In vitro approaches to cosmetic safety assessment

Paul Walker, Cyprotex Discovery Ltd.
Overview of the presentation

In vitro approaches to cosmetic safety assessment: ASCCT

- High Content Imaging (HCI) introduction.
- HCI in 3D cellular models.
- Designing a cell stress panel based on cell signalling pathways.
- Cell stress panel example data and images using HCI.
- Recent publication in collaboration with Unilever - Identifying and characterising stress pathways of concern for consumer safety in next generation risk assessment – Hatherell et al., 2020.
- Concluding remarks.
High Content Imaging (HCl)

Data capture and analysis

- HCl instruments used to image cells
  - Live cell chambers
  - Confocal imaging: 2D and 3D
  - Automated platform

- Cellular quantification: Organelles, nuclear, perinuclear and cytoplasmic regions.

- Automated fluorescence imaging and image analysis

- Multi-parametric indicators of cell toxicity (multiplexed in a single well).
Confocal HCI in ULA spheroid microplates

3D liver model imaging

- Hanging drop technique compared with Ultra-Low Attachment microplates (ULA).
- High content imaging compatibility in 3D.
Prediction of 3D DILI using HCl Assay

Comparison of hLiMTs and HepaRG spheroids using HCl

55 Reference compounds, HCl and ATP endpoints: hLiMTs and HepaRG spheroids

<table>
<thead>
<tr>
<th>Compound</th>
<th>DILI severity category</th>
<th>hLiMTs</th>
<th>HepaRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetaminophen</td>
<td>Most +ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>amiodarone</td>
<td>Most +ve</td>
<td>+ve</td>
<td>+ve</td>
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<tr>
<td>...</td>
<td></td>
<td></td>
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<tr>
<td>MEC &lt;25x Avg Cmax cutoff</td>
<td></td>
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</tbody>
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Confocal (CF)

Nuclei

Mitochondria

ROS formation

GSH content

Combined

DMSO 10 µM CPZ

Positive in hLiMTs only

Negative in both

HepaRG spheroids only

Combined Assay (MEC/25xCmax)

sensitivity 87%
specificity 100%

hLiMTs

sensitivity 89%
specificity 100%

HepaRG spheroids

>90% Accuracy
Selection of Cellular Stress Pathways

Collaboration with Unilever to develop NGRA cell stress panel

36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.
Cell Stress Panel Assay development

Cellular Markers identified for each mechanism, multiplexed were possible
Cell Stress Panel Design

Design Involves cellular dyes, antibodies & ELISA’s
Example data: Metal Stress

Metallothionein response to metal stress

Excess metals  ➔ MTF1 activation  ➔ Metallothionein expression
Example data: Endoplasmic Reticulum (ER) Stress

ER disruption, BiP & CHOP signalling pathway

Vehicle

Tunicamycin 10 µM

Hoechst  Endoplasmic Reticulum  Hoechst  BiP  Hoechst  CHOP

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ER disruption

Unfolded proteins

Increased BiP

PERK & ATF4 activation

Increased CHOP
Example data: Mitochondrial Oxidative Stress

Mitochondrial ROS, PGC\textsubscript{1}\textalpha{} & TNFAIP3 signalling pathway

Hoechst  MitoSox  Hoechst  PGC\textsubscript{1}\textalpha{}  TNFAIP3

Vehicle

Rotenone 0.1 µM

Rotenone 40 µM

mROS formation

PGC\textsubscript{1}\textalpha{} activation

TNFAIP3 expression
Cell stress panel development steps

Joint publication with Unilever accepted

- Stage 1: Development of cell stress panel
- Stage 2: Benchmark substance selection
- Stage 3: Does selection based on $C_{\text{max}}$

Data generated for 13 benchmark substances – mix of substances known to cause adverse effects in humans due to cellular stress (e.g. doxorubicin) or history of safe use (e.g. caffeine).

Hatherell et al., 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. in Press.
Evaluation of the 13 Benchmark Chemicals/drugs

PoD established for each biomarker in the cell stress panel

For all the chemical-exposures categorized as low-risk (except triclosan), the estimated $C_{\text{max}}$ was below the minimum PoD detected or no response was detected.

By contrast, for chemical-exposures categorized as high risk (except diclofenac), the estimated $C_{\text{max}}$ values were above the minimum PoD.

Using the in vitro cellular stress panel and statistical approach described in Hatherell et al. (2020) it was possible to identify substance exposures that may be associated with adverse health effects due to cellular stress.
Concluding remarks

Cell stress panel developed for NGRA

• We have presented the development and initial characterisation of a Cell Stress Panel Assay for NGRA. The panel consists of multiple cellular stress signalling pathways using both live cell organelle dyes (e.g. ER tracker, MitoSox) alongside specific antibodies e.g. transcription factors (ATF4) and chaperone proteins (BiP), 36 biomarkers in total.

• Initial validation of the cell stress panel in press (Hatherell et al., 2020) whereby this predominately high content imaging (HCI) strategy has the potential to improve our understanding of chemical exposure outcomes using PoD in relation to $C_{\text{max}}$ with a set of 13 benchmark substances.

• In combination with other cellular assays and in silico approaches this panel could provide a powerful NGRA tool to use in non-animal safety decision making.
QUESTIONS AND ANSWERS