**Introduction**

The consequence of drug-induced irregular heart beat (pro-arrhythmia) and/or changes in contractility (isotropy liability) can limit the utility of potential novel therapeutics. Since abnormal ventricular repolarization can cause not only electrical disorders (pro-arrhythmia), but also affect the heart's contractile function, the main motivation of this investigation was to develop a human cardiomyocyte-based model that uses adult human primary cardiomyocytes to provide a novel preclinical approach for the simultaneous prediction of drug-induced isotropic and pro-arrhythmia risks. In order to facilitate the scalability of the model, we recorded fractional sarcomere shortening and then used changes in the contractility transients to infer both isotropic (sarcomere shortening) as well as pro-arrhythmia risk (aftercontraction, AC; contractility escape (CE) and time to 90% relaxation (TR90)). To address the clinical relevance of this approach, we performed a pilot study to test the effects of a large set of reference drugs with well-characterized clinical outcomes. Both positive and negative controls were selected, including torsadogenic and non-torsadogenic drugs. We found that the isolated cardiomyocytes exhibited drug-induced contractility changes and pro-arrhythmia markers that are consistent with the known clinical safety profiles of the drugs tested.

**Methods and Selection of drugs**

Adult human primary ventricular myocytes isolated from ethically consented donor's hearts were used to measure fractional sarcomere shortening in 1Hz field-stimulation recording using the IsoOptix® system. The stability of sarcomere shortening was assessed by continuous recording for 2 min. in Tyrode's solution establishing the baseline vehicle control (0.1% dimethyl sulfoxide) condition. Test articles were applied for a maximum 250 sec period or when a steady-state effect was achieved. Four ascending concentrations were examined for each test article. Known torsadogenic as well as non-torsadogenic drugs were used in this study. Historical inconsistencies in drug categorization among different studies has created some uncertainty in the interpretation of past results. Therefore, we selected molecules from a series of studies where consensus existed with regards to their pro-arrhythmia risk from both the GIPA and the JCSA initiatives.

**Isolated adult human primary ventricular myocytes**

Studies has established a novel protocol for the isolation of adult human primary cardiomyocytes. Each isolation pools 3-4 ventricular cardiomyocytes from 4-5 hearts. Each isolation yields 1-3 million cardiomyocytes, exhibit excellent atrophy and contractility in response to electrical field stimulation.

**Stability of the contractility transients over time**

![Stability of the contractility transients over time](chart)

- Change in TR90 and IC in isolated and CCl induced by sequential addition of vehicle V; X in human cardiomyocytes at the pacing frequency 40 Hz, 300 V. (B) Vertical axes depict the sarcomere shortening, V1, V2, V3 and V4 correspond to the 1st, 2nd, 3rd and 4th application of vehicle.

**Human cardiomyocytes predict torsadogenic potential**

![Human cardiomyocytes predict torsadogenic potential](chart)

(A) Typical contractility transients recorded from an adult human primary ventricular myocyte in the presence of vehicle control and after exposure to control V1 (51.1 Hz and 300 V) and 224 μM ERP151 (ERP20) (n=4). (B, C) Changes in TR90 and AC of a single isolated human primary cardiomyocyte with different concentrations and order of sequential application of vehicle. (B) ERP151 (10 μM) and 224 μM ERP151 (ERP20) (C) ERP151 (10 μM). IC50 values were determined as the concentration which produced a 50% decrease in the reference compound.

**Human cardiomyocytes predict safety of verapamil**

![Human cardiomyocytes predict safety of verapamil](chart)

(A) Typical contractility transients recorded from an adult human primary ventricular myocyte in the presence of vehicle control and after exposure to control V1 (51.1 Hz and 300 V) and 224 μM ERP151 (ERP20) (n=4). (B, C) Changes in TR90 and AC of a single isolated human primary cardiomyocyte with different concentrations and order of sequential application of vehicle. (B) ERP151 (10 μM) and 224 μM ERP151 (ERP20) (C) ERP151 (10 μM). IC50 values were determined as the concentration which produced a 50% decrease in the reference compound.

**Human cardiomyocyte-based model differentiates between torsadogenic and non-torsadogenic drugs and has excellent sensitivity (95%) and specificity (100%)**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Clinical TdP risk</th>
<th>ERP151</th>
<th>ERP151</th>
<th>ERP151</th>
<th>ERP151</th>
<th>ERP151</th>
<th>ERP151</th>
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</thead>
<tbody>
<tr>
<td>Drug name</td>
<td>Clinical TdP risk</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult human primary cardiomyocyte model</td>
<td>Torsadogenic</td>
<td>Torsadogenic</td>
<td>Torsadogenic</td>
<td>Torsadogenic</td>
<td>Torsadogenic</td>
<td>Torsadogenic</td>
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</table>

**Table 1. Pro-arrhythmia prediction of the adult human primary cardiomyocyte-based model**

**Table 2. Sarcomere shortening effects for reference drugs measured in adult human primary cardiomyocytes**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Top test concentration (μM)</th>
<th>Human cardiomyocyte</th>
<th>IC50 (μM)</th>
<th>Ratio (IC50/IC100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajmaline</td>
<td>0.25</td>
<td>No effect</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Astemizole</td>
<td>0.25</td>
<td>No effect</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Clozapine</td>
<td>0.25</td>
<td>No effect</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>0.25</td>
<td>No effect</td>
<td>0.009</td>
<td>0.009</td>
</tr>
</tbody>
</table>

**Summary**

1. Adult human primary cardiomyocytes can simultaneously predict risks associated with pro-arrhythmia and isotropic activity.
2. The human primary cardiomyocyte model enables the generation of reliable and predictive data for human-focused cardiac safety assessment at early stages in drug discovery.
3. The adult human primary cardiomyocyte model appears to be more predictive of drug-induced cardiotoxicity than the stem cell-derived cardiomyocyte models.

**References**