High Content Toxicology: Cytotoxicity Screening Panel

Background Information

- Drug toxicity is often a combination of multiple mechanisms. A single experimental approach is unlikely to capture the complexity involved in cellular toxicity.

- High Content Screening uses automated fluorescence imaging to simultaneously analyse multi-parametric indicators of cellular toxicity. It can detect general cell death and/or mechanisms of cell death within the same cell population within the same well, and it can cover a wide spectrum of cytopathological changes.

- Cyprotex have the most advanced High Content Screening equipment available, including four Thermo Scientific Cellomics ArrayScan® VTI’s and a Thermo Scientific Cellomics ToxInsight.

- Cyprotex offer a cytotoxicity panel to evaluate key toxicity markers including cell number, nuclear condensation, total nuclear intensity, cell permeability, mitochondrial membrane potential and cytochrome c release.

Protocol

**Instruments**
- Cellomics ArrayScan® VTI or Cellomics ToxInsight (Thermo Scientific)

**Analysis Method**
- High Content Screening

**Toxicity Markers**
- Cell loss
- Nuclear size
- Nuclear morphology
- Cell membrane permeability
- Mitochondrial membrane potential
- Mitochondrial mass
- Cytochrome c release

**Cell Type**
- HepG2 (others available on request)

**Test Article Concentration**
- 8 point dose response curve up to 500 µM or solubility limit (different concentrations available)

**Number of Replicates**
- 3 replicates per concentration

**Quality Controls**
- 0.5% DMSO (vehicle control)
- Chlorpromazine (positive control)
- Valinomycin (positive control)

**Test Article Requirements**
- 3-5 mg solid (depending on molecular weight) or equivalent DMSO solution

**Data Delivery**
- Minimum toxic concentration
- Dose response curves
The potential of HCS today lies in its versatility. HCS can be used for primary screening, basic research, target identification, biomarkers, cytotoxicity, and helping to predict clinical outcomes (Hoffman and Garippa, 2007).

Cells were incubated with a number of known toxic and non-toxic compounds at a range of different concentrations. At the end of the incubation period the cells were loaded with the relevant dye/antibody and scanned using an automated cell imager (Cellomics ArrayScan® VTI HCS Reader) to determine a panel of cell health markers. As expected all toxic compounds exhibited an effect on one or more endpoints whereas dexamethasone, a non-toxic compound, had no effect up to a concentration of 100 µM.

**Figure 1**
Simultaneous monitoring of multiple indicators of cell health in HepG2 cells using High Content Screening (HCS) technology. The images for treated cells (exposed to paclitaxel, cerivastatin or amiodarone over 24 hrs) are representative of an adverse effect on cell health as determined by monitoring nuclear morphology, cell membrane permeability, mitochondrial membrane potential and cytochrome c release. Data are compared with those from vehicle control wells (exposed to 0.2% DMSO over 24 hrs).

Using HCS technology, multiple observations can be detected from a single analysis. In the example in Figure 1, cell loss, nuclear size and nuclear intensity, cell membrane permeability, mitochondrial membrane potential and cytochrome c release from mitochondria were all observed in a single well.

**Figure 2**
Effect of different concentrations of amiodarone, paclitaxel and valinomycin on a panel of cytotoxicity markers.

<table>
<thead>
<tr>
<th>Cell health parameter</th>
<th>Amiodarone</th>
<th>Paclitaxel</th>
<th>Valinomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear intensity increase</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
<tr>
<td>Nuclear size decrease</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
<tr>
<td>Cell membrane permeability</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
<tr>
<td>Mitochondrial membrane potential</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
<tr>
<td>Cytochrome release</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
<tr>
<td>Cell loss</td>
<td>vehicle</td>
<td>0.2µM, 0.4µM, 2µM</td>
<td>vehicle</td>
</tr>
</tbody>
</table>

In Figure 2, six different markers of cytotoxicity are evaluated, including nuclear size and intensity, cell membrane permeability, mitochondrial membrane potential, cytochrome c release and nuclear count. Using a concentration-dependent approach it is possible to distinguish early-stage events from later-stage events, and to uncover the mechanism of toxicity leading up to cell death. For example, in the case of amiodarone, cytochrome c release and nuclear intensity are observed at low concentrations (0.2 µM) and a greater range of cytotoxic effects, including cell membrane permeability and cell loss observed at higher concentrations (20 µM).

**References**