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Development and evaluation of 3D in vitro models for the prediction of tissue specific toxicities

Overview of Presentation

3D In Vitro Models

- Overview of compound attrition in drug discovery
- Fundamentals of high content screening in *in vitro* toxicology
- Development and characterisation of human 3D microtissues
- Development of key areas to assess potential safety liabilities:
  - Hepatotoxicity
  - Cardiotoxicity
  - Neurotoxicity
  - Nephrotoxicity

Causes of drug failure in development and clinic

In Vitro Toxicology and Safety Assessment

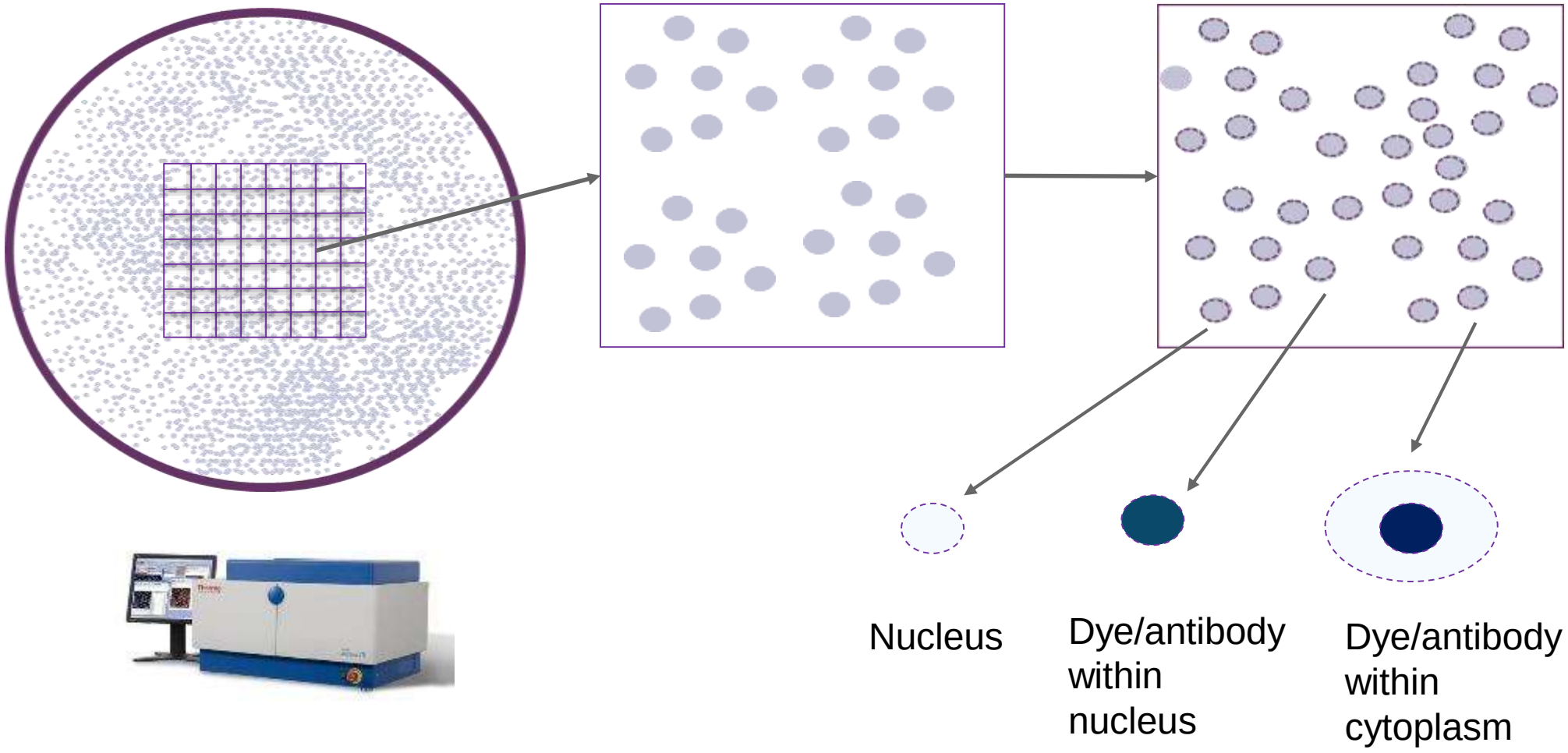
- Toxicity remains a leading source of attrition in Drug Dx both pre-clinically but also in clinical development
- The industry has responded to this failure by developing early *in vitro* screen to minimize liabilities.
- Initial focus has been in the CV area (hERG) but that has divested into additional organ specific areas such as hepatic and CNS
- Approaches to *in vitro* safety models require physiologically relevant models and predictive endpoints

Phase	Preclinical	Preclinical	Phase I-III	Phase I-III	Post Approval
Information	Causes of attrition	Causes of attrition	Causes of attrition	Causes of attrition	Withdrawl
Source	ABPI (2008)	Car (2006)	ABPI (2008)	Olson et al. (2000)	Stevens & Baker (2008)
Sample size	155 CD	88 CD	63 CD	82 CD	47 drugs
Cardiovascular	25%	27%	35%	21%	45%
Hepatotoxicity	15%	8%	29%	21%	32%
CNS	12%	14%	2%	21%	2%
Immunotox	7%	7%	10%	11%	2%
GI	5%	3%	2%	5%	2%
Reprotox	9%	13%	5%	1%	2%
Renal	6%	2%	5%	9%	0%
Carnogenicity	0%	3%	3%	0%	0%

Adapted fro, Redfern WS et al. SOT Poster 1081 and provided by Tim Hammond

Overview of high content screening

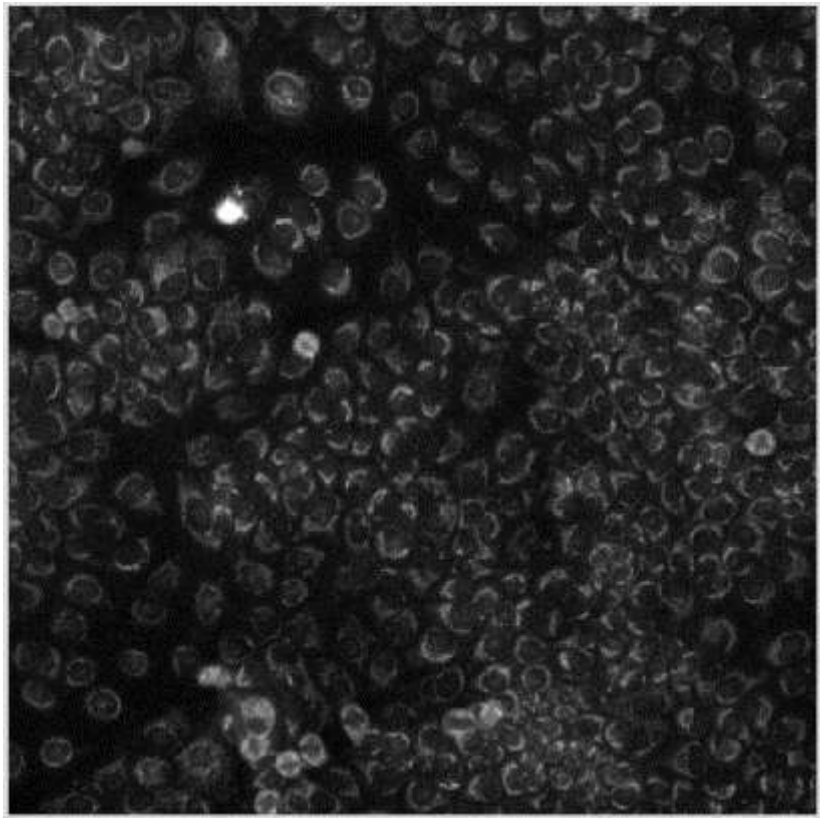
Data Analysis

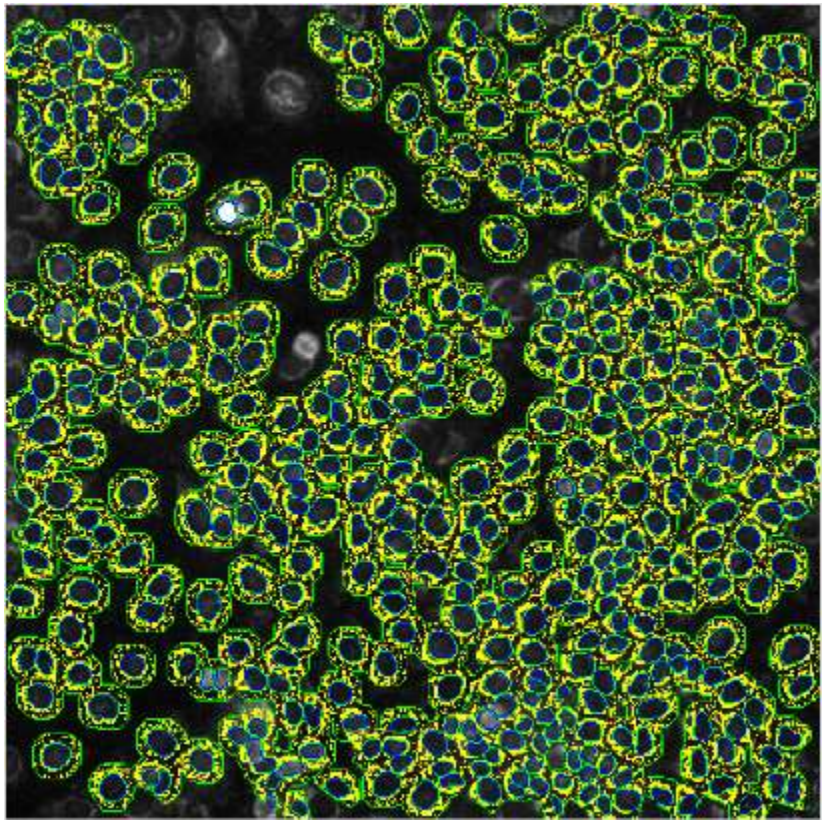


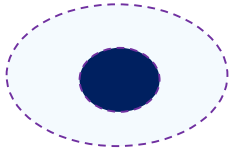
Adapted fro, Redfern WS et al. SOT Poster 1081 and provided by Tim Hammond

Overview of high content screening

Image analysis



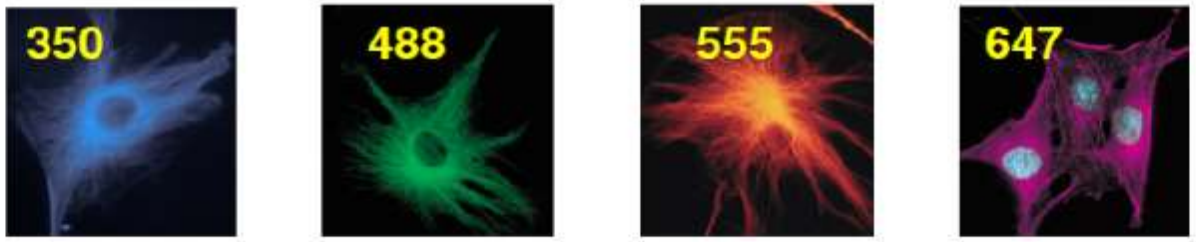




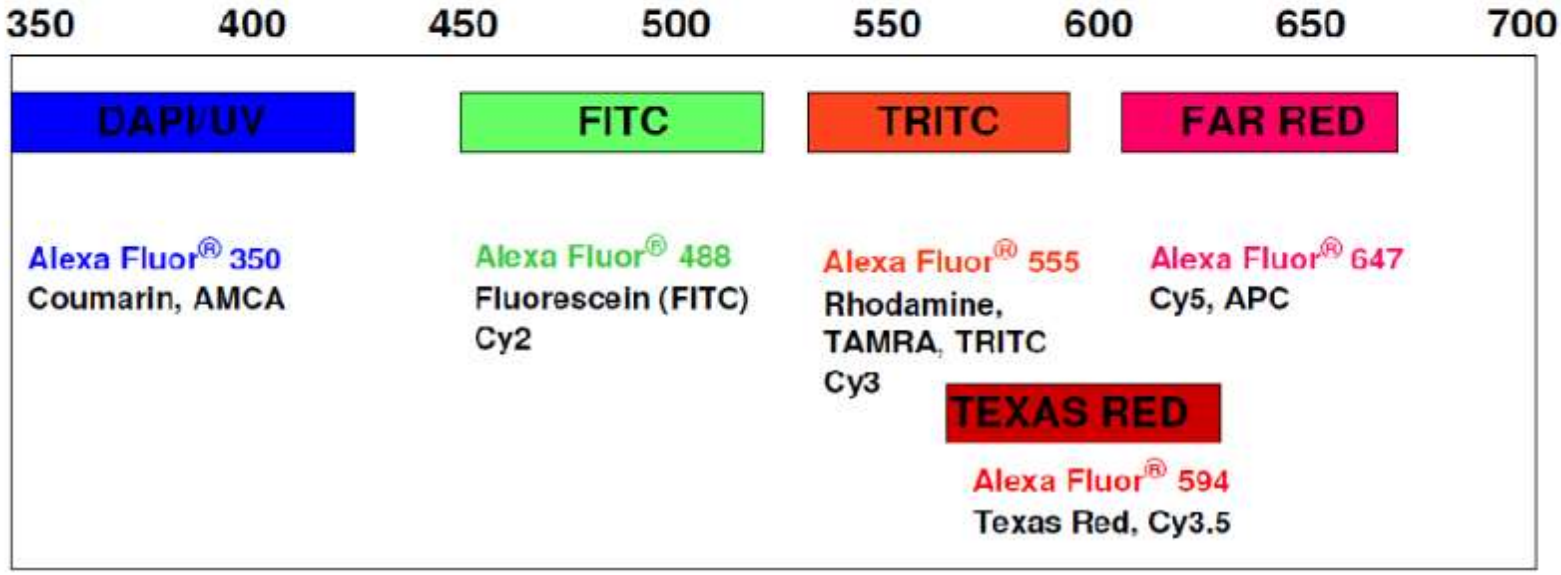
Dye/antibody within cytoplasm

Overview of high content screening

Validated channels and colours

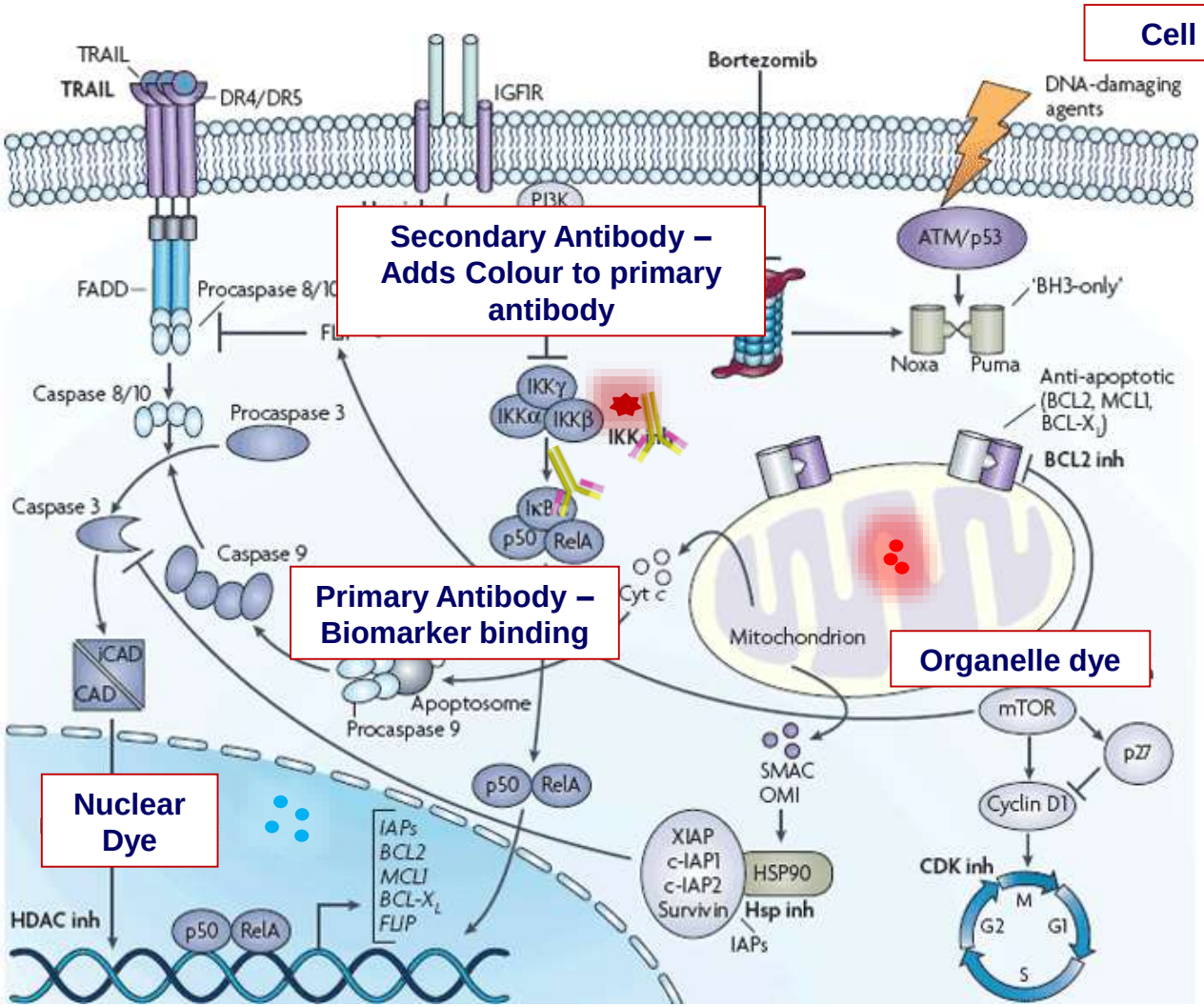


Emission wavelengths: Blue green orange/red far red



Overview of high content screening

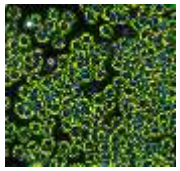
Multiplexing dyes and antibodies

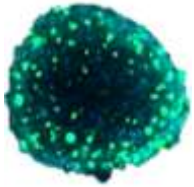


Development of Cell Culture Models

Transition from simple to complex

2D Cell Culture → 3D Cell Culture → Organ on a chip





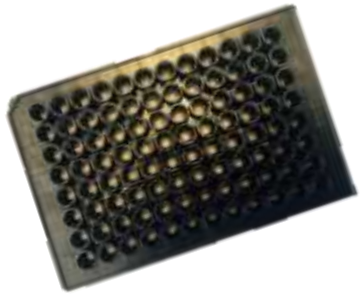
Optimal throughput and predictivity

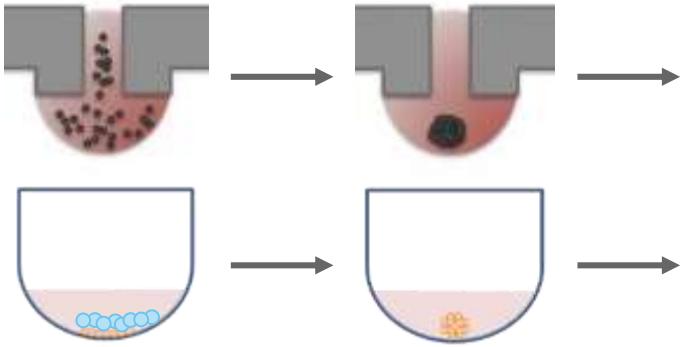
- Industry acceptance
- Throughput
- Organotypic response
- Cost
- Human like response

Organ on a chip image from EmulateBio.com

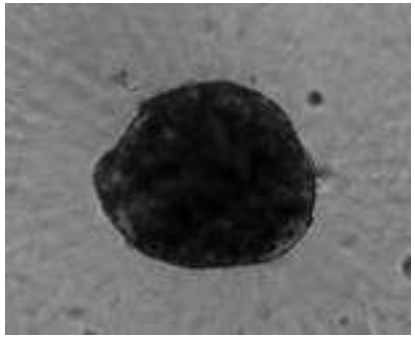
In vitro 3D Cell Culture

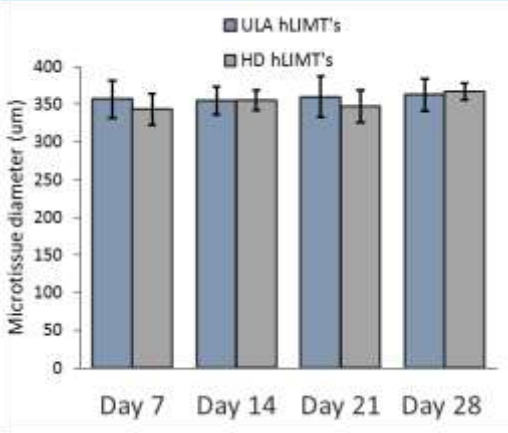
- *In vitro* three-dimensional (3D) cell cultures more accurately **reflect the complex *in vivo* microenvironment** than simple two-dimensional (2D) cell monolayers
- Spheroids are a popular 3D cell culture choice due to **cost effective** cell usage and **scaffold free**
- Spheroid formation utilised the **hanging drop technique** can be used whereby cells are suspended in droplets of medium to promote cell aggregation
- Alternatively **ultra-low adhesion microplates** can be used which are assay amenable plate formats for high content screening





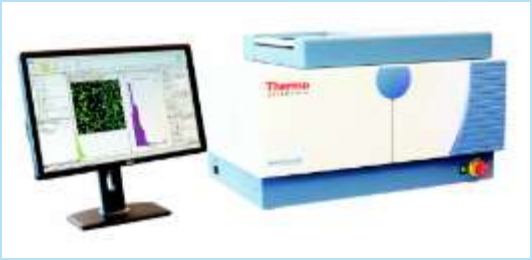
Cell suspension seeding **Single spheroid formation**



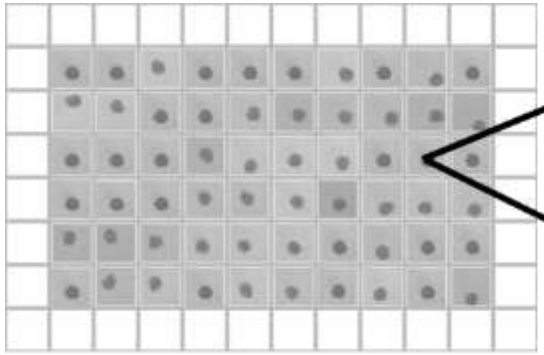


Confocal HCS Imaging of Microtissues

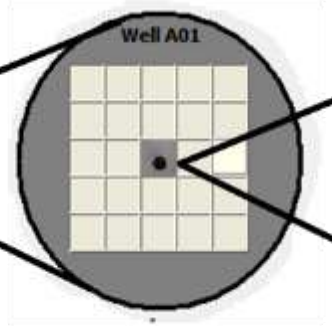
Confocal High Content Imaging (using ArrayScan XTI)



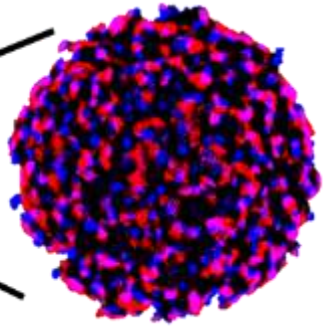
- Confocal imaging to allow us to **analyse more complicated tissue** models using high content screening techniques
- 3D models represent more ***in vivo* relevant** *in vitro* tissue model
- Combined with HCS to determine **sensitive and mechanistic cell health parameters**
- Microtissue in **ULA or transwell** imaging possible



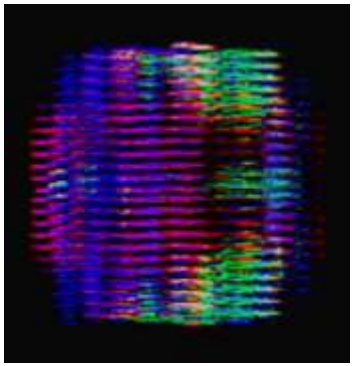
Single MT per well



Single field of view

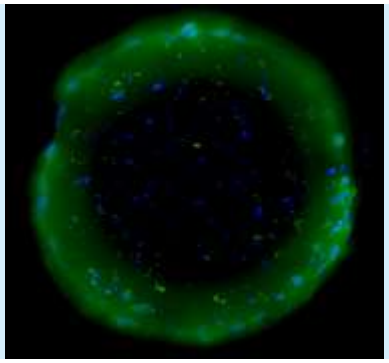
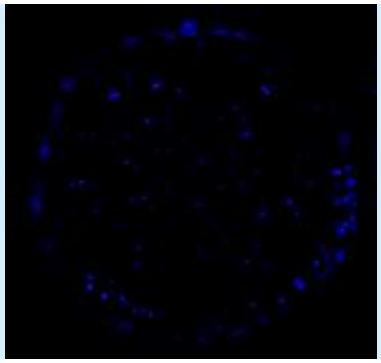
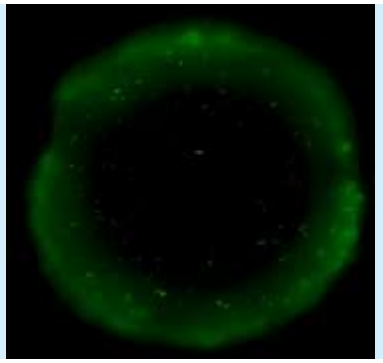
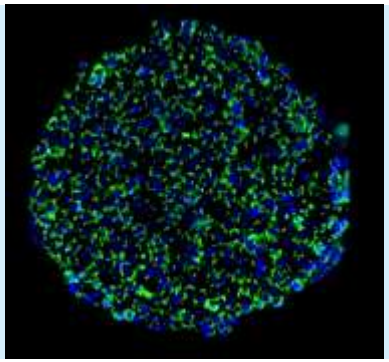
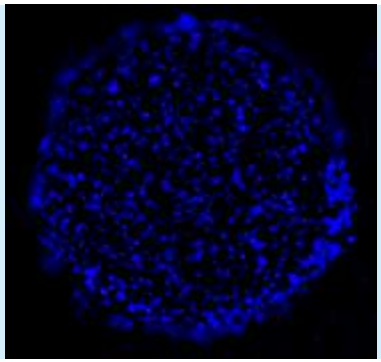
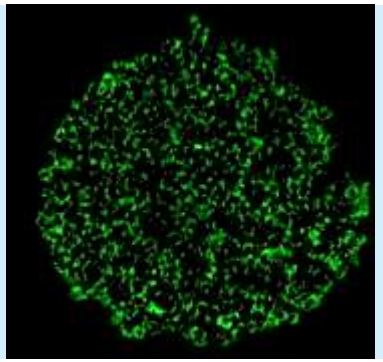


Flexibility of antibodies and dyes



Confocal and Z-Stack imaging

Confocal vs. Widefield Imaging

	Composite	Hoechst	Mitochondria
Widefield			
Confocal			

Maximum projection image capture

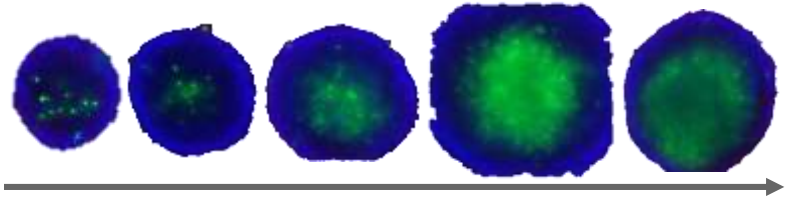
350µm microtissue

Spheroid and Microtissue Development Stages

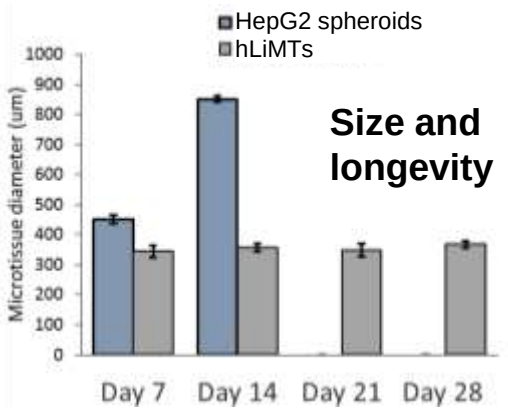
Single cell model = spheroid. Co-culture cell model = microtissue

1. Optimisation of **seeding density** involves HCS imaging of membrane permeability (**PI**) as a marker of necrotic core combined with a measure of cellular **ATP and DNA correlation** as a marker of cell health
2. Co-cultured models re-quire **additional optimisation** of cell to cell ratios
3. Characterisation of longevity and **tissue specific functions**

Spheroid seeding density

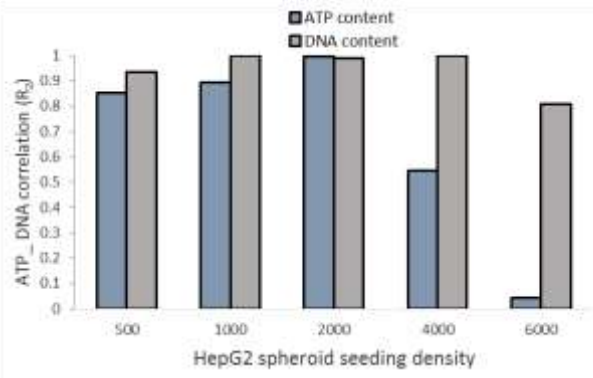


Increasing seeding density

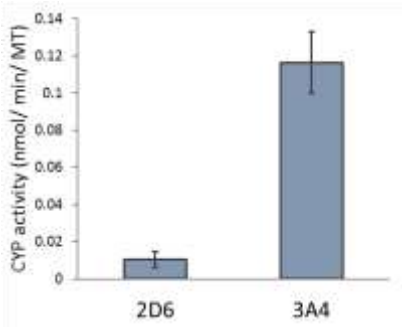


Size and longevity

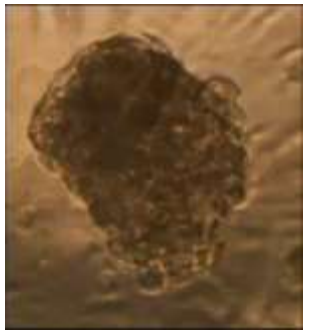
Spheroid ATP content



Tissue specific characteristics



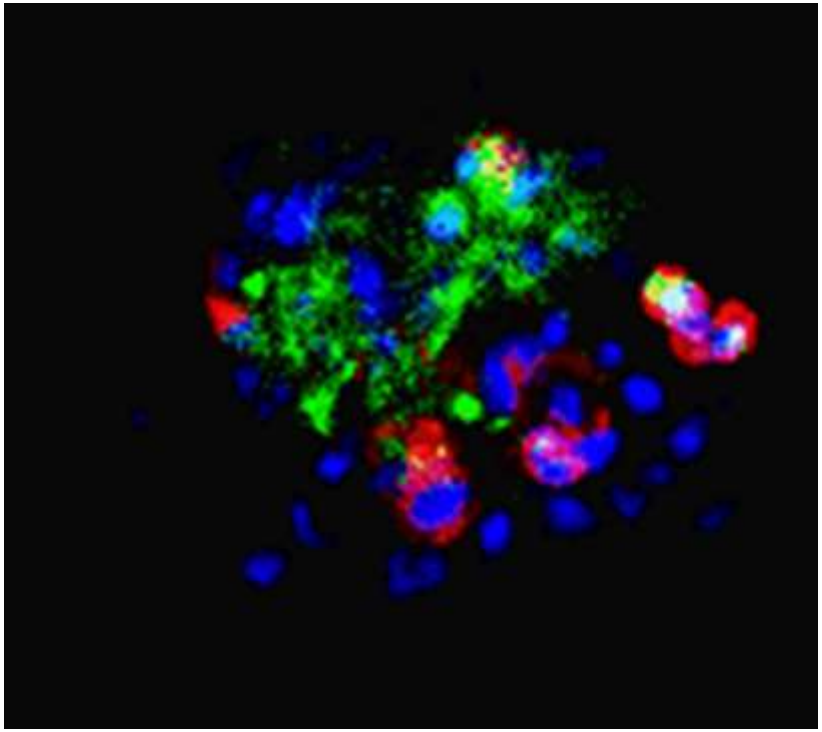
hLIMT's



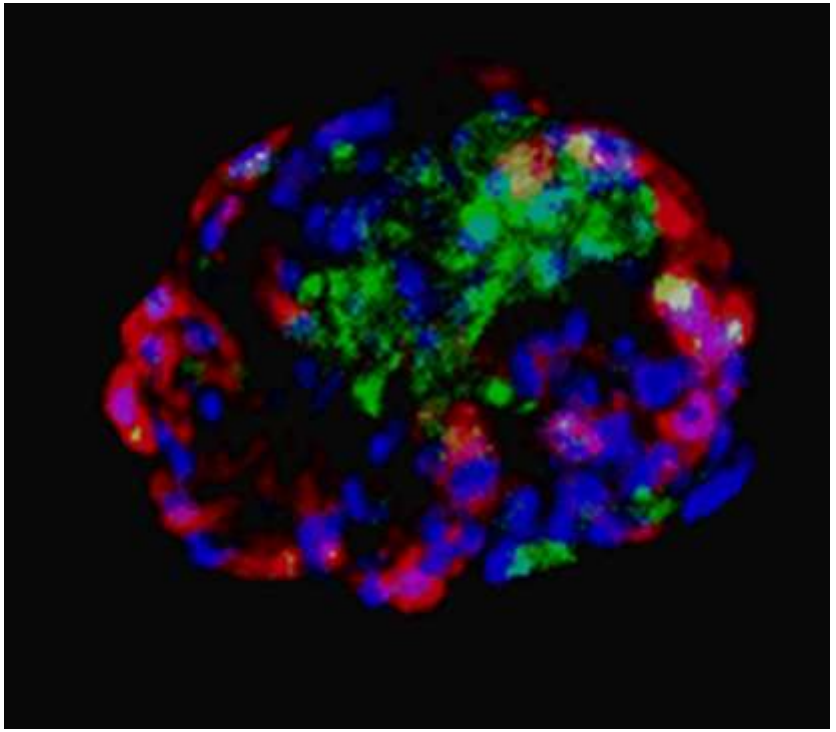
Cardiac microtissue

Confocal 3D Imaging

Z stacks through a microtissue



Z-plane rotation



Development of 3D Capabilities

Focus on tissue specific models and predictive endpoints

Spheroids and Microtissues (MTs)

Platforms	Confocal high content imaging (XTI Thermofisher)
Spheroids/Microtissues	HepG2, H9c2, HepaRG, Cryopreserved human and rat primary hepatocytes, iPSC cardiomyocytes tri culture, Brain (iPSC astrocytes and neurons), Kidney
Non-parenchymal cells (NPCs)	+/- NPCs including Kupfer cells
Assay Endpoints / endpoint combinations	GSH content, ROS formation, phospholipidosis, steatosis, mitochondrial potential, mitochondrial mass, DNA damage, cell stress, calcium homeostasis, CYP activity, ATP content, LDH release
Time points	Live cell imaging, 4, 16, 72 and 14 day repeat dose [1)]

3D Approaches for Drug Induced Liver Injury (DILI)

Genentech and AstraZeneca 2017 Publication

Historical published pharmaceutical DILI strategies (GSK, AZ, Pfizer, Lundbeck, Astellas, Roche) focused on 2D multi-parametric endpoints many including HCS based approaches.

Recent publications have focused on transition to 3D approaches.

Arch Toxicol
DOI 10.1007/s00204-017-2002-1

IN VITRO SYSTEMS

Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury

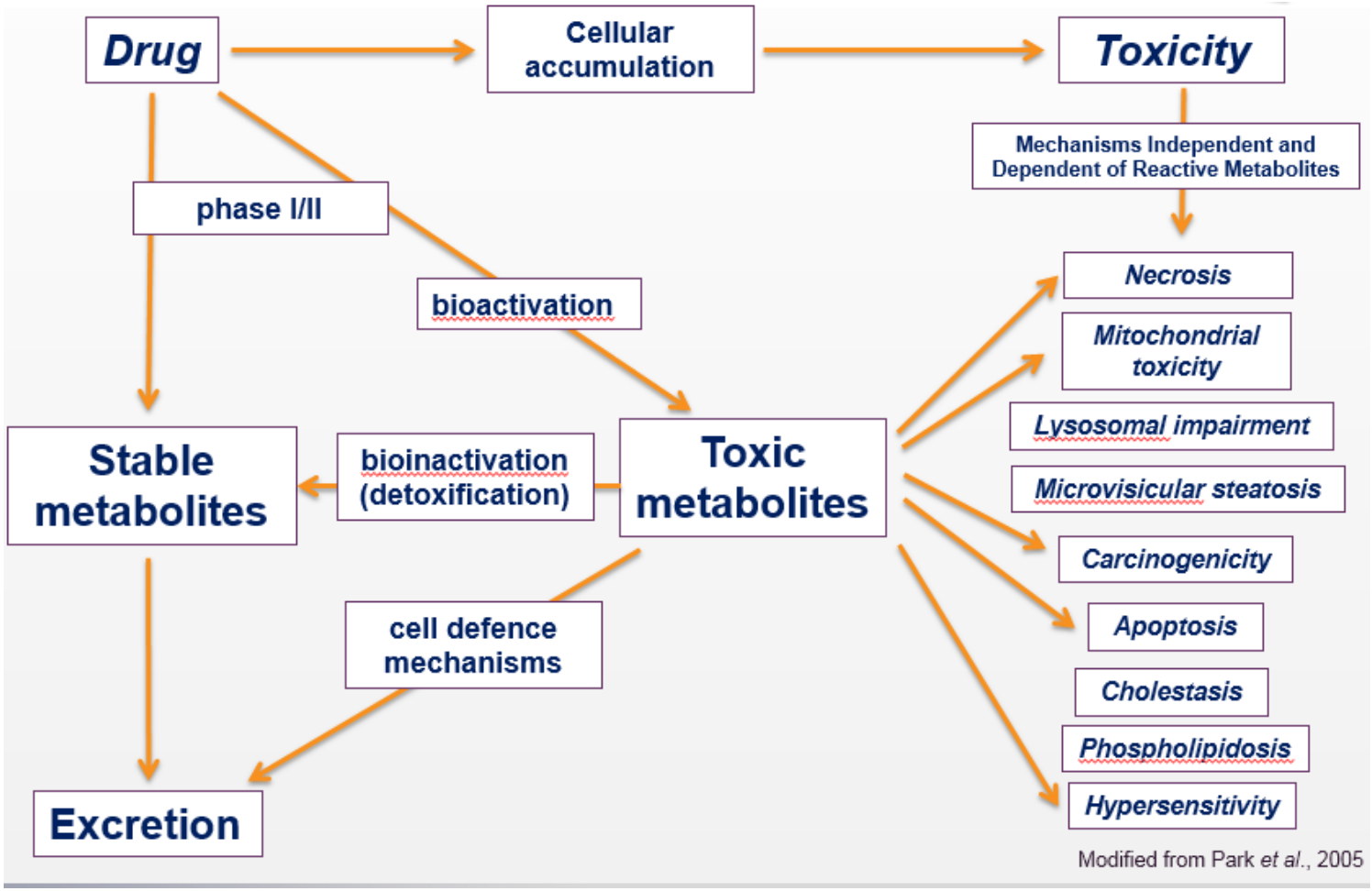
William R. Proctor[1] · Alison J. Foster[2,4] · Jennifer Vogt[1] · Claire Summers[2,4] · Brian Middleton[3,4] · Mark A. Pilling[3,4] · Daniel Shienson[5] · Monika Kijanska[6] · Simon Ströbel[6] · Jens M. Kelm[6] · Paul Morgan[2,4] · Simon Messner[6] · Dominic Williams[2,4]

MIP-DILI

Mechanism-Based Integrated Systems for the Prediction of Drug-Induced Liver Injury

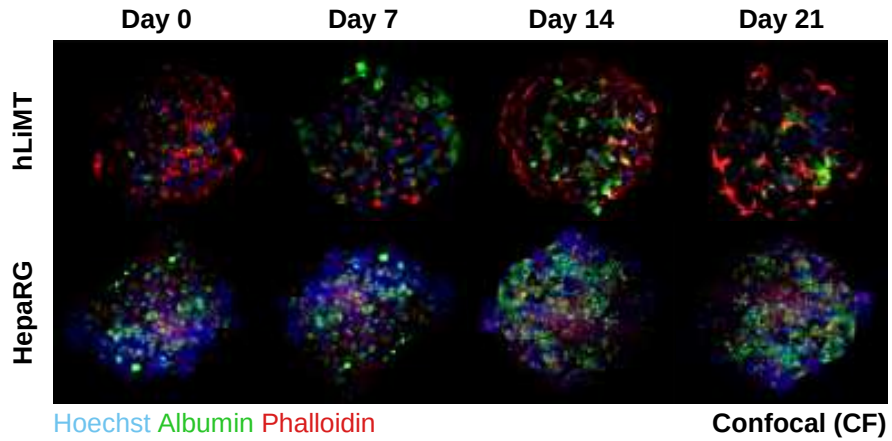
Complexity of Modelling Hepatotoxicity

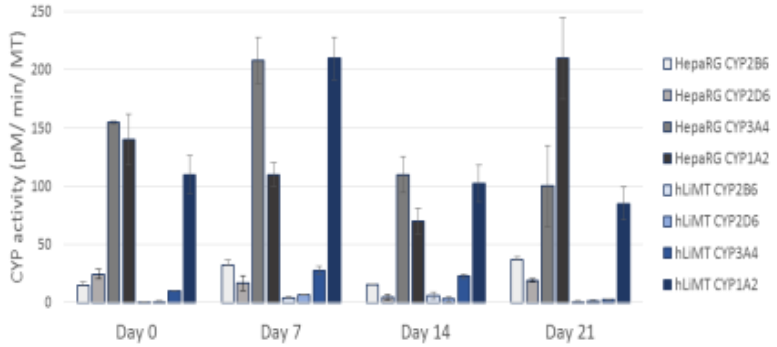
Combination of model and relevant endpoints

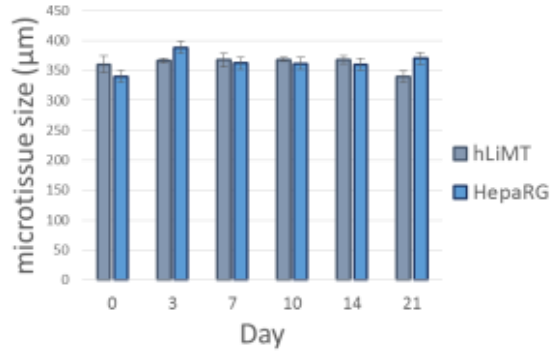


Human Liver Microtissues and HepaRG Spheroid Characterisation

Microtissue size, CYP450 activity and bile caniculi





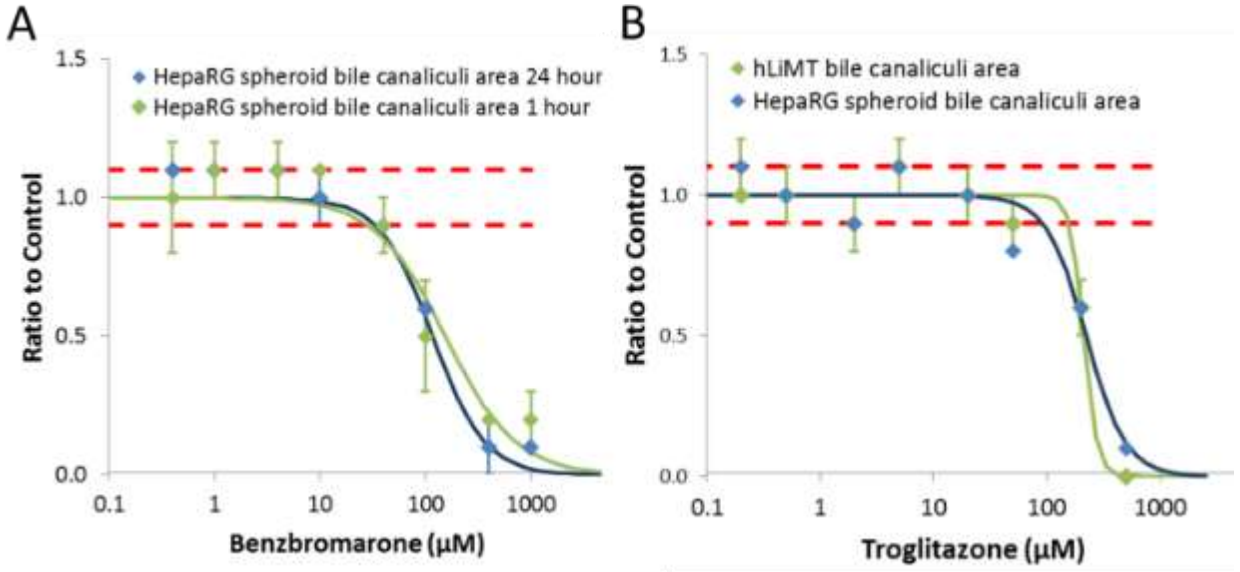


- hLiMTs and HepaRG spheroids display uniform size, shape and improved longevity
- Both models show albumin production, functional bile canaliculi and cytochrome P450 activity

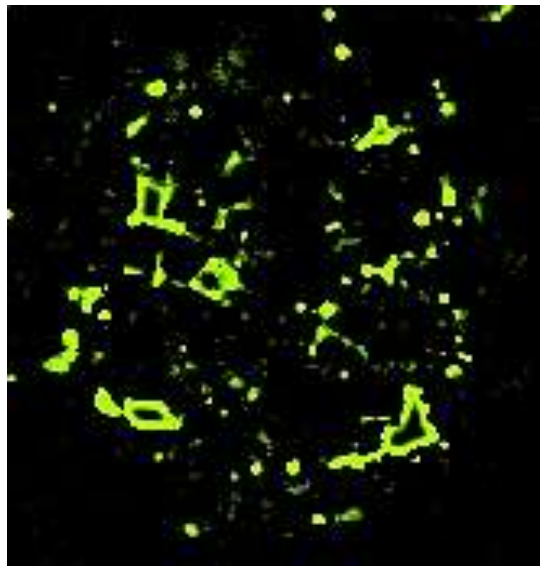
Efflux Transporter Activity in Human Liver Microtissues

Active MRP2 in hepatocytes and HepaRG microtissues

- Human liver microtissues (hLIMT's) establish **bile canaliculi networks with active MRP2 transporters** as highlighted by the efflux of **CMFDA dye** into bile canaliculi
- HCS imaging of CMFDA dye in liver microtissues allows efflux transporter activity to be studied by **quantifying bile canaliculi area**



Confocal (CF)



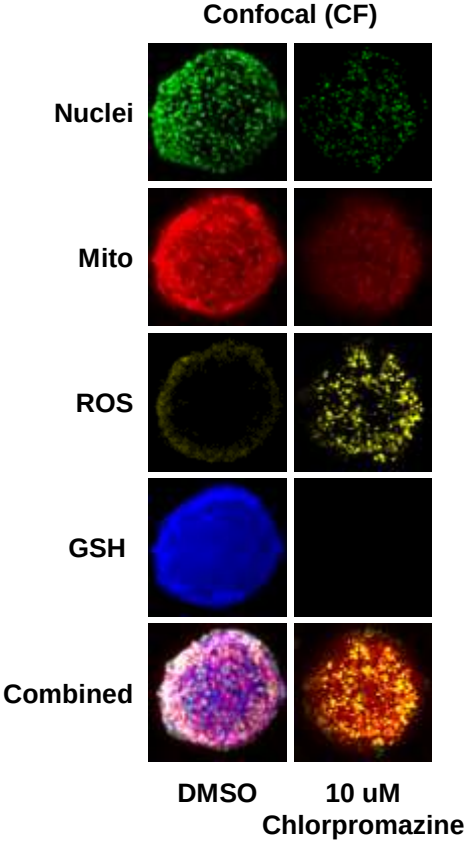
Comparison of HepaRG Spheroids and hLiMTs

Response to hepatotoxicants

In this small set of reference compounds with a 5x C_{max} cut-off HepaRG spheroids are more sensitive to DILI com-pounds than hLiMTs

Drug	C_{max} (µM)	DILI category	Cyprotex hLiMT DILI prediction using MEC (µM)	Cyprotex hLiMT DILI prediction using ATP MEC (µM)	Most Sensitive Feature	Cyprotex 3D HepaRG spheroid DILI prediction using MEC (µM)	Cyprotex 3D HepaRG DILI prediction using ATP MEC (µM)	Most Sensitive Feature
Amiodarone	5.3		0.51	15.4	ROS	2.41	5.12	DNA
Trovafloxacin	19.7		45.2	54.4	GSH	7.27	7.27	ATP
Diclofenac	10.1		50	78.1	DNA	30.5	38.8	DNA
Flutamide	5.4		3.63	8.72	ROS	7.43	7.75	SIZE
Lapatinib	19.2		1.79	12.6	ROS	0.27	1.21	GSH
Nitrofurantoin	21		24.7	51.2	ROS	4.89	9.27	SIZE
Carbamazepine	50.8		49.2	73.6	DNA	81.5	81.5	ATP
Sunitinib	0.25		0.24	0.417	MMP	0.28	1.1	GSH
Troglitazone	6.29		0.99	1.55	MMP	1.69	25	DNA
Fialuridine	1		11.5	11.5	ATP	1.41	1.41	ATP
Nefazodone	4.3		13.7	13.7	ATP	11.6	11.6	DNA
Perhexiline	2.16		1.03	1.49	ROS	1.69	1.76	DNA
Tolcapone	21.96		21.9	21.9	ATP	18.2	20.5	MMP
Acetaminophen	165.4		302	302	ATP	240	342	SIZE
Bosentan	4.7		12.3	29.4	DNA	16.4	35.2	DNA
Ticlopidine	8.1		17.5	27.2	DNA	34	36.2	MitoMass
Azathioprine	2.22		2.48	2.48	ATP	0.28	0.278	ATP
Chlorpromazine	0.94		0.34	0.347	ROS	1.07	3.48	SIZE
Tamoxifen	1.18		1.54	1.98	ROS	3.52	10.8	SIZE
Buspirone	0.01	N	3.12	3.12	ATP	NR	-	-
Entacapone	3.276	N	40.2	40.2	ATP	45.4	45.5	GSH

≤ 1x Cmax
≤ 5x Cmax
>5x Cmax

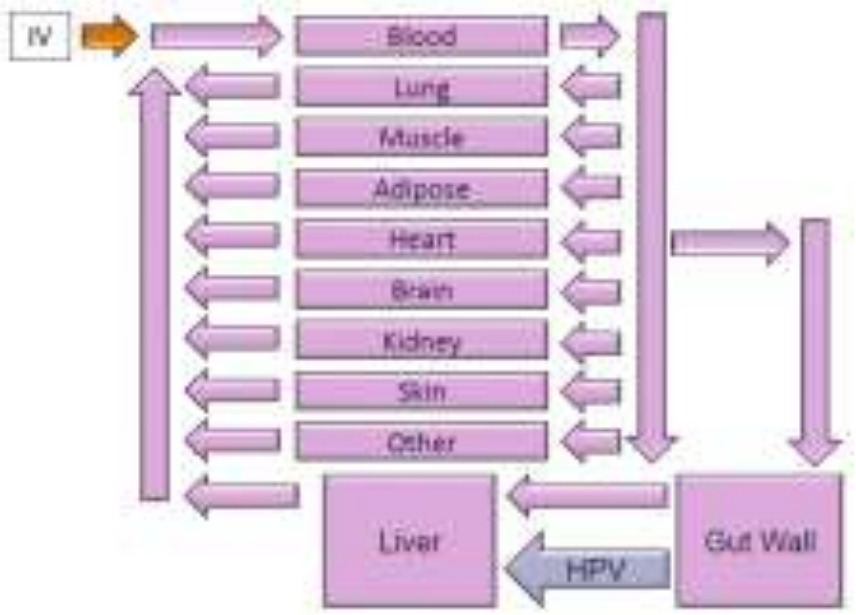


HepaRG MTs Normalised to Human Liver Concentrations (Tissue C_{max})

Dose normalisation of toxicity

- PBPK model to predict tissue C_{max} from total plasma C_{max}
- Improves IVIVE of hits within 1x C_{max}

Compartmental PBPK Model



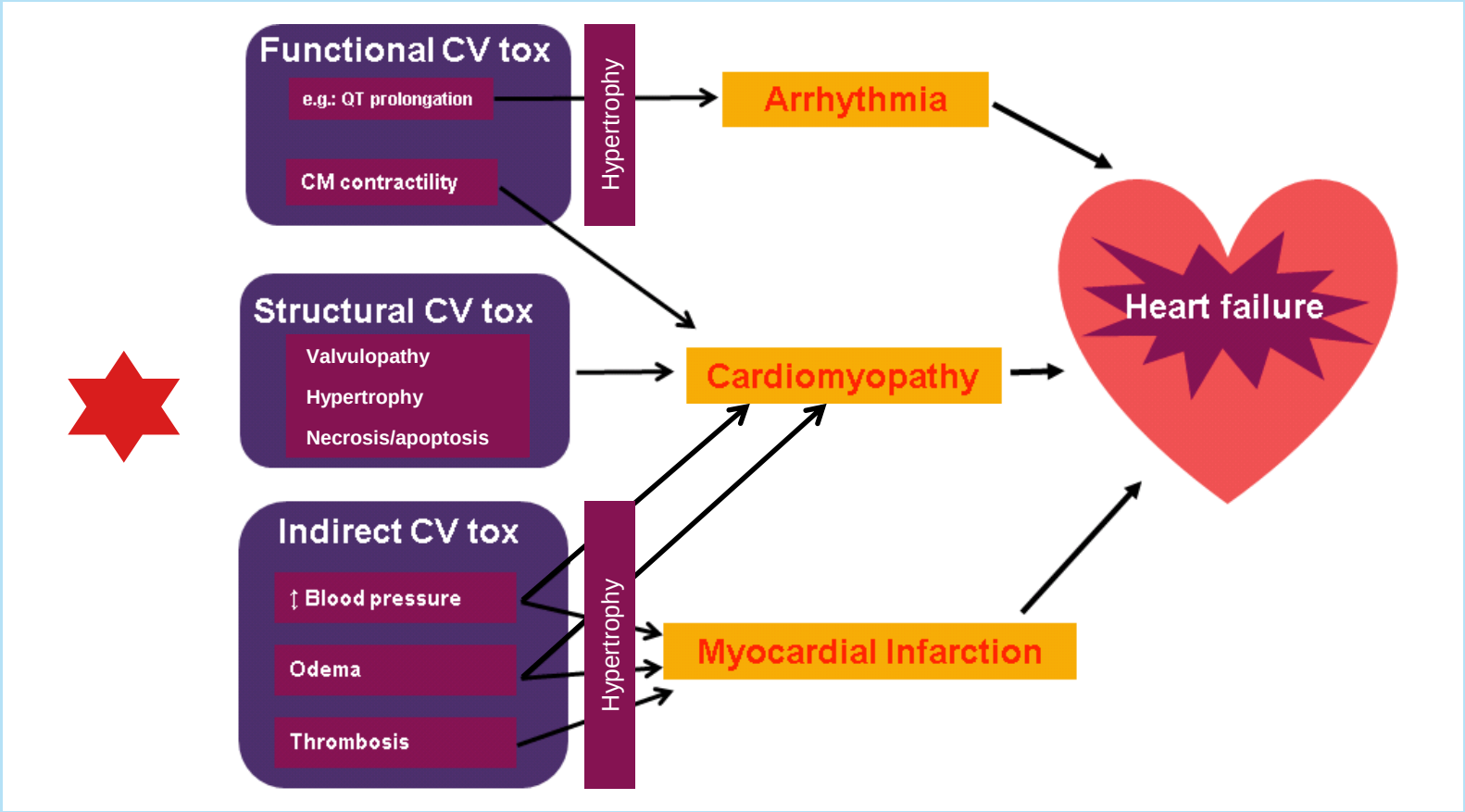
Drug	DILI category	Plasma C_{max} (µM)	Liver_kP C_{max} (µM)	Lowest MEC (µM)	
				Plasma C_{max} normalisation	Liver_kP C_{max} normalisation
Amiodarone	P	5.3	49.5	2.41	2.41
Trovafloxacin	P	19.7	28.3	7.27	7.27
Diclofenac	P	10.1	2.7	30.5	30.5
Flutamide	P	5.4	9.7	7.43	7.43
Lapatinib	P	19.2	152.8	0.77	0.77
Nitrofurantoin	P	21	13.0	4.89	4.89
Carbamazepine	P	50.8	41.3	81.5	81.5
Sunitinib	P	0.25	1.7	0.28	0.28
Troglitazone	P	6.29	37.5	1.69	1.69
Fialuridine	P	1	0.9	1.41	1.41
Nefazodone	P	4.3	22.4	11.6	11.6
Perhexiline	P	2.16	53.6	1.69	1.69
Tolcapone	P	21.96	16.8	18.2	18.2
Acetaminophen	P	165.4	360.8	240	240
Bosentan	P	4.7	4.3	10.4	10.4
Ticlopidine	P	8.1	38.4	34	34
Azathioprine	P	2.22	3.0	0.28	0.28
Chlorpromazine	P	0.94	19.3	1.07	1.07
Erythromycin	P	11	78.0	125	125
Tamoxifen	P	1.18	20.8	3.52	3.52
Buspirone	N	0.01	0.1	NR	NR
Entacapone	N	3.276	4.1	45.4	45.4
Donepezil	N	0.028	0.4	6.49	6.49
Clotrimazole	N	0.06	0.2	5.13	5.13
Betaine	N	940	539.0	NR	NR
Metformin	N	7.74	8.0	NR	NR
			Within 1x C_{max} =	10	14

≤ 1x Cmax
≤ 5x Cmax
>10x Cmax

Drug-induced Cardiovascular Toxicity

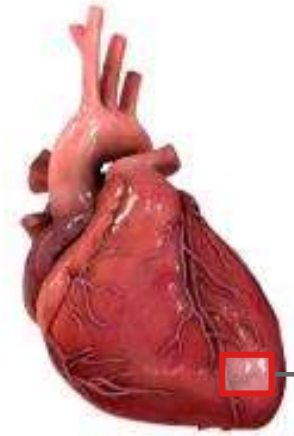
Focus on structural CV toxicity

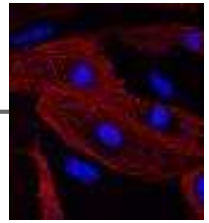
Cardiovascular toxicity can arise from **direct or indirect effects** upon the heart resulting in **structural and/or functional changes** and ultimately reduced cardiac efficiency



Role of non-cardiomyocytes in Cardiotoxicity

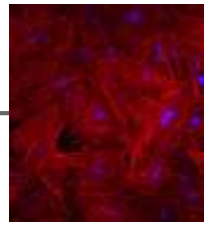
Importance of co-culture cardiac in vitro models





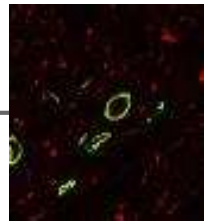
Cardiomyocytes

Provide contractility and rhythmicity



Fibroblasts

Provide extracellular matrix (ECM) and cardiac remodelling



Endothelial cells

Provide vascular system to deliver O_2 and free fatty acids

Targets of cardiovascular toxins

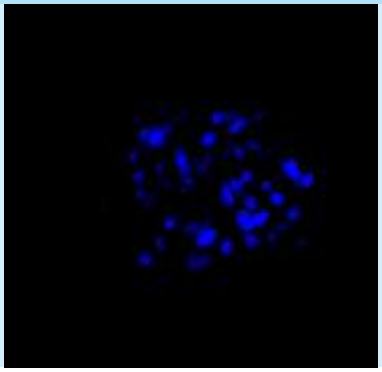
- Current cardiac drug safety tests **primarily focus on cardiomyocytes** in isolation, ignoring other cellular components of the myocardium
- The myocardial tissue comprises 30% cardiomyocytes and **70% non-myocytes**, the majority of which are **endothelial and fibroblast cells**
- These **non-myocytes are essential** to myocardial structure and function with emerging evidence suggesting **important roles within drug induced cardiovascular toxicity**

Brutsaert DL (2003). *Phys Revs* **83**; 59-115.
Souders CA *et al.*, (2009). *Circ Res* **105**; 1164-1176.
Mikaelian I *et al.*, (2010). *Tox Sci* **117(1)**; 144-151

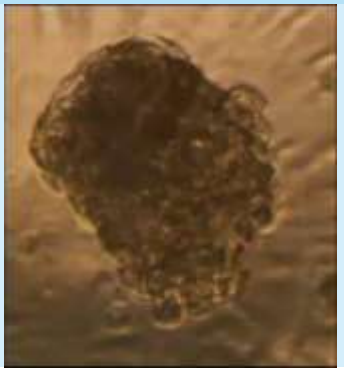
Spontaneously Beating Cardiac Spheroids

Co-culture of cardiomyocytes, endothelial cells and fibroblasts

Hoechst Nuclear Staining



Brightfield



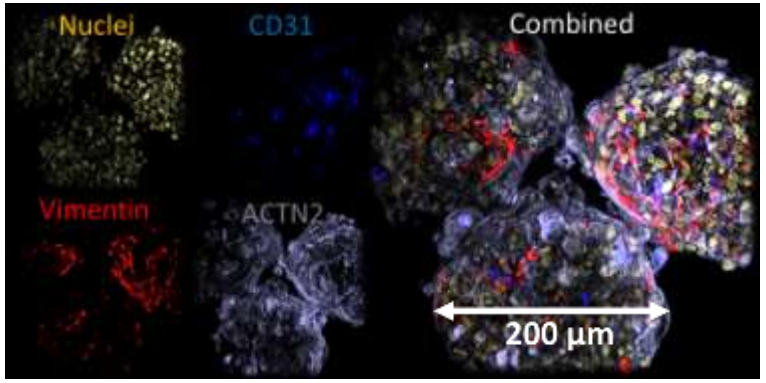
- Cardiac spheroids are a 3D monoculture formed from iPSC derived cardiomyocytes
- Spontaneous contractions are observed following 5 days in culture
- Functionality is maintained for at least 28 days in culture
- Amenable to repeat dosing (chronic) or acute exposures

CD31; endothelial marker

Vimentin; fibroblast marker

ACTN2 (sarcomeric α actinin); cardiomyocyte marker

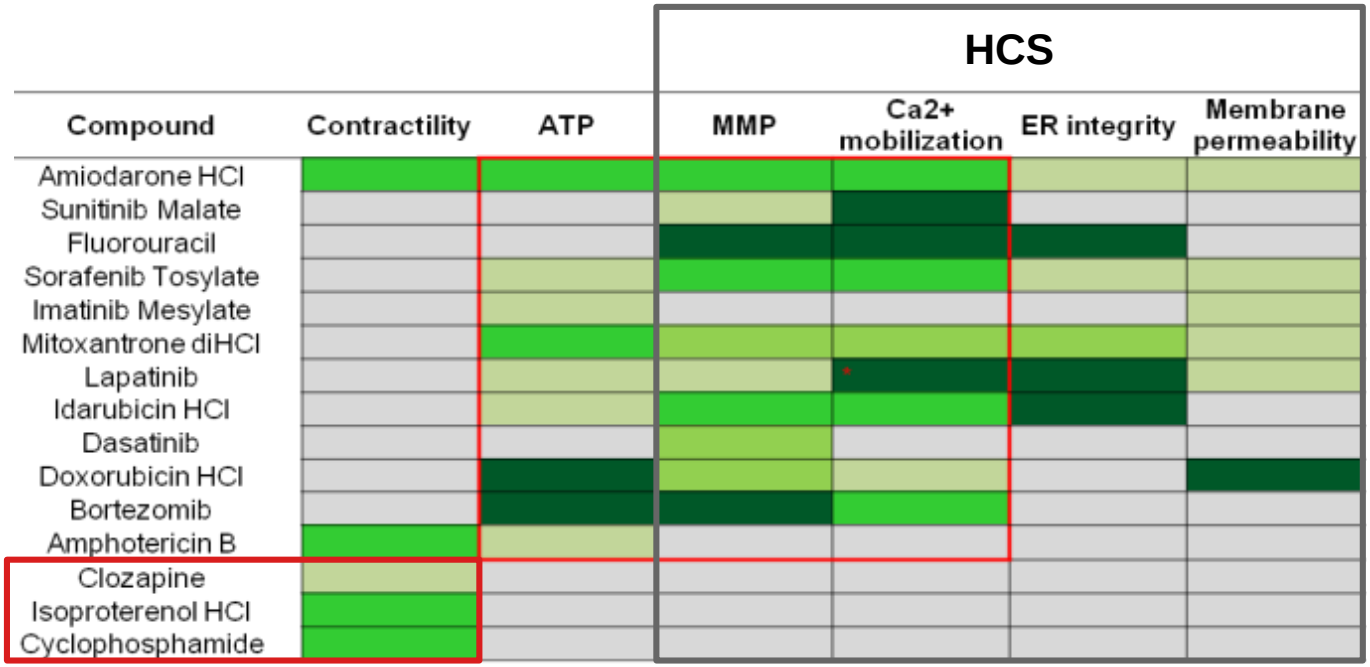
- Cardiac tri-cultured MT's display uniform size and shape with a diameter of 200 µm
- Whole mount immunofluorescence displays even distribution of key cell markers throughout MT's



HCS – Structural Cardiac Toxicity

Historical structural CV toxicity dataset

- Structural cardiotoxicants were analysed using hESC cardiomyocytes (Pointon *et al*., 2013)
- HCS parameters combined with a measure of ATP content improves structural cardiotoxicity assay sensitivity
- Identified mitochondrial disruption and calcium mobilization as major targets for structural cardiotoxins
- Clozapine, isoproterenol and cyclophosphamide only picked up by IonOptix (contractility)



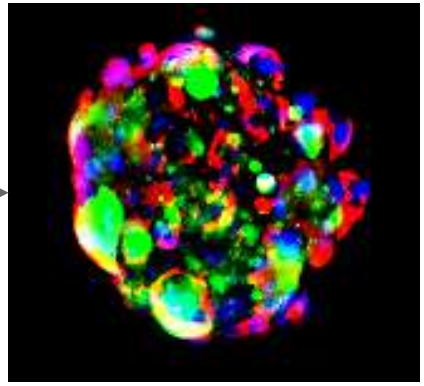
Pointon *et al.*, (2013). *Tox Sci*. **132(2)**: 317-326

Modified 3D Structural Cardiotoxicity Assay

Plate cells in 96/ 384 well plate

Incubate at 37°C until microtissue or monolayer formation

Compound treat for desired time period



Fluorescently label cells (TMRE, Fluo-4 AM, Hoechst)

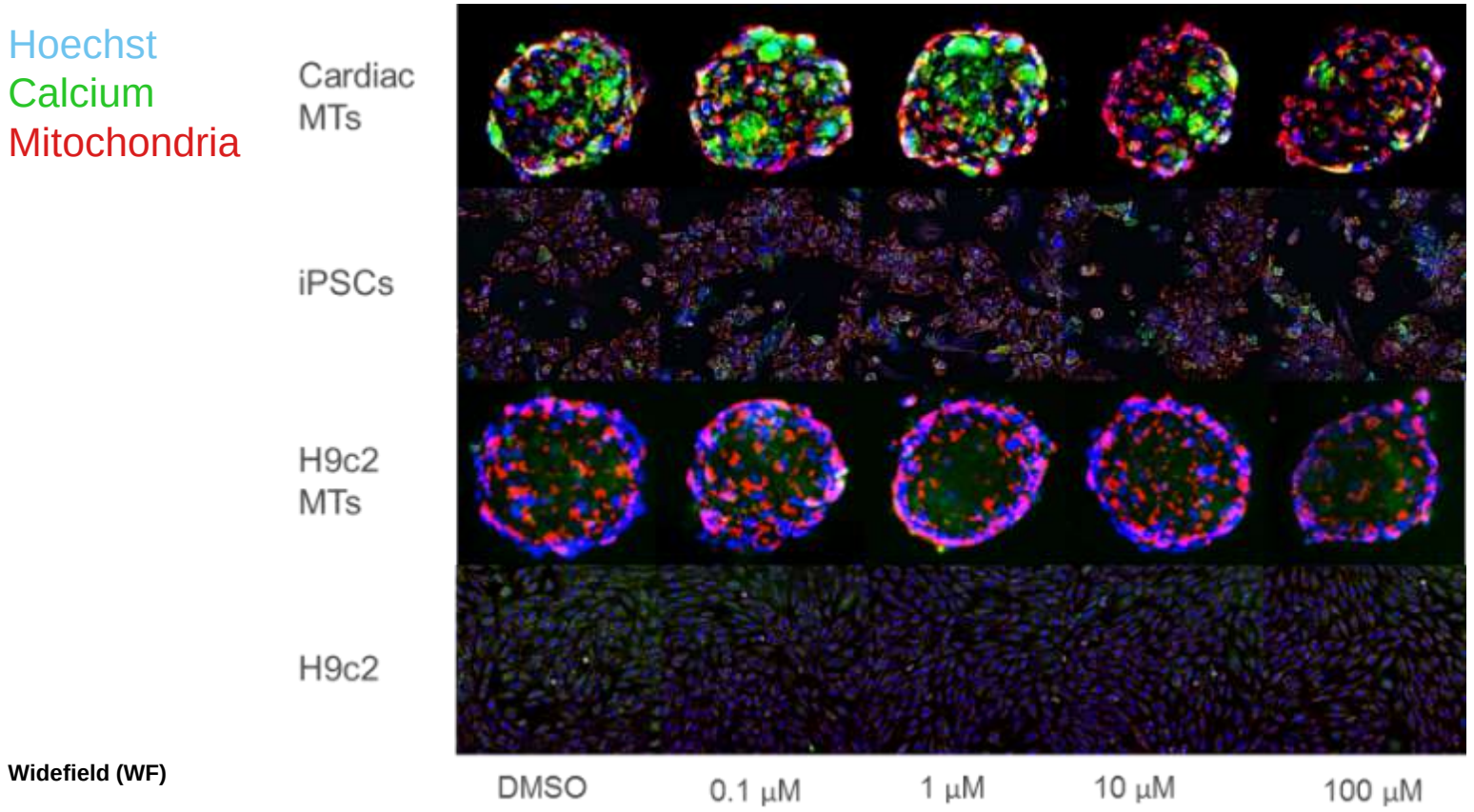
3D confocal live cell (37°C, 5% CO_2) imaging using ArrayScan XTI (ThermoScientific)

Promega CellTiter-Glo® ATP assay

- Modified structural cardiotoxicant assays (Pointon *et al*., 2013) to analyse cardiac microtissues

Isoproterenol Calcium Dyshomeostasis – in Cardiac Microtissues (MTs)

- Isoproterenol as previously shown undetected by structural cardiotoxicant assays (Pointon *et al*., 2013)
- Calcium dyshomeostasis shown in cardiac MTs



Cardiac Cell Model Comparison (72h Exposure)

Comparison on different cell models (2D/3D)

- iPSC derived cardiac MTs are more predictive than either 2D or H9c2 spheroids
- Cyclophosphamide and isoproterenol only detected in iPSC derived cardiac MTs using HCS

Compound	C_{max}	*In vivo* toxicity	*In vivo* cardiac	Reference	H9c2 monolayer	H9c2 MTs	iPSC-CM's monolayer	Cardiac Spheroids	Cardiac MTs	Feature
					MEC µM					
Cyclophosphamide	153.20	Structural cardiotoxin	Acute cardiac toxicity, CHF, myocarditis, myocardial necrosis	Floyd *et al* . 2005	NR	NR	NR	3710	30.8	Mito Mass
Dasatinib	0.72		QT prolongation, CHF, LVD and MI, cardiomyopathy, arrhythmia, cardiomegaly	Force *et al* . 2007	0.04	0.0771	7.32	6.58	0.208	Mito Mass
Doxorubicin HCl	15.34		CHF, decreased LVEF, sinus tachycardia, myocarditis, cardiomyopathy	Minotti *et al* . 2004	0.04	0.115	0.04	<0.04	0.04	ATP
Fluorouracil	4.61		HF, MI, ventricular dysfunction, cardiac fibrillation, arrhythmia	Schimmel *et al* . 2004	1.88	NR	1.4	77.4	0.0407	Ca^{2+}
Idarubicin HCl	0.12		CHF, arrhythmia, cardiomyopathy, decreased LVEF	Anderlini *et al* . 1995	<0.04	<0.04	0.04	<0.04	<0.04	ATP
Imatinib Mesylate	3.54		CHF, decreased LVEF	Kerkela *et al* . 2006	13.7	3.53	29.3	0.558	22.6	ATP
Isoproterenol HCl	0.01		Tachycardia, palpitations, ventricular arrhythmias, myocarditis	Zhang *et al* . 2008	NR	NR	NR	NR	2.1	Ca^{2+}
Lapatinib	4.18		Decreased LVEF and HF, QT interval prolongation	Force *et al* . 2007	4.57	8.33	0.04	8.95	5.9	ATP
Sunitinib Malate	0.25		Decreased LVEF and HF, QT interval prolongation and TdP, cardiomyopathy	Chu *et al* . 2007	0.896	0.114	0.04	1.2	0.817	Ca^{2+}
Acyclovir	6.66	Non-structural cardiotoxins	No report	-	NR	NR	NR	NR	NR	-
Buspirone HCl	0.03		Nonspecific chest pain	-	NR	0.237	NR	NR	NR	MT size

Positive response
Negative response

Cardiac Cell Model Comparison (72h Exposure)

Comparison to Cmax values

- iPSC derived cardiac MTs are more predictive than either 2D or H9c2 spheroids
- Cyclophosphamide and isoproterenol only detected in iPSC derived cardiac MTs using HCS

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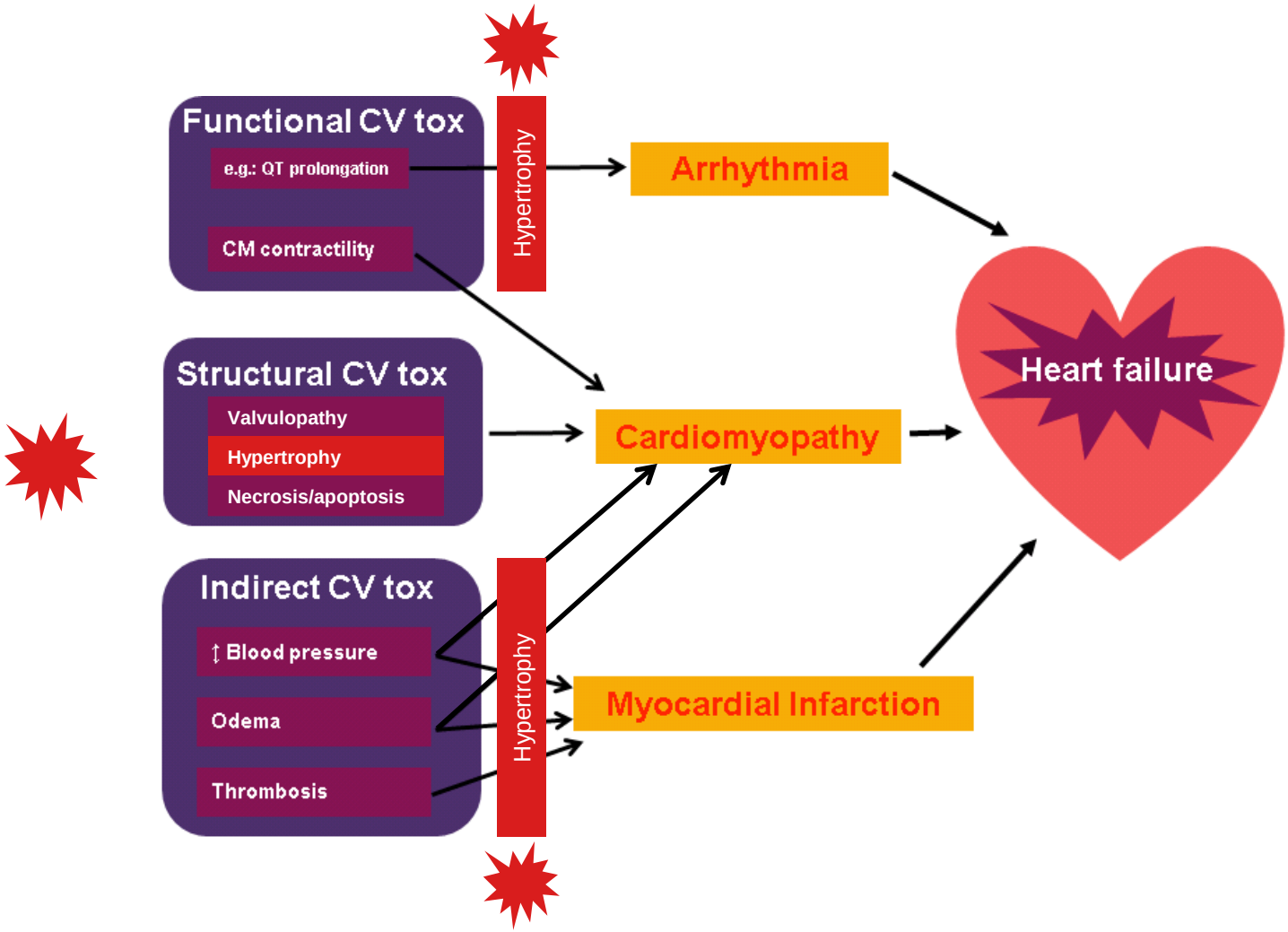
Acyclovir	6.66	Non-structural cardiotoxins	No report	-	NR	NR	NR	NR	NR	-
Buspirone HCl	0.03		Nonspecific chest pain	-	NR	0.237	NR	NR	NR	MT size

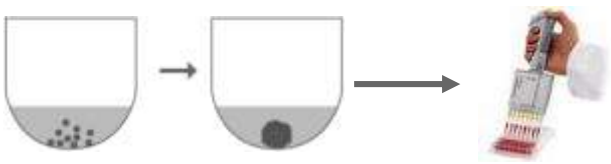
5-10x Cmax
<5x Cmax
Negative response

Drug-induced Cardiomyocyte Hypertrophy

Key even in cardiotoxicity



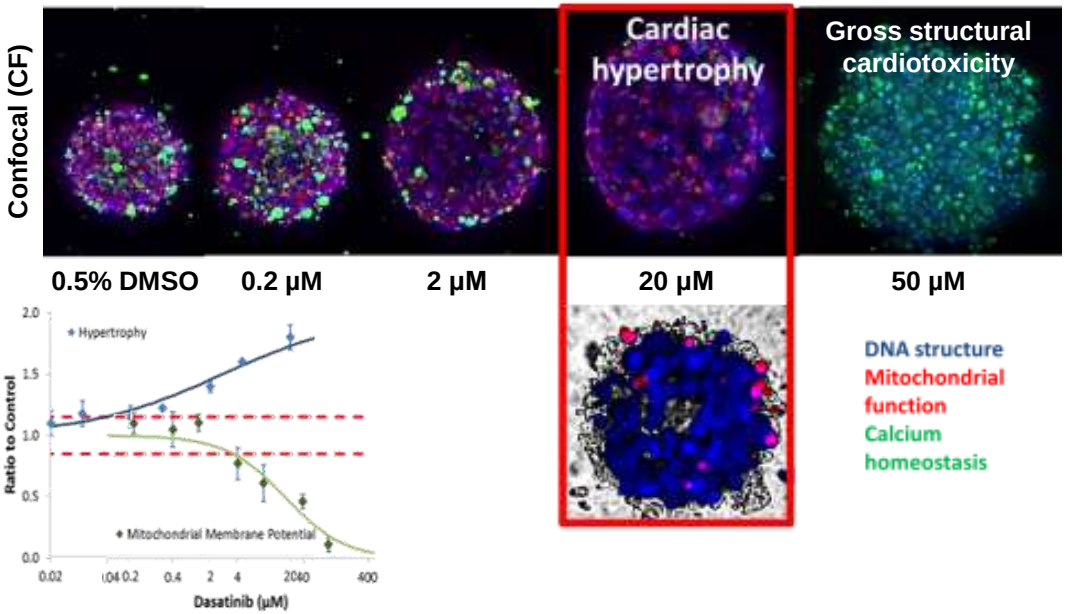
Detecting Cardiomyocyte Hypertrophy in Spheroids

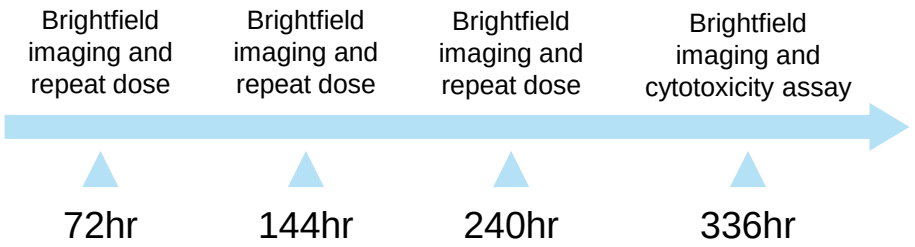


Day 5 post seeding (0hr)

336hr

Dasatinib





- Pathophysiological **hypertrophy ultimately leads to cell death**
- Brightfield **time course** imaging of MT area with repeat dose chronic compound exposure allows the improved detection of pathophysiological hypertrophy
- An **exposure period of 336hrs** displays increased microtissue area followed by a decrease in MT area and increased cell membrane permeability
- Increase in spheroid size is not observed in **cardiac microtissues**

Predicting Pathophysiological Hypertrophy in Cardiac Spheroids (336h Exposure)

3D cardiomyocyte spheroid models allow for the improved *in vitro* prediction of structural cardiotoxins with patho-physiological hyper-trophic potential

Drug	Human exposure (C_{max}; µM)	*In vivo* structural toxicity (P/N)	*In vivo* patho-physiological hypertrophy (P/N)	Most sensitive structural MEC (µM)	Most sensitive hypertrophy MEC (µM)	Lowest combined assay MEC (µM)	Most sensitive structural mechanism
sunitinib	0.25	P	P	0.38	0.16	0.16	Calcium
dasatinib	0.72	P	P	0.15	0.02	0.02	ATP
imatinib	3.54	P	P	0.04	0.05	0.04	ATP
doxorubicin	15.34	P	P	0.01	1.46	0.01	ATP
norepinephrine	0.17	P	P	0.10	0.06	0.06	ATP
amphotericin B	9.00	P	P	7.85	0.25	0.25	DNA
lapatinib	4.18	P	P	0.19	37.40	0.19	ATP
clozapine	2.40	P	P	32.40	6.67	6.67	DNA
isoproterenol	0.01	P	P	0.10	26.30	0.10	ATP
cyclophosphamide	153.20	P	P	381.00	NR	381.00	ATP
amiodarone	5.30	P	N	7.76	3.51	3.51	MMP
mitomycin C	3.12	P	N	0.21	NR	0.21	ATP
idarubicin	0.12	P	N	0.004	1.45	0.004	ATP
fluorouracil	4.61	P	N	10.30	NR	10.30	ATP
acyclovir	6.66	N	N	NR	NR	NR	-
buspirone	0.03	N	N	NR	NR	NR	-

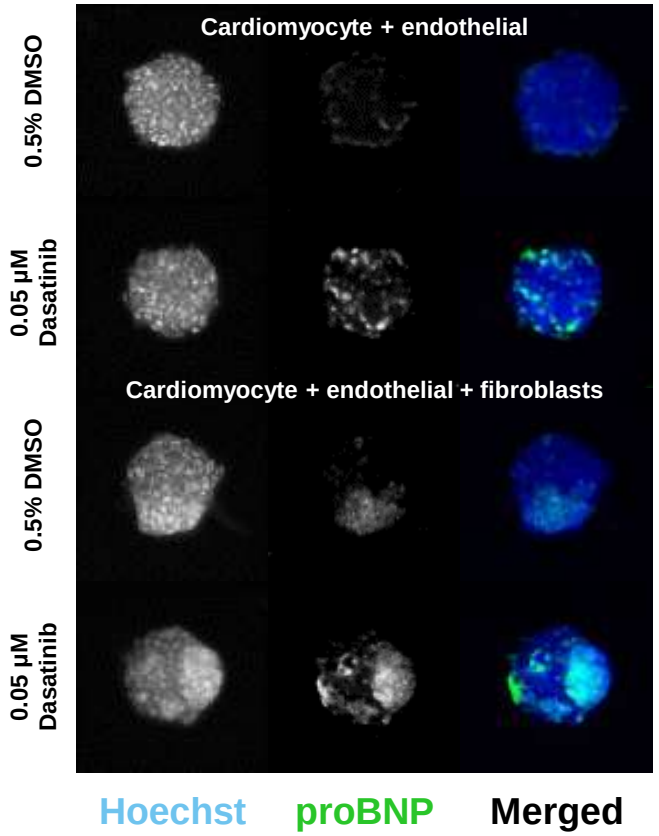
≤ 1x C_{max}
≤ 3x C_{max}
≤ 10x C_{max}
≥ 10x C_{max}

Effect of non-myocytes on Drug-induced Cardiomyocyte Hypertrophy

Importance of model characterisation

Microtissue model cell composition	Hypertrophy responses (Size increase MEC; µM)			
	Pathophysiological hypertrophins			Cytotoxin
	Dasatinib	Clozapine	Sunitinib	Mitomycin C
Cardiomyocytes	0.02	6.67	0.16	NR
Cardiomyocytes + endothelial cells	0.168	5.44	NR	NR
Cardiomyocytes + fibroblasts	NR	NR	NR	NR
Cardiomyocytes + endothelials + fibroblasts	NR	NR	NR	NR

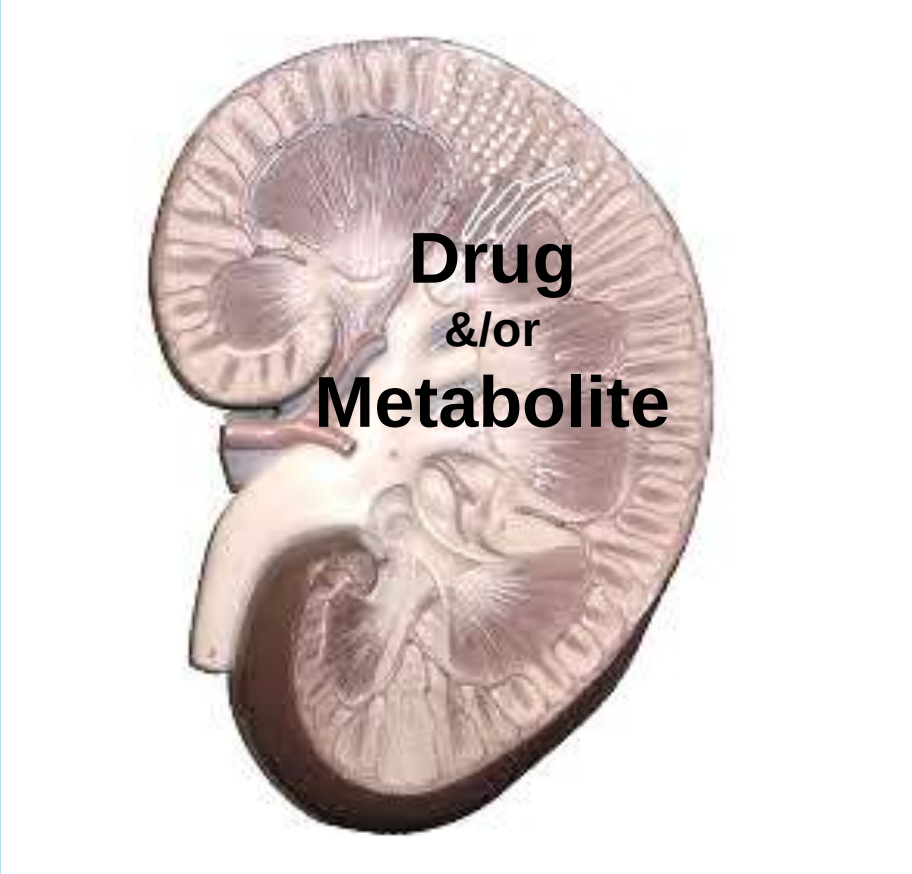
- Only the cardiomyocyte model exhibits hypertrophy phenotypic response
- Cardiomyocyte + endothelial model detects dasatinib (however at a 10x higher MEC) and clozapine, but not sunitinib
- Once cardiac fibroblasts are introduced no hypertrophic responses are detected, using MT size parameters
- Interestingly the hypertrophy marker anti-proBNP shows increased staining following compound exposure



Drug-induced Nephrotoxicity

Research Model

- Drug-induced toxicity is the cause of approximately 20% of renal failure hospital admissions
- The kidney comprises a highly structured filtration net-work which is difficult to replicate *in vitro*
- *In vitro* nephrotoxicity assays are currently limited in their *in vivo* predictivity

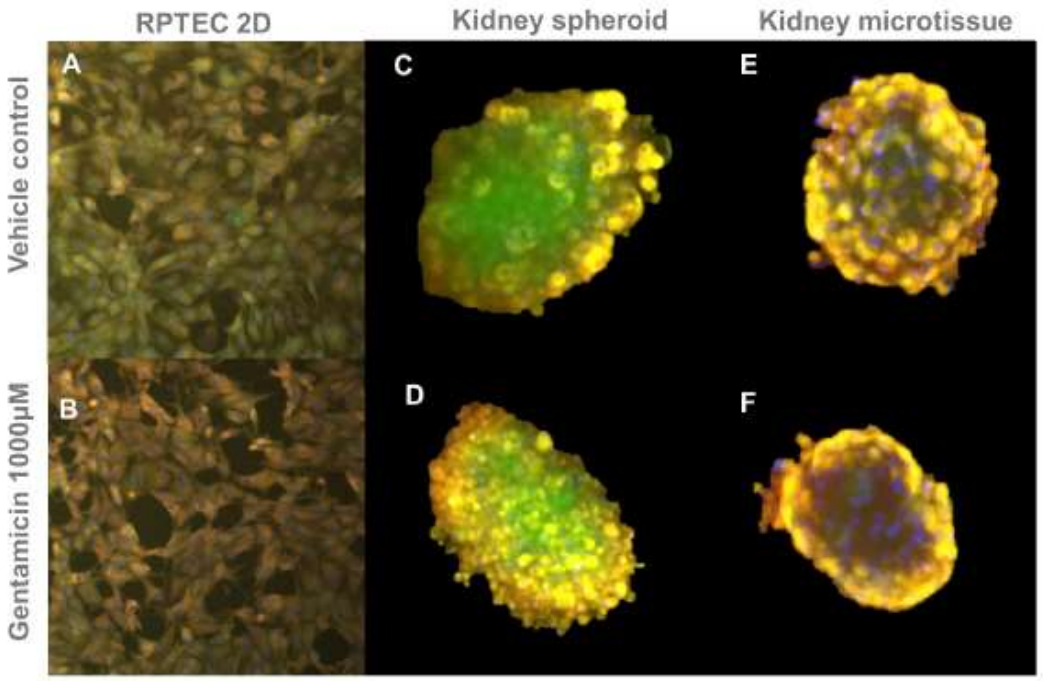


Development of *in vitro* kidney models

Comparison 2D and 3D cell culture models

- Renal Models Developed:
- 2D culture of renal proximal tubule epithelial cells (RPTEC)
- 3D culture of RPTEC in spheroids
- 3D co-culture of renal microtissues consisting of RPTEC, renal cortical epithelial cells and renal fibroblasts

- Exposure to nephrotoxins (7 positive + 3 negative compounds) for 72hrs, 216hrs and 336hrs
- Assessed for changes in DNA structure, ATP content, mitochondrial function and GSH content



Development of *in vitro* kidney models

Response to nephrotoxins of 2D and 3D cellular models

- The longevity of *in vitro* human kidney MTs permits the study of chronic compound exposure
- Extended incubation times and repeat dosing improved accuracy of prediction of nephrotoxicity
- *In vivo* nephrotoxicity can be predicted with an accuracy of 100% and 90% in 2D and 3D cell models respectively
- Increases in phospholipids and changes in ATP and glutathione content are common observations

	Cmax [µM]*	RPTEC 72hr		RPTEC 216hr		MT 72hr		MT 216hr	
(S)-(+)camptothecin	0.083	0.102	PLD	0.18	GSH	0.263	MM	0.00746	MMP
acetaminophen	165.4	NR		1830	ATP	NR		1880	ATP
cisplatin	2	0.513	GSH	0.479	GSH	7.51	DNA	62.2	ATP
cyclosporin A	11	0.665	PLD	0.798	PLD	7.09	MMP	0.379	MMP
diclofenac	10.1	32.5	PLD	136	ATP	96.5	ATP	24.7	ATP
gentamycin	13	249	PLD	189	PLD	971	ATP	255	ATP
tobramycin	16	1020	PLD	128	PLD	13.9	MMP	768	DNA
buspirone	0.009	NR		NR		NR		NR	
piroxicam	12.79	NR		NR		NR		NR	
tacrine	0.077	19	PLD	25.7	GSH	NR		1.19	MM

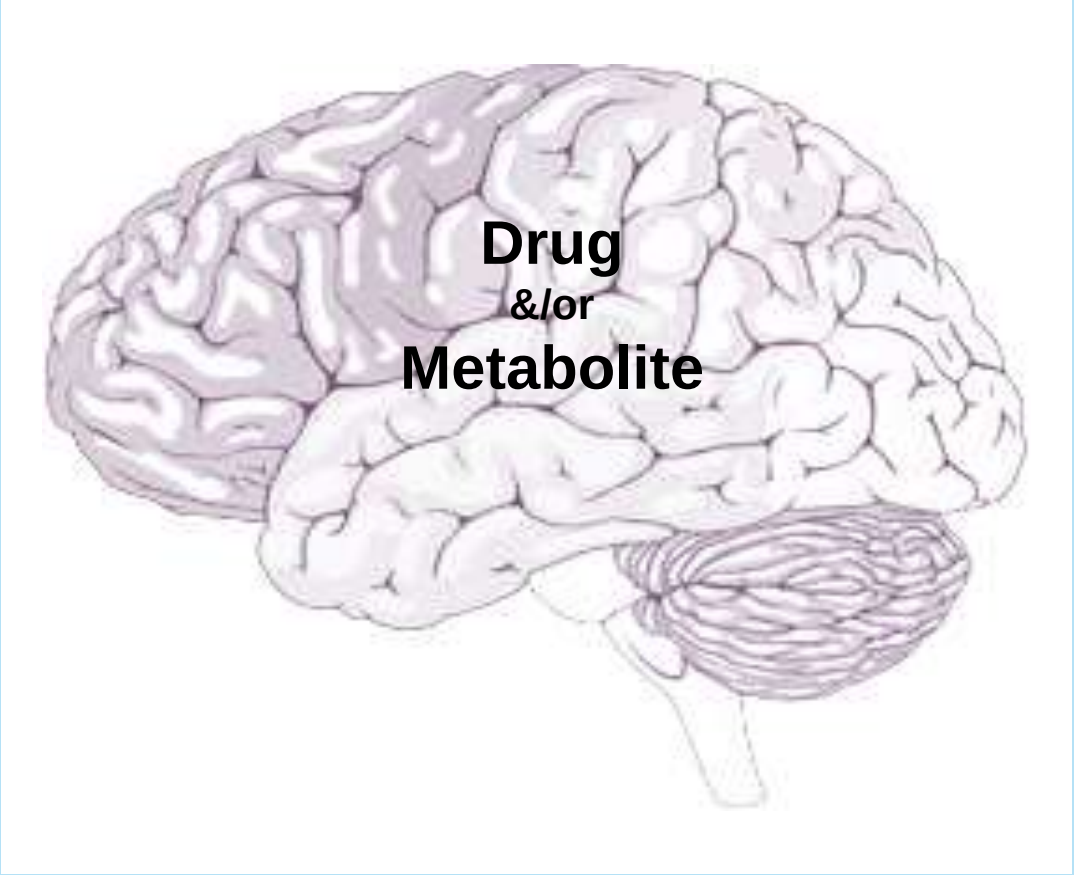
<1x Cmax
<10 Cmax
<30x Cmax
30-50
>50

MEC = Minimal effective concentration

Drug-induced Brain Neurotoxicity

Research Model

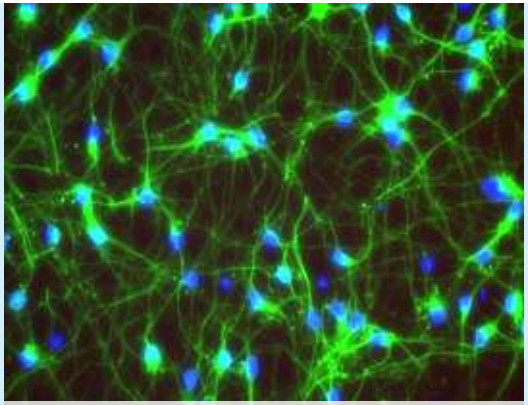
- Current pre-clinical *in vitro* neurotoxicity models often focus on neurons alone, in a restrictive 2D environment with acute compound exposures and display very little *in vivo* toxicity correlation
- *In vitro* neurotoxicity models are currently are reliably predictive of *in vivo* CNS responses



iPSC Cellular Dynamic Human Neural Microtissue

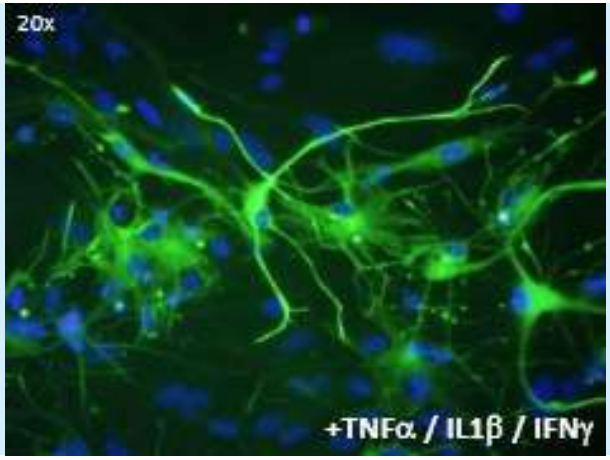
Development of 3D CNS Model

iCell Neurons

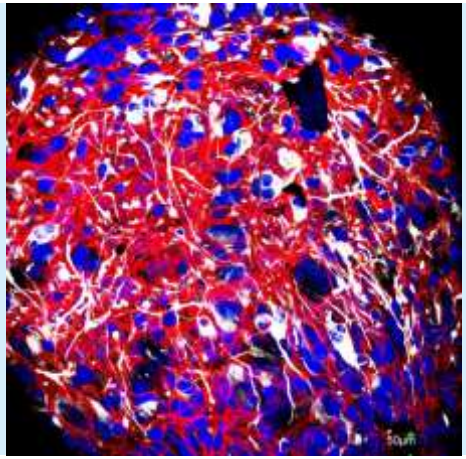


ßIII-Tubulin / Nestin / Hoechst

iCell Astro-cytes



Brain Micro-tissue



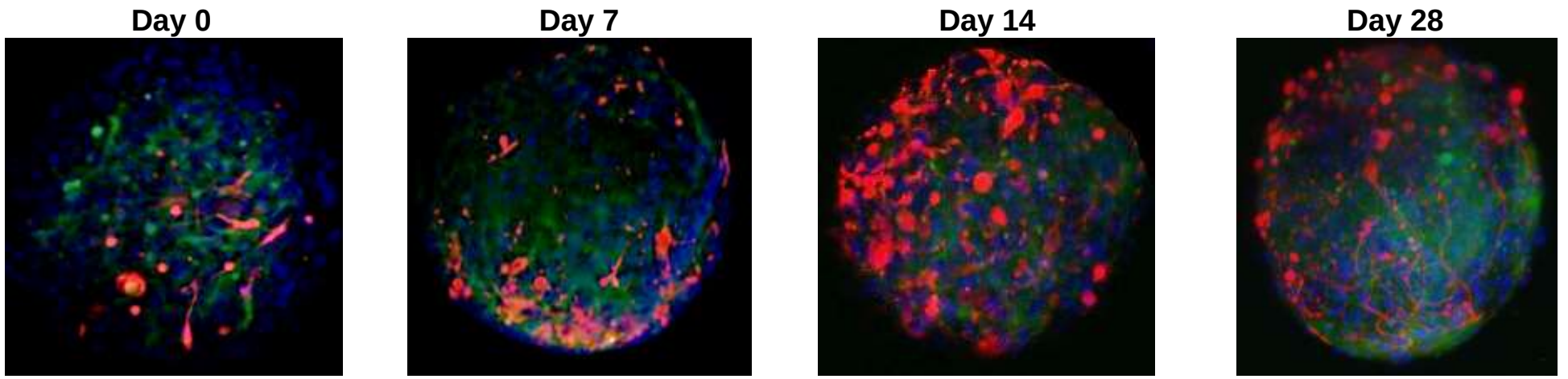
GFAP and βIII-Tubulin immunofluorescence

Brain Microtissue Structure

Development of 3D CNS Model

7000 cells/ MT

ßIII-Tubulin / GFAP / Hoechst

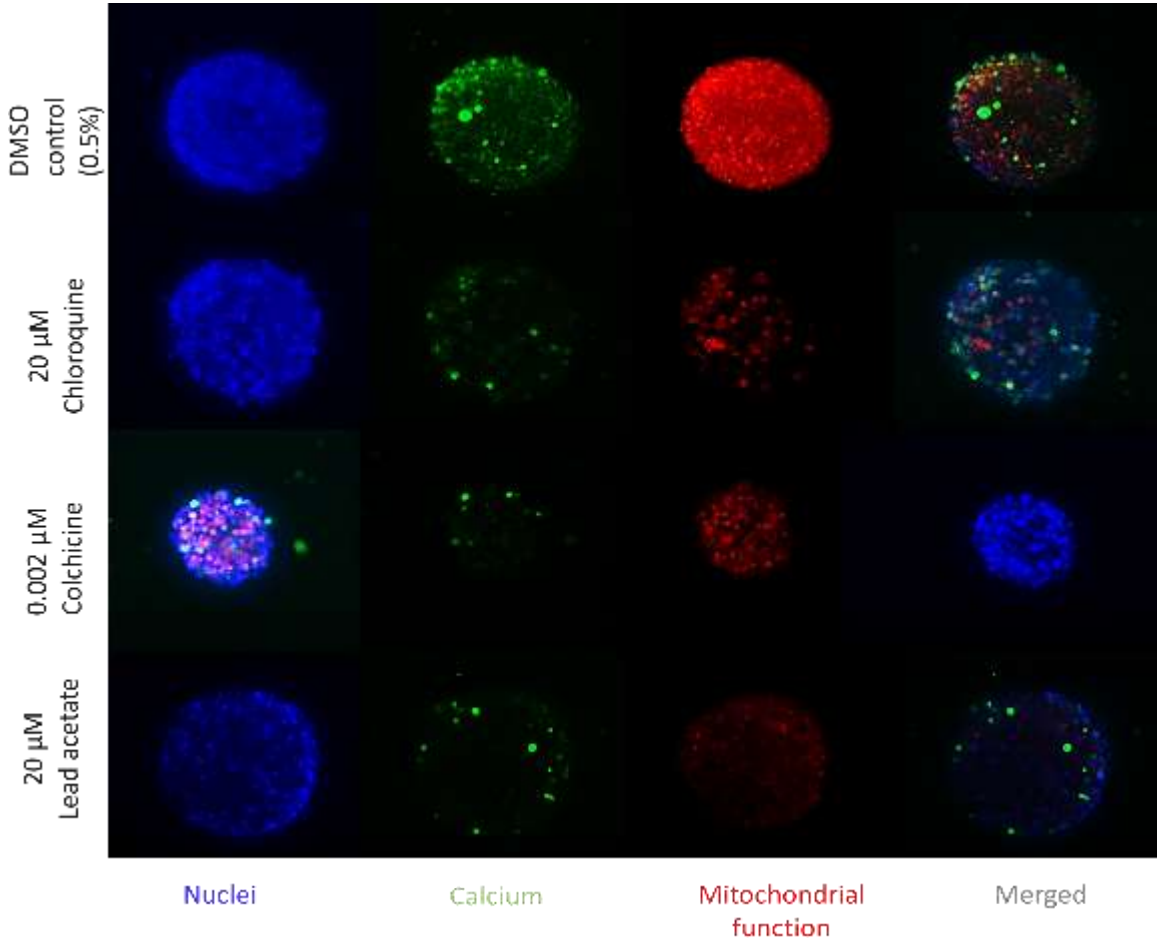


- Astrocyte cell number increases with microtissue age and/or astrocytes migrate to the outer surface until day 14
- At day 28 astrocytes show networking of processes throughout the microtissues
  - In the mature brain, there are 5 to 10 times as many astrocytes as there are neurons and they form spindle like processes of around 50 µm which extend throughout the CNS
  - Literature also suggests in the mature brain astrocytes form a thick layer on the surface of the CNS

High Content Screening of Neurotoxicity

Development of 3D CNS Model

- Response of 3D CNS microtissues to colchicine, lead acetate and chloroquine
- Measurement of calcium levels, mitochondrial function and nuclear cell count



Predicting Neurotoxicity by Normalisation of Data to Brain Specific Exposure Levels (tsC_{max})

- Following, 72 hour exposure brain micro-tissues correctly predicted 60% of the compound panel with normalisation to either plasma C_{max} or brain tsC_{max}
- Following 14 day exposure, 60% of the compound panel correctly predicted neurotoxicity when normalised to plasma C_{max}. However, with normalisation to brain tsC_{max} 80% pre-diction was observed.

Compound	Expected outcome	C_{max} (µM)	tsC_{max} (µM)	72 hr MEC (µM)	72 hr MEC (µM)	14 day MEC (µM)	14 day MEC (µM)
Amoxicillin	Non-toxic	0.87	0.07	NR	NR	NR	NR
Acetaminophen	Non-neurotoxic	165	183	NR	NR	804	804
Acrylamide	Neurotoxic	0.03	0.037	NR	NR	NR	NR
Chloroquine disphosphate	Neurotoxic	1.62	4.7	13	13	7.22	7.22
Colchicine	Neurotoxic	0.015	0.008	0.00326	0.00326	0.0008	0.0008
Lidocaine	Neurotoxic	25.6	121.2	2120	2120	1540	1540
Paclitaxel	Neurotoxic	2	0.023	6.07	6.07	0.08	0.08
Tamoxifen	Neurotoxic	0.083	9.69	6.33	6.33	4.74	4.74
Lead acetate	Neurotoxic	1.3	1.03	NR	NR	12.3	12.3
Vinblastine sulfate	Neurotoxic	0.24	0.04	0.008	0.008	0.008	0.008
				C_{max}	tsC_{max}	C_{max}	tsC_{max}

≤ 15x C_{max}/ tsC_{max}

≥ 15x C_{max}/ tsC_{max}

MEC = minimum effective concentration

Conclusions

Development of 3D Human Focused Models

- Human derived microtissues allow **better reconstitution of *in vivo* cellular physiology**, improved longevity *in vitro* and display organ specific characteristics
- Multiplexed 3D high content imaging (HCS) enables **multiple toxicity endpoints** to be predict organ specific toxicity
- *In silico* modelling of tissue exposure levels can improve *in vitro* toxicity prediction
- Aim to drive *in vitro* responses towards **human relevant dose** (C_{max})

Human derived microtissues **3D high content screening (HCS)** **Compartmental PBPK Model**