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The “Rule of 2” – Do Drug Properties Predict Drug-Induced Liver Injury (DILI)?

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Views expressed in this presentation are those of the presenter and not necessarily those of the U.S. FDA.

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Translational Challenges in DILI Risk Assessment

Preclinical toxicology often failed to predict DILI risk in humans

- “High-dosing of healthy animals” test identified < 50% DILI liability in humans
- Conventional \textit{in vitro} assays have very limited prediction for DILI risk assessment
- No biomarker is available to identify the susceptible patients prior to drug treatment

Liver Toxicity Knowledge Base (LTKB)

Search “LTKB” @ Google

Assess DILI risk of new chemicals

Chemical structure & similarity search

Query interface

Link to external resources, e.g., PubMed, Wiki, LiverTox

Excel-like spreadsheet

Drug specific data
- Japanese TGX Project
  >130 drugs
  4 testing systems
  Multi-doses and times
  >20,000 arrays
- DrugMatrix
  ~700 chemicals
  ~5000 arrays
- Others (e.g., in-house)
  - In house *in vitro* data
    - Human primary hepatocytes
    - Rat primary hepatocytes
    - HepG2
  - Tox21 and ToxCast
  - Others

- Chemical structure (SAR/QSAR)
- Daily dose (Cmax)
- Lipophilicity
- Reactive Metabolites
- P450 activities

- Therapeutic uses
- Side effects
- Pharmacological class
  - ATC
  - FDA classification
- DILI types
- DILI annotation based on FDA drug label
- Severe DILI
- Case reports

How to Assess DILI Risk in Humans for a Drug?

• Three attributes of a drug are important for its DILI assessment:
  ❖ Causality: was liver injury caused by drug or other cause
  ❖ Incidence: how many case reports are considered significant
  ❖ Severity: elevated ALT; Hy’s law; disability and hospitalization, liver failure; liver transplantation or death

• Risk = (How likely) x (How many) x (How severe)

This is opinion based !!!
Drug Labeling is not Perfect but Probably the Best and Mostly Consistent Information We Have

- Drug labeling is not perfect
  - Opinion based, not based on pre-defined criteria
  - Not according to a consistent or scientifically justified master plan
  - “Guilty by Association”...carryover from other drugs
    - Adverse Reactions: “This section must list the adverse reactions that occur with the drug and with drugs in the same pharmacologically active and chemically related class, if applicable”

- Can we use drug labeling to identify more dangerous drugs?
  - The question is how to mitigate the inherent defects in labeling!
Drugs assigned to labeling sections

- Box Warnings
- Warnings & Precautions
- Adverse Reactions
- None of above

DILI Classification

- Most-DILI-concern
- Less-DILI-concern
- No-DILI-concern

Withdrawn drugs

DILI severity

Keywords for text-mining & manual reading

- fatty liver
- steatosis
- steatohepatitis
- cholestatic hepatitis
- cholestasis
- hepatopathy
- hepatomegaly
- veno-occlusive disease

- ALT/AST
- SGPT/SGOT
- liver enzyme transaminase
- aminotransferase
- liver/hepatic injury
- liver/hepatic function test
- liver/hepatic dysfunction

- hepatotoxicity
- liver/hepatic effect
- liver/hepatic toxicity
- liver/hepatic reaction
- liver/hepatic damage
- liver/hepatic warning
- liver/hepatic disorder
- liver/hepatic impairment

- bilirubinemia
- hyperbilirubinemia
- jaundice
- liver/hepatic failure
- liver/hepatic necrosis
- liver/hepatic decompensation
- liver/hepatic encephalopathy
- liver/hepatic transplantation

The Rule of 2 ('RO2'): High Lipophilicity ($\log P \geq 3$) + High Daily Dose ($DD \geq 100$ mg) Predicts DILI

Observed in 164 drugs
Verified by 179 drugs
Demonstrated on 5 drug pairs
Applied to co-medication

### Assess ‘RO2’ Model by Drug Pairs

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Daily dose (mg/day)</th>
<th>AlogP</th>
<th>‘RO2’ test</th>
<th>Label Section</th>
<th>DILI Concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macitentan</td>
<td>10</td>
<td>3.5</td>
<td>Negative</td>
<td>Warning &amp; Precaution</td>
<td>Less-DILI-Concern</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>7.5</td>
<td>3.66</td>
<td>Negative</td>
<td>Adverse Reaction</td>
<td>Less-DILI-Concern</td>
</tr>
<tr>
<td>Bosentan</td>
<td>250</td>
<td>3.95</td>
<td>Positive</td>
<td>Box Warning</td>
<td>Most-DILI-Concern</td>
</tr>
<tr>
<td>Sitaxsentan</td>
<td>100</td>
<td>3.7</td>
<td>Positive</td>
<td>Withdrawn</td>
<td>Most-DILI-Concern</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Daily dose (mg/day)</th>
<th>AlogP</th>
<th>‘RO2’ test</th>
<th>Label Section</th>
<th>DILI Concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zolpidem</td>
<td>10</td>
<td>3.36</td>
<td>Negative</td>
<td>Adverse reaction</td>
<td>Less-DILI-concern</td>
</tr>
<tr>
<td>Alpidem</td>
<td>150</td>
<td>5.46</td>
<td>Positive</td>
<td>Withdrawn</td>
<td>Most-DILI-Concern</td>
</tr>
<tr>
<td>Piglitazone</td>
<td>6</td>
<td>3.91</td>
<td>Negative</td>
<td>Warnings &amp; Precautions</td>
<td>Less-DILI-concern</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>30</td>
<td>3.36</td>
<td>Negative</td>
<td>Warnings &amp; Precautions</td>
<td>Less-DILI-concern</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>400</td>
<td>5.1</td>
<td>Positive</td>
<td>Withdrawn</td>
<td>Most-DILI-Concern</td>
</tr>
</tbody>
</table>
### More of ‘RO2’ Successful and Failed Cases

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Daily dose (mg/day)</th>
<th>AlogP</th>
<th>‘RO2’ test</th>
<th>Label Section</th>
<th>DILI Concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entacapone</td>
<td>1000</td>
<td>1.66</td>
<td>Negative</td>
<td>No mentioned</td>
<td>No-DILI-concern</td>
</tr>
<tr>
<td>Tolcapone</td>
<td>450</td>
<td>3.13</td>
<td>Positive</td>
<td>Box warning</td>
<td>Most-DILI-Concern</td>
</tr>
<tr>
<td>Trazodone</td>
<td>300</td>
<td>2.42</td>
<td>Negative</td>
<td>Adverse reaction</td>
<td>Less-DILI-concern</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>400</td>
<td>4.42</td>
<td>Positive</td>
<td>Withdrawn</td>
<td>Most-DILI-Concern</td>
</tr>
</tbody>
</table>

### ‘RO2’ failed cases (30-35% sensitivity and 90-95% specificity)

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Daily dose (mg/day)</th>
<th>AlogP</th>
<th>‘RO2’ test</th>
<th>DILI concern</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trovafloxacin</td>
<td>200</td>
<td>-0.91</td>
<td>False Negative</td>
<td>Withdrawn</td>
<td></td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>48</td>
<td>1.16</td>
<td>False Negative</td>
<td>Withdrawn</td>
<td></td>
</tr>
<tr>
<td>Aliskiren</td>
<td>150</td>
<td>3.32</td>
<td>False Positive</td>
<td>No-DILI-concern</td>
<td>Bioavailability (2.6%)</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>300</td>
<td>3.38</td>
<td>False Positive</td>
<td>No-DILI-concern</td>
<td>Antihistamine, Modulate immune</td>
</tr>
</tbody>
</table>
‘RO2’ Validation by FDA-Approved Oral Medications

748 oral drugs

168 most-DILI-concern
Sensitivity = 72/168 (43%)

193 no-DILI-concern
Specificity = 1 - 11/193 (94%)

387 less-DILI-concern
RO2 pos% = 50/387 (13%)

Withdrawn
RO2 pos%= 51%

Box warning
RO2 pos%= 38%

Warnings
RO2 pos%= 40%

Immune modulation:
diphenhydramine, orphenadrine, chlorcyclizine, flavoxate, benzogetamine, mifepristone, pentazocine, ursodeoxycholic acid

Poor bioavailability(%): aliskiren (3%)

Unknown: retigabine, tapentadol 12

* 23 of 50 RO2 positives were assigned as DILI positives by at least one literature
Selected Failed Drug Development Due to Hepatotoxicity

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Daily Dose (mg/day)</th>
<th>AlogP</th>
<th>RO2 test</th>
<th>Safety Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP260</td>
<td>3000*</td>
<td>5</td>
<td>Positive</td>
<td>Liver toxicity were found in beagle dog</td>
</tr>
<tr>
<td>Casopitant</td>
<td>150</td>
<td>4.73</td>
<td>Positive</td>
<td>Liver toxicity were found in Phase III patients</td>
</tr>
<tr>
<td>Tasosartan</td>
<td>200</td>
<td>3.051</td>
<td>Positive</td>
<td>Phase III clinical trials showed elevated transaminases</td>
</tr>
<tr>
<td>Fiduxosin</td>
<td>100</td>
<td>4.215</td>
<td>Positive</td>
<td>Discontinued due to liver toxicity</td>
</tr>
<tr>
<td>Alaproclate</td>
<td>100*</td>
<td>2.695</td>
<td>Negative</td>
<td>Observed liver complications in rodent studies</td>
</tr>
<tr>
<td>Pafuramidine</td>
<td>100</td>
<td>2.77</td>
<td>Negative</td>
<td>Liver abnormalities identified in healthy volunteers</td>
</tr>
<tr>
<td>Aplaviroc</td>
<td>1200</td>
<td>1.386</td>
<td>Negative</td>
<td>Severe hepatotoxicity observed in Phase III studies</td>
</tr>
<tr>
<td>Pralnacasan</td>
<td>300</td>
<td>0.132</td>
<td>Negative</td>
<td>Liver abnormality observed at one species of animals</td>
</tr>
<tr>
<td>Fialuridine</td>
<td>15</td>
<td>-1.228</td>
<td>Negative</td>
<td>Unexpected fulminant liver failure in patients</td>
</tr>
</tbody>
</table>

* Equivalent human dose

Call for the sharing of the failed drugs!
# Improve ‘RO2’ by Integrating High-Content Screening (HCS) Assays

## Human hepatotoxicity

<table>
<thead>
<tr>
<th>Model</th>
<th>Test result</th>
<th>Human hepatotoxicity</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th># drugs requiring HCS test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (N=49)</td>
<td>Negative (N=21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO2-HCS</td>
<td>Positive</td>
<td>27</td>
<td>1</td>
<td>67%</td>
<td>55%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>22</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCS</td>
<td>Positive</td>
<td>19</td>
<td>0</td>
<td>57%</td>
<td>39%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>30</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO2</td>
<td>Positive</td>
<td>13</td>
<td>1</td>
<td>47%</td>
<td>27%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>36</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Accuracy, Sensitivity, Specificity

- **RO2-HCS**: 67% accuracy, 55% sensitivity, 95% specificity
- **HCS**: 57% accuracy, 39% sensitivity, 100% specificity
- **RO2**: 47% accuracy, 27% sensitivity, 95% specificity

## Total positives (N=49)

- **HCS**: 13 positives
- **RO2**: 6 positives
- **RO2-HCS**: 7 positives

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Drug Properties or Host Factors?

Finally, they stop talking, start listening and collaborate to "see" the full elephant.
Take-home Messages

• Along with host factors, drug properties also contribute to the prediction of DILI

• LTKB provides a centralized repository of diverse DILI-related drug properties data.

• Several predictive models were developed
  ✷ The ‘RO2’ has added value in identifying idiosyncratic DILI
  ✷ *In vitro* assays can be enhanced by integrating the ‘RO2’

• Improvements have been made, but still a long way to go.
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