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
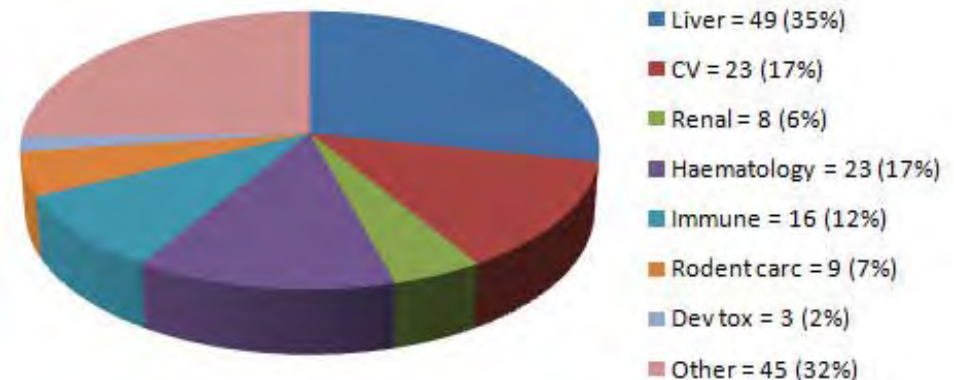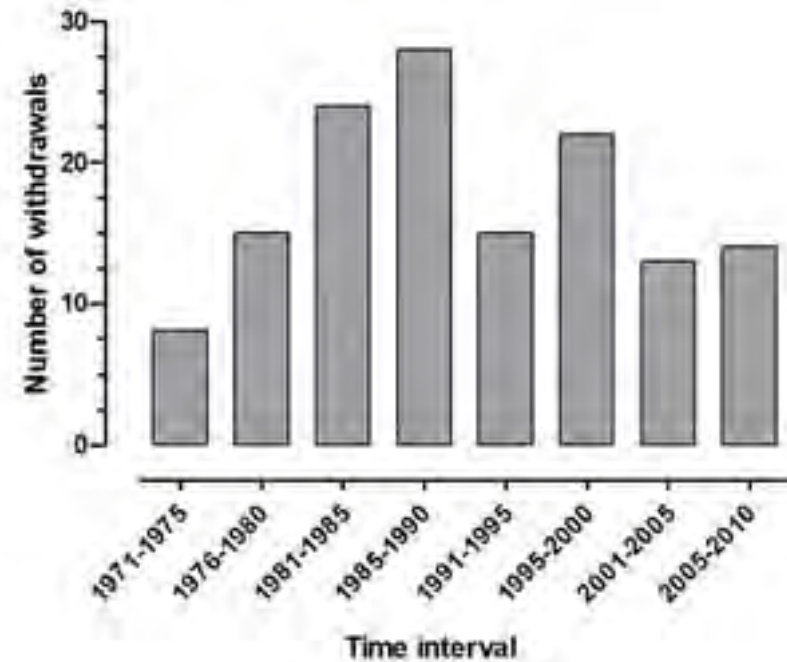
What is the problem?

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

Liver = 49 (35%)
CV = 23 (17%)
Renal = 8 (6%)
Haematology = 23 (17%)
Immune = 16 (12%)
Rodent carc = 9 (7%)
Dev tox = 3 (2%)
Other = 45 (32%)

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
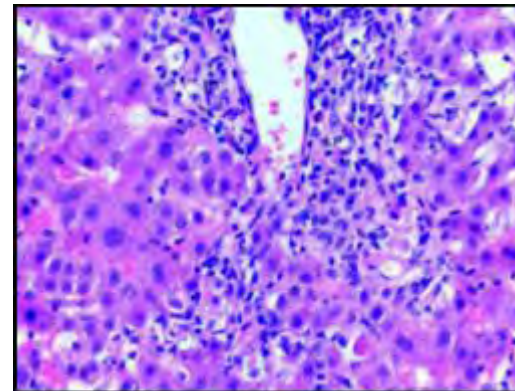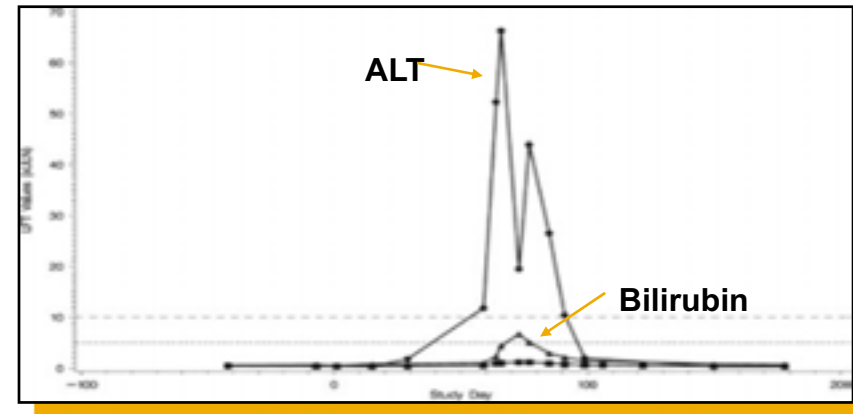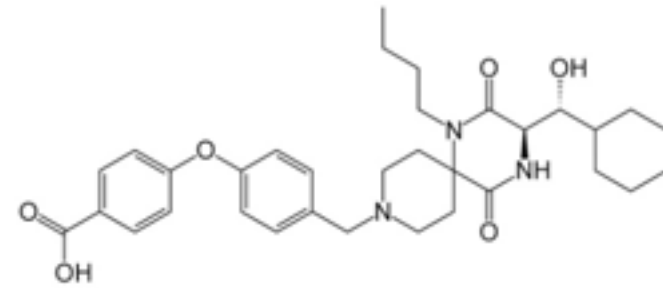- Relatively few fail to adapt and develop DILI (typically $\leq$1%)
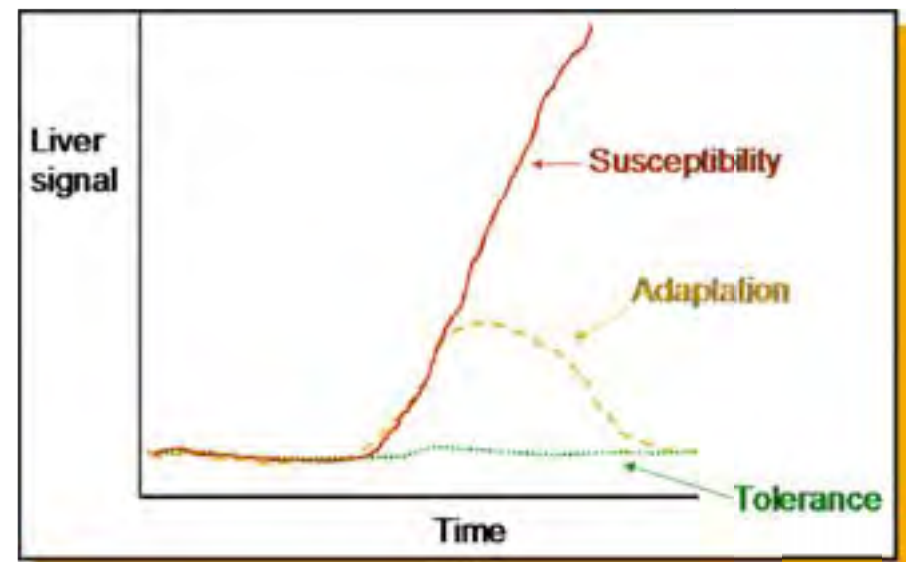
Rawlins & Thompson, Textbook of adverse drug reactions, Oxford University Press 1991: 18–45.
Gruchalla, Lancet 2000, 356: 1505-11.

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
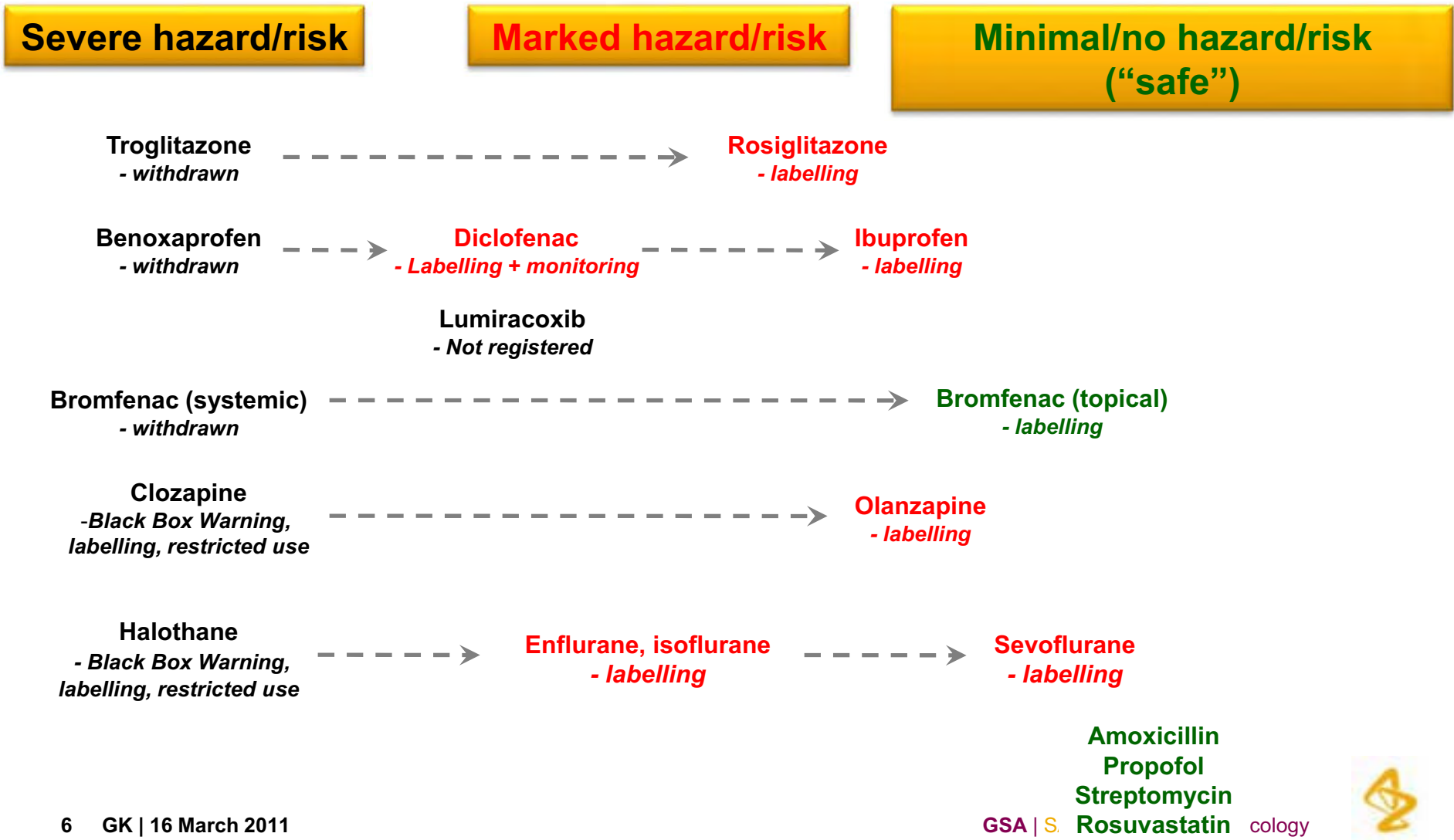
- Clinical development stopped

ALT

Bilirubin

Portal tract inflammation on biopsy

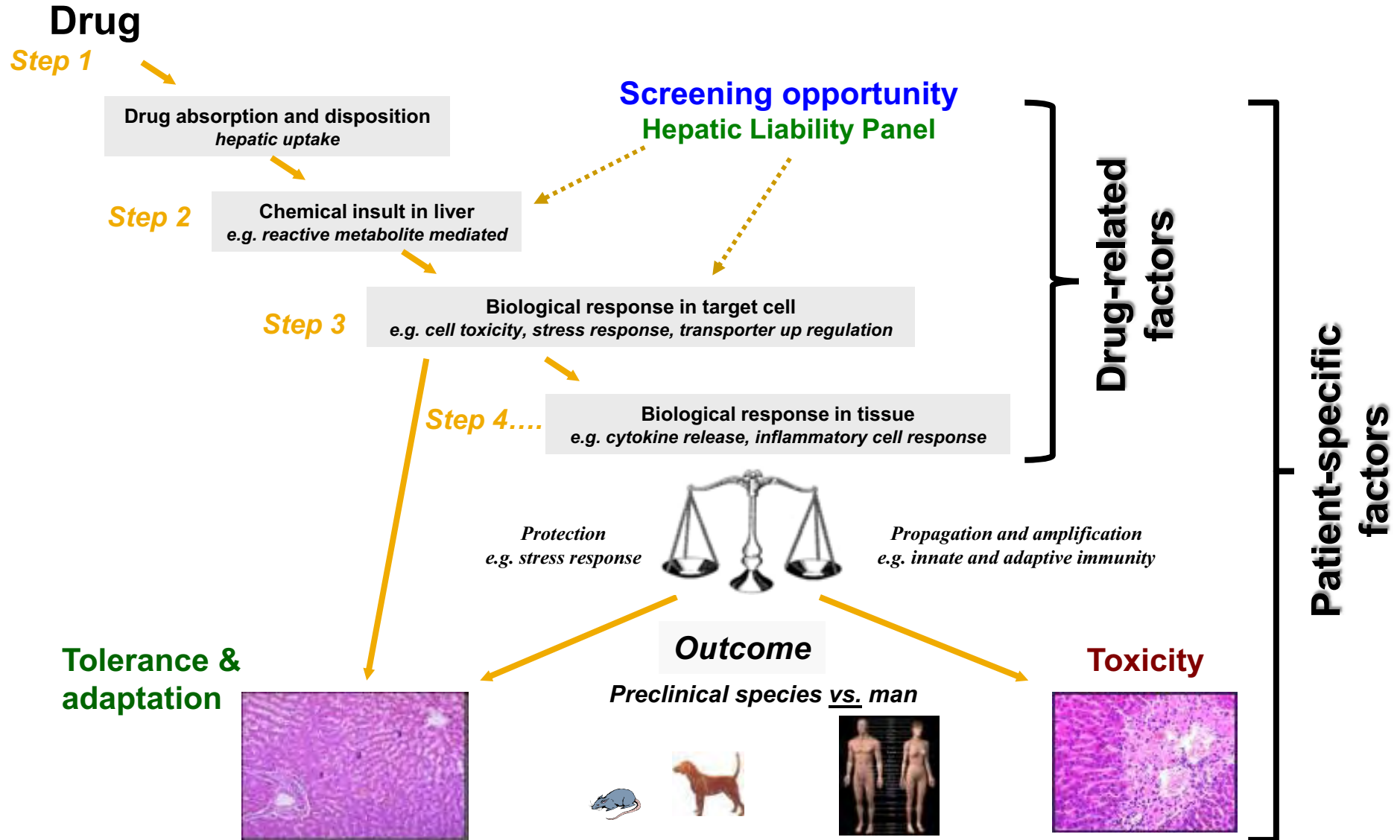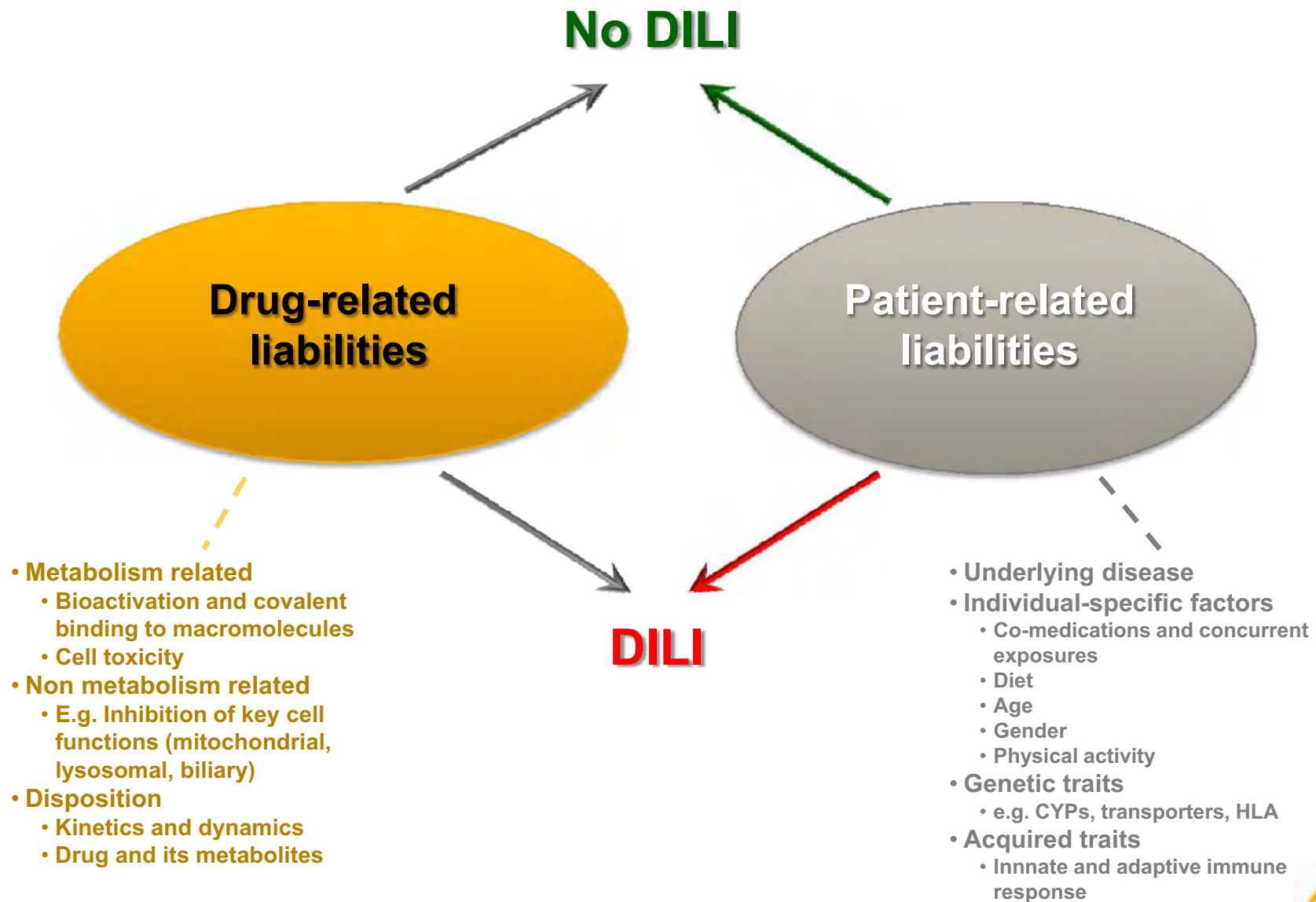Nichols et al., Antimicrob. Agents. Chemother. 2008; 52:858–865.

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DILI risk ranking

Some drugs pose a much greater risk than others

Severe hazard/risk	Marked hazard/risk	Minimal/no hazard/risk ("safe")

Troglitazone
- withdrawn → **Rosiglitazone**
- labelling

Benoxaprofen
- withdrawn → **Diclofenac**
- Labelling + monitoring → **Ibuprofen**
- labelling

Lumiracoxib
- Not registered

Bromfenac (systemic)
- withdrawn → **Bromfenac (topical)**
- labelling

Clozapine
-Black Box Warning, labelling, restricted use → **Olanzapine**
- labelling

Halothane
- Black Box Warning, labelling, restricted use → **Enflurane, isoflurane**
- labelling → **Sevoflurane**
- labelling

Amoxicillin
Propofol
Streptomycin
Rosuvastatin

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DILI Mechanisms
Multiple steps

Drug

Step 1

Drug absorption and disposition
hepatic uptake

Screening opportunity
Hepatic Liability Panel

Step 2

Chemical insult in liver
e.g. reactive metabolite mediated

Step 3

Biological response in target cell
e.g. cell toxicity, stress response, transporter up regulation

Step 4....

Biological response in tissue
e.g. cytokine release, inflammatory cell response

Drug-related factors

Patient-specific factors

Protection
e.g. stress response

Propagation and amplification
e.g. innate and adaptive immunity

Outcome

Preclinical species vs. man

Tolerance & adaptation

Toxicity

No DILI

Drug-related liabilities

Patient-related liabilities

DILI

- **Metabolism related**
  - **Bioactivation and covalent binding to macromolecules**
  - **Cell toxicity**
- **Non metabolism related**
  - **E.g. Inhibition of key cell functions (mitochondrial, lysosomal, biliary)**
- **Disposition**
  - **Kinetics and dynamics**
  - **Drug and its metabolites**

- **Underlying disease**
- **Individual-specific factors**
  - **Co-medications and concurrent exposures**
  - **Diet**
  - **Age**
  - **Gender**
  - **Physical activity**
- **Genetic traits**
  - **e.g. CYPs, transporters, HLA**
- **Acquired traits**
  - **Innnate and adaptive immune response**

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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?

Reactive metabolite toxicity
➡ **hepatocyte necrosis**
➡ **immunoallergic toxicity**

Mitochondrial impairment
➡ **hepatocyte necrosis**
➡ **microvesicular steatosis**

Inhibition of BSEP (MRP2)
➡ **Intrahepatic cholestasis**

AZ Hepatic Liability Panel

| Molecular Toxicology
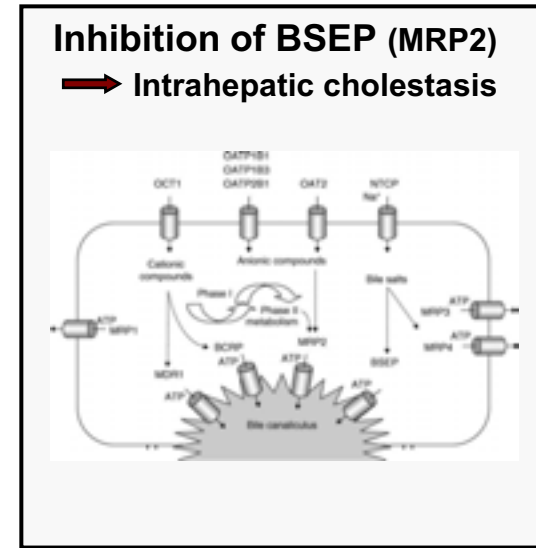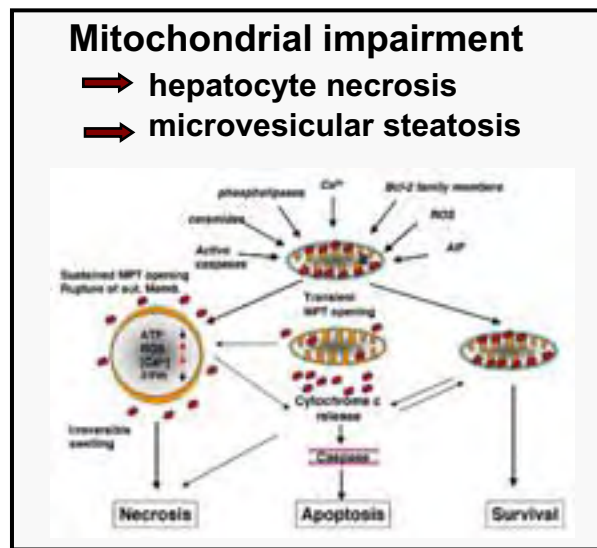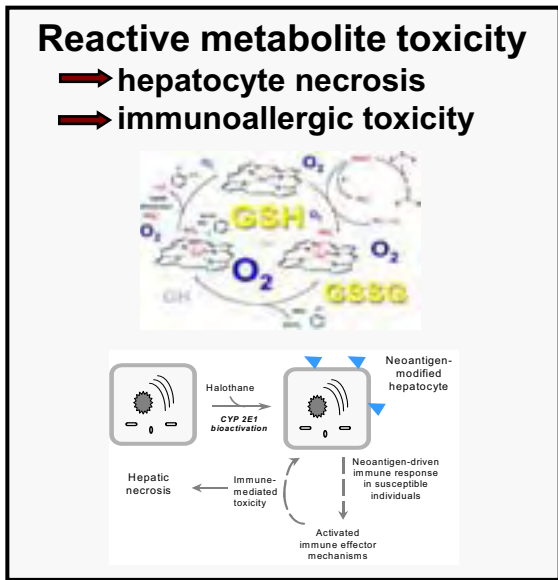
Cell Cytotoxicity Assessment

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 transfectionwith 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)

P450 cDNA

CMV promoter

Selection marker (Hygromycin)
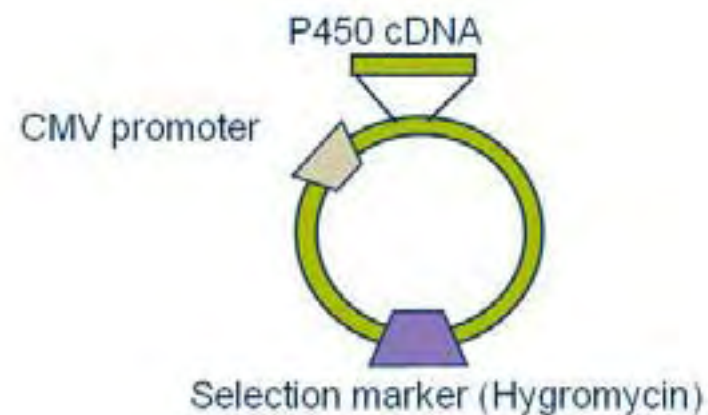
THLE assessment of DILI liability - I

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 or 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%
	Sensitivity = 72%	Specificity = 99.7%	

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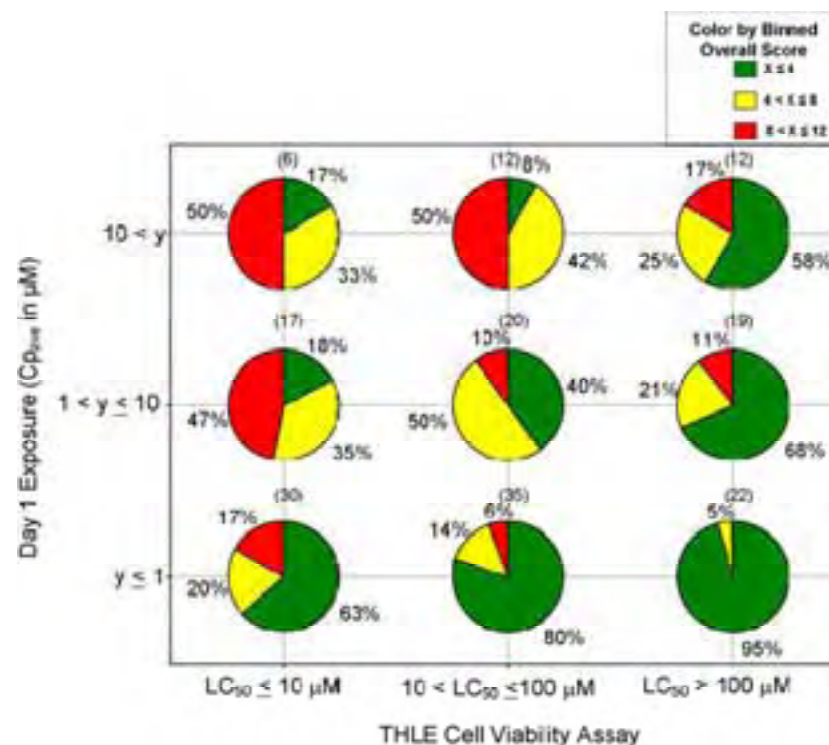
Fig. 2. Correlation of THLE cytotoxicity assay to composite safety scores from rat in vivo exploratory toxicity studies as a function of exposure.

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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 ($EC_{50} \leq 200$ μM) was exhibited by approx 30% marketed drugs that caused DILI, but very infrequently by non-DILI drugs
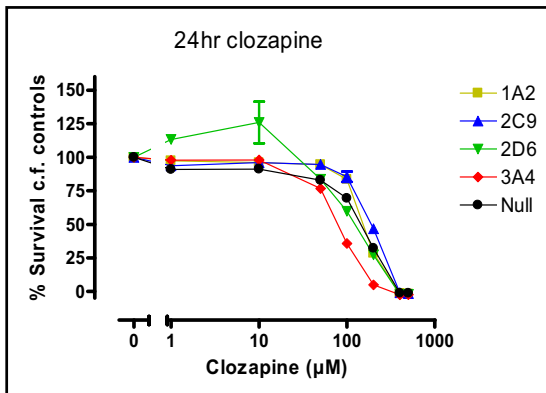

THLE-3A4 cell toxicity

...Molecular Toxicology
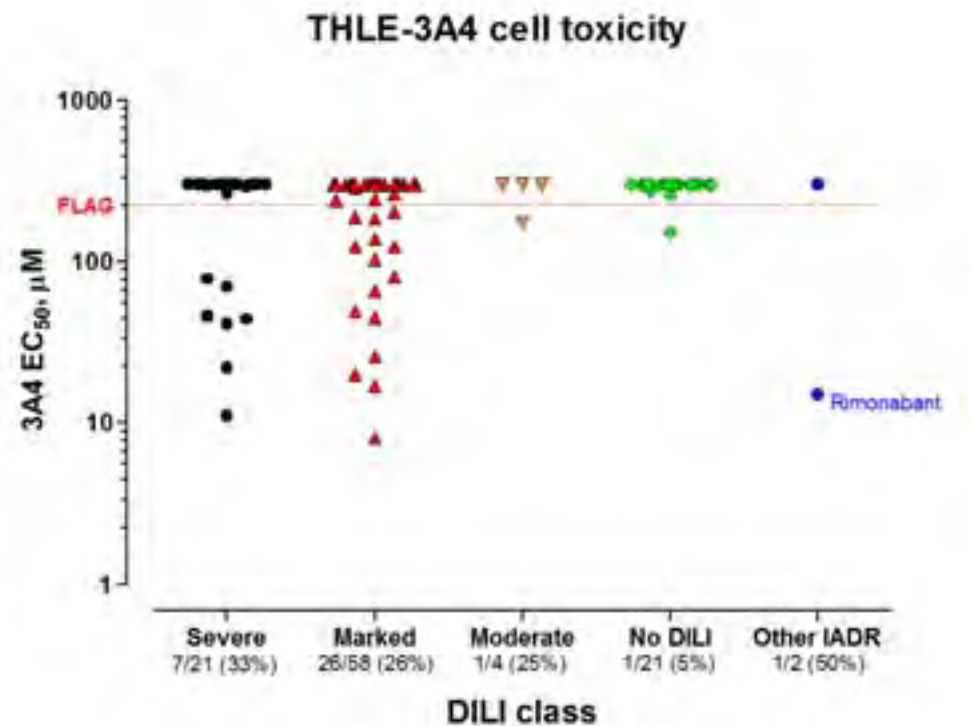
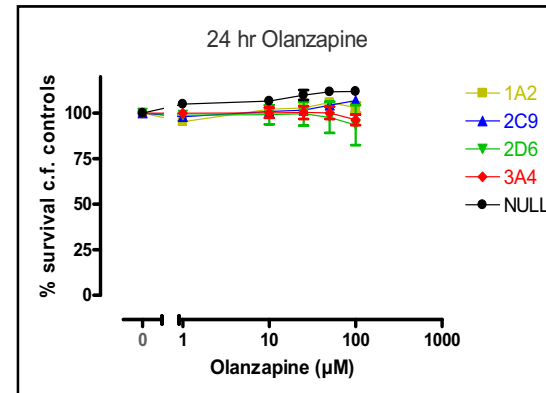# THLE assessment of DILI liability - IV
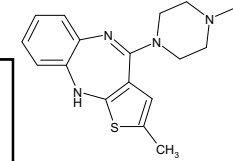
AstraZeneca compound comparison
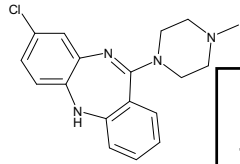
CLOZAPINE
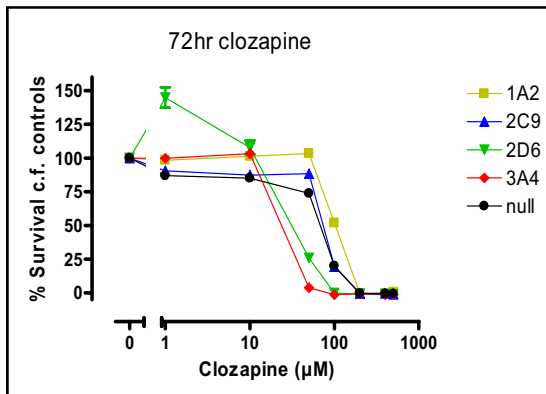
OLANZAPINE

- High dose (300-450 mg/day)
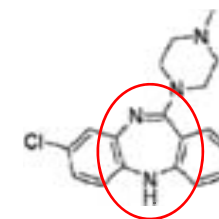- 1-2% incidence of agranulocytosis
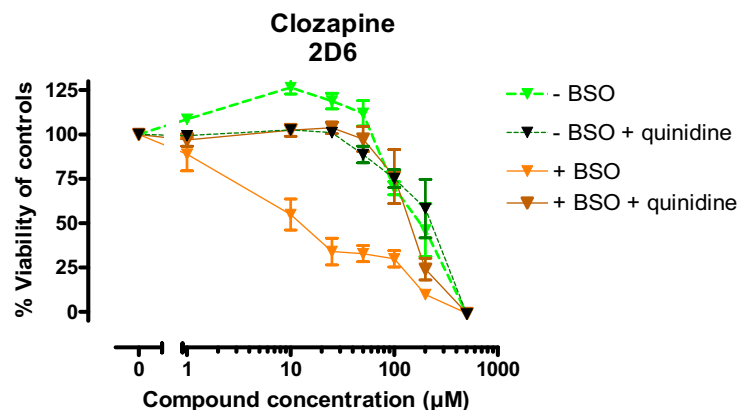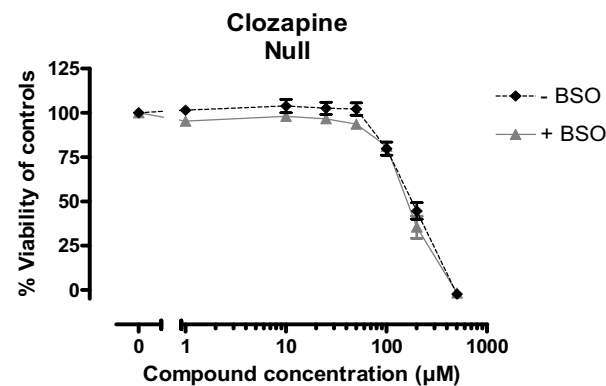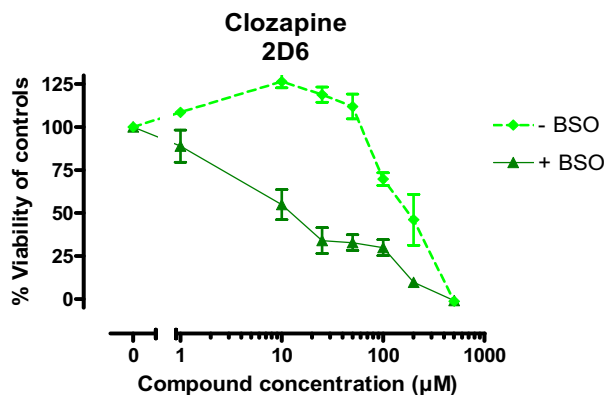- <0.1% incidence of hepatotoxicity
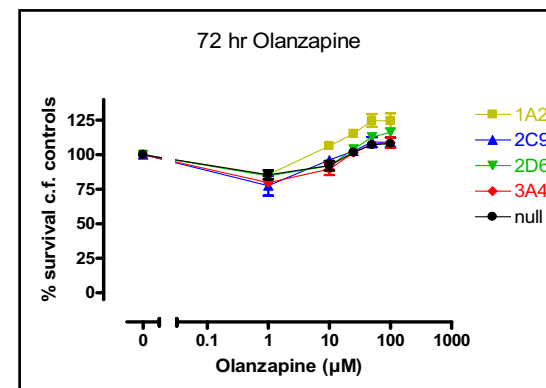
- Low dose (max. 20mg/day)
- Minimal ADRs in man

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

Clozapine 2D6 (top left): % Viability of controls vs Compound concentration (µM); legend: – BSO, + BSO

Clozapine Null (top right): % Viability of controls vs Compound concentration (µM); legend: – BSO, + BSO

Clozapine 2D6 (bottom): % Viability of controls vs Compound concentration (µM); legend: – BSO, – BSO + quinidine, + BSO, + BSO + quinidine

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)**

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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
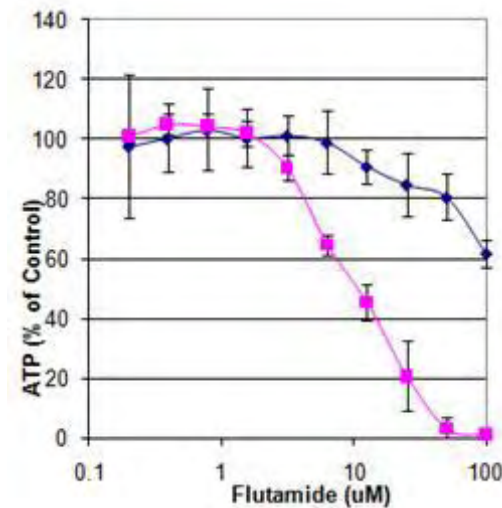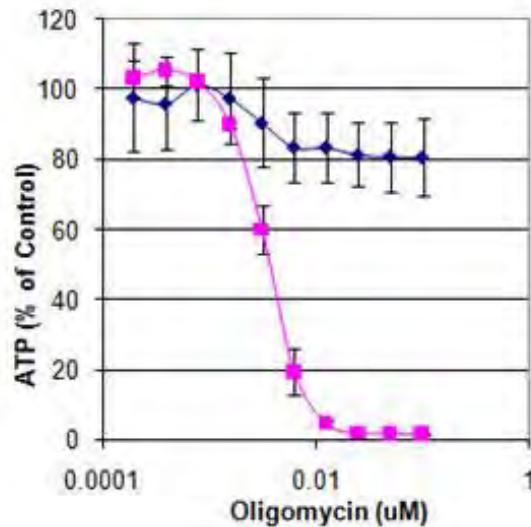
Biliary Transport Inhibition

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, ...

S. aureus Sav1866
Multidrug exporter
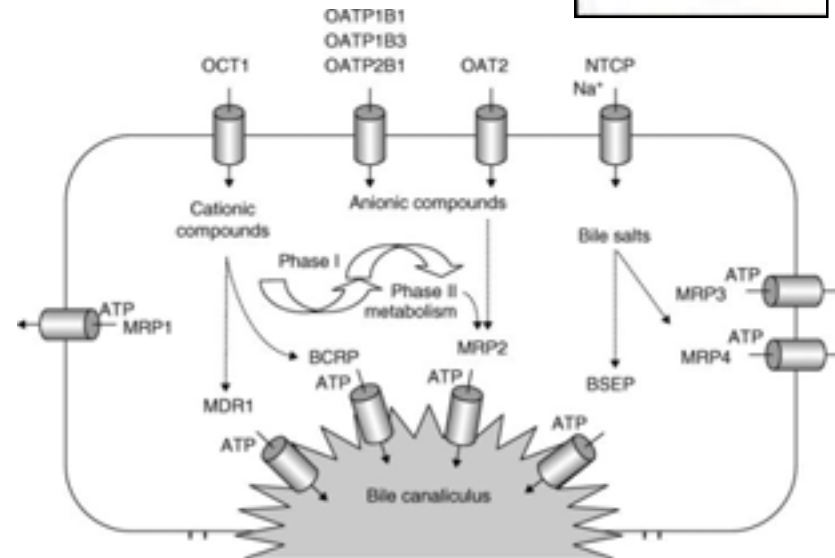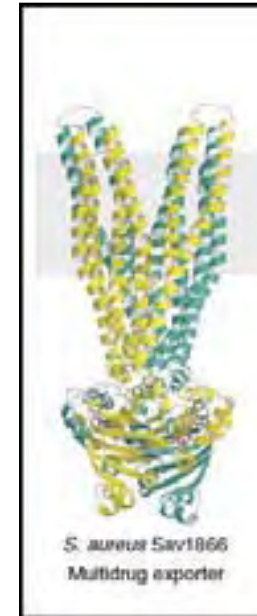
Analysis of BSEP inhibition *in vitro*

Membrane vesicle assay

- Inverted plasma membrane vesicles derived from *Sf*21 insect cells over-expressing BSEP

- Quantify inhibition of ATP-dependent uptake of probe substrate ([3H]-taurocholate of NBD-taurocholate)

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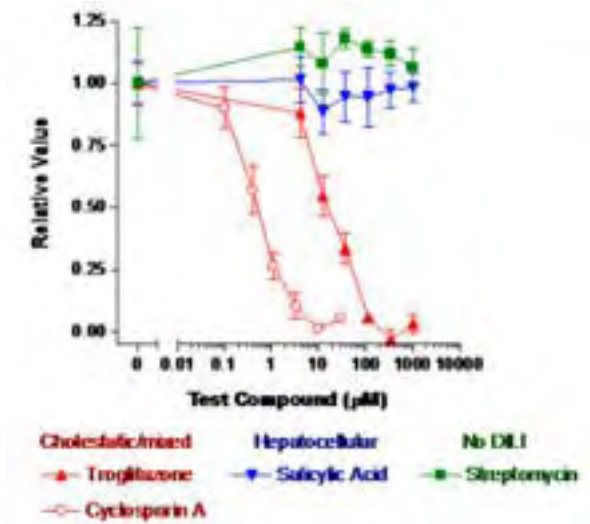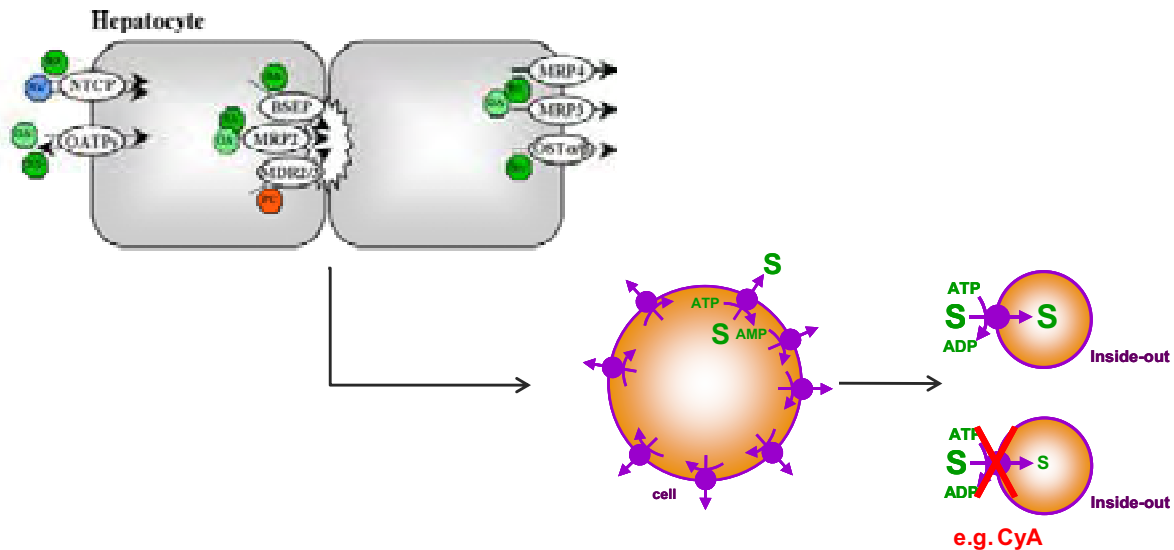
BSEP inhibition *in vitro*

Human BSEP inhibition by marketed drugs

- 85 marketed drugs tested

- $IC_{50} < 300$ µM 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
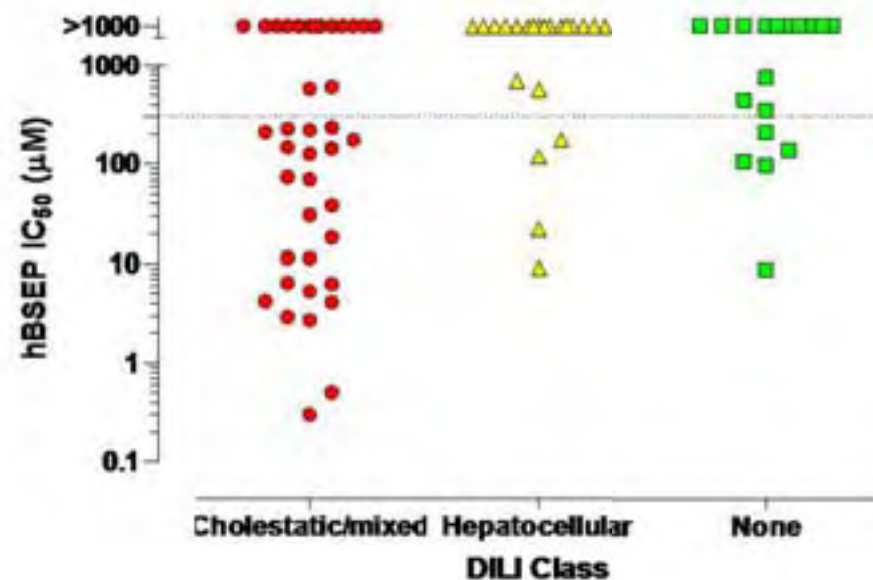
Assessment of Reactive Metabolite Liability

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*
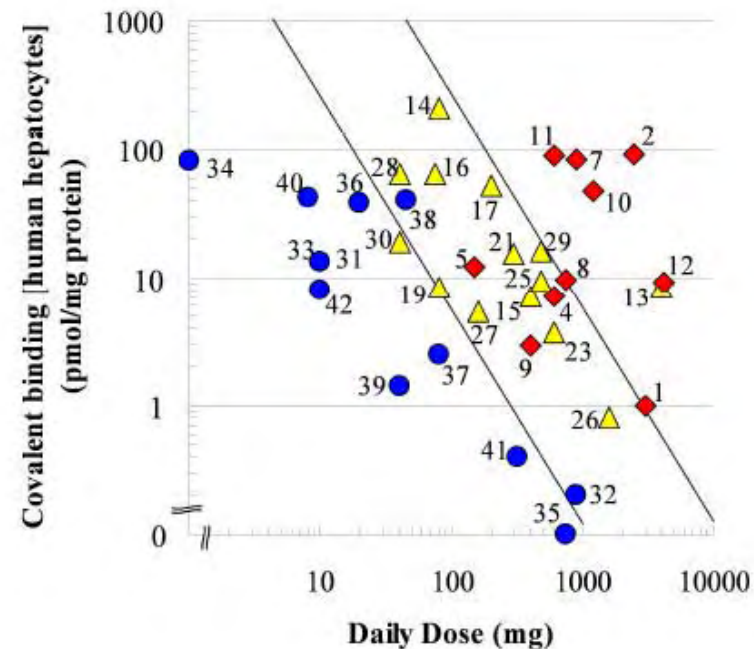
Data Integration

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

- Individual *in vitro* assays rank **<u>relative</u>** 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
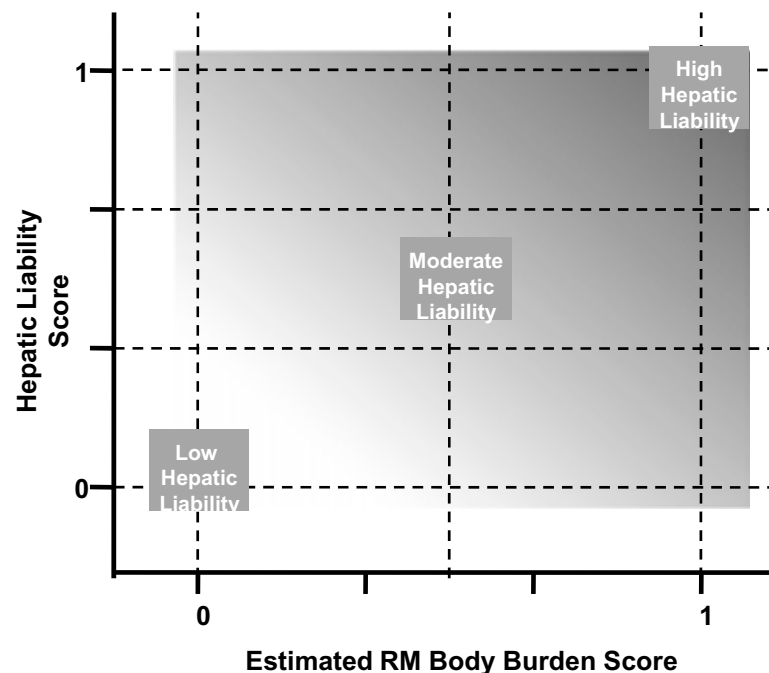
Data Integration

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

In vitro Hepatic Liability (screens) vs. DILI Class
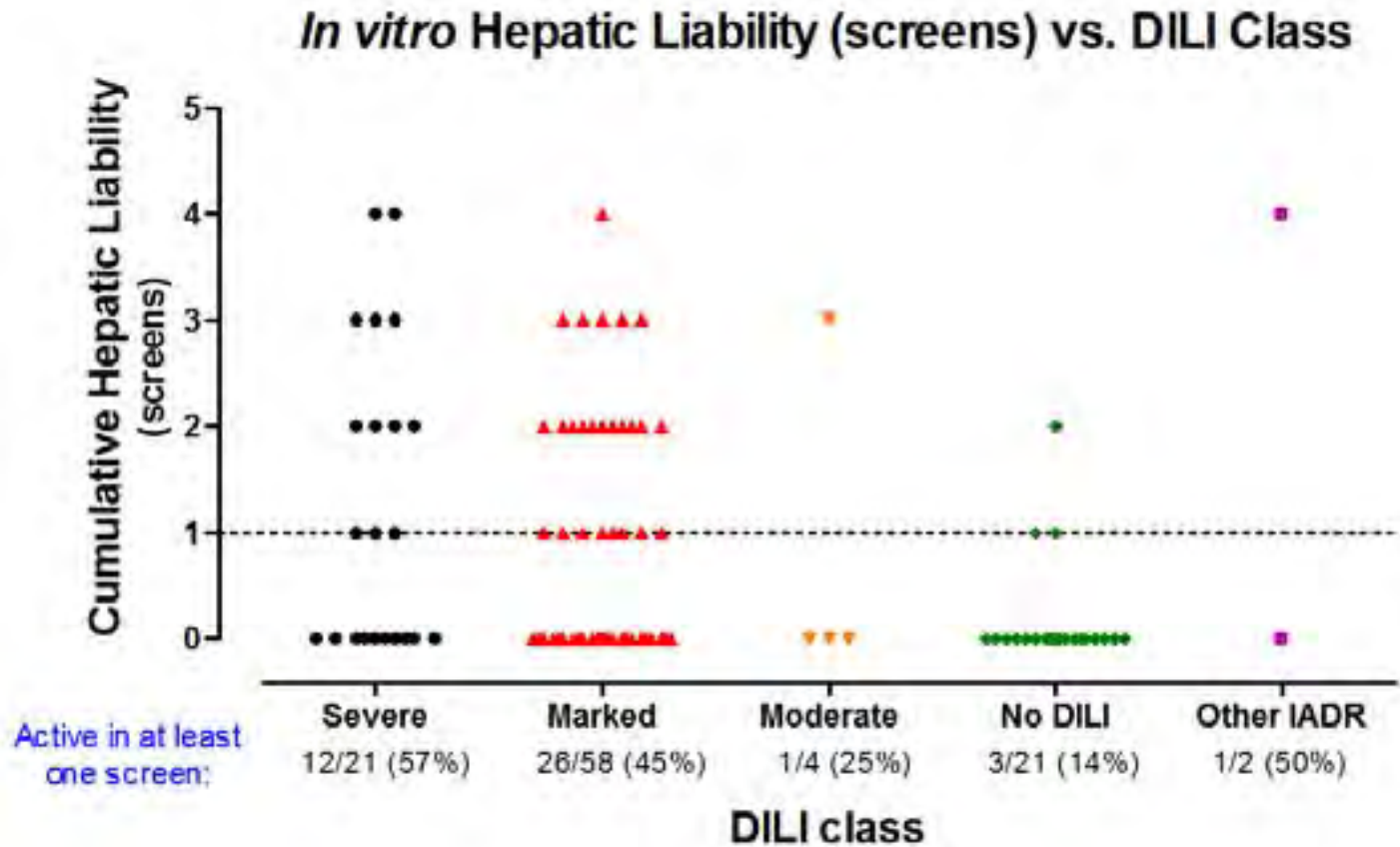
Nonclinical *in vitro*/*in vivo* Translation

Risk assessment of compounds that exhibit *in vitro* liabilities

- *In vitro* assays **<u>cannot</u>** 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_{50}) 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)
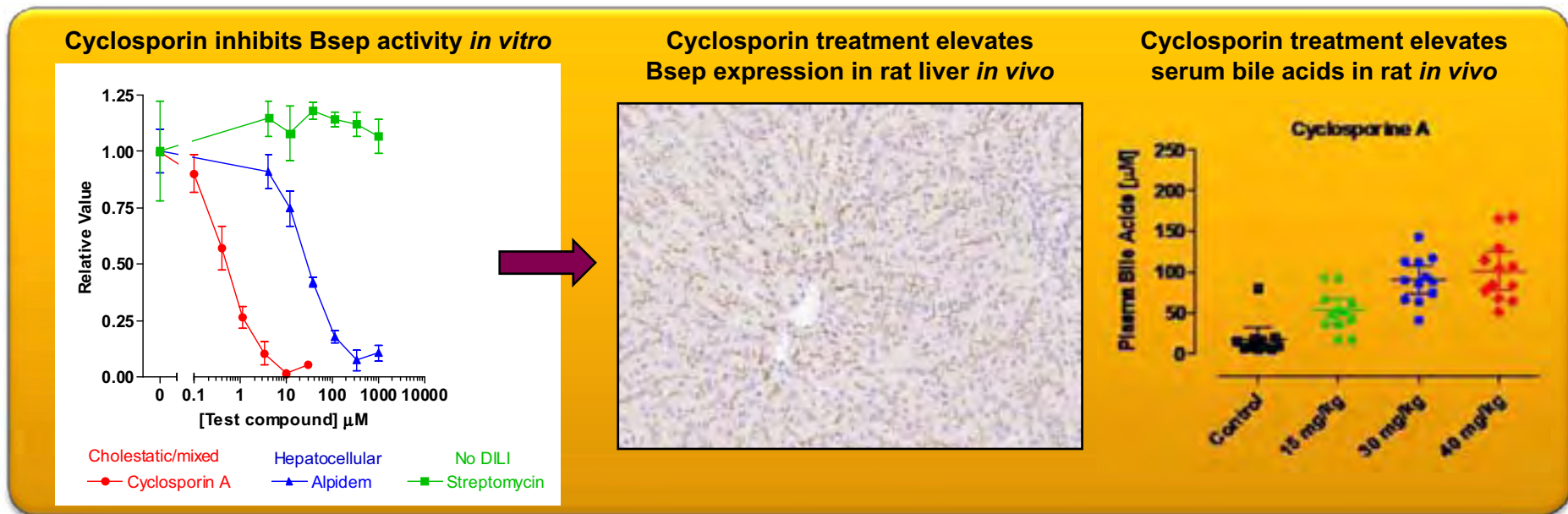 - prediction of plasma exposure may be incorrect

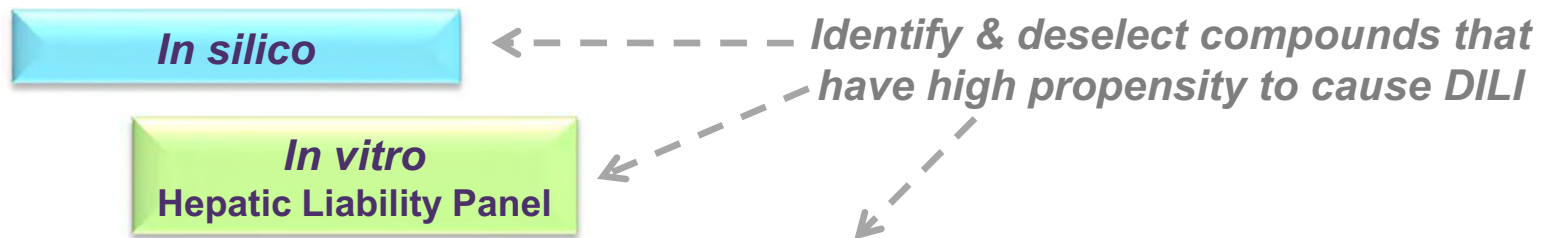# Nonclinical *in vitro*/*in vivo* Translation

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

Cyclosporin inhibits Bsep activity *in vitro*

Cholestatic/mixed — Cyclosporin A
Hepatocellular — Alpidem
No DILI — Streptomycin

Cyclosporin treatment elevates Bsep expression in rat liver *in vivo*

Cyclosporin treatment elevates serum bile acids in rat *in vivo*

Cyclosporine A

AZ Hepatotoxicity Target Organ Strategy

Target Selection → Lead Gen → Lead Optimization → Pre clinical → Phase I → Phase IIa → Phase IIb → Phase III/ Launch → Product Maint.

In silico

In vitro
Hepatic Liability Panel

Identify & deselect compounds that have high propensity to cause DILI

Preclinical Safety Evaluation – *in vivo*
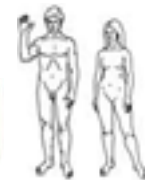
Clinical Safety Evaluation

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

- Safety Screening Centre
  - Helen Garside, Matt Bridgland-Taylor, Malcolm Haddrick, Jo Bowes

- DMPK
  - Richard Thompson, Ian Wilson and the ARMS team

- 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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