In vitro ADME & PK

Low Clearance Hepatocyte Stability Assay

Background Information

- Reducing the metabolic clearance of new chemical entities is a common goal in drug discovery projects in order to reduce dose, improve exposure and prolong the half-life. However, accurately predicting the clearance of stable compounds is challenging using standard in vitro suspension methods.

- Prolonged incubation times are restricted using suspended primary hepatocytes due to activity and viability issues. This can lead to inaccuracies in the intrinsic clearance values.

- New methods are being developed to address this concern through extension of the incubation time, which in turn is able to provide a more accurate estimation of the intrinsic clearance.

- Through its parent company, Evotec, Cyprotex are able to offer a low clearance method which utilises plated primary human hepatocytes, and matrix overlay to extend the time course for up to 5 days.

Protocol

Optimization of clearance is one of the more significant challenges for a drug discovery project. Identification of the rate in preclinical species and optimization in human are major goals in most projects."

1 Grime KH, Barton P & McGinnity DF (2013) Mol Pharm 10, 1191-1206

Cells
Primary human hepatocytes

Test Compound Concentration
1 μM (different concentrations available)

Overlay Matrix
Geltrex®

Incubation Time
0, 1, 2, 4, 8, 22, 26, 30 h

Replicates
n=2

Compounds Requirements
20 μL of 10 mM solution

Analysis Method
LC-MS/MS quantification

Assay Controls
Disopyramide (low clearance)
Metoprolol (moderate clearance)
Sildenafil (high clearance)

Data Delivery
Intrinsic clearance
Half life
Correlation of scaled in vitro human intrinsic clearance (using Evotec’s low clearance model) with in vivo human intrinsic clearance for a set of 12 known drugs. The data generated by Evotec is consistent to those reported by Bonn et al., 2016 as illustrated in Table 1. Further, the scaled in vitro human intrinsic clearance data from the Evotec model demonstrates a strong correlation with in vivo human intrinsic clearance showing the advantages of this approach as illustrated in Figure 1.

### Table 1
Comparison of human in vitro intrinsic clearance data generated by Evotec (Cyprotex’s parent company) and a publication by Bonn et al., 2016 where plated human hepatocytes and a co-culture model were used.

<table>
<thead>
<tr>
<th>Ion Class</th>
<th>Major Drug Metabolising Enzyme</th>
<th>Bonn et al., 2016 PHH CL\text{int} (µL/min/10^6 cells)</th>
<th>Bonn et al., 2016 Hurel CL\text{int} (µL/min/10^6 cells)</th>
<th>Evotec CL\text{int} (µL/min/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>Base CYP2B6, CYP1A2, CYP2A6, CYP3A4, CYP2E1</td>
<td>Not reported</td>
<td>Not reported</td>
<td>5.4</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Base CYP2D6, CYP2C9</td>
<td>26.3</td>
<td>34.2</td>
<td>14.5</td>
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<tr>
<td>Diazepam</td>
<td>Neutral CYP2C19, CYP3A4</td>
<td>0.8</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Acid CYP2C9, UGT2B7</td>
<td>Not reported</td>
<td>Not reported</td>
<td>4.7</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>Base CYP3A4</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Ethinylestradiol</td>
<td>Acid UGT1A1, CYP3A4</td>
<td>Not reported</td>
<td>Not reported</td>
<td>3.3</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Base CYP1A2, CYP2C19, CYP2D6</td>
<td>8.6</td>
<td>1.7</td>
<td>8.5</td>
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<tr>
<td>Metoprolol</td>
<td>Base CYP2D6, CYP3A4</td>
<td>2.2</td>
<td>0.8</td>
<td>0.9</td>
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<td>Midazolam</td>
<td>Neutral CYP3A4</td>
<td>Not reported</td>
<td>Not reported</td>
<td>5.1</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>Base CYP3A4, CYP2C9, CYP3A4</td>
<td>7.0</td>
<td>6.2</td>
<td>9.0</td>
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<tr>
<td>Tolbutamide</td>
<td>Acid CYP2C9</td>
<td>Not reported</td>
<td>Not reported</td>
<td>0.8</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Neutral CYP2C9, CYP3A4</td>
<td>BLQ</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Figure 1**
Correlation of scaled in vitro human intrinsic clearance (using Evotec’s low clearance model) with in vivo human intrinsic clearance for a set of 12 known drugs.

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References: