Modeling Hepatic Drug Metabolism and Toxicity: Where are We Heading?

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Overview

• Outline adverse drug reactions
• Liver - pathogenesis & mechanisms
• AZ - *in vitro* hepatic safety screens
  • Evaluation of CRO model
  • Collaborative development of in-house model
• Quantitative predictive hepatic safety model
• Summary
Adverse Drug Reactions

- Cause patient sickness, death & prevent effective drug therapy
- 4th-6th leading cause of death in USA
- Responsible for drug withdrawal & attrition
- Affect any major organ system – liver, skin, blood, cardiovascular

Classification

- **ON TARGET ADRs**
  - Predictable from the known primary or secondary pharmacology of the drug
  - Exaggeration of the pharmacological effect of the drug
  - Clear dose-dependent relationship

- **OFF TARGET ADRs**
  - Not predictable from a knowledge of the basic pharmacology of the drug
  - Exhibit marked inter-individual susceptibility (idiosyncratic)
  - Complex dose dependence

\[ \text{ADR} = f_1 \left( \text{Chemistry of drug} \right) + f_2 \left( \text{Biology of individual} \right) \]
Prediction of Adverse Drug Reactions

\[ \text{ADR} = f_1\left( \text{Chemistry of drug} \right) + f_2\left( \text{Biology of individual} \right) + f_3\left( \text{capability of in vitro model} \right) \]

What we CAN risk assess preclinically:
- Chemotype vs target
- Endpoints
- Cell type
- Culture method

What we CANNOT risk assess preclinically:
- Genetic susceptibility (HLA)
- Co-morbidity (NAFLD, diabetes, infection)
- Co-medication

Improved predictivity for FTIM risk assessment
- Appropriate assays & endpoints for patient-centric risk assessment
- Understand in vitro limitations
- Wide spectrum of possible mechanisms

Clinical positive/negative control compounds

Cell Health Endpoints, cell type, 2D, 3D

Patient phenotype
- Predict clinical outcome
- Biological capability (metabolism, function)
Cardiovascular and liver toxicity are major causes of drug attrition

<table>
<thead>
<tr>
<th>Phase</th>
<th>‘Nonclinical’</th>
<th>Phase I</th>
<th>Phase I-III</th>
<th>Phase I-III</th>
<th>Phase III/Marketing</th>
<th>Post-Marketing</th>
<th>Post-Marketing</th>
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<tbody>
<tr>
<td>Information:</td>
<td>Causes of attrition</td>
<td>Serious ADRs</td>
<td>Causes of attrition</td>
<td>Causes of attrition</td>
<td>ADRs on label</td>
<td>Serious ADRs</td>
<td>Withdrawal from sale</td>
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<td>Sample size:</td>
<td>88 CDs stopped</td>
<td>1,015 subjects</td>
<td>63 CDs stopped</td>
<td>82 CDs stopped</td>
<td>1,138 drugs</td>
<td>21,298 patients</td>
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<td>9%</td>
<td>35%</td>
<td>21%</td>
<td>36%</td>
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<td>7%</td>
<td>29%</td>
<td>21%</td>
<td>13%</td>
<td>0%</td>
<td>32%</td>
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<td>Haematology/BM:</td>
<td>7%</td>
<td>2%</td>
<td>3%</td>
<td>4%</td>
<td>16%</td>
<td>10%</td>
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<td>Nervous system:</td>
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<td>21%</td>
<td>67%</td>
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<td>10%</td>
<td>11%</td>
<td>25%</td>
<td>34%</td>
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<td>5%</td>
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<td>14%</td>
<td>2%</td>
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<td>Reprotox:</td>
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<td>0%</td>
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<td>1%</td>
<td>10%</td>
<td>0%</td>
<td>2%</td>
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<td>Musculoskeletal:</td>
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<td>0%</td>
<td>5%</td>
<td>1%</td>
<td>28%</td>
<td>3%</td>
<td>2%</td>
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<tr>
<td>Respiratory:</td>
<td>2%</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
<td>32%</td>
<td>8%</td>
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<td>Renal:</td>
<td>2%</td>
<td>0%</td>
<td>5%</td>
<td>9%</td>
<td>19%</td>
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<td>0%</td>
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<tr>
<td>Genetic tox:</td>
<td>5%</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Carcinogenicity:</td>
<td>3%</td>
<td>0%</td>
<td>3%</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Other:</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>4%</td>
<td>16%</td>
<td>2%</td>
<td>2%</td>
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</tbody>
</table>

Sample size: 88 CDs stopped, 1,015 subjects, 63 CDs stopped, 82 CDs stopped, 1,138 drugs, 21,298 patients, 47 drugs

The various toxicity domains have been ranked first by contribution to products withdrawn from sale, then by attrition during clinical development. Note general agreement between pairs of equivalent studies.

Laverty et al. 2011
How can we improve safety in discovery pipelines

- Safety is just another drug property
  - Fail early – fail cheaply
  - Failing early does not equate to increased success

- At CDID, all molecular properties are fixed, including those causing
  - Target (mode of action)-related toxicity
  - Off-target side effects
  - Compound/chemistry-related toxicity

- Safety has to be designed into the molecular entities before CD investment
  - Just like other drug properties

- Safety as an integrated part of drug discovery
  - Identify potential liabilities early on and steer away from them
  - Do not just provide go / no go decisions ("fail early")
  - Provide direction to the teams ("increase likelihood to not fail")
Drug-Induced Liver Injury

- Leading cause of acute liver failure\(^1\)
  - Drugs cause 58% of all ALF

- High morbidity & mortality\(^2\)
  - 20% survival without transplant

- Main reason for late stage termination or withdrawal\(^2\)

- 76 drugs found to be significant cause of hepatotoxicity across 3 DILI Registries (US, Sweden, Spain)\(^3\)
  - Cause of liver injury ≥ 5 cases/registry

- 137 drugs withdrawn or BBW\(^4\) - LTKB dataset

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Preclinical hepatic safety - Relative positioning of hepatic assays in overall discovery phase decision-making

- Target safety assessment
  - In silico tools
- Secondary Pharmacology / Off-target panel
  - hERG & other in vitro cardiotoxicity screens
- General/Hepatic toxicity
  - Bespoke hepatic safety assays
- ADME / PK screens
  - Bespoke ADME / PK
- Genotoxicity
  - CVS in vivo
- Bespoke investigative toxicity in vivo
  - MTD/D RF rat, dog
Chemical insults & pathogenic mechanisms of DILI

Diverse Clinical Presentations of DILI
- Acute fatty liver with lactic acidosis
- Acute hepatic necrosis
- Acute liver failure
- Acute viral hepatitis-like liver injury
- Autoimmune-like hepatitis
- Bland cholestasis
- Cholestatic hepatitis
- Cirrhosis
- Immuno-allergic hepatitis
- Nodular regeneration
- Nonalcoholic fatty liver
- Sinusoidal obstruction syndrome
- Vanishing bile duct syndrome

DILI can present with multiple: varying phenotypes
clinical & histopathological features
A single ‘hepatotoxicity signature’ is unlikely
DILI patients provide mechanistic clues
Pre-clinical hepatic safety questions

**Project Team questions**
- Do I have an hepatic signal taking my compound into GLP toxicology studies?
- Do I have a risk of an hepatic signal in early clinical development?
- How does hepatobiliary disposition of compound/metabolites influence hepatic safety?

- Difficult to answer with current tools

**Generic issues**
- the physiological gap between incubations and liver
- the lack of physiological integration for amplification/adaptation
- inability to assess how minor chemical stress leads to major toxicity in susceptible patients
- lack of consideration of systemic effects in humans

**Generic discovery phase issues**
- Integrated hazard assessment *NOT* integrated biology
- Weighting of individual hazards for risk assessment
- Limited models with competent Phase I, II drug metabolism & transporters
- Limited mitochondrial mechanism coverage
- No immune (innate or adaptive) or non-parenchymal cell component
- Human cell based – won’t address species specific toxicity
- Target expression unknown in the *in vitro* system (particularly for oncology)
AZ hepatic safety assay toolbox

**Hepatic Safety Panel:**
1. hBSEP (vesicle) \([I]/IC_{50} > 0.1\)
2. Glu/Gal (HepG2) \(>2.5 \text{ fold}\)
3. hLiMT (primary+NPC) \(IC_{50}/[I] < 30 \text{ (2015)}\)
4. HepG2 C3A SSC spheroids \(\text{(2016)}\)
5. HCA \(\text{(2017)}\)

**Early Toxicology:**
- **Rat**
- **Dog** \(\text{(MTD / DRF)}\)
- Clinical chemistry
  - ALT, AST, GLDH, Bili, BA
- Hepatic pathology
- Biomarkers (miR122 etc)

**Regulatory Studies:**
- Toxicology:
  - **Rat** 28 day
  - **Dog** 28 day
- Clinical chemistry
- Hepatic pathology
- Biomarkers

**Bespoke/mechanistic assays**
- Primary hepatocytes +/- CYP inhibitors with cytotoxicity endpoints
- hLiMT (primary+NPC; \(IC_{50}/C_{\text{max}} >30\))
- Covalent Binding to protein
- Transporters (MRP2,3,4)
- Rat, dog, NHP 2D & 3D models
- Integration of DMPK/safety models
- Compound washout

**Toolbox of routine & bespoke assays: biochemical & cell based**
- Influences chemical design & decision making
- Assays monitored for performance vs emerging novel systems

**Phase I studies**
In vitro models for hazard assessment: Spheroids / Microtissues

- Spheroids are a versatile in vitro model
- Do not rely on proprietary technology
  - this will lead to variability
- High level of interest – Pharma, academics, consortia
- Demonstrate functional drug metabolism
- Much longer usage period (+28d)
- ATP appears a more sensitive endpoint in 3D
- Evidence of clinical pathological endpoints (steatosis, phospholipidosis)
- In Sphero had the most reproducible larger scale spheroid production system (2015)
  - 1000 cells – 10% NPC
- Many more providers in 2017
Primary Human Hepatocyte Microtissues

- KC Stained for CD68+
- Functionality assessed by IL-6 release

Bell et al., 2015; Messner et al., 2013
3D InSight™ Human Liver Microtissues

- Retrospective analysis (69 DILI +ve & 41 DILI -ve)
- Same validation compounds and hepatocyte donor
- Sen. & Spec. expressed as ratio to total Cmax
- ~20 more false -ve’s with 2D PHH

- Cmax agreement with Shah et al., 2015
  - Total Cmax >1.1µM = ↑DILI liability (70 cpds, 55 non-DILI)
  - Sensitivity 80%, Specificity 73%

- Hanging drop spheroids - adopted & used during 2015
- Primary human hepatocytes + 10% NPC

- Used primarily at late stage (216 compounds)
  - Due to cost and turnaround time

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Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury; Proctor et al., 2017
3D Hepatotoxicity Assay to High Throughput Screen Transfer – 2015 / 2016

- HepG2, C3a clone (cell line, immortalised, derived from liver HCC)
- Contact limited proliferation
- Unremarkable 2D hepatic profile

- AZ performed PoC work with HepG2 C3a spheroids
- Progressed this to launch decision 2Q 2016
- Using Ultra Low Attachment (ULA) plates, not hanging drop

- Collaboration with Liverpool Uni, LJMU & Sheffield Uni – probed C3A spheroid biology while AZ developed higher throughput screen

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**Develop internal screen with equivalent sensitivity & specificity**

- Increased use throughout Discovery, not just late stage
- Faster turnaround
- Higher throughput
- Equivalent data quality at reduced cost

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**Characterization of a functional C3A liver spheroid model**

*Harriet Gaskell, Parveen Sharma,* Helen E. Colley, Craig Murdoch, Dominic P. Williams & Steven D. Webb* *Toxicol Res., (2016)*

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**A 3D in vitro model of differentiated HepG2 cell spheroids with improved liver-like properties for repeated dose high-throughput toxicity studies.** Sreenivasa C. Ramaiyahari · et al. (2014)
**3D Hepatotoxicity SSC Assay: metabolic functionality**

- HepG2 C3A spheroids (seeded 1500 cells per well)
- 7 probe substrate cocktail
- 1, 2, 3, 4, 7 days and for 48 h on days 12-14, 19-21, 26-28.

- Metabolites determined by LC/MS and quantified using a standard curve.

<table>
<thead>
<tr>
<th>CYP Isoform</th>
<th>Probe Substrate</th>
<th>Metabolite</th>
<th>Probe Substrate conc (µM)</th>
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</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin</td>
<td>Acetaminophen</td>
<td>15</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion</td>
<td>Hydroxybupropion</td>
<td>20</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Amodiaquine</td>
<td>n-Desethylamodiaquine</td>
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</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>4-Hydroxydiclofenac</td>
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<tr>
<td>CYP2C19</td>
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<td>4-Hydroxymephenytoin</td>
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<td>1-Hydroxybufuralol</td>
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<tr>
<td>CYP3A4/5</td>
<td>Midazolam</td>
<td>1-Hydroxymidazolam</td>
<td>1.5</td>
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<table>
<thead>
<tr>
<th>CYP</th>
<th>Metabolism detected</th>
<th>IHC detected</th>
<th>Metabolism achieved (nM)</th>
<th>Metabolism achieved (fmoles/spheroid/h)</th>
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</thead>
<tbody>
<tr>
<td>1A2</td>
<td>✓</td>
<td>✓</td>
<td>22.63 ± 1.86 (14 d)</td>
<td>94.31 ± 7.75 (14 d)</td>
</tr>
<tr>
<td>2B6</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2C8</td>
<td>✓</td>
<td>-</td>
<td>24.80 ± 5.95 (2 d)</td>
<td>103.33 ± 24.80 (2 d)</td>
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<tr>
<td>2C9</td>
<td>✓</td>
<td>✓</td>
<td>0.50 ± 0.02 (2 d)</td>
<td>2.10 ± 0.06 (2 d)</td>
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<tr>
<td>2C19</td>
<td>x</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2D6</td>
<td>✓</td>
<td>✓</td>
<td>0.16 ± 0.05 (7 d)</td>
<td>0.19 ± 0.06 (7 d)</td>
</tr>
<tr>
<td>3A4/5</td>
<td>✓</td>
<td>x</td>
<td>0.19 ± 0.01 (2 d)</td>
<td>0.79 ± 0.06 (2 d)</td>
</tr>
</tbody>
</table>

**CYP1A2 Activity & IHC**
3D Hepatotoxicity SSC Assay: predictivity

- HepG2 C3a form spheroids from day 2
- Spheroids imaged, quantified, QC’d fully automated procedure
- IHC - absence of a necrotic core (to 28d) and limited proliferation for HepG2 C3a cells
- Close alignment of the C3a spheroids and previous supplier data (90 cpds)
- Assay launched 2Q 2016 – 4 day incubation time
- Risk assessment in absence of Cmax: IC50<30uM = greater risk

<table>
<thead>
<tr>
<th>Primary spheroid</th>
<th>C3A Spheroids</th>
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<tr>
<td>+ Cmax</td>
<td>-Cmax</td>
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<tr>
<td>Sensitivity</td>
<td>60 53</td>
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<tr>
<td>Specificity</td>
<td>93 75</td>
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</table>

**Chlorpromazine IC50 ref = 3.14 µM ± SD 1.58; n= 52**

**IMPACT:**
- 56 cpds every 2 weeks
- 2 week TAT
  - previous average = 5 weeks
- Cost reduction
  - 50x in 96 well and 450x 384 well

**Table:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>DILI Description</th>
<th>C3A</th>
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<tbody>
<tr>
<td>Perhexazine</td>
<td>Severe</td>
<td>0.8</td>
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<tr>
<td>Amiodarone</td>
<td>Severe**</td>
<td>6.89</td>
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<tr>
<td>Chlorpromazine</td>
<td>Low concern</td>
<td>7.04</td>
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<tr>
<td>Flucitidine</td>
<td>Severe**</td>
<td>11.4</td>
</tr>
<tr>
<td>Troxiflavinaxin</td>
<td>Severe</td>
<td>14.2</td>
</tr>
<tr>
<td>Piroxicamazone</td>
<td>Low concern</td>
<td>22.3</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>Severe</td>
<td>25</td>
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<tr>
<td>Tolcapone</td>
<td>Severe**</td>
<td>27</td>
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<tr>
<td>Troglitazone</td>
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<td>Entacapone</td>
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<tr>
<td>Ximelagatran</td>
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<tr>
<td>Diclofenac</td>
<td>High concern</td>
<td>168</td>
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<tr>
<td>Buspinone</td>
<td>Liver enz elev</td>
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<tr>
<td>Acetaminophen</td>
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<td>1000</td>
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<tr>
<td>Metformin</td>
<td>No DILI</td>
<td>inactive</td>
</tr>
<tr>
<td>Bosentan</td>
<td>Severe**</td>
<td>inactive</td>
</tr>
</tbody>
</table>

**Graph:**

- Graph of log10(Y) vs log10(X)
- Scatter plot of difference vs mean
3D Hepatotoxicity SSC Assay: gain of function from 2D to 3D

miR122 biomarker release:

Reports on chronic hepatotoxicity liability:
Acetaminophen (APAP) cross species study:  
*Primary Hepatic Spheroids*

**Human:**
- Can cause hepatic necrosis $>4g$/day
- Liver failure with overdosing\(^1\)

**Rats**
- Resistant to APAP toxicity *in vivo*\(^2\)
- Lower CYP2E1 activity

**Dogs**
- Sensitive to methaemoglobinaemia *in vivo*
- Large variation in hepatic effects *in vivo*:
  - After 1g/kg dose;
  - AST range from 65 – 8000U/L\(^5\)
  - Centrilobular necrosis, congestion, hydropic degeneration, and biliary stasis\(^3,4\)

**Expected:** Human $\geq$ Dog $>>$ Rat

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human predicted total Cmax (µM)</th>
<th>hLiMT IC(_{50}) (µM)</th>
<th>rLiMT IC(_{50}) (µM)</th>
<th>dLiMT IC(_{50}) (µM)</th>
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<tr>
<td>APAP</td>
<td>165.4</td>
<td>654</td>
<td>19407</td>
<td>4260</td>
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</table>

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**Questions**

**Will project compounds cause liver toxicity in animals?**

**Can we predict hepatic issues *in vitro* for preclinical species?**

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\(^1\) Antoine et al., 2012; \(^2\) Davis et al., 1974; \(^3\) Green et al., 1984; \(^4\) Savides et al. 1984; \(^5\) Gazzard et al., 1975
Which mechanisms should we focus on?

- Mechanisms with greatest validity
  - Top 200 marketed drugs
  - 78-86% drugs associated with toxicity have a structural alert
  - Of these, 62-69% have RM causative factor in toxicity
  - Several instances of BSEP & mitochondrial liabilities
  - Differentiating factor is daily dose

- Recommendation
  - Integration of chemical hazard with cell health
    - RM formation potential
    - Cytotoxicity
    - BSEP inhibition
    - Mitochondrial toxicity

- Reality
  - Lacking models with competent drug metabolism capability
  - Difficulty with complete integration

- Relative Importance RM >> Bsep or mito hazard > MRP
Predicting Hepatotoxicity vs idiosyncratic hepatotoxicity

- Increasingly sensitive in vitro assays detect minor chemical stress that most patients will adapt to
- Reactive metabolites are the oldest of 4 hypotheses resulting in idiosyncratic toxicity (hapten, superantigen, p-i, altered repertoire)
- Reactive metabolites are not always toxic, however, it is good practice to design out where possible
- True idiosyncratic hepatotoxicity has a high patient susceptibility factor
- Idiosyncratic toxicity becomes hypersensitivity when the adaptive immune system becomes involved

Exemplar compounds:
- Ticlopidene A*33:03; 4% ↑ALT
- Flucloxacillin B57:)01; 0.003% ALF
- Lapatinib DQA1*02:01; 5 - 15% ↑ALT
- Nevirapine DRB1*01:01; 4 - 20% ↑ALT
- Ximelagatran DRB1*07; 7.9% ↑ALT

Exemplar compounds:
- Troglitazone (1:5000)
- Bromfenac (53 cases/6 mo)
- Trovaflloxacin (1:18,000)
- Nefazodone (1:300,000 patient years)
- Fenclozic acid (10% jaundice)
- Tienilic acid (500 cases/25 fatalities)
- Sitaxsentan (Phase III)

The spectrum of idiosyncratic DILI: what should we aim at predicting?

- Differentiate prediction of toxicity in healthy volunteer vs patient
- Aim at predicting idiosyncratic toxicity which has a high chemotype component vs patient component
- Not immunoallergic / HLA associated hepatotoxocities
- Redesign in vitro models
  - mechanistic accuracy
  - patient characteristics

**Reactive Metabolites**

- Cytotoxicity
- Hapten formation
- Antigenicity
- Immunogenicity
- HLA association
- Cannot assess preclinically (yet)

**Stable Metabolites**

**Drug**

- CYP

**Toxicity**

- Dose related in appropriate model
- CRM coverage in animal model (?)
- Aim to assess preclinically

**Examples**

- Troglitazone (1:5000)
- Bromfenac (53 cases/6 mo)
- Trovafloxacin (1:18,000)
- Nefazodone (1:300,000 patient years)
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Prediction of DILI at CDID based on current science

- Predicted Cmax supersedes fCVB in predicting liver toxicity \(^2,3,4\)
- Most hepatic ADRs are dose-related \(^5,6\)
- Reactive metabolite formation associated with many drugs causing DILI
- Reactive metabolites are not always toxic, non-bioactivatable drugs are not always safe

- Clarity over what is being risk assessed by covalent binding assays
  - bioactivation leading to irreversible binding to protein
  - Increased risk through binding ‘critical’ proteins for cell health
  - high volume occupancy of cysteine-containing protein targets \(^7\)
  - Selected instances & therapy areas where CVB assessment is helpful

- There are no in vitro assays which risk assess for idiosyncratic toxicity
  - High degree of patient susceptibility factors in ADR
  - No host factors present in in vitro assay
    - Inflammation, infection, disease, co-meds, lifestyle, HLA genes
  - No estimation of risk through antigenicity or immunogenicity

- AZ accelerated development of in vitro models with patient characteristics

1, Kullak-Ublick 2017; 2, Shah et al., 2015; 3, Obach et al., 2006-12; 4, Chen et al., 2016; 5, Lammert et al., 2008 & 2010; 6, Stepan et al., 2011; 7, Whitby et al., 2017
Predictive Hepatic Safety: observations from published models

- Very well-mined chemical space (+200 publications - *in silico, in vitro*, QSAR, mode/mechanism of action)
- One DILI prediction model does not perform equally well across all drug classes and DILI mechanisms
- Drugs with different mechanisms may exhibit different DILI properties and should be predicted by mechanism-specific models
- A single drug may have several mechanisms final predictions should be a consensus of several mechanism based models
- Improvements in exposure prediction contributes more to overall prediction capability vs *in vitro* assays
- Models range from simple (FDA rule of 2) to complex (DILIsim) with multiple inputs
- No agreed consensus approach for classification of DILI in humans suitable for evaluating *in vitro* assays

Prediction of DILI relies on clinical DILI Severity Categories

- No agreed consensus approach for classification of DILI in humans suitable for evaluating *in vitro* assays
- Contributes to variation across models

**AZ’s Published Categories:**

**Severity Category 1:** Severe clinical DILI
Withdrawn from clinical use due to DILI
Black Box warnings for DILI in the US product labels

**Severity Category 2:** High clinical DILI concern associated with acute liver failure in humans, not withdrawn or given DILI Black Box warning

**Severity Category 3:** Low clinical DILI concern cause symptomatic liver injury, but not liver failure

**Severity Category 4:** Enzyme elevations in clinic associated with raised serum liver enzymes indicative of DILI not been reported to cause symptomatic DILI

**Severity Category 5:** No DILI not been associated with evidence of liver dysfunction

(Literature, FDA labels, FDA LTKB)

**FDA’s Published Categories:**

(Chen et al., 2016)

**Other Published Categories:**

Gustafsson et al., 2014; Garside et al., 2014; Tong W et al 2005-present
Four parts of the problem

- **Assays** → which assays or readouts to use?
- **Model** → which model or machine learning method?
- **Visualisation** → how to present the results/predictions?
- **Decision** → which decision criteria to use?
Risk of DILI is on a continuous scale

We observe a 3 level DILI severity categorization

We can predict a risk of DILI based on assay data, \( c \text{LogP} \), \( C_{\text{max}} \) and other variables

To estimate risk:

- estimate the influence or weight (\( \beta \)'s) of each assays (or variables) association with the DILI severity score
- assay values are multiplied by their \( \beta \)'s and summed together, giving continuous risk score
- score can be converted to a probability value for easier interpretation

DILI category discrimination thresholds estimated

- allows prediction of DILI severity category from continuous risk value

Bayesian – cross validation, with a prior on \( \beta \)'s to prevent overfitting

Bayesian hepatic safety (DILI) model
AZ Predictive Model: DILI probability & uncertainty from human-relevant *in vitro* models
Probability data visualisation for hepatic safety

- Data for 96 compounds (33 low, 40 med, 23 high risk)
- Variation for each compound going from safest to most toxic
- Statistical thresholds & proportion of data are calculated
- Uncertainty calculation means compounds often span risk thresholds
Paired DILI compounds

• Observe right shift as probability of DILI become greater which aligns with DILI severity categories for these molecules

Ambrisentan

Nefazodone

Increasing DILI severity in humans
Paired DILI compounds

<table>
<thead>
<tr>
<th>Pioglitazone</th>
</tr>
</thead>
</table>

- Separation similar to FDA model
- Troglitazone
- Rosiglitazone
- Pioglitazone
- Olanzapine
- Clozapine

Increasing DILI severity in humans
Modelling hepatic drug metabolism & toxicity: where are we heading?

- **In Vitro 2D/simple**
- **In Vitro 3D/complex**
- **In preclinical species**
- **In humans**

**Chemistry of the drug**
**Biology of the system**
**Systemic response**

- **Translatable biomarkers**

**Species / strain responses**

- **Disease/patient phenotype cells/co-cultures**
- **Hepatic Microphysiological systems (MPS)**
- **Novel/emerging Hepatic Biomarkers**
  - Retrospective translation to animals
  - /in vitro

- **Predictive machine learning / AI**
- **Preclinical animal MPS**
- **Linked MPS**
In Vitro Modelling of Disease Phenotypes: NAFLD

- Underlying disease does not necessarily lead to increased risk of DILI
- Likely higher risk for complicated progression and severity outcome if DILI occurs
- NAFLD to NASH disease spectrum
- Progression of NAFLD is highly variable
  - Interaction between several different pathways
  - Multiple cells types required in model
- Minority of patients progress to end stage liver disease
- Risk factors include obesity and diabetes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epidemiology</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>0.1–0.7%</td>
<td>Anti-TB drugs: methimazole(^2)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>0.13% and 3.26%</td>
<td>Anti-TB(^2), HAART(^2), ibuprofen(^3)</td>
</tr>
<tr>
<td>HIV</td>
<td>~0.8% of adults aged between 15-49 years</td>
<td>Anti-TB(^2,5), HAART(^2,5), paracetamol, trimethoprim(^5)</td>
</tr>
<tr>
<td>NAFLD</td>
<td>25% of general population NASH uncommon(^8)</td>
<td>APAP, halothane, isoflurane, losartan, ticlopidine and omeprazole(^6), methotrexate(^7)</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>8.5% of adults aged 18 years and above (^9)</td>
<td>Methotrexate(^7)</td>
</tr>
</tbody>
</table>

Phenotypic alterations during NAFLD

1. Tujios and Fontana 2011, 2Gupta and Lewis 2008, 3Bell and Chalasani et al., 2009, 4Blachier et al., 2013, 5Akhtar et al., 2007, 6Fromenty et al., 2013, 7Tarantino et al., 2007, 8Rosenburg et al., 2007, 9WHO website.
In Vitro Modelling of Patient Phenotypes: Monocyte-derived hepatocytes from patients with idiosyncratic DILI

- Monocytes from patients with idiosyncratic DILI
- Converted into hepatocytes
- Re-exposed to same drugs the patient was taking
- Cytotoxic response strongest in likely causative drug
- Only 4 / 84 co-meds showed +ve result
- Assay could be used to identify causative drug in complex DILI cases

**Translatable DILI Biomarkers**

**FDA, EMA, C-PATH support exploratory DILI biomarkers**
- **Novel mechanistic DILI biomarkers, prognostic for idiosyncratic DILI**
- Cytokeratin 18 (cleaved + full), HMGB1 (Ac & total), Osteopontin, CSF1
- miR122, GLDH due to proven benefit in acetaminophen overdose

**Gaps**
- Sparse pre-clinical knowledge (rat, dog, *in vitro*);
- Limited in-house experience

**Areas where Industry could benefit by having in-house quantification:**
- Prognostic capability - biomarker profiles indicate likely severity outcome
- Mechanistic understanding (link to clinic)
- Earlier & more sensitive detection of liver injury

**Translational understanding needed:**
- Cross-species reporting ability, particularly rat, dog & monkey
- Ability of *in vitro* model to release these biomarkers

**Further work needed:**
- Robust in-house SOPs & QC
- Acetylated HMGB-1 was best performing biomarker in stratifying liver injury outcome in cohort of idiosyncratic hepatotoxicity patients - only detection method is through proteomic MS
AstraZeneca approach: Quantitative & translational understanding at safety and ADME interface

Hepatic Organ Safety

ADME / PK

Gut
Liver
Systemic Circulation
Tissue

First pass metabolism
Targeted delivery
Protein binding
Distribution

Solubility
Soluble drug
Free drug
Bound drug
Elimination

In Vitro & In Vivo Models

Hepatic ADME/DILI modelling

In vitro
Animal toxicology studies
In silico
**MIP-DILI Roadmap - Stratification of In Vitro systems – aligned with Industrial needs**

### Tier 1: Single cell 2D systems
- **PHH**
- Cell-lines
- Reporter-signalling

**Key Features:**
- high throughput
- separate ‘worst offenders’ – risk/no risk
- clear risk threshold based on large validation set
- allows SAR / STR
- Relevant ADME/PK assays in parallel
- **aim to choose best chemistry**

### Tier 2: Multi-dimensional 3D systems
- **Multi-cell culture**
- Spheroids
- Long term culture

**Key Features:**
- lower throughput
- physiological alignment of hepatic function & ADME
- *in vivo*-like mechanisms
- **aim at predicting early clinical hepatotoxicity risk**

### Tier 3: Complex systems
- **Patient-dependent factors**
  - Viral infection
  - HLA restriction
  - T cells

**Key Features:**
- consider patient genotype / phenotype
- easy to combine with ADME/PK/DDI data
- **aim at predicting patient specific risks**
Summary

• Validation of a novel 3D culture model
• Successfully modified & brought in-house (cheaper & higher throughput)
• Significant gain of function of cell line in 3D – metabolism, transport, biomarkers
• Towards integrated hepatotoxicity assessment *in vitro*
• Be realistic about what we can predict
• Continuously improving models of hepatic safety

• Future:
  • iPSC hepatocytes derived from patients with idiosyncratic DILI
  • Patient-centric *in vitro* models for risk assessment (HLA, NASH, etc..)
  • *In vitro* models with integrated endpoints/biomarkers
  • Machine learning models of predictive safety
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R&D | Innovative Medicines | Drug Safety and Metabolism
Back Ups
Summary of outliers from Bayesian DILI Model

Category 1 compounds in Cat. 2

Zomepirac, Rimonabant & Dexamethasone - show flags for DILI assays, however very low Cmax
Meclofenemate - high clogP, low Cmax

Category 3 compound in Cat. 1

Ximelagatran: mechanism currently unknown
no *in vitro* DILI assays available
## Bayesian DILI Predictive Model: biological & statistical improvements

<table>
<thead>
<tr>
<th></th>
<th>Previous model</th>
<th>DILI Bayesian model</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation compounds</td>
<td>36</td>
<td>96</td>
<td>Includes more diverse compounds</td>
</tr>
<tr>
<td>Assays</td>
<td>THLE, THP1, Mitotox, BSEP, CVB</td>
<td>3D Spheroid, THP1, BSEP, mitotoxicity + combinations (HCA)</td>
<td>Addition of more physiologically relevant assays</td>
</tr>
<tr>
<td>Predicted exposure</td>
<td>dose</td>
<td>Cmax</td>
<td>Systemic concentration</td>
</tr>
<tr>
<td>Incorporates bioactivation</td>
<td>Covalent binding</td>
<td>GSH-, CN-adduct formation, in vivo evidence (defined by FDA)</td>
<td>Aligns with FDA recommendations (Chen et al., 2016)</td>
</tr>
<tr>
<td>Data integration</td>
<td>Assays given equal weight &amp; combined - “bin &amp; sum” calculation (above threshold in x assays)</td>
<td>Uses a statistical model and cross validation to optimally combine assay data</td>
<td>Flexible framework for addition/removal of assays based on emerging science</td>
</tr>
<tr>
<td>Prediction output</td>
<td>Prediction is a number from 0-4</td>
<td>Prediction is a continuous probability of DILI risk with uncertainty</td>
<td>More quantitative and includes uncertainty</td>
</tr>
<tr>
<td>Visualisation</td>
<td>Visualisation is a point in a quadrant</td>
<td>Visualisation is full probability distribution of DILI risk + comparisons with other compounds</td>
<td>Know when predictions are uncertain</td>
</tr>
</tbody>
</table>
Why do we use bioactivation as a predictive variable?

- 78-86% top 200 marketed drugs have structural alert; 62-69% have RM as causative factor in toxicity (Stepan et al., 2011)
- Good practice to steer away from RM where possible

**FDA approach**: binary +/− for bioactivation based on
  - covalent binding *in vitro* or *in vivo* animal studies
  - thioether adducts and/or conjugates detected
  - Literature keywords “drugs AND (RM OR CVB OR bioactivation OR glutathione conjugate OR active metabolites)”
  - Compounds causing GSH depletion / ROS formation were removed from analysis

**FDA Recommendation**: Factoring RM formation into FDA ‘Rule of 2’ increased predictivity (Chen et al., 2016)

- CVB is one consequence of bioactivation, any evidence of bioactivation adds predictive capability
Why don’t we use covalent binding as a standalone predictive variable?

- Retrospective analyses show no correlation between incidence of liver toxicity observed in preclinical studies and the level of covalent binding (Park et al., 2011; Bauman, Obach et al., 2009)

- AZ have moved CVB to a bespoke assay for hepatic safety (selected instances & therapy areas of use)

<table>
<thead>
<tr>
<th>Category</th>
<th>Average Dose (mg/day)</th>
<th>Average fCVB</th>
<th>Average Body Burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe DILI</td>
<td>990</td>
<td>0.012</td>
<td>8.78</td>
</tr>
<tr>
<td>Marked DILI</td>
<td>550</td>
<td>0.009</td>
<td>3.06</td>
</tr>
<tr>
<td>Low DILI</td>
<td>144</td>
<td>0.012</td>
<td>0.93</td>
</tr>
</tbody>
</table>

- Drugs in severe & low category have the same level of fCVB - the dose provides discrimination

*Thompson et al., 2012; acyclovir & bromfenac outliers removed from analysis