cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

Structural 3D cardiotoxicity assay

Background Information

'Numerous studies have shown that cell responses to drugs in 3D culture are improved from those in 2D, with respect to modeling *in vivo* tissue functionality, which highlights the advantages of using 3D-based models for preclinical drug screens'

[6]Nam KH, Smith AS, Lone S, Kwon S and Kim DH (2015) *J Lab Autom*; In press

- Drug induced cardiovascular toxicity is the leading cause of attrition during drug development. Drugs can exert functional toxicities such as arrhythmia and morphological (structural) damage to the myocardium[1]. Evaluation of the potential for both types of cardiotoxicity by novel compounds is essential for the discovery of safe drugs.
- 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[2,3] with emerging evidence suggesting important roles within drug induced cardiovascular toxicity[4].
- Mitochondrial disruption, calcium dyshomeostasis and cellular ATP content have been identified as major targets for structural cardiotoxins[5].
- Three dimensional (3D) confocal HCS allows the simultaneous detection of each cell health parameter in combination with a measure of cellular ATP.

Protocol

Microtissue
Human induced pluripotent stem cell derived cardiomyocytes (iPSC-CM's), cardiac endothelial cells and cardiac fibroblasts

Analysis Platform
Confocal Cellomics ArrayScan® XTI (Thermo Scientific)

Test Article Concentrations
8 point dose response curve with top concentration based on 100x C_{max} or solubility limit. 3 replicates per concentration.

Test Article Requirements
50 µL of a DMSO solution at a concentration of 200x top concentration (top concentration = 100x C_{max}) or equivalent amount in solid compound

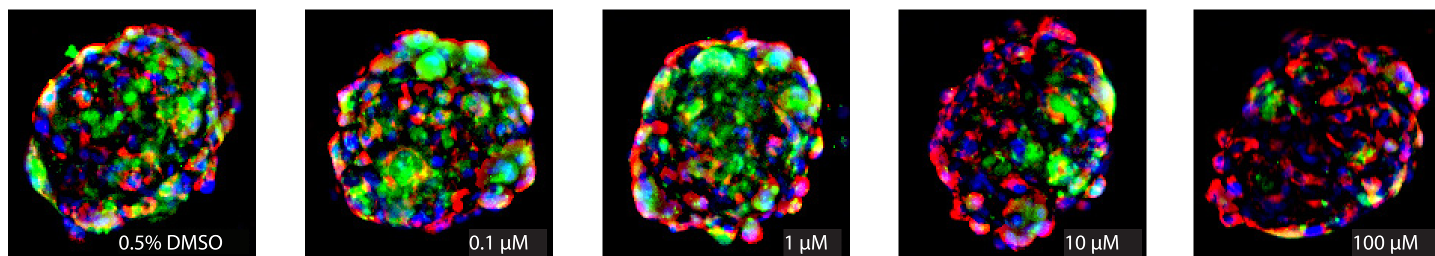
Time Points
72 hours (Others available on request)

Quality Controls
Negative control: 0.5% DMSO (vehicle)
Positive controls: Sunitinib (Ca^{2+} homeostasis) and dasatinib (mitochondrial membrane potential)

Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for each measured parameter (microtissue count, microtissue size, DNA structure, calcium homeostasis (Ca^{2+}), mitochondrial mass (Mito Mass), mitochondrial membrane potential (MMP) and cellular ATP content)

Figure 1

Representative 3D confocal high content screening (HCS) images of isoproterenol calcium dyshomeostasis in spontaneously beating cardiac 3D microtissues labelled with Hoechst (blue) to detect DNA structure, Fluo-4 AM (green) to detect calcium dyshomeostasis and TMRE (red) to detect mitochondrial disruption.



Compound	C_{max} (µM)	*In vivo* toxicity	hESC-CM prediction (Pointon *et al* 2013)[5]	H9c2 monolayer	H9c2 MTs	iPSC-CM's monolayer	Tri-cultured cardiac MTs	Most sensitive feature
				MEC (µM)				
Dasatinib[7]	0.72	Structural cardiotoxin	Positive structural cardiotoxin	0.529	NR	7.32	2.08	MMP
Doxorubicin[8]	15.34	Structural cardiotoxin	Positive structural cardiotoxin	0.04	0.115	0.04	0.04	ATP
Fluorouracil[9]	4.61	Structural cardiotoxin	Positive structural cardiotoxin	1.88	NR	1.4	0.0407	Ca^{2+}
Idarubicin HCl[10]	0.12	Structural cardiotoxin	Positive structural cardiotoxin	<0.04	<0.04	0.04	<0.04	ATP
Imatinib Mesylate[11]	3.54	Structural cardiotoxin	Positive structural cardiotoxin	13.7	3.53	29.3	22.6	ATP
Lapatinib[7]	4.18	Structural cardiotoxin	Positive structural cardiotoxin	4.57	8.33	0.04	5.9	ATP
Sunitinib Malate[12]	0.25	Structural cardiotoxin	Positive structural cardiotoxin	0.896	0.114	0.04	0.817	Ca^{2+}
Cyclophosphamide[13]	153.20	Structural cardiotoxin	Negative structural cardiotoxin	NR	NR	NR	30.8	Mito Mass
Isoproterenol HCl[14]	0.01	Structural cardiotoxin	Negative structural cardiotoxin	NR	NR	NR	2.1	Ca^{2+}
Acyclovir	6.66	Non-structural cardiotoxin	Negative structural cardiotoxin	NR	NR	NR	NR	-
Buspirone HCl	0.03	Non-structural cardiotoxin	Negative structural cardiotoxin	NR	0.237	NR	NR	Microtissue size

Table 1

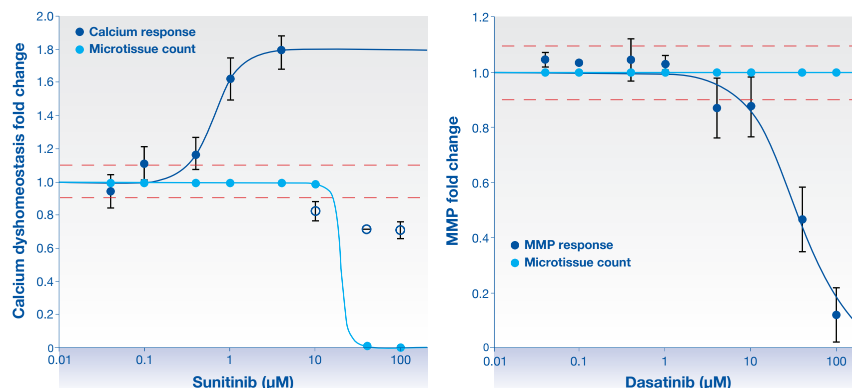
Structural cardiovascular toxicity prediction of 12 reference compounds categorised according to literature data.

Tri-culture cardiac 3D microtissues (MTs), H9c2 3D microtissues (MTs) and H9c2 monolayers were incubated with test compound for 72 hours. The cell models were analysed using the confocal mode of Cellomics ArrayScan® XTI (Thermo Scientific) following which cellular ATP content was measured using CellTiterGlo® (Promega).

MEC = minimum effective concentration
NR = no response

Figure 2

Graphical representation of (a) sunitinib induced calcium dyshomeostasis and (b) dasatinib induced mitochondrial membrane potential disruption in spontaneously beating cardiac 3D microtissues.



All reference compound toxicities were correctly predicted in the spontaneously beating cardiac tri-culture 3D microtissue model including isoproterenol (MEC 2.1µM, calcium dyshomeostasis (Table 1 and Figure 2)) and cyclophosphamide (MEC 30.8µM, mitochondrial mass (Table 1)) which previously went undetected by Pointon *et al* (2013)[5] and Cyprotex's in-house H9c2 data.

Control compound sunitinib displays cytosolic calcium increase (calcium dyshomeostasis) followed by gross cytotoxicity (microtissue loss) (Figure 2a) while control compound dasatinib displays mitochondrial membrane potential loss without gross cytotoxicity (microtissue loss) (Figure 2b).

The combination of an *in vitro* 3D model that better recapitulates the *in vivo* cellular physiology of the myocardium with a multiparametric HCS and cytotoxicity assay presents a viable screening strategy for the accurate detection of novel therapeutics that cause drug induced structural cardiovascular toxicity early in drug development.

References

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