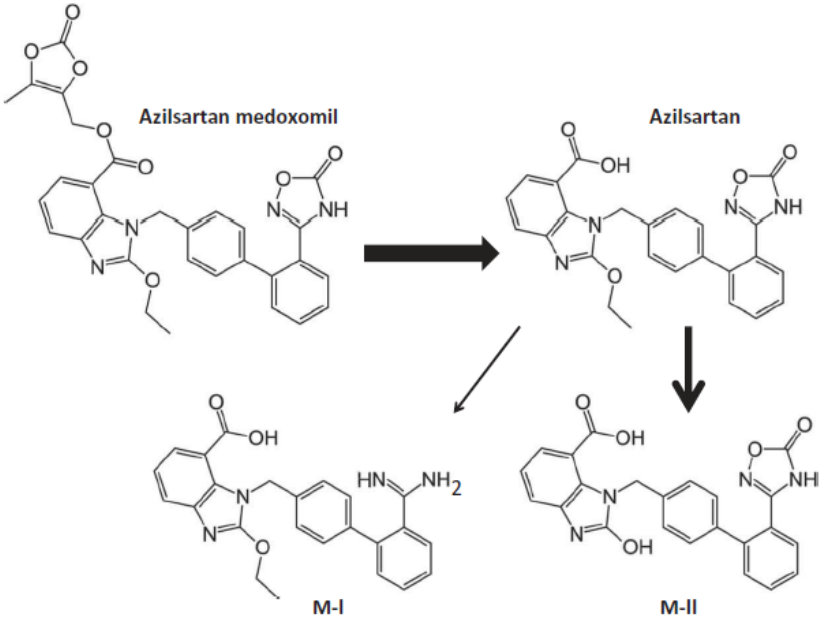


AXCELEAD Drug Discovery Partners

# Case-1: Example for human ADME study -azilsartan medoxomil-

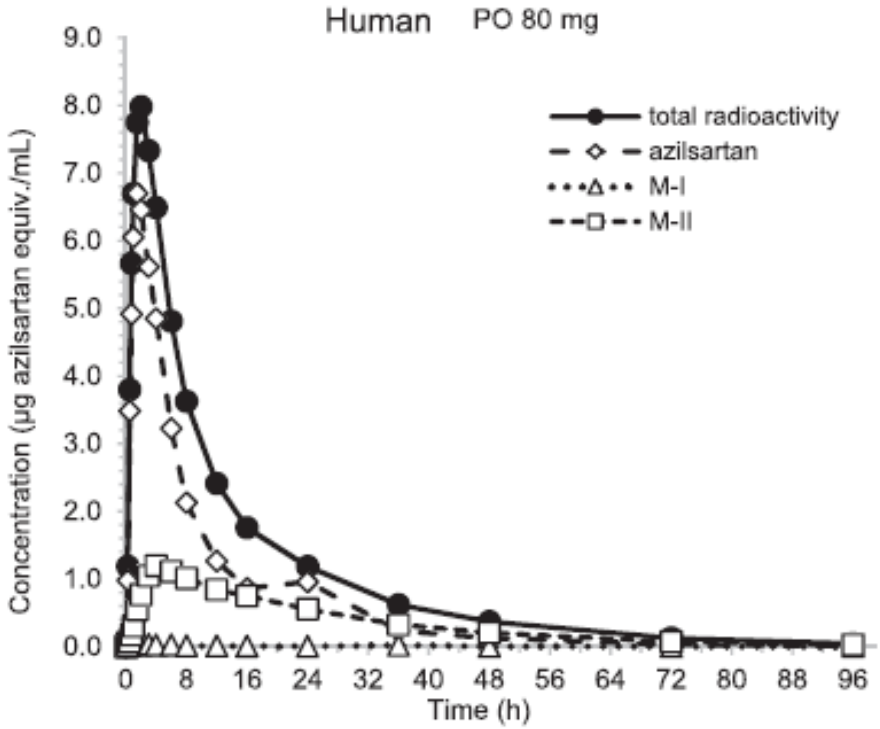
A single-center, open-label, absorption, distribution, metabolism, and excretion study of a single oral dose of [$^{14}$C]azilsartan medoxomil, containing a target dose of azilsartan medoxomil 80 mg with 100 μCi of radioactivity, administered to 8 healthy male subjects aged 18 to 55 years, inclusive.

Structures of azilsartan medoxomil prodrug, azilsartan, and two primary metabolites (M-I and M-II)




Ref: EXPERT OPINION ON DRUG METABOLISM & TOXICOLOGY, 2017 VOL. 13, NO. 9, 897–900

Mean plasma concentrations of total radioactivity, azilsartan, M-I, and M-II after oral administration of [$^{14}$C] azilsartan medoxomil suspension in healthy male subjects (n = 8).




Ref: Drug Metab Dispos 46:865–878, June 2018





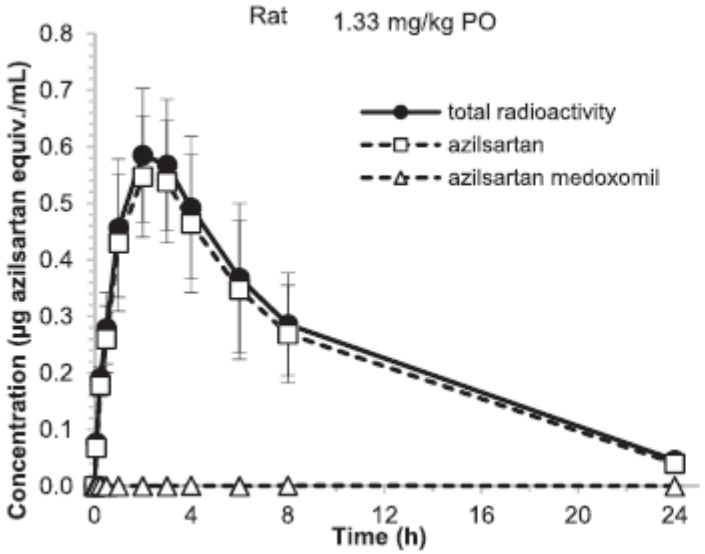
AXCELEAD Drug Discovery Partners

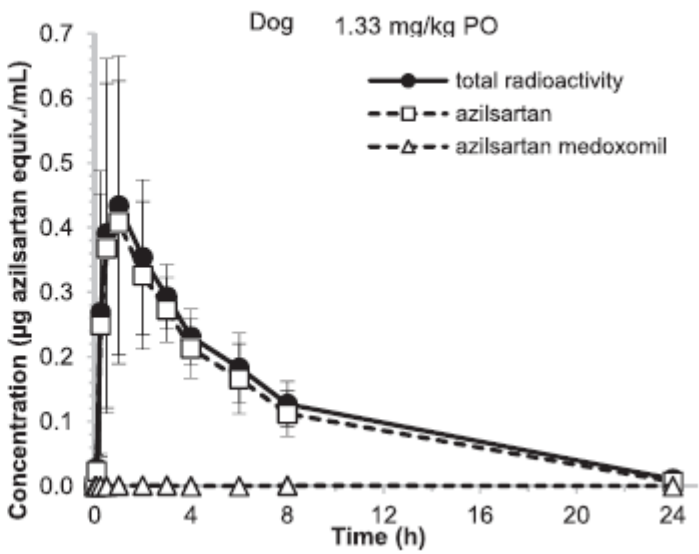
# Plasma exposure of the disproportional metabolite M-II in rats, dogs and humans

| Species | Dose | AUC (0–24 h) ng-h/ml[a] |
|---|---|---|
| Rat (male) | 20 mg/kg per day | 424 |
| Rat (female) | 200 mg/kg per day | 1762 |
| Dog (male) | 60 mg/kg per day | 704 |
| Dog (female) | 12 mg/kg per day | 188 |
| Human | 80 mg | 22,793 |

**Exposure of M-II in tox species could not approach that in human**

[a]NOAEL doses for rats and dogs and the highest prescribed dose in the human.




Ref: Drug Metab Dispos 46:865–878, June 2018





AXCELEAD Drug Discovery Partners

# Case-1: Example for human ADME study
## -azilsartan medoxomil-

***Human disproportionate metabolite, M-II, was detected as over 10% of total drug related metabolite. The exposure of M-II in tox species could not approach exposure of M-II in human.***

**Tox studies with M-II were required.**

**Preparation of GLP bulk of M-II**

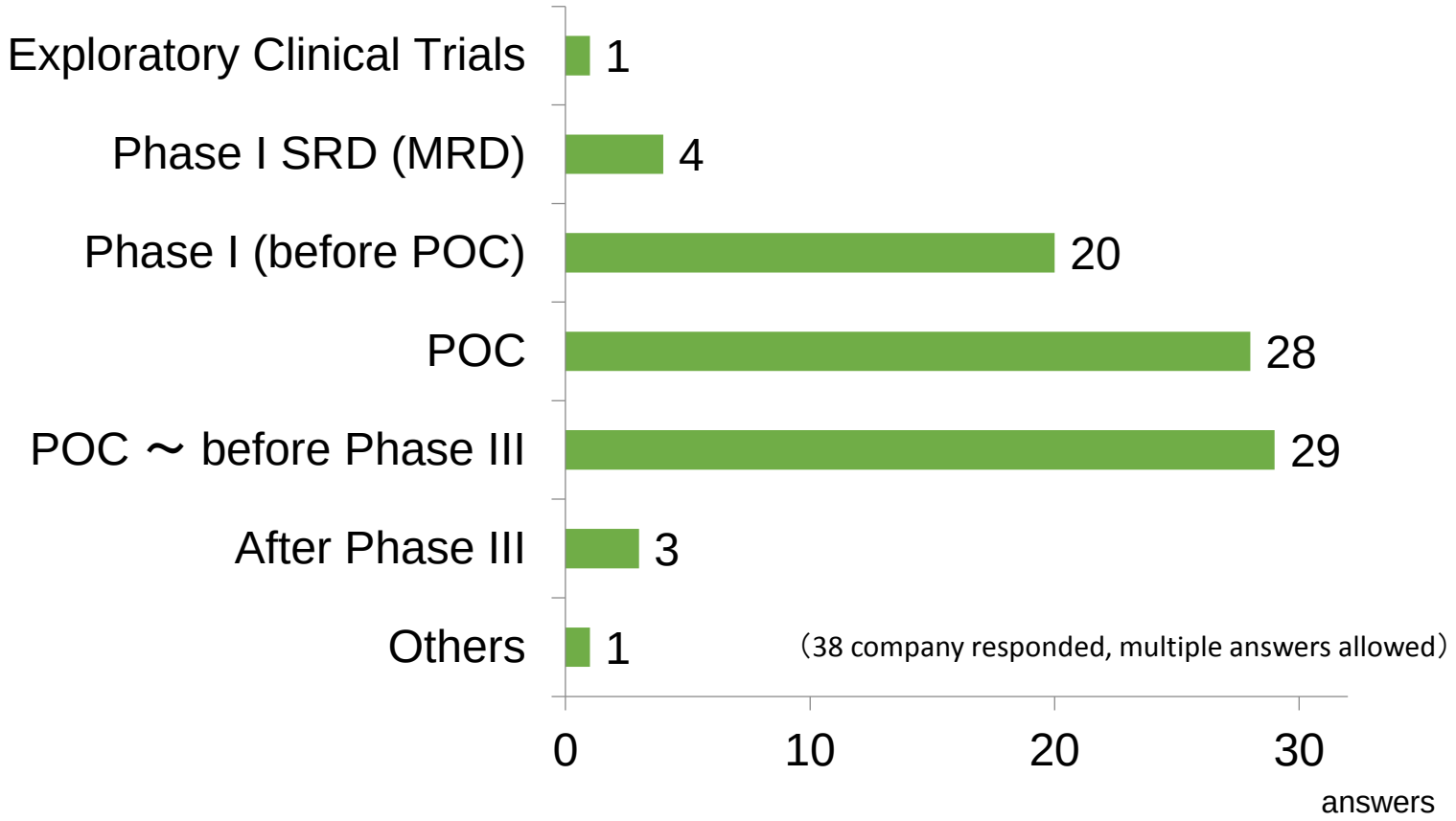
**Tox studies dosing M-II**

26week Tg.rasH2 mouse, 2-year rat studies, Ames reverse mutation assay, Chinese hamster ovary cell forward mutation assay, mouse lympoma gene assay, in vivo mouse and/or rat bone marrow micronucleus assay

Ref: Drug Metab Dispos 46:865–878, June 2018

**Unidentified metabolites which were highly exposure in human much affect the development schedule and NDA plan.**
**→ It's recommended to conduct human ADME study as early as possible.**

# Timing to be conducted a human ADME study




*Jpn J Clin Pharmacol Ther*：2015; 46(6): 265-272
Survey from pharmaceutical companies affiliated with Japan Pharmaceutical Manufactures Association in 2013





AXCELEAD Drug Discovery Partners

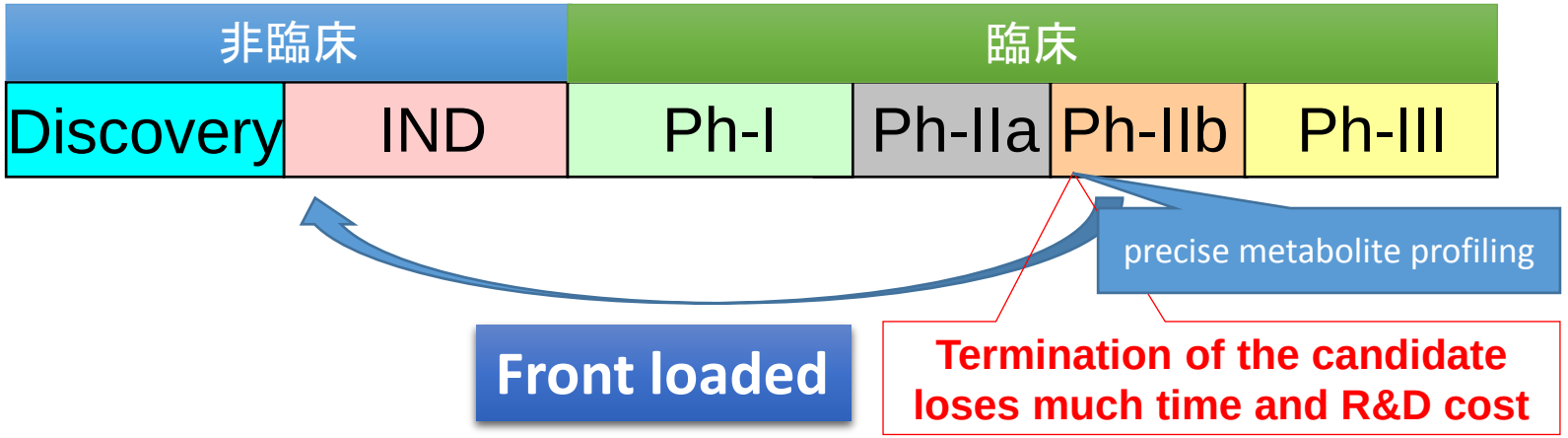
# FDA MIST Guidance encourages a human ADME study as early as possible

We encourage the identification of any differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process. The discovery of disproportionate drug metabolites late in drug development can potentially cause development and marketing delays.

Human ADME study is essential to confirm comprehensive and definitive answer about metabolites exposure in human.

**Early implementation of a human ADME study using RI compound is very important for strategy toward NDA.**

# Case-2: Microtracer human ADME study




**+ human ADME with microtracer and AMS analysis**

**Microtracer human ADME**
- ✓Approx. **100 nCi / human**
- ✓To conduct as **non-RI compound**
- ✓The possible to conduct **earlier** than a conventional human ADME study
- ✓Requirement of **AMS analysis**
- ✓**Regulatory requirement** of the RI compound (as GMP?)

**Conventional human ADME**
- ✓Maximum radioactivity **100 µCi /human**
- ✓To conduct as **RI compound**
- ✓Necessity of **permission** of the human ADME study and nonclinical ADME study using RI compound for **the dosimetry**
- ✓Requirement of **time** for permission and preparation of the study

- ☐ Nonclinical ADME studies using RI compound are conducted in parallel.
- ☐ Simple comparison of the metabolite profiling between human and animals can be made by the same analytical method.



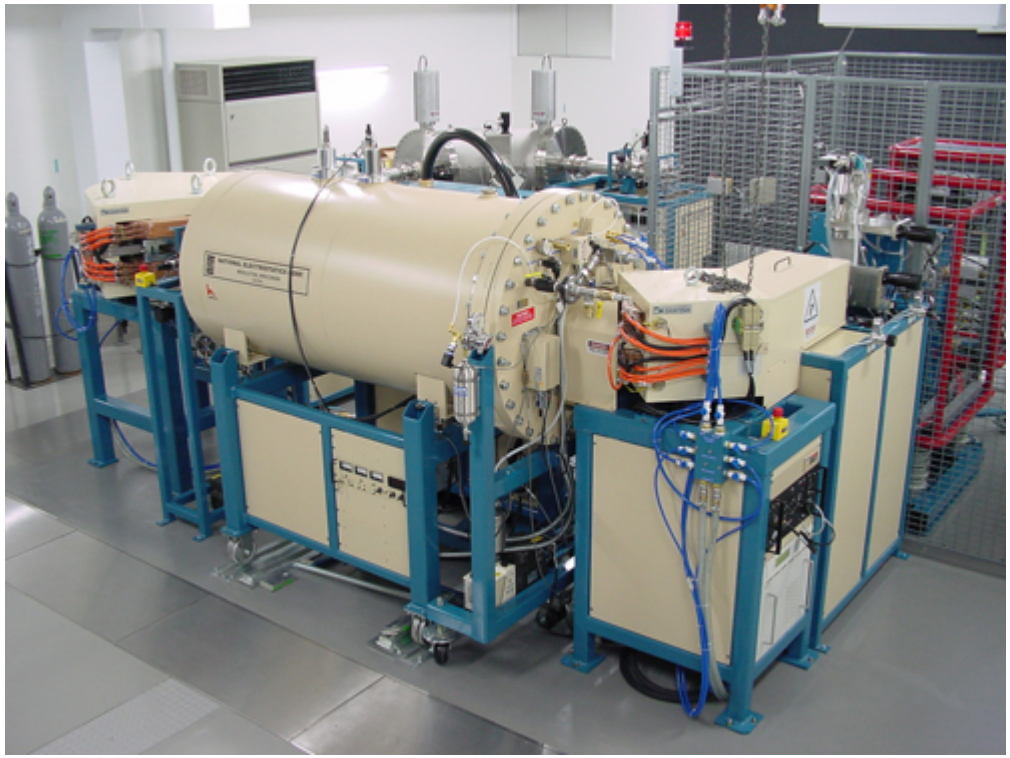


AXCELEAD Drug Discovery Partners

# Ultrasensitive analysis of an *in vivo* radioactive compound using AMS

AMS has allowed the measurement of radioactive compound in plasma, feces, tissue, etc. at low level, which conventional liquid scintillation counters cannot achieve. It is an especially useful analysis method in human pharmacokinetic studies on microdosing.

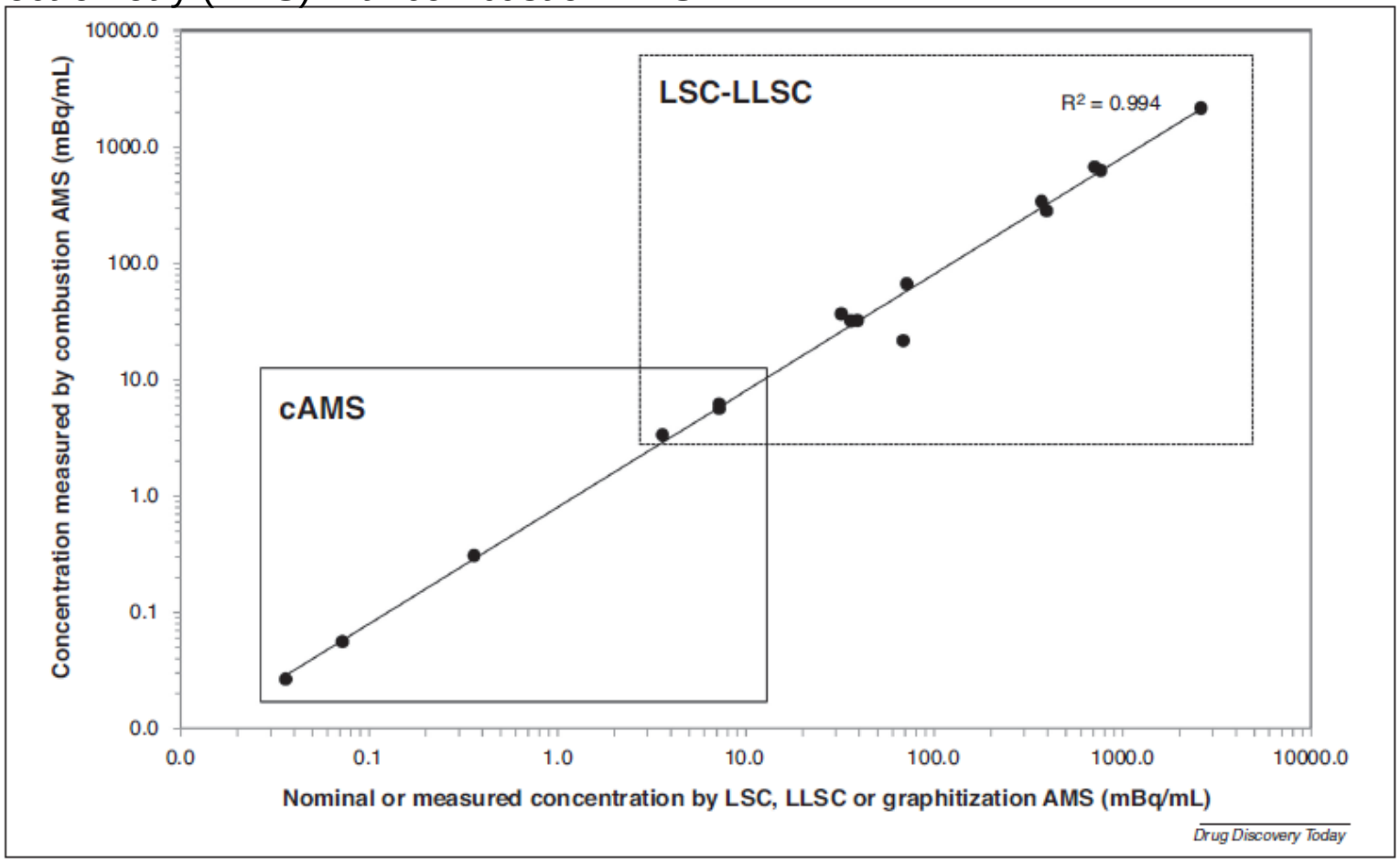
*Accelerator Mass Spectrometry



https://www.sekisuimedical.jp/english/business/adme_tox/business/drug/invivo.html

# Sensitivity by AMS and LSC analysis

A head-to-head comparison of measured $^{14}C$ concentrations in plasma by liquid scintillation counters (LSC), low-level scintillation counting (LLSC), and graphitization accelerator mass spectrometry (AMS) with combustion AMS.




Ref: Swart P. *et al*. Drug Discov Today 2016 Jun; 21(6)873





AXCELEAD Drug Discovery Partners

# Clinical study design

Compound: Compound A and Compound B

Timing: Exploratory early phase I trial

Dose level: 60 mg including ≤250 nCi of [$^{14}$C]Compound A

100 mg including ≤250 nCi of [$^{14}$C]Compound B

Plasma samples (AUC proportional†): 1, 2, 4, 6 and 8 hour postdose (N=6)

†Hamilton method

**Analytical methods**

- ✓ Protein precipitation
- ✓ HPLC fraction collection with AMS analysis

| Recovery (%) | Compound A | Compound B |
|---|---|---|
| Through extraction | 94.5 | 99.3 |
| Through HPLC | 102.4 | 111.1 |

# Case-2: Microtracer human ADME study

Results: Microtracer human ADME studies for Compound A and B:

- ✓ A human disproportionate metabolite observed at greater than 10% of total drug-related exposure was newly detected in each compound.
- ✓ The exposure of the metabolites at NOAEL in tox species were lower than those in human.

As the results, the development of the candidates were terminated, although GLP bulk syntheses were considered for additional tox studies

NOAEL: non observed adverse effect level

***Beneficial Information by front loaded***

**Human ADME with microtracer and AMS analysis**

- ✓ **Comprehensive metabolite profile in human at earlier stage**
- ✓ **Confirmation of exposure of the metabolites at pharmacological active dose**
- ✓ **Selection of a candidate that has better pharmacokinetics profile for development**

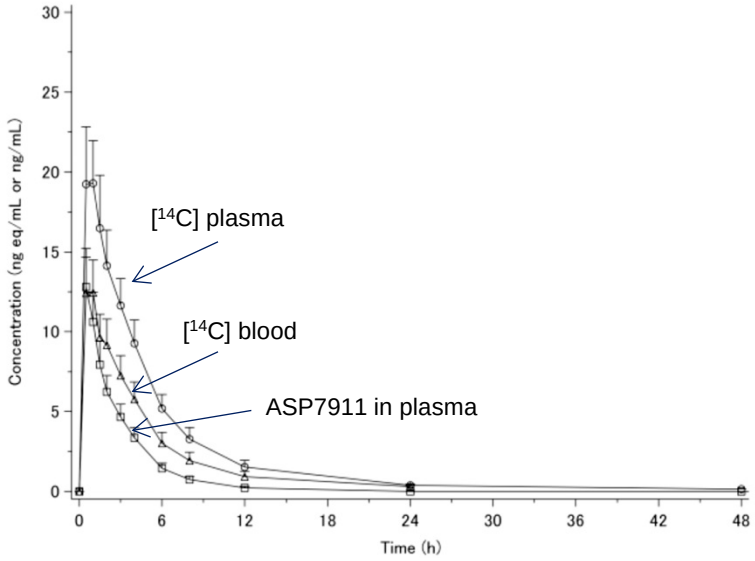
- ➢ **Development can be proceeded with no concern about exposure of human metabolites**
- ➢ **Tox studies can be conducted more efficient as planned.**

***Effective development plan and reduction of risk for NDA***

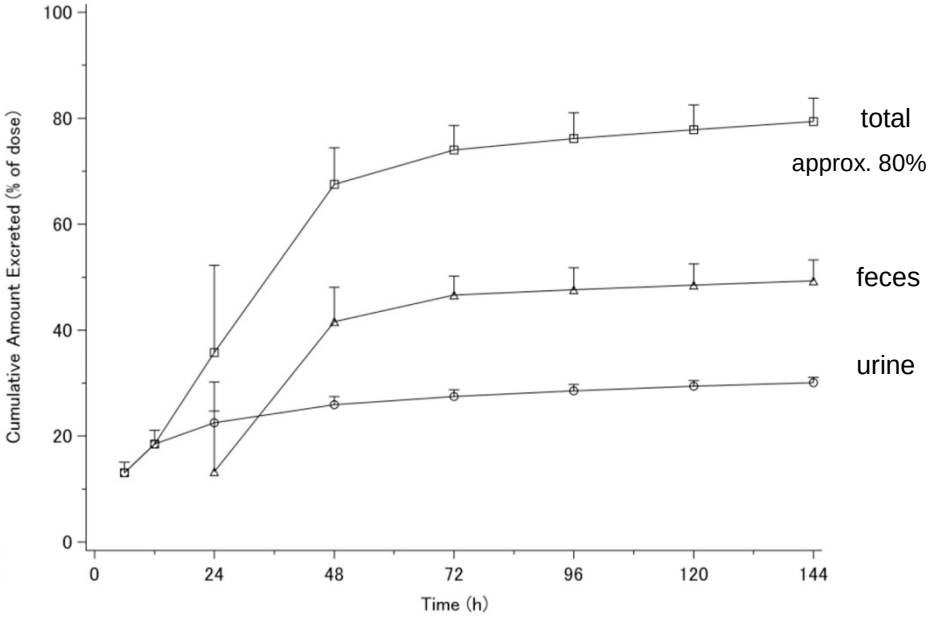
# (Information) Microtracer human ADME study @ Japan

| | |
|---|---|
| Design: | open-label study |
| Subjects: | 6 Japanese healthy adult male |
| Compound: | [$^{14}$C]ASP7991 |
| Dose: | 1mg-500nCi/man |
| Route: | oral |

Concentration-time profiles of radioactivity in plasma and blood, and ASP7991 in plasma. Circle: [$^{14}$C] in plasma, triangle: [$^{14}$C] in blood, square: ASP7991 in plasma.




Cumulative excretion-time profiles of radioactivity. Circle: urine excretion, triangle: feces excretion, square: total recovery.




Ref: Miyatake D, *et al*, Drug Metabolism and Pharmacokinetics 33 (2018) 118-124

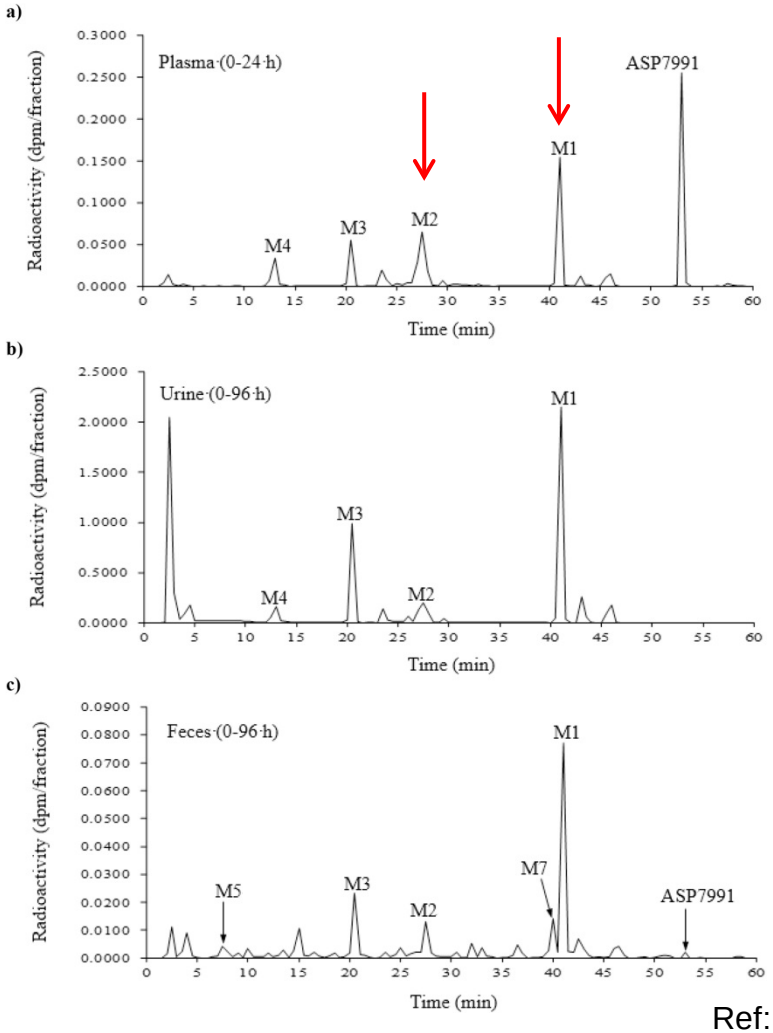




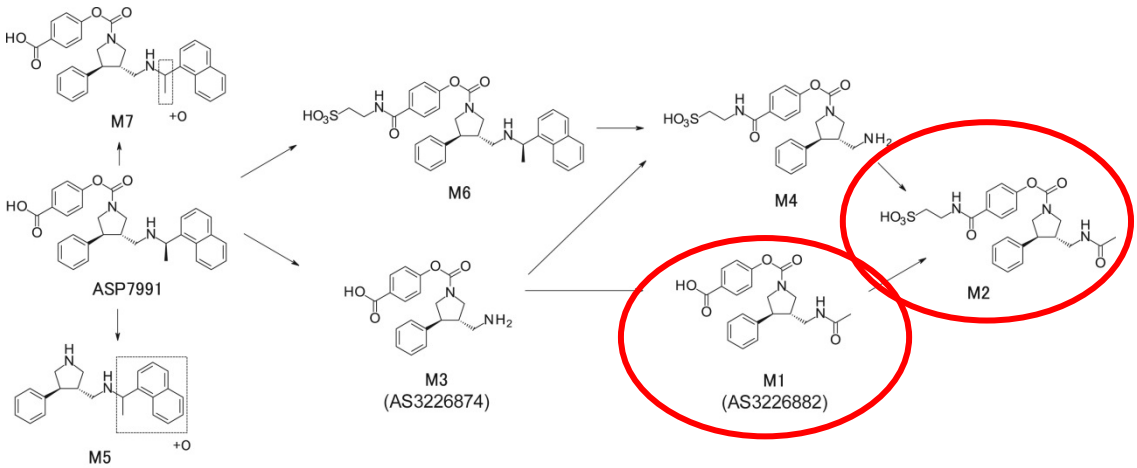
AXCELEAD Drug Discovery Partners

# (Information) Microtracer human ADME study @ Japan

HPLC-radiochromatograms of ASP7991 and its metabolites in biological samples after a single oral administration of [$^{14}$C]ASP7991 to humans. (a) Plasma (0–24 h), (b) urine (0–96 h), (c) feces (0–96 h).




Postulated metabolic pathways of ASP7991 in humans




M1, and M2 were more than 10% of the total drug related exposure in human plasma

Ref: Miyatake D, *et al*, Drug Metabolism and Pharmacokinetics 33 (2018) 118-124





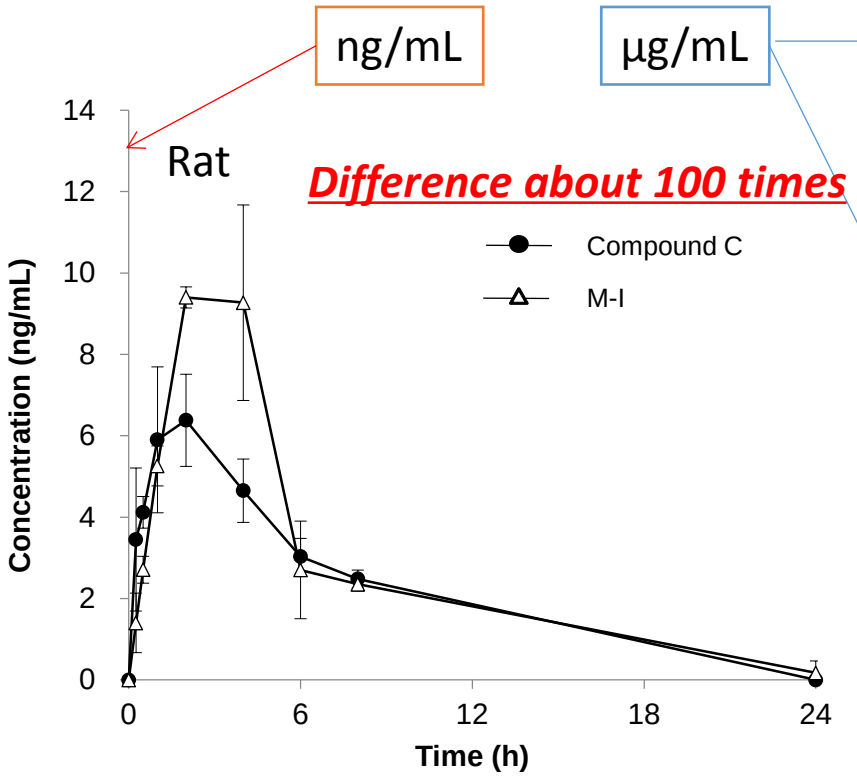
AXCELEAD Drug Discovery Partners

# Case-3: Nonclinical studies using RI compound for IND

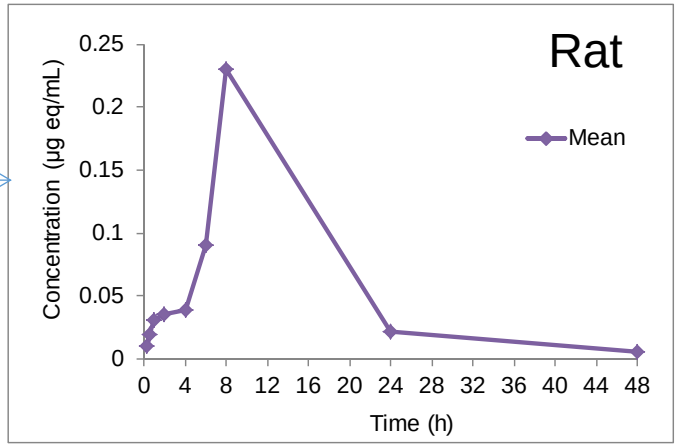
Compound C

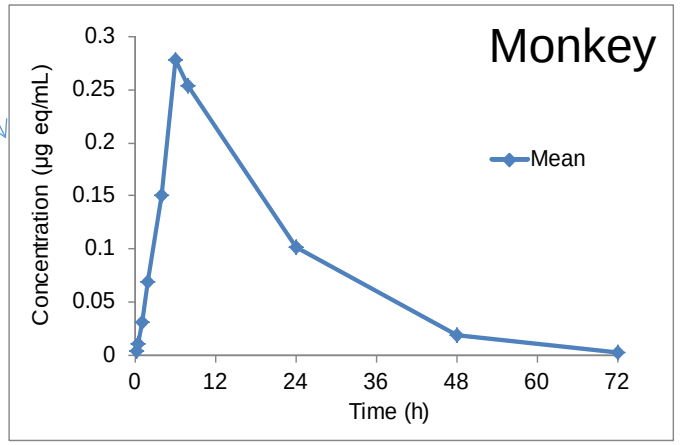
## PK profile: *Cold* vs *Hot*

PK profile of unchanged compound and M-I in rats plasma by LC/MS analysis (n=3)




Radioactivities in plasma of rats and monkeys after administration of the radiolabeled compound（mean of n=2）








AXCELEAD Drug Discovery Partners

# Results：Metabolite profile using RI compound

- ✓ PK-C-1 was hardly detected using non-RI compound, but it was newly detected in samples using the RI compound.
- ✓ PK-C-1 was detected as the major metabolite in *in vivo* samples, but it was not detected in *in vitro* samples.
- ✓ Exposure of PK-C-1 was much higher than the unchanged compound in *in vivo* animal samples.
  - The dose level of the studies using RI compound were pharmacological active dose.

**Evaluation of exporsure of PK-C-1 in nonclinical safety study is required at non observed adverse effect level (NOAEL)**

- ➢ Identification of chemical structure for PK-C-1
- ➢ Synthesis of the reference standard of PK-C-1 and its internal standard
- ➢ Validation studies for TK methods of PK-C-1 for GLP-Tox studies
- ➢ Evaluation of PK-C-1 exposure at NOAEL in non-clinical safety studies

**Concern about the schedule of IND submission!**

# Strategy for IND

## Determination of the exposure of the metabolite at NOAEL using the RI compound

- ✓ To evaluate exposure of PK-C-1 in nonclinical tox studies, ***RI*** compound C were administered to rats at a NOAEL level.
- ✓ IND submission was made using the exposure (AUC) of PK-C-1, and other results of tox studies.

Quantification by radioactivity: no reference standard and no bioanalytical method were necessary.

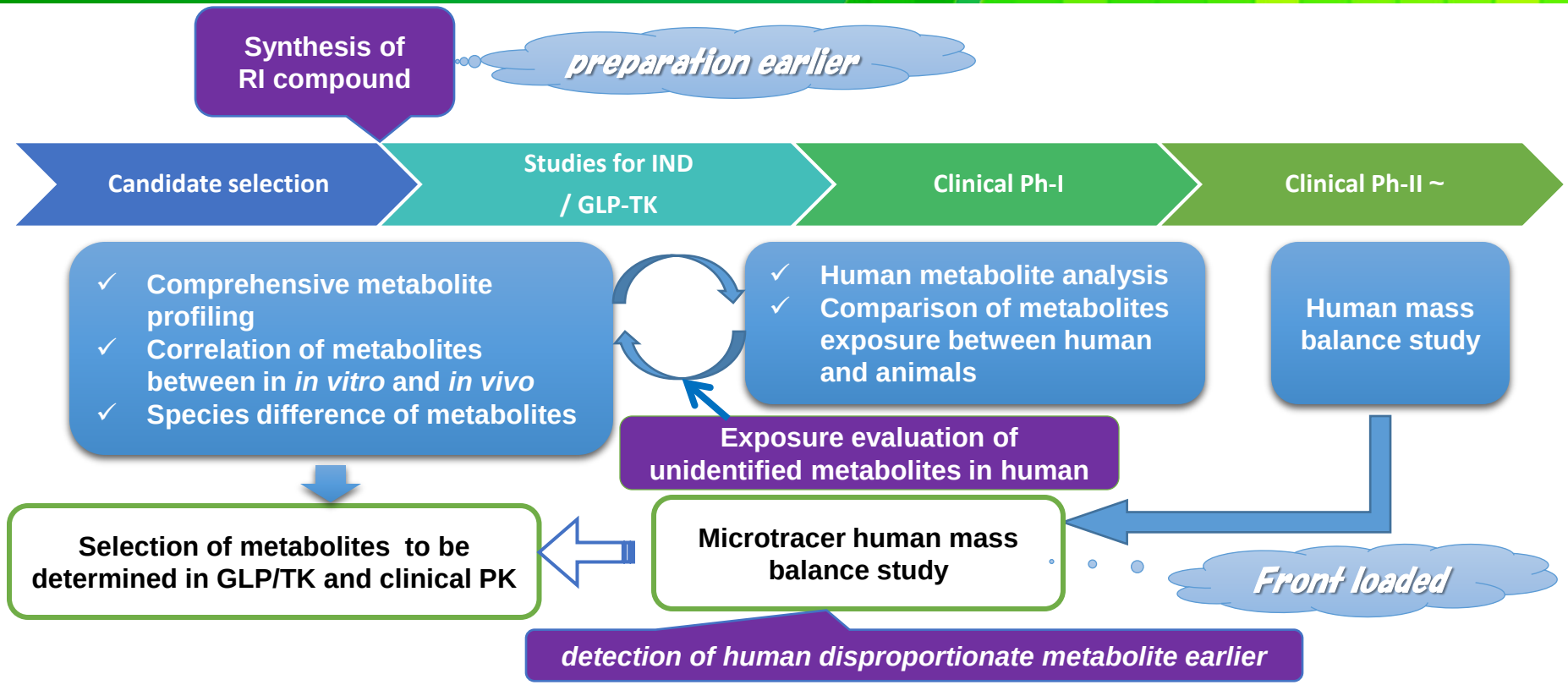
**This was a case that delay of IND submission was avoided by determination of the metabolite exposure using RI compound.**

Subsequently, in parallel with IND submission

- ■ Synthesis of reference standard of PK-C-1
- ■ Determination of PK-C-1 in GLP TK studies

→ Comparison of exposure of PK-C-1 between human and animals were made during phase-I

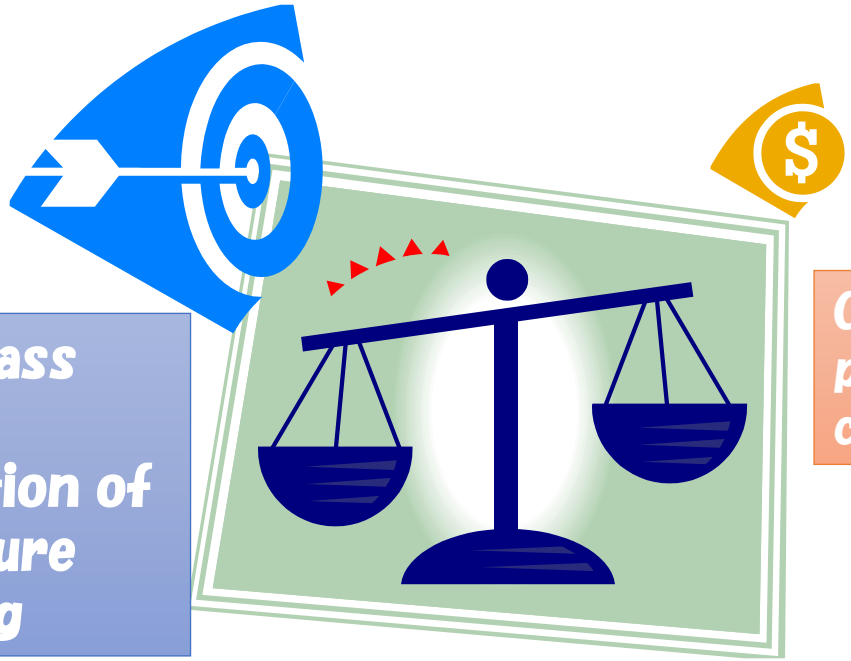
# Strategy for metabolites exposure using *RI compound*




- ***Strategy from nonclinical to clinical :***
  - ✓ Evaluation of metabolite exposure in nonclinical ADME studies using RI compound before or around IND contributes making effective strategy for evaluation of metabolites exposure in human.
- ***Human mass balance study at earlier stage :***
  - ✓ Human ADME study using RI compound should be conducted as early as possible so that the risk for development of a candidate can be reduced by obtaining comprehensive and definitive information of metabolite exposure in human.
- ***Lean strategy for selection of target metabolite for NDA :***
  - ✓ Information from mass balance study using RI compound can elicit efficient study plan of clinical and nonclinical about evaluation of metabolite exposure for NDA.

# Conclusion: Strategy for evaluation of metabolite exposure in human

- ☐Strategy from non-clinical to clinical
- ☐Human mass balance study at earlier stage
- ☐Lean strategy regarding metabolite targeting for NDA



**Information from mass balance study**
- ✓ Effective evaluation of metabolite exposure
- ✓ strategy planning

**Cost and Time for preparation of RI compound**





AXCELEAD Drug Discovery Partners

# 경청 해 주셔서 감사합니다

Thank you for your attention.


KRCC
KOREA
RADIOCHEMICALS
CENTER

SEKISUI