cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

Spontaneously beating cardiac spheroids: 3D combined hypertrophy and 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'

[5]Nam KH, Smith AS, Lone S, Kwon S and Kim DH (2015) *J Lab Autom* **20(3)**; 201-215

- Drug-induced cardiovascular toxicity is the leading cause of attrition during drug development. Drugs can exert functional toxicities such as arrhythmia or morphological (structural) damage including changes 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 only 30% cardiomyocytes, despite this they comprise the majority of the cardiac tissue mass. These terminally differentiated cardiomyocytes can only respond with hypertrophic growth (increased muscle mass) to external stimuli[2].
- Various stimuli are known to induce cardiac hypertrophy including mechanical and oxidative stress as well as neurohormonal perturbation and metabolic hypoxia[2]. Hypertrophy can be physiologically induced or a pathophysiological response to toxicity.
- Mitochondrial disruption, calcium dyshomeostasis and cellular ATP content have been previously identified as major targets for structural cardiotoxins[3] and are used to indicate pathophysiological hypertrophy.
- Three dimensional (3D) high content screening (HCS) allows temporal monitoring of cardiomyocyte spheroid hypertrophy over a 14 day repeat dose period with a terminal measure of mitochondrial function, calcium homeostasis, DNA structure and cellular ATP at day 14.

Protocol

Spheroid
Induced pluripotent stem cell (iPSC) derived cardiomyocytes

Analysis Platform
Brightfield & 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
150 µL of a DMSO* solution to achieve 100x C_{max} (200x top concentration to maintain 0.5% DMSO) or equivalent amount in solid compound.

Time Points
Spheroid hypertrophy: day 3, 7, 10 & 14*
Structural cardiotoxicity HCS & ATP: day 14*

Quality Controls
Negative control: 0.5% DMSO (vehicle)*

Positive controls: dasatinib (structural cardiotoxin with pathophysiological hypertrophic potential) and mitomycin C (structural cardiotoxicity without hypertrophic potential)

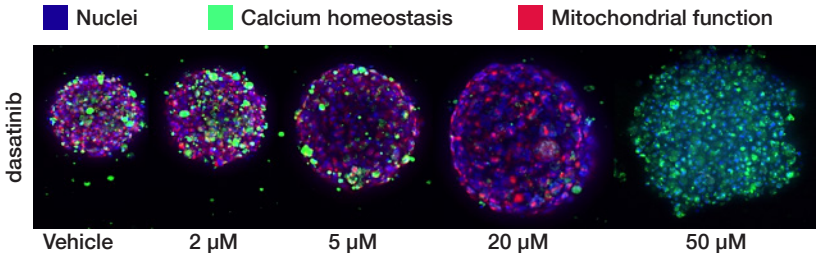
Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for each measured parameter; spheroid count and spheroid size (day 3, 7, 10 & 14) and DNA structure (DNA), calcium homeostasis (Ca^{2+}) mitochondrial mass (Mito Mass), mitochondrial membrane potential (MMP) and cellular ATP content (ATP) (day 14)*

*Other options available on request.

To find out more contact **enquiries@cyprotex.com**

Figure 1

Representative 3D confocal high content screening (HCS) images of dasatinib, a known structural cardiotoxin with hypertrophic potential, labelled with Hoechst (Blue) to detect DNA structure, Fluo-4 AM (Green) to detect calcium homeostasis and TMRE (Red) to detect mitochondrial function.



Drug	Human exposure (C_{max}; µM)	*In vivo* cardiac structural toxicity (P/N)	*In vivo* cardiac patho-physiological hypertrophy (P/N)	Most sensitive structural MEC (µM)	Most sensitive hypertrophy MEC (µM)	Most sensitive 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}

Table 1

Combined structural cardiotoxicity and hypertrophic potential prediction of 16 reference compounds categorised according to literature data[4].

Cardiac spheroids were exposed to test compound for 14 days. During the 14 day period re-dosing occurred on 3 occasions. Spheroid hypertrophy was measured on day 3, 7, 10 and 14 using the brightfield live cellular imaging mode of a Cellomics ArrayScan® XTI (Thermo Scientific). On day 14 the cell model was analysed by using the confocal mode of Cellomics ArrayScan® XTI (Thermo Scientific) following incorporation of fluorescent dyes. Cellular ATP content was subsequently measured using CellTiterGlo® (Promega).

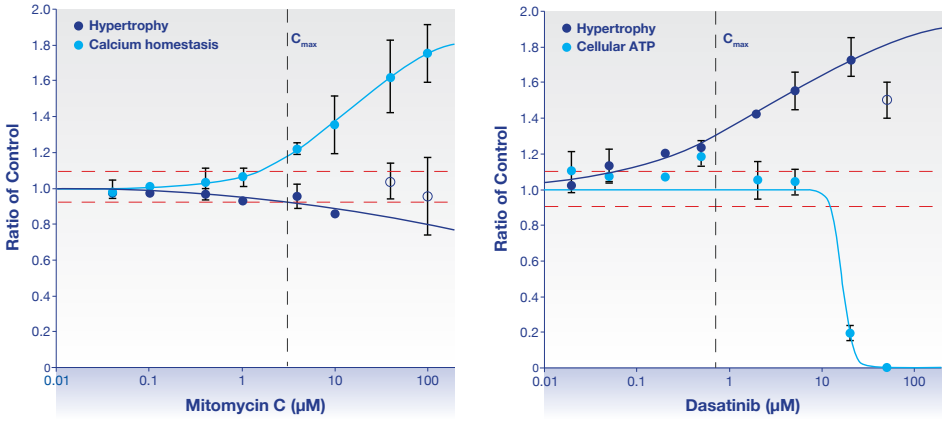
MEC = minimum effective concentration.

P = Positive, N = Negative

	Structural toxicity potential	Patho-physiological hypertrophy model	Cardiac toxicity
Correct prediction with a 10x C_{max} cut off (%)	94%	81%	100%

Figure 2

Graphical representation of (a) hypertrophy and cellular ATP response to dasatinib and (b) hypertrophy and calcium homeostasis response to mitomycin C in cardiac spheroids following 14 day exposure.



Utilising the 3D cardiac combined assay approach all reference compound toxicities were correctly predicted within a 10x C_{max} cut off. Structural cardiotoxicity was correctly predicted for 94% and pathophysiological hypertrophy potential (PHP) for 81% of the compound set within a 10x C_{max} cut off.

The combination of an *in vitro* 3D model that better recapitulates the *in vivo* cellular physiology of cardiac tissue with multiparametric temporal HCS and a cytotoxicity assay presents a viable screening strategy for the accurate *in vivo* relevant detection of novel therapeutics that cause structural cardiotoxicity with pathophysiological hypertrophy potential early in drug development.

References

[1] Laverty H *et al.*, (2011). How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? *Br J Pharmacol* **163(4)**; 675-693
[2] Brutsaert DL (2003). Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Phys Rev* **83(1)**; 59-115
[3] Pointon A *et al.*, (2013) Phenotypic profiling of structural cardiotoxins *in vitro* reveals dependency on multiple mechanisms of toxicity. *Toxicol Sci* **132(2)**; 317-326
[4] Cross MJ *et al.*, (2015) Physiological, pharmacological and toxicological considerations of drug-induced structural cardiac injury. *Br J Pharmacol* **172(4)**; 957-974
[5] Nam KH *et al.*, (2015) Biomimetic 3D tissue models for advanced high-throughput drug screening. *J Lab Autom* **20(3)**; 201-215