

# Approaches that can be applied in drug discovery to minimize the likelihood of drug induced liver injury in man

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# Overview

- What is the problem?
- DILI Screening Rationale
- AZ Non-Clinical Strategy
  - Hepatic Liability Panel
- The translational challenge
- IMI Predictive DILI Project



# What is the problem?

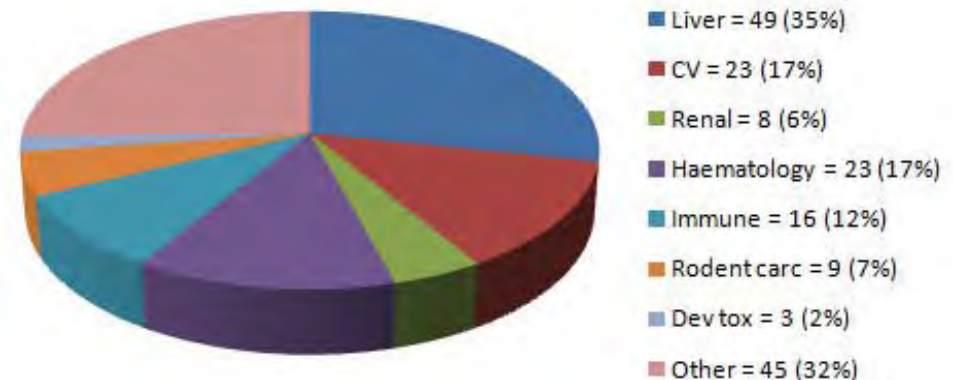
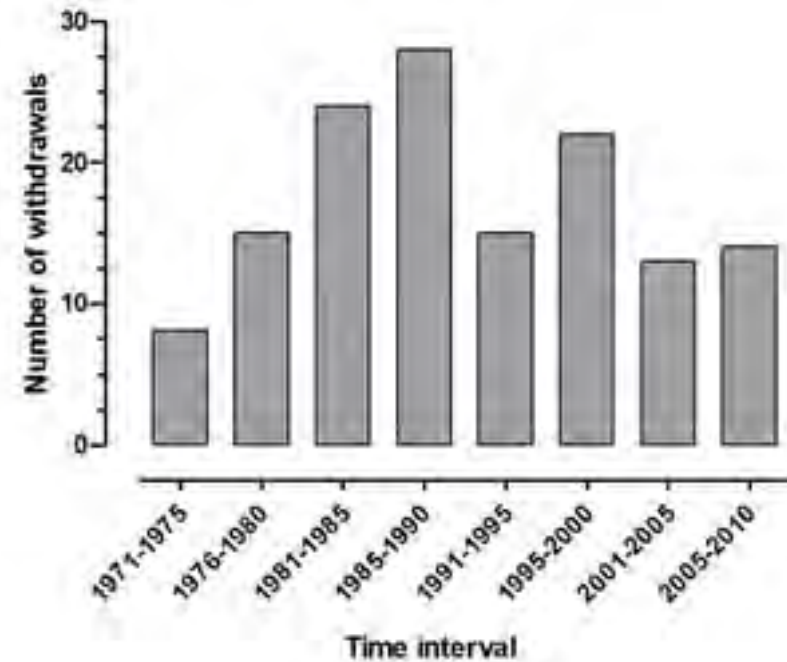
## Drug Induced Liver Injury (DILI)

A leading cause of:

- Drug attrition due to preclinical toxicity
- Drug attrition due to toxicity in man in late clinical trials
- Failed drug registration (cf. Exanta: > one case in entire clinical trial population is ominous).
- Drug withdrawal post-licensing
- Cautionary and restrictive labelling
- Serious ill health in man

Very challenging regulatory position (FDA Guidance)

- > One case in an entire clinical trial population considered ominous



# What is the problem?

## Patterns of DILI in man

### Type A

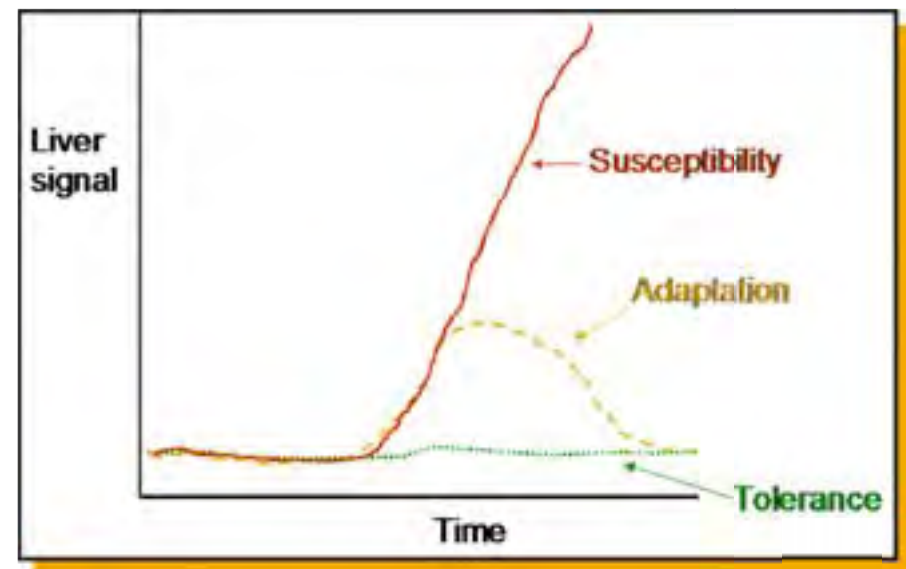
- Reproducible, overtly dose dependent,
- Evident in preclinical species and/or man.
- Detected during safety testing of many intended candidate drugs
- An important cause of compound attrition or restricted (dose capped) clinical exposure.

### Idiosyncratic

- Infrequent, not overtly dose dependent
- Evident in man in late clinical trials or after licensing, not in animals
- A major cause of late attrition, failed licensing or drug withdrawal

When dosed with drugs that can cause DILI:

- Most individuals “tolerate” (typically  $\geq 90\%$ )
- A small proportion sustain initial injury, then adapt
- Relatively few fail to adapt and develop DILI (typically  $\leq 1\%$ )



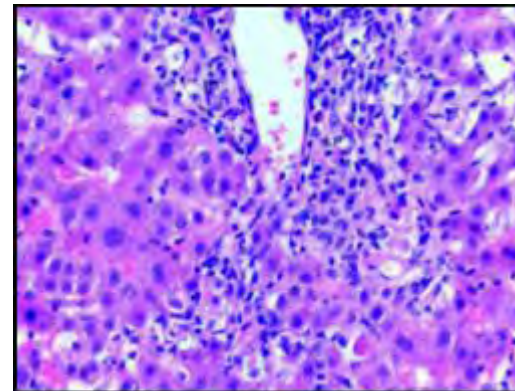
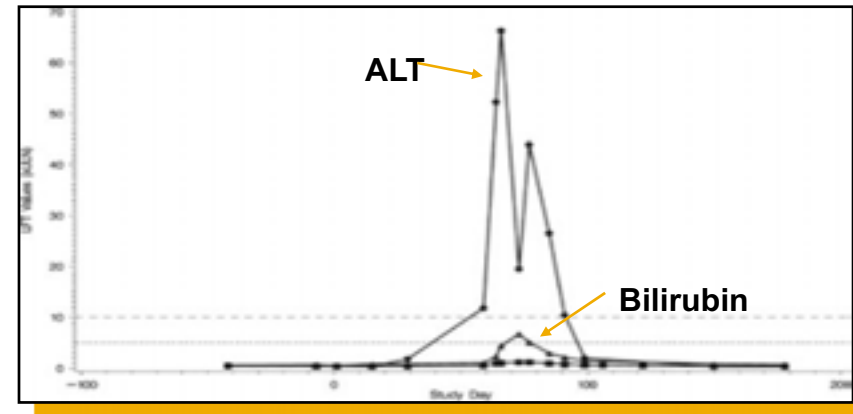
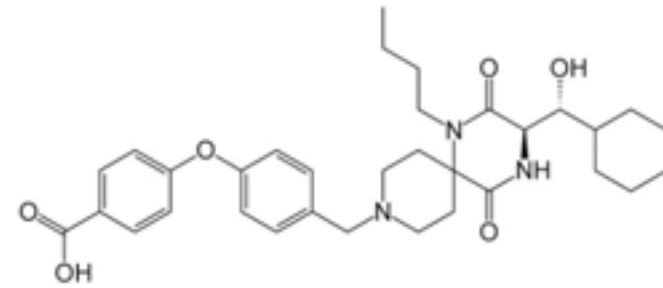
*Rawlins & Thompson, Textbook of adverse drug reactions, Oxford University Press 1991: 18–45.*

*Gruchalla, Lancet 2000, 356: 1505-11.*

# What is the problem?

## Aplaviroc

- CCR5 antagonist, intended for treatment of HIV infection
- Elevated LFTs in 10% (of 281) patients in Phase IIb
- Symptomatic DILI observed in 2 cases
- Clinical development stopped



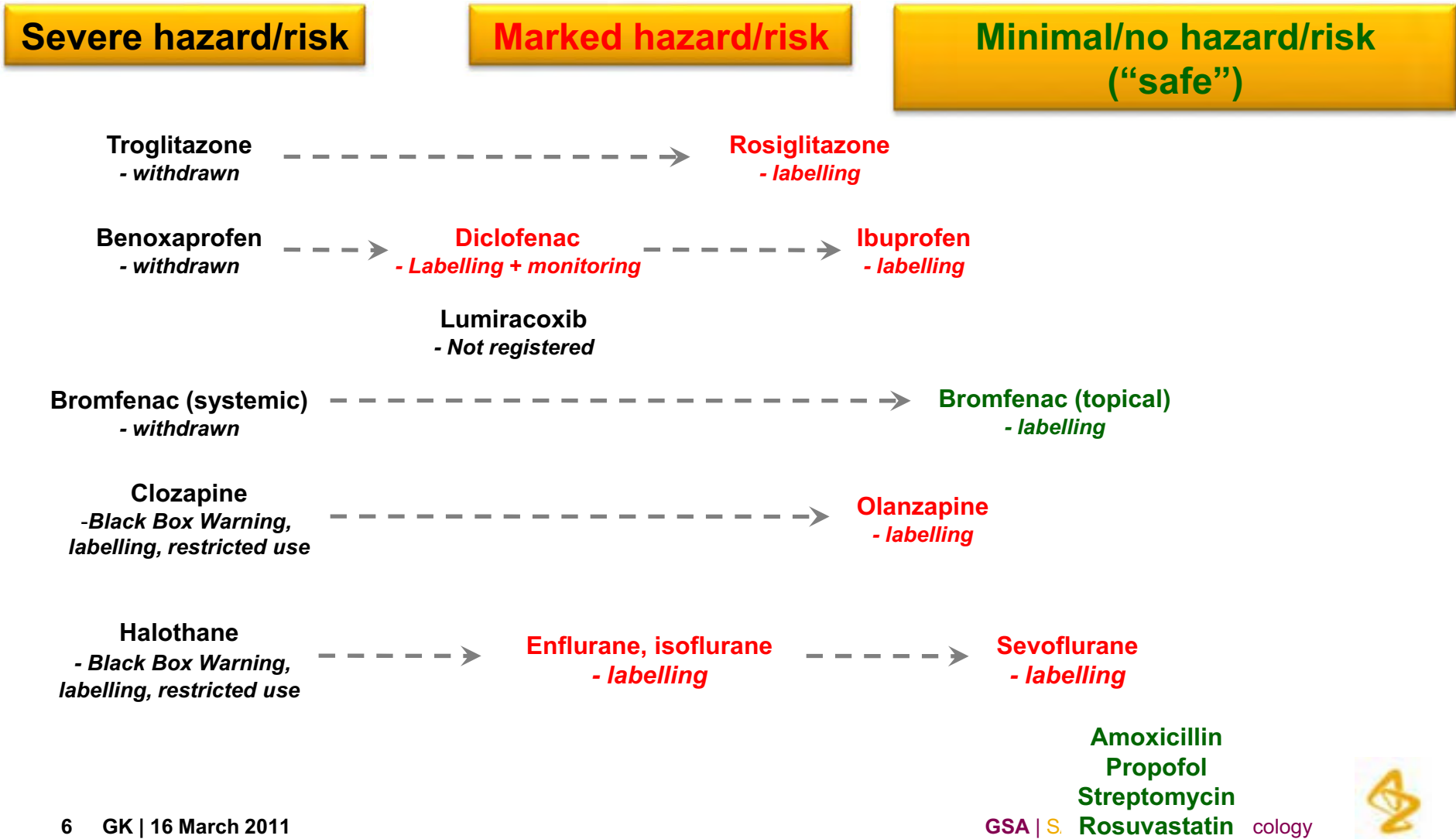
Portal tract inflammation on biopsy

Nichols et al., *Antimicrob. Agents. Chemother.* 2008; 52:858–865.



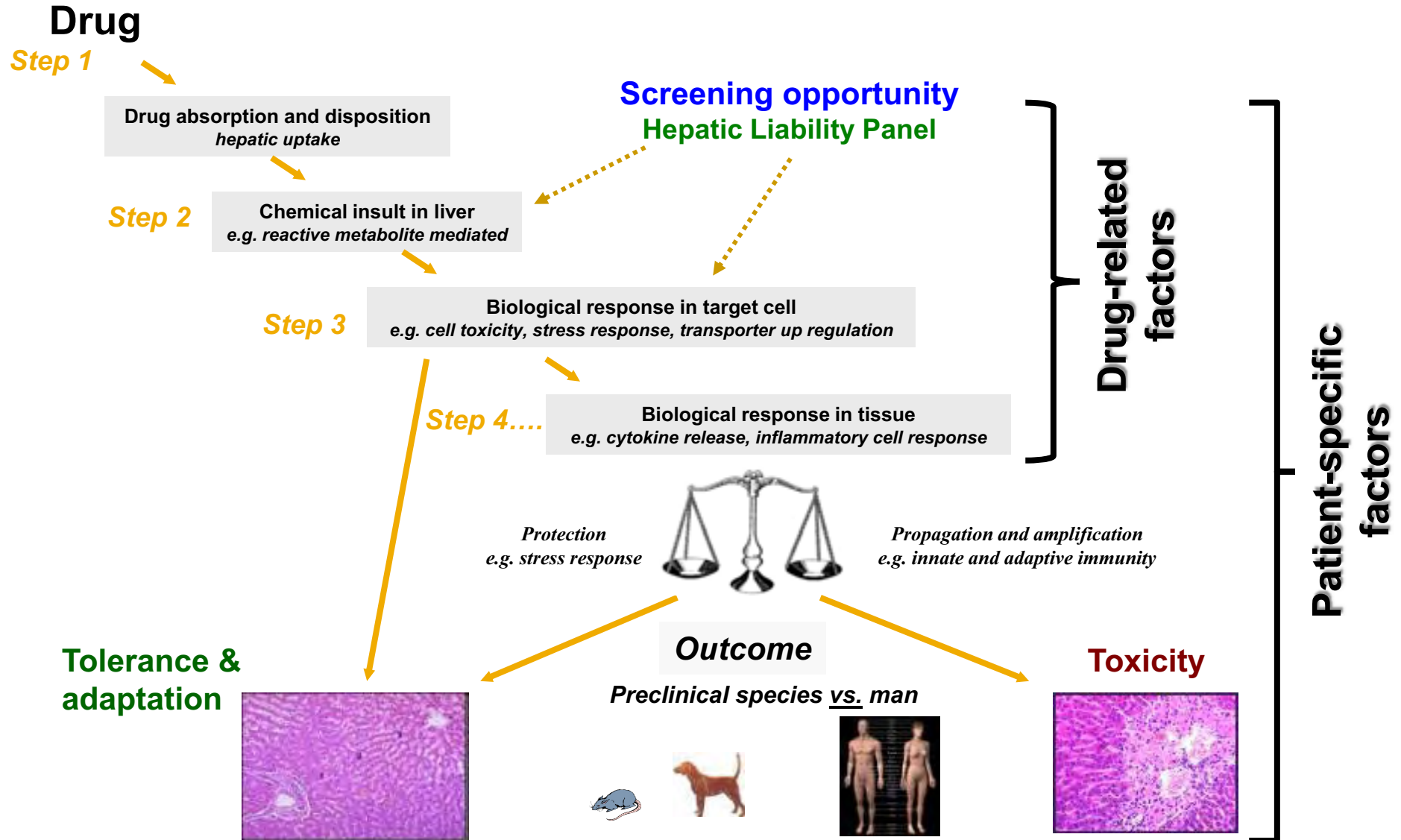
# DILI risk ranking

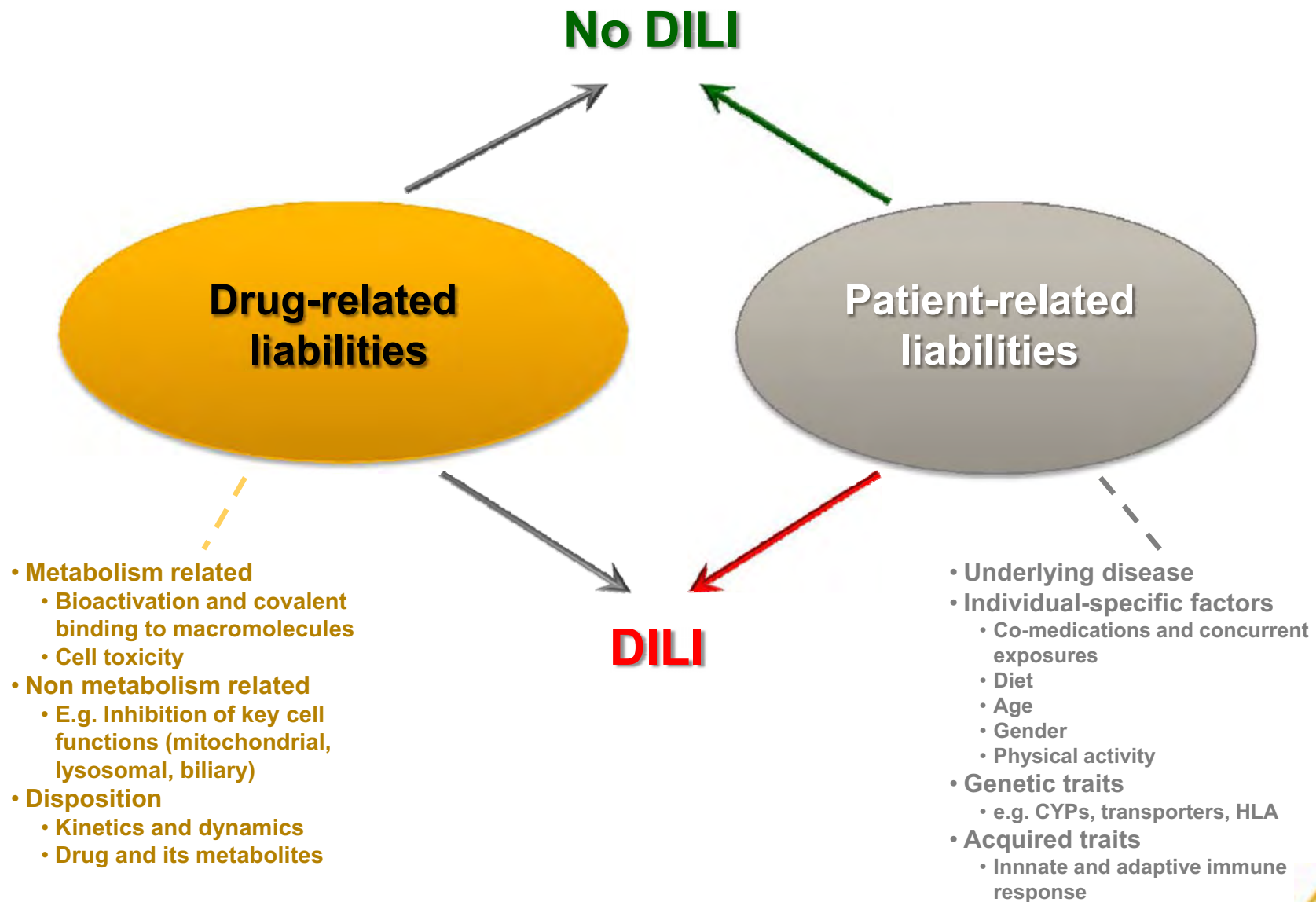
Some drugs pose a much greater risk than others



# DILI Mechanisms

## Multiple steps





# DILI Screening Rationale

In drug Discovery:

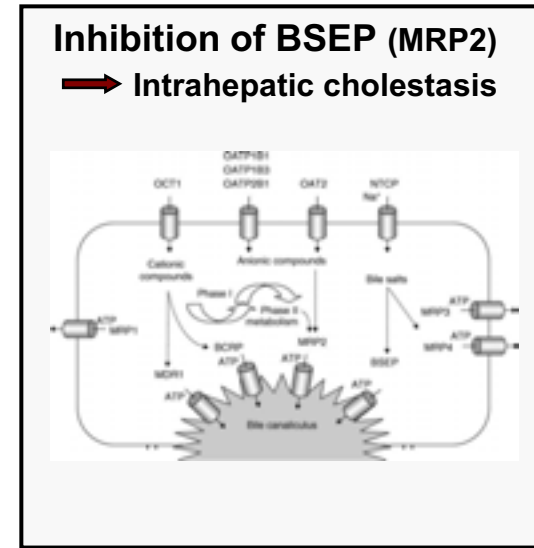
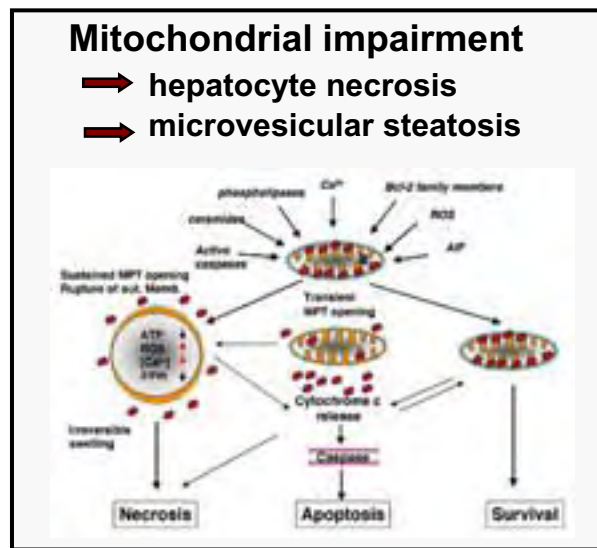
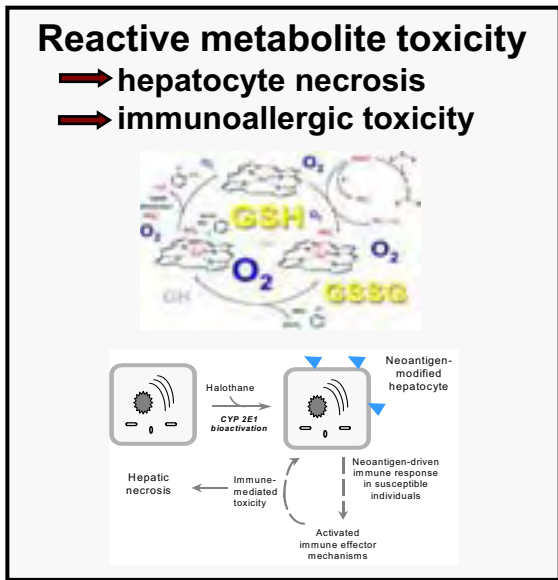
- We **cannot** assess or influence Patient-related Hazard/Risk factors
  - which determine likelihood that a molecule will cause DILI in an individual patient
- We **can** assess and influence Drug-related Hazards
  - which influence likelihood that a molecule will cause DILI in the human population



# DILI Screening

## Which drug-related liabilities and which assays?

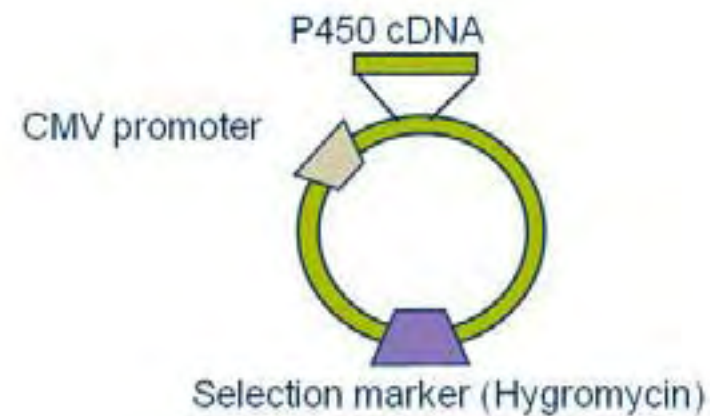
- Multiple potential mechanisms, some DILI-specific and some not
- Many possible *in vitro* assays
- Currently there is no consensus on:
  - Which mechanisms and assays comprise an “ideal” test cascade
  - How to select and validate assays – e.g. which test compounds?
  - How to interpret assay data – e.g. alongside reactive metabolite data?



# Cell Cytotoxicity Assessment

## THLE cells: SV40 - T antigen immortalised Human Liver Epithelial Cells

- Immortal, stable cell background, excellent growth properties.
  - No CYP expression/activity
  - Retain most phase II activities (GST, ST, EH), but not UGT. (Pfeifer et al. PNAS USA 90: 5123, 1996)
- HumanCYP expressing sub-lines were prepared by transfection with pCMV-CYP constructs (Mace et al. Carcinogenesis 18: 1291, 1997)
  - No CYP construct = null
  - Individual 1A2, 2C9, 2C19, 2D6, 3A4 lines
- Sub-lines have been used to evaluate role of individual human CYPs in:
  - Genetic toxicity (e.g. Mace et al. Carcinogenesis 18: 1291, 1997)
  - Drug metabolism (e.g. Molden et al. Eur J Clin Pharmacol 56: 575, 2000)
  - *In vitro* cytotoxicity of drugs that cause DILI (Dambach et al. Toxicol. Pathol. 33: 17, 2005)



# THLE assessment of DILI liability - I

**BMS: Drug Metab Rev 35: 201, 2003**

- Toxicity of 679 marketed drugs - 92 DILI, 587 no DILI
  - 5 CYP cell lines - Null, 3A4, 2C9, 2C19, 2D6
  - Alamar Blue assay:  $IC_{50} \leq 50 \mu M$  = toxic
- Data distinguish between drugs that cause DILI in man and non-DILI drugs with extremely high specificity and high sensitivity

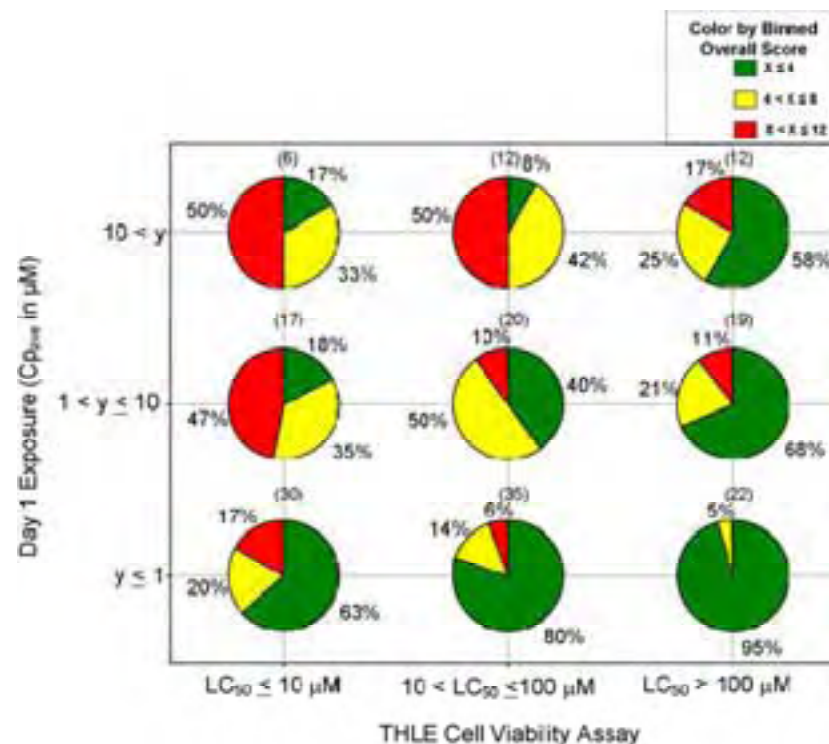
Minimum $IC_{50}$ in Null <u>or</u> CYP expressing cells	Hepatotoxic drugs	Non hepatotoxic drugs	
Positive: $IC_{50} \leq 50 \mu M$	66	2	PPV= 97%
Negative: $IC_{50} > 50 \mu M$	26	585	NPV= 96%
	<b>Sensitivity = 72%</b>	<b>Specificity = 99.7%</b>	



# THLE assessment of DILI liability - II

**Pfizer:** Benbow *et al.*, Toxicology Letters 197 (2010) 175–182

- Evaluation of THLE-Null cell toxicity of compounds tested in repeat dose safety studies in animals
- 50% of drugs causing organ toxicity (primarily DILI) with poor exposure margins were cytotoxic
- “In summary, cytotoxicity screening can be used to approximate, not define, the safety characteristics of lead pharmaceutical series early in the drug discovery process”



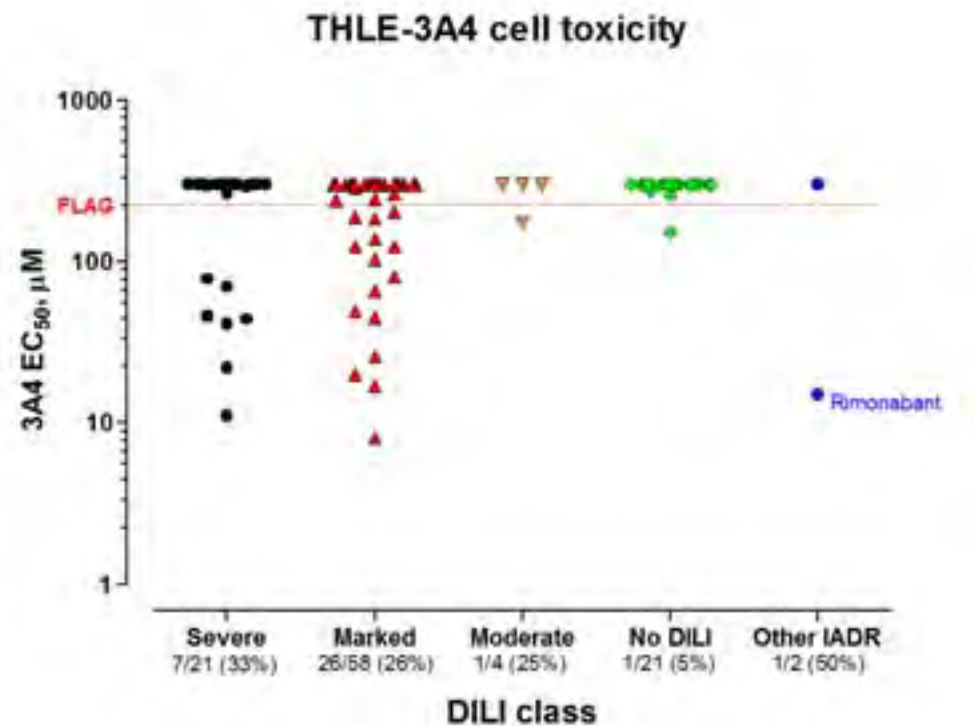
**Fig. 2. Correlation of THLE cytotoxicity assay to composite safety scores from rat in vivo exploratory toxicity studies as a function of exposure.**



# THLE assessment of DILI liability - III

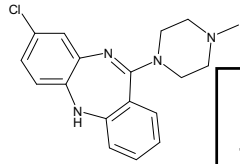
## AstraZeneca screen

- Cytotoxicity of 85 marketed drugs to Null and 3A4 lines (MTS assay)
- “Activity” in the THLE screen ( $EC_{50} \leq 200 \mu\text{M}$ ) was exhibited by approx 30% marketed drugs that caused DILI, but very infrequently by non-DILI drugs

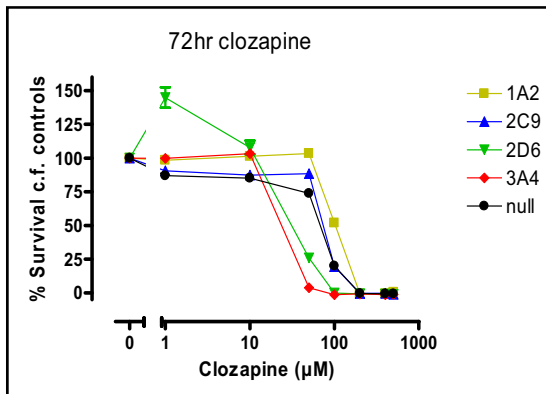
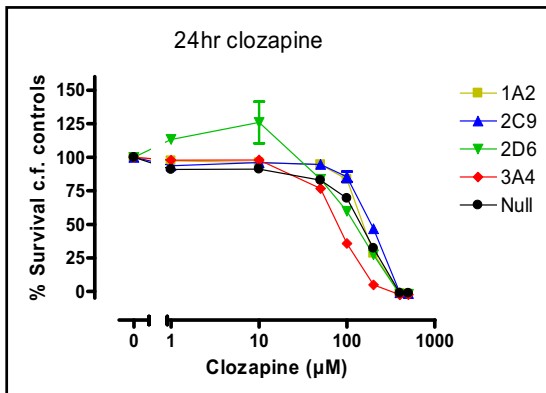


# THLE assessment of DILI liability - IV

## AstraZeneca compound comparison

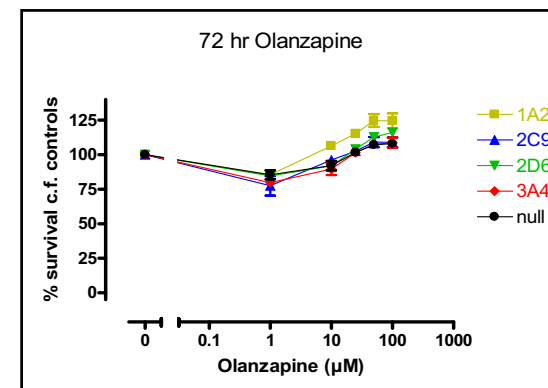
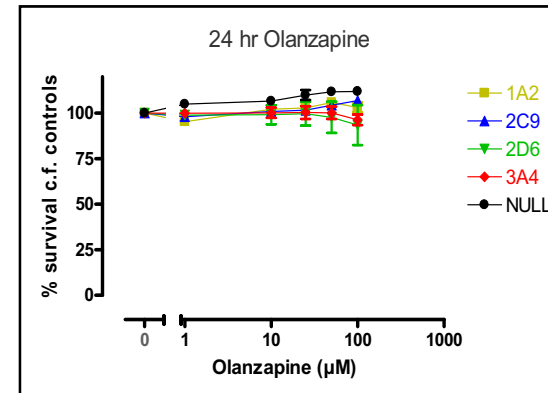
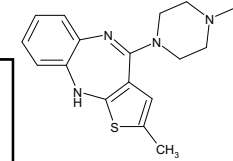


### CLOZAPINE



- High dose (300-450 mg/day)
- **1-2% incidence of agranulocytosis**
- **<0.1% incidence of hepatotoxicity**

### OLANZAPINE



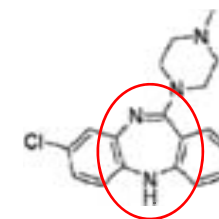
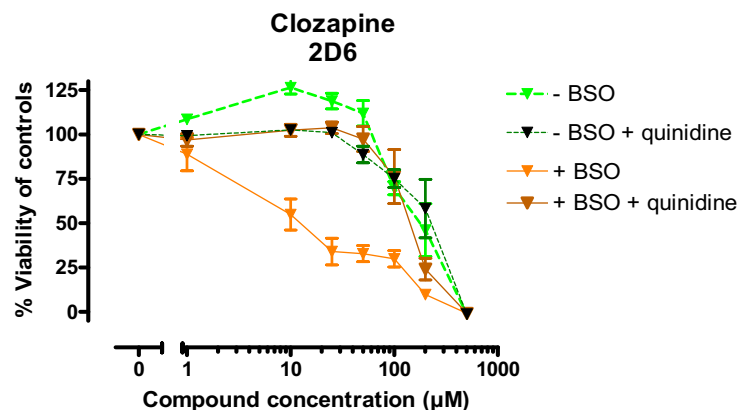
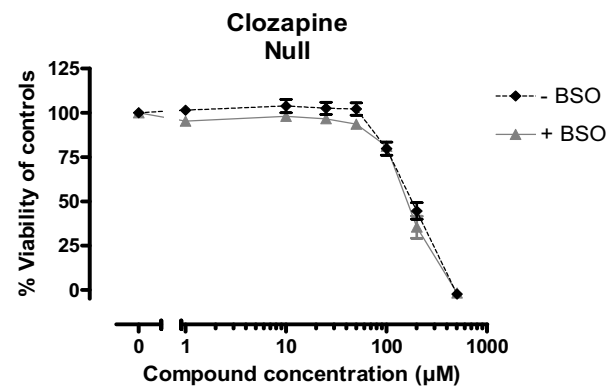
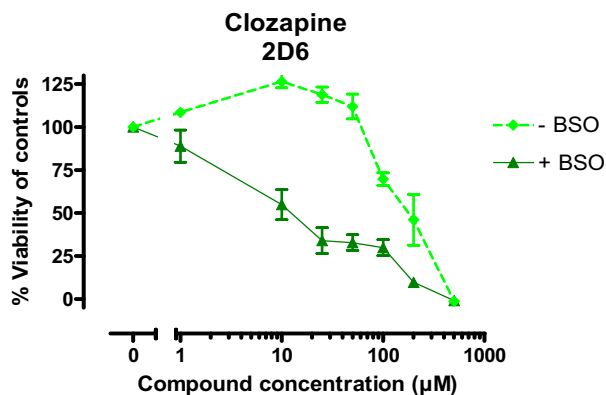
- Low dose (max. 20mg/day)
- **Minimal ADRs in man**

Both drugs form RMs and exhibit similar levels of covalent binding to proteins *in vitro*



# THLE assessment of DILI liability - V

## AstraZeneca: Reactive metabolite mediated clozapine cytotoxicity



Nitrenium ion

- GSH conjugates detected in THLE-2D6 cells and 2D6 supersomes
- MS fragmentation consistent with nitrenium ion RM
- THLE-2D6 toxicity is accompanied by covalent binding to protein

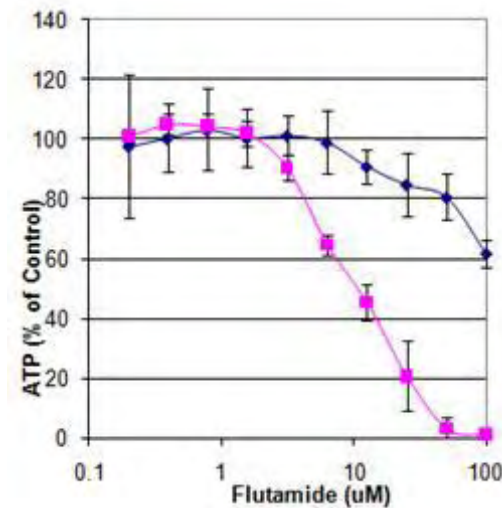
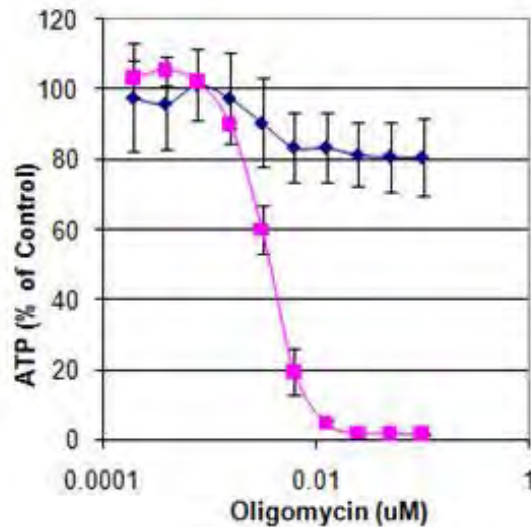
- Selective clozapine toxicity in GSH-depleted THLE-2D6 cell line
- Reversed by quinidine (CYP2D6 inhibitor)



# Mitochondrial Impairment

## HepG2 “Crabtree effect” toxicity assay

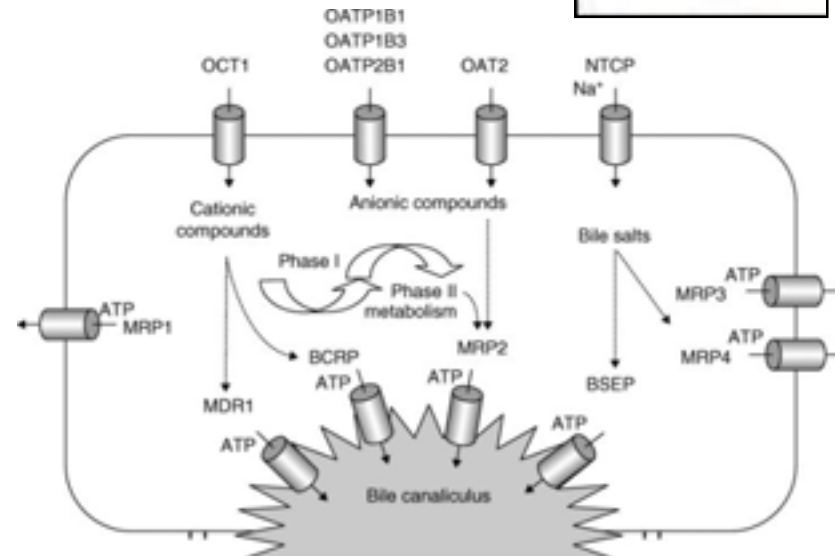
- Cells cultured in galactose utilise mitochondrial ox phos, not glycolysis, for energy production
- Greater cellular sensitivity to mitochondrial injury in galactose vs. high glucose medium
- Valuable for early identification of some mechanisms of mitochondrial injury



# Biliary Transport Inhibition

## Hepatic transporters and DILI

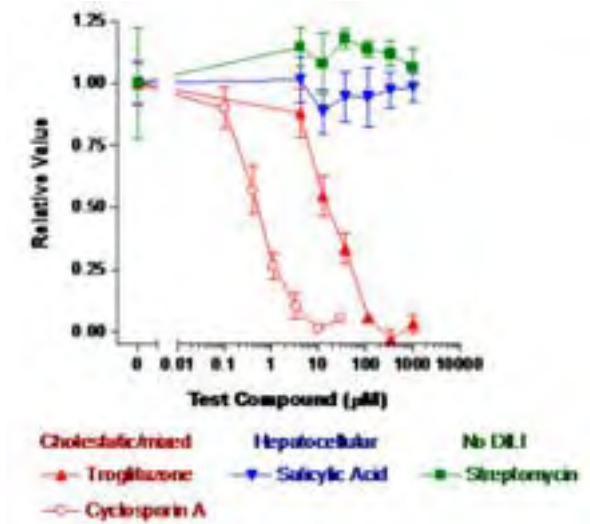
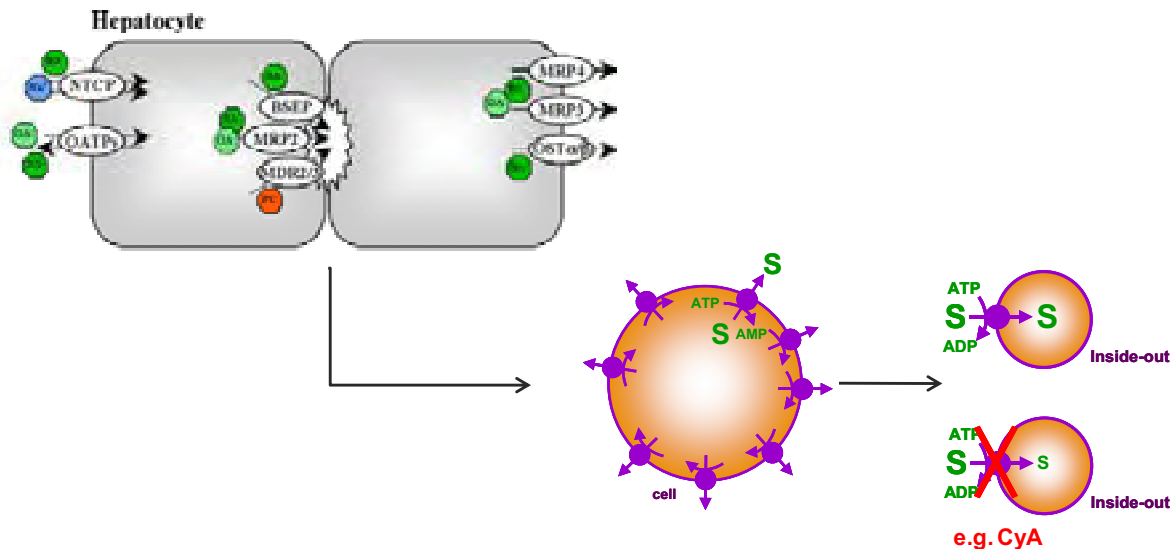
- Cholestasis = functional impairment of bile flow
  - Reduced bile secretion/flow, intracellular accumulation of bile constituents and overflow into blood plasma
- Genetic deficiencies in BSEP, MRP2 and MDR3 in humans cause cholestatic liver injury and/or hyperbilirubinaemia
  - PFIC, BRIC, Dubin-Johnson syndrome etc.
- Cholestatic liver injury is an important form of DILI (includes mixed hepatocellular/cholestatic)
- Many drugs that cause cholestatic DILI inhibit BSEP in vitro
  - e.g. troglitazone, bosentan, ketoconazole, nefazodone, chlorpromazine, erythromycin, glibenclamide, cyclosporine A, ...



# Analysis of BSEP inhibition *in vitro*

## Membrane vesicle assay

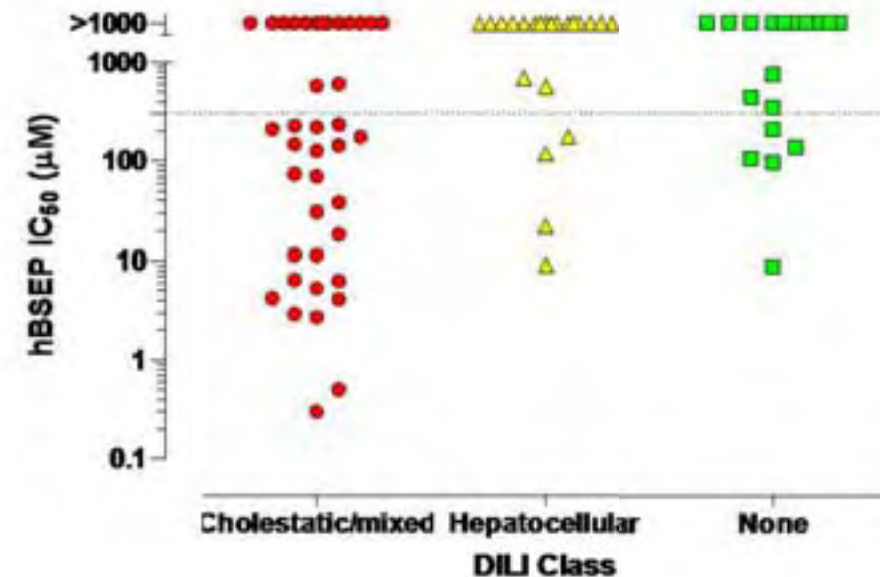
- Inverted plasma membrane vesicles derived from *Sf21* insect cells over-expressing BSEP
- Quantify inhibition of ATP-dependent uptake of probe substrate ([<sup>3</sup>H]-taurocholate or NBD-taurocholate)



# BSEP inhibition *in vitro*

## Human BSEP inhibition by marketed drugs

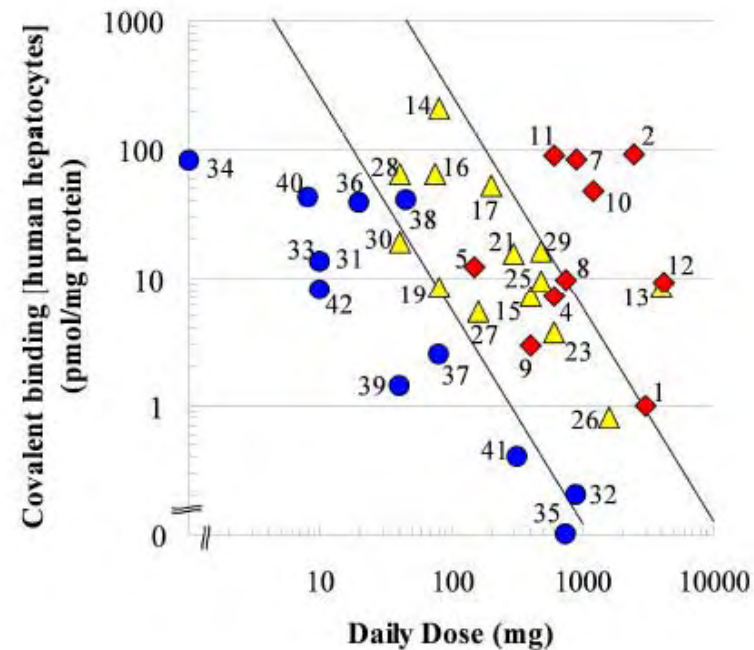
- 85 marketed drugs tested
- $IC_{50} < 300 \mu\text{M}$  observed with:
  - 24/42 (57%) cholestatic or mixed DILI
  - 4/22 (18) hepatocellular DILI
  - 5/21 (24%) no DILI
- Data support a relationship between DILI inhibition by drugs and DILI risk
- No apparent relationship between BSEP inhibition potency and DILI severity or incidence
  - e.g. rosiglitazone vs. pioglitazone



# Assessment of Reactive Metabolite Liability

**Daiichi Sankyo:** Nakayama, *et al.*, Drug Metab. Dispos. 2009; 37:1970

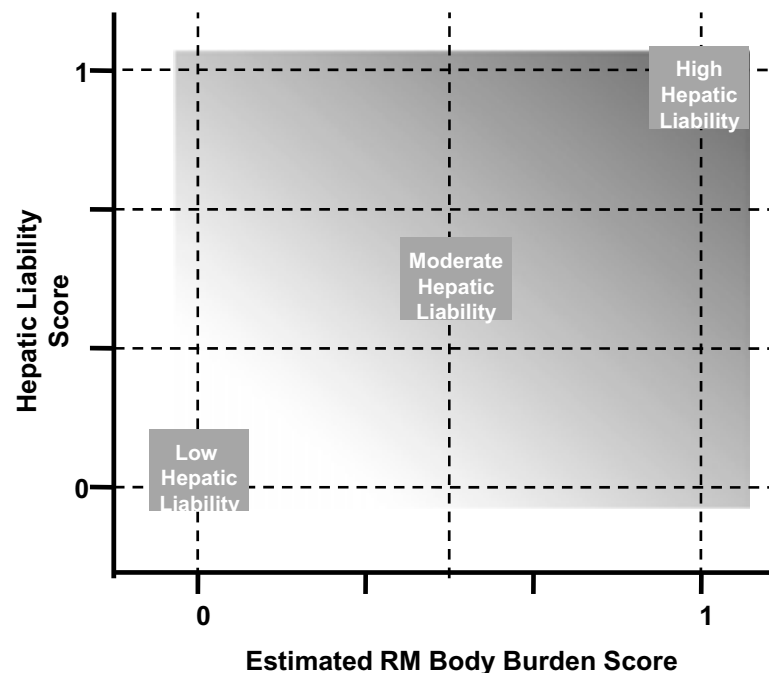
- Covalent binding of radiolabelled drugs to human hepatocyte protein *in vitro*
- 42 drugs tested:
  - 4 withdrawn due to ADRs
  - 8 ADR Black Box warning
  - 18 ADR warnings
  - 12 no ADR
- CVB plotted against dose
- Zone classification correctly classified most drugs
- In principle, the data analysis will be improved by adjusting for metabolic turnover *in vitro*



# Data Integration

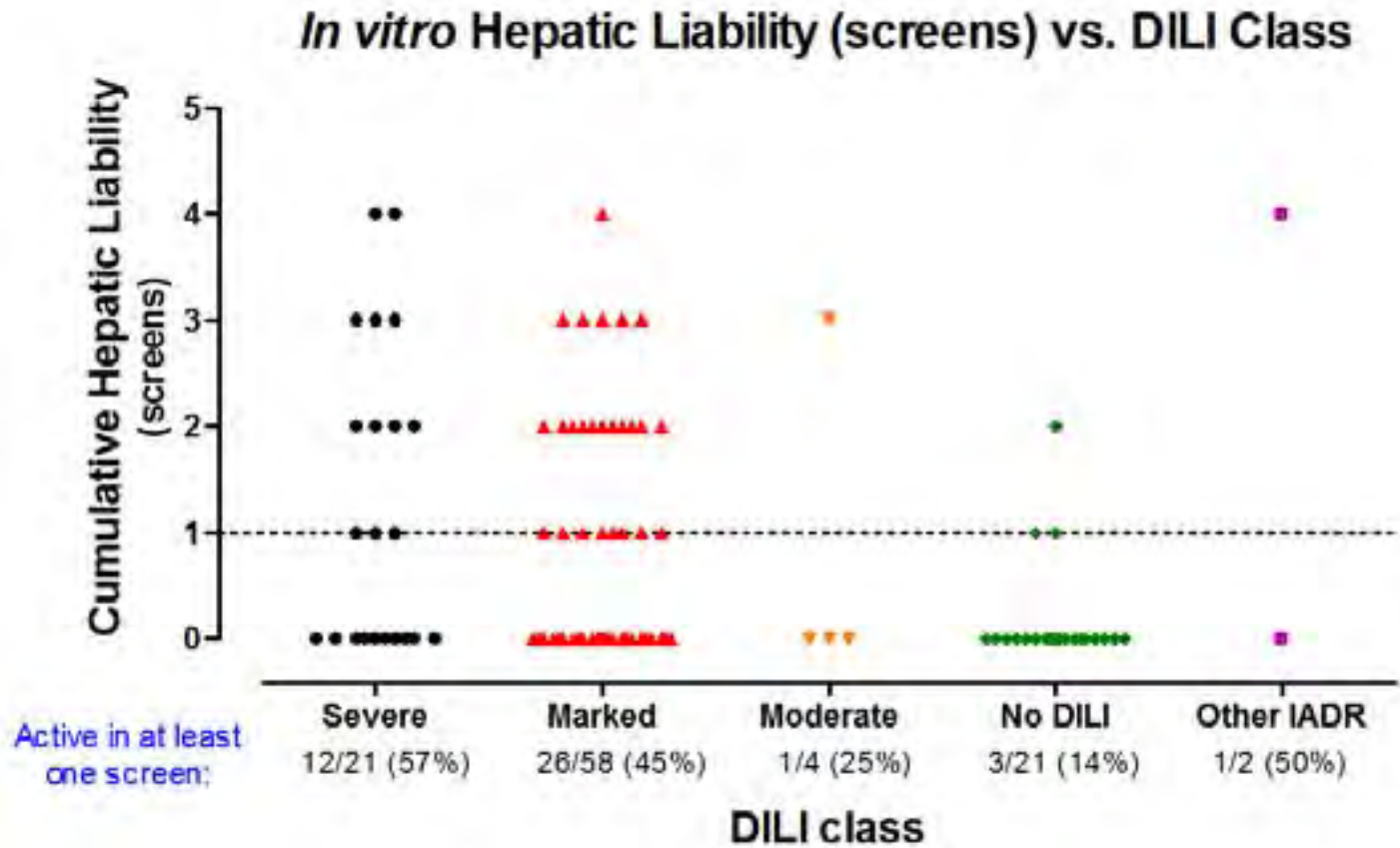
## Proposed DILI Hazard Matrix: Thompson et al., 2010, Chemico-Biol. Interact. ePub

- Individual *in vitro* assays rank **relative** DILI liability hazard of compounds and series
  - enabling choice of compounds with reduced potential to cause DILI during drug discovery, when there is chemical choice
  - i.e. internal decision making within projects, and across a project portfolio
- Combination of assays has the potential to improve prediction of DILI propensity



# Data Integration

Many drugs that cause DILI exhibit multiple *in vitro* liabilities



# Nonclinical *in vitro/in vivo* Translation

## Risk assessment of compounds that exhibit *in vitro* liabilities

- *In vitro* assays **cannot** be used to quantify “absolute” safety hazard or risk posed by novel compound series in man. This requires translation from:
  - chemical insult to biological response (e.g. Nrf2 induction)
  - *in vitro* models to relevant *in vivo* preclinical (animal) models
  - preclinical (animal) models to man
  - non-susceptible to susceptible humans
- The value of risk assessments that compare *in vitro* assay potency values (e.g. IC<sub>50</sub>) with predicted plasma exposure is questionable:
  - *in vitro* toxicity potency may not be equivalent to *in vivo* potency
  - assays quantify potency of parent compounds and have minimal metabolic capacity
  - plasma exposure is unlikely to accurately reflect exposure within liver cells (e.g. active hepatocyte uptake and biliary excretion)
  - prediction of plasma exposure may be incorrect

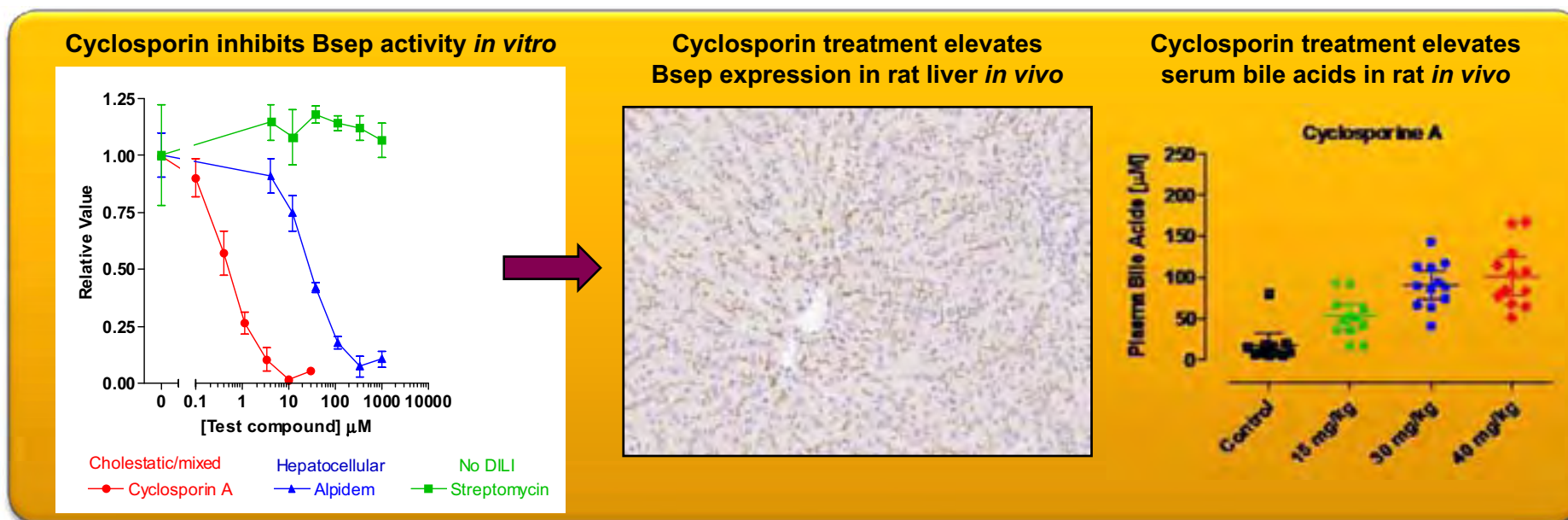


# Nonclinical *in vitro/in vivo* Translation

## Risk assessment of compounds that inhibit BSEP

A follow-on cascade is required to enable *in vivo* risk assessment for compounds exhibiting *in vitro* BSEP inhibition (or other liabilities)

- Evaluation in preclinical species of hepatic Bsep and Mrp2 expression, and serum bile acids, demonstrates whether Bsep inhibition occurs *in vivo* and provides safety margins
- Serum bile acids provide a potential translational biomarker which can be measured in man



# AZ Hepatotoxicity Target Organ Strategy



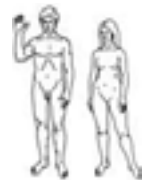
*In silico*

*In vitro*  
Hepatic Liability Panel

Preclinical Safety Evaluation – *in vivo*

Clinical Safety Evaluation

Identify & deselect compounds that have high propensity to cause DILI



Opportunities for improvement are being explored via in house activities (e.g. Imaging) and consortia (biomarkers: PSTC, IMI SAFE-T)

Liver monitoring and data interpretation in clinical trials is undertaken in accordance with FDA Clinical Guidance



# Innovative Medicine Initiative (IMI) Project

## Improved early prediction of Drug Induced Liver Injury (DILI) in man

- Primary goal is:  
“to identify new assays and models, which can be used during drug discovery and early non-clinical development to support design, ranking and selection of drugable candidates that have low propensity to cause DILI in man”
- A pre-competitive, industry-led project comprising (currently) 11 EFPIA pharma companies, plus an academic consortium selected by open competition in February 2011
- A detailed project plan is being developed
- Aim is to gain IMI approval and initiate work on a 5 year project in 2012



# IMI Predictive DILI Project

## Project goals

1. To identify and validate an improved panel of in vitro “best practice assays” for predicting DILI in the human population (**major objective**)
2. To explore and understand the relationship between in vitro assay signals and DILI in vivo, in preclinical test species and in man (**supportive**)
3. To develop and validate novel Systems Modelling approaches that integrate multiple preclinical data types to improve prediction of DILI in man (**supportive**)
4. To enhance shared understanding, between academia, pharma and regulatory agencies, of the value and limitations of new and existing approaches for DILI hazard identification and risk assessment (**supportive**)



# My thanks to many AZ colleagues

- Molecular Toxicology
  - Simone Stahl, Clare Walker, Sarah Dawson, Mhairi Greer, Alison Foster, Frida Gustafsson, Irene Edebert, Ina Schuppe-Koistinen
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- DECS Imaging
  - Jose Ulloa, Paul Hockings, John Waterton
- GSA/AZ
  - Hepatotoxicity TOS Steering Group
- Clinical
  - John Pears, Debra Silberg and the Hepatotoxicity Safety Knowledge Group



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