Novel Assays for Assessing Cardiotoxicity, Contractility, and ECG-Like Aberrations in Stem Cell-Derived Cardiomyocytes

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FDA CiPA Initiative

Comprehensive *In Vitro* Proarrhythmia Assay (CiPA)

- **Functional Effects on Multiple Cardiac Currents**
  - Voltage Clamp (HT or Manual)

- **In Silico Cellular Simulations**
  - Proarrhythmic Liability

- **Proarrhythmia Score**
  - Mechanism-based, Continuous Scale, Rank-ordered Comparisons, Contextual Data

- **Integrated Human Cellular Studies**
  - Confirmatory Electrophysiology Data

Multi-Electrode Array (MEA) vs. xCELLigence: complementary, but unique technologies

**MEA**
- Field Potential Duration “QT”
- Na⁺ Block

**xCELLigence**
- Contraction
- Subacute/Chronic Dosing
- Cellular Toxicity

Axion Biosystems Maestro MEA

ACEA Biosciences xCELLigence Cardio
Human iPSC cardiomyocytes

- Derived from non-embryonic source
- High purity (>99%) cardiomyocyte population
- Normal cardiomyocyte physiology
  - ventricular, atrial, and nodal cell types
  - electrically coupled activity
  - integrated ion channel function
- Spontaneously beating
- Sensitive to arrhythmogenic drugs
- Viable in culture for months
Multi-Well Maestro MEA System

Axion MEA system–monitors spontaneous electrical activity in cardiomyocyte monolayers

Maestro
• Recording
• Heating
• A/D

Middleman
• Power
• Pre-processing
• IO

MEA
• 768 electrodes,
• 16 electrodes/well
• 48 well assay plate

Detects effects of test compounds on electrical activity in stem cell-derived human cardiomyocytes
• extracellular field potential (ECG related signal)
• beat prolongation
• beat irregularities
xCELLigence: Impedance Assay

xCELLigence RTCA Cardio (ACEA) continuously monitors electrical impedance in cardiomyocyte monolayers during spontaneous beating.

- Detects effects on cardiomyocytes in 96-well format
  - contractility
  - beat rate
  - beat irregularities
  - cytotoxicity

Analyzer
Cardio Station

Contraction

Relaxation

Microelectrode array fabricated in the bottom of the well

electrodes without cells

electrodes with cells attached

\[ Z = Z_0 \]

\[ Z = Z_{cell} \]
**MEA** - measures extracellular field potentials yielding ECG-like recordings. Na⁺ spike amplitude, field potential duration (FPD), beat period/rate in stem cell-derived human cardiomyocytes.

**xCELLigence** - measures electrical impedance changes resulting from myocyte contraction. Detects amplitude, rise and decay times, beat period/rate, cell index.
Moxifloxacin prolongs FPD and induces arrhythmic events.

**A:** Line graph (green line) shows percent change in FPD vs. [Moxifloxacin], µM.

**B:** Waveform comparison of baseline versus moxifloxacin treatment. Arrows mark the peak of each T wave (FPD). Early arrhythmic events were observed with 300 µM moxifloxacin after 10 minutes (white trace).
Dofetilide, moxifloxacin, risperidone and ibutilide induce arrhythmic events. FPD prolongation, EADs, and arrhythmias associated with *torsades de pointes* were reproducibly observed. Ibutilide induced arrhythmias at the effective therapeutic plasma concentration (ETPC), while risperidone induced pre-arrhythmic activity at concentrations ≥ 30X the ETPC.
hIPSC cardiomyocytes recapitulate the multiple ion channel effects (MICE) model

- hERG endpoint analysis is not sufficient to predict arrhythmias.
- Integrated ion channel effects must be evaluated for accurate arrhythmia prediction.

\[1\]


*EADs expected at concentrations > 100X ETPC*
Nitrendipine – Ca\textsuperscript{2+} channel blocker shortens FPD and decreases beat interval

FPL 64176 – Ca\textsuperscript{2+} channel activator lengthens FPD, increases beat interval and induces afterpotentials

*Cor.4U human iPSC Cardiomyocytes, Axiogenesis AG
MEA detects Na⁺ channel block

Tetrodotoxin reduces the Na⁺ spike
Line graph (green line) shows percent change in spike amplitude vs. [Tetrodotoxin], μM.

Human iPSC Cardiomyocytes, Axiogenesis AG
**MEA: Effect of Na⁺ channel block on Na⁺ spike amplitude**

Effect of Na⁺ channel blockers on Na⁺ amplitude. hiPSC cardiomyocytes were treated with test compounds at multiple concentrations in duplicate wells. The heat map shows that addition of Na⁺ channel blockers decrease the Na⁺ spike amplitude (peak QRS voltage) detected in each electrode/well.
MEA Assay Data Reporting

Typical Report Figures
- Statistical summary of each compound
- Line graph summarizing change in experimental parameters
- +/- Arrhythmic event
- Representative waveforms

Figure 8. Effect of Quinidine (at indicated concentrations) on ECG parameters. A) Line graph depicting those values seen in the accompanying table. Values are normalized to vehicle control ± SEM, n = number of wells averaged, (#/e) = total electrodes in use. Statistical significance (from vehicle control, Student’s T test, p < 0.05) is signified by a closed circle • (graph) or * (table).

*Cor.4U human iPSC Cardiomyocytes, Axiogenesis AG
A. xCELLigence

- DMSO 0.1%
- Blebbistatin 1 µM

B. MEA

Baseline 0.3 μM FPL 64176
Baseline 1 µM Blebbistatin
Baseline FPL 64176 + Blebbistatin

**A:** Impedance records. Blebbistatin (myosin II inhibitor, at 1 µM) suppresses contraction in control (0.1% DMSO) or in 1 µM FPL64176, a calcium channel agonist, that slows the beat rate.

**B:** MEA traces. Blebbistatin has no effect on spontaneous electrical activity.
xCELLigence sensitivity to calcium modulators

Nitrendipine – Ca\(^{2+}\) channel blocker reduces contractile amplitude and increases beat rate. FPL 64176 – Ca\(^{2+}\) channel activator slows the decay rate, increases beat period, and amplitude.
xCeLLigence: Identifies afterdepolarizations and arrhythmic events

Inset panels show impedance recordings from pre-treatment baseline and post-treatment dofetilide wells

<table>
<thead>
<tr>
<th>(µMol/L)</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
<th>Dose 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-4031</td>
<td>0.003</td>
<td>0.009</td>
<td>0.03</td>
<td>0.09</td>
<td>0.3</td>
</tr>
<tr>
<td>FPL64176</td>
<td>0.01</td>
<td>0.03</td>
<td>0.1</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Flecaïnide</td>
<td>0.75</td>
<td>2.26</td>
<td>7.5</td>
<td>22.6</td>
<td>75.3</td>
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<tr>
<td>Donepezil</td>
<td>0.0025</td>
<td>0.0075</td>
<td>0.025</td>
<td>0.076</td>
<td>0.25</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>0.0020</td>
<td>0.0060</td>
<td>0.020</td>
<td>0.060</td>
<td>0.20</td>
</tr>
<tr>
<td>Droperidol</td>
<td>0.016</td>
<td>0.047</td>
<td>0.16</td>
<td>0.47</td>
<td>1.6</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>0.0030</td>
<td>0.0090</td>
<td>0.030</td>
<td>0.090</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Pentamidine prolongs the cardiac action potential by inhibiting hERG trafficking to the cell surface. “Fall Time” indirectly measures hERG channel activity. Loss of surface hERG expression prolongs the falling time.

Doxorubicin induces dose-dependent cytotoxicity (decline in cell index).

Kuryshev YA et al. (2005) J. Pharmacol Exp. Ther. 312(1):316-23

Cor.4U human iPSC Cardiomyocytes, Axiogenesis AG

Each data point represents the mean ± SEM for ≥ 3 wells per condition.
xCELLigence Data Reporting

Typical Report Figures
✓ Visual summary for each compound data set (with statistics)
✓ Line graph summarizing changes in each experimental parameter over time
✓ Single .xls summary file
✓ Acute to chronic dosing/time points
✓ Representative impedance traces

*Cor.4U human iPSC Cardiomyocytes, Axiogenesis AG
Human iPSC cardiomyocyte assays: protocols and reports

**xCELLigence Summary**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Plating Option</th>
<th># Test Cpds</th>
<th>Typical Protocol</th>
<th>Positive Controls</th>
<th>End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>xCELLigence</td>
<td>96 well</td>
<td>1 - 5</td>
<td>4 concentrations, n=4 wells/conc. Positive &amp; vehicle controls included</td>
<td>Customer-specified e.g. Pentamidine, E-4031</td>
<td>ΔCell index Δ Beat Rate Δ Amplitude Δ Fall Time</td>
</tr>
</tbody>
</table>

**MEA Summary**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Plating Option</th>
<th># Test Cpds</th>
<th>Typical Protocol</th>
<th>Concentrations</th>
<th>Positive Controls</th>
<th>Typical End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA</td>
<td>48 well</td>
<td>1 - 9</td>
<td>5 concentrations, n=4 Sequential dosing Positive &amp; vehicle controls Turnaround time, 3-4 weeks</td>
<td>2 log dosing recommended e.g., ETPC, 3X, 10X, 30X, 100X Serum-free conditions</td>
<td>Moxifloxacin (or customer-specified)</td>
<td>Δ FPD (&quot;QT&quot;) Δ Beat Period Δ Spike Amp ± Arrhythmia</td>
</tr>
</tbody>
</table>
**xCELLigence and MEA – complementary assays for integrated cardiac risk assessment**

<table>
<thead>
<tr>
<th>xCELLigence</th>
<th>Multiple Electrode Array (MEA)</th>
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<tbody>
<tr>
<td>Measures impedance changes due to spontaneous contractile activity in cardiomyocyte monolayers</td>
<td>Measures electrical field potentials in spontaneously beating cardiomyocytes monolayers</td>
</tr>
<tr>
<td>Allows subacute or chronic exposure to test compounds in serum-containing media</td>
<td>Allows acute exposure to test compounds in serum-free media</td>
</tr>
<tr>
<td>Impedance transients are correlated to contraction</td>
<td>Field potentials (&quot;ECG-like&quot; signal) are correlated with electrical activity</td>
</tr>
<tr>
<td>Detects prolongation of contraction (indirectly related to APD prolongation)</td>
<td>Detects FPD prolongation (directly related to QT interval and APD prolongation)</td>
</tr>
<tr>
<td>Detects irregular beating</td>
<td>Detects changes sodium spike amplitude (directly related to QRS amplitude and AP Vmax)</td>
</tr>
<tr>
<td>Detects cytotoxicity</td>
<td>Detects EADs and irregular beating as proarrhythmic signals</td>
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Acknowledgements

**ChanTest Scientists**

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