Integrating data from multiple *in vitro* methods enable prediction of serious human adverse drug reactions

Gerry Kenna  
Safer Medicines Trust  
Drug Safety Consultant  
gerry@safermedicines.org
Safer Medicines Trust

• An independent charity.

• Our goal is to replace poorly performing animal studies with more predictive human biology-based methods, for human efficacy and safety testing of pharmaceuticals and other chemicals.

• See: www.SaferMedicines.org
Outline

• Adverse Drug Reactions
• *In vitro* screening strategy
• Assay data generation and interpretation
• Data integration challenge
• Taking account of drug exposure *in vivo*
• Conclusions and future prospects
Adverse Drug Reactions (ADRs)

• Type A
  – Dose dependent, common
  – Occur reproducibly in humans and test animals
  – Often, but not always, due to exaggerated pharmacology

• Type B
  – Dose independent, infrequent
  – Occur only in susceptible humans, not in animals
  – Unrelated to drug pharmacology
  – Termed “idiosyncratic”
Consequences of ADRs

- Serious human ill health
- Failed drug development

Drug withdrawal 1971 - 2010 data

Inefficient development

![Graph showing cost in millions for preclinical and clinical stages from 1970s to 2010s]

Cautionary labelling, e.g. bosentan

---

**WARNING: RISKS OF LIVER INJURY and TERATOGENICITY**

*See full prescribing information for complete boxed warning.*

Tracleer can be prescribed and dispensed only through a restricted distribution program (Tracleer Access Program) because of these risks:

Elevations of liver aminotransferases (ALT, AST) and liver failure have been reported with Tracleer (5.1).

- Measure liver aminotransferases prior to initiation of treatment and then monthly (5.1).
- Discontinue Tracleer if aminotransferase elevations are accompanied by signs or symptoms of liver dysfunction or injury or increases in bilirubin ≥2 x ULN (2.2, 5.1).

Based on animal data, Tracleer is likely to cause major birth defects if used during pregnancy (4.1, 8.1).

- Must exclude pregnancy before and during treatment (4.1, 8.1).
- To prevent pregnancy, females of childbearing potential must use two reliable forms of contraception during treatment and for one month after stopping Tracleer (2.4, 8.1).
Toxic vs. nontoxic drugs

• Many drugs cause serious human ADRs
  – e.g. halothane, troglitazone, sitaxentan, bromfenac etc.

• But many “similar” drugs do not
  – e.g. desflurane, pioglitazone, ambrisentan, ibuprofen etc.

How can safe new drugs be designed and selected?
How drugs cause ADRs

1. **Drug ADME**
2. **Chemical insult to target cells**
3. **Biological response in cell**
4. **Biological response in tissue**

**Protection**
- e.g. stress response

**Compromise**
- e.g. innate and adaptive immunity

**Outcome**
- **Preclinical species vs. man**
- **Toxicity**

**Compound related effects**
- Can be explored using simplified “in vitro” model systems

**Patient related effects**
- Can be explored only *in vivo*

**No toxicity:**
- *tolerance & adaptation*
ADR screening strategy

- Focus on early biochemical processes that can initiate human ADRs.
- Select *in vitro* assays that can be used in drug discovery (robust, high volume, reasonable cost).
- Generate validation data using toxic and non-toxic drugs.
- Take account of *in vivo* drug exposure when interpreting assay data.
**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
Predictive toxicity challenges

• Which *in vitro* assays and endpoints?
  – Mechanistic relevance?
  – Robustness, throughput, turnaround time, cost?

• How to interpret the data the assays provide?
  – How to evaluate and validate them?

> Many divergent views, scientific consensus not yet achieved
Many possible assays

**Simple**
- Cultured liver cell lines
- Membrane vesicles

**Intermediate**
- Supportive Stromal Fibroblasts
- Micropatterned Hepatocytes

**Complex**
- Bioreactors
- Spheroids

**Complexity**
- Low
- High

**Cost**
- Low
- High

**Volume**
- High
- Low

**Turnaround time**
- High
- Low
Multiple chemical insults

Cell death pathways
- apoptosis
- necrosis

Reactive metabolites
- necrosis
- immunoallergic toxicity

Mitochondrial impairment
- apoptosis, necrosis
- microvesicular steatosis

BSEP inhibition
- Intrahepatic cholestasis

Immunoallergic toxicity: Neoantigen-modified hepatocyte

Activated immune effector mechanisms:
- Hepatic necrosis
- Immunoallergic toxicity

Immune-mediated toxicity:
- Activated immune effector mechanisms
- Necrosis, Apoptosis, Survival

Halothane bioactivation:
- CYP 2E1
- Reactive metabolites

Neoantigen-driven immune response in susceptible individuals

Hepatic necrosis

Cell death pathways:
- Apoptosis
- Necrosis

Hepatic necrosis: GSH → GSSG

Mitochondrial impairment:
- Apoptosis, Necrosis
- Microvesicular steatosis

BSEP inhibition:
- Intrahepatic cholestasis

Immunoallergic toxicity: Neoantigen-modified hepatocyte

Activated immune effector mechanisms

Hepatic necrosis

Cell death pathways:
- Apoptosis
- Necrosis

Hepatic necrosis: GSH → GSSG

Mitochondrial impairment:
- Apoptosis, Necrosis
- Microvesicular steatosis

BSEP inhibition:
- Intrahepatic cholestasis

Immunoallergic toxicity: Neoantigen-modified hepatocyte

Activated immune effector mechanisms

Hepatic necrosis
Some useful assays

<table>
<thead>
<tr>
<th>Chemical insult</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cytotoxicity</td>
<td>THLE-Null cell toxicity</td>
</tr>
<tr>
<td>Reactive metabolite toxicity</td>
<td>THLE-3A4 cell toxicity</td>
</tr>
<tr>
<td>Mitochondrial injury</td>
<td>Covalent binding to human hepatocyte proteins</td>
</tr>
<tr>
<td></td>
<td>HepG2 cell toxicity in glucose vs. galactose media</td>
</tr>
<tr>
<td>Membrane transporter inhibition</td>
<td>Bile Salt Export Pump (BSEP) inhibition</td>
</tr>
</tbody>
</table>

Drug Metab Dispos 2012; 40:130
Toxicol Sci 2014;137:189
Increased frequency and potency of BSEP inhibition amongst drugs which cause human cholestatic DILI

Numerous drugs inhibited BSEP but did not cause DILI

Concern cut-off value = 300 µM

No in vitro signal

in vitro signal
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

Frida Gustafsson, Alison J. Foster, Sanil Sarda, Matthew H. Bridgland-Taylor, and J. Gerry Kemna

*Global Safety Assessment and Discovery Sciences, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom*

To whom correspondence should be addressed at 23S37-69, Molecular Toxicology, Safety Assessment, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. Fax: +44(0)1625 513779. E-mail: frida.gustafsson@astraZeneca.com.

Present address: Safety Science Consultant, Macclesfield, United Kingdom.

No *in vitro* signal

![Graph showing in vitro signal](image)

*P450 independent cell toxicity*
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

Frida Gustafsson,§,1 Alison J. Foster,§ Sunil Sarda,† Matthew H. Bridgland-Taylor,‡ and J. Gerry Kennard＊

*Global Safety Assessment and †Discovery Sciences, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom

†To whom correspondence should be addressed at 23S37-69, Molecular Toxicology, Safety Assessment, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. Fax: +44(0)1625 513779. E-mail: frida.gustafsson@astrazeneca.com.

§Present address: Safety Science Consultant, Macclesfield, United Kingdom.

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

*P450 3A4 potentiated cell toxicity*
Integrating *in vitro* toxicity data


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

In vitro Panel score: Min 0, Max 5

Each Y scores 1, each N scores 0
## 36 Test drugs

<table>
<thead>
<tr>
<th>Severe concern</th>
<th>Marked concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopyrine</td>
<td>Acetaminophen</td>
<td>Acyclovir</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>Amlodipine</td>
<td>Caffeine</td>
</tr>
<tr>
<td>Benzbromarone</td>
<td>Celecoxib</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Bromfenac</td>
<td>Diclofenac</td>
<td>Flumazenil</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Indomethacin</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Ritonavir</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>Fenclozic Acid</td>
<td>Rosiglitazone</td>
<td>Pioglitazone</td>
</tr>
<tr>
<td>Flutamide</td>
<td>Tacrine</td>
<td>Rimonabant</td>
</tr>
<tr>
<td>Ibufenac</td>
<td>Tamoxifen</td>
<td>Warfarin</td>
</tr>
<tr>
<td>Imiloxan</td>
<td>Tolmetin</td>
<td></td>
</tr>
<tr>
<td>Nefazodone</td>
<td>Verapamil</td>
<td></td>
</tr>
<tr>
<td>Suprofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticlopidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tienilic Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troglitazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zomepirac</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Classification based on reported serious human idiosyncratic ADRs (IADRs):**
  - DILI (most frequent)
  - Blood dyscrasias (*e.g.* agranulocytosis)
  - Skin reactions (*e.g.* Stevens-Johnson syndrome)
Aggregated *in vitro* toxicity data


- **High IADR specificity, but modest sensitivity**
- **Analysis took no account of drug exposure *in vivo***

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

<table>
<thead>
<tr>
<th></th>
<th>Severe &amp; Marked concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Signals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or less</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Signals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>(13/27) = 48%</td>
<td>Specitivity (8/9) = 89%</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value
Including dose bioactivation data

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,‡ Richard Weaver,‡ and J. Gerry Kenna‡

†DMPK Innovative Medicine, AstraZeneca, Mölndal, 431 83, Sweden
‡Discovery DMPK, AstraZeneca, Wilmington, Delaware, United States
§DMPK Innovative Medicine, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom
Global Safety Assessment, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom
Discovery Sciences, AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom
Discovery DMPK, AstraZeneca, Loughborough, Leicestershire LE11 1RH, United Kingdom

Summary so far

• The Hazard Matrix discriminated very well between 27 IADR drugs and 9 safe drugs: 100% sensitivity, 78% specificity.

• A useful drug discovery tool, aiding design and selection of “safe” drug candidates prior to their clinical development.

- Can the approach be improved, to enhance specificity without compromising sensitivity?
- Can it be applied to other drugs?
Improvement 1: Mitochondrial injury

- Potentiated toxicity in galactose/glucose media is convenient, but lacks sensitivity

- Current methods of choice is Seahorse bioanalyzer
  - Quantifies cellular oxygen consumption and acidification
  - Good sensitivity
  - Provides mechanistic insight
Improvement 2: Exposure adjusted toxicity data

e.g. BSEP inhibition: data from Dawson et al. 2012, DMD 40:130–138

- Requires accurate determination of *in vivo* plasma drug concentrations
- Not possible when in vivo human drug exposure is unknown
## 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 • 1 liver transplantation</td>
<td>Withdrawn 2010</td>
</tr>
<tr>
<td>Bosentan - Tracleer®</td>
<td>250</td>
<td>80,000</td>
<td>• Elevated LFT common • Cases of severe liver injury</td>
<td>Black box warning</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>

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
Assessed 82 drugs, annotated in the US NCTR Liver Toxicity Knowledge Base (NCTR-LTKB):
http://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/ucm2024036.htm

- 24 Most-DILI
- 28 Less-DILI
- 20 No-DILI

Dual inhibition of mitochondrial function and BSEP activity, plus “high” drug exposure, was highly associated with more severe human DILI and more restrictive DILI safety labelling.
Conclusions

• Mechanistically relevant in vitro assays can discriminate between non-toxic drugs and drugs that cause idiosyncratic human ADRs.

• These assays have the potential to aid selection of safe new drugs.

• Many drugs that cause serious ADRs exhibit multiple liabilities, hence data from multiple assays must be integrated.

• When undertaking risk assessment, in vivo drug exposure needs to be considered.
The future

• Improved *(ideally fewer!)* toxicity assays
  – Exhibiting biotransformation

• PBPK based exposure simulations, *cf. DILIsym.*

• Mechanistic biomarkers that can be evaluated *in vivo* in humans - *Adverse Outcome Pathways.*

• Test more drugs, causing a broad range of ADRs – *DILI, cardiac, hypersensitivities etc.*

• Explore value for prediction of non-idiosyncratic toxicities.

• Incorporate patient susceptibility factors – *adaptive immunity etc.*
Acknowledgements

Many AstraZeneca ex-colleagues, especially:

Richard Thompson
Simone Stahl
Alison Foster
Mhairi Greer
Jane Barber
Sarah Dawson
Clare Walker
Julie Eakins
Peter Webborn
Manfred Ismail