**Background Information**

- 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.

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**Protocol**

**Microtissue**

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

**Analysis Platform**

Confocal Cellomics ArrayScan\textsuperscript{\textregistered} XTI (Thermo Scientific)

**Test Article Concentrations**

8 point dose response curve with top concentration based on 100x C\textsubscript{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\textsubscript{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\textsuperscript{2+} homeostasis) and dasatinib (mitochondrial membrane potential)

**Data Delivery**

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

All reference compound toxicities were correctly predicted in the spontaneously beating cardiac tri-culture 3D microtissue model including isoproterenol (MEC 2.1 µM, calcium dyshomeostasis (Table 1 and Figure 2)) and cyclophosphamide (MEC 30.8 µM, mitochondrial mass) which previously went undetected by Pointon et al. (2013) and Cyprotec’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 disruption (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.

### Table 1

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>C&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>In vivo toxicity</th>
<th>hESC-CM prediction (Pointon et al. 2015)</th>
<th>H9c2 monolayer</th>
<th>H9c2 MTs</th>
<th>iPSC-CMs monolayer</th>
<th>iPSC-CMs MTs</th>
<th>Most sensitive feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idarubicin&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.12</td>
<td>Structural cardiotoxin</td>
<td>Positive structural cardiotoxicity</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>MMP</td>
</tr>
<tr>
<td>Doxorubicin&lt;sup&gt;3&lt;/sup&gt;</td>
<td>15.34</td>
<td>Structural cardiotoxin</td>
<td>Negative structural cardiotoxicity</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyclophosphamide&lt;sup&gt;11&lt;/sup&gt;</td>
<td>0.01</td>
<td>Non-structural cardiotoxic</td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acyclovir&lt;sup&gt;4&lt;/sup&gt;</td>
<td>6.66</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Microtissue size</td>
</tr>
<tr>
<td>Buspirone HCl&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.03</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Microtissue size</td>
</tr>
</tbody>
</table>

### Figure 1

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

### References