

In vitro approaches to cosmetic safety assessment

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Overview of the presentation

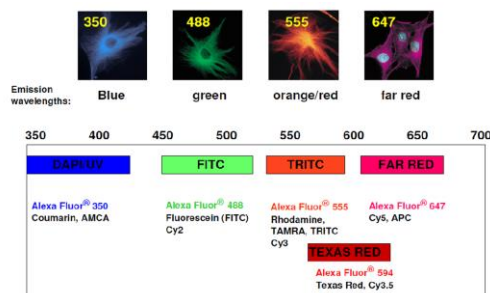
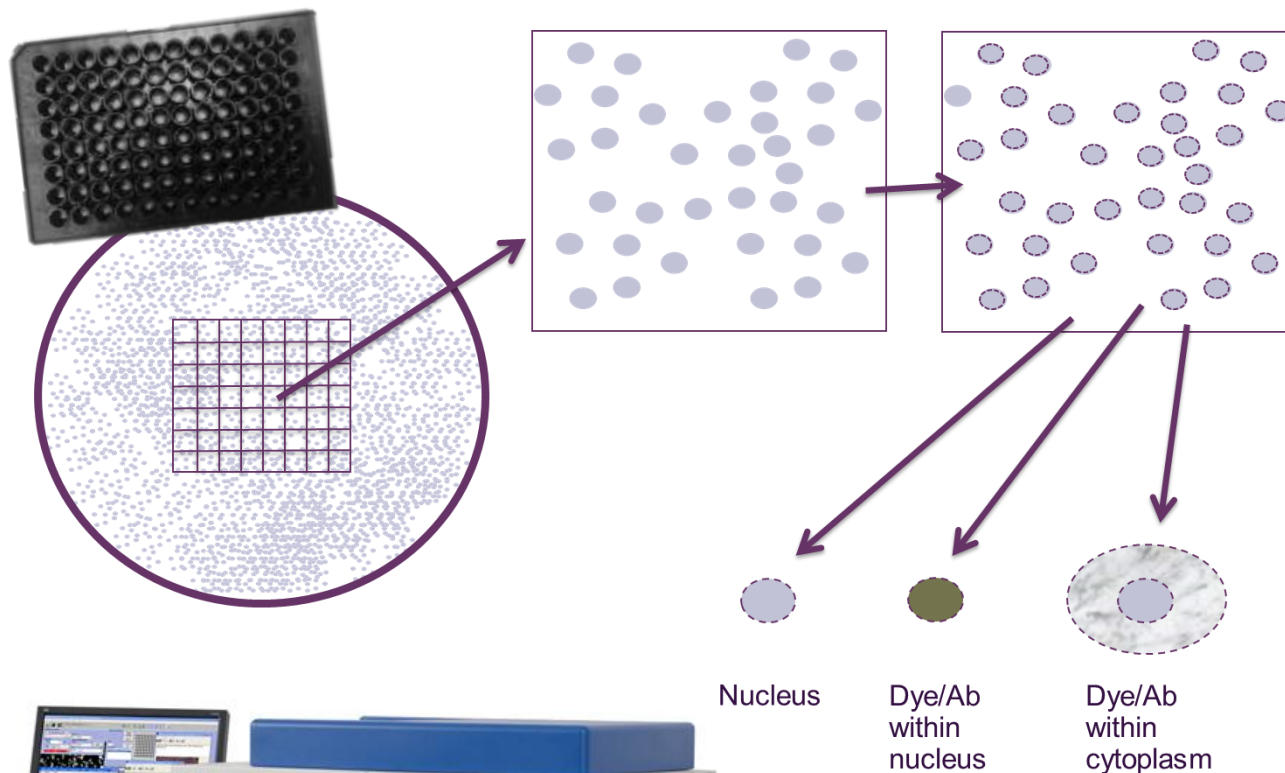
In vitro approaches to cosmetic safety assessment: ASCCT

- 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 (HCI)

Data capture and analysis

- HCI 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).

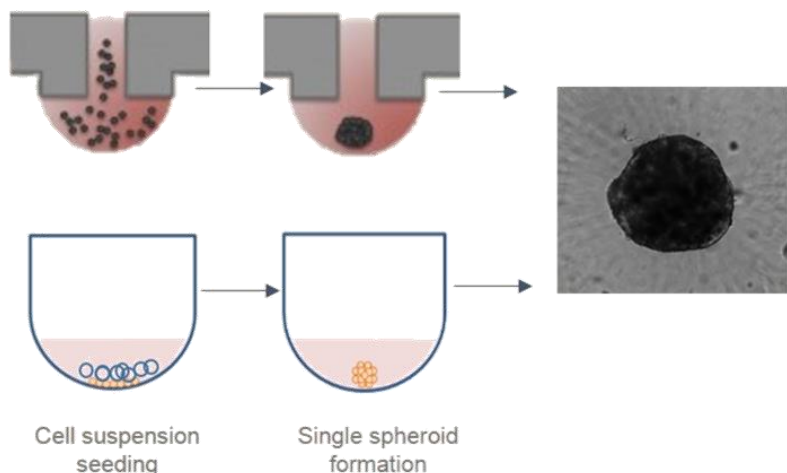


Confocal HCI in ULA spheroid microplates

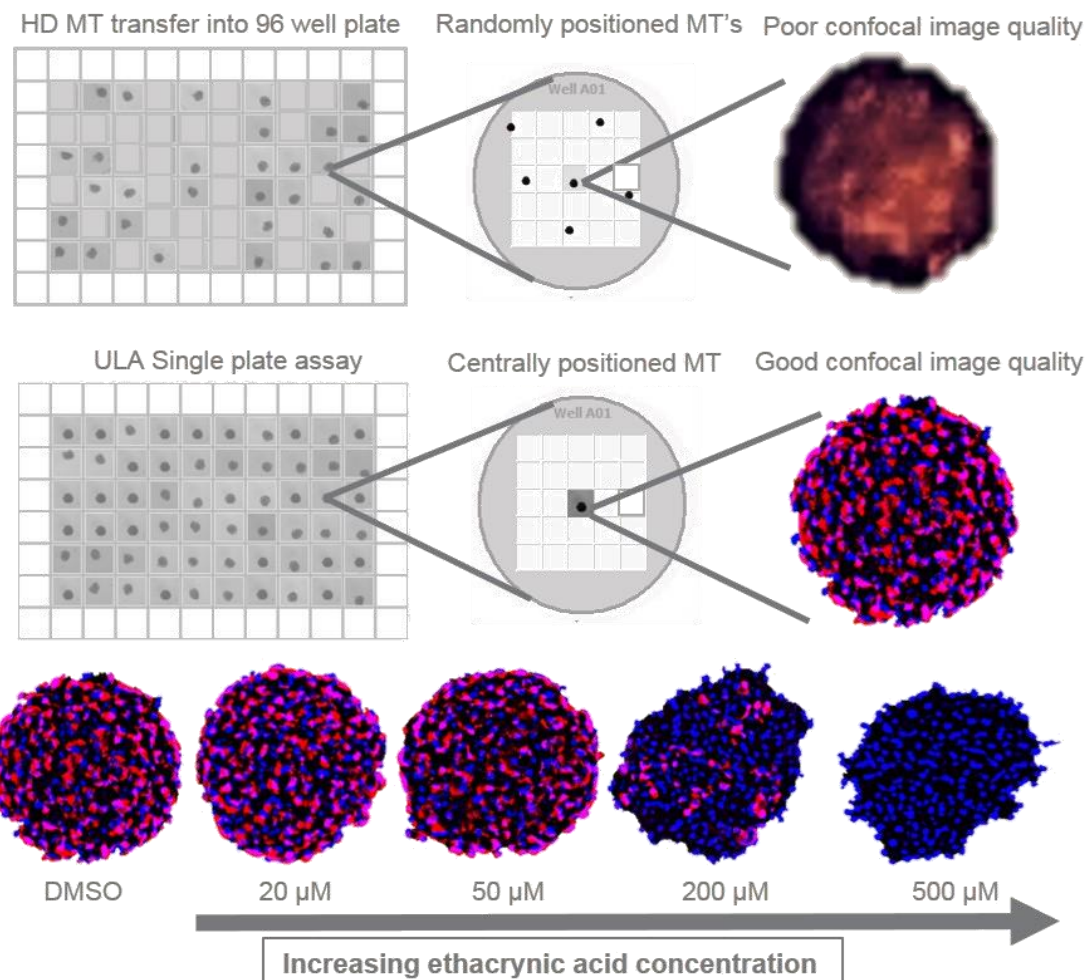
3D liver model imaging



Live Cell Confocal
High Content Imaging
(using ArrayScan XTI
or CX7)



- Hanging drop technique compared with Ultra-Low Attachment microplates (ULA).
- High content imaging compatibility in 3D.

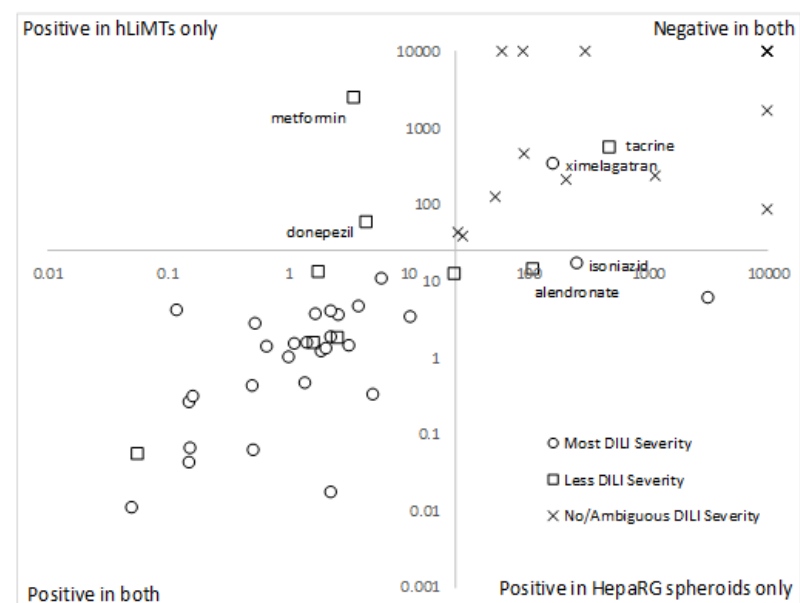
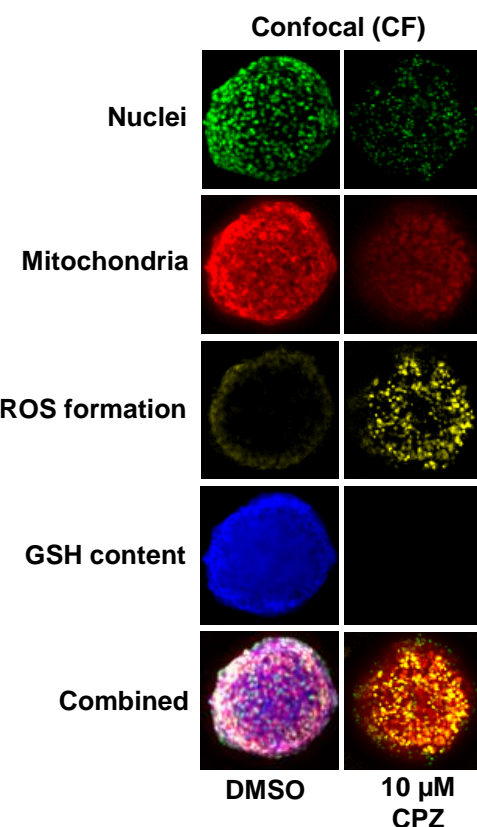


Prediction of 3D DILI using HCl Assay

Comparison of hLiMTs and HepaRG spheroids using HCl

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

Compound	DILI severity category	hLiMTs			HepaRG		
		MEC <25x Avg C _{max} cutoff			<25x Avg C _{max}		
		All	ATP	HCS	All	ATP	HCS
acetaminophen	Most	+ve	+ve	+ve	+ve	+ve	+ve
amiodarone	Most	+ve	+ve	+ve	+ve	+ve	+ve
azathioprine	Most	+ve	+ve	+ve	+ve	+ve	+ve
bosentan	Most	+ve	+ve	+ve	+ve	+ve	+ve
carbamazepine	Most	+ve	+ve	+ve	+ve	+ve	+ve
clozapine	Most	+ve	+ve	+ve	+ve	+ve	+ve
Dantrolene	Most	+ve	+ve	+ve	+ve	+ve	+ve
diclofenac	Most	+ve	+ve	+ve	+ve	+ve	+ve
Fluoridine	Most	+ve	+ve	+ve	+ve	+ve	+ve
flutamide	Most	+ve	+ve	+ve	+ve	+ve	+ve
Indomethacin	Most	+ve	+ve	+ve	+ve	+ve	+ve
Isoniazid	Most	-ve	-ve	-ve	+ve	+ve	-ve
ketoconazole	Most	+ve	+ve	+ve	+ve	+ve	+ve
lapatinib	Most	+ve	+ve	+ve	+ve	+ve	+ve
methotrexate	Most	+ve	+ve	+ve	+ve	+ve	+ve
nefazodone	Most	+ve	+ve	+ve	+ve	+ve	+ve
nitrofurantoin	Most	+ve	+ve	+ve	+ve	+ve	+ve
perhexiline	Most	+ve	+ve	+ve	+ve	+ve	+ve
sitaxsentan	Most	+ve	+ve	+ve	+ve	+ve	+ve
sunitinib	Most	+ve	+ve	+ve	+ve	+ve	+ve
tamoxifen	Most	+ve	+ve	+ve	+ve	+ve	+ve
ticlopidine	Most	+ve	+ve	+ve	+ve	+ve	+ve
tolcapone	Most	+ve	+ve	+ve	+ve	+ve	+ve
trogilazone	Most	+ve	+ve	+ve	+ve	+ve	+ve
trovaflaxacin	Most	+ve	+ve	+ve	+ve	+ve	+ve
valproic acid	Most	+ve	+ve	+ve	+ve	+ve	+ve
ximelagatran	Most	-ve	-ve	-ve	+ve	+ve	-ve
acetylsalicylic acid	-	+ve	+ve	+ve	+ve	+ve	+ve
erythromycin	-	+ve	+ve	+ve	+ve	+ve	+ve
alendronate	Less	-ve	-ve	-ve	+ve	+ve	-ve
chlorpromazine	Less	+ve	+ve	+ve	+ve	+ve	+ve
cyclophosphamide	Less	+ve	+ve	+ve	+ve	+ve	+ve
donepezil	Less	+ve	+ve	+ve	+ve	+ve	+ve
entacapone	Less	+ve	+ve	+ve	+ve	+ve	+ve
Metformin	Less	+ve	+ve	+ve	+ve	+ve	+ve
mitomycin C	Less	+ve	+ve	+ve	+ve	+ve	+ve
pioglitazone	Less	+ve	+ve	+ve	+ve	+ve	+ve
tacrine	-	-ve	-ve	-ve	-ve	-ve	-ve
betaine	No	-ve	-ve	-ve	-ve	-ve	-ve
chlorpheniramine	No	-ve	-ve	-ve	-ve	-ve	-ve
Flavoxate	No	-ve	-ve	-ve	-ve	-ve	-ve
flumazenil	No	-ve	-ve	-ve	-ve	-ve	-ve
Liothyronine	No	-ve	-ve	-ve	-ve	-ve	-ve
Mecamylamine	No	-ve	-ve	-ve	-ve	-ve	-ve
minoxidil	No	-ve	-ve	-ve	-ve	-ve	-ve
Oxybutynin	No	-ve	-ve	-ve	-ve	-ve	-ve
Phenoxymethylamine	No	-ve	-ve	-ve	-ve	-ve	-ve
Phentolamine	No	-ve	-ve	-ve	-ve	-ve	-ve
Propantheline bromide	No	-ve	-ve	-ve	-ve	-ve	-ve
Streptomycin	No	-ve	-ve	-ve	-ve	-ve	-ve
buspirone	Ambiguous	-ve	-ve	-ve	-ve	-ve	-ve
nadolol	Ambiguous	-ve	-ve	-ve	-ve	-ve	-ve
neostigmine	-	-ve	-ve	-ve	-ve	-ve	-ve
pargyline	-	-ve	-ve	-ve	-ve	-ve	-ve
Sensitivity		87	71	87	89	84	84
Specificity		100	100	100	100	100	100
Accuracy		91	80	91	93	89	89



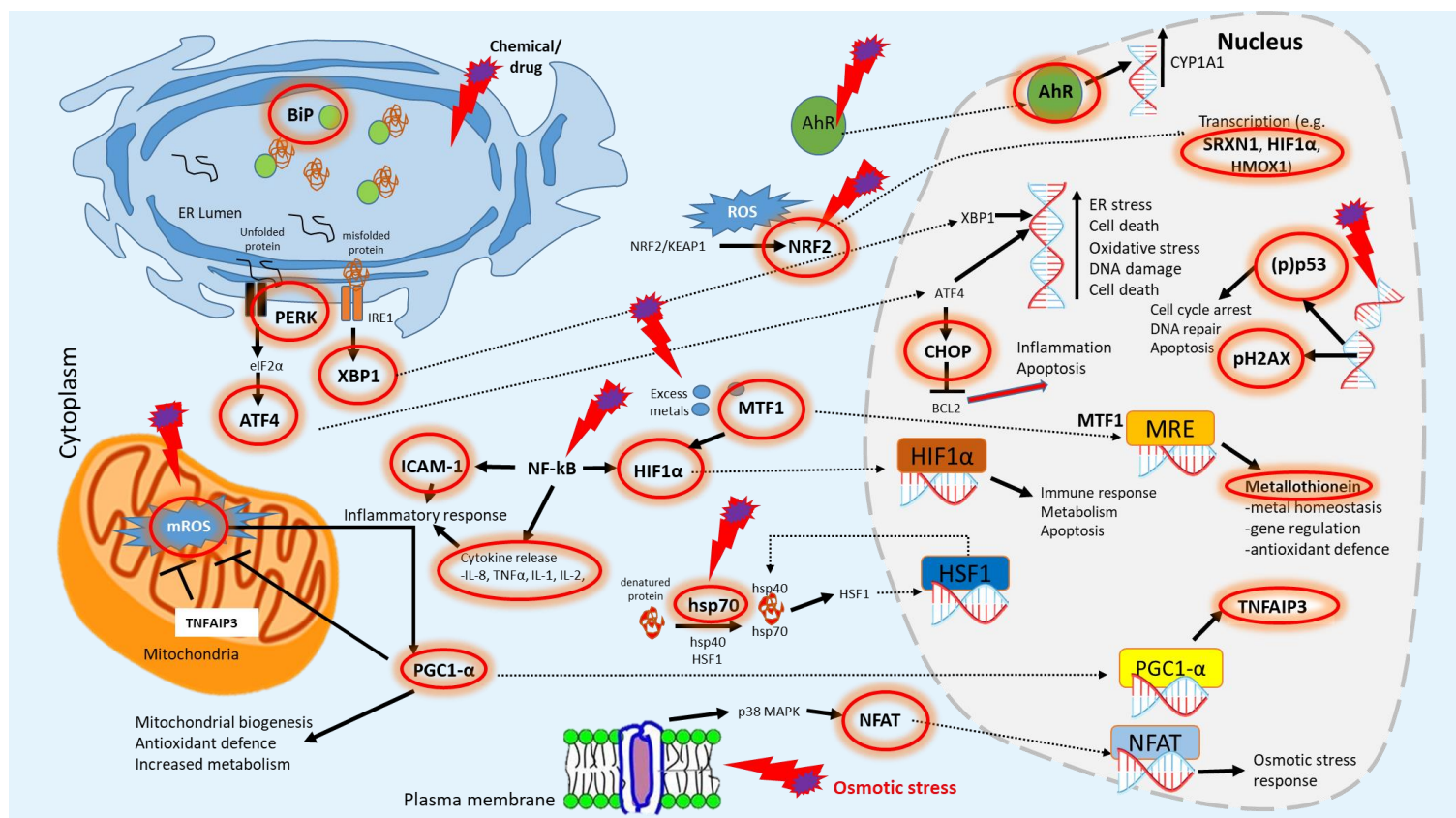
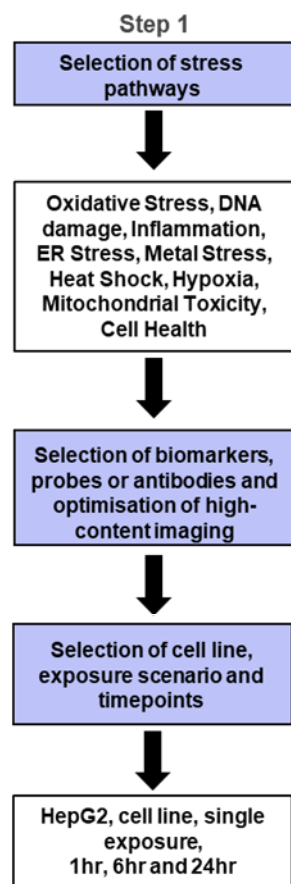
MEC <25x Median C_{max} cut-off

	Combined Assay (MEC/25xC _{max})	
	hLiMTs	HepaRG spheroids
sensitivity	87%	89%
specificity	100%	100%

>90% Accuracy

Selection of Cellular Stress Pathways

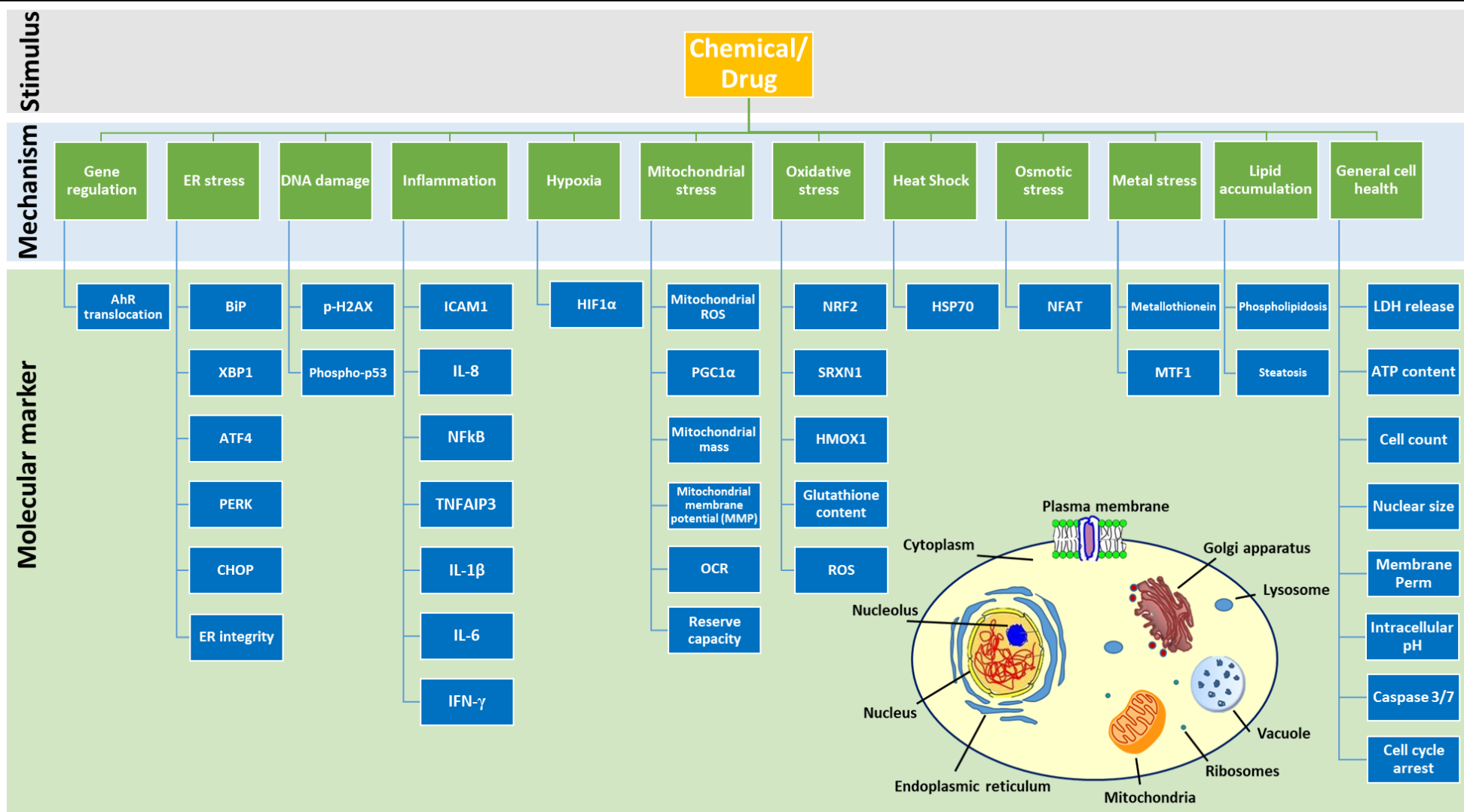
Collaboration with Unilever to develop NGRA cell stress panel



36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

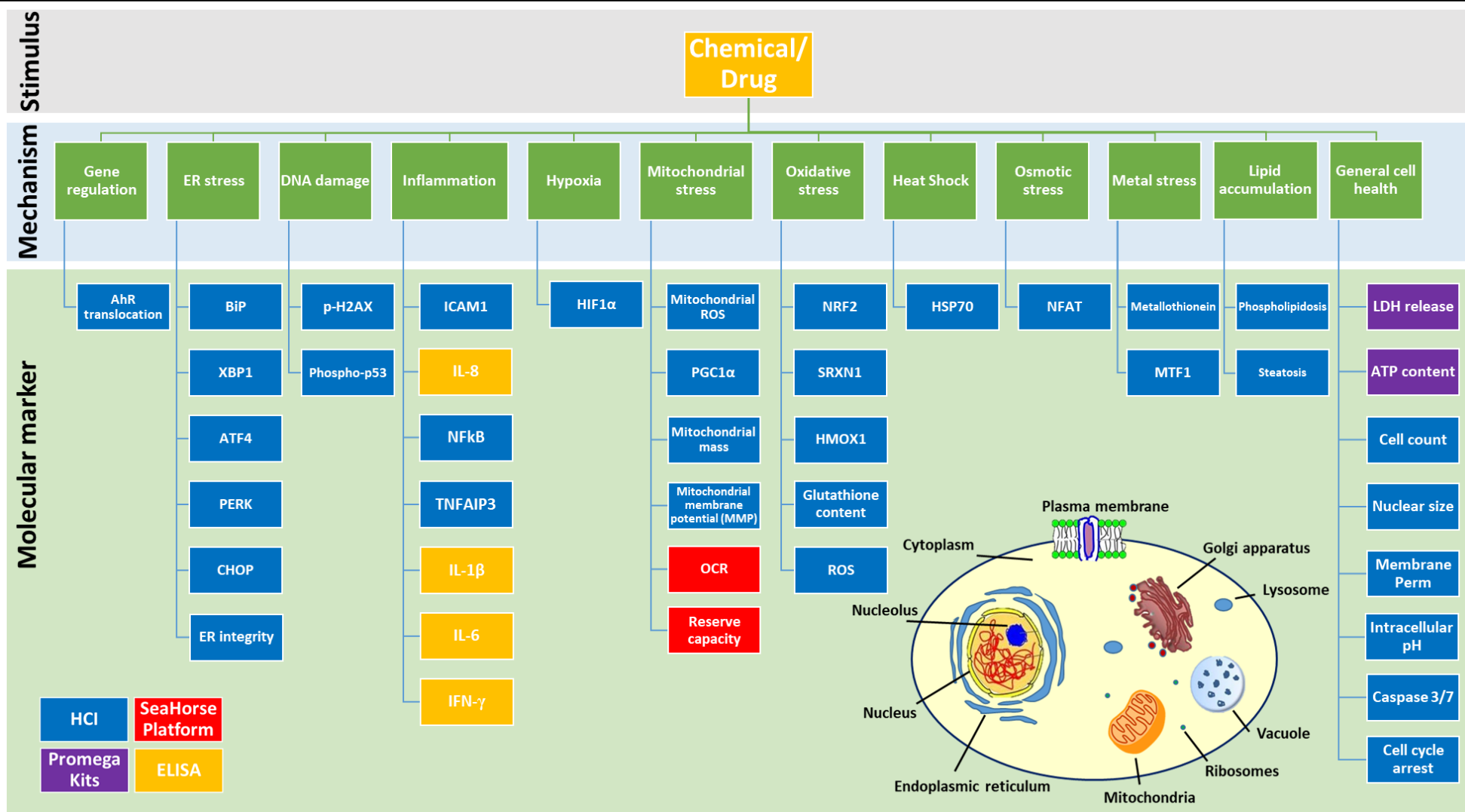
Cell Stress Panel Assay development

Cellular Markers identified for each mechanism, multiplexed were possible



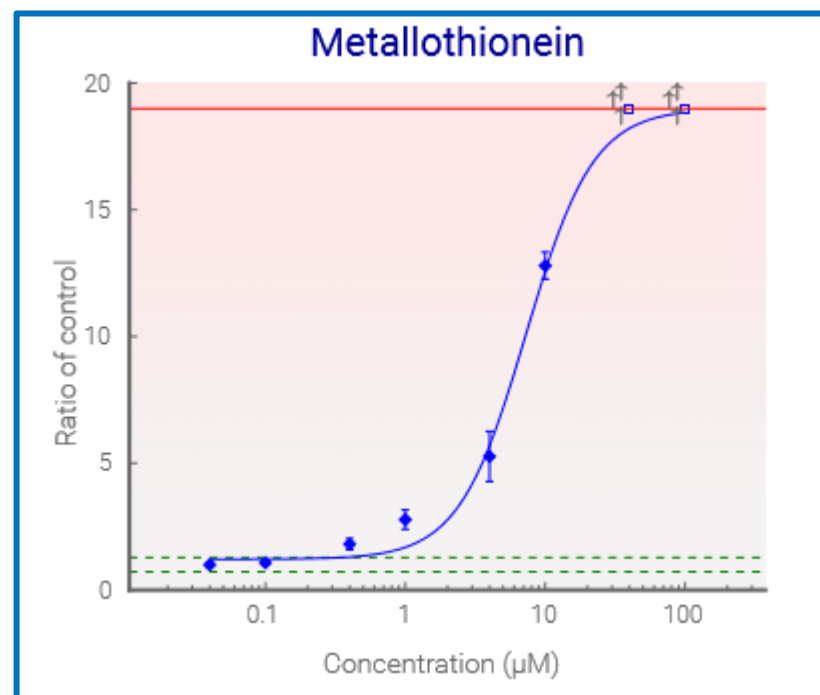
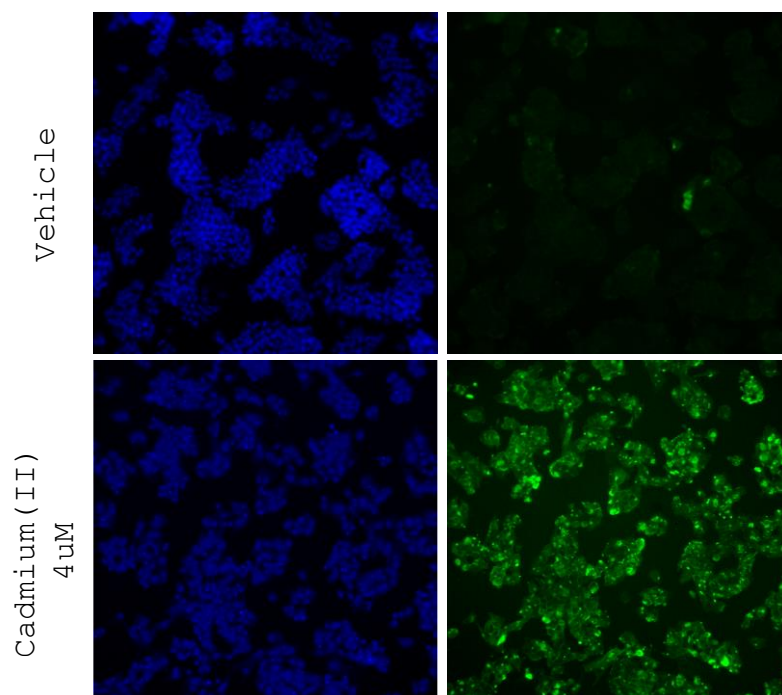
Cell Stress Panel Design

Design Involves cellular dyes, antibodies & ELISA's

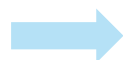


Example data: Metal Stress

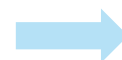
Metallothionein response to metal stress



Excess metals



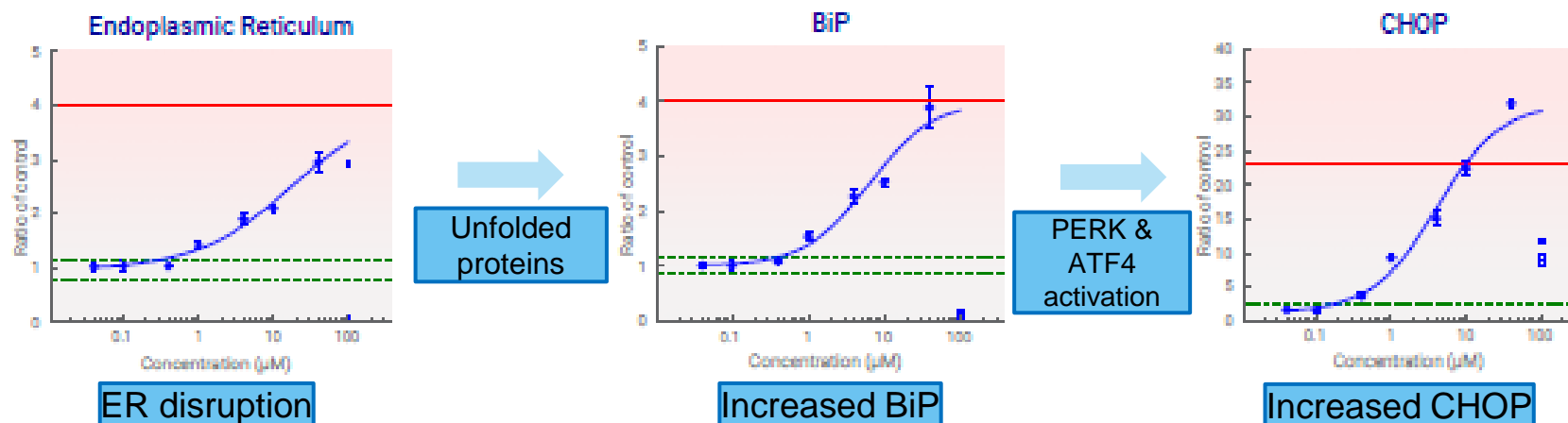
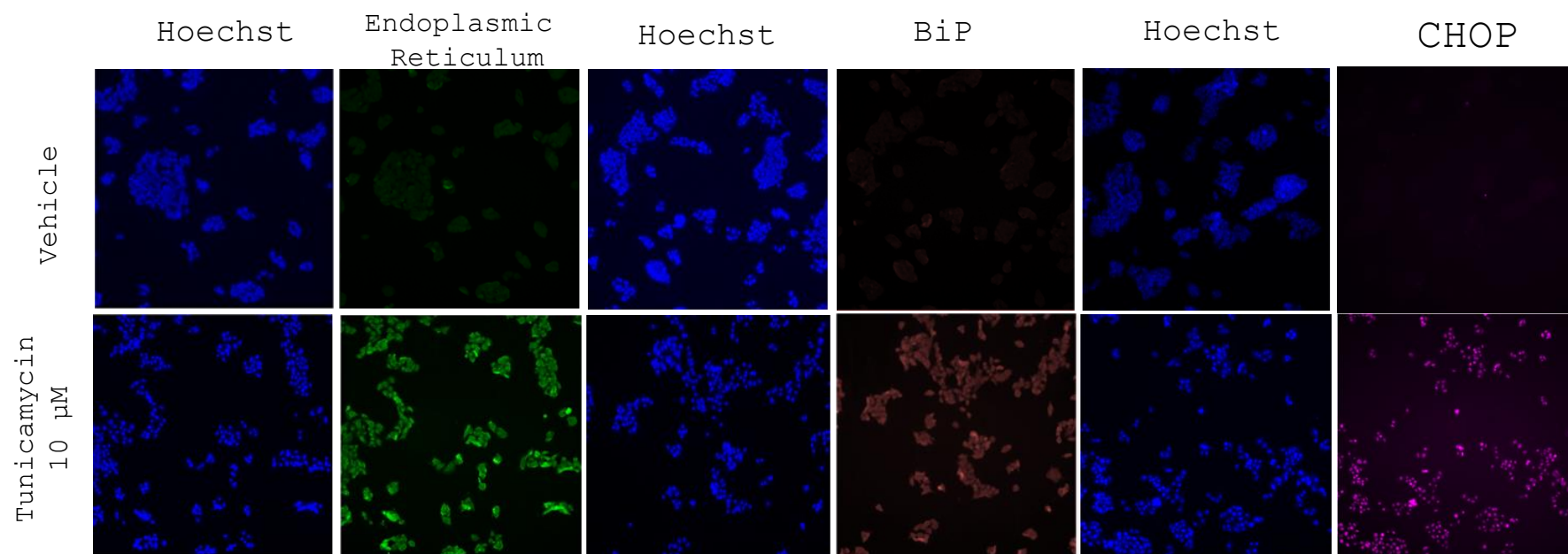
MTF1 activation



Metallothionein expression

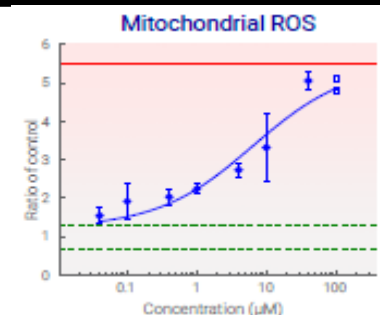
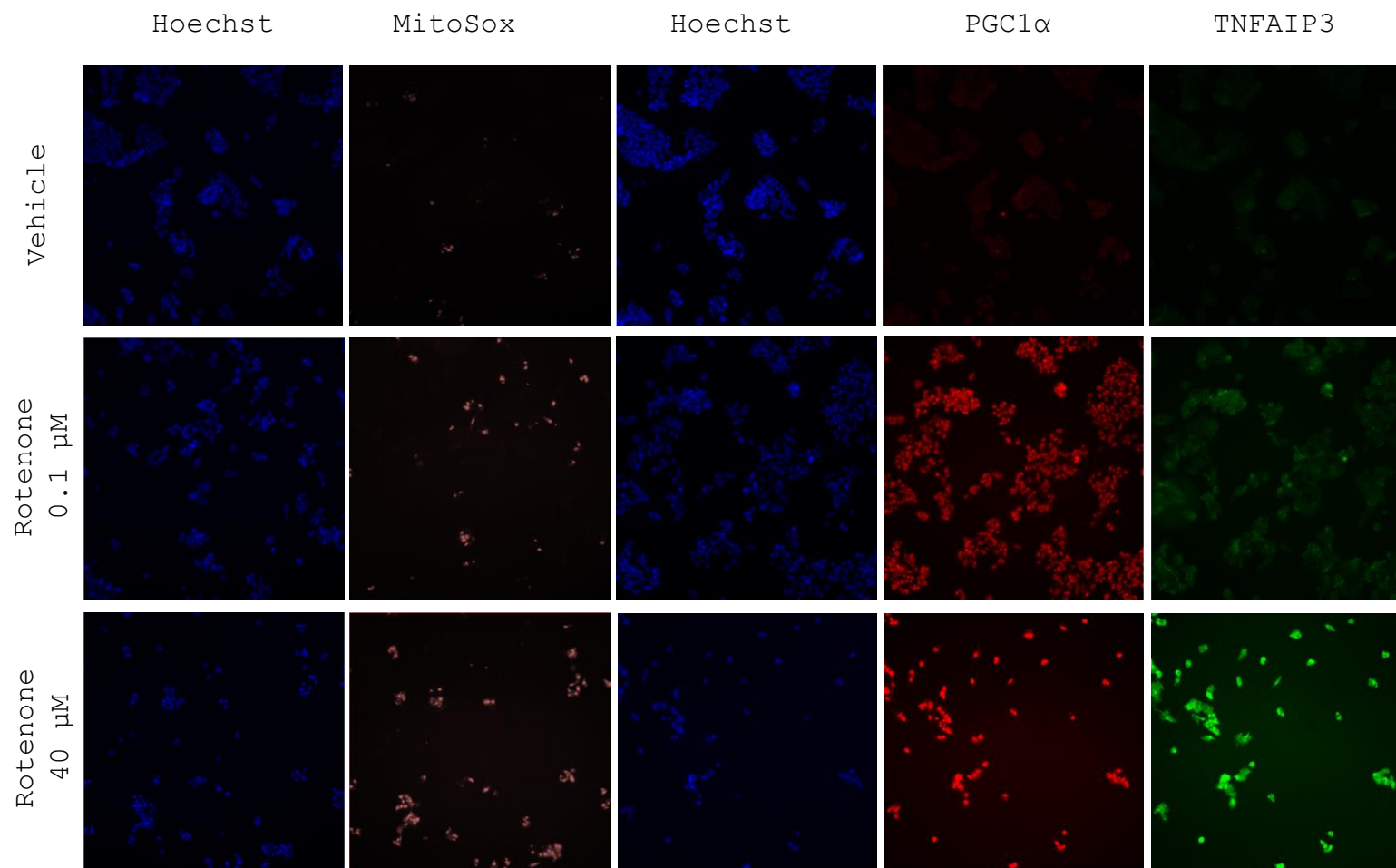
Example data: Endoplasmic Reticulum (ER) Stress

ER disruption, BiP & CHOP signalling pathway

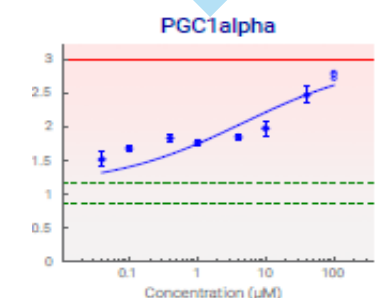


Example data: Mitochondrial Oxidative Stress

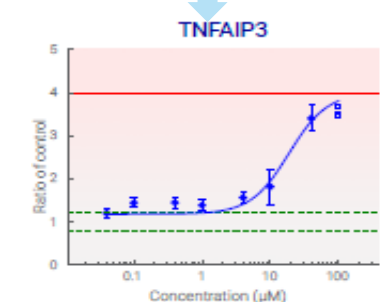
Mitochondrial ROS, PGC1 α & TNFAIP3 signalling pathway



mROS formation



PGC1 α activation



TNFAIP3 expression

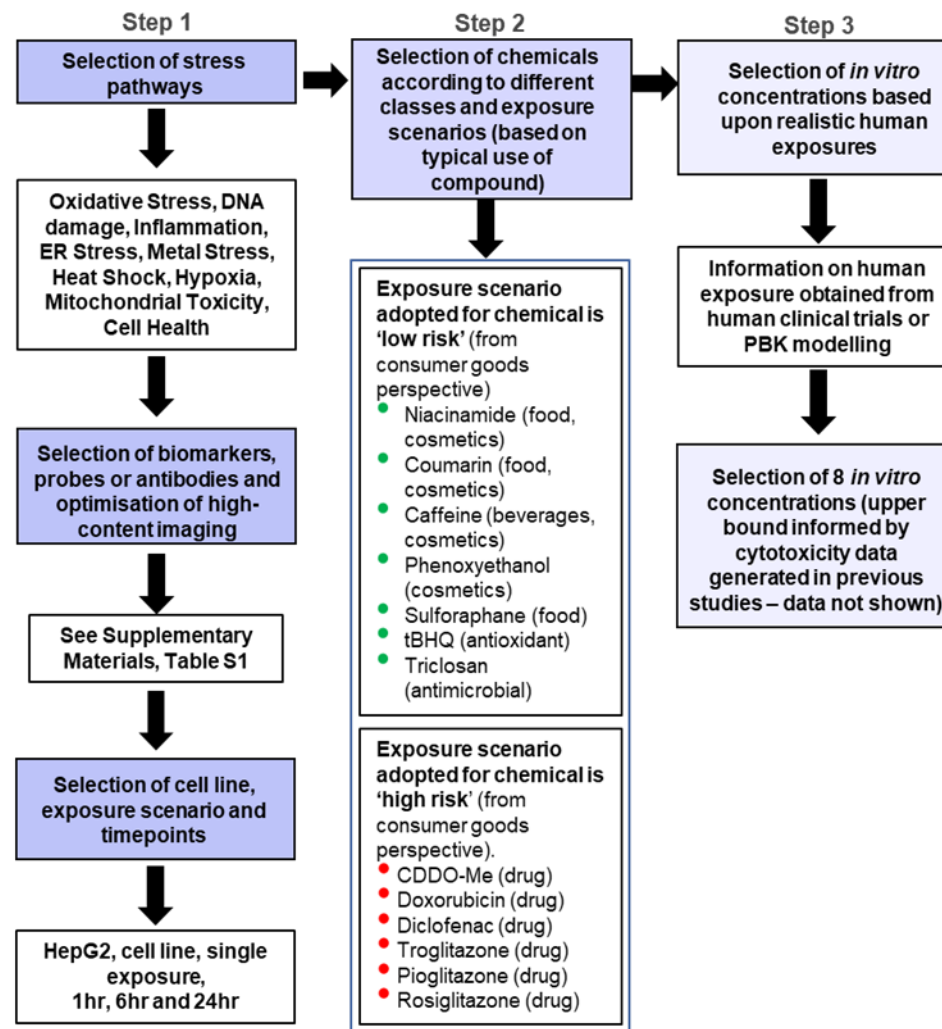
Cell stress panel development steps

Joint publication with Unilever accepted

- Stage 1: Development of cell stress panel
- Stage 2: Benchmark substance selection
- Stage 3: Does selection based on C_{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).

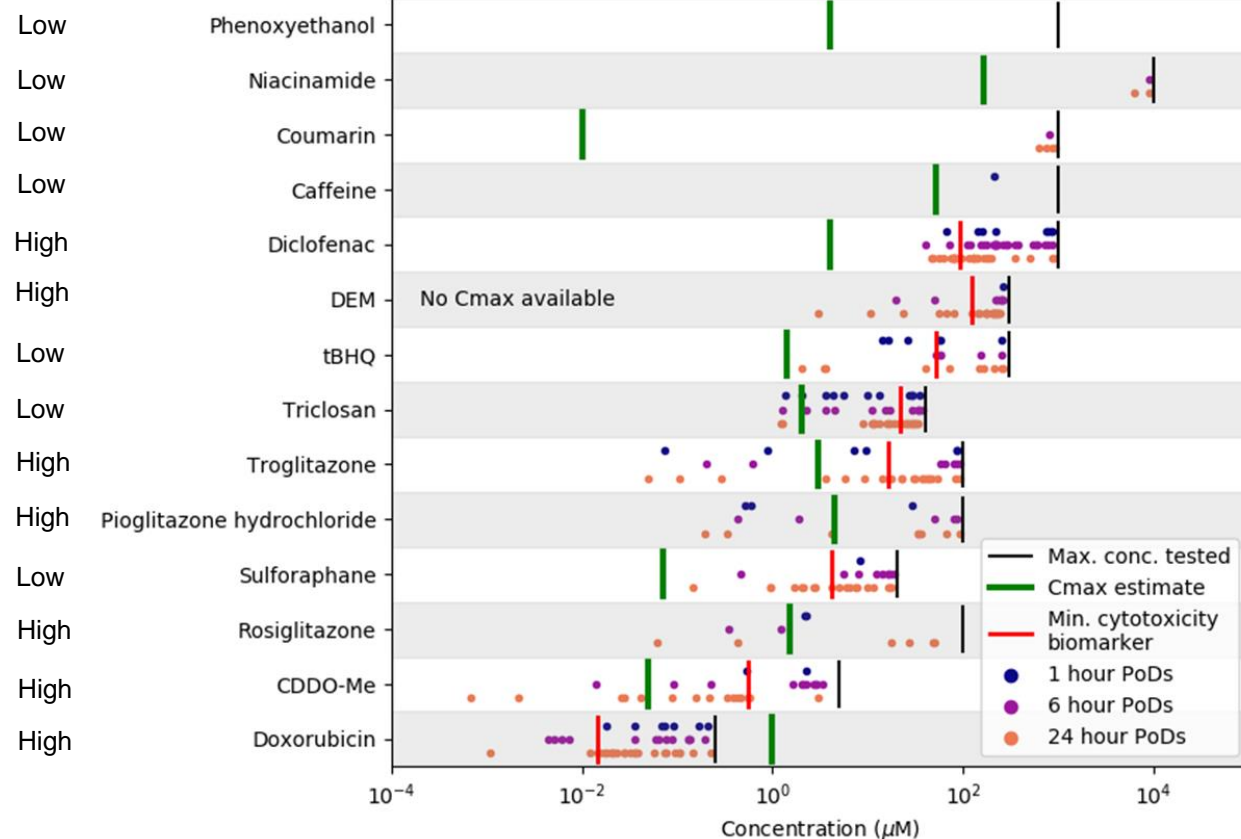
Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. in Press.



Evaluation of the 13 Benchmark Chemicals/drugs

PoD established for each biomarker in the cell stress panel

Chemical-
exposure
classification (risk)



Overview of PoD modes and associated mean C_{max} estimates for each substance.

- For all the chemical-exposures categorized as low-risk (except triclosan), the estimated C_{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_{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_{\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

