Integrating DILI hazards enables prediction, assessment and management of human risk

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Presentation outline

- What we have learned so far
- Hazard and risk
- DILI prediction reality check
- Data integration challenge
  - One proposed approach: *in vitro* Hazard Matrix
  - Exposure adjusted *in vitro* Hazard Matrix
- The future: PBPK based simulation
- Gaps and other opportunities
What we have learned so far

- DILI is complicated
  - Multiple pathologies
  - Dose dependent in animals
  - Dose-dependent or idiosyncratic in humans

- DILI arises multiple steps
  - Multiple initiating drug related mechanisms
  - Drug related and patient related susceptibility factors

- Reactive metabolites, mitochondrial injury and transporters play important roles
How DILI arises

Drug-related properties

- Disposition
  - Kinetics and dynamics of drug and its metabolites
- Metabolism related
  - Bioactivation and covalent binding to macromolecules
  - Adaptive immunity to haptens
- Non metabolism related
  - Mitochondrial injury
  - BSEP and other transporters
  - Innate immunity

Patient-related properties

- Inherited traits
  - Genetic (CYPs, transporters, HLA etc.)
  - Epigenetic
- Acquired traits
  - Underlying disease
  - Infections
  - Co-medications and concurrent exposures
  - Diet (inc. smoking, drinking)
  - Age, gender
  - Physical activity

Dose dependent: most patients given the toxic dose develop DILI, e.g. paracetamol
Idiosyncratic: most patients tolerate the drug or adapt, e.g. halothane, ximelagatran
Hazard and risk

**Hazard** = any source of potential adverse health effect, harm or damage

**Risk** = the likelihood that a person exposed to a hazard will be harmed

**Exposure** = the extent to which someone is subjected to a hazard

**HAZARD + EXPOSURE = RISK**
DILI prediction reality check

- *In vitro* assays assess drug-related liabilities, not patient-related factors
  - Therefore they cannot quantify DILI risk of DILI to individual patients (or animals)

- However, they ought to be able to provide useful discrimination between drugs with high vs. low DILI propensity
  - Providing account is taken of *in vivo* drug exposure

- An ideal *in vitro* assay should assess all the most important DILI initiating mechanisms

- Alternatively, need to integrate data from a panel of assays that each assess different DILI liabilities
Chemical Research in Toxicology

In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs

Richard A. Thompson,† Emre M. Isin,‡ Yan Li,§ Lars Weidolf,† Ken Page,§ Ian Wilson,§ Steve Swallow,‖ Brian Middleton,‡ Simone Stahl,‖ Alison J. Foster,‖ Hugues Dolgos,‖ and J. Gerry Kenna‖

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§DMPK Innovative Medicine, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom
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- Multi-parametric evaluation of several in vitro safety endpoints, plus covalent binding to hepatocyte proteins
### In vitro assay panel

<table>
<thead>
<tr>
<th>Assay</th>
<th>Endpoint</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>THLE-3A4 toxicity</td>
<td>Cytotoxicity</td>
<td>CYP3A4 mediated toxicity</td>
</tr>
<tr>
<td>THLE-Null toxicity</td>
<td>Cytotoxicity</td>
<td>Parent drug toxicity</td>
</tr>
<tr>
<td>HepG2 Glu/Gal assay</td>
<td>Cytotoxicity</td>
<td>Mitochondrial inhibition</td>
</tr>
<tr>
<td>BSEP vesicle</td>
<td>BSEP inhibition</td>
<td>Bile acid excretion</td>
</tr>
<tr>
<td>Mrp2 vesicle</td>
<td>Mrp2 inhibition</td>
<td>Bile salt transport</td>
</tr>
<tr>
<td>Human hepatocyte covalent binding (CVB)</td>
<td>CVB to proteins</td>
<td>Bioactivation</td>
</tr>
</tbody>
</table>
- Inverted plasma membrane vesicles from BSEP-transfected Sf21 cells
- Inhibition of ATP-dependent probe substrate ([3H]-taurocholate) uptake

BSEP inhibition

- Transport of substrate S under control conditions
- Reduced substrate uptake in presence of inhibiting drug D

Test Compound (μM)

Relative Value

Cholestatic/mixed
- Troglitazone
- Salicylic Acid
- Cyclosporin A

Hepatocellular
- Streptomycin

No DILI
Concern cut-off value = 300 μM

- Increased frequency and potency of BSEP inhibition amongst drugs which cause human cholestatic DILI
- Numerous drugs inhibited BSEP but did not cause DILI
THLE cell toxicity

- THLE = SV40 - T antigen immortalised Human Liver Epithelial Cells
- Immortal and stable cell background, excellent growth properties
  - No CYP expression/activity
  - Retains phase II activities (GST, ST, EH), not UGT. (Pfeifer et al. PNAS USA 1996;90: 5123)

- Sub-lines prepared by transfection with constructs, encoding individual human P450s (Macé et al.1997, Carcinogenesis 18:1291)
  - No CYP construct = “THLE-Null”
  - Individual cell lines expressing CYP 1A2, 2E1, 2C9, 2C19, 2D6, 3A4 = “THLE-CYP”
THLE-null cell toxicity

- CYP-independent THLE-Null cytotoxicity caused by numerous drugs which caused DILI
- One “false positive”: rimonabant, a very low dose/exposure drug

Gustafsson et al. 2014, Toxicol. Sci. 137:189-211.
Potentiated THLE-3A4 cell toxicity

Ratio = \frac{\text{THLE-Null IC}_{50}}{\text{THLE-3A4 IC}_{50}}

Gustafsson et al. 2014, Toxicol. Sci. 137:189-211.
Integrating *in vitro* assay data


<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP inhibition</td>
<td>Inhibition of human BSEP transport activity</td>
</tr>
<tr>
<td>Mrp2 inhibition</td>
<td>Inhibition of rat Mrp2 transport activity</td>
</tr>
<tr>
<td>HepG2 MitoTox</td>
<td>HepG2 toxicity in glucose vs galactose media (mito-independent) (mito-dependent)</td>
</tr>
<tr>
<td>THLE toxicity</td>
<td>Toxicity to THLE-Null (CYP independent)</td>
</tr>
<tr>
<td></td>
<td>THLE-3A4 (CYP3A4 potentiated) toxicity</td>
</tr>
</tbody>
</table>

![Binary scores diagram](image)
Aggregated *in vitro* assay data


**Selectivity and Specificity for the in vitro Panel**

<table>
<thead>
<tr>
<th>Severe &amp; Marked concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more Signals</td>
<td>13</td>
</tr>
<tr>
<td>1 or less Signals</td>
<td>14</td>
</tr>
</tbody>
</table>

PPV (13/14) = 93%

NPV (8/22) = 58%

Sensitivity (13/27) = 48%
Specificity (8/9) = 89%

Correct = 58%

PPV, positive predictive value; NPV, negative predictive value
Human hepatocyte CVB


- High human idiosyncratic adverse reaction concern
- Medium concern
- No concern

- Combination of CVB and daily dose discriminated between High Concern and No/Low Concern drugs
Human hepatocyte $f_{cvb}$ vs. Dose

$\text{Maximum daily dose (mg/day)}$

$\text{Severe}$  
$\text{Marked}$  
$\text{Low}$

$ f_{cvb} = \text{fraction of metabolised dose resulting in CVB}$

- Could be split into 3 zones, cf. Nakayama et al.
- Slope of lines approx 1
- High $f_{CVB}$ and dose = high adverse reaction concern
- But middle zone provides inadequate discrimination for use as a compound selection tool

Integrating dose adjusted $f_{CVB}$ ("CVB burden") with other *in vitro* liabilities


<table>
<thead>
<tr>
<th></th>
<th>Inhibition of human BSEP transport activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP inhibition</td>
<td></td>
</tr>
<tr>
<td>Mrp2 inhibition</td>
<td>Inhibition of rat Mrp2 transport activity</td>
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<td>HepG2 MitoTox</td>
<td>HepG2 toxicity in glucose vs galactose media (mito-independent) (mito-dependent)</td>
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<td>THLE toxicity</td>
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</tr>
<tr>
<td></td>
<td>THLE-3A4 (CYP3A4 potentiated) toxicity</td>
</tr>
<tr>
<td>Covalent binding (CVB)</td>
<td>CVB of radiolabelled drug to human hepatocyte proteins</td>
</tr>
<tr>
<td></td>
<td>$F_{cvb} = \text{Fraction of metabolism leading to CVB}$</td>
</tr>
<tr>
<td></td>
<td>CVB Burden = $f_{cvb} \times \text{Daily dose (mg/day)}$</td>
</tr>
</tbody>
</table>

*Binary scores*
Integrated *in vitro* Hazard Matrix

**Zone 1** = CVB + multiple safety assay signals

**Zone 2** = Multiple assay signal signals

**Zone 3** = CVB

**Zone 4** = No CVB or safety signal concerns

*Chem Res Toxicol 2012; 25:1616*
Value of *in vitro* Hazard Matrix

- Provides objective evidence of whether a candidate drug exhibits properties that raise human safety concern
  - If Low/No Concern, can progress into the clinic with confidence
  - Individual assays can be used to address liabilities of High Concern compounds
- But cannot be used to predict toxicity in individual patients.

A tool to evaluate and address risk, where previously there would only have been endless discussions……
**Case study:**

**Endothelin Receptor Antagonists (ETRAs)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose, mg/day</th>
<th>Number of patients treated</th>
<th>Human DILI observed</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan-Thelin®</td>
<td>100</td>
<td>2,000</td>
<td>• 4 deaths</td>
<td>Withdrawn 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 1 liver transplantation</td>
<td></td>
</tr>
<tr>
<td>Bosentan-Tracleer®</td>
<td>250</td>
<td>80,000</td>
<td>• Elevated LFT common</td>
<td>Black box warning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Cases of severe liver injury</td>
<td></td>
</tr>
<tr>
<td>Ambrisentan-Letairis™ (US), -Volibris® (EU)</td>
<td>10</td>
<td>10,000</td>
<td>None, but precautionary label when licensed</td>
<td>Safe drug, no DILI label</td>
</tr>
</tbody>
</table>

Can the *in vitro* assay panel correctly rank the ETRAs?

- Sitaxentan: sulfonamide, 1,3-benzodioxazole, High
- Bosentan: sulfonamide, High
- Ambrisentan: propanoic acid, None

DILI concern
**BSEP and MRP2 inhibition, plus THLE cell cytotoxicity**

<table>
<thead>
<tr>
<th></th>
<th>hBSEP IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>hMRP2 IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Binary score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>14</td>
<td>67</td>
<td>2</td>
</tr>
<tr>
<td>Bosentan</td>
<td>28</td>
<td>157</td>
<td>2</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>285 Activator*</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* >1000 in rat Mrp2

<table>
<thead>
<tr>
<th></th>
<th>THLE-Null EC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>THLE-Null/3A4 EC&lt;sub&gt;50&lt;/sub&gt; ratio (μM)</th>
<th>Binary score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>160</td>
<td>1,8</td>
<td>2</td>
</tr>
<tr>
<td>Bosentan</td>
<td>&gt;300</td>
<td>1,5</td>
<td>1</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>&gt;300</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total in vitro panel score**

- Sitaxentan: 4
- Bosentan: 3
- Ambrisentan: 1

*Kenna et al. 2015, J. Pharmacol. Exp. Ther. 352(2):281-90*
## CVB to human hepatocyte proteins

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/day)</th>
<th>CVB (pmol/mg protein)</th>
<th>$f_{cvb}$</th>
<th>Estimated RM Body Burden (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>100$^1$</td>
<td>200</td>
<td>0.07</td>
<td>7</td>
</tr>
<tr>
<td>Bosentan</td>
<td>250 (2x125)</td>
<td>39</td>
<td>0.006</td>
<td>1.5</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>10</td>
<td>7</td>
<td>0.003</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1. Dose is expressed as the daily dose divided by the body weight (mg/kg/day).

The compounds are represented with their corresponding molecular structures:

- [3$^3$H]Bosentan
- [4-3$^3$H]Sitaxentan
- [3$^3$H]Ambrisentan
- [7-3$^3$H]Sitaxentan
ETRA Integrated in vitro Hazard Matrix

Kenna et al. 2015, J. Pharmacol. Exp. Ther. 352(2):281-90
Opportunity for improvement: Drug exposure adjustment of assay data

- All tested drugs with BSEP IC$_{50}$ < 300 µM and C$_{max}$ > 2 µM caused DILI

Dawson et al. 2012, DMD 40:130–138
See also: Morgan et al. 2013, Tox Sci 136:216-41
A simple way to take account of potency of BSEP inhibition plus plasma drug concentration ($C_{\text{max}}$, or $C_{ss}$)

- Requires accurate determination of *in vivo* plasma drug concentrations

*Data from: Dawson et al. 2012, DMD 40:130–138*
Considering *in vivo* human plasma exposure markedly improves the correlation between in vitro BSEP inhibition and DILI risk

Hence exposure adjusted binary scoring (cf. FDA DDI Guidance):

- Plasma $[C]_{max,ss}$ / *in vitro* IC$_{50}$ or EC$_{50}$ ≥ 0.1 = 1
- Plasma $[C]_{max,ss}$ / *in vitro* IC$_{50}$ or EC$_{50}$ < 0.1 = 0

Data from Dawson et al. 2012, *Drug Metab. Dispos.* 40:130
Multiple compound related adverse properties contribute to liver injury caused by endothelin receptor antagonists

J. Gerry Kenna, Simone H. Stahl, Julie A. Eakins, Alison J. Foster, Linda C. Andersson, Jonas Bergare, Martin Billger, Marie Elebring, Charles S. Elmore, Richard A. Thompson

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JPET Fast Forward. Published on December 2, 2014 as DOI: 10.1124/jpet.114.220491

**Improved in vitro ranking of ETRA human DILI propensity**

- **Sitaxentan (withdrawn):**
  - High CVB
  - Cytotoxic metabolites
  - Mitochondrial impairment
  - Intrinsic cell cytotoxicity
  - BSEP, MRP2 inhibition

- **Bosentan (BBW) exhibited:**
  - CVB
  - BSEP inhibition

- **Ambisentan (safe)**
  - No signals

*J Pharmacol Exp Ther. 2015 Feb;352(2):281-90*
The future: PBPK based exposure scaling

Other future opportunities

- An optimal tiered test cascade
  - Simple low cost/low complexity volume tier 1 assays (e.g. THLE cells, BSEP inhibition?) plus higher cost/more complex organotypic tier 2 assays which address immune responsiveness?

- Enhanced *in vitro* / *in vivo* scaling and data integration
  - Quantification of drug and key metabolite concentrations *in vitro* and *in vivo*?
  - PBPK based toxicity simulations and systems models?

- Mechanistically relevant translational biomarkers
  - *In vitro*/*in vivo*, nonclinical species/man

- Human idiosyncratic DILI susceptibility factors

- Novel sources of differentiated human cells – iPS technology?
References


Gustafsson F, Foster AJ, Sarda S, Bridgland-Taylor MH, Kenna JG. A correlation between the in vitro drug toxicity of drugs to cell lines that express human P450s and their propensity to cause liver injury in humans. Toxicol Sci. 2014 Jan;137(1):189-211.


Backup slides
<table>
<thead>
<tr>
<th>Drug No.</th>
<th>Drug Name</th>
<th>Daily dose (mg)</th>
<th>Pharmacology</th>
<th>DILI pattern reported in humans</th>
<th>Other significant adverse drug reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aminopyrine</td>
<td>3000</td>
<td>Analgesic</td>
<td>Cholestatic and hepatocellular</td>
<td>Withdrawn due to agranulocytosis</td>
</tr>
<tr>
<td>2</td>
<td>Amodiaquine</td>
<td>600</td>
<td>Antimalarial</td>
<td>Most often hepatocellular, also cholestatic acute liver failure</td>
<td>Agranulocytosis</td>
</tr>
<tr>
<td>3</td>
<td>Benzbromarone</td>
<td>150</td>
<td>Uricosuric</td>
<td>Withdrawn due to DILI; hepatocellular, occasional acute liver failure</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Bromfenac</td>
<td>50</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Withdrawn due to DILI; hepatocellular, occasional acute liver failure</td>
<td>Black Box warning: severe dermatological reactions, aplastic anemia, agranulocytosis</td>
</tr>
<tr>
<td>5</td>
<td>Carbamazepine</td>
<td>1200</td>
<td>Anticonvulsant and mood stabilizer</td>
<td>Cholestatic, mixed or primarily hepatocellular</td>
<td>Black Box warning: agranulocytosis, seizures, myocarditis, other cardiovascular and respiratory effects</td>
</tr>
<tr>
<td>6</td>
<td>Clozapine</td>
<td>900</td>
<td>Atypical antipsychotic</td>
<td>Cholestatic hepatitis, very rare liver failure</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Fenclozic Acid</td>
<td>400</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Development terminated due to cholestatic jaundice in clinical trials</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Flutamide</td>
<td>750</td>
<td>Nonsteroidal anti-androgen</td>
<td>Black Box Warning: hepatic necrosis and cholestasis</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ibufenac</td>
<td>2400</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Withdrawn due to cholestatic or hepatocellular DILI</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Imiloxan</td>
<td>500</td>
<td>Antidepressant, alpha2 adrenoceptor antagonist</td>
<td>Not reported</td>
<td>Development terminated due to hypersensitivity in clinical trials</td>
</tr>
<tr>
<td>11</td>
<td>Nefazodone</td>
<td>600</td>
<td>Anti-depressant, 5HT antagonist</td>
<td>Withdrawn due to rare but severe hepatitis</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Suprofen</td>
<td>800</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Rare hepatocellular DILI</td>
<td>Withdrawn due to renal toxicity</td>
</tr>
<tr>
<td>13</td>
<td>Ticlopidine</td>
<td>600</td>
<td>Antiplatelet, adenosine diphosphate receptor inhibitor</td>
<td>Cholestatic, with macrovesicular steatosis and bile ductular damage</td>
<td>Black Box warning: life-threatening hematological adverse reactions (neutropenia/agranulocytosis, thrombotic thrombocytopenic purpura, aplastic anemia)</td>
</tr>
<tr>
<td>14</td>
<td>Tienilic Acid</td>
<td>500</td>
<td>Uricosuric diuretic</td>
<td>Withdrawn due to rare hepatocellular DILI, acute liver failure</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Troglitazone</td>
<td>600</td>
<td>Anti-diabetic, thiazolidinedione</td>
<td>Withdrawn due to DILI; primarily hepatocellular, occasional cholestatic</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Zomepirac</td>
<td>600</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Not reported</td>
<td>Withdrawn due to anaphylaxis; renal toxicity also reported.</td>
</tr>
</tbody>
</table>
## Drug classification

### Marked IADR concern

<table>
<thead>
<tr>
<th>Drug No.</th>
<th>Drug Name</th>
<th>Daily dose (mg)</th>
<th>Pharmacology</th>
<th>DILI pattern reported in humans</th>
<th>Other significant adverse drug reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Acetaminophen</td>
<td>4000</td>
<td>Analgesic</td>
<td>Acute dose dependent hepatocellular necrosis, liver failure. DILI warning on label.</td>
<td>Rare adverse effects include gastrointestinal, cardiovascular plus severe haematopoetic, allergic and dermal reactions.</td>
</tr>
<tr>
<td>18</td>
<td>Amlodipine</td>
<td>10</td>
<td>Calcium channel antagonist</td>
<td>Rare acute cholestatic DILI, very rare fatal liver failure, DILI warning on label.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>19</td>
<td>Celecoxib</td>
<td>400</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Cholestatic DILI, very rare liver failure, DILI warning on label and periodic monitoring of liver function advised.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>20</td>
<td>Diclofenac</td>
<td>200</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Rare DILI, predominantly hepatocellular, mixed and cholestatic also reported, very rare liver failure, DILI warning on label, periodic monitoring of liver function advised.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>21</td>
<td>Indomethacin</td>
<td>200</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Rare hepatocellular or cholestatic DILI, very rare fatal liver failure, DILI warning on label.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>22</td>
<td>Ritonavir</td>
<td>1200</td>
<td>Antiviral, HIV protease inhibitor</td>
<td>Raised liver enzymes, infrequent jaundice, and rare fatal liver injury. DILI warning on the label.</td>
<td>Asthenia, gastrointestinal and neurological disturbances.</td>
</tr>
<tr>
<td>23</td>
<td>Rosiglitazone</td>
<td>8</td>
<td>Anti-diabetic, thiazolidinedione</td>
<td>Rare cholestatic or hepatocellular DILI, very rare liver failure, DILI warning on label.</td>
<td>Withdrawn due to risk of rare but severe cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>24</td>
<td>Tacrine</td>
<td>160</td>
<td>Cholinesterase inhibitor</td>
<td>Relatively frequent liver enzyme elevations, rare hepatocellular DILI. DILI warning on label.</td>
<td>Black box warning: life threatening uterine malignancies, stroke and pulmonary embolism.</td>
</tr>
<tr>
<td>25</td>
<td>Tamoxifen</td>
<td>40</td>
<td>Nonsteroidal antiestrogen</td>
<td>Rare cholestatic or mixed cholestatic/hepatic liver injury, very rare liver failure, DILI warning on the label.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>26</td>
<td>Tolmetin</td>
<td>1800</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Liver enzyme elevations, very rare hepatocellular DILI, very rare liver failure, DILI warning on label.</td>
<td>Black Box warning: cardiovascular and gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>27</td>
<td>Verapamil</td>
<td>480</td>
<td>Calcium channel inhibitor</td>
<td>Infrequent liver enzyme elevations, rare hepatocellular DILI; DILI warning on label and periodic monitoring of liver function is advised.</td>
<td>Rare adverse effects include cardiovascular, digestive, nervous system and skin.</td>
</tr>
</tbody>
</table>
## Drug classification

### Low IADR concern

<table>
<thead>
<tr>
<th>Drug No.</th>
<th>Drug Name</th>
<th>Daily dose (mg)</th>
<th>Pharmacology</th>
<th>DILI pattern reported in humans</th>
<th>Other significant adverse drug reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>Acyclovir</td>
<td>4000</td>
<td>Antiviral, guanosine analogue</td>
<td>Elevated liver function tests, very rare hyperbilirubinemia, jaundice, hepatitis.</td>
<td>Rare adverse effects include gastrointestinal, CNS, cardiovascular, hematopoietic and dermal.</td>
</tr>
<tr>
<td>29</td>
<td>Caffeine</td>
<td>900</td>
<td>CNS stimulant</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>30</td>
<td>Dexamethasone</td>
<td>10</td>
<td>Corticosteroid</td>
<td>Not reported</td>
<td>Adverse effects attributable to corticosteroid activity.</td>
</tr>
<tr>
<td>31</td>
<td>Flumazenil</td>
<td>5</td>
<td>Benzodiazepine antagonist</td>
<td>Not reported</td>
<td>Black Box warning: seizures.</td>
</tr>
<tr>
<td>32</td>
<td>Ibuprofen</td>
<td>1800</td>
<td>Nonsteroidal anti-inflammatory</td>
<td>Very rare liver injury, primarily mainly hepatocellular, cholestatic/mixed also reported.</td>
<td>Rare but severe allergic reactions, stomach bleeding.</td>
</tr>
<tr>
<td>33</td>
<td>Olanzapine</td>
<td>20</td>
<td>Atypical antipsychotic</td>
<td>Very rare cholestatic or mixed DILI</td>
<td>Somnolence, weight gain, metabolic abnormalities; infrequent blood dyscrasias.</td>
</tr>
<tr>
<td>34</td>
<td>Pioglitazone</td>
<td>45</td>
<td>Anti-diabetic, thiazolidinedione</td>
<td>Very rare mixed, cholestatic or hepatocellular DILI, very rare.</td>
<td>Black Box warning: congestive heart failure.</td>
</tr>
<tr>
<td>35</td>
<td>Rimonabant</td>
<td>20</td>
<td>Cannabinoid type-1 receptor antagonist</td>
<td>Not reported.</td>
<td>Withdrawn due to severe depression and suicide.</td>
</tr>
<tr>
<td>36</td>
<td>Warfarin</td>
<td>10</td>
<td>Vitamin K antagonist</td>
<td>Very rare cholestatic liver injury.</td>
<td>Black Box warning: risk of fatal bleeding.</td>
</tr>
</tbody>
</table>
Why *in vitro*/*in vivo* “BSEP exposure scaling” is problematic

- Actual *in vitro* drug concentrations are unknown
  - Apparent IC$_{50}$ values assume all added drug is available in solution
  - True values likely to be much lower, due to binding to proteins and lipids
- Drug concentrations within human hepatocytes *in vivo* are unknown
  - Likely to be much higher than plasma concentrations
- BSEP inhibition by drug metabolites not evaluated
  - May be markedly more potent than parent e.g. troglitazone sulfate
- Short *in vitro* assay time interval, focussed on competitive inhibition
  - Time dependent / irreversible inhibition not addressed
- 1% DMSO vehicle, required to solubilize many compounds

Unsurprising that apparent *in vitro* hBSEP IC$_{50}$ values are much lower than unbound plasma C$_{max}$ values