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Current trends in BSEP inhibition and perturbation to bile acid homeostasis as mechanisms of drug-induced liver injury

Session 6: Future directions

A Predictive Safety Testing Consortium Webinar Series

CRITICAL PATH INSTITUTE

Session 6: Agenda

All participant lines are muted

#	Title	Presenter	Time
1	Introduction to Series	John-Michael Sauer (Critical Path Institute)	5 minutes
2	Introduction to session theme and speaker biosketches)	Gerry Kenna, Drug Safety Consultant	5
3	Combining BSEP inhibition data with mathematical modeling to impact decisions in drug development	Brett Howell, DILIsym Services	20
4	Recommendation to the regulatory agencies concerning BSEP screening in drug discovery and development	Gerry Kenna	20
5	The future: How can we improve DILI prediction based on alterations in bile acid homeostasis.	Paul Watkins, UNC School of Pharmacy	20
6	Q&A and general discussion	John-Michael Sauer, C-Path	35
7	Meeting summary, closing remarks, next steps	John-Michael Sauer	15

Series Overview

	Session Title	Date	Time
1	Clinical examples of BSEP inhibition and hepatotoxicity	Thursday, May 26, 2016	
2	Clinical examples of BSEP inhibition and hepatotoxicity & *In vivo* tools for confirming perturbation to bile acid homeostasis	Friday, June 3, 2016	
3	*In vivo* tools for confirming perturbation to bile acid homeostasis	Tuesday, June 7, 2016	10:00 – 12:00 US ET (14:00 – 16:00 UTC)
4	*In vitro* tools for identifying a BSEP liability	Wednesday, June 15, 2016	
5	*In vitro* tools for identifying a BSEP liability	Friday, June 22, 2016	
6	Future direction and next steps	Thursday, July 14, 2016	

Session 6: Agenda

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Combining BSEP Inhibition Data with Mathematical Modeling to Impact Decisions in Drug Development

PSTC BSEP Webinar Series, Session 6

July 14, 2016

Brett A. Howell, Ph.D,
Associate Director, DILI-sim Initiative
Chief Executive Officer, DILIsym Services Inc.

Conflict of Interest Declaration

Brett A. Howell is an employee and stockholder of DILIsym Services Inc., which serves as the Coordinating Member of the DILI-sim Initiative.

Outline of Primary Discussion Points

- Key BSEP questions to address moving forward
  - Exposure
  - Type or mode of inhibition
  - Complexity of multiple transporters and pathways
- Approaches taken within the DILI-sim Initiative
  - Experimental
  - Mathematical modeling
- Summary

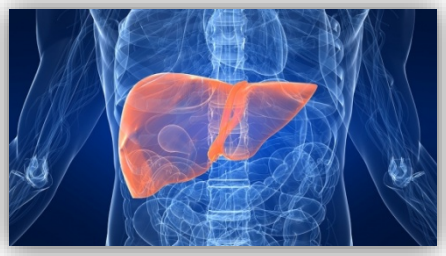
Key BSEP Questions to Address Moving Forward in Drug Development

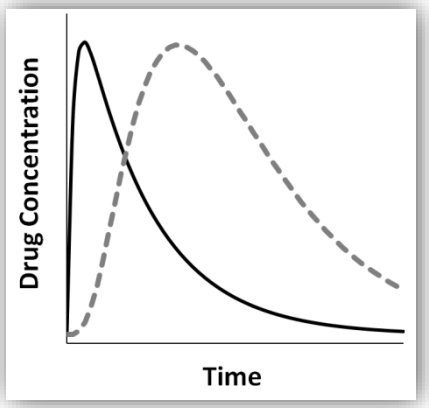
- Overwhelming evidence suggest bile acid transporter inhibition is important within drug safety
- What factors can be leveraged to add understanding and support decision making?

1. **Exposure** – organ versus systemic, kinetics (short and long term), free versus total, etc.
2. **Type or mode of inhibition** – how does the inhibitor inhibit?
3. **Complexity of multiple transporters and pathways** – can we focus on one transporter or should we be more holistic?

Relevant Drug Exposures for Transporter Effects are Complex and Dynamic

- Site of action – organ versus systemic?
  - Many compounds are present in tissues at much higher concentrations than plasma
- Kinetics of exposure at the site of action
  - Exposure effect will be a combination of magnitude and duration (peak and AUC)
  - Clinical dosing protocols vary significantly in duration
  - Organ concentration relationship to systemic concentration may be complex for active transport substrates
  - Dosing regimen may also play a role (QD, BID, etc.)
- Protein binding considerations
  - Free versus total
  - Cytosolic versus localized
  - Tight binding versus loose binding

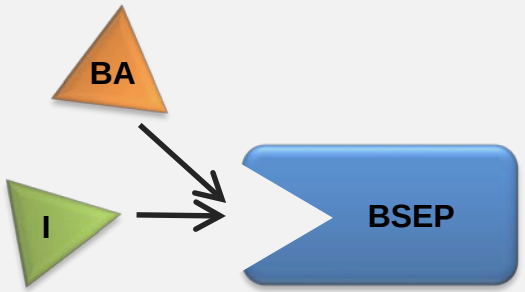




Competitive Inhibition Alters Affinity Whereas Noncompetitive Alters Capacity

- Competitive inhibition involves drug and bile acids competing for same active site on a transporter
  - Affects *affinity* for the bile acid, i.e. K_m
- Noncompetitive inhibition involves drug preventing bile acid from binding on the transporter altogether
  - Affects *capacity* with respect to bile acid, i.e. V_{max}
- Mixed inhibitors display characteristics of both types (α value indicative of extent for each)

Competitive Inhibition



$$\frac{d[BA]}{dt} = \frac{V_{max}[BA]}{K_m(1+\frac{[I]}{K_i})+[BA]}$$

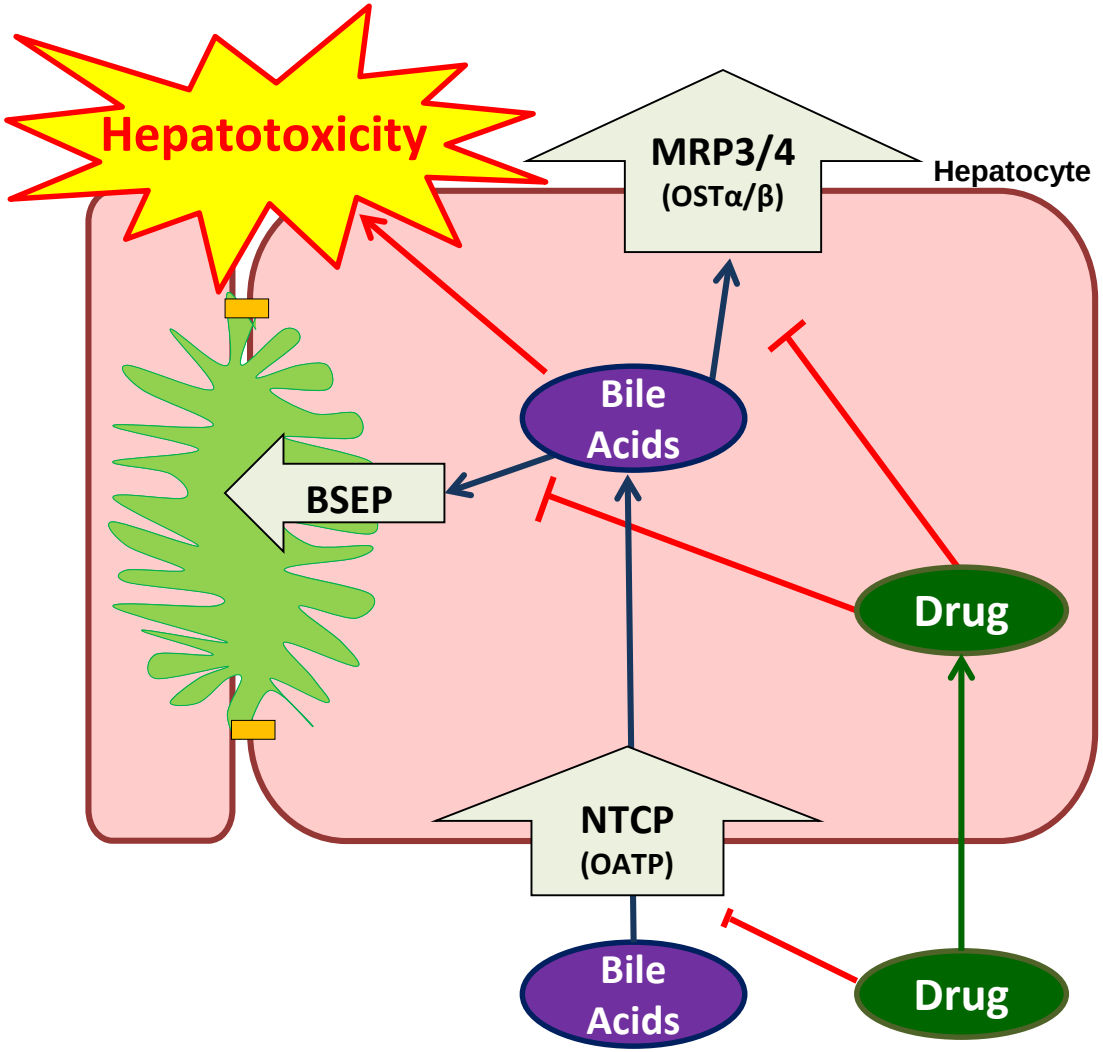
Noncompetitive Inhibition

$$\frac{d[BA]}{dt} = \frac{\frac{V_{max}}{(1+\frac{[I]}{K_i})}[BA]}{K_m+[BA]}$$

**BA – bile acids; I – inhibitor*

DILI-sim Initiative

Multiple Efflux and Uptake Transporters Contribute to Bile Acid Build-up



- Drugs can interfere with multiple bile acid transport processes such as uptake, canalicular efflux, and basolateral efflux
- Metabolites can also contribute, complicating the picture
- Regulation of transporter expression over time further complicates outcomes

Outline of Primary Discussion Points

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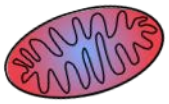


DILI-sim Initiative

The DILI-sim Initiative has Focused on Development of a QSP Framework of DILI to Address Complex Questions

- *Overall Goals*
  - *Improve patient safety*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *16 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*

FDA

DILI-sim Initiative

The DILIsym Team Has Begun Recommending Ki Assessments When Simulations Suggest Sensitivity to Type

Inhibition constant	IC_{50}	K_i
Definition	Inhibitor concentration at the half maximal activity	Affinity of the inhibitor to the probe substrate binding site
Experimental methods	Transport assays with one substrate concentration & multiple inhibitor concentrations	Transport assays with multiple substrate concentrations & multiple inhibitor concentrations
Robustness	Varies depending on the substrate concentrations IC_{50} will approach K_i, if [S] << K_m	A more robust parameter
Provide information on the type of inhibition?	No	Yes
Cost	$	$$$
Comment	Commonly measured	Recommended for reliable prediction of hepatotoxicity

A Case Study: Use of BSEP Vesicles to Assess AMG 009 Ki and Type of Inhibition

- Vesicles: Sf9-BSEP
  - Control vesicles not used because TC transport in control vesicles was negligible
- Substrate
  - TC: 0.46, 1.4, 4.2, 12.6, 37.8 µM
- Inhibitors
  - Vehicle control: 1.3% DMSO (v/v)
  - AMG 009: 0.007, 0.02, 0.06, 0.18, 0.55, 1.64, 4.93, 14.8, 44.3, 133 µM
- Incubation conditions: 15 min, 24°C
- Assay groups

Assay group	Number of wells	
	ATP	No ATP Control
AMG 009	3 X10 (AMG 009) X 5 (TC)	-
Vehicle control	3 X 5 (TC)	3 X 5 (TC)

Van Staden (2012) Current Protocols in Toxicology
Morgan (2013) Toxicological Sciences
Experiments done by Ryan Morgan, Amgen

A Case Study: Use of BSEP Vesicles to Assess AMG 009 Ki and Type of Inhibition

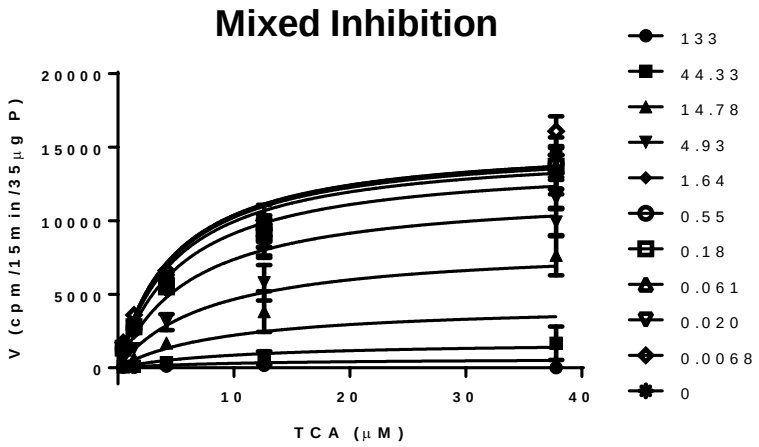
- ATP-dependent TCA transport data are presented as substrate concentration versus transport velocity for each inhibition concentration
- The kinetic parameters (K_m, V_{max}, and K_i) and type of inhibition were determined by fitting competitive, noncompetitive, uncompetitive, and mixed models to the untransformed data by nonlinear regression analysis
  - The best-fit model was assessed from visual inspection of the observed versus predicted data and Akaike Information Criterion (AIC)

Competitive: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S}$

Noncompetitive: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{K_i}\right)}$

Uncompetitive: $V = \frac{V_{max} \times S}{K_m + S \times \left(1 + \frac{I}{K_i}\right)}$

Mixed: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{\alpha \times K_i}\right)}$

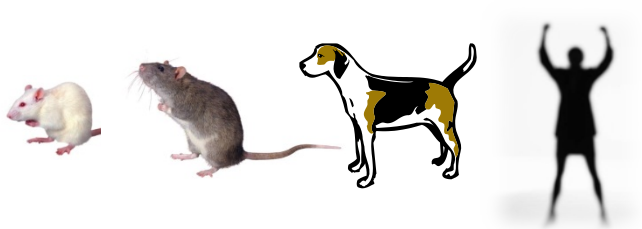


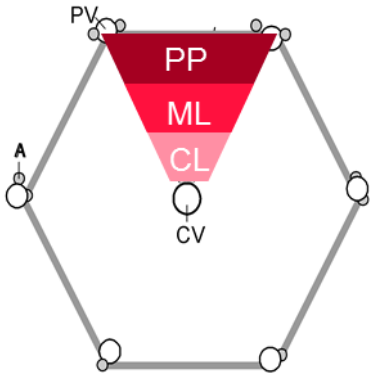
AMG 009 Ki = 2.4 µM
Type of Inhibition: mixed (α = 2.4)

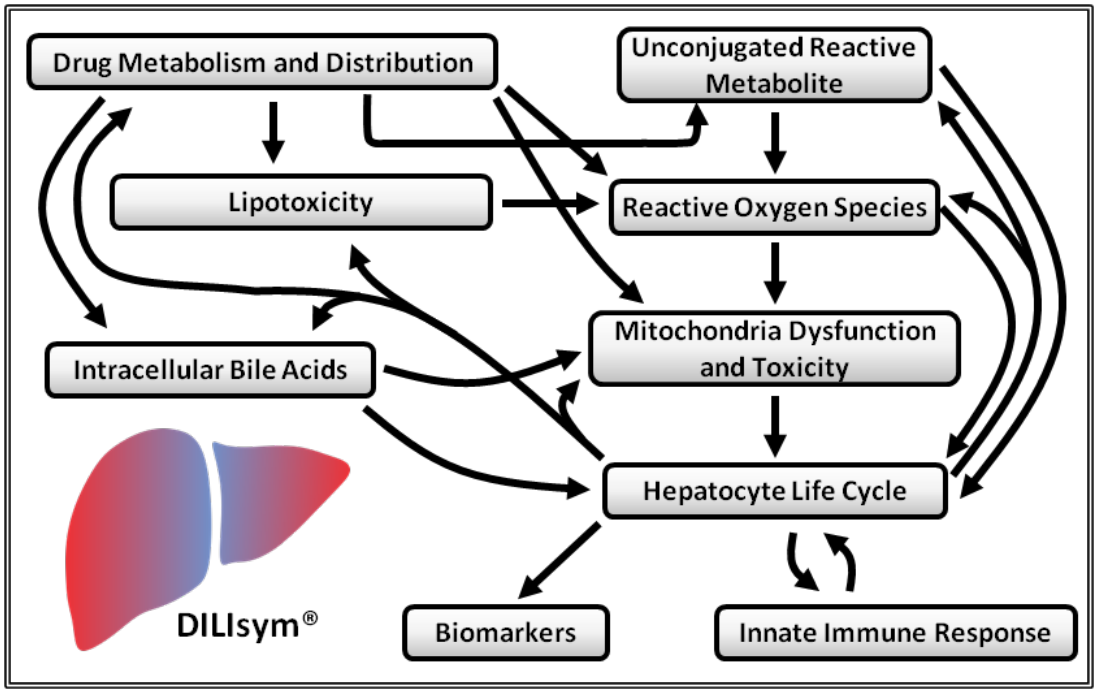
DILI-sim Initiative

QSP Tool Overview - DILIsym

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential processes represented to multiple scales in interacting sub-models**
  - **Pharmacokinetics**
  - **Injury pathways**
  - **Hepatocyte life cycle**
  - **Immune response**
  - **Biomarkers**







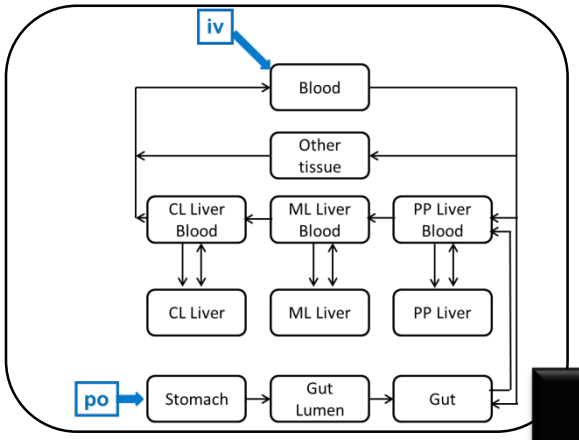
DILI-sim Initiative

AMG 009 and AMG 853 Represented as a Lead/Backup Drug Candidate Pair in DILIsym

- Amgen's AMG 009 and 853 were lead and backup drug candidates for the same target for the treatment of asthma and are included in DILIsym as exemplar compounds
- Dose-dependent hepatotoxicity signals observed for AMG 009 during phase I multiple dose studies, but not in preclinical studies
  - Serum transaminase elevations observed in 5 out of 8 subjects administered 100 mg bid; returned to normal upon cessation of treatment
  - No hepatotoxicity observed at 25 mg and 50 mg bid
- No hepatotoxicity signals observed for AMG 853 during clinical studies
  - Drug failed due to lack of efficacy
- Bile acid transporter inhibition was measured by Amgen for both compounds and other mechanisms were assessed and ruled out
- ***How can mechanistic modeling of DILI utilizing PK data and in vitro transporter data (including Ki values) be used?***

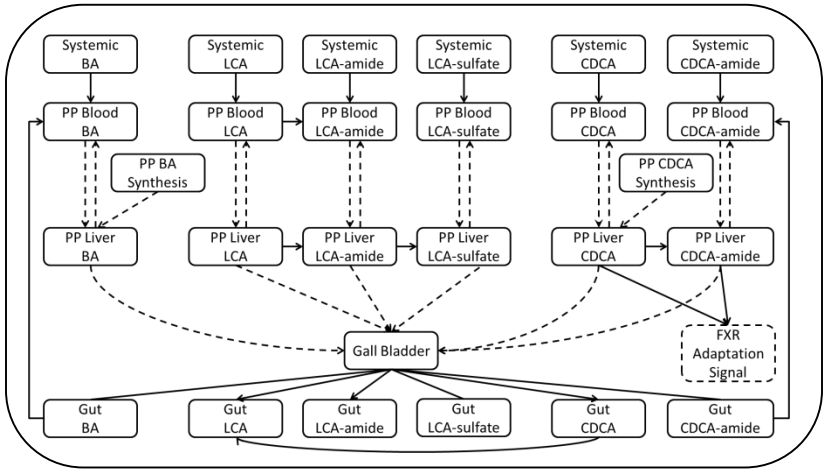
Bile Acid Transport Inhibition Model

Drug PBPK model



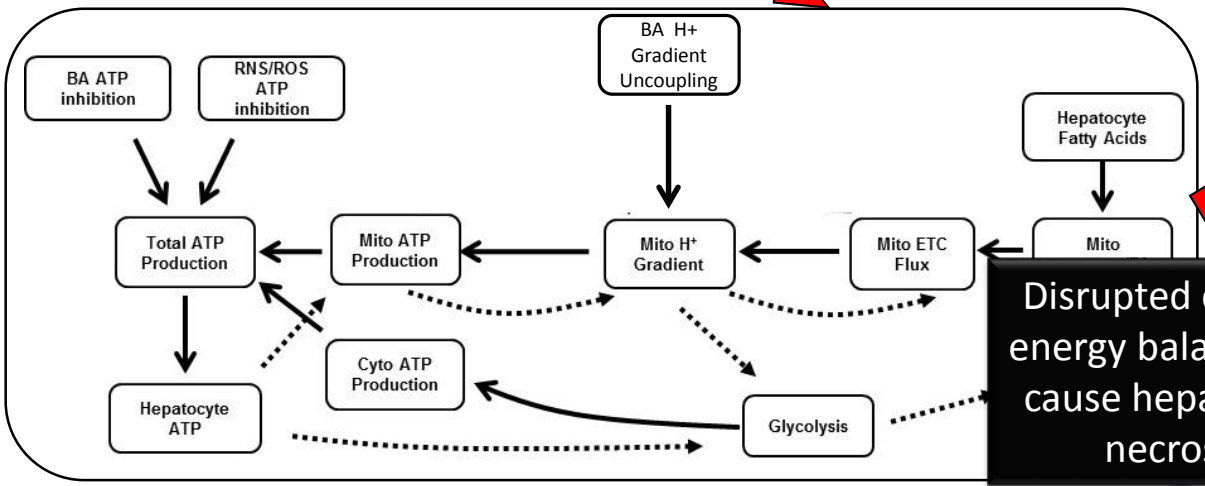
Drug inhibits BA transport

Bile Acid Homeostasis Model

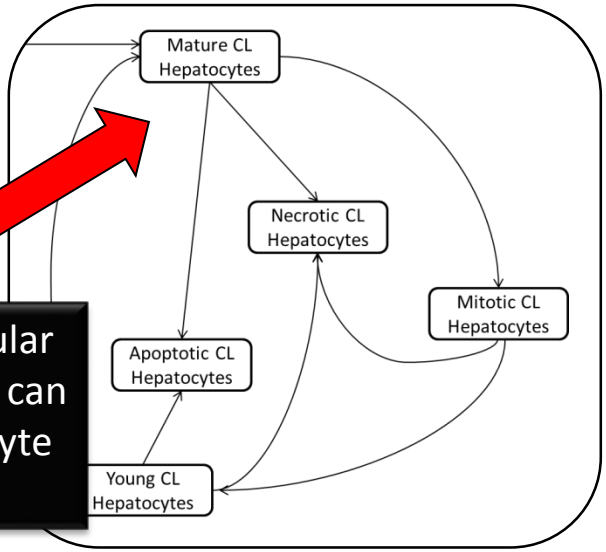


Bile acid accumulation disrupts cellular energy balance

Cellular ATP Model



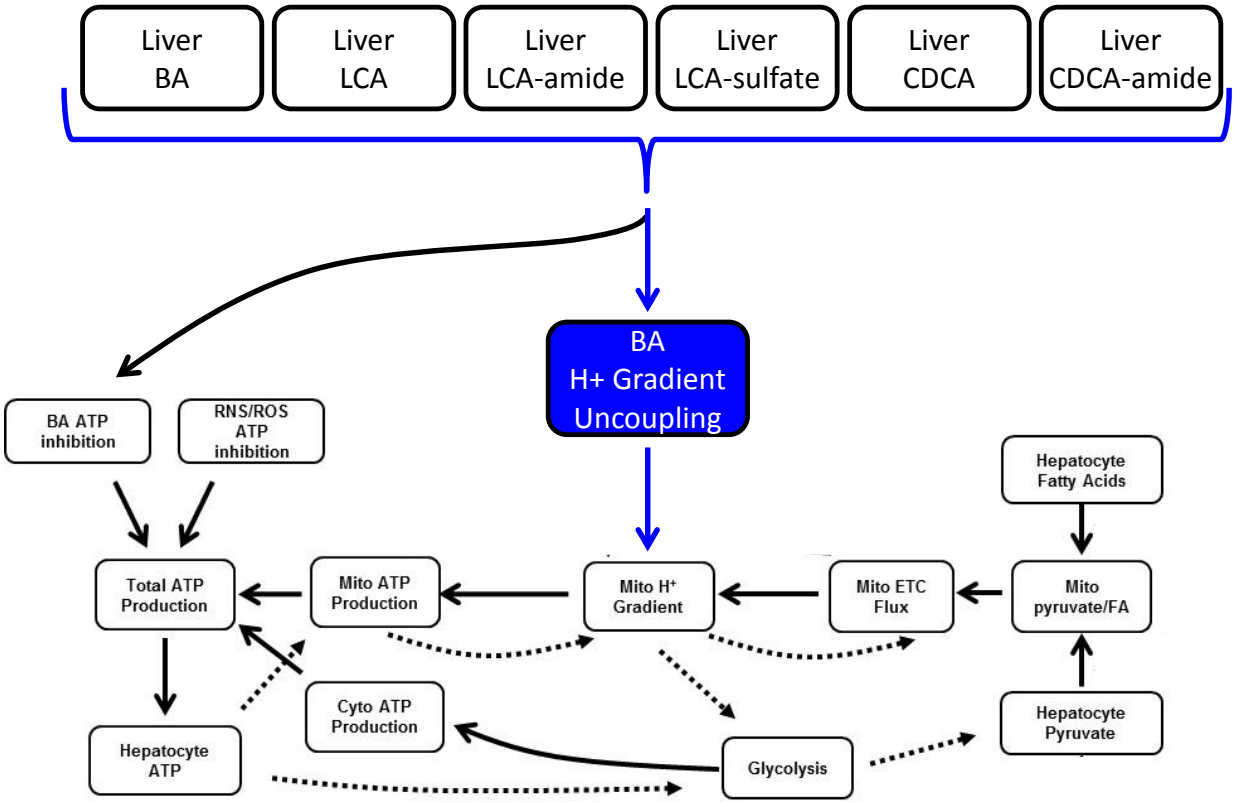
Hepatocyte Life-Cycle



Disrupted cellular energy balance can cause hepatocyte necrosis

DILI-sim Initiative

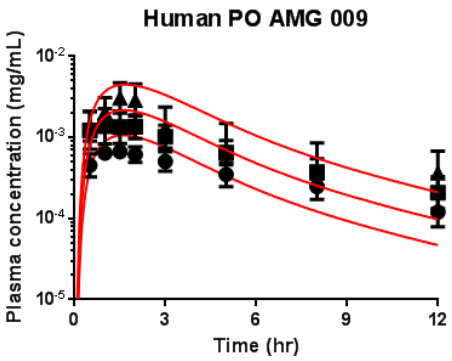
Bile Acid Accumulation Affects Mitochondrial Function in DILIsym



- Hepatic bile acids cause reductions in mitochondria proton gradient
  - BA H+ gradient uncoupling
- Parameter optimization based on Yang et al. hepatocyte studies (Yang, 2016)
  - Measured ATP and cell death following exposure to different levels of bile acids
  - Supported by DILI-sim Initiative
- Alternate representation still available as an option
  - Direct effect on ATP synthesis

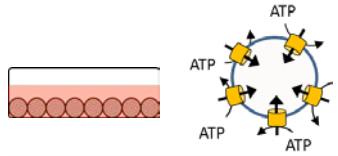
DILI-sim Initiative

AMG 009/853 Modeling Approach



PBPK Modeling
- *In vitro* PK data
- *In vivo* PK profile
- Parameter optimization

Mechanistic Toxicity Data
- Bile acid inhibition for AMG 009 and 853 (Ki, type of inhibition)



Transporter	Ki (μM)	Type
Human BSEP	2.4	Mixed (α = 2.4)
Rat Bsep	5.6	Mixed (α = 34)
Human MRP4	12.9	Mixed (α = 2.1)
Human NTCP	126.5*	-
Rat Ntcp	48.4*	-

*AMG 009 transporter data shown

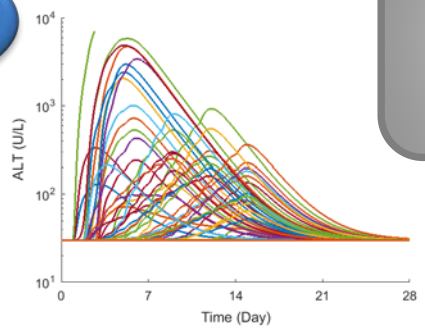
PBPK model and toxicity data inputs were not modified after hepatotoxicity simulations

Combine PK and *in vitro* Tox

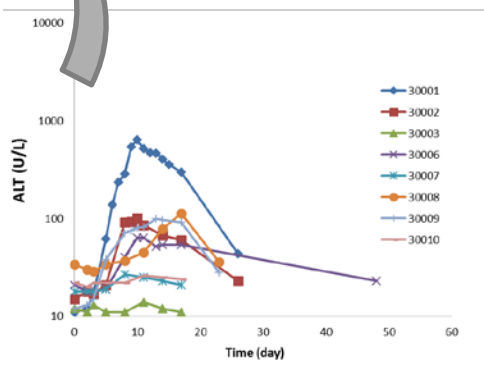
Further optimization using AMG 009/853 clinical toxicity data was not necessary

Hepatotoxicity Simulation
- Baseline
- Population (SimPops™)

Stop criteria used*



Compare to clinical data



*ALT screening before AM dosing on days 2,3,4,5,8,11, and 14
If ALT>3X ULN, dosing discontinued after 24 h
Employed to recapitulate the clinical protocol of AMG 009

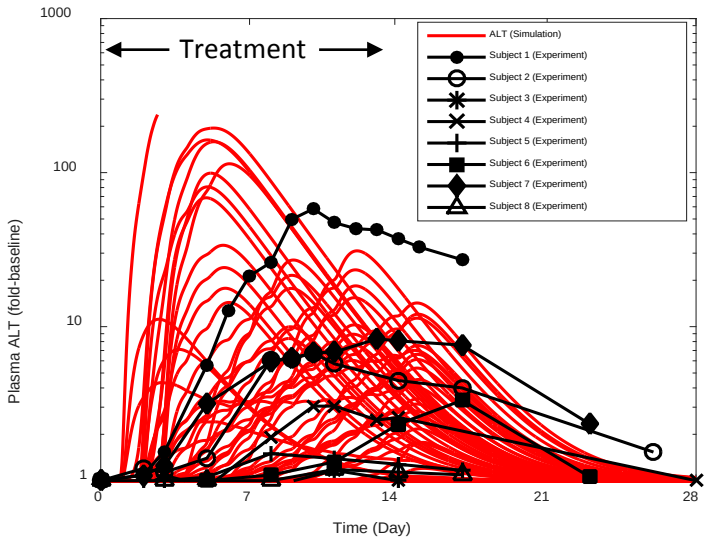
Clinical Data and Simulation Results

DILI-sim Initiative

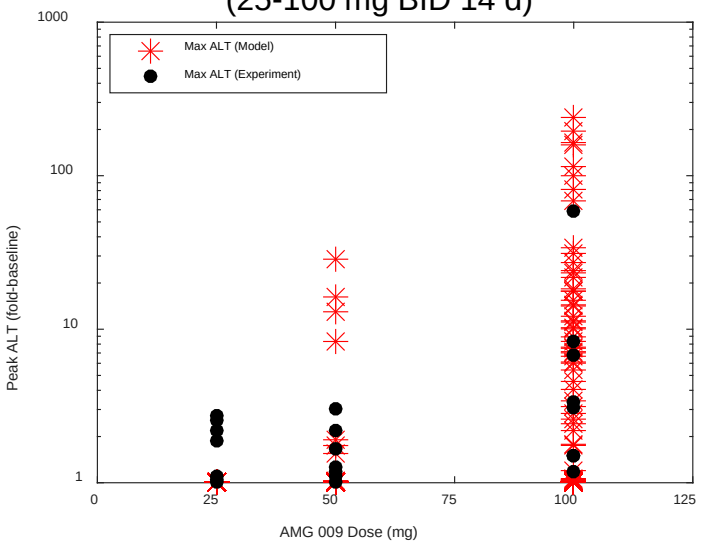
DILIsym Predicts Dose-Dependent AMG 009 Hepatotoxicity in Human

HUMAN

100 mg BID 14 d



Dose-Response
(25-100 mg BID 14 d)



	Clinical Trial	DILIsym Simulation		
	100 mg	25 mg	50 mg	100 mg
ALT > ULN	5/8 (62.5%)	0/212 (0%)	7/212 (3.3%)	44/212 (20.8%)
ALT > 3X ULN	1/8 (12.5%)	0/212 (0%)	4/212 (1.9%)	37/212 (17.5%)
ALT > 10X ULN	1/8 (12.5%)	0/212 (0%)	2/212 (0.9%)	16/212 (7.5%)
Bili > 2X ULN	0/8 (0%)	0/212 (0%)	0/212 (0%)	4/212 (1.9%)
Hy's Law	0/8 (0%)	0/212 (0%)	0/212 (0%)	4/212 (1.9%)

*Mechanistic bile acid toxicity model used
*Human_mito_BA_v3A_6 SimPops™ used for simulation (17 NASH-like patients were excluded)

- DILIsym predicts dose-dependent, delayed presentation of AMG 009 hepatotoxicity and recovery after discontinuation

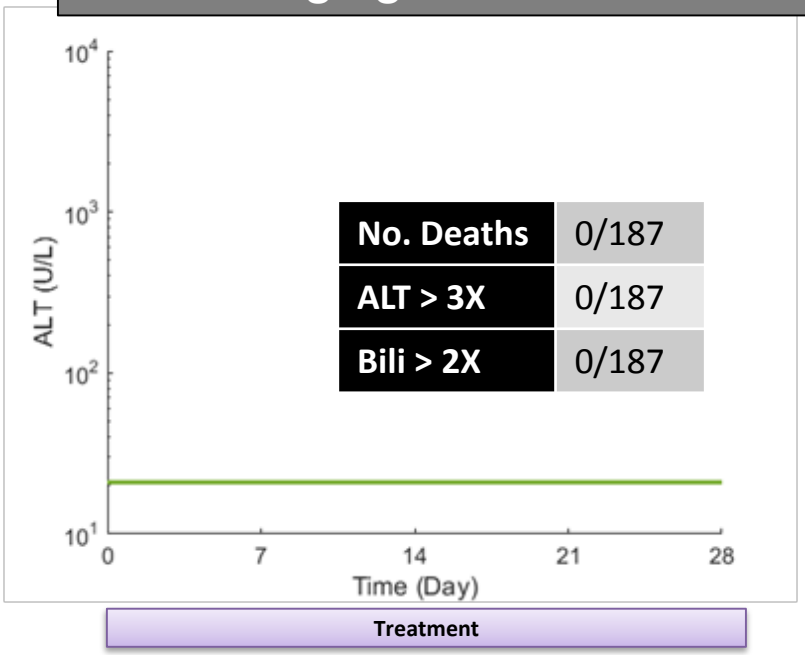
Stop criteria used

Clinical Data and Simulation Results

DILI-sim Initiative

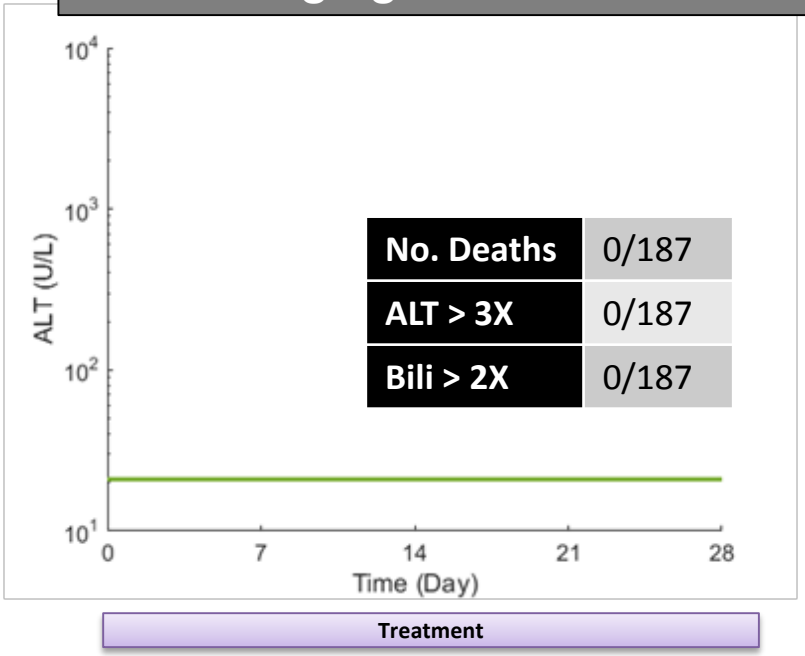
No Hepatotoxicity Predicted in the Rat SimPops™ Administered AMG 009

100 mg/kg IV for 1 month



No. Deaths	0/187
ALT > 3X	0/187
Bili > 2X	0/187

1500 mg/kg PO for 1 month



No. Deaths	0/187
ALT > 3X	0/187
Bili > 2X	0/187

RATS

- No hepatotoxicity predicted in the rat SimPops™ administered AMG 009, consistent with pre-clinical data

†Asbt inhibition by AMG 009 incorporated to explore extra-hepatic effects of AMG 009 on BA disposition observed in rats

Preclinical Data and Simulation Results

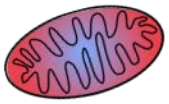
DILI-sim Initiative

DILIsym Correctly Predicted the Lack of Hepatotoxicity for AMG 853

- Amgen also assessed bile acid transporter inhibition for AMG 853, including full velocity curve studies to assess mode of inhibition
- AMG 853 was a more potent BSEP inhibitor than AMG 009 with a Ki = 1.8 µM; however inhibition was competitive
- AMG 009 was a mixed inhibitor with Ki= 2.4 µM and α=2.4
- Additional studies performed to assess direct mitochondrial toxicity and oxidative stress for both compounds showed no *in vitro* signals
- DILIsym predicted relative safety of AMG 853 in simulated humans (SimPops™)
- Exposure to AMG 853 is lower compared to AMG 009 at comparable doses; however, higher doses were simulated in DILIsym up to 50X the clinical dose and AMG 853 remained safe

Compound	Clinical DILI	BA Inhibition	Mito tox	RM	ROS/ RNS	DILIsym prediction
AMG 009	Dose-dependent hepatotoxicity	Yes	No liability	No liability	No liability	Dose-dependent hepatotoxicity
AMG 853	No	Yes	No liability	No liability	No liability	No

Clinical Data and Simulation Results



DILI-sim Initiative

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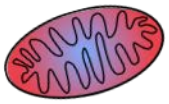

DILI-sim Initiative

Summary of Key Points

- Overwhelming evidence suggest bile acid transporter inhibition is important within drug safety
- Several factors can be better understood or considered to add context and support decision making
  - Exposure
  - Type or mode of inhibition
  - Complexity of multiple transporters and pathways
- Quantitative systems pharmacology combined with the proper experimental data can be powerful for considering multiple factors in parallel – DILIsym currently being applied in this way
  - AMG009 versus AMG853 example shows how type of BSEP/transporter inhibition can influence outcomes

Acknowledgements

Ryan Morgan - Amgen

DILI-sim members for continued support and guidance

DILIsym Development Team

DILI-sim Initiative

Session 6: Agenda

All participant lines are muted

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7	Meeting summary, closing remarks, next steps	John-Michael Sauer	15

Recommendation to the regulatory agencies concerning BSEP screening in drug discovery and development

Gerry Kenna

Pharmaceutical Director, Safer Medicines Trust

www.SaferMedicines.org

Drug Safety Consultant

SAFER MEDICINES TRUST

Disclaimer

The opinions expressed are mine and are not an agreed consensus position of C-Path, or C-Path's partners and collaborators.

They are intended to stimulate debate and to enable the scientific community to develop a position on an important, rapidly evolving topic.

I hope that my views and opinions are reasonable and are factually accurate. If you disagree, please let me know.

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Overview

- BSEP and Drug Induced Liver Injury (DILI)
  - What we know
  - What we don’t know
- Views expressed by the International Transporter Consortium
- Current EMA recommendations
- The case for pro-active BSEP screening
- A possible BSEP screening cascade
- Possible next steps

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BSEP and DILI – what we know

- Genetically inherited BSEP defects cause human liver injury (PFIC2 etc.).
- Many drugs that cause human idiosyncratic DILI inhibit BSEP activity *in vitro* and *in vivo.*
- When adjusted for in vivo exposure, BSEP inhibition by drugs correlates well with DILI propensity
  - **"Guilt by Inference"**
- For some drugs, BSEP inhibition appears to be the only plausible explanation for human DILI.
- Other drugs that cause DILI exhibit one or more additional liabilities in addition to BSEP inhibition.
- Drugs that inhibit BSEP and cause human DILI typically do not cause DILI in animals.

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BSEP and DILI – what we don't know

- Why BSEP inhibition by drugs causes DILI only infrequently in humans.
- The susceptibility factors that explain why some humans dosed with drugs that inhibit BSEP develop, but most do not.
  - DILI-sim Simpops simulations?
- Biomarkers that can accurately identify at risk patients in clinical trials, or post-licensing.
- Why DILI does not arise in animals dosed with many potent BSEP inhibitors.
  - Species differences in bile acid composition and cytotoxicity?
- Whereas DILI in animals is caused by some BSEP inhibitors, e.g. AZ-CKA.

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International Transporter Consortium

- Has acknowledged emerging data linking BSEP inhibition with cholestatic DILI
- However, pro-active testing of BSEP inhibition is not recommended during drug development because:
  - "Currently (in 2013) it is impossible to define a value for a BSEP inhibition constant that will realistically predict significant BSEP-mediated DILI…."
  - A strategy which enables accurate assessment of the clinical relevance of BSEP inhibition has not yet been devised
- If clinical studies or pre-clinical safety studies provide evidence of cholestatic DILI, the potential contribution of BSEP inhibition should be considered:
  - When such compounds inhibit BSEP, serum bile acid levels should be evaluated *in vivo* in preclinical species (alongside serum alkaline phosphatase and transaminases)
  - If such investigations indicate Bsep inhibition in animals *in vivo*, evaluation of serum bile acids is recommended to aid ongoing clinical safety assessment

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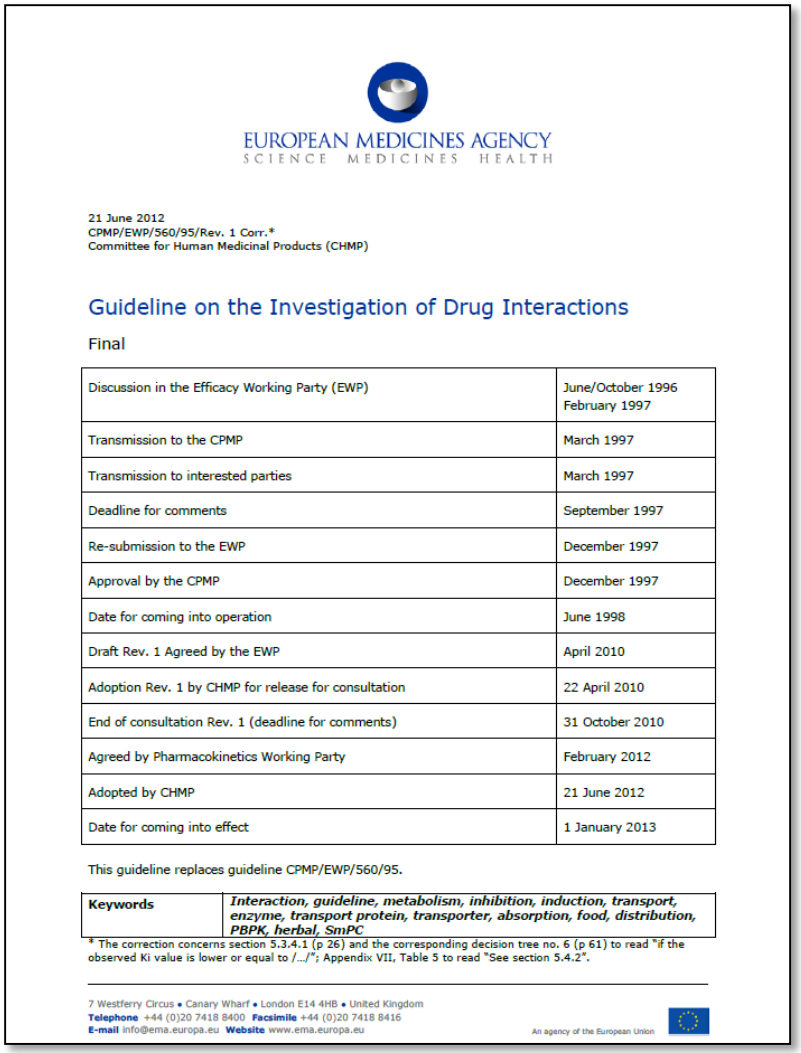
Hillgren *et al.* 2013, Clin Pharmacol Ther 94:52-63

EMA Regulatory Guidance

- Highlights need to consider transporter inhibition
  - P-g, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, BCRP (plus OCT1, MATE1, MATE2)
- Inhibition of BSEP should also preferably be investigated
  - If *in vitro* studies indicate BSEP inhibition, adequate biochemical monitoring including serum bile salts is recommended
- *In vitro* data on transporter inhibition should preferably be available before initiating Phase III



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 June 2012
CPMP/EWP/560/95/Rev. 1 Corr.*
Committee for Human Medicinal Products (CHMP)

Guideline on the Investigation of Drug Interactions

Final

Discussion in the Efficacy Working Party (EWP)	June/October 1996 February 1997
Transmission to the CPMP	March 1997
Transmission to interested parties	March 1997
Deadline for comments	September 1997
Re-submission to the EWP	December 1997
Approval by the CPMP	December 1997
Date for coming into operation	June 1998
Draft Rev. 1 Agreed by the EWP	April 2010
Adoption Rev. 1 by CHMP for release for consultation	22 April 2010
End of consultation Rev. 1 (deadline for comments)	31 October 2010
Agreed by Pharmacokinetics Working Party	February 2012
Adopted by CHMP	21 June 2012
Date for coming into effect	1 January 2013

This guideline replaces guideline CPMP/EWP/560/95.

Keywords	***Interaction, guideline, metabolism, inhibition, induction, transport, enzyme, transport protein, transporter, absorption, food, distribution, PBPK, herbal, SmPC***

* The correction concerns section 5.3.4.1 (p 26) and the corresponding decision tree no. 6 (p 61) to read "if the observed Ki value is lower or equal to /.../"; Appendix VII, Table 5 to read "See section 5.4.2".

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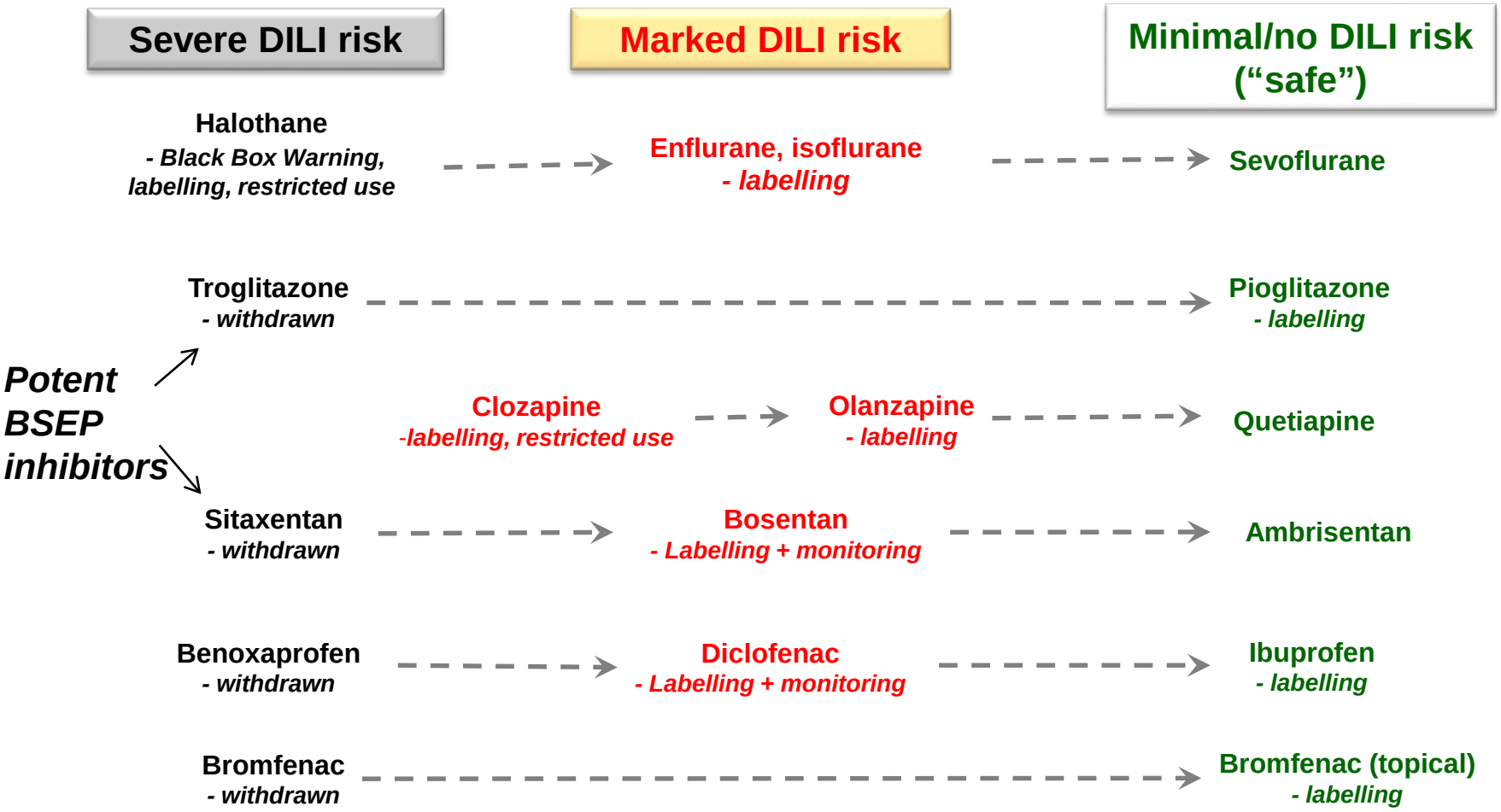
An agency of the European Union

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf

DILI screening need



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DILI screening purpose

Goals are to:

1. Reduce the incidence and severity of liver injury in animals, in preclinical safety studies
2. Reduce the likelihood that drugs which enter clinical trials will cause DILI in humans

- "Flagging" likely problem compounds early, when chemical choice is available and their de-selection is relatively cheap

Current ITC and EMA recommendations do not address this need

DILI screening opportunity

Adapted from: Ulrich (2007) Annu Rev Med 58:17
Thompson et al (2010) Chem-Biol Interact 192:65

Drug

Step 1 **Hepatic uptake**

Step 2 **Chemical insult in liver** ⬅ **e.g. BSEP inhibition**

Step 3 **Biological response in target cell**
e.g. cell toxicity, stress response, transporter up-regulation

Steps 4… **Biological response in tissue**
e.g. cytokine release, inflammatory cell response

Drug related properties

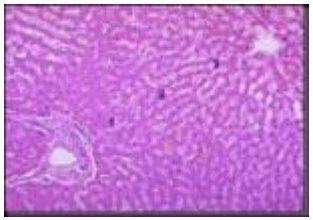
Patient related properties

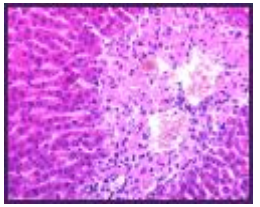
Protection
e.g. stress response

Propagation and amplification
e.g. innate and adaptive immunity

Outcome

Preclinical species <u>vs.</u> man

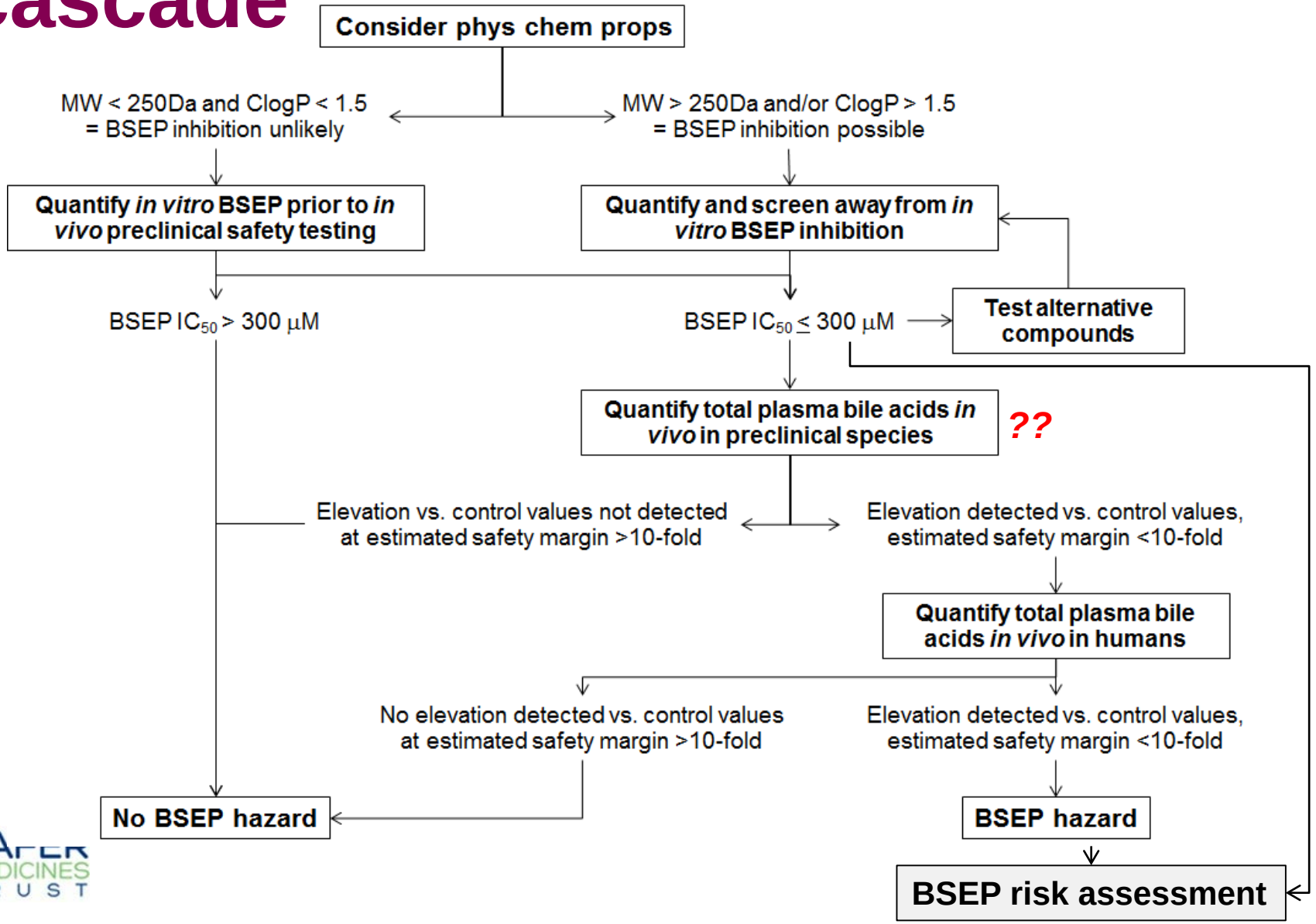




Tolerance & adaptation

Toxicity

A possible BSEP screening cascade



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BSEP risk assessment

- *In vit*ro BSEP potency alone is insufficient –also need to take account of *in v*ivo drug exposure.
- Unbound plasma drug concentrations are uninformative (cf. transporter mediated DDIs).
- Total plasma drug concentrations (bound plus unbound) are more useful.
- Many drugs that inhibit also BSEP also exhibit other DILI liabilities. Best to evaluate these in parallel, wherever possible.
- PBPK based simulations (cf. DILI-sym) will be part of the solution.

No consensus view currently on how best to do this.

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Pros and cons of prospective BSEP screening

Pros

- Human health: enables design and selection of safer drugs.
- Commercial: Competitive advantage via reduced likelihood of expensive late dev failure, failed registration, restrictive labelling or withdrawal post-licensing.
- 3Rs: Tackling a problem not addressed by conventional animal safety testing.

Cons

- Cost of BSEP screening
- Uncertainties:
  - How best to take stop/go decisions and undertake risk assessment?
  - We don't yet know for sure that BSEP screening will reduce human DILI risk. Might we throw out the baby with the bathwater?
  - BSEP inhibition by drugs correlates with human population DILI risk, not DILI risk in individual patients.

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Proposed next steps

- Form an expert group to discuss and recommend a BSEP discovery/development screening cascade
  - hosted by C-Path?
- Draft publications (White Papers?) that:
  - Set out the scientific case for BSEP inhibition as a cause of human DILI (what we know and what we don't yet know)
  - Provide Guidance on how best to generate and interpret *in vitro* and *in vivo* BSEP inhibition data
- Engage with ITC and regulators
- Continue ongoing scientific investigations

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Backup slides

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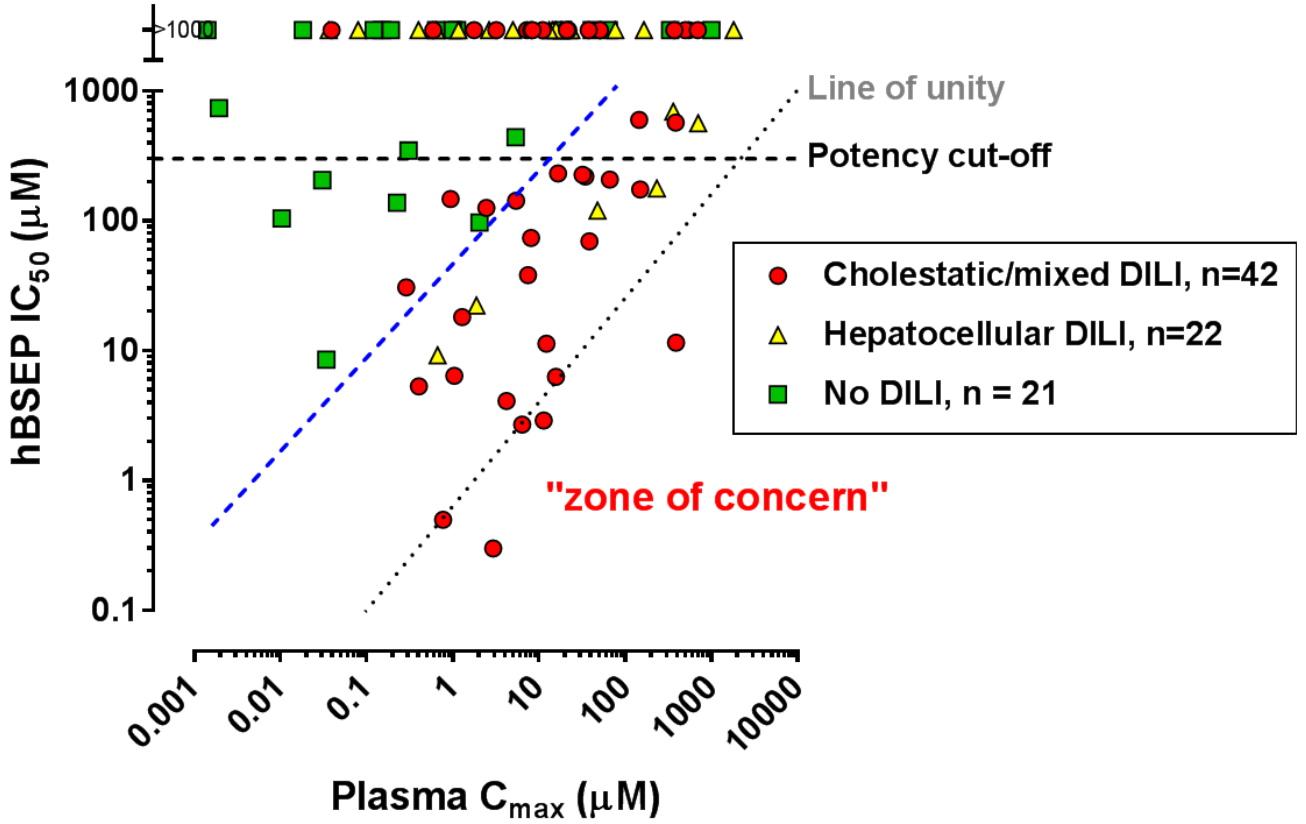
DILI risk is due to potency of BSEP inhibition plus *in vivo* drug exposure



- All tested drugs with BSEP IC_{50} < 300 μM **and** C_{max} > 2 μM caused DILI

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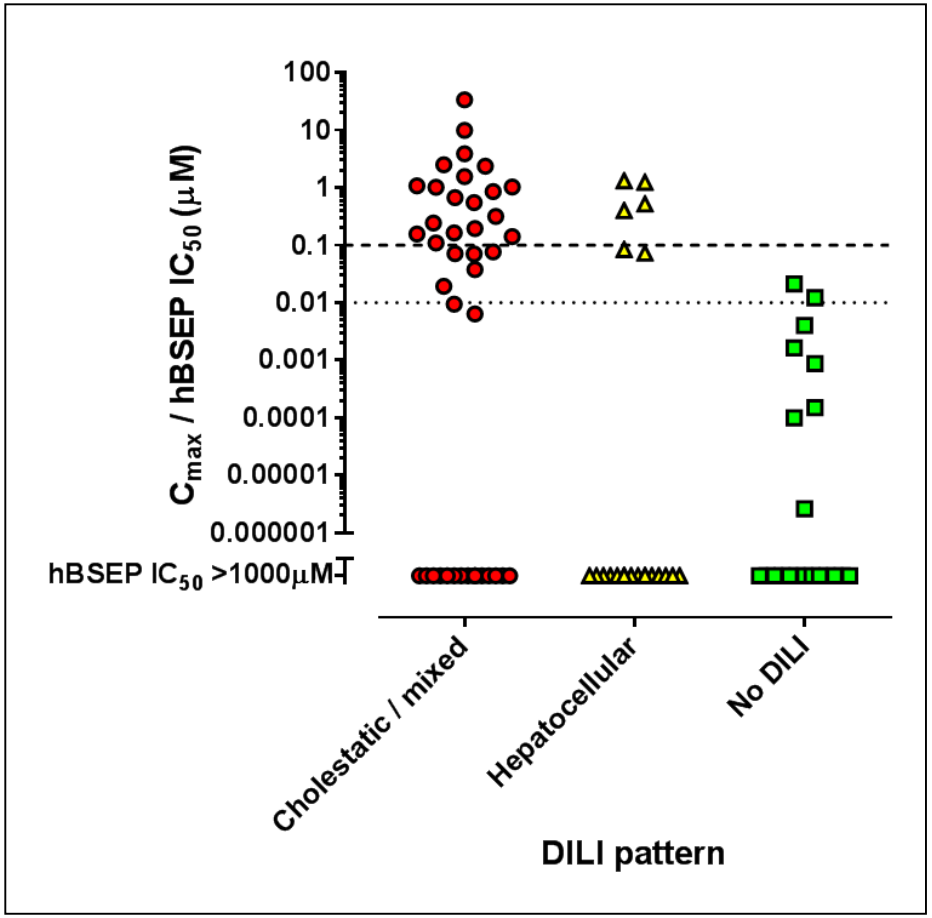
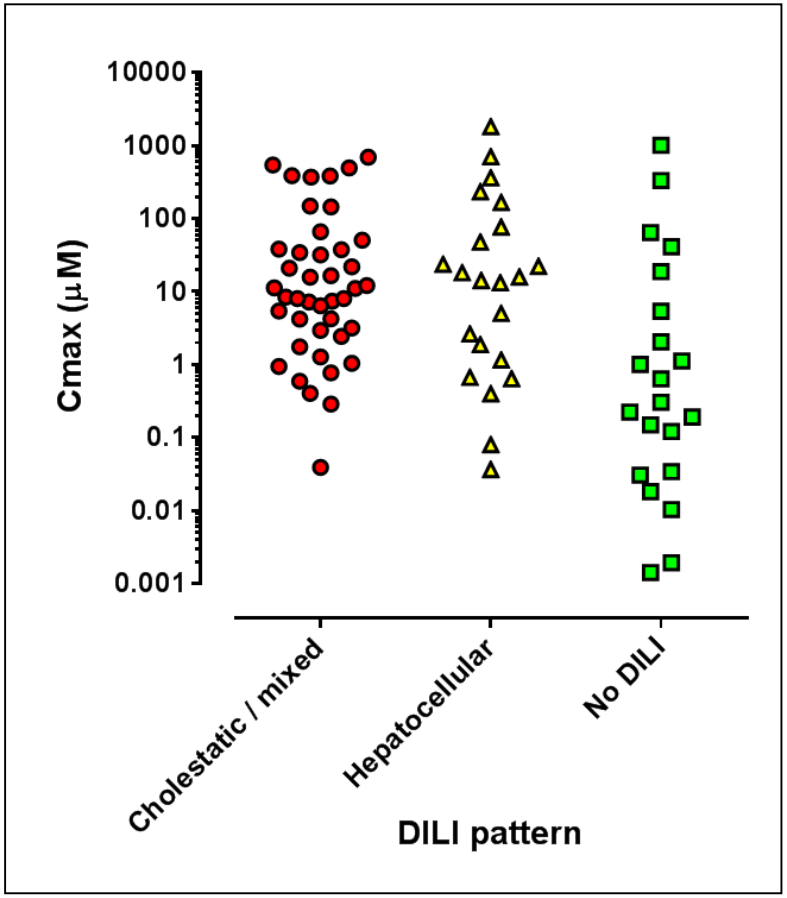
Dawson *et al*. 2012, DMD 40:130–138
See also: Morgan et al. 2013, Tox Sci 136:216-41

BSEP risk assessment: value of [C]/IC_{50}





- A simple way to take account of potency of BSEP inhibition plus total (bound plus unbound) plasma drug concentration (C_{max}, or C_{ss})
- Requires accurate determination of *in vivo* plasma drug concentrations

Data from: Dawson *et al*. 2012, DMD 40:130–138

International Transporter Consortium

Hillgren et al. (2013) Emerging transporters of clinical importance: an update from the International Transporter Consortium. Clin Pharmacol Ther 94(1):52-63.

" **Currently, it is impossible to define a value for a BSEP inhibition constant that will realistically predict significant BSEP-mediated DILI** because:

1) While a trend between low IC_{50} values of BSEP inhibition and DILI was described, no correlation between $C_{max,unbound}$, the potency of BSEP inhibition and DILI was observed.
2) Many drugs form metabolites, some of which may be even more potent BSEP inhibitors than the parent drug and act synergistically.
3) Some drugs may in addition to interference with BSEP also impair mitochondrial function and/or form reactive intermediates. Such a situation may lead to a synergism in toxicity, as elevated intracellular bile salts may aggravate mitochondrial toxicity of a metabolite.
4) There are BSEP inhibitors that require parallel transport activity of BSEP and MRP2 in the same membrane. This was first demonstrated for estradiol 17β-glucuronide and later for drug metabolites.
5) Drugs have been identified that are BSEP inhibitors but are not associated with DILI.

……. **prospective BSEP testing cannot be endorsed at this moment without a strategy to assess clinical relevance of such inhibition**, but *in vitro* characterization of BSEP-drug interactions is certainly warranted after the appearance of cholestatic issues in clinical trials or safety studies."

Session 6: Agenda

All participant lines are muted

#	Title	Presenter	Time
1	Introduction to Series	John-Michael Sauer (Critical Path Institute)	5 minutes
2	Introduction to session theme and speaker biosketches)	Gerry Kenna, Drug Safety Consultant	5
3	Combining BSEP inhibition data with mathematical modeling to impact decisions in drug development	Brett Howell, DILIsym Services	20
4	Recommendation to the regulatory agencies concerning BSEP screening in drug discovery and development	Gerry Kenna	20
5	The future: How can we improve DILI prediction based on alterations in bile acid homeostasis.	Paul Watkins, UNC School of Pharmacy	20
6	Q&A and general discussion	John-Michael Sauer, C-Path	35
7	Meeting summary, closing remarks, next steps	John-Michael Sauer	15

The Future

July 14, 2016

Paul B. Watkins, M.D.
Institute for Drug Safety Sciences
Eshelman School of Pharmacy
University of North Carolina- Chapel Hill

Disclosure

I direct the DILIsim Initiative and own equity in DILIsym Services Inc.

Some Challenges to the Field

1). Need for standardization of transporter assays.

2). Need for physiologically relevant cell-based system(s) to

a). Identify bile acid mediated DILI (e.g. addition of bile acids to media) and also other mechanisms, high content imaging, probes to measure OCR, etc.)

b). Account for metabolites.

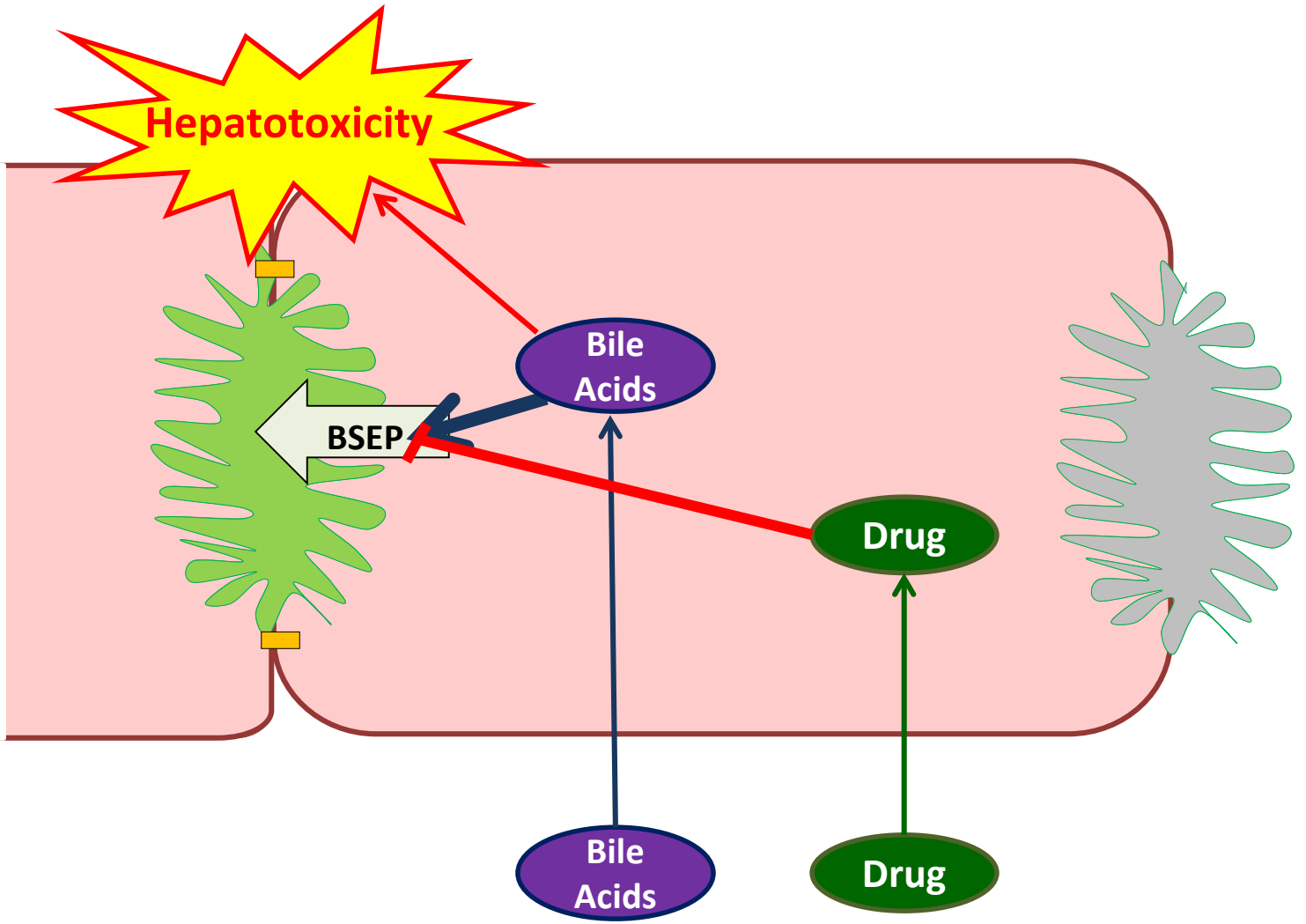
Troglitazone and Pioglitazone

	Troglitazone	Pioglitazone
Hepatotoxicity	Significant	Minimal
Marketing status	Withdrawn due to hepatotoxicity	still on the market
Clinical dose	200-600 mg/day, PO	15-45 mg/day, PO
Cmax	2.82 µg/ml	1.5 µg/ml
Route of elimination	Mostly excreted into bile as metabolites	Mostly excreted into bile as metabolites
BSEP inhibition	TGZ Ki = 1.3 µM TS Ki = 0.23 µM	IC_{50}= 0.4 µM
MRP3 inhibition	TGZ IC_{50} = 31 µM	IC_{50}= 133 µM
MRP4 inhibition	TGZ IC_{50} = 61 µM TS Ki = 8 µM	IC_{50}= 49.5 µM
NTCP inhibition	IC_{50} = 2.3 µM	Ki = 4.04 uM

What I’m going to talk about

The need to characterize and define the role for serum bile acids as translational biomarkers of bile acid mediated DILI.

Hepatotoxicity can be caused by BSEP inhibition – does this always lead to increases in serum bile acids?



Assumption

When BSEP is inhibited, serum bile acids should rise…..

or

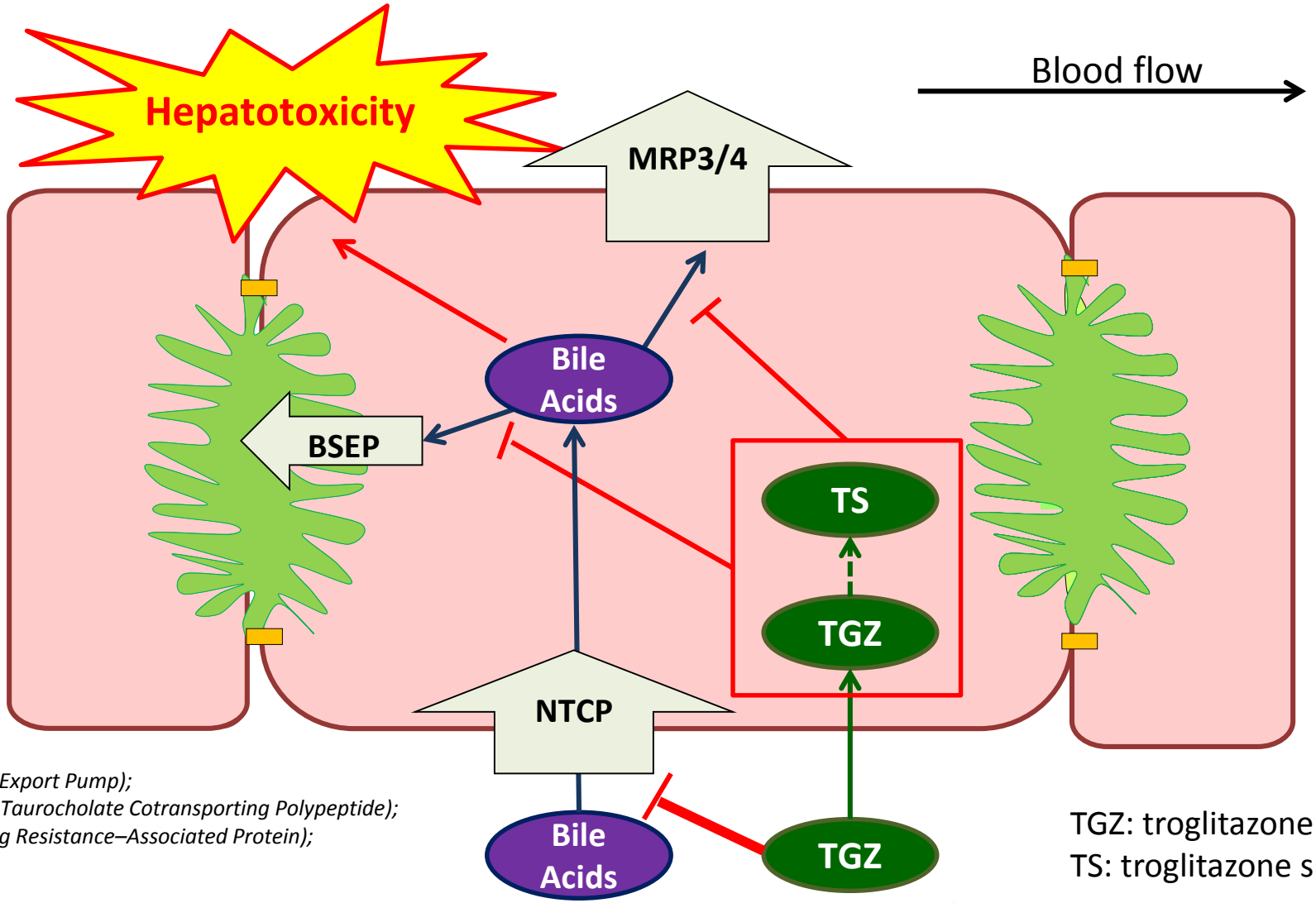
If serum bile acids don't rise, BSEP is not inhibited.

Systems Pharmacology Modeling Predicts Delayed Presentation and Species Differences in Bile Acid–Mediated Troglitazone Hepatotoxicity

K Yang[1], JL Woodhead[2], PB Watkins[1,2], BA Howell[2] and KLR Brouwer[1,3]

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 96 NUMBER 5 | NOVEMBER 2014

Mechanism of Troglitazone DILI



BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);

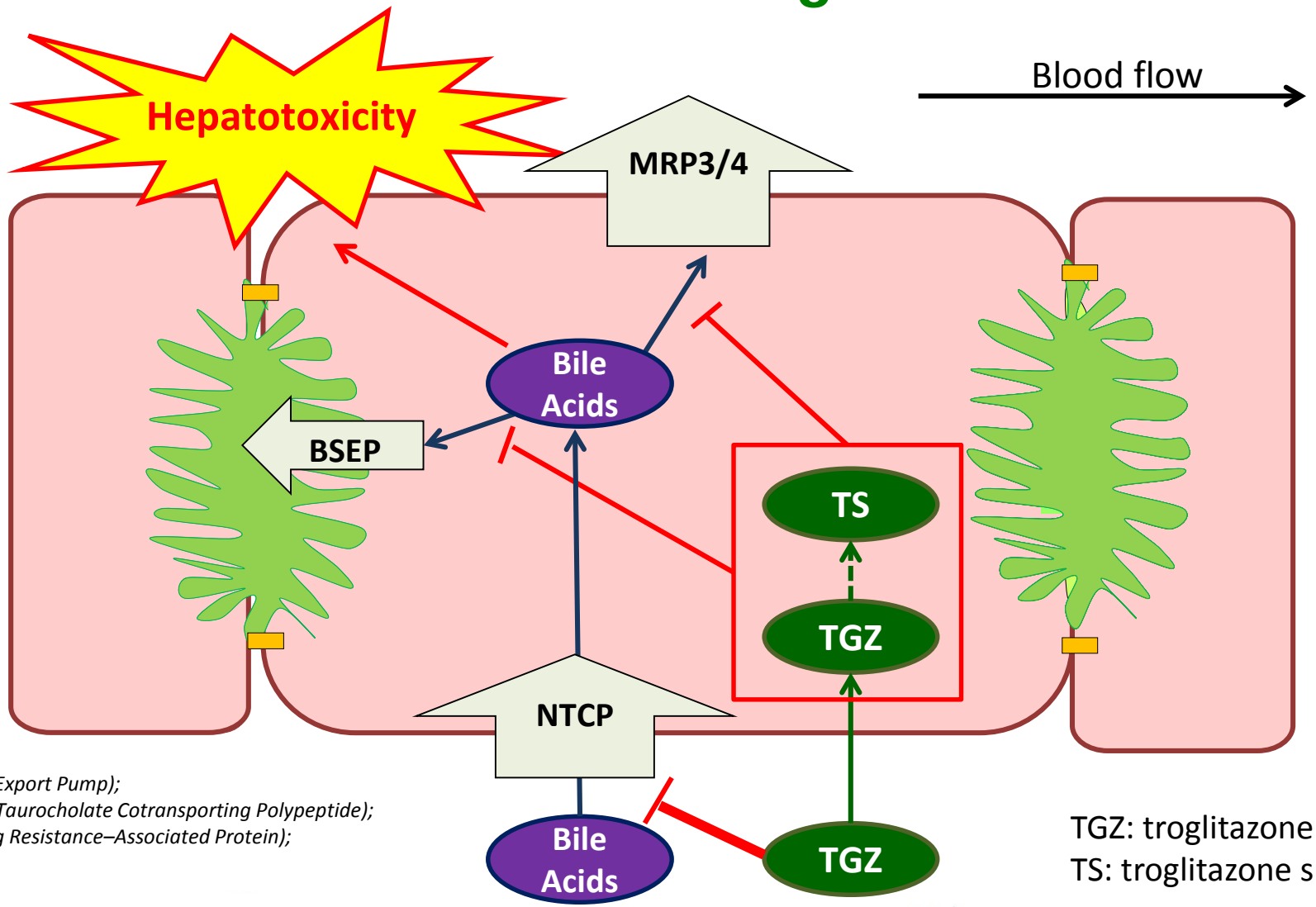
TGZ: troglitazone
TS: troglitazone sulfate

Summary of Modeling Results

Dilisym ® modeling based on TGZ-mediated alterations in bile acid homeostasis adequately predicted the incidence, delayed presentation, potential susceptibility factors, and species differences of TGZ hepatotoxicity.

Yang et al Clin Pharmacol Ther 96 (5) 2014

Mechanisms of DILI: Transport Protein-Mediated Bile Acid-Drug Interaction



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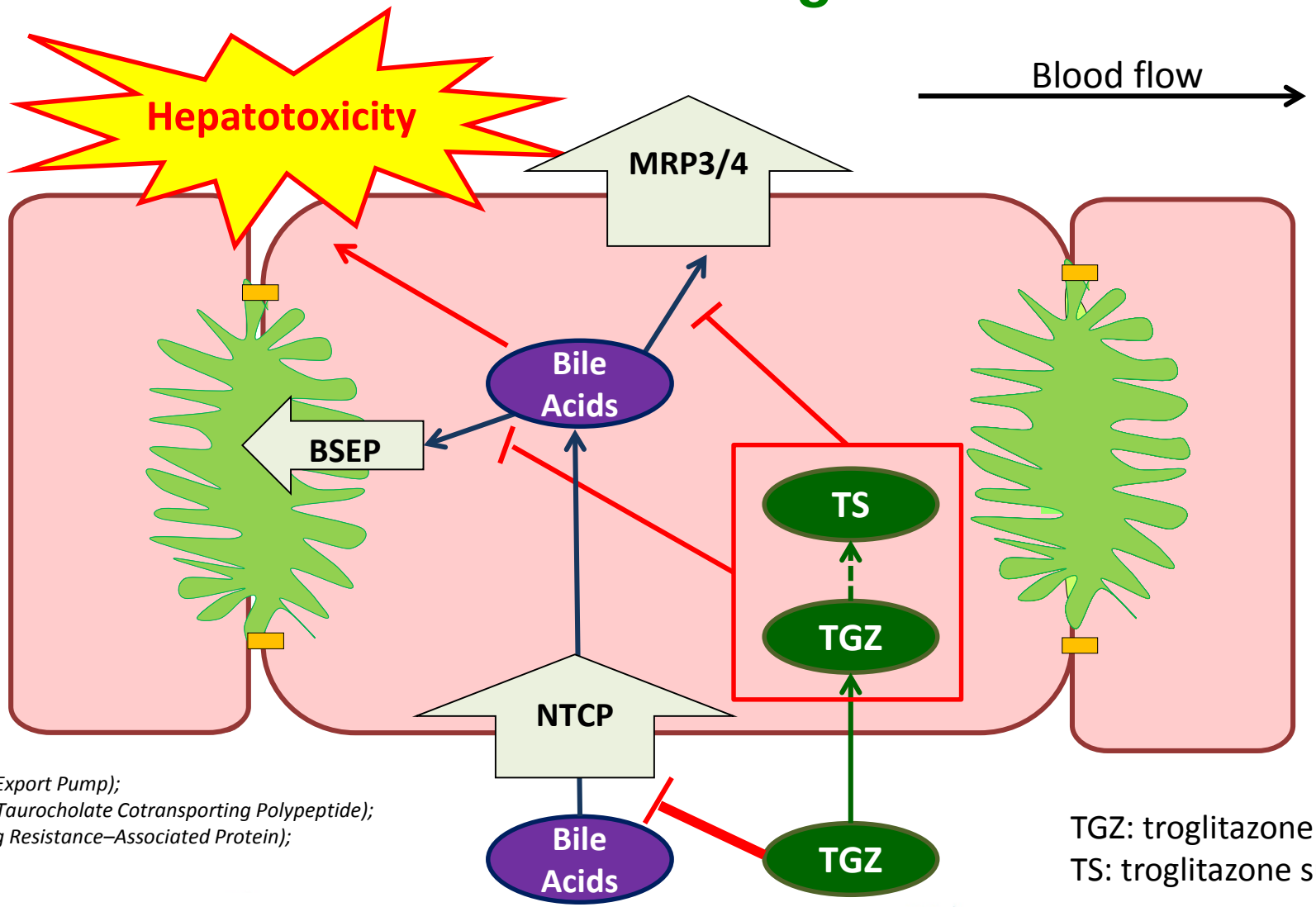

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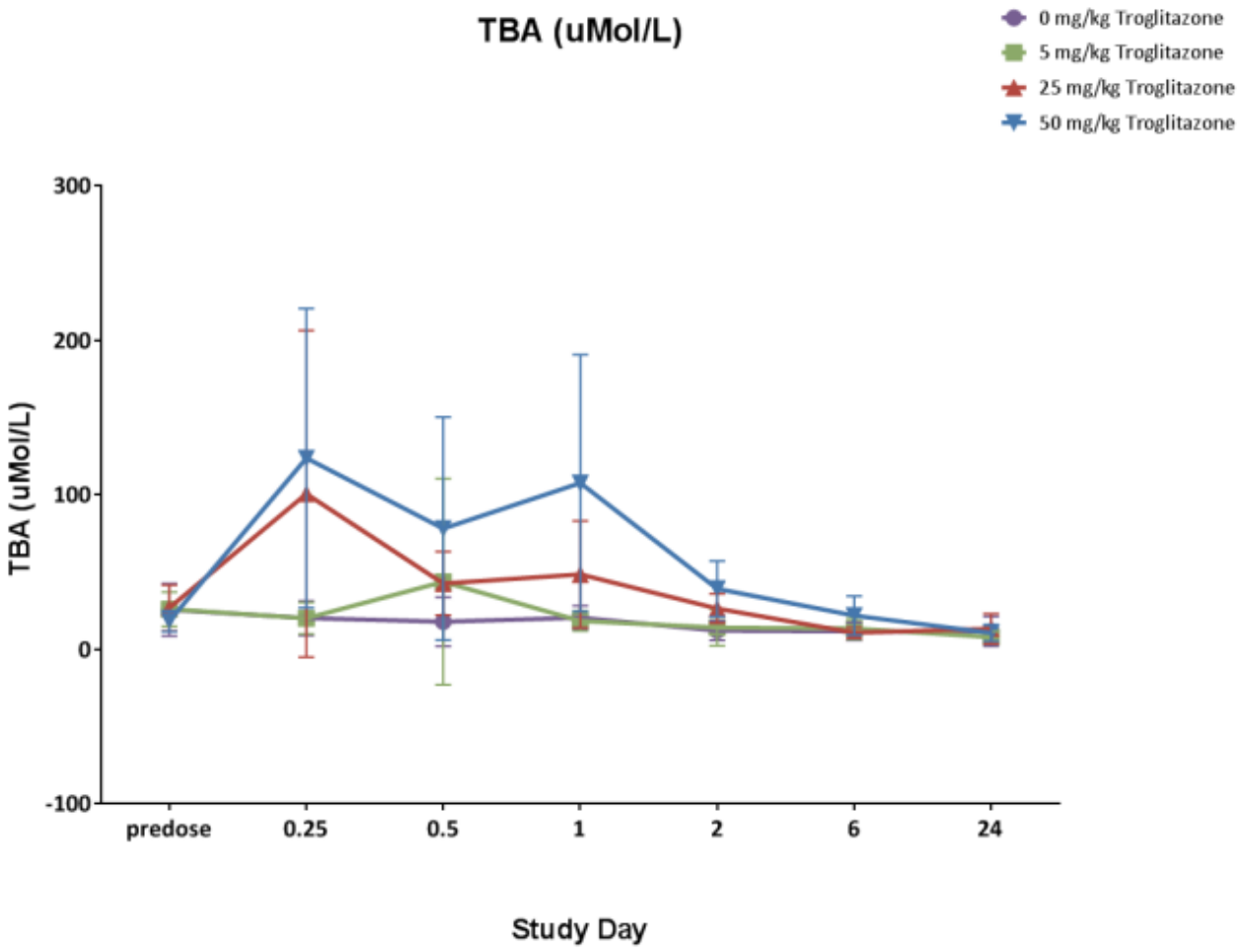
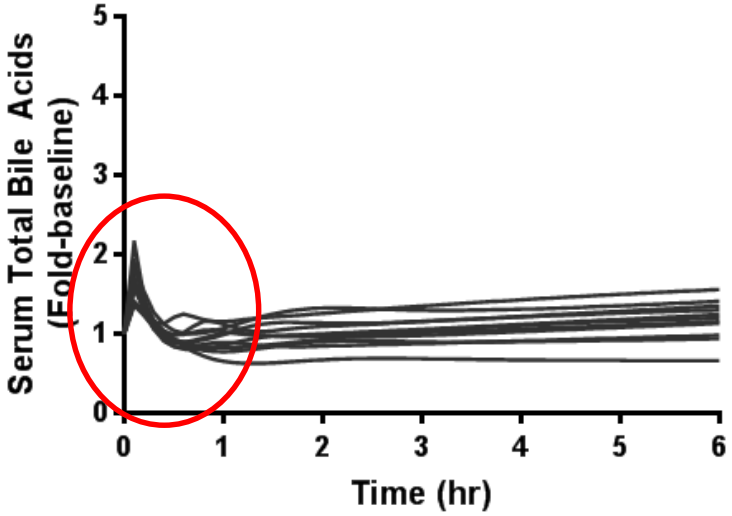
Observed Total Serum Bile Acids after Troglitazone – intravenous dosing (rat)



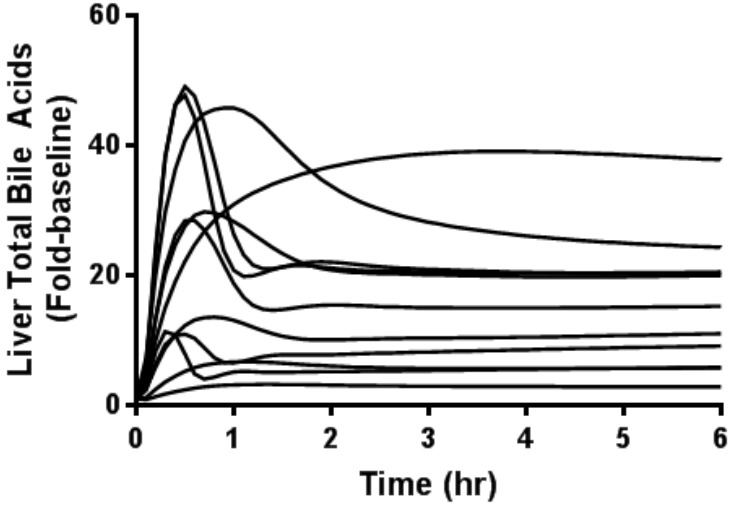
Actual Data – Jonathan Maher and PSTC, May 26, 2016 presentation

Simulated Serum Bile Acids in Rat Troglitazone Model (50 mg/iv)

Serum Bile Acids

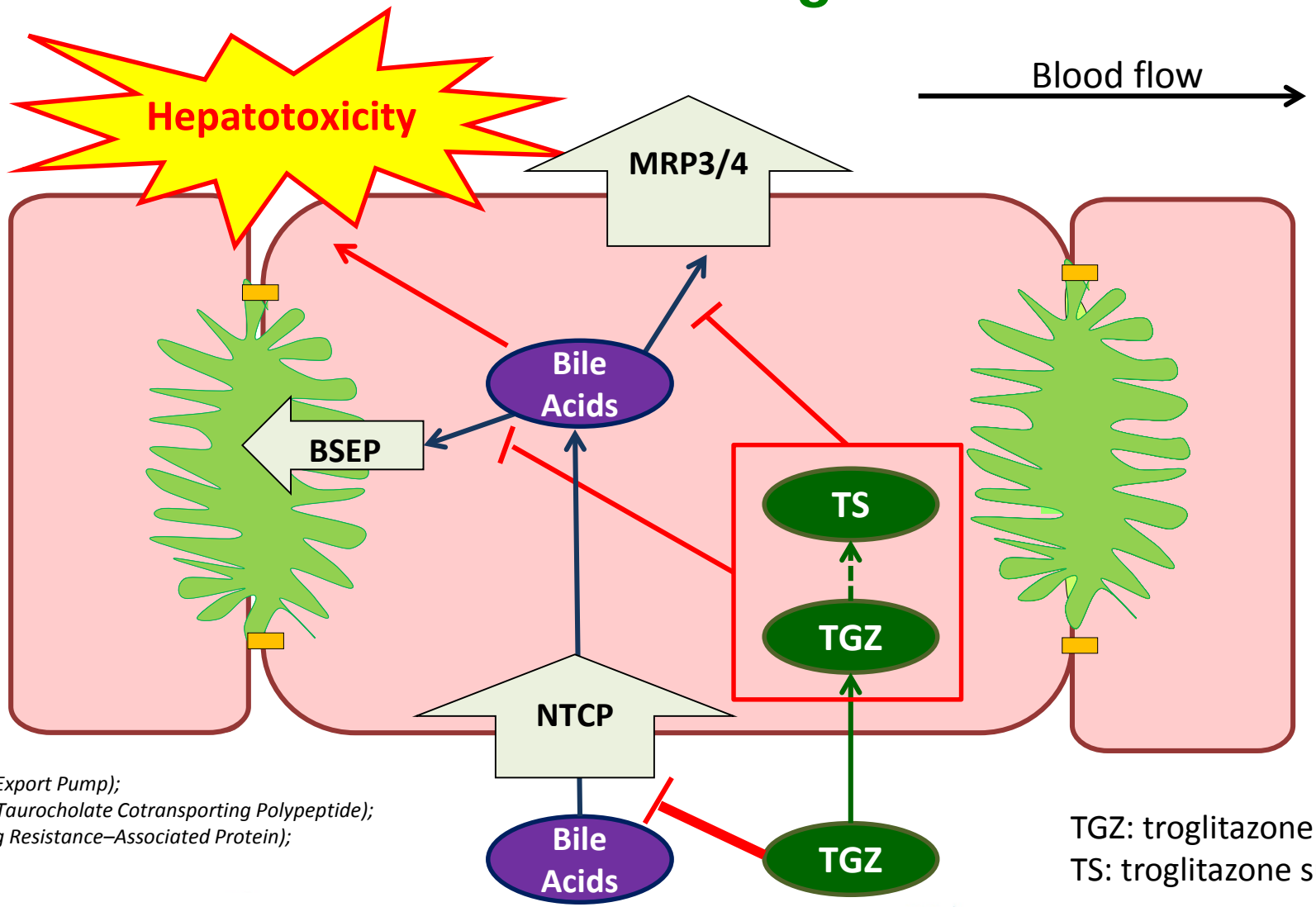


Liver Bile Acids



Simulation – DILIsym® modeling – KyungheeYang, unpublished results

Mechanisms of DILI: Transport Protein-Mediated Bile Acid-Drug Interaction



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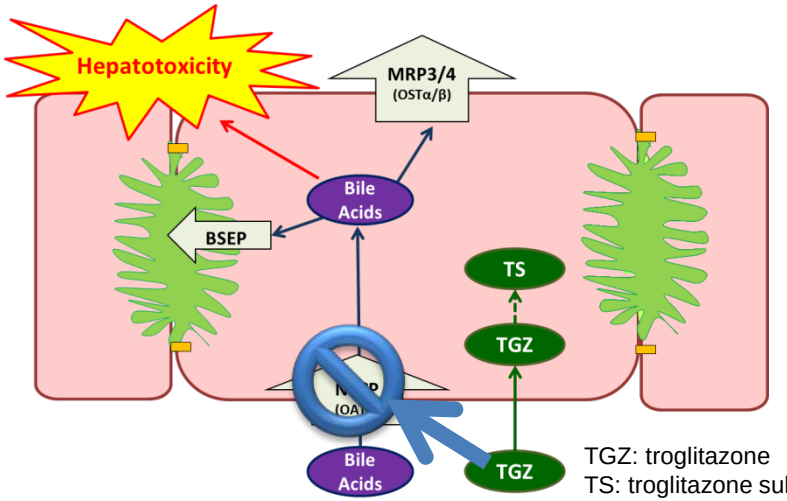
MRP (Multidrug Resistance–Associated Protein);

TGZ: troglitazone
TS: troglitazone sulfate

Simulated Changes in Serum and Liver Bile Acids are not always in Parallel

- Troglitazone **rat** model removing BSEP and MRP3/4 inhibition (but keeping NTCP inhibition)

Serum bile acids increased, but liver bile acids decreased



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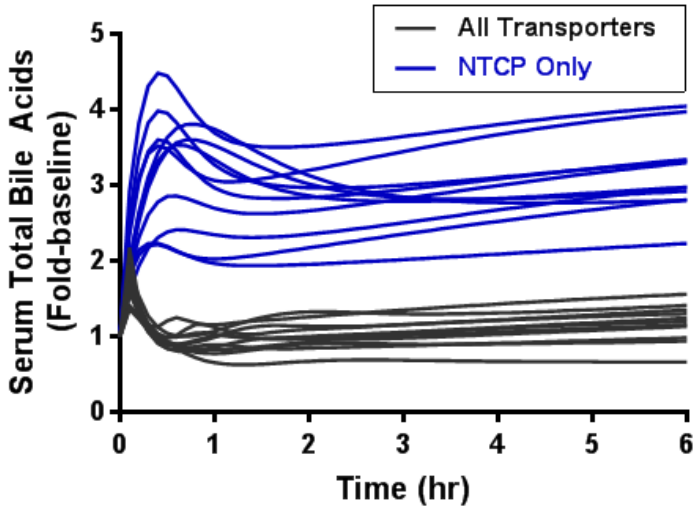

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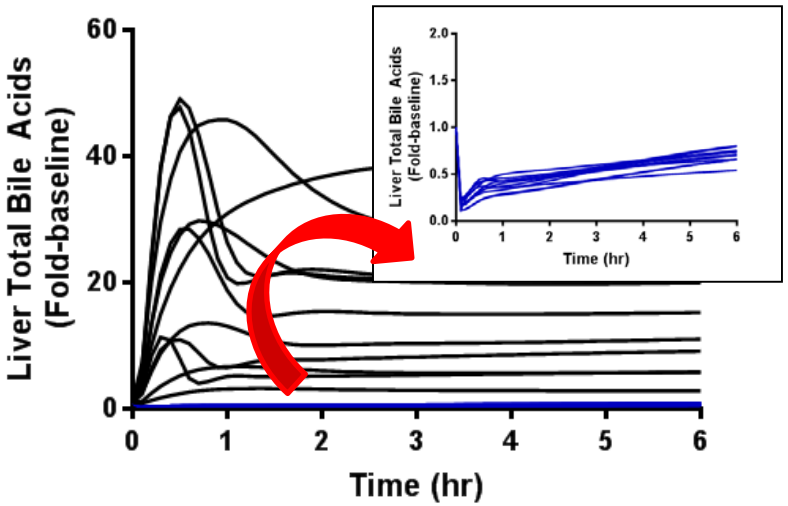
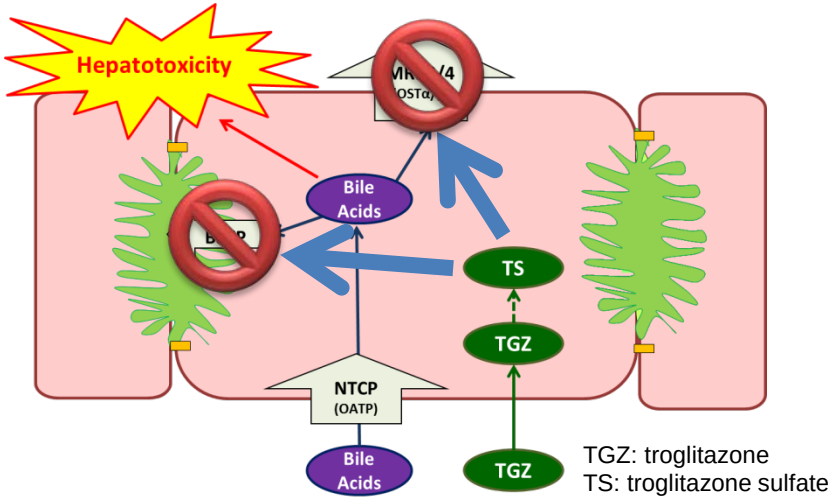


Kyunghee Yang-unpublished results

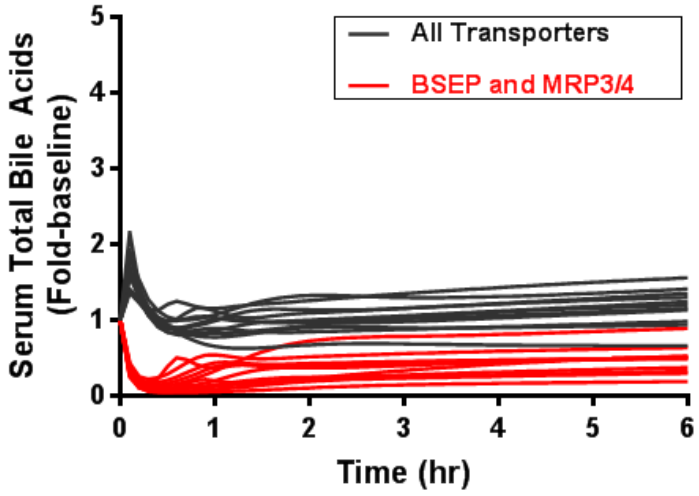
Simulated Changes in Serum and Liver Bile Acids are not Always in Parallel

- Troglitazone rat model removing NTCP inhibition but leaving BSEP and MRP3/4 inhibition

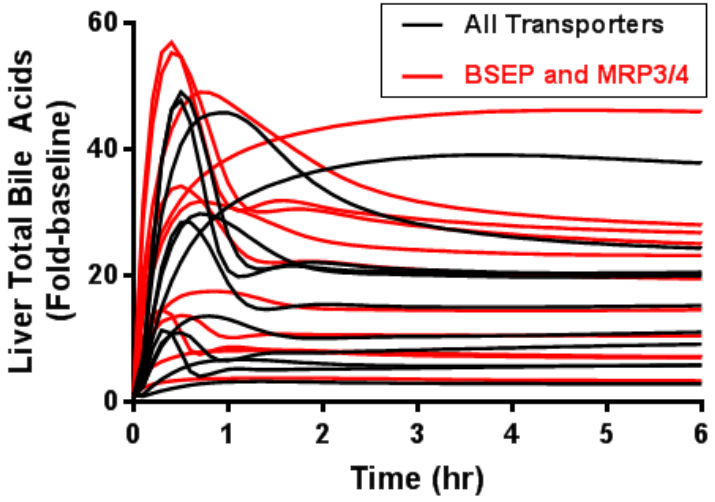
Liver bile acids increased but serum bile acids actually decreased.



Serum Bile Acids



Liver Bile Acids



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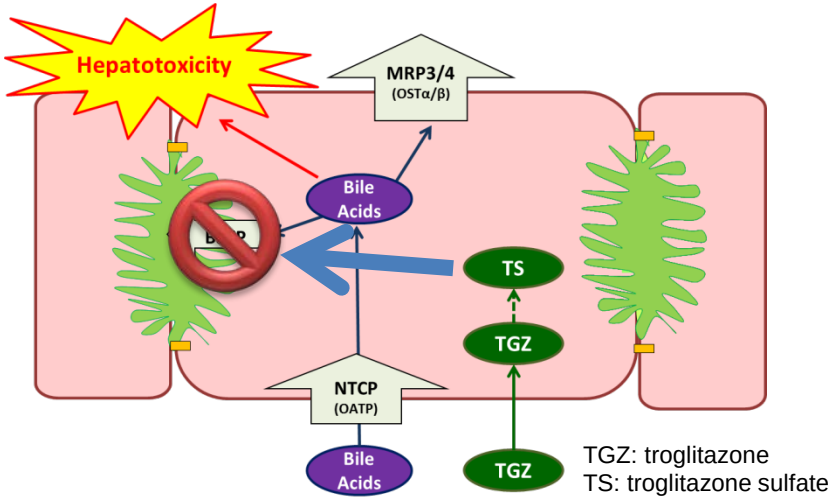
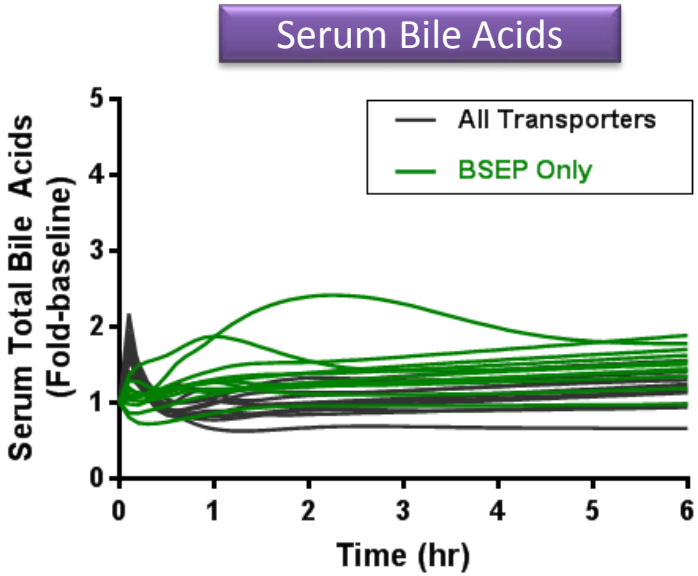
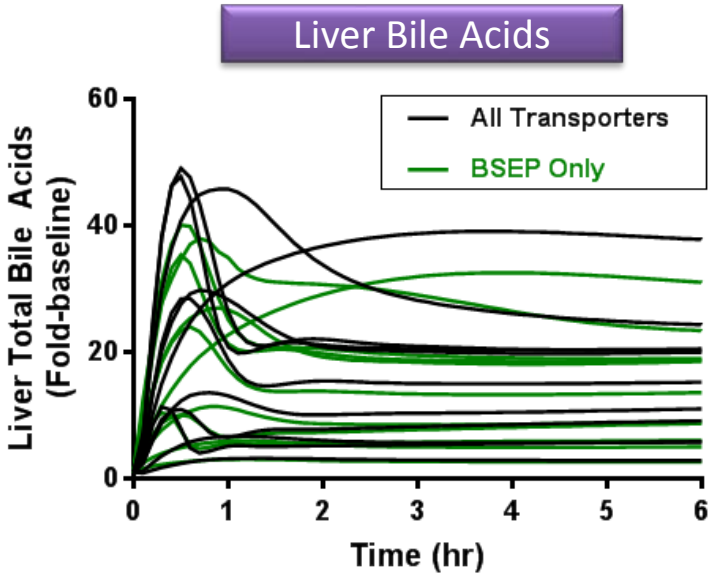
Liver bile acids increased but serum bile acids minimally changed.





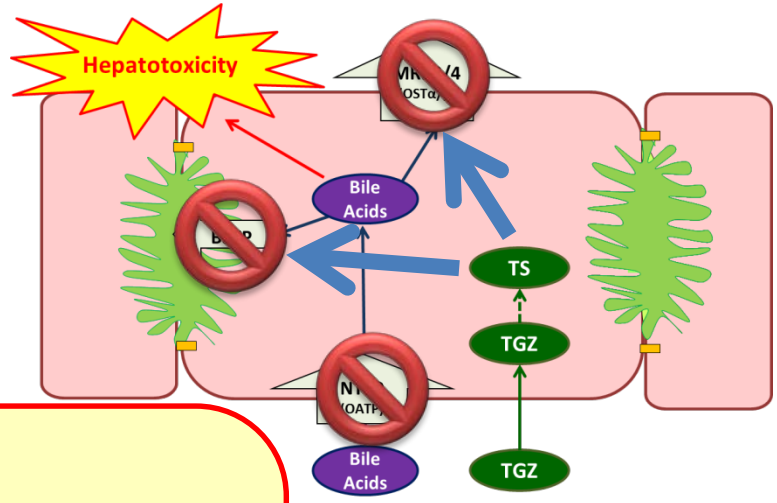


Kyunghee Yang-unpublished results

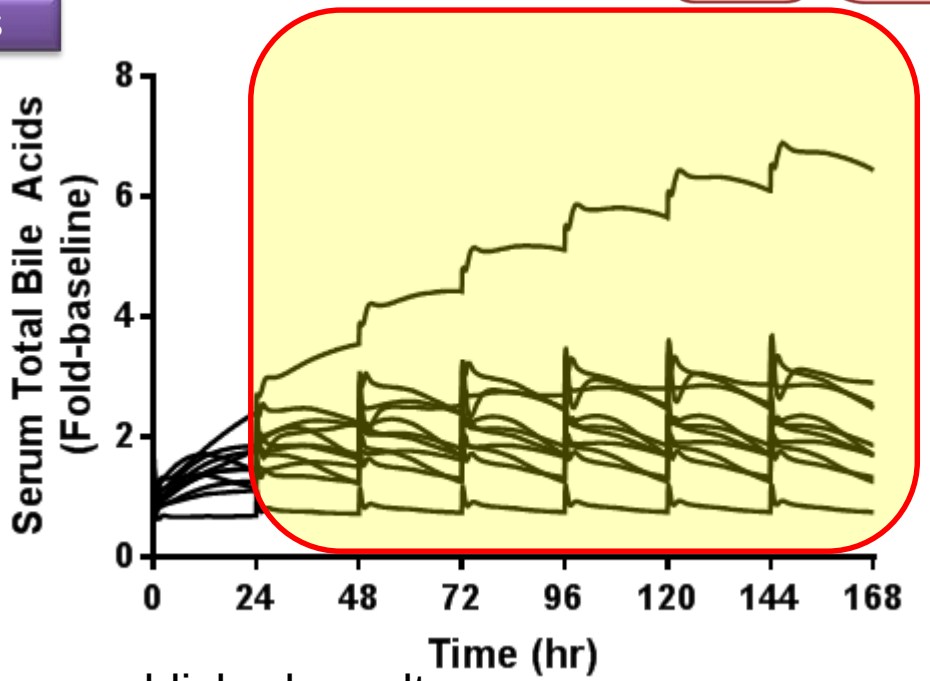
Simulated Impact of Feedback Regulation on Serum TBA Levels (rat, 50 mg iv/day)

Full model with all transporters inhibited

Feedback regulation raises hepatocyte bile acid concentrations over time



Serum Bile Acids



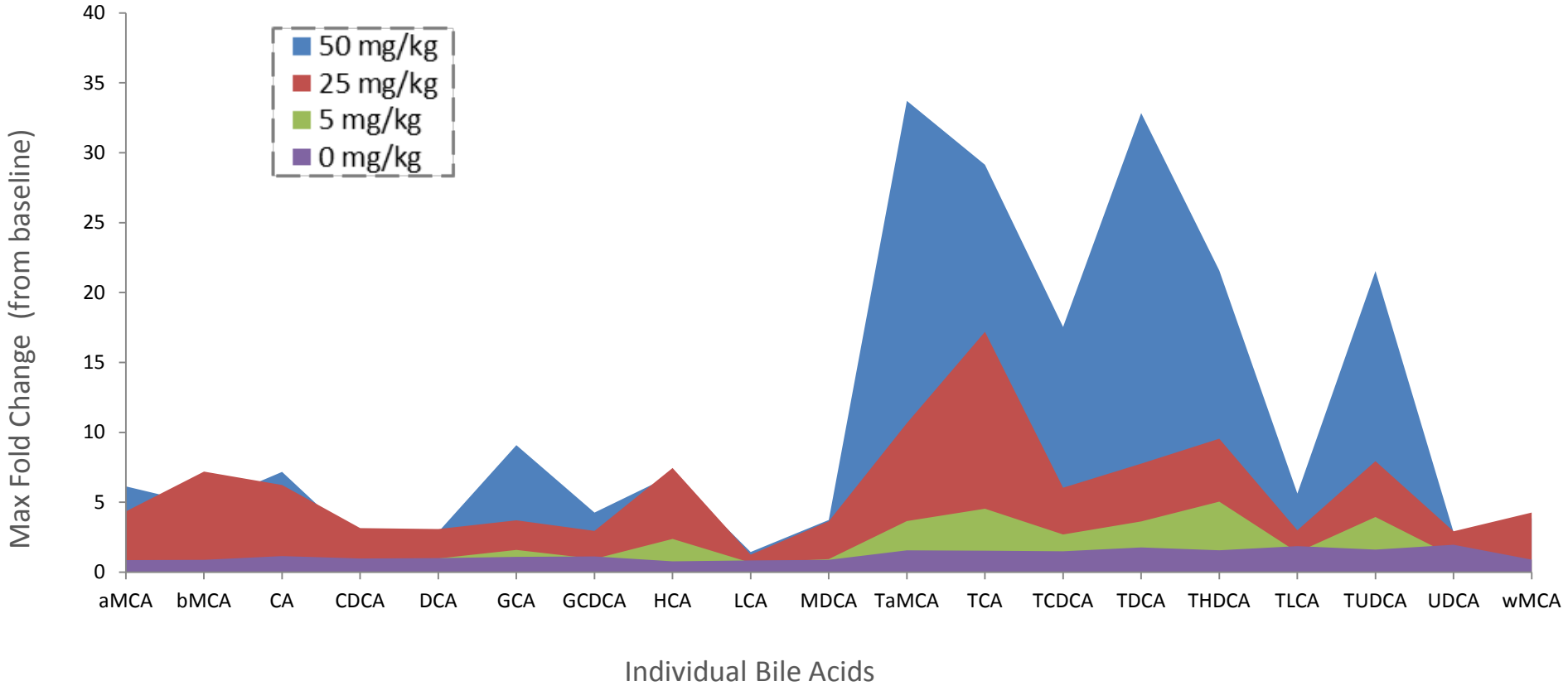
Kyunghee Yang – unpublished results

Conclusion

- **The modeling suggests counter intuitive short term changes in serum bile acids with combined inhibition of BSEP and MRP3/4 (but not NTCP).**

- **Feedback regulation (e.g., FXR activation) should contribute to temporal changes in serum bile acid profiles.**

Increases in Individual Bile Acids $AUC_{0\text{-}6hr}$ after Troglitazone Administration in Male Rats



From Jonathan Maher PSTC presentation

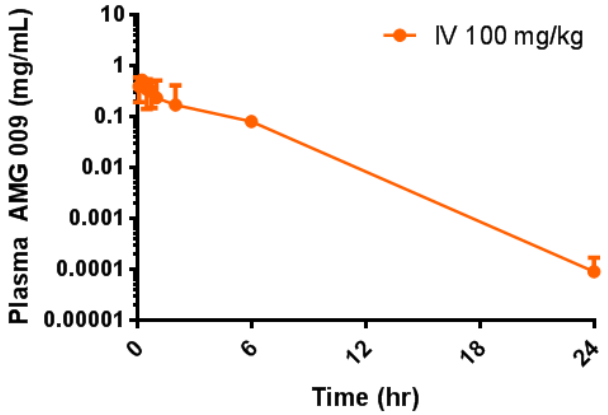
Conclusion

Individual bile acids may be more sensitive and informative as translational biomarkers, but the biological rationale for general use will need to be established.

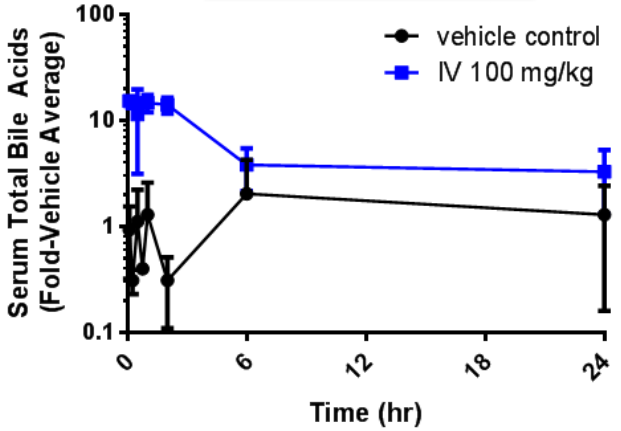
Rat Serum Total Bile Acids and TCA After IV and PO AMG 009

IV

AMG 009



Total Bile Acids



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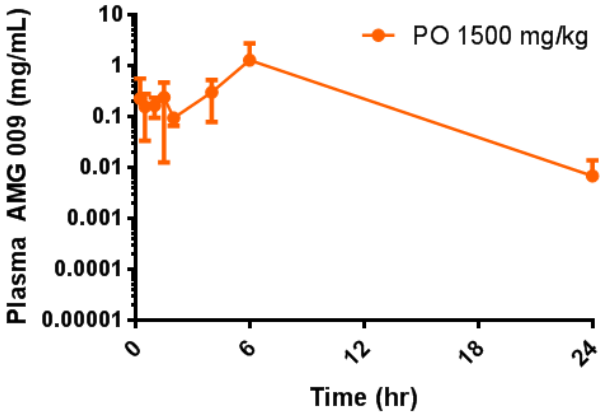
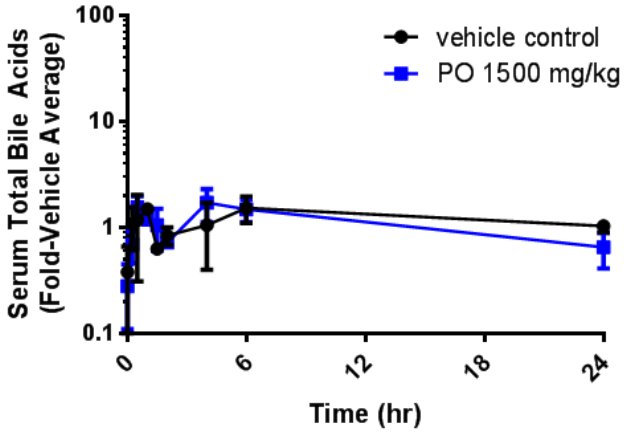
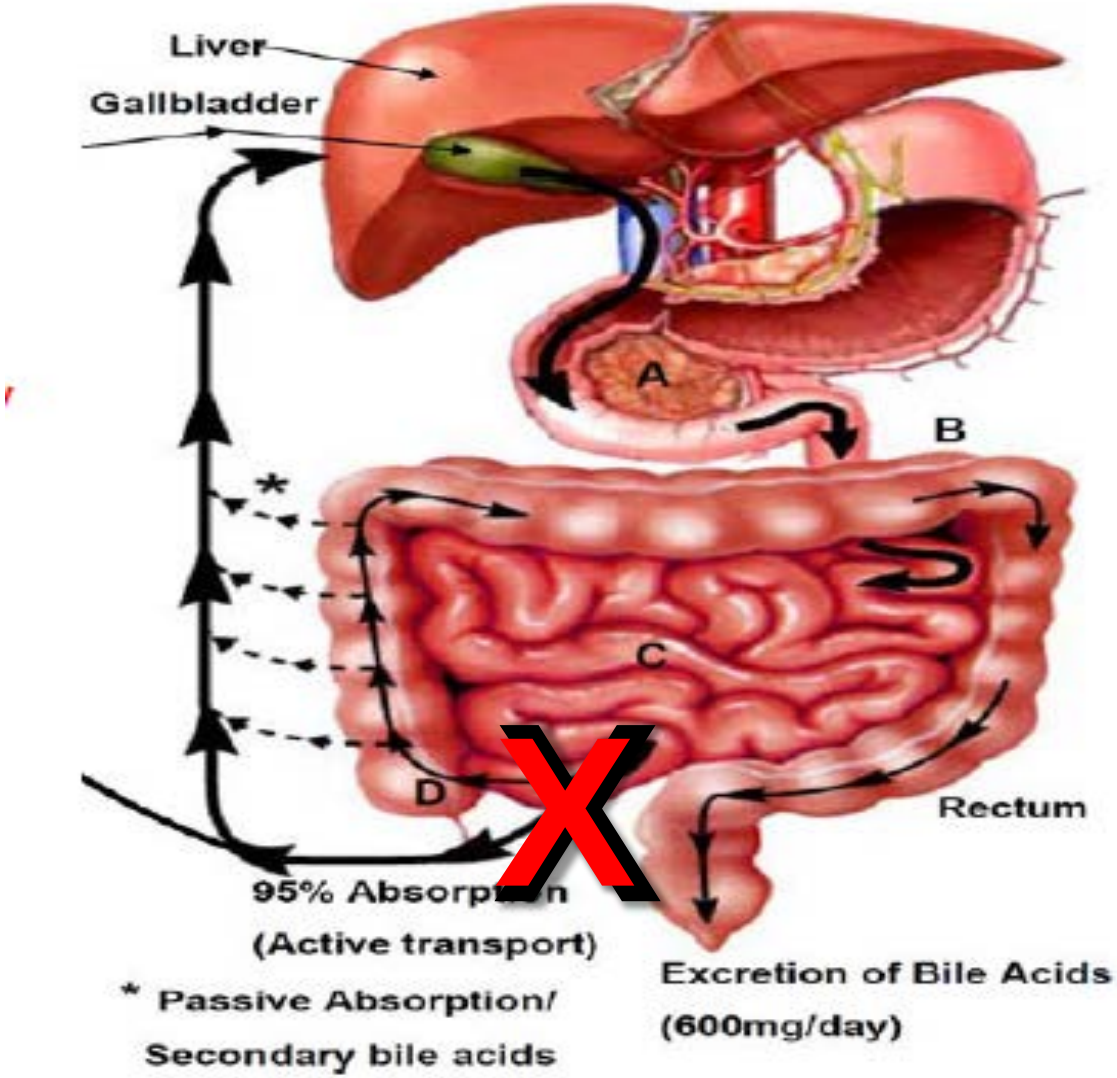




Ryan Morgan unpublished data

Could inhibition of intestinal bile acid uptake explain the oral AMG009 results?



Conclusion

Interpretation of changes in the profile of serum bile acids will be complex and involve modeling to sort out.

Take home points:

1). Interpreting-drug induced serum bile acid changes is an exciting work in progress.

2). Serum bile acids should be profiled when BSEP inhibition is suspected and the data contributed to precompetitive research efforts.

3). The PSTC is the logical organization to continue to lead pre-competitive efforts in this area.

The DILI-sim Team Sept 2015

Session 6: Agenda

All participant lines are muted

#	Title	Presenter	Time
1	Introduction to Series	John-Michael Sauer (Critical Path Institute)	5 minutes
2	Introduction to session theme and speaker biosketches)	Gerry Kenna, Drug Safety Consultant	5
3	Combining BSEP inhibition data with mathematical modeling to impact decisions in drug development	Brett Howell, DILIsym Services	20
4	Recommendation to the regulatory agencies concerning BSEP screening in drug discovery and development	Gerry Kenna	20
5	The future: How can we improve DILI prediction based on alterations in bile acid homeostasis.	Paul Watkins, UNC School of Pharmacy	20
6	Q&A and general discussion	John-Michael Sauer, C-Path	35
7	Meeting summary, closing remarks, next steps	John-Michael Sauer	15

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Discussion

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