Can we use *in vitro* data to predict human hazard?

*A comparison of three evidence streams for troglitazone and rosiglitazone*

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Outline

• EBTC and the drug safety challenge
• The Tox21 project
• Data obtained
  – Animal and human *in vivo*
  – ToxCast Tox21 *in vitro*
  – Human *in vivo*
• Conclusions and next steps
About EBTC

What is the Evidence Based Toxicology Collaboration (EBTC)?
Ann international collaboration of science, regulatory and industry leaders that is formed to establish and coordinate evidence-based, transparent toxicology and safety assessment methods to improve the risk assessment standards for regulatory decision making.

EBTC's Vision:
Evidence-based toxicology is the standard used to ensure public health, a healthy environment and a sustainable future.

EBTC’s Mission:
Bring together the international toxicology community to facilitate use of evidence-based toxicology to inform regulatory, environmental and public health decisions.

EBTC Funding:
Anonymous Charitable Foundation (91%), Beagle Freedom Prize, ARDF, Safer Medicines Trust

Where is EBTC?
www.EBTox.org

In-kind contributions towards the projects:
ToxStrategies, Norwegian Institute of Public Health, CAAT, Sciome
The drug safety problem

- Discovery and development of new drugs is slow, expensive and inefficient.
- Many licensed drugs cause human adverse drug reactions (ADRs) which limit their use.

**POST-MARKETING**

- ADRs cause human ill health and lead to cautionary labelling, or drug withdrawal.
- Many ADRs are not observed in animal safety studies, or in early clinical trials.

https://www.slideshare.net/rahul_pharma/drug-discovery-and-development-10698660
The challenge

• How can we:
  - Improve efficiency, cost and predictivity of preclinical drug safety testing?

• Currently it is unclear:
  – Which new test methods provide the most useful safety data
  – How best to analyse and interpret the new data
  – Where new safety tests should be used, especially in a regulatory setting

• An objective, reproducible evaluation workflow is needed.
**GOAL:** to conduct an objective, transparent and reproducible evaluation of the human safety relevance of preclinical drug safety data

**EBTC Tox 21 project**

**Evidence Stream 1**  
Systematic literature review of published animal and human trials  
- **Gunn Vist**  
  (Norwegian Institute of Public Health)

**Evidence Stream 2**  
Tox21 / Toxcast in vitro data  
- **Sricharan Bandhakavi**  
  (Northeastern University)

**Evidence Stream 3**  
Human adverse effects reported in Vigibase (WHO)  
- **Jyotsna Mehta**  
  (KEVA Health)

**Evidence Integration**
### Evidence stream 1

**Why systematic reviews?**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Narrative review</th>
<th>Systematic review</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Research question</strong></td>
<td>Often unclear or broad</td>
<td>Precise PECO question</td>
</tr>
<tr>
<td><strong>Protocol</strong></td>
<td>N/A</td>
<td>Published beforehand</td>
</tr>
<tr>
<td><strong>Literature search</strong></td>
<td>Not described</td>
<td>Explicit broad search strategy</td>
</tr>
<tr>
<td><strong>Study selection</strong></td>
<td>Not described</td>
<td>Explicit selection criteria</td>
</tr>
<tr>
<td><strong>Quality assessment</strong></td>
<td>Not usually done</td>
<td>Appraisal with explicit criteria</td>
</tr>
<tr>
<td><strong>Data synthesis</strong></td>
<td>Often qualitative</td>
<td>Qualitative or quantitative</td>
</tr>
</tbody>
</table>

Table adapted from de Vries et al., The ILAR Journal, 2016
Systematic Review Steps

1. Identify a problem
2. Formulate question
3. Write protocol
4. Search for evidence
5. Appraise the evidence
6. Integrate evidence
7. Apply evidence to policy
8. Engage all stakeholders to implement and change habits

P – Population
I – Intervention
C – Comparator
O – Outcome(s)

Use validated risk of bias and study quality tools

Publish!

Broadly!

Qualitatively or quantitatively

PROSPERO 2018 CRD42018112353
Evidence stream 1

Systematic review of animal and human studies

Main study question:
How well do in vitro tests and animal tests (rats, Beagle dogs, non-human primates) predict liver outcomes in humans?

– Case study: exposure to drug pairs
Pilot: Troglitazone and Rosiglitazone

**Troglitazone**
- **Withdrawn in 2000** due to human drug-induced liver injury (MOST-DILI Concern) (US FDA Liver Toxicity Knowledgebase, TLKB)

**Rosiglitazone**
- **Licensed since 1999**
- **LESS-DILI concern** (US FDA LTKB)
- **Black Box Warning** for cardiotoxicity

Both drugs are antidiabetic PPARγ agonists, which stimulate insulin function.

Evidence stream 1
Systematic Review Results – PRISMA Diagram

Records identified through database searching
(n = 8,380)
Databases searched: PubMed, Embase, and Web of Science

Additional records identified through other sources
(n = )

Records after duplicates removed
(n = 6,805)

Records screened
(n = 6,805)

Records excluded – out of scope
(n = 6,165)

Full-text articles assessed for eligibility
(n = 640)

Full-text articles excluded, (n = 282)
122 No primary data
66 No liver outcomes
31 Drug Combination
24 No included compounds
9 Case report
8 Duplicate
7 Altered animal model
7 Excluded language
6 No in vivo data

Studies being extracted
(n = 357)

Studies included in quantitative synthesis (meta-analysis)
(n = )

PROSPERO 2018 CRD42018112353

2 reviewers/paper
## Evidence stream 1

### Systematic Review-extracted data

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Strain / population</th>
<th>Drug</th>
<th>Endpoints</th>
<th>Duration</th>
<th>Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>Fujimoto, 2009</td>
<td>Mouse B6.129S7-Sod2tm1Leb/J</td>
<td>Troglitazone</td>
<td>Liver weight, ALT, AST, ALOP, histopathology</td>
<td>28 days</td>
<td>300 mg/kg</td>
</tr>
<tr>
<td>13</td>
<td>Anandhanjan, 2009</td>
<td>Mouse C57BL/6J</td>
<td>Rosiglitazone</td>
<td>Liver weight, ALT, AST</td>
<td>10 days</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>307</td>
<td>Watanabe, 2000</td>
<td>Rat Wistar</td>
<td>Troglitazone</td>
<td>ALT, AST, ALP, bilirubin (total) and bilirubin (direct), histopathology</td>
<td>94 days</td>
<td>0, 100, 400 mg/kg</td>
</tr>
<tr>
<td>121</td>
<td>Herman, 2002</td>
<td>Rat Wistar</td>
<td>Troglitazone</td>
<td>Liver weight, histopathology (valuation)</td>
<td>104 weeks</td>
<td>0, 50, 400, 800 mg/kg</td>
</tr>
<tr>
<td>144</td>
<td>Kanimushi-Kiyota, 2011</td>
<td>Rat Wistar</td>
<td>Troglitazone</td>
<td>Liver weight (relative, absolute)</td>
<td>4 weeks</td>
<td>0, 5, 200 mg/kg</td>
</tr>
<tr>
<td>115</td>
<td>Rostubsky, 2001</td>
<td>Rat Wistar</td>
<td>Troglitazone</td>
<td>Liver weight, histopathology (valuation)</td>
<td>2.5, 36 hrs</td>
<td>200 mg/kg</td>
</tr>
<tr>
<td>136</td>
<td>Xia, 2009</td>
<td>Rat LETO, OLE</td>
<td>Rosiglitazone</td>
<td>Liver weight, ALT, AST</td>
<td>10 days</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>187</td>
<td>Li, 2009</td>
<td>Rat Wistar</td>
<td>Troglitazone</td>
<td>Liver weight, histopathology</td>
<td>4 weeks, 60 weeks</td>
<td>2000 mg/kg</td>
</tr>
<tr>
<td>246</td>
<td>Rothwell, 2001</td>
<td>NHP Cynomologus monkey</td>
<td>Troglitazone</td>
<td>ALT, AST, ALP, bilirubin (total), liver weight</td>
<td>0, 3, 6, 9, 12 months</td>
<td>300, 600, 1200 mg/kg</td>
</tr>
<tr>
<td>21</td>
<td>Azziz, 2001</td>
<td>Human Female</td>
<td>Troglitazone</td>
<td>ALT, AST, study withdrawal due to abnormal LFT</td>
<td>44 weeks</td>
<td>150, 300, 600 mg/day</td>
</tr>
<tr>
<td>316</td>
<td>Yale, 2009</td>
<td>Human Male-Female</td>
<td>Troglitazone</td>
<td>ALT, AST, LFT</td>
<td>24 and 48 weeks</td>
<td>300 mg/day</td>
</tr>
<tr>
<td>160</td>
<td>Knower, 2005</td>
<td>Human Obs Male-Female</td>
<td>Troglitazone</td>
<td>ALT, AST, LFT</td>
<td>24 and 48 weeks</td>
<td>400 mg/day</td>
</tr>
<tr>
<td>270</td>
<td>St. Peter, 2001</td>
<td>Human Obs Male-Female</td>
<td>Troglitazone</td>
<td>ALT, AST, LFT</td>
<td>412.7 +/- 255.6 days</td>
<td>not reported</td>
</tr>
<tr>
<td>20</td>
<td>Aizoglu, 2000</td>
<td>Human Male-Female</td>
<td>Troglitazone</td>
<td>ALT, AST, histopathology</td>
<td>6 months</td>
<td>200 mg/day</td>
</tr>
<tr>
<td>101</td>
<td>Jegrick, 2004</td>
<td>Human Obs Male-Female</td>
<td>Troglitazone</td>
<td>AST x 3 x ULN</td>
<td>12 months</td>
<td>8 mg/day</td>
</tr>
<tr>
<td>36</td>
<td>Beylon, 2008</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST</td>
<td>20 weeks</td>
<td>8 mg/day</td>
</tr>
<tr>
<td>16</td>
<td>Aramwit, 2009</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST</td>
<td>12 weeks</td>
<td>2 mg/day</td>
</tr>
<tr>
<td>85</td>
<td>Chiang, 2000</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST, ALP</td>
<td>12 months</td>
<td>2-4 mg/day</td>
</tr>
<tr>
<td>214</td>
<td>Nolan, 2000</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>Hepatic transaminases &gt; 3, &gt; 1.5 x ULN, GGT</td>
<td>8 weeks</td>
<td>4, 8, 12 mg/day</td>
</tr>
<tr>
<td>232</td>
<td>Phay Ming, 2011</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST, GGT</td>
<td>1 year, 4 years</td>
<td>2, 4, 8 mg/day</td>
</tr>
<tr>
<td>78</td>
<td>Dorel, 2005</td>
<td>Human Female</td>
<td>Rosiglitazone</td>
<td>SGOT, SGPT</td>
<td>3 months, 8 months</td>
<td>2, 4 mg/day</td>
</tr>
<tr>
<td>58</td>
<td>Chalasani, 2005</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST, bilirubin</td>
<td>12 months</td>
<td>400 mg/day</td>
</tr>
<tr>
<td>233</td>
<td>Phillips, 2009</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>ALT, AST</td>
<td>26 weeks</td>
<td>8 mg/day</td>
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<tr>
<td>76</td>
<td>Oebbrock, 2003</td>
<td>Human Male-Female</td>
<td>Rosiglitazone</td>
<td>Study withdrawal due to hepatotoxicity</td>
<td>2-16 months</td>
<td>4 mg/day</td>
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<tr>
<td>42</td>
<td>Biswas, 2001</td>
<td>Human Male-Female</td>
<td>Troglitazone</td>
<td>ALT, AST, GGT</td>
<td>3 months</td>
<td>unreported</td>
</tr>
</tbody>
</table>

### Differences:

- **Different species**
- **Different doses**
- **Different group sizes**
- **Different endpoints**
- **Different treatment times**
- **Incomplete reporting**
Evidence stream 1, Troglitazone:

**Endpoint example: Rat serum ALT**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Troglitazone Mean</th>
<th>SD</th>
<th>Total</th>
<th>Control Mean</th>
<th>SD</th>
<th>Total</th>
<th>Weight</th>
<th>Mean Difference IV, Random, 95% CI</th>
<th>Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.1 ALT U/L Troglitazone 100 mg/kg 3 weeks</td>
<td>10.6</td>
<td>1</td>
<td>5</td>
<td>11</td>
<td>1.5</td>
<td>5</td>
<td>100.0%</td>
<td>-0.40 [-1.98, 1.18]</td>
<td>-0.40 [-1.98, 1.18]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
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</tr>
<tr>
<td>Heterogeneity: Not applicable</td>
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<tr>
<td>Test for overall effect: Z = 0.50 (P = 0.62)</td>
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<tr>
<td>3.1.2 ALT U/L Troglitazone 100 mg/kg 12 weeks</td>
<td>72.2</td>
<td>11.3</td>
<td>5</td>
<td>88.8</td>
<td>10.9</td>
<td>5</td>
<td>64.5%</td>
<td>-16.60 [-30.36, -2.84]</td>
<td>-16.60 [-30.36, -2.84]</td>
</tr>
<tr>
<td>Watanabe 2000 Wistar female</td>
<td></td>
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</tr>
<tr>
<td>Watanabe 2000 Wistar male</td>
<td>53.4</td>
<td>9.3</td>
<td>5</td>
<td>75.6</td>
<td>19</td>
<td>5</td>
<td>35.5%</td>
<td>-22.20 [-40.74, -3.66]</td>
<td>-22.20 [-40.74, -3.66]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td></td>
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</tr>
<tr>
<td>Heterogeneity: Chi² = 0.23, df = 1 (P = 0.63); I² = 0%</td>
<td></td>
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<tr>
<td>Test for overall effect: Z = 3.30 (P = 0.0010)</td>
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</tr>
<tr>
<td>3.1.3 ALT U/L Troglitazone 400 mg/kg 12 weeks</td>
<td>69.2</td>
<td>11.9</td>
<td>5</td>
<td>88.8</td>
<td>10.9</td>
<td>5</td>
<td>59.3%</td>
<td>-19.60 [-33.74, -5.46]</td>
<td>-19.60 [-33.74, -5.46]</td>
</tr>
<tr>
<td>Watanabe 2000 Wistar female</td>
<td></td>
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</tr>
<tr>
<td>Watanabe 2000 Wistar male</td>
<td>52</td>
<td>4.3</td>
<td>5</td>
<td>75.6</td>
<td>19</td>
<td>5</td>
<td>40.7%</td>
<td>-23.60 [-40.68, -6.52]</td>
<td>-23.60 [-40.68, -6.52]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
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</tr>
<tr>
<td>Heterogeneity: Chi² = 0.00, df = 1 (P = 0.72); I² = 0%</td>
<td></td>
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<tr>
<td>Test for overall effect: Z = 3.82 (P = 0.0001)</td>
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</tr>
<tr>
<td>3.1.4 ALT U/L Troglitazone 500 mg/kg 3 weeks</td>
<td>11.8</td>
<td>1.9</td>
<td>5</td>
<td>11</td>
<td>1.5</td>
<td>5</td>
<td>100.0%</td>
<td>0.80 [-1.32, 2.92]</td>
<td>0.80 [-1.32, 2.92]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td></td>
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<tr>
<td>Heterogeneity: Not applicable</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.74 (P = 0.46)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Evidence stream 1, **Troglitazone**:

**Endpoint example: ALT cross-species**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Rat</th>
<th>NHP</th>
<th>Human</th>
</tr>
</thead>
</table>

No clear liver toxicity signals observed in animals, or in human clinical trials.
Evidence stream 1, Rosiglitazone:
Endpoint example: Human serum ALT and AST

No clear liver toxicity signals observed in animals, or in human clinical trials
Evidence Stream 1

Results summary

Systematic review of published literature provided no evidence of adverse liver effects of Troglitazone or Rosiglitazone in vivo in animal studies or human studies.

Limitations of Published Literature:

- Publication bias:
  - Regulatory preclinical and clinical studies tend not to be published
  - Need incentives for industry and regulatory agencies to make data public

- Publication quality:
  - Lack of detailed protocols
  - Lack of individual animal data
  - Insufficient details in the methods section
  - Selective reporting

- Publication standards:
  - Lack of structured abstracts
Evidence stream 2: ToxCast and Tox21 *in vitro* data sets

**Toxicology in the 21st Century (Tox21)**

- Largest public database of *in vitro* test results
- A US federal collaboration between EPA, NIH, NCATS, EPA’s NTP and FDA.
- >700 high-throughput assays, which cover ~ 300 signalling pathways.
- Data can be downloaded for 1,800 chemicals.
- [https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21](https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21)
Evidence stream 2: ToxCast *in vitro* data

**Workflow**

ToxCast dataset

For chosen drugs, identify *all tests with results* (i.e., those with \( \text{AC50} \neq \text{NA} \))

From common (437) tests, identify “positives” (i.e., those with \( \text{AC50} < 10^6 \))

**Results**

Venn diagrams not drawn to scale

- **Positive** w/Rosiglitazone Maleate ONLY: 61 tests
- **Positive** w/Troglitazone ONLY: 78 tests
- **Positive** w/both drugs: 51 tests
- **Positive** w/Rosiglitazone Maleate: 129 tests
- **Positive** w/Troglitazone: 10 tests

Higher number of “positive” ToxCast test data for Troglitazone than for Rosiglitazone Maleate
Evidence stream 2: Biological processes affected

Venn diagrams not drawn to scale

Cell cycle or Cell morphology  
* n = 21

Regulation of gene expression  
* n = 32

Regulation of transcription factor activity  
* n = 43
Evidence stream 2: 
**Human in vivo drug exposure normalization**

- If $C_{\text{max}} < EC_{50}$, lower potential for activation
  - Potency: Drug A > Drug B > Drug C > Drug D
- If $C_{\text{max}} > AC_{50}$ (for any in vitro/cellular assay), higher potential for *in vivo* relevance effect
- If $C_{\text{max}} << AC_{50}$, lower potential for *in vivo* relevance

- $C_{\text{max}}$ is closest to toxic levels of drug
Evidence stream 2: Human exposure normalization

(Normalized) Activation Score

\[ NAS = \frac{C_{\text{max}} - AC_{50}}{C_{\text{max}}} \]

\( AC_{50} = \) (drug) concentration at 50% max response

<table>
<thead>
<tr>
<th>Normalized activation score (NAS) level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAS(\geq 0)</td>
<td>(C_{\text{max}} \geq AC_{50}) = likely relevance</td>
</tr>
<tr>
<td>0(\geq NAS &gt; -4)</td>
<td>(C_{\text{max}} &lt; AC_{50}) = possible relevance</td>
</tr>
<tr>
<td>NAS(&lt; -4)</td>
<td>(C_{\text{max}} \ll AC_{50}) = unlikely relevance</td>
</tr>
</tbody>
</table>

For the 437 tests conducted on both Rosiglitazone and Troglitazone:
\(\rightarrow NAS\) values were generated and compared
Evidence stream 2: In vitro Tox21 normalized activation scores

**Cmax (Troglitazone) = 2.82 μg/mL (at 600mg/day dose) = 6.38 μmoles/L

* (Normalized) Activation Score = \( \frac{C_{\text{max}} - AC50}{C_{\text{max}}} \)
Evidence Stream 2:
Exposure-normalized clustering of all ToxCast assays

- Assays affected by Rosiglitazone (Maleate) alone
- Assays affected by Troglitazone alone
- Assays affected by both drugs

Target: PPAR-γ

Rosiglitazone (Maleate) Normalized Activation Score
Troglitazone Normalized Activation Score

Duarte S et al., 2015; Saiman Y & Friedman SL., 2013; Bohm F et al., 2010; Rudraiah S et al., 2016, Zimmerman HW et al., 2011, Gomex-Ospina N et al., 2016, Jadeja RN et al., 2016, Zuniga S et al., 2011, Tan W et al., 2017
Evidence Stream 2: Results from US EPA ToxCast assays

- Troglitazone triggered 7-fold more off-target biological effects than Rosiglitazone.
- Drug exposure adjustment aided identification pathways that could be relevant to DILI caused by Troglitazone \textit{in vivo}.

Limitations of ToxCast data:
- Missing data (not all compounds were tested in each assay)
- Complex data processing is needed
- Several key mechanisms important for liver injury are not present in ToxCast:
  - e.g. formation of reactive metabolites, BSEP inhibition
Evidence Stream 3: Human adverse event monitoring via Vigibase

- World’s largest spontaneous adverse event reporting system
- 8.4 million reports from 104 countries since the WHO International Drug Monitoring Programme started in 1968
- Main use by regulatory agencies and pharmaceutical companies:
  - Post-marketing surveillance of adverse effect data from patients treated with licensed drugs
- Pioneering use in EBTC Tox21 study:
  - “Gold standard” real human data for comparison of animal, in vitro and human randomized clinical trials
  - Linking MedDRA terms with in vitro mechanistic endpoints

Ref: Magnus Wallberg, UMC
Evidence Stream 3:
Troglitazone-related liver adverse effect frequency
## Evidence Stream 3:

### Top 5 MedDRA terms

<table>
<thead>
<tr>
<th>Troglitazone</th>
<th>Possible link to mechanistic data</th>
<th>Rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic failure</td>
<td>Hepatic failure</td>
<td></td>
</tr>
<tr>
<td>Hepatic function abnormal</td>
<td>Liver injury</td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>Jaundice</td>
<td>Hepatic function abnormal</td>
</tr>
<tr>
<td>Liver function tests abnormal</td>
<td></td>
<td>Liver disorder</td>
</tr>
</tbody>
</table>
Evidence Stream 3:
MedDRA grouped term comparison after approval

Vigibase: number of adverse events occurrences

<table>
<thead>
<tr>
<th>Category</th>
<th>Troglitazone</th>
<th>Rosiglitazone adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER FUNCTION TESTS</td>
<td>951</td>
<td>79</td>
</tr>
<tr>
<td>HISTOLOGICAL CHANGES</td>
<td>626</td>
<td>35</td>
</tr>
<tr>
<td>BILIARY TRACT DISORDER</td>
<td>335</td>
<td>50</td>
</tr>
<tr>
<td>MEDICAL DIAGNOSES</td>
<td>1153</td>
<td>144</td>
</tr>
<tr>
<td>GENERAL</td>
<td>95</td>
<td>62</td>
</tr>
</tbody>
</table>

*Count for Number of occurrences may differ slightly due to the limitation for segregation in the Vigibase output data set (Initial case/ Follow up case/ number of instance )
Evidence Stream 3: Observations on Vigibase data

• Vigibase provides access to post-marketing adverse effect data from many thousands of patients treated with licensed drugs

• Unexplored use: bridge between in vitro endpoints and in vivo human outcomes.

Limitations of post-marketing data:

• Potential confounding of the relationship between treatment and adverse effect by underlying diseases, co-medications and co-morbidities
  
  • E.g., might the liver failure cases in rosiglitazone treated patients have been secondary to heart failure?

• Defining causal relationships requires establishment of biological plausibility and /or mechanistic understanding
Overall conclusions

Evidence Stream 1:
Confirmed existing knowledge:
• Animal tests cannot differentiate liver effects of Troglitazone from Rosiglitazone
• Human randomized controlled trials have insufficient power to detect rare events

Evidence Stream 2:
New finding:
ToxCast \textit{in vitro} tests demonstrate 7-fold more off-target effects for Troglitazone compared to Rosiglitazone

Evidence Stream 3:
Confirmed existing knowledge:
Higher relative frequency of severe liver adverse effects in patients treated with Troglitazone, compared with Rosiglitazone

Overall conclusions:
Animal tests did not detect Troglitazone as a MOST-DILI drug
Some \textit{in vitro} assays discriminated between Troglitazone and Rosiglitazone
What we learned

• The Evidence Streams we evaluated have important limitations
  – **Stream 1 (Systematic literature review):** publication bias, lack of published regulatory animal tox studies, lack of published human RCTs, positive results bias, reporting deficiencies
  – **Stream 2 (ToxCast):** Limited set of assays subjectively selected ~ 2005, varied \( AC_{50} \) calculation not always relevant for all endpoints missing data
  – **Stream 3 (Vigibase):** Observational, non-randomized studies (by definition), no data on the number of prescriptions, reporting incompleteness and potential biases.

• New developments will facilitate Systematic Reviews
  – New literature review and analysis AI-powered software (Distiller, SysRev, Sciome)
  – Machine learning and AI - *being developed for full-text screening and data extraction*
Next steps

Immediate:

– Summarize all animal and human RCTs data with traditional liver endpoints
– Finish test-specific analysis of ToxCast data
– Publish the systematic review (draft in progress)
– Finish data extraction and analysis of additional drug pairs

Follow-up studies:

– In vivo:
  • Expand the endpoints (gene expression, other omics biomarkers)
  • Request preclinical and clinical data from FDA
– In vitro:
  • Conduct systematic literature review of in vitro data
  • Explore connection of mechanistic data to MeDRA terms
  • Enhanced informatics analysis

We welcome collaborations and are happy to make available the literature database of literature for additional investigations!
Thanks for listening.
Any questions?

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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
  - Gerry@SaferMedicines.org

**EBTC Protocols:**

https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=112353
https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=96120
https://zenodo.org/record/2529091#.XKZQAetKhTY
https://zenodo.org/record/2528922#.XKZQoKZ7mAw
https://zenodo.org/record/1493498#.XKZQ4aZ7mAw