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ELEVATING SCIENCE™

Prediction of Cholestatic Hepatotoxicity: Integration of Transporter Regulation and Adaptive Response

Hepatocyte Transporter Network
(Les Diablerets, Switzerland)
2 – 4 September, 2019

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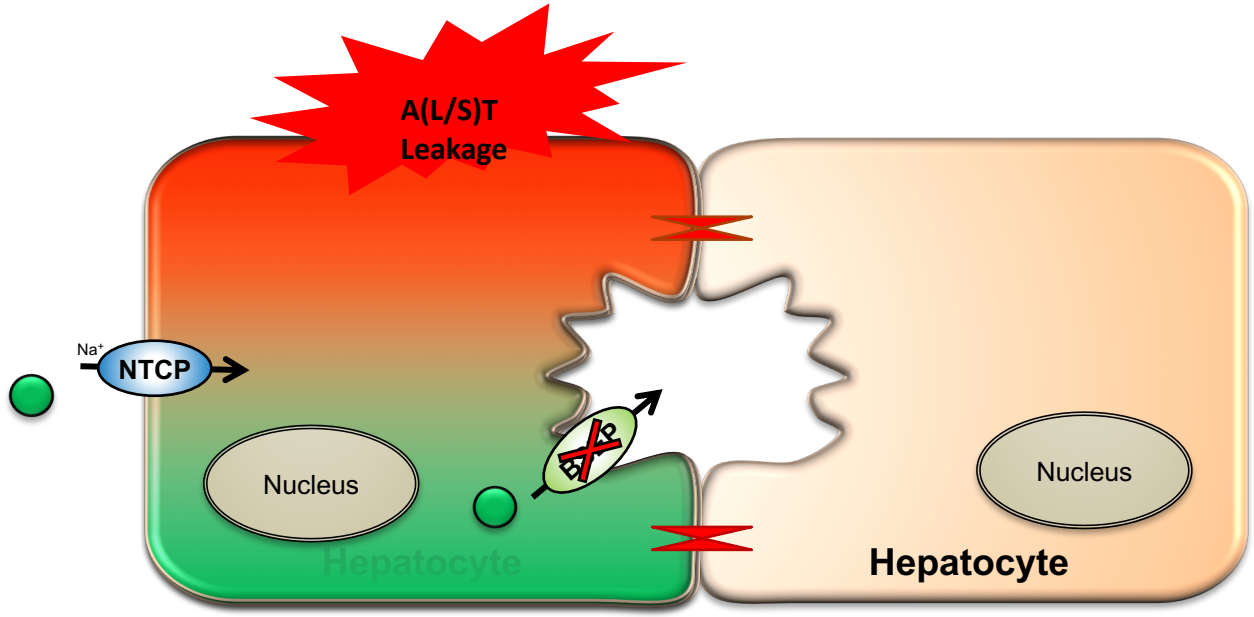
BioIVT

Drug Induced Liver Injury (DILI)

- DILI is the leading cause of acute liver failure in the US, and a major reason for liver transplantation.[1]
  - Approximately 55,000 cases/year in the US [2]
- DILI is the #1 cause of regulatory actions
  - drug failure in clinical trials
  - drug withdrawal
- Herbals and dietary supplements are the second leading cause for liver injury [3]
- Numerous DILI Mechanisms
- Cholestatic-DILI
  - Drug exposure disrupts bile acid homeostasis within hepatocytes
  - Accumulation of bile acids within hepatocytes lead to bile acid-induced hepatotoxicity

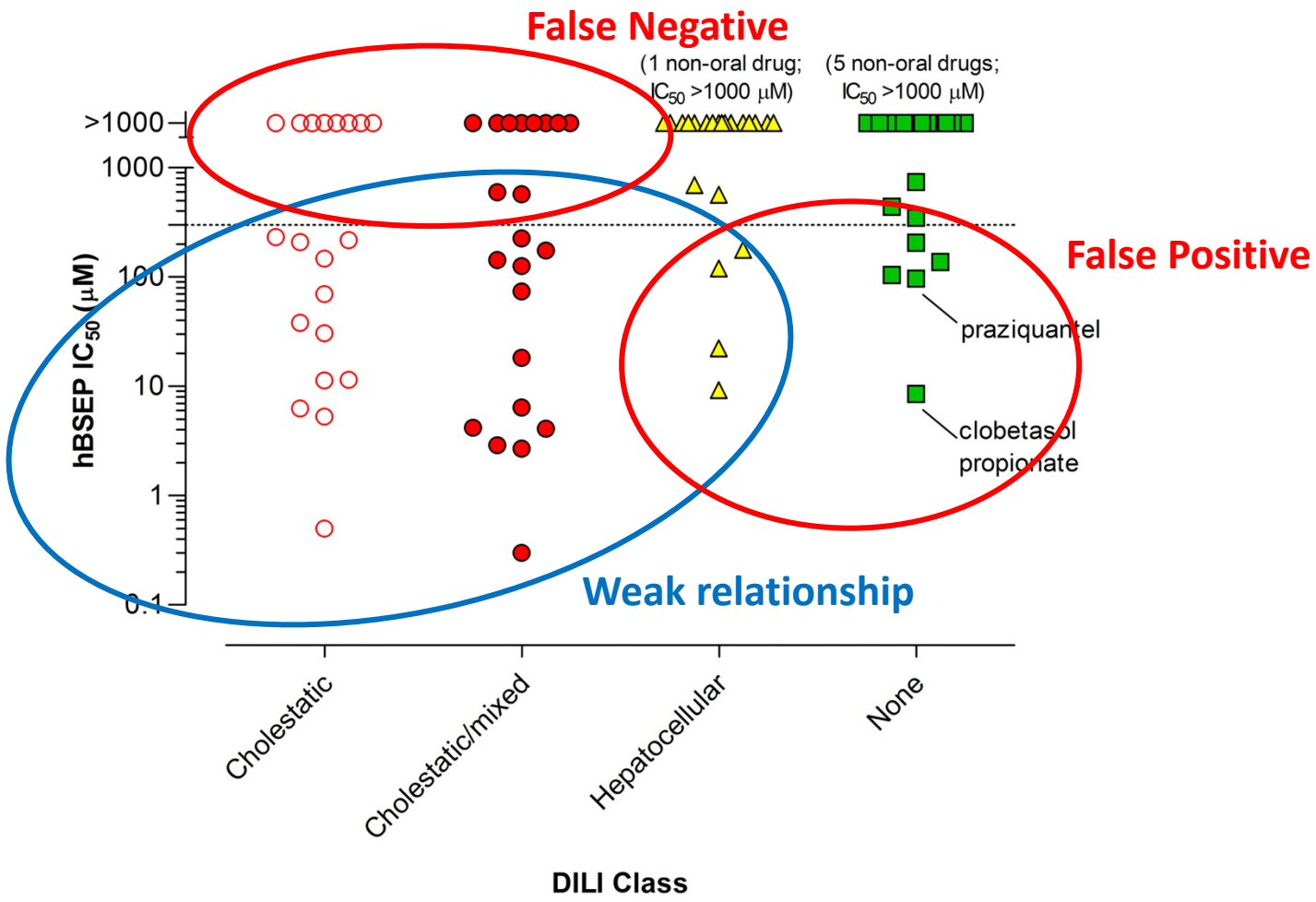
[1] Reuben et al. Hepatology 2010:52: 2065-2076
[2] Fontana. Gastroenterology 2013;314: 1818
[3] Chalasani et al. Gastroenterology 2008;135:1924-1934, 1934.e1-

Historical Cholestatic DILI Hypothesis



- Normal Vectoral Flow of Bile Acids
  - Uptake (NTCP) into hepatocyte
  - Excreted (BSEP) out of hepatocyte to bile canaliculi
- BSEP inhibition results in build up of bile acids (detergents) which can “dissolve” membranes at high intracellular concentrations, leading to hepatotoxicity
- BSEP inhibition = Hepatotoxicity
  - Progress familial intrahepatic cholestasis II (PFIC II)
    - Rare genetic disorder caused by mutations in ABCB11 (BSEP)
    - Progress liver disease beginning at infancy usually ending with liver failure

In Vitro Potency of BSEP Inhibition and Cholestatic Drug Induced Liver Injury



Dawson et al., *Drug Metab Dispos* 40:130, 2012

Predictive Power of BSEP Inhibition for Liver Injury

Compound
Cyclosporin
Pioglitazone
Rosiglitazon
Troglitazone
Ketoconazol
Imatinib
Simvastatin
Fluvastatin
Deferasirox

Table 1 Comparison of various assays measuring key mechanisms of toxicity endpoints associated with DILI (adapted from ref. 15)

Chan & Benet Toxicol. Research 2018, 7, 358-370

Criteria	% Correct (positive predictive value, PPV)	% DILI missing (false negative rate, FNR)	% Accuracy (ACC) (true positive + true negative)/106
GSH	71.9%	52.1%	69.1%
TDI	75.0%	81.3%	61.8%
Cytotoxicity (3T3 cells)	48.3%	70.8%	55.5%
Mitotox	71.4%	79.2%	61.8%
BSEP	69.2%	62.5%	65.5%
All assays	65.1%	14.6%	73.6%
BDDCS Class 1	33.3%	75.0%	45.5%
BDDCS Class 2	64.6%	35.4%	69.1%
GSH and BDDCS Class 2	89.5%	64.6%	70.0%
BSEP and BDDCS Class 2	87.5%	70.8%	67.3%
BSEP and Mitotox	50.0%	95.8%	56.4%

ensitivity: 60%
pecificity: 50%

ccuracy: 22%

osed Threshold of 25 µM
gan et al. (2010) Toxicol Sci
):118;485–500

False Positives and False Negatives are a serious issue

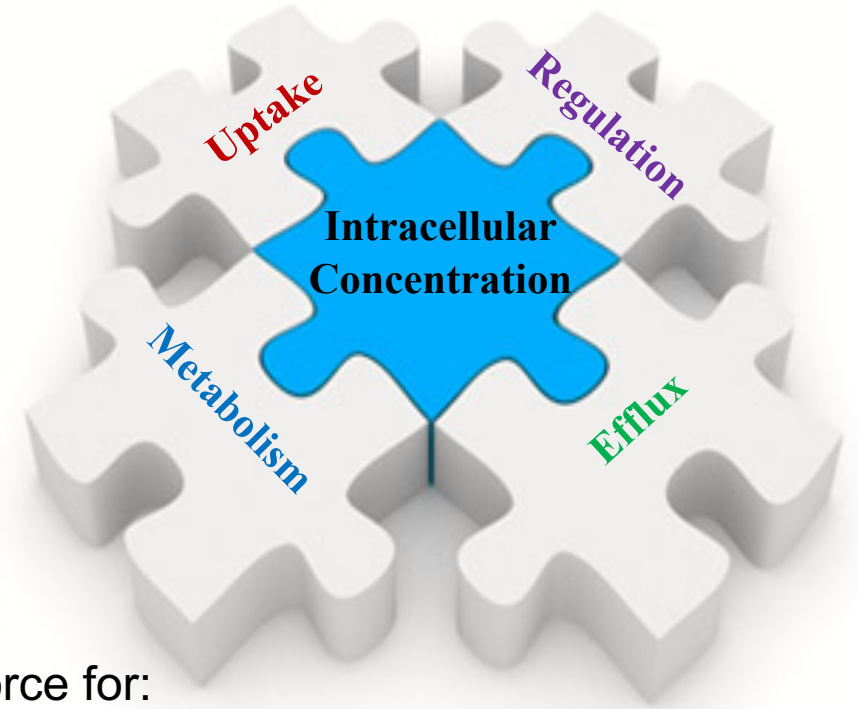
Not Much Better than a Coin Toss!

Findings consistent with Dawson et al., *Drug Metab Dispos* 40:130, 2012

Requirements for an In Vitro Model

Integrate Key Components for a *Predictive* Hepatic Model:

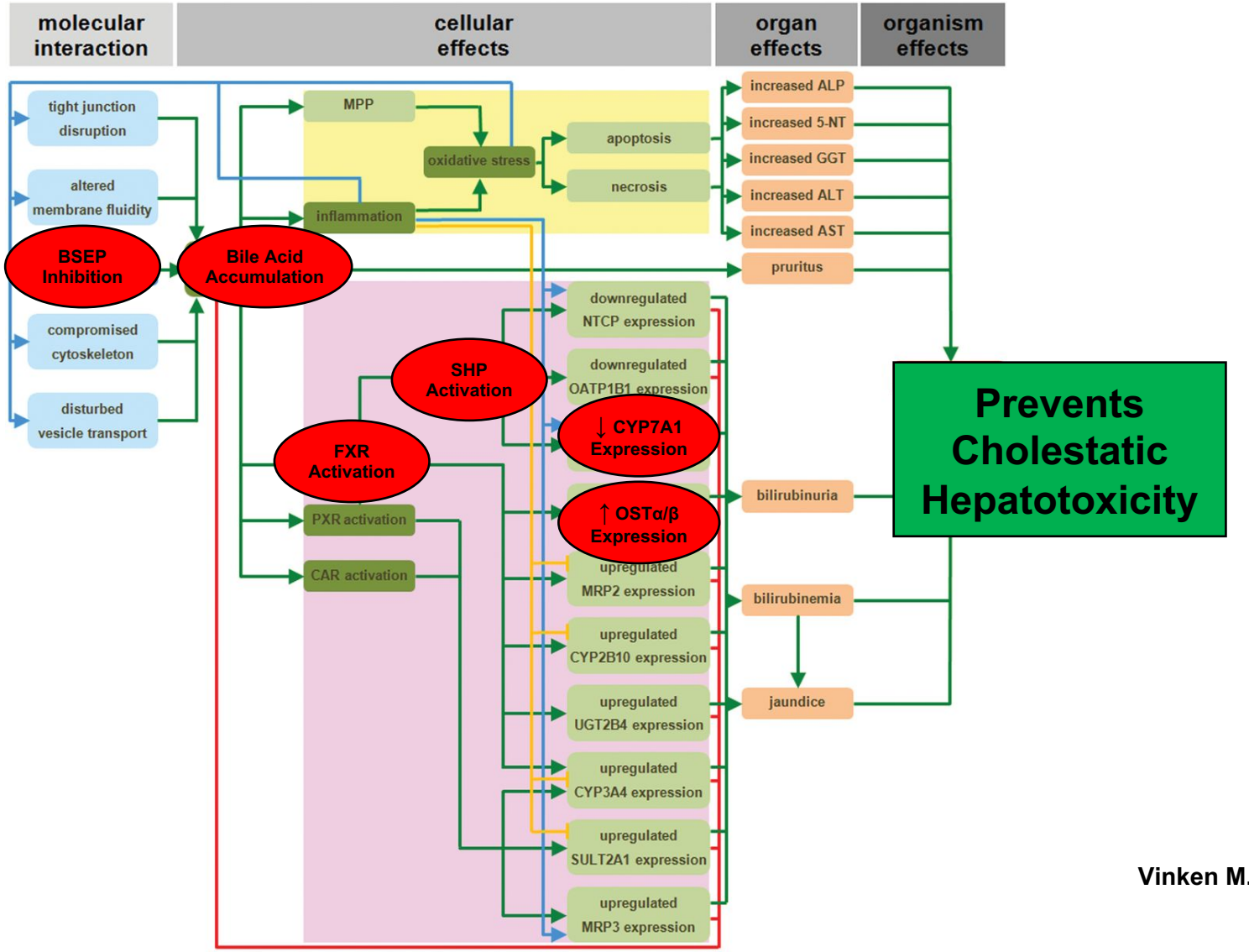
- ✓ **Uptake**
  - *Sinusoidal uptake transport proteins*
- ✓ **Efflux**
  - *Biliary and/or basolateral transport proteins*
- ✓ **Metabolism**
  - *Metabolic enzymes for elimination, or generation of active/toxic metabolites*
- ✓ **Regulation**
  - *Induction of transport and metabolism*



The **Intracellular Concentration** is the driving force for:

- Hepatotoxicity
- Efflux based interactions
- Metabolism – induction/inhibition

Adverse Outcomes Pathway: Integration of the Adaptive Response to Predict Cholestasis



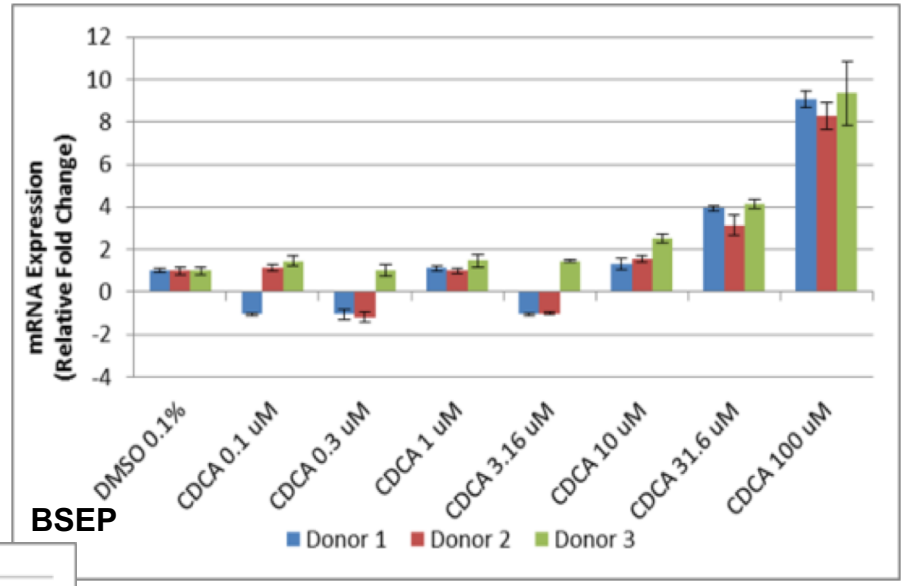
Vinken M. (2013) Toxicology 312 158-165

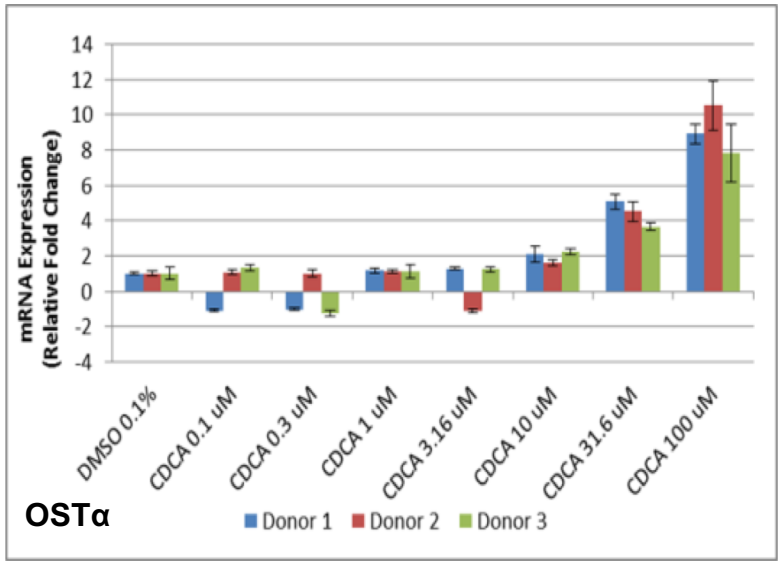
Increased Intracellular Bile Acid Concentrations - Adaptive Response

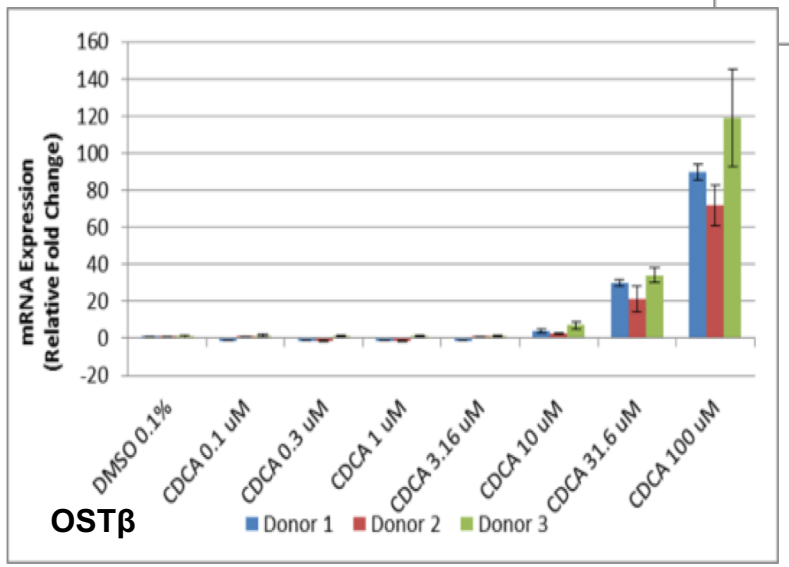
- In response to high intracellular concentrations of bile acids:
  - Decreased expression of CYP7A1
  - Increased expression of BSEP
  - Increased expression of OSTα and OSTβ
- Increase in mRNA expression of transporters linked to **function**
- The **Net Effect** of the Adaptive Response is a **decrease** in the intracellular concentration of bile acids

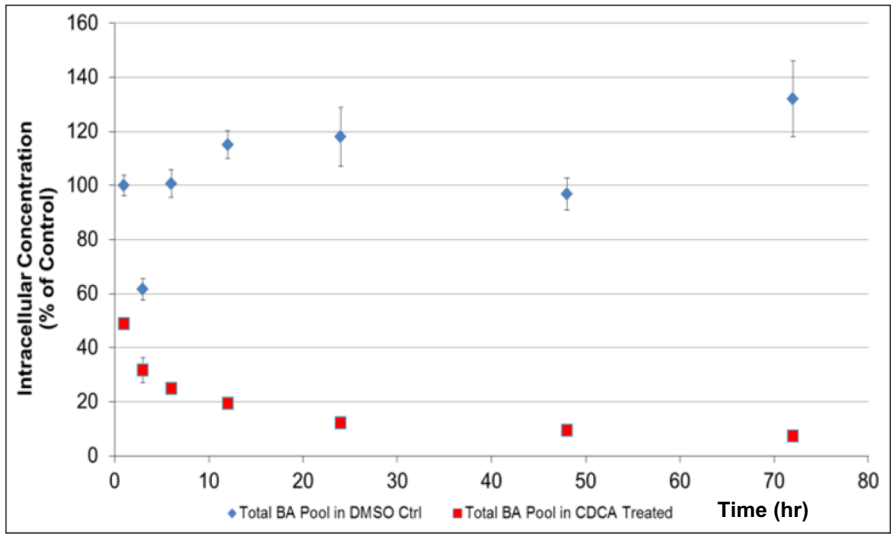
All studies in Transporter Certified™ Human Hepatocytes

CDCA ≡ chenodeoxycholic acid



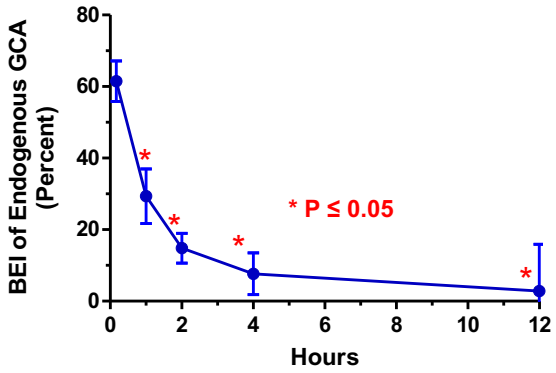




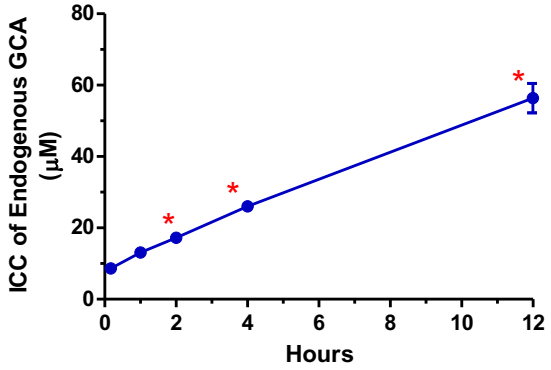


BSEP Inhibition "Triggers" Adaptive Response

Exposure to Cyclosporine A (10 µM), a potent BSEP inhibitor leads to a **rapid, time dependent decrease** in biliary excretion of endogenous bile acids.

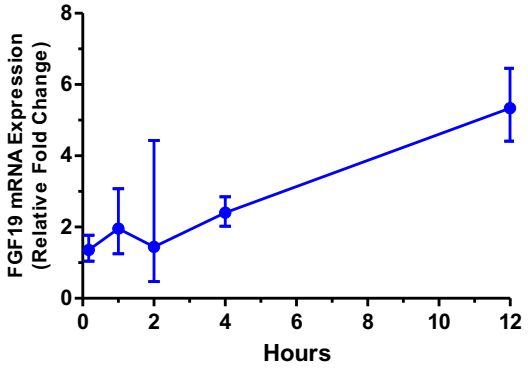


Inhibition of biliary excretion leads to an increase in the **intracellular concentration** of endogenous bile acids.



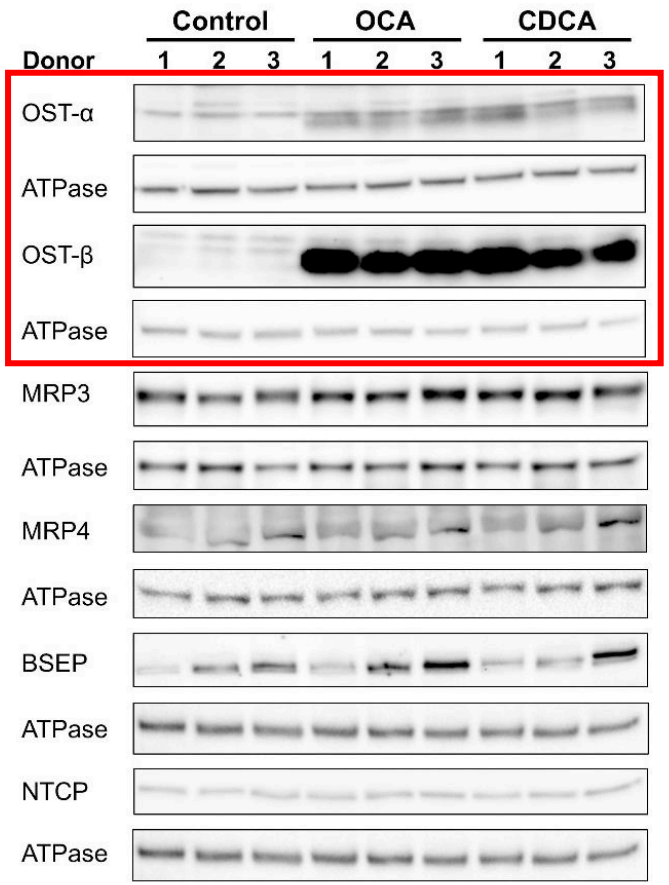
Increased intracellular concentrations of bile acids **activate FXR** (increased FGF19)

- This leads to **suppression of CYP7A1** (bile acid synthesis), and **induction of OST α/β** (basolateral efflux transporter)

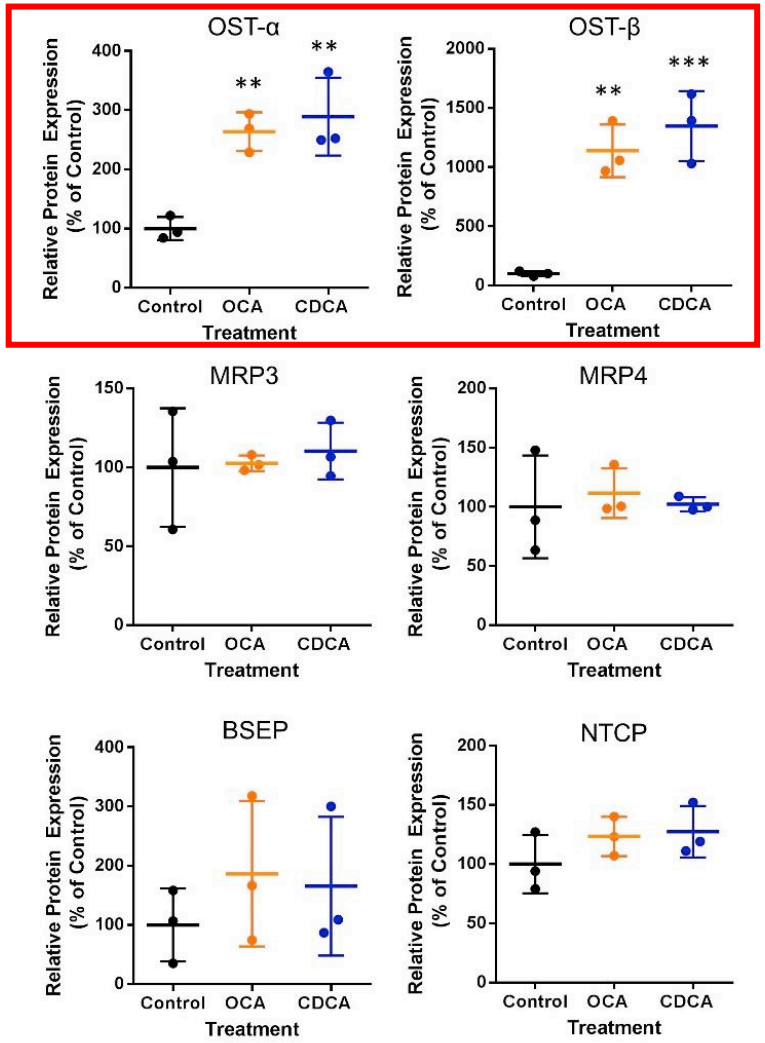


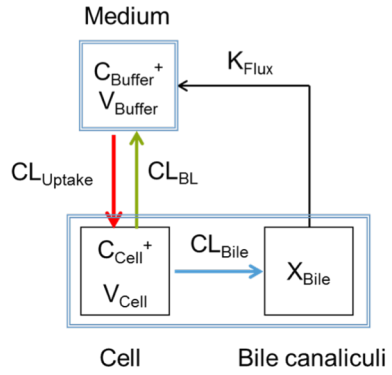
Jackson JP, Freeman KM, St. Claire III RL, Black CB, and Brouwer KR. Cholestatic DILI: A Function of BSEP Inhibition and FXR Antagonism. Applied In Vitro Toxicology, Vol 4, No 3, 2018

Change in mRNA Translates to Changes in Protein and Function

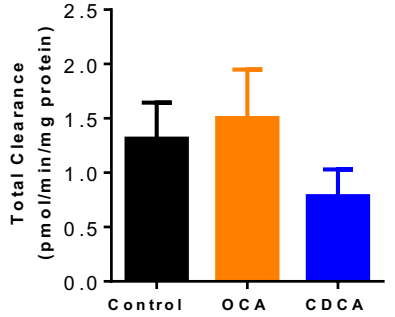


OCA ≡ Obeticholic Acid





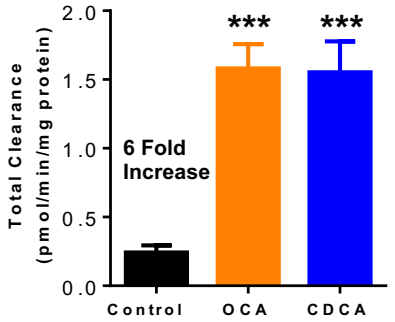
Uptake Clearance



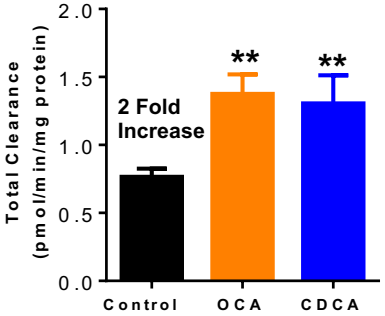
Mean ± S.D. (n=3 hepatocyte donors)
**p<0.01; ***p<0.001 (treated vs. control)

OCA: Obeticholic Acid
CDCA: Chenodeoxycholic Acid

Basolateral Efflux Clearance



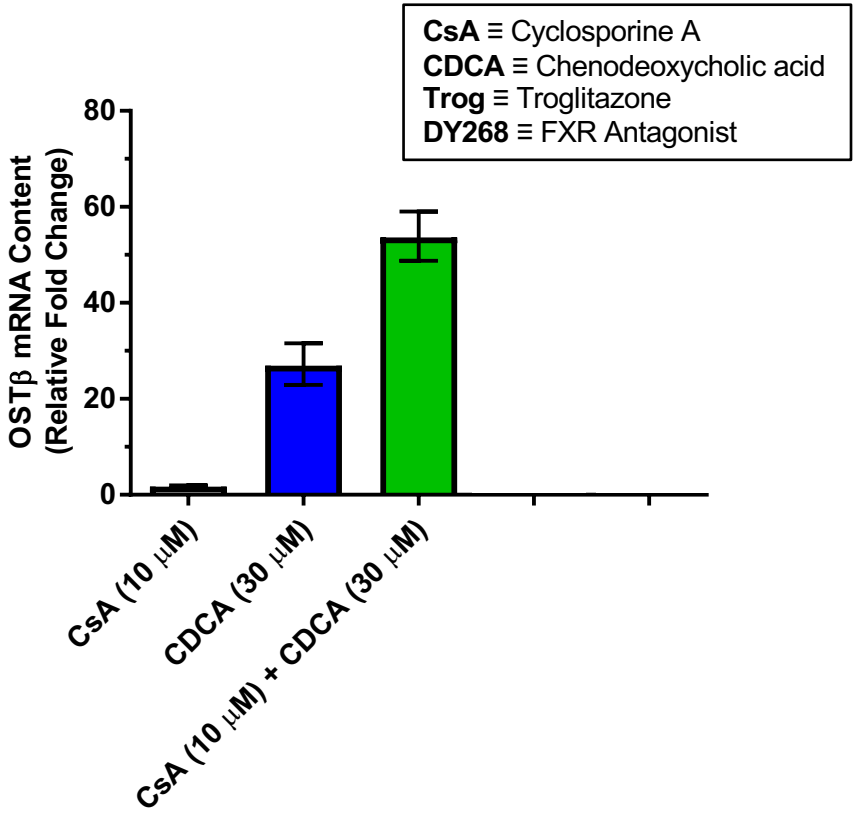
Biliary Clearance



Cen Guo, Carl LaCerte, Jeffrey E. Edwards, Kenneth R. Brouwer, and Kim L. R. Brouwer Farnesoid X Receptor Agonists Obeticholic Acid and Chenodeoxycholic Acid Increase Bile Acid Efflux in Sandwich-Cultured Human Hepatocytes: Functional Evidence and Mechanisms. J Pharmacol Exp Ther 365:413–421, May 2018.

Impact of FXR Antagonism on the Adaptive Response

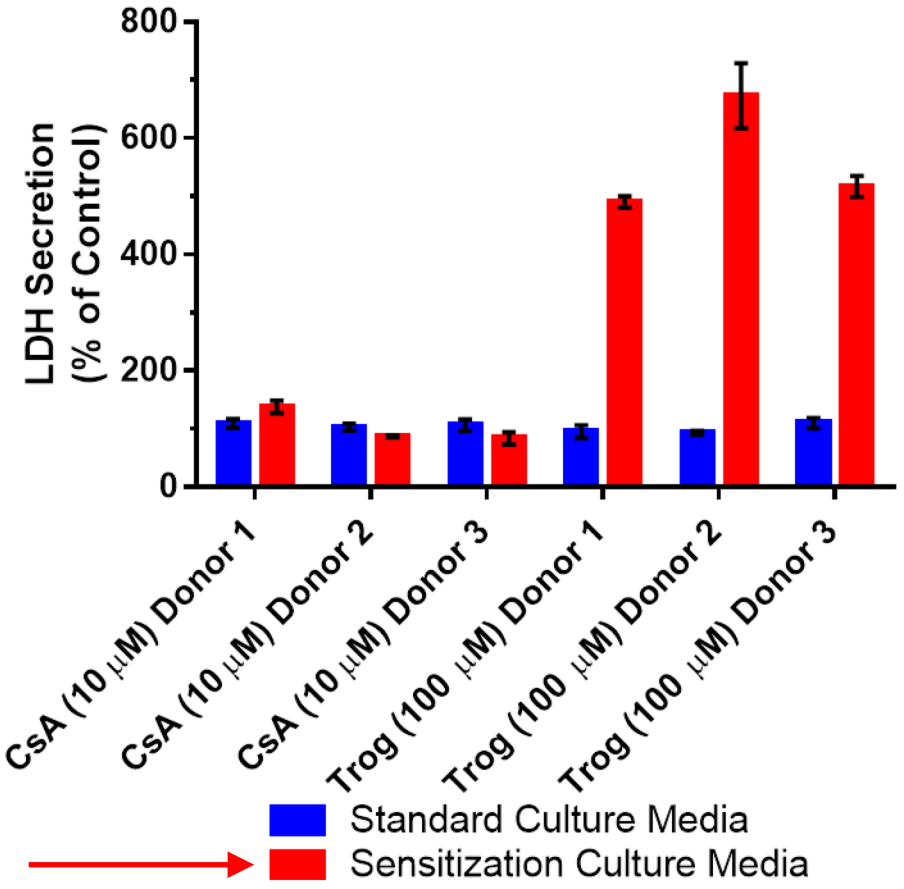
- Synergistic effect on activation of FXR in the presence of CDCA and CDCA + CsA
- Troglitazone (weak FXR antagonist) response decreased to 46.8 % of control
- DY268 (strong FXR antagonist) response decreased to 5.6 % of control
- **FXR antagonism prevents the hepatocyte from responding to high intracellular concentrations of bile acids**



Experimental: 24 hours exposure, Transporter Certified™ human hepatocytes in sandwich configuration (24-well) using QualGro™ media

Jackson JP, Freeman KM, St. Claire III RL, Black CB, and Brouwer KR. Cholestatic DILI: A Function of BSEP Inhibition and FXR Antagonism. Applied In Vitro Toxicology, Vol 4, No 3, 2018

Integration of Multiple Mechanisms to Produce Hepatotoxicity

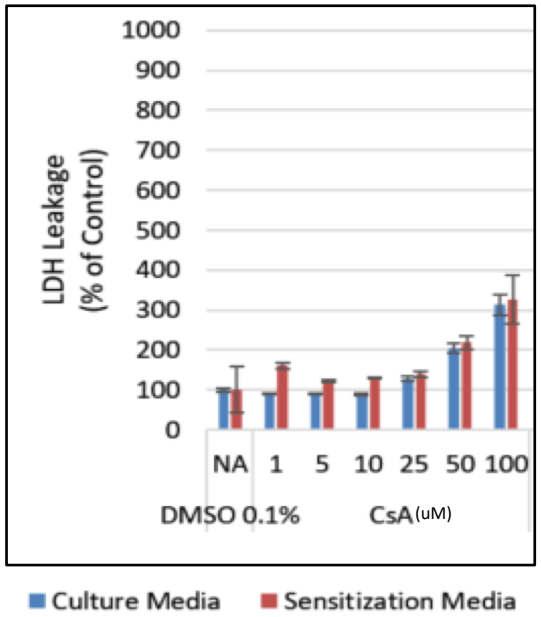


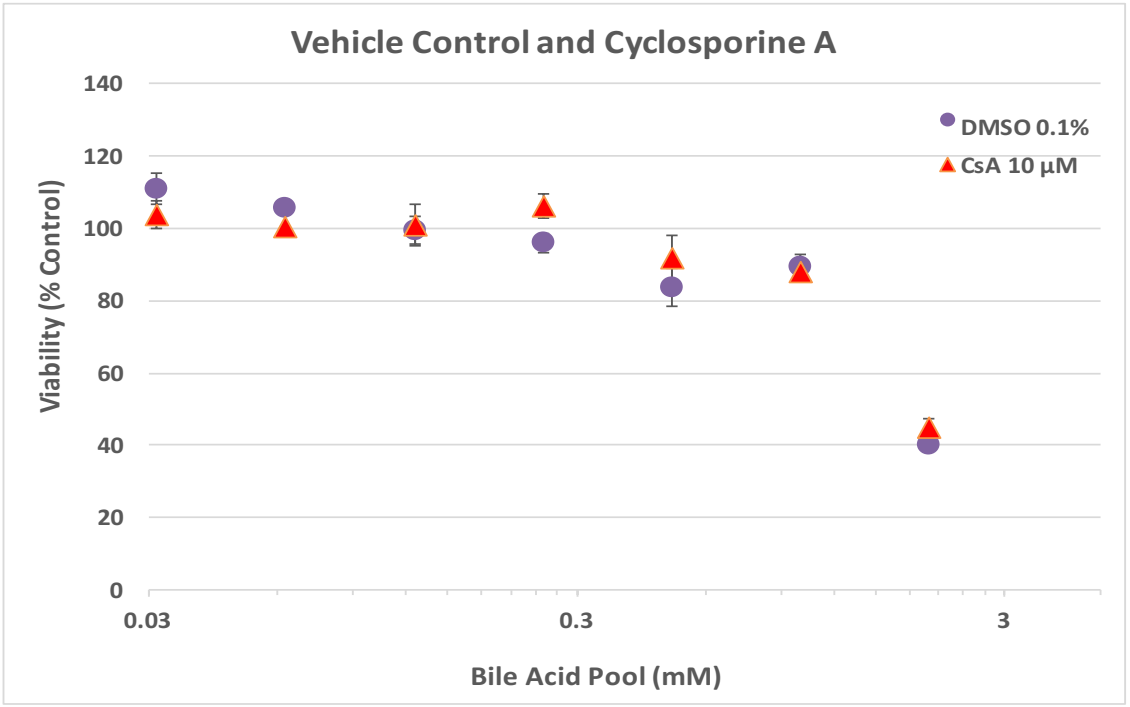
Contains physiologic concentrations of multiple bile acids, fatty acids, and glucose

- Troglitazone and it's sulfate metabolite inhibit BSEP
- Troglitazone is a weak FXR antagonist
- Troglitazone sulfate is also an inhibitor of the basolateral efflux transporters OSTα/β *
- Toxicity is **only observed when compounds impact multiple pathways**
  - Inhibition of BSEP and/or basolateral efflux
  - FXR gene regulation (e.g. FXR antagonists)

* Malinen et.al., Organic Solute Transporter OSTα/ß is Over-Expressed in Nonalcoholic Steatohepatitis and Modulated by Drugs Associated with Liver Injury. American Journal of Physiology-Gastrointestinal and Liver Physiology - 8 Feb 2018 https://doi.org/10.1152/ajpgi.00310.2017

Negative Control: Toxicity of Bile Acids with Cyclosporine A





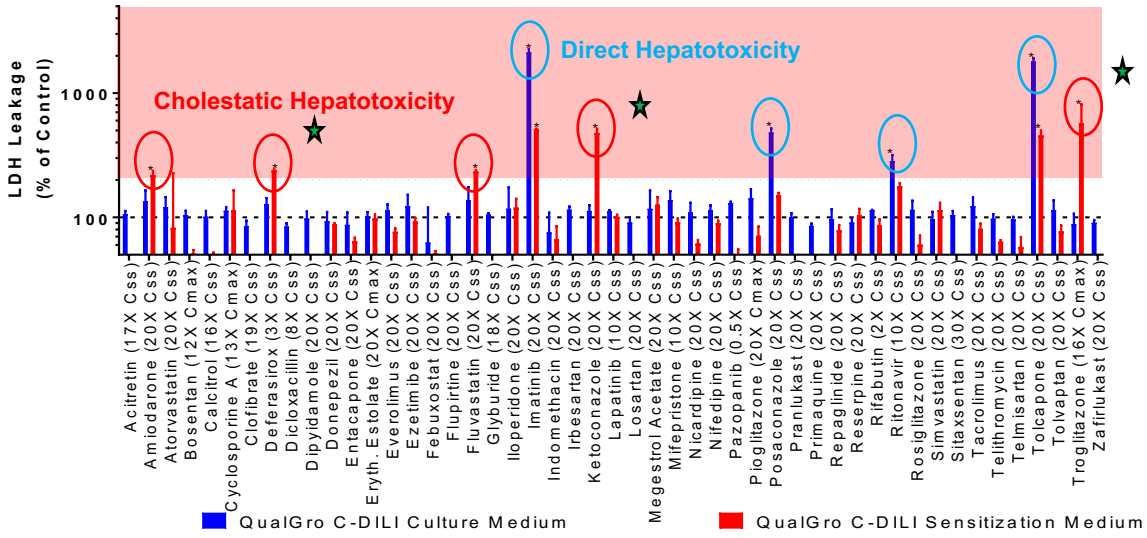
Transporter Certified™ Human Hepatocytes

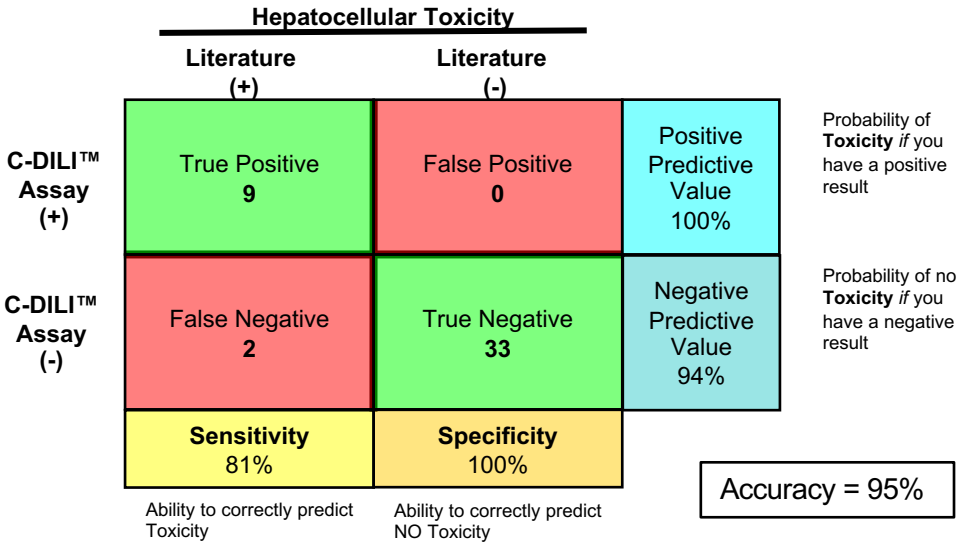
- At high concentrations cyclosporine A is toxic
- Increasing Bile Acid concentration leads to hepatotoxicity
- Cyclosporine A, a potent BSEP inhibitor (IC_{50} ~ 0.5µM) does **NOT** show toxicity greater than DMSO control

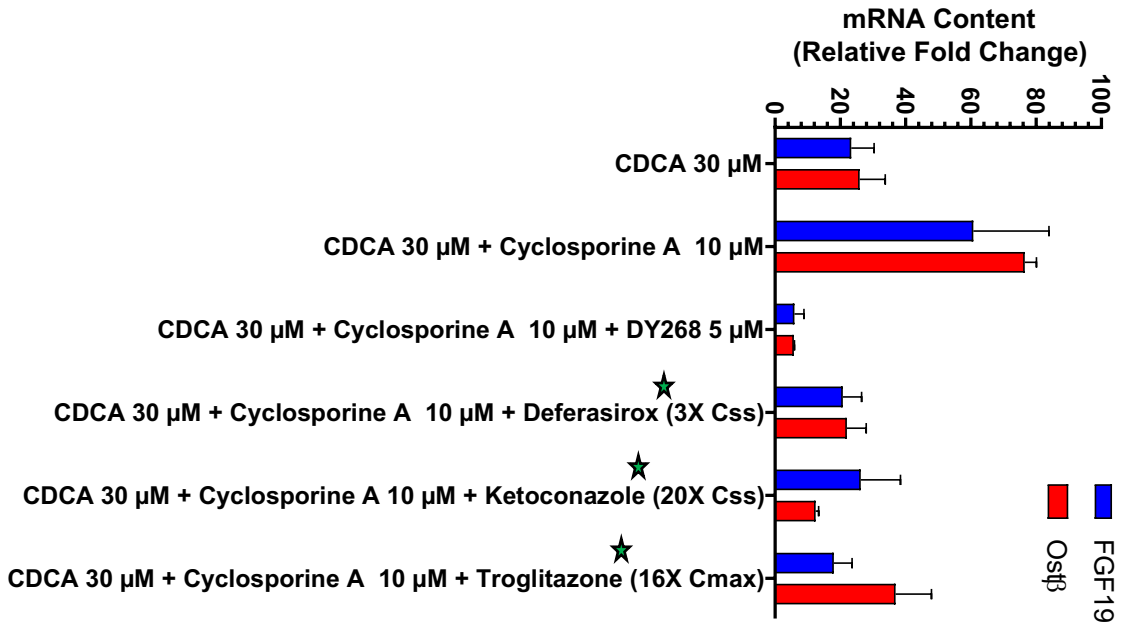
The C-DILI™ Assay: Key Features

- Transporter Certified™ human hepatocytes
- 96-well plate format
- Optimized culture conditions
  - 5 days in culture: optimizes formation of bile pockets and efflux transporter function
  - QualGro™ Sensitization Media: Creates a sensitized cellular environment using lipids and bile acids
- Standard Culture Media (control)
  - Non-sensitized cells to account for direct compound toxicity
- Positive, negative and direct toxicity controls
- 24-hour incubation with test article
  - Integrates metabolism and FXR gene expression changes (Adaptive Response)
- LDH and ATP readout for toxicity
- Validation:
  - Test set of @ 50 drugs selected from Morgan et al. (2010) and Dawson et al. (2012) with hBSEP vesicle IC_{50} values ranging from 0.3 to 78 µM
  - Drug concentrations were 10X to 20X systemic C_{max} to account for higher portal vein concentrations
  - NIH LiverTox Database was used to identify and rank compounds with clinical hepatotoxicity potential

Improved Predictability and Mechanistic Links







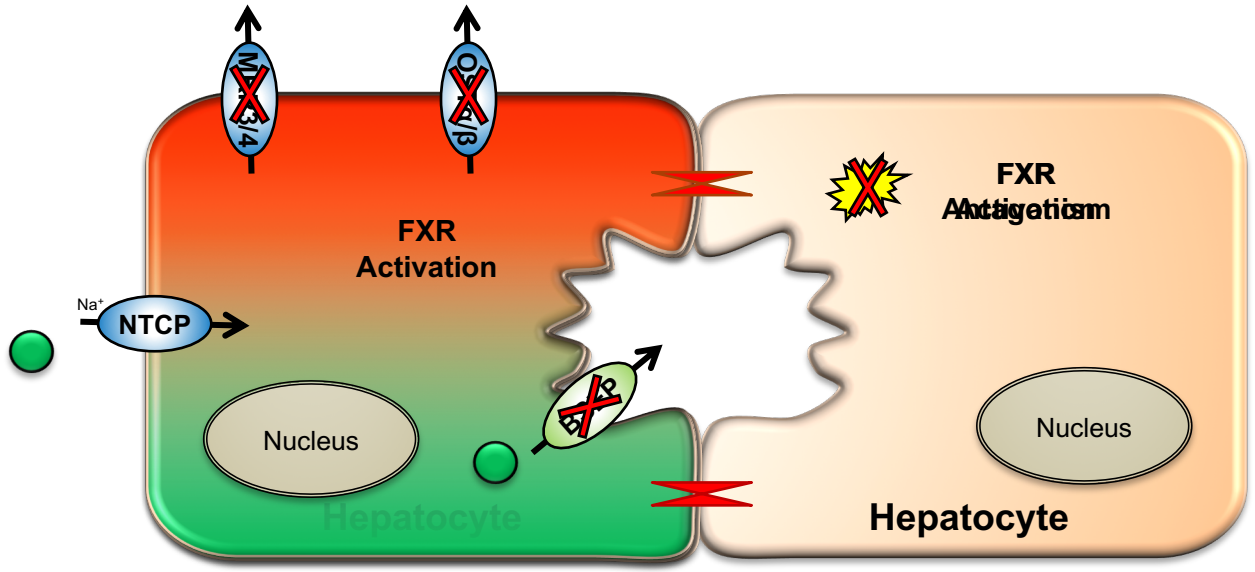
Bile-induced Hepatotoxicity (C-DILI)

- Compounds that inhibit bile acid efflux and antagonize FXR or block basolateral efflux
- Ketoconazole, deferasirox, troglitazone **reduce the effectiveness** of the FXR-dependent compensatory mechanism

Cholestatic DILI: Hepatocellular Injury Need to integrate multiple mechanisms

Initiating Insult
- BSEP Inhibition

Secondary Insult
- FXR Antagonism and/or
- Basolateral Efflux Inhibition

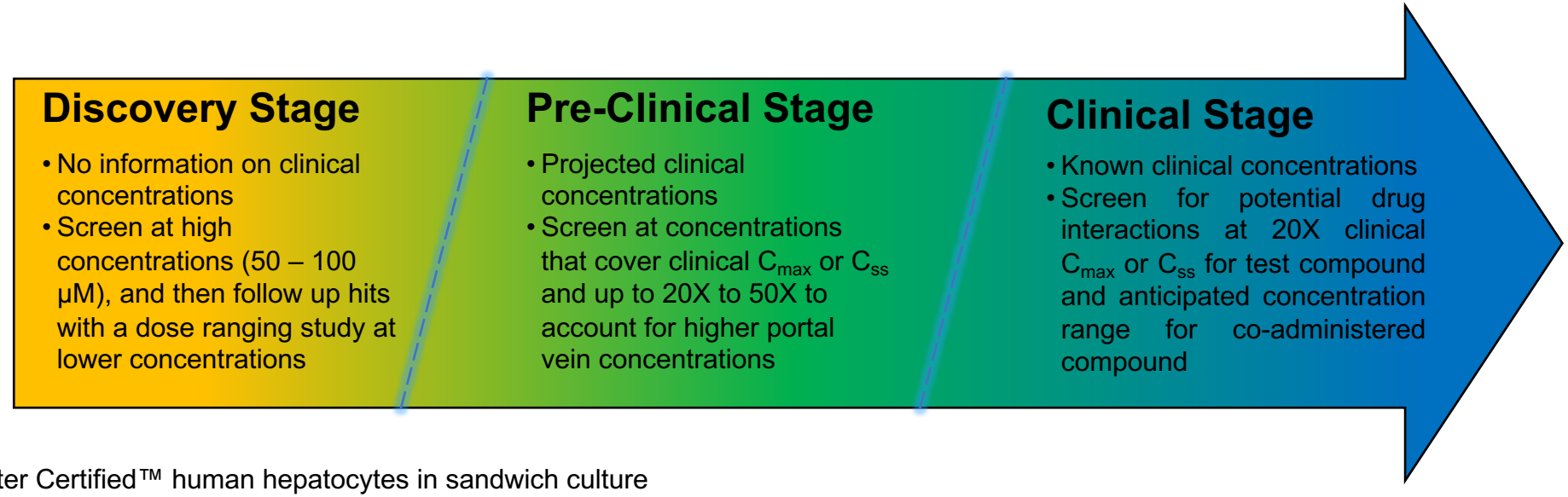


Compounds can Increase the Intracellular Concentration of Bile Acids through:

- BSEP Inhibition plus
- Basolateral Efflux Inhibition (MRP3/4 and/or OSTα/β) and/or
- FXR Antagonism

Jackson JP, Freeman KM, St. Claire III RL, Black CB, and Brouwer KR. Cholestatic DILI: A Function of BSEP Inhibition and FXR Antagonism. Applied In Vitro Toxicology, Vol 4, No 3, 2018

The C-DILI™ Assay: Applications and Summary



- **System**
  - Transporter Certified™ human hepatocytes in sandwich culture
  - 96 well format
  - 24 hour exposure
  - LDH readout

C-DILI™ Assay Integrates:

- Acute Effects
  - Metabolism (endogenous and exogenous)
  - Uptake and/or Efflux (basolateral and canalicular) Transporter Inhibition
- Chronic Effects (adaptive response)
  - Regulation (induction – transporters and metabolism)
  - Synthesis of endogenous bile acids

It is the **NET effect** of all these processes on bile acid disposition (adaptive response) that determine the cholestatic drug induced liver injury potential of a compound.

Acknowledgements:

BioIVT:

- Jonathan Jackson, Ph.D.
- Robert St. Claire, Ph.D.
- Kimberly Freeman, M.S.
- Matt Palmer, B.S.

UNC Eshelman School of Pharmacy:

- Kim Brouwer, Pharm.D., Ph.D.

Pfizer:

- Cen Guo, Ph.D.

Intercept Pharmaceuticals:

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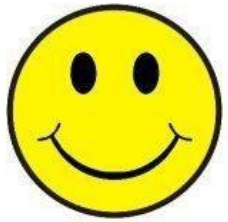
Optimist



The Glass is Half Full

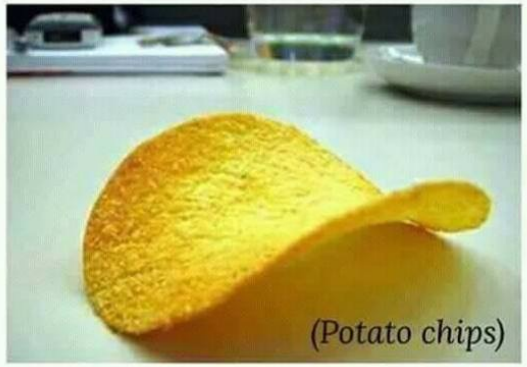
Pessimist

The Glass is Half Empty

Chemist

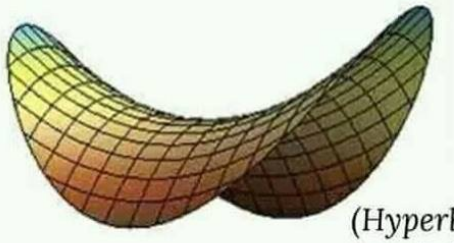
The Glass Contains
50% $H_2O(l)$
39% $N_2(g)$
10.5% $O_2(g)$
.44% Ar(g)
.06% $CO_2(g)$

What others see...



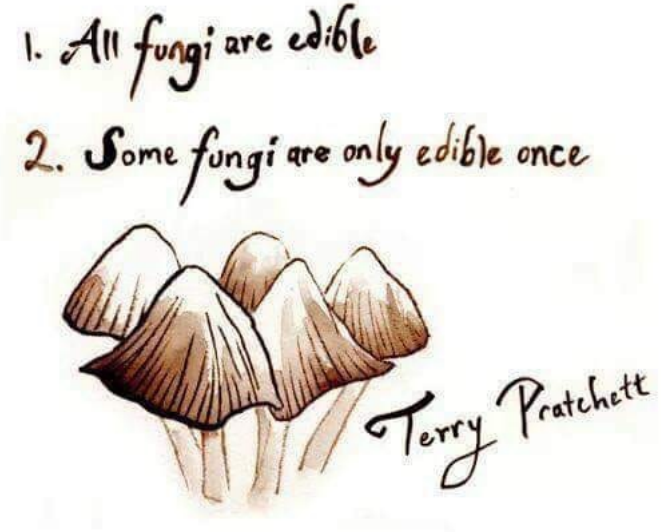
(Potato chips)

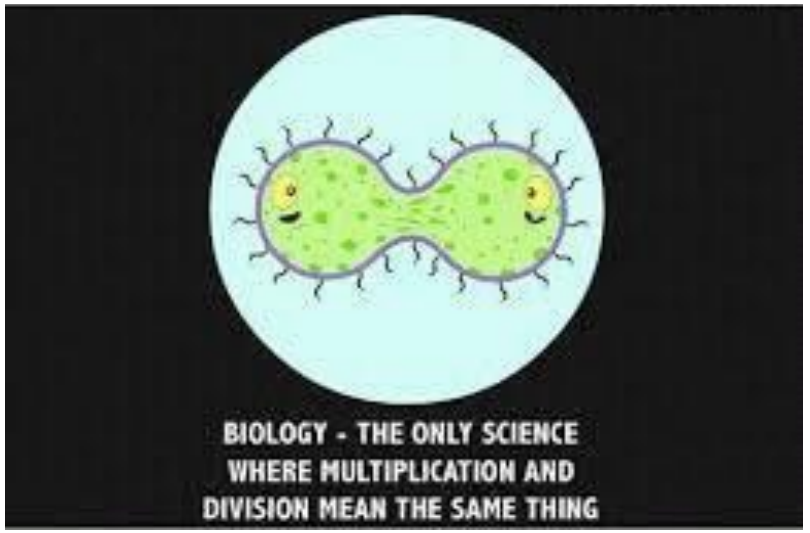
What I see...



(Hyperbolic paraboloid)

$$\frac{x^2}{a^2} - \frac{y^2}{b^2} = cz$$





Backup Slides

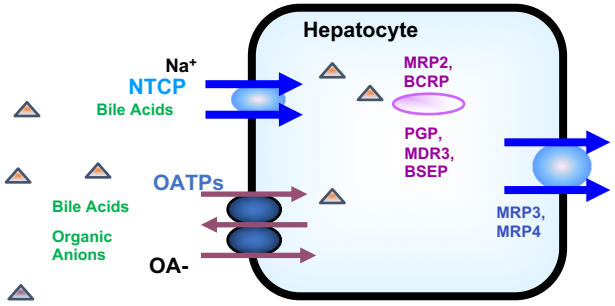
Rationale for Evaluating C-DILI™ Assay: Data for a Proof-of-Concept Study

- Drugs were selected from extensive work published by Morgan et al. (2010) and Dawson et al. (2012) based on hBSEP vesicle IC_{50} data
  - IC_{50} ranged from 0.3 to 78 µM
- For orally administered drugs, portal circulation concentration (10-50X) > systemic concentration
- Need to test concentrations greater than systemic concentrations (e.g. C_{max} or C_{ss})
- Test concentrations were 10X to 20X systemic C_{max}
  - Solubility limited max. testable concentration in some instances
- NIH LiverTox Database was used to identify and rank compounds with clinical hepatotoxicity potential

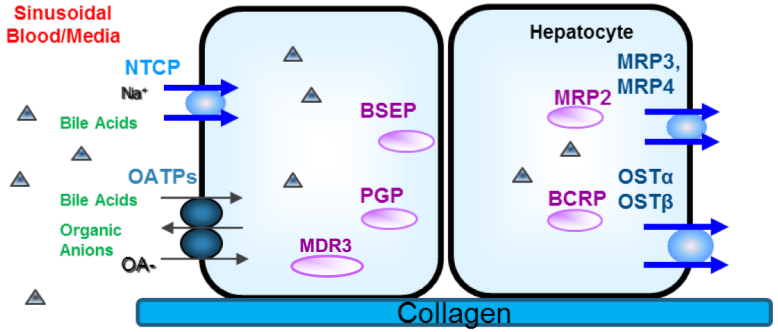
A Polarized System is Critical for *In Vivo* Relevant Transporter Function

- Systems are **not polarized**
- Canalicular efflux transporters are internalized and **NOT** functioning
- Uptake and basolateral efflux transporters only
- Limited regulation

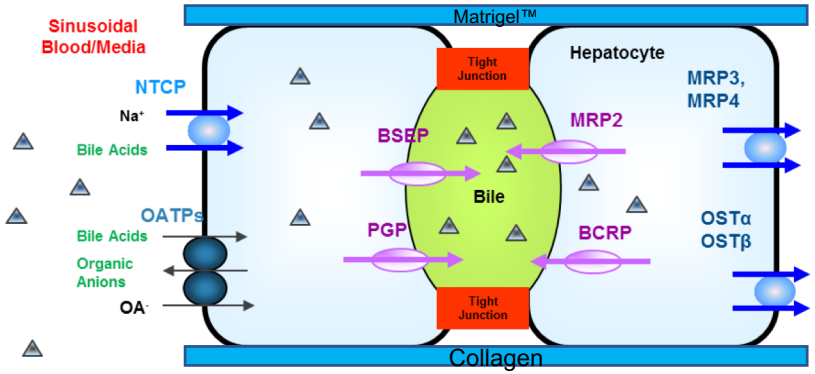
Suspended Hepatocytes



Plated Hepatocytes

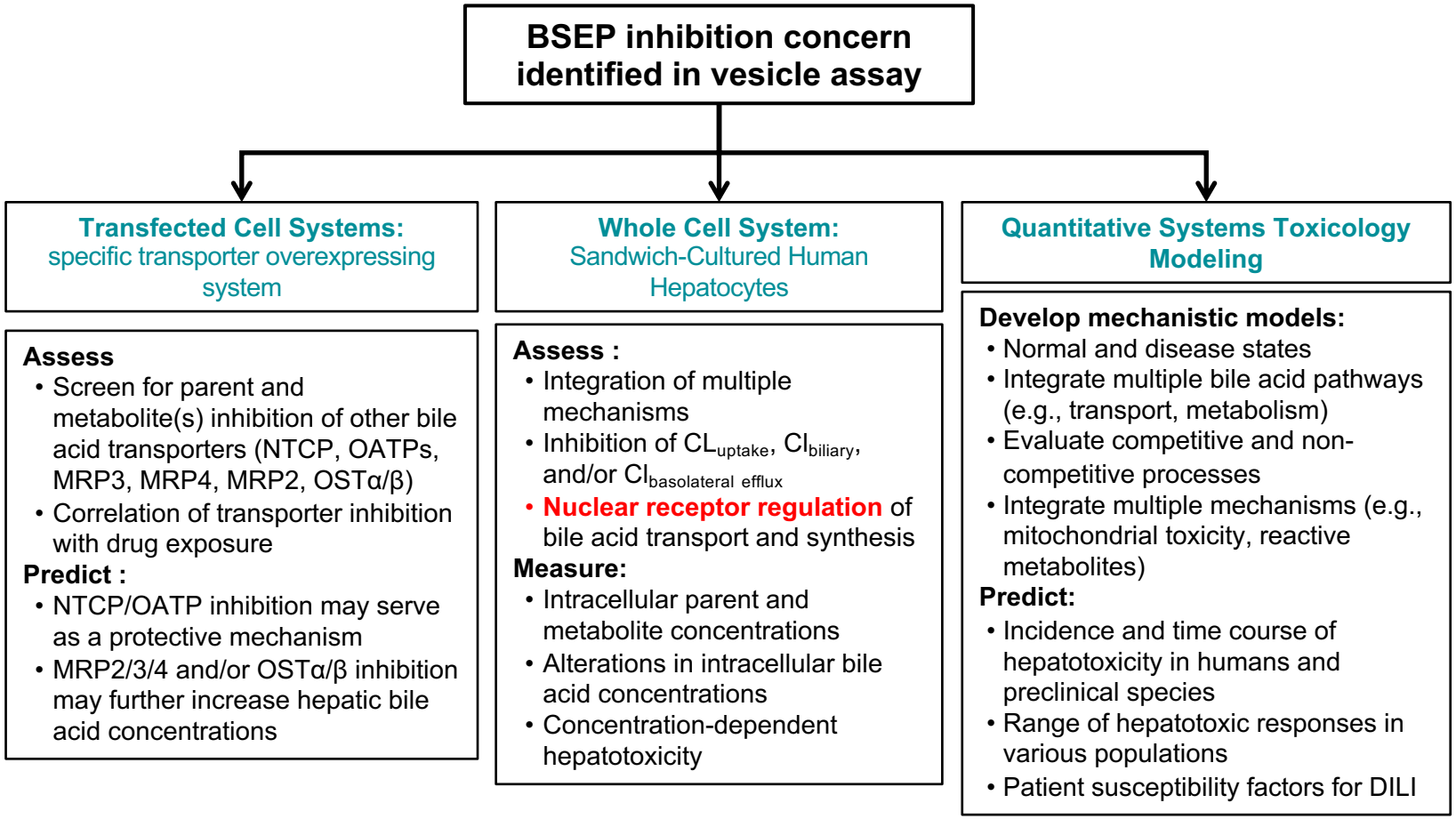


B-CLEAR® Sandwich-Cultured Hepatocytes



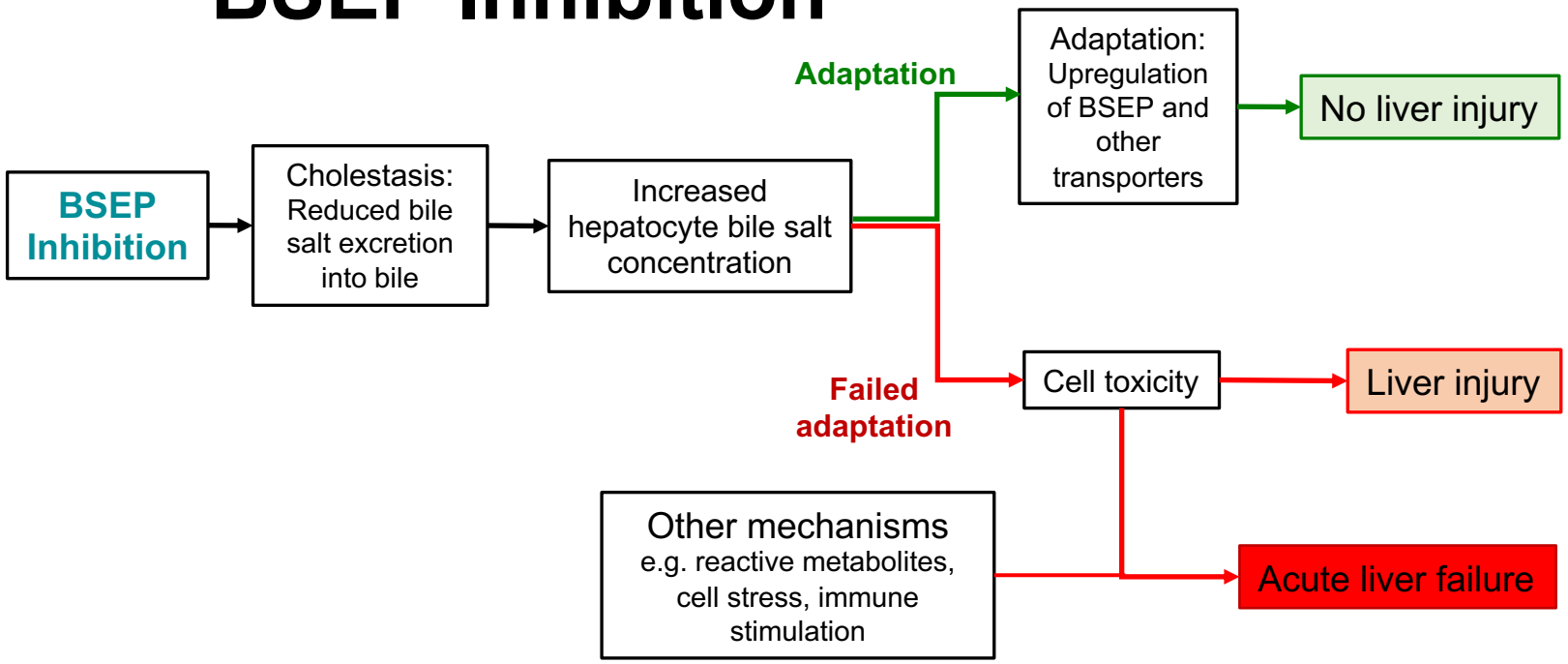
- Normal **cell polarity re-established**
- Uptake **and** efflux transporters functioning
- Regulatory pathways are intact and functioning

Changing Opinions: International Transporter Consortium Perspective



J. Gerry Kenna, Kunal S. Taskar, et. al. Can Bile Salt Export Pump Inhibition Testing in Drug Discovery and Development Reduce Liver Injury Risk? An International Transporter Consortium Perspective. Clinical Pharmacology & Therapeutics, Vol 104, No 5, November 2018

Importance of the Adaptive Response to BSEP Inhibition



Inclusion of the **adaptive response** improves DILI prediction accuracy

- BSEP inhibition "triggers" the adaptive response
- A secondary insult required to cause cholestatic DILI such as:
  - Basolateral Efflux Inhibition (MRP3/4 and/or OSTα/β) and/or
  - FXR Antagonism

It is the **NET effect** of all these processes on bile acid disposition (adaptive response) that determine the cholestatic drug induced liver injury potential of a compound.

Summary

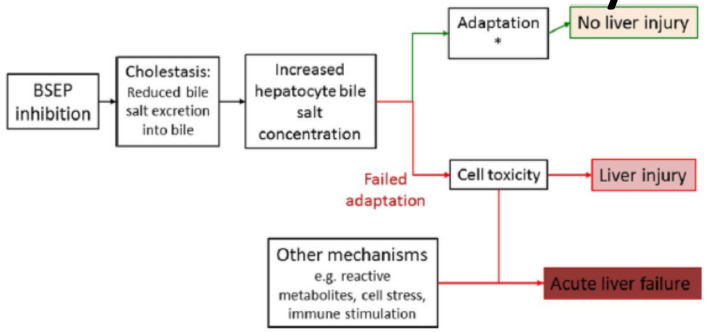


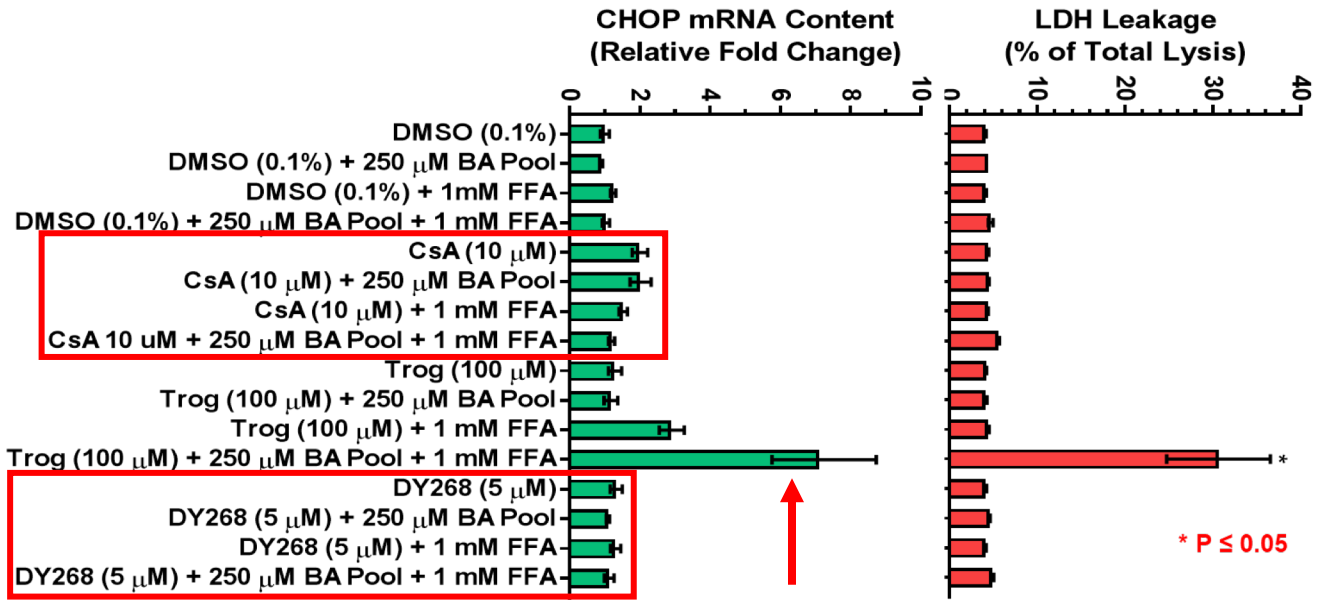
Figure 2 Proposed role of bile salt export pump (BSEP) inhibition in drug-induced liver injury. *Adaptation may arise via upregulation of BSEP expression and upregulation or downregulation of other hepatic plasma membrane efflux or uptake transporters, respectively, plus intracellular mechanisms that include farnesoid X receptor (FXR)-mediated downregulation of bile acid synthesis (see text for details).

- Inclusion of the adaptive response improves C-DILI potential prediction accuracy
  - BSEP inhibition "triggers" the adaptive response
  - A secondary insult required to cause cholestatic DILI such as:
    - Basolateral Efflux Inhibition (MRP3/4 and/or OSTα/β) and/or
    - FXR Antagonism
- Increasing acceptance of the new paradigm within scientific community (e.g. ITC, AOP)
- DILI prediction accuracy improves with the use of more physiological-relevant in vitro models
  - Understanding of the MOA is important to developing assay
  - Properly characterized model to ensure recapitulation of "normal" function
  - "Sensitization" of model to create diseased/susceptible phenotype
    - Steatosis/NASH individuals may be more susceptible to bile-acid induced hepatotoxicity
- C-DILI Assay provides mechanistic information
  - BA-dependent (cholestatic) or independent (general mechanism e.g. reactive metabolite)
- C-DILI Assay is hepatocyte focused
  - Bile-acid induced injury can also occur down-stream of the hepatocyte
    - Bile duct blockage due to inflammation or "sludge" formation

Linking Cell Death Initiation with Cytotoxicity

CCAAT/enhancer-binding protein homologous protein (CHOP) is a key marker of ER stress and early initiator of cell death

- ER stress initiates bile acid induced programmed cell death
- CsA (BSEP inhibition) and DY268 (FXR antagonist) were negative
  - Each only has one of the required characteristics for bile-induced hepatotoxicity



- **Troglitazone** has **BSEP** inhibition**, FXR** antagonism**, and OSTα/β** inhibition
- Concomitant **increases** of **CHOP mRNA** and **LDH leakage** only in hepatocytes treated with Troglitazone under sensitization conditions