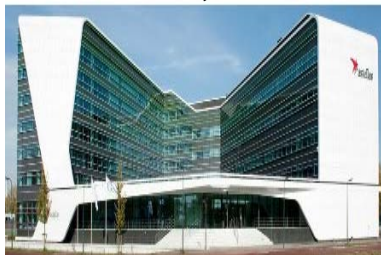


What I Learned from Drug Discovery ADME Research.

–Challenges to Aldehyde Oxidase Metabolism in Humans–

Leiden, NL



Tsukuba

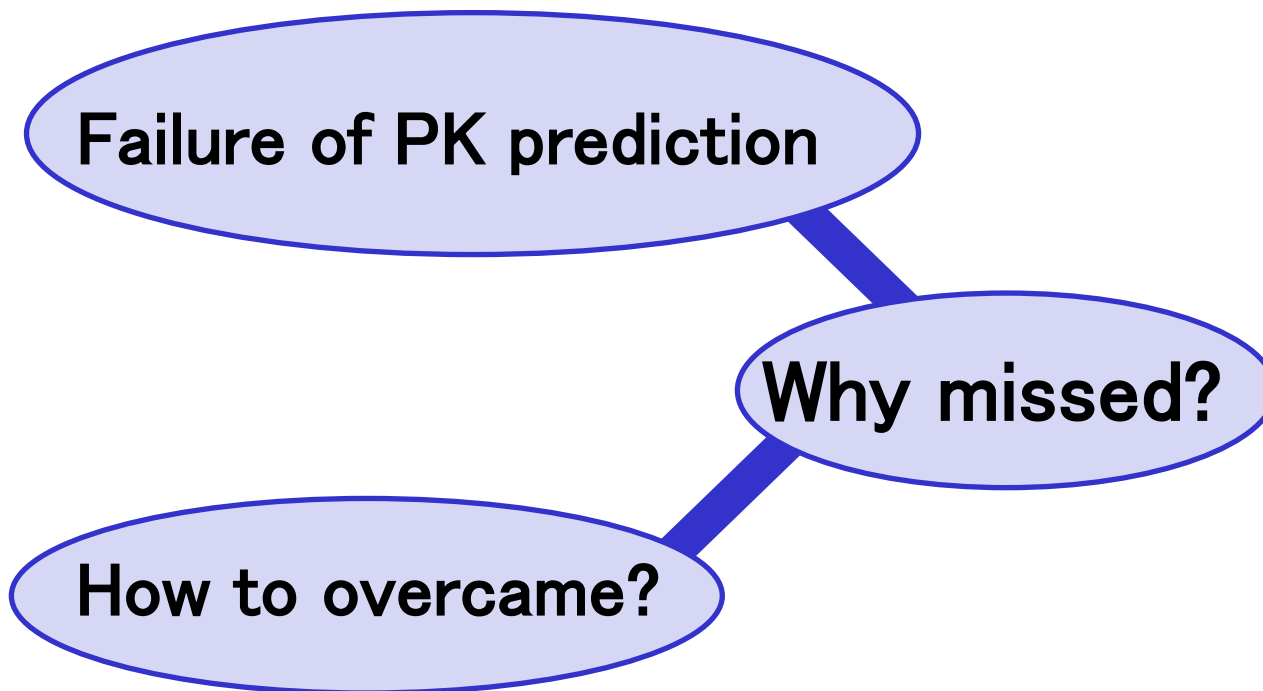


Skokie, IL USA



Takafumi AKABANE, PhD

Astellas Pharma Inc.



It began with a big mistake
それは失敗から始まった

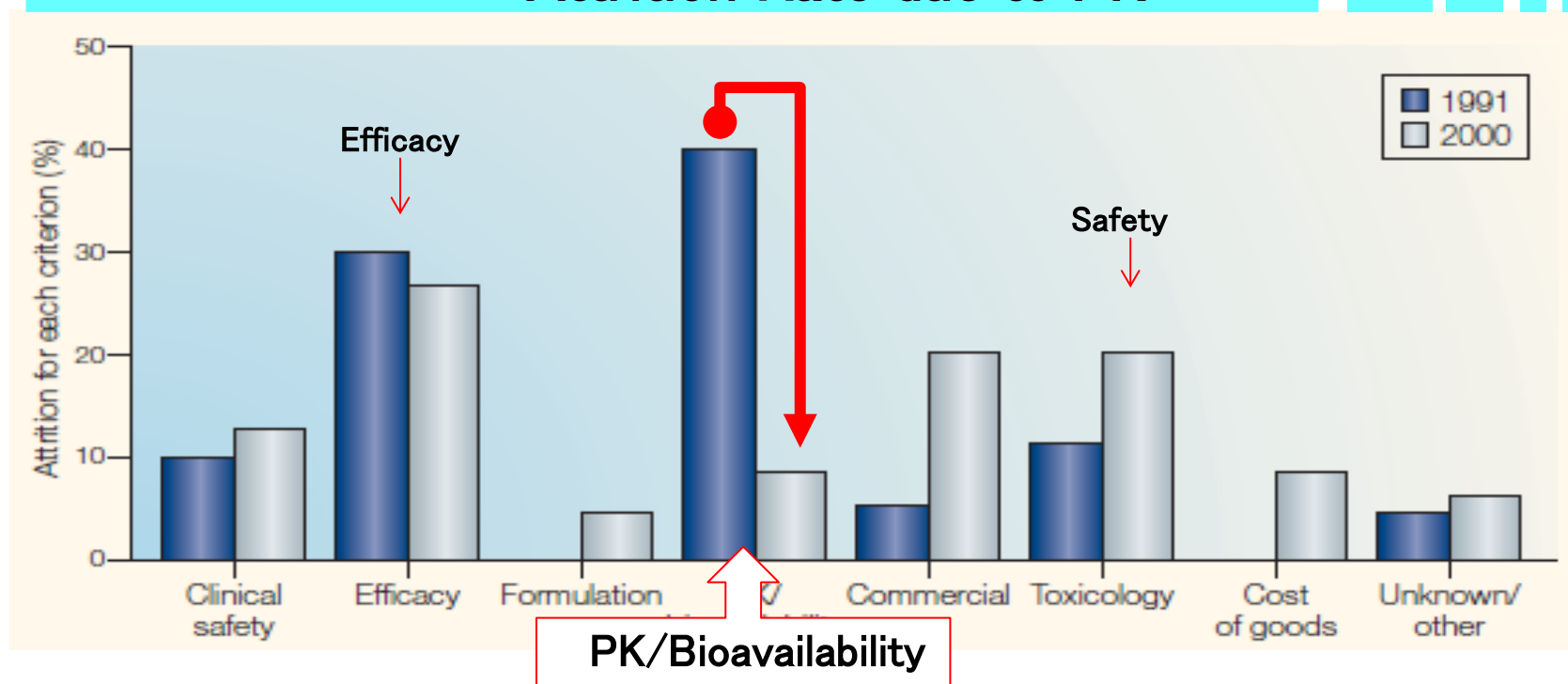
Learn from Ancient Wisdom

先人の知恵から学ぶ

Discovery ADME research in 2000' s

Past ——— 1990 ——— 2000 ——— 2010 ——— Future

Attrition Rate due to PK



Nat. Rev. Drug Discov. (2004): 3 (8) 711–716.

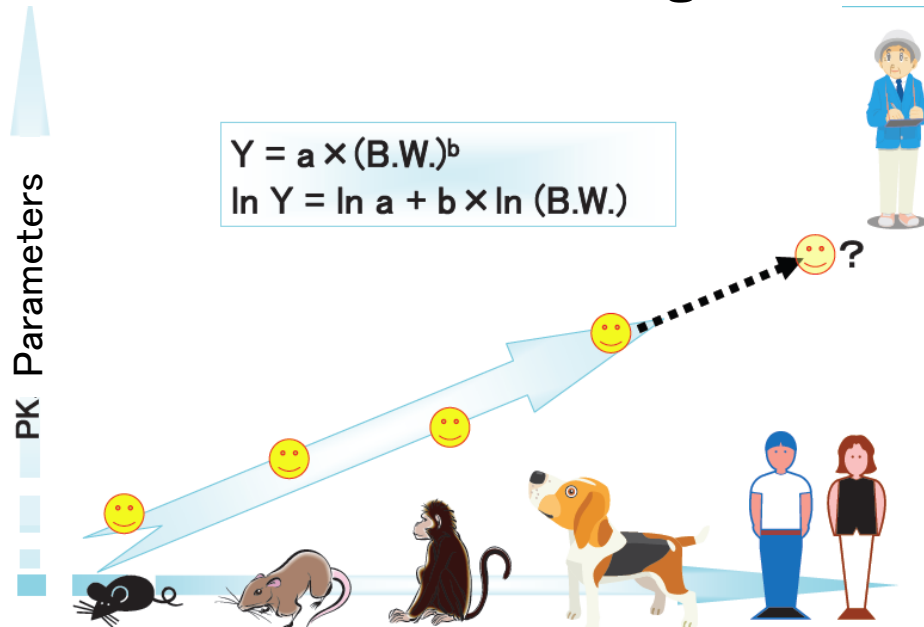
Overcome fears of P1 failure by fist-pass metabolism

What I learned from Ancient Wisdom

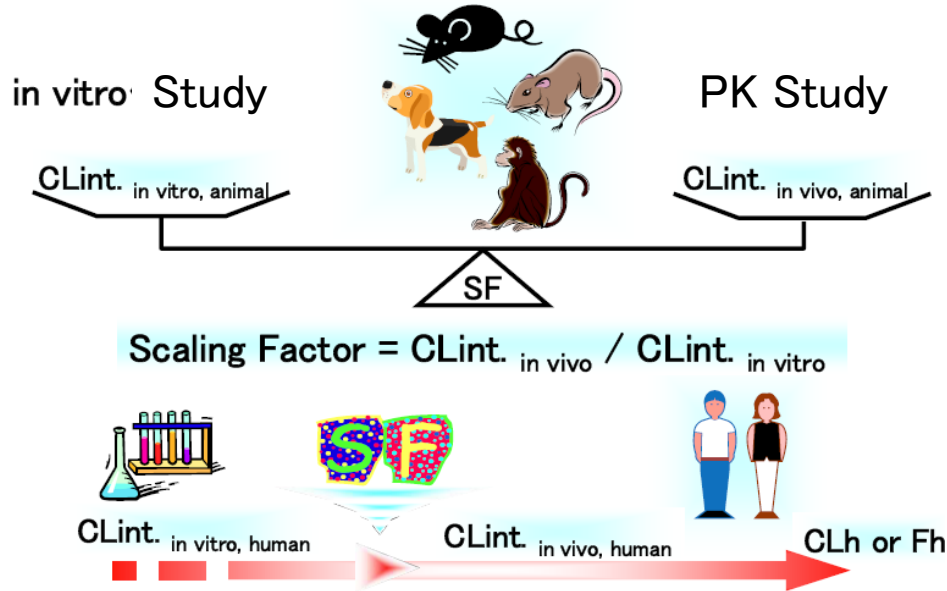
Allometric Scaling

$$Y = a \times (B.W.)^b$$

$$\ln Y = \ln a + b \times \ln (B.W.)$$



In vivo-in vitro extrapolation



In vivo animal PK + In vitro human liver extracts

**Development of human PK prediction method
(based on CYP metabolism in the liver)**

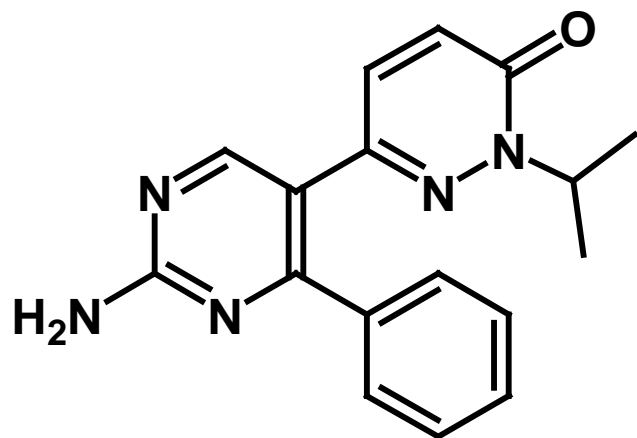
Learn from Failures

失敗から学ぶ

Failure of human PK prediction in FK3453

Mechanism ; Adenosine A1/2 dual inhibitor

Indication ; Parkinson's disease



| | |
|-------------------|--------------|
| MW; | 307 |
| Log D7.4; | 1.54 |
| Solubility (pH1); | >100 ug/mL |
| Solubility (pH6); | 10-100 ug/mL |
| Permeability; | Good |

Favorable physicochemical profile

Preclinical PK Profiles of FK3453

| | In vitro | | In vivo | |
|--------------|----------------------|--|-----------------|-----------|
| | Microsomal Stability | | CLt (mL/min/kg) | BA (%) |
| | CLint (mL/min/kg) | | CLt (mL/min/kg) | BA (%) |
| Rat | 42.3 | | 10.8–14.9 | 30.5–41.9 |
| Dog | 14.5 | | 5.0 | 87.1 |
| Human | 1.1 | | (<1*) | (>97*) |



Stable to CYP metabolism



Low Clearance

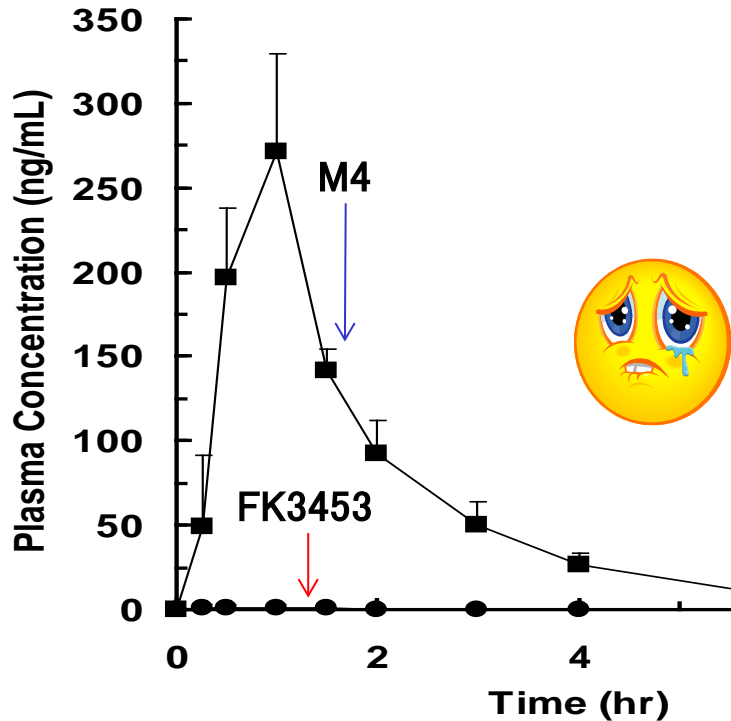


Good BA

*Predicted values by IVIVE

Favorable PK in humans was expected.

Plasma Concentration Profile of FK3453 in Humans



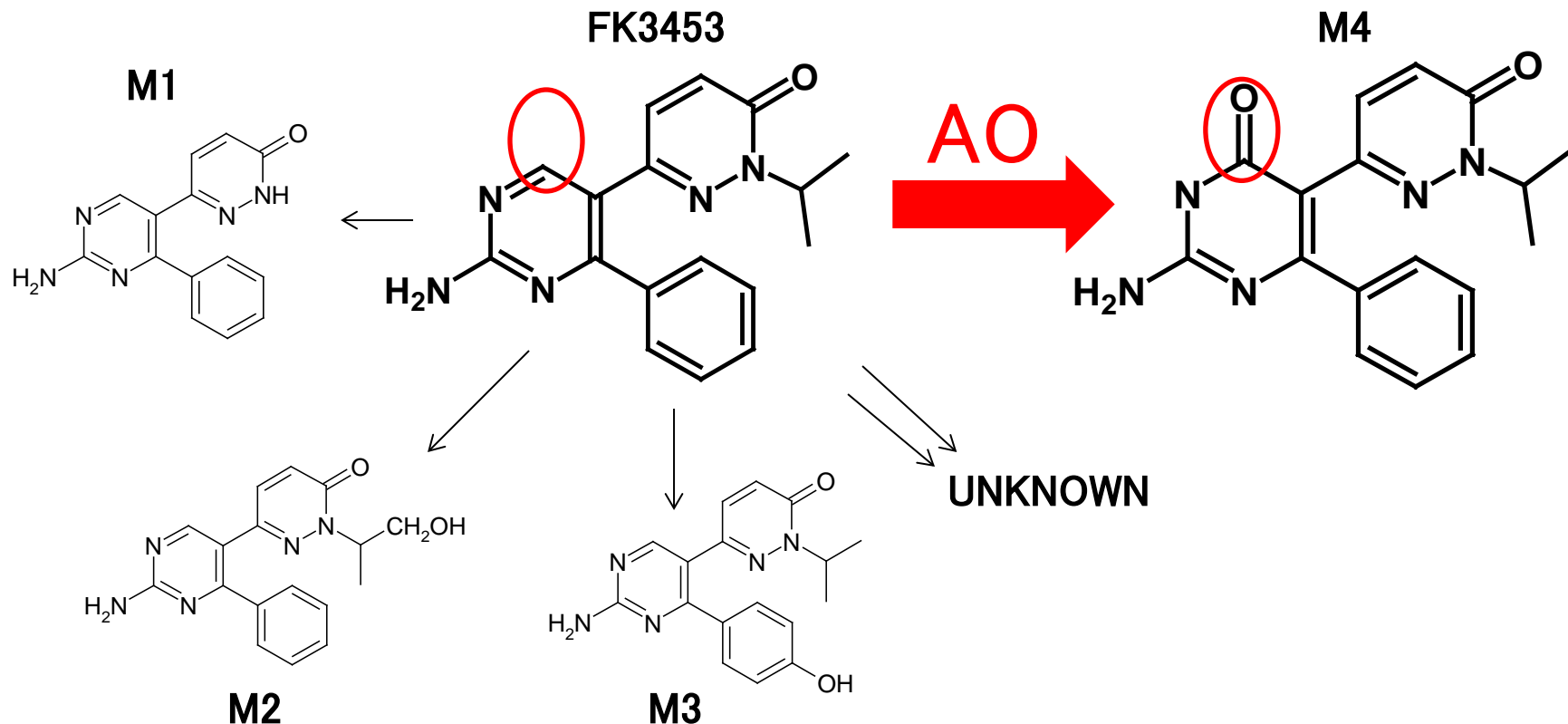
PK Parameters after administration at 10 mg/head

| Compound | Cmax (ng/mL) | AUC (ng·hr/mL) |
|----------|--------------|----------------|
| ● FK3453 | 1.5 ± 2.1 | 2.2 ± 3.1 |
| ■ M4 | 275.9 ± 54.6 | 477.5 ± 39.0 |

(Mean ± SD, n=6)

Akabane et al, Xenobiotica. 2011; 41(5):372-84

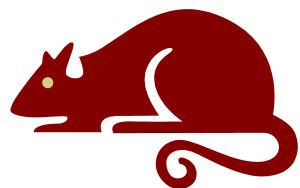
Suspended P1 trail due to extremely low PK.



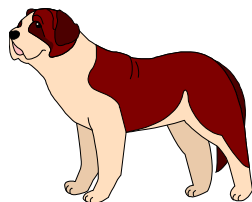
Aldehyde Oxidase

is responsible for FK3453 metabolism in humans.

What I learned from Failures

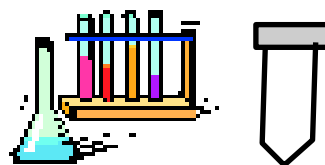


Large variations
in AO activity



Deficiency of
AO expression

Human liver cytosol

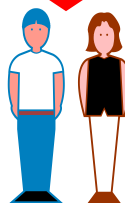


Low AO activity.

Conventional method



Conventional methods do not predict AO
metabolism in human.



Consider Non-CYP metabolism as well as CYP

Learn from Challenges & Experiences

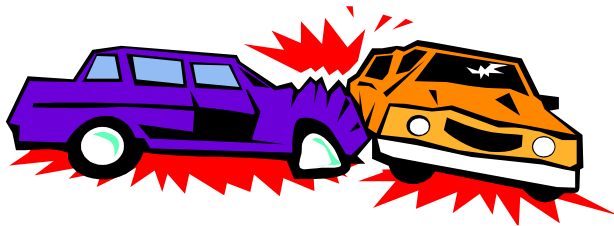
挑戦と経験から学ぶ

Develop novel approach to overcome under-estimation risk for AO metabolism

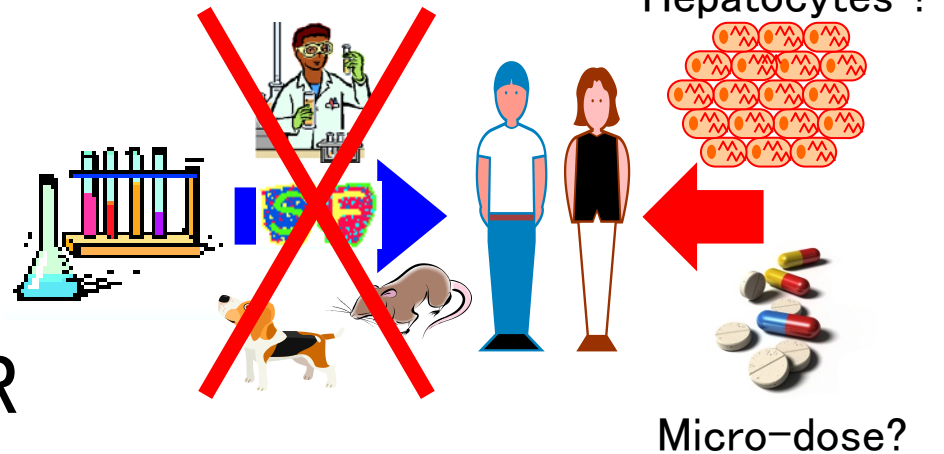
Stand at a crossroad...(1/2)

If compound is metabolized by AO,

Accident?



New Ideas?



OR

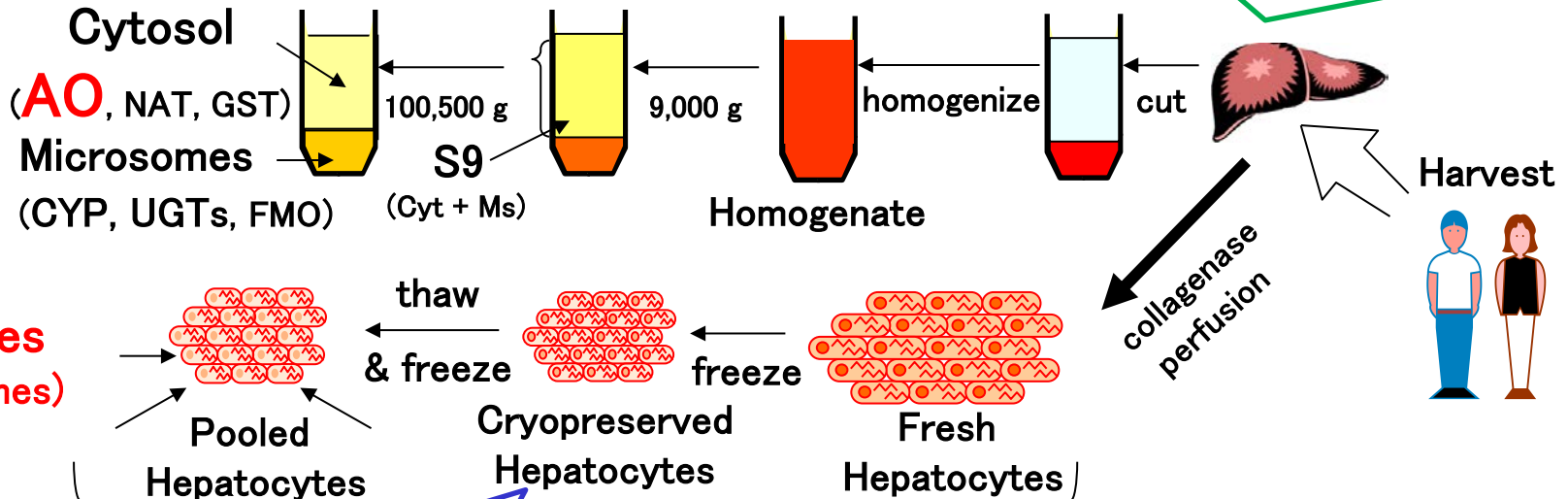
Challenge: Establish novel approach

Stand at a crossroad... (2/2)

Cytosol?
Hepatocytes?

More often?
(Improve supply situation)

Liver
Extracts

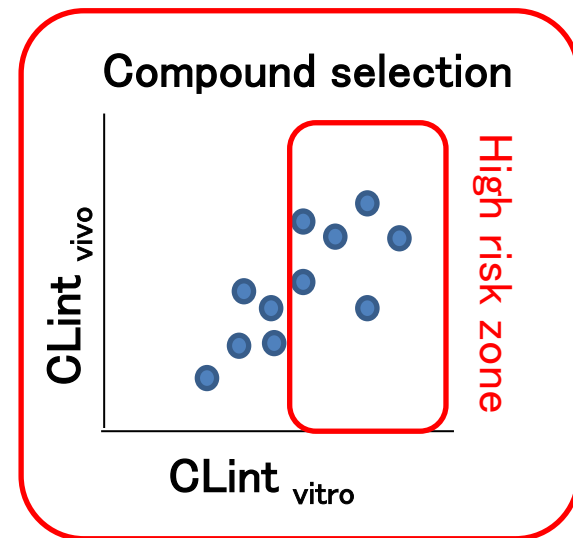
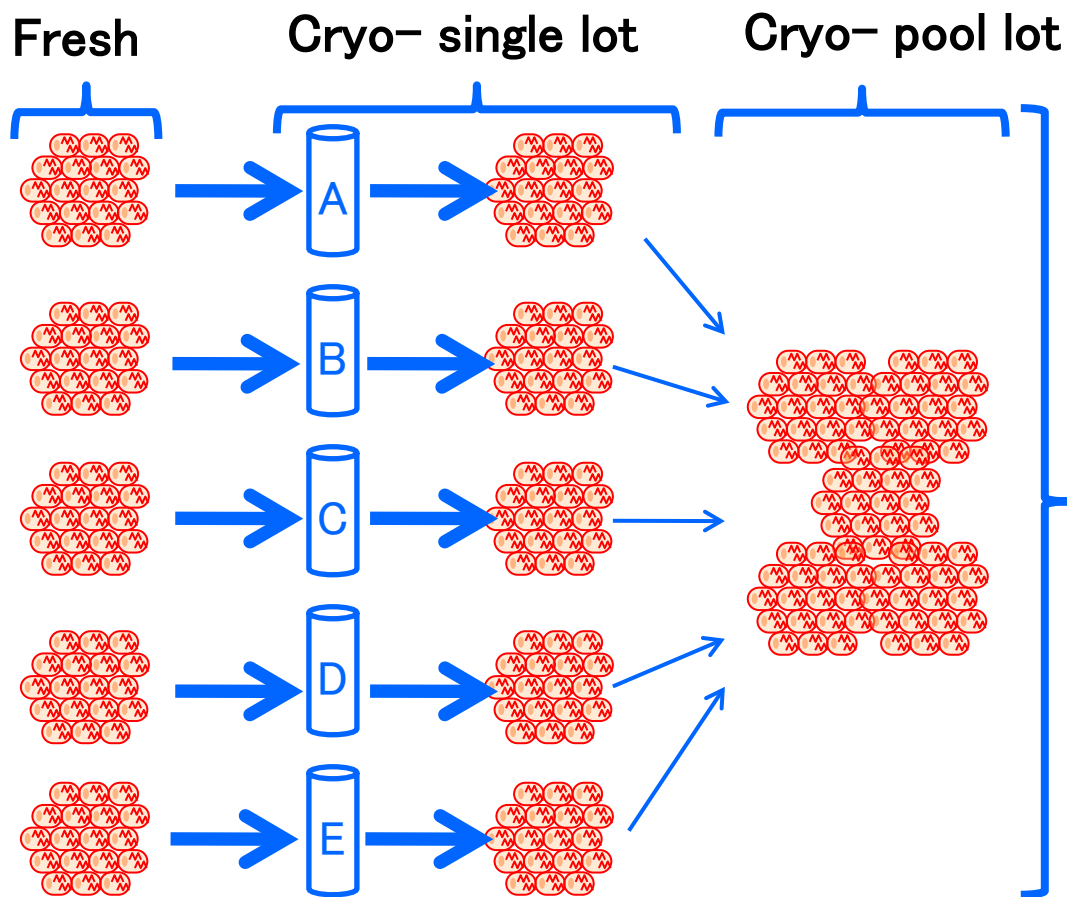


Hepatocytes
(Whole enzymes)

High activity?
(Improve preparation process)

Whether to use? & Where to begin?

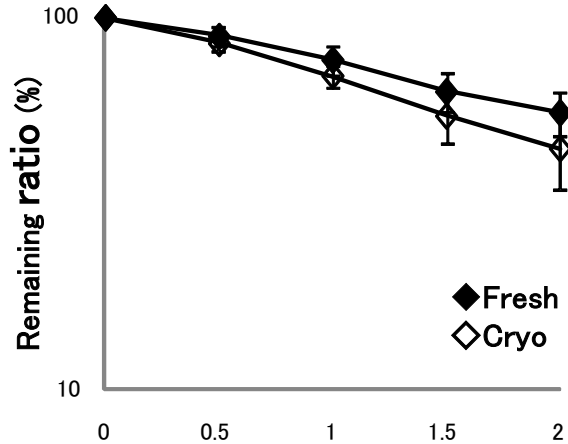
Cont: Stand at a crossroad... (2/2)



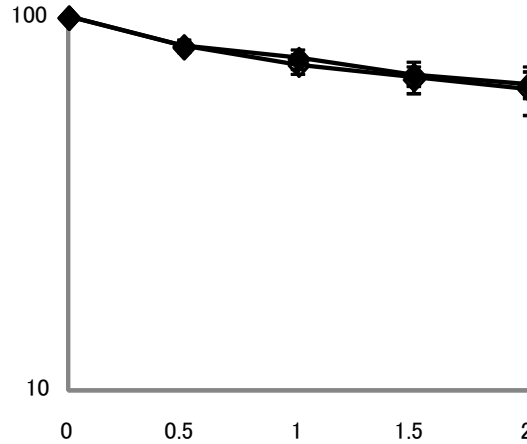
| Hepatocytes | Fresh | Cryopreserved | |
|-----------------------|-------|---------------|--------|
| | | Individual | Pooled |
| AO activity | ? > | ? > | ? |
| Use in drug discovery | × | △ | ○ |

Fresh vs cryopreserved hepatocytes

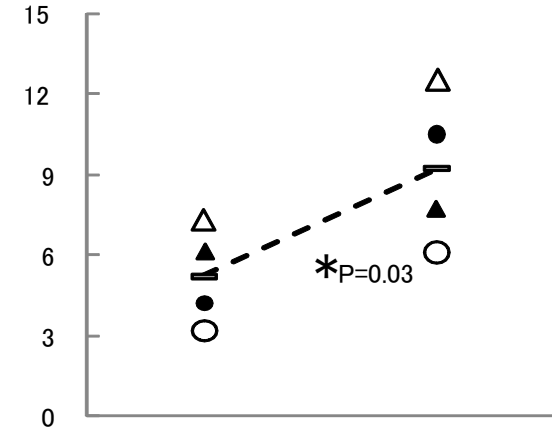
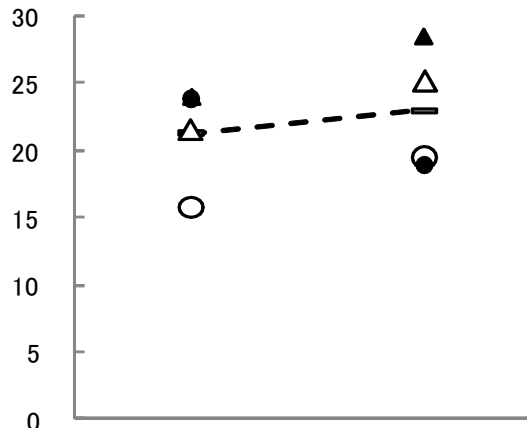
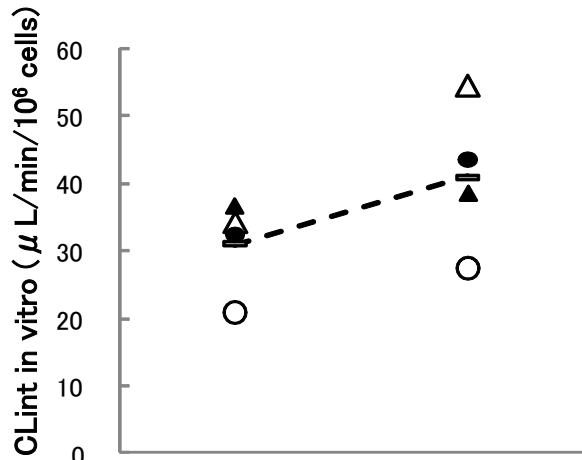
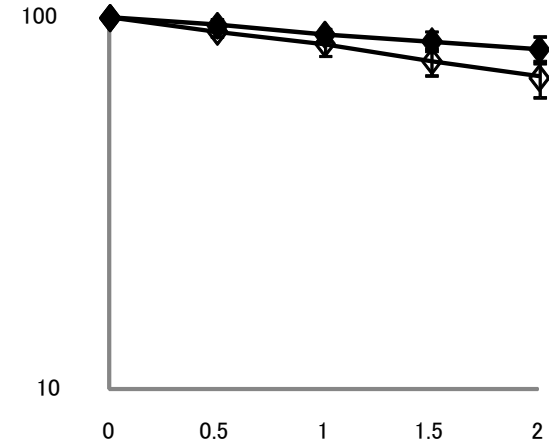
FK3453



O6-benzylguanine



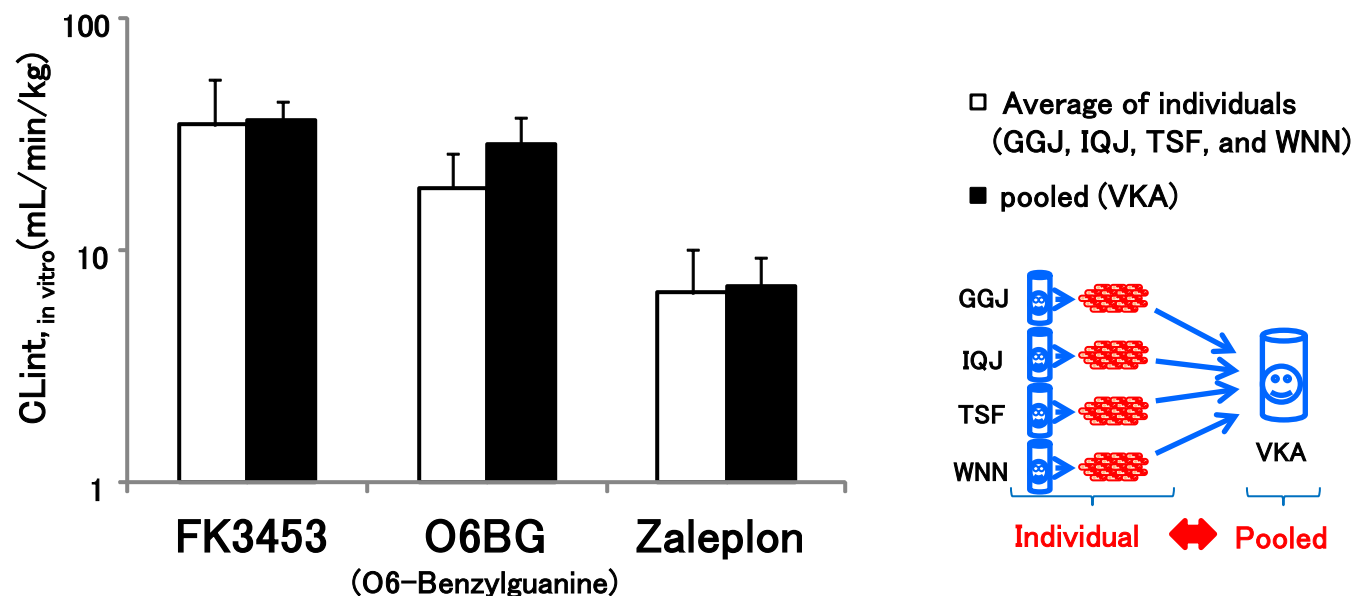
Zaleplon



▲ Hu1186 △ SLH ● Hu1197 ○ EXG - - - Average

CLint: Fresh ≅ Cryopreserved Hepatocytes

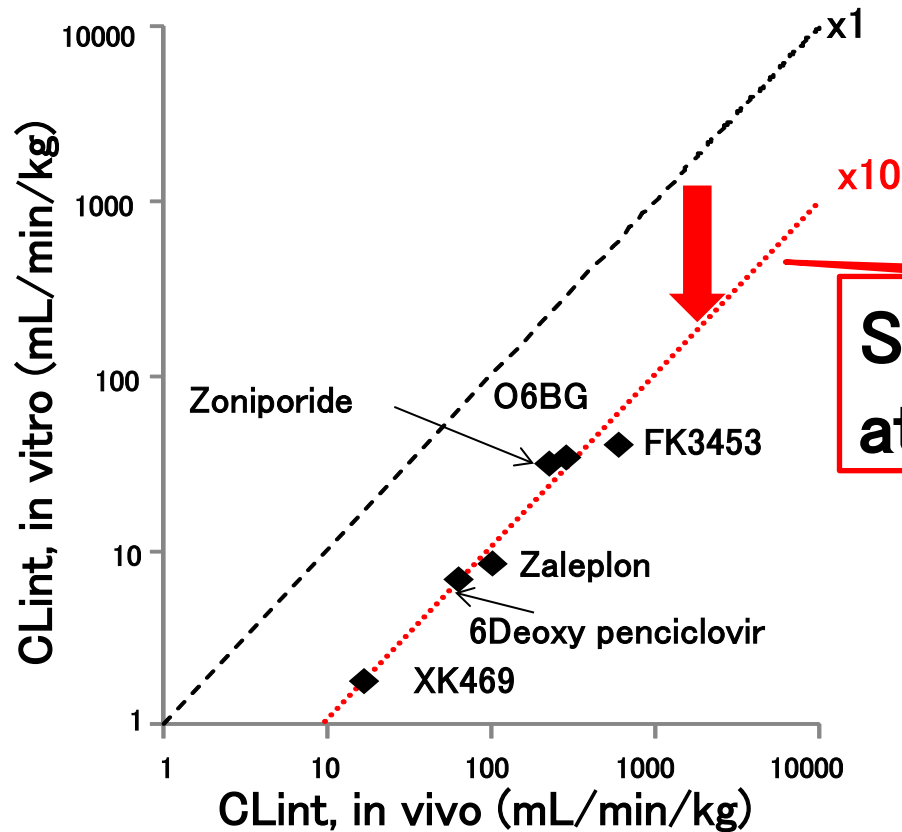
Individual vs pooled hepatocytes



| Compounds | Individual lots | | | | | VKA (Mean ± SD, n=3) | | Ratio |
|-----------|-----------------|------|------|------|------------------|----------------------|------|-------|
| | GGJ | IQJ | TSF | WNN | Mean ± SD (CV) | Mean ± SD (CV) | | |
| | mL/min/kg | | | | % | | % | |
| FK3453 | 22.2 | 30.0 | 23.1 | 64.6 | 35.0 ± 20.1 (57) | 36.4 ± 7.8 (21) | 1.04 | |
| O6BG | 13.7 | 22.2 | 11.4 | 27.7 | 18.8 ± 7.5 (40) | 29.1 ± 8.6 (30) | 1.55 | |
| Zaleplon | 3.4 | 5.9 | 5.2 | 11.8 | 6.6 ± 3.7 (56) | 7.1 ± 2.3 (32) | 1.07 | |

Akabane et al., Xenobiotica. 2012 ;42(9):863-71

$$CLint_{pool} \doteq CLint_{average\ of\ individual}$$



**Systemic under-prediction
at comparable rate.**

**‘Why’ remains unclear, but
‘Selection’ becomes possible.**

What I learned from Challenges & Experiences.

- / Confirm AO activity in hepatocytes (fresh = pooled).
- / Underestimate, but good correlation at a steady rate.



Avoid candidates attrition at P1 trial due to **high AO metabolism** in humans.



Facilitating more efficient candidate **selection** and **optimization** in drug **discovery** research.

Learned from

Drug Discovery ADME Research

創薬動態研究から学んだこと

**Only those who have the courage to
take on a challenge make failure.**

「失敗をすることができるのは、
挑戦をする勇気を持った者だけだ」

Leaned from Great Colleagues.

Special thanks to

Astellas Research Institute of America, LLC.

Nicolas Gerst
Bindu Upadhyay
Kazu Tetsuka
Jeffrey N Masters
Kouichi Tamura



Dr. Tamura

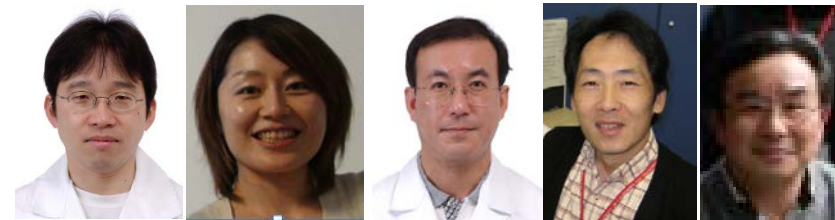


Members involved in AO project at Astellas Pharma Inc.

Kohichiro Tanaka
Megumi Irie
Yoichi Naritomi
Kenji Tabata
Shigeyuki Terashita
Toshio Teramura



Dr. Teramura



Hepatocytes provider: Celsis IVT, Invitrogen, and Xenotech