

2015 JSSX Award for Young Industrial Scientists

**薬物の肝代謝とその後の胆管/血管側排泄を評価する*in vitro*試験系の構築と創薬への利用**

Establishment of an *in vitro* assay for hepatic metabolism and subsequent sinusoidal/canalicular efflux

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## Acknowledgements

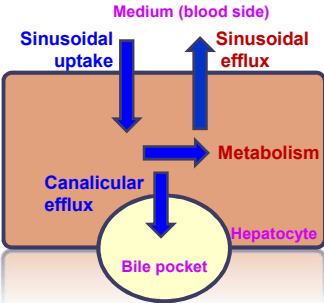



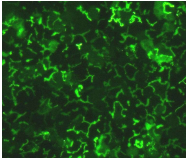
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## Sandwich-cultured hepatocytes (SCH) as an *in vitro* liver model



- ◆ **Historical uses**
  - Biliary excretion
  - IVIVE







Images from Qualyst Transporter Solution, LLC

- ◆ **Expansive uses; objectives**
  - Biliary excretion of metabolites
  - Sinusoidal efflux in addition to canalicular efflux of metabolites
- Utilization to in-house compound
  - ◆ Characterization of ADME profiles
  - ◆ Demonstration of advantages over competitors

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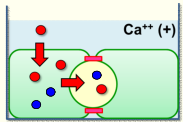
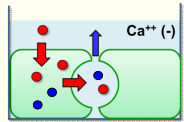
## Assay procedures



### Cell measurement

**Biliary excretion and cellular accumulation**

- ◆ Pre-conditioning cells with Ca<sup>+</sup>/(-)
- ◆ Loading substrate
  - Possible metabolism

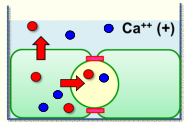
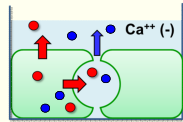



Liu et al., DMD 27:637-644 (1999)

### Buffer measurement

**Sinusoidal and canalicular efflux**

- ◆ Loading substrate
  - Possible metabolism
- ◆ Replacement buffer to Ca<sup>+</sup>/(-)

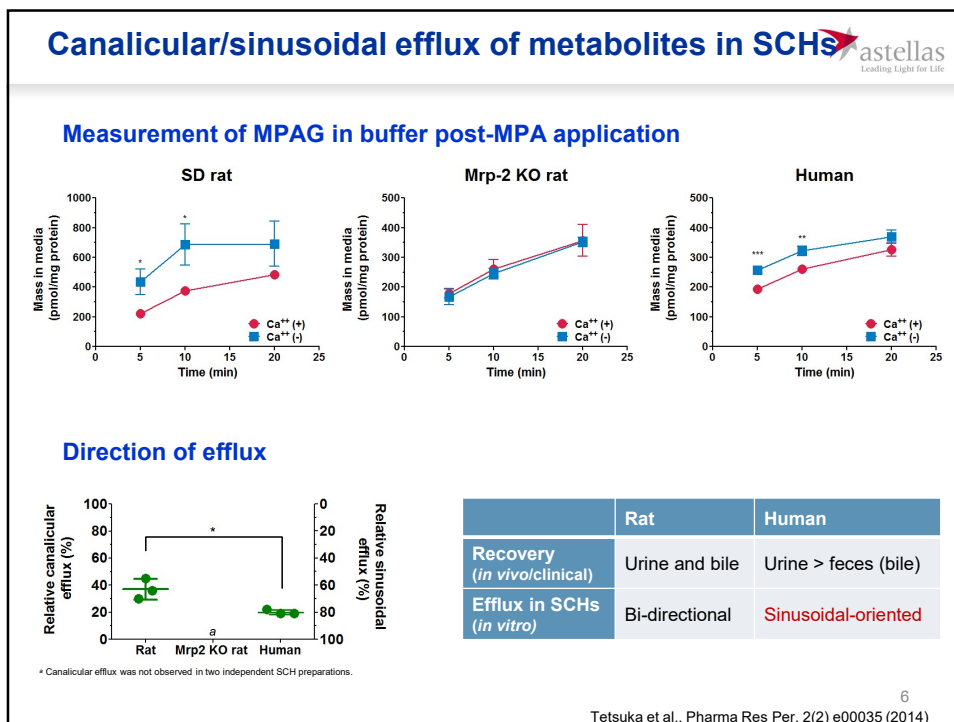
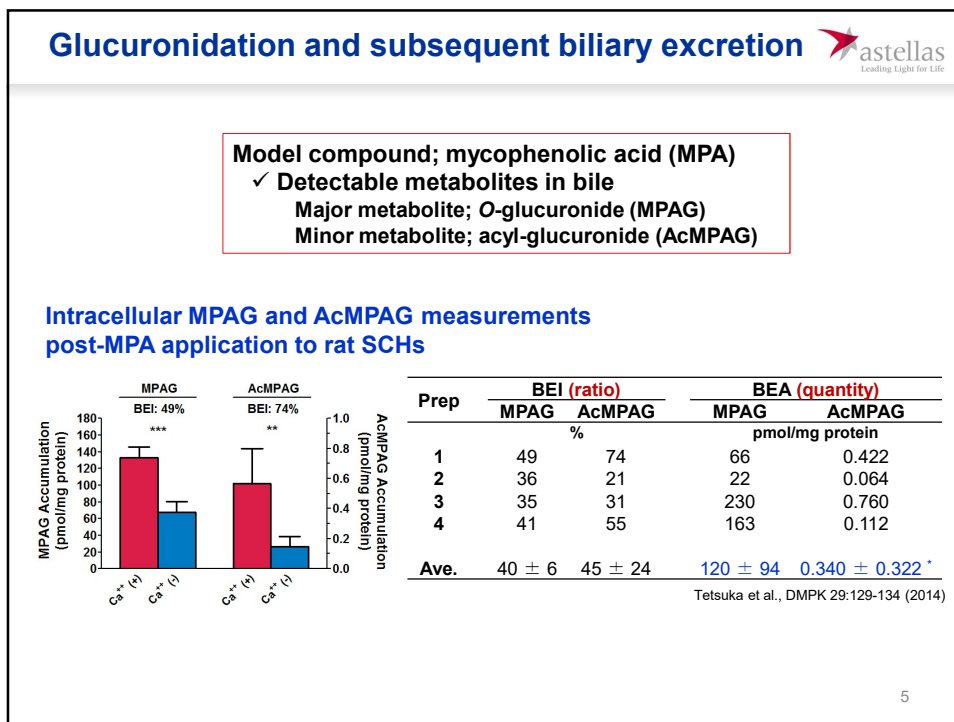




Pfeifer et al., JPET 347:727-736 (2013); for unchanged drugs  
Tetsuka et al., 28th JSSX (2013, poster), Pharma Res Per, 2(2) e00035, (2014, publication) for metabolites

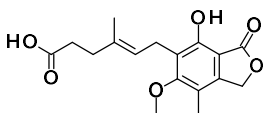
論文未掲載の為グラフを削除

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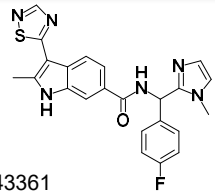
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**Demonstrating advantages of AS2643361 over MPA** 



Mycophenolic acid



AS2643361

◆ **Known profiles**


- **Metabolism; glucuronidation**
- **Biliary excretion**
  - ✓ Glucuronide
  - ✓ Entero-hepatic circulation
- **Toxicology; diarrhea**

↔

◆ **Investigation of ADMET profiles**

- **Metabolism; microsomes**
- **Biliary excretion; SCHs**
- **Toxicology; animal studies**

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**Biliary excretion of AS2643361** 

**Lewis rat SCHs**

論文未掲載の為  
グラフを削除

論文未掲載の為  
グラフを削除

**Human SCHs**

論文未掲載の  
テーブルを削除

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Tetsuka et al., 19th North American ISSX/29th JSSX, symposium (2014)

## Efficacy and adverse effects



### Effective dose: rat heart transplantation model

	Rat heart transplantation model (Mono-therapy)
<b>MMF</b>	20 mpk (MST: 14.5 d) 40 mpk (MST: 18.5 d)
<b>AS2643361</b>	10 mpk (MST: 16 d) 20 mpk (MST: 19 d)

Index; prolongation of graft survival  
MST; median survival time (day)

### GI-toxicity: diarrhea score

	Dose	Mean
<b>MMF</b>	Vehicle	0
	40 mpk	0
	60 mpk	1.63*
	80 mpk	1.96*

	Dose	Mean
<b>AS2643361</b>	Vehicle	0
	15 mpk	0
	30 mpk	0
	60 mpk	0.08

Status of each stool scored on 0-3 scale: 0, normal stool; 1, stool with soft surface; 2, soft stool; 3, watery stool

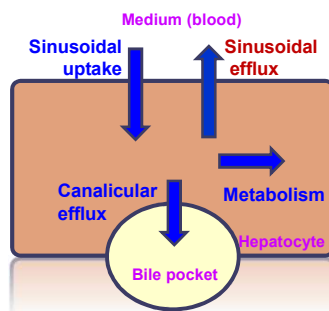
Nakanishi et al. Euro J Pharmacol 674: 58-63 (2012) 9  
Nakanishi et al. Int Immunopharmacol 10:91-97 (2010)

## Conclusions



- ◆ **Expansive uses of sandwich-cultured hepatocytes**
  - **Biliary excretion (canalicular efflux) of metabolites**
  - **Sinusoidal efflux of metabolites**
- ◆ **Future challenges**
  - **Plasma metabolite appearance; species differences (e.g. MIST\*)**

\*Metabolites in Safety Testing



*SCHs give us opportunities to light the 'shadow' part of hepatic disposition.*<sup>10</sup>