Evaluation of Drug Mediated Changes in Action Potentials Recorded from Adult Human Stem Cell-derived Cardiomyocytes

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The Problem

- Cost associated with late withdrawal is $1 billion and ≥ 15 years\textsuperscript{2}

- **Warning/Withdrawal of approved drugs**
  - Two million adverse drug reactions per year\textsuperscript{3}
  - 100,000 deaths per year\textsuperscript{3}
  - cardiac effects of non-cardiac drugs (40%)\textsuperscript{4}
  - 2012: Celexa, Zofran, Zithromax warnings

- **Premature termination of safe/effective drugs**
  - Inadequate research and development tests
  - US FDA/HESI/Cardiac Research Consortium

- Better testing must be done sooner in the pipeline to select safe, effective drugs.
• Ionic Transport Assays uses adult human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes.

• The FDA stated these “iCell® Cardiomyocytes are...a reliable source of human cardiomyocytes suitable for use in targeted drug discovery and toxicity testing.”

• FDA Director, Dr. Norman Stockbridge, stated a goal to revise ICH S7B guidelines using stem cells by July 2016.

• Ionic Transport Assays generates unique stem cell data to satisfy ICH S7B guidelines for current and new drug cardiac safety testing.
Solution: Ionic Transport Assays

- Ion channels (purple/pink protein) regulate the movement of Ca++, K+ and Na+ through cell membranes.

![Diagram of ion transport through cell membrane]

Record action potential/ion channels in cardiac cells derived from adult human stem cells (hiPSC)

- Discovery Pharmacology - What is the Mechanism of Action?
- Preclinical Cardiac Safety Pharmacology - Is it safe?
The Action Potential is the ECG of the hiPSC Ventricular-like Cardiomyocyte

- Prolonged action potential duration \((A \rightarrow B)\) is a surrogate for Q-T prolongation \((T_{(A)} \rightarrow T_{(B)})\) both result from disruption of hERG function.
- QT prolongation increases the probability that a patient will develop life threatening cardiac arrhythmias.
Atrial-like & Ventricular-like Adult hiPSC-CM vs. Human Atrial & Ventricular Cells

Adult hiPSC Cardiomyocytes

Atrial-like

Adult Human Cardiomyocytes *

A Atrium

0 mV

50 mV

100 ms

B Ventricle

0 mV

50 mV

100 ms

Terfenadine, Unlike Fexofenadine, Increases APD$_{90}$ in Ventricular-Like hiPSC-CM

![Graph A](Terfenadine Graph)

![Graph B](Fexofenadine Graph)

Control, 0.1 µM, 0.3 µM, 1.0 µM, 3.0 µM
Dofetilide Increases APD$_{90}$ in Ventricular-like hiPSC-CM
E-4031 Increases APD_{90} in Ventricular-like hiPSC-CM
30 nM E-4031 Produces EADs in Ventricular-like hiPSC-CM
Flecainide Inhibits Upstroke & Depolarizes Resting Membrane Potential in Ventricular-like hiPSC-CM

<table>
<thead>
<tr>
<th>µM</th>
<th>Rate of Rise (V/s)</th>
<th>APD_90 (ms)</th>
<th>APD_99 (ms)</th>
<th>RMP (mV)</th>
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<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>363</td>
<td>485</td>
<td>-79</td>
</tr>
<tr>
<td>0.1</td>
<td>45</td>
<td>353</td>
<td>475</td>
<td>-79</td>
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<tr>
<td>0.3</td>
<td>43</td>
<td>364</td>
<td>509</td>
<td>-77</td>
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<tr>
<td>1</td>
<td>35</td>
<td>396</td>
<td>579</td>
<td>-76</td>
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<tr>
<td>3</td>
<td>30</td>
<td>463</td>
<td>710</td>
<td>-75</td>
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</table>
Verapamil Shortens, then Increases APD_{90} in Ventricular-like hiPSC-CM
An action potential is the interactive summary of transmembrane ion currents to produce electrical activation of the myocyte leading to an release/increase in cytoplasmic $\text{Ca}^{++}$, the internal biochemical stimulation of myocyte contraction.\textsuperscript{6}
Nifedipine Shortens Action Potential Duration in Ventricular-like hiPSC-CM
Nifedipine Blocks L-Type ICa in the Membrane of Ventricular-like Cardiomyocytes

IC$_{50}$ - 0.038 μM
hiPSC-CM Calcium Fluorescence*

- Peak Count & Frequency
- Peak Position (time) and Amplitude
- Peak Width (FWHM)
- Rise Time (10% to 90%)
- Decay Time (90% to 10%)

*SpectraMax i3 (96/384 well), SoftMax Pro 6.4, Molecular Devices, Sunnyvale, CA
96 Well HTS Calcium Fluorescence*

*SpectraMax i3 (96/386 well), SoftMax Pro 6.4
Molecular Devices, Sunnyvale, CA
hiPSC-CM Isoproterenol: 1 Hour Exposure

A. 0.5 % DMSO Control (02 E3)

B. 0.1 µM Isoproterenol (01 E4)

C. 10 µM Isoproterenol (02 E5)
hiPSC-CM Sotalol: 1 Hour Exposure

A. 0.5 % DMSO Control (02 E3)

B. 1.0 µM Sotalol (01 E6)

C. 100 µM Sotalol (02 E7)

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<tr>
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<th>DMSO</th>
<th>1 µM</th>
<th>100 µM</th>
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<tbody>
<tr>
<td>Rate (bpm)</td>
<td>26</td>
<td>23</td>
<td>4</td>
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<tr>
<td>Rise (sec)</td>
<td>0.312</td>
<td>0.311</td>
<td>0.420</td>
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<tr>
<td>Peak (X 10³ RFU)</td>
<td>156</td>
<td>157</td>
<td>97</td>
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<tr>
<td>Width (sec)</td>
<td>0.648</td>
<td>0.668</td>
<td>11</td>
</tr>
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</table>
hiPSC-CM Nifedipine: 2 Hour Exposure

A. 0.5 % DMSO Control (04 G3)

B. 0.03 μM Nifedipine (01 B10)

C. 0.1 μM Nifedipine (02 E10)

<table>
<thead>
<tr>
<th></th>
<th>N = 3</th>
<th>DMSO</th>
<th>0.03 μM</th>
<th>0.1 μM</th>
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<tbody>
<tr>
<td>Rate (bpm)</td>
<td>28 ± 1.7</td>
<td>27 ± 11</td>
<td>0 ± 0</td>
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<tr>
<td>Rise (sec)</td>
<td>0.32 ± 0.04</td>
<td>0.45 ± 0.42</td>
<td>0 ± 0</td>
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<tr>
<td>Peak (X 10^3 RFU)</td>
<td>169 ± 6</td>
<td>138 ± 15</td>
<td>0 ± 0</td>
<td></td>
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<tr>
<td>Width (sec)</td>
<td>0.60 ± 0.02</td>
<td>0.18 ± 0.13</td>
<td>0 ± 0</td>
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</table>
Cell Toxicity

Cor.4U®
IC$_{50}$ = 8.2 µM

iCell®
IC$_{50}$ = 9.8 µM
• Using hiPSC cardiomyocytes, Ionic Transport studies:
  o Spontaneous/paced action potentials and ion channels
  o Cardiac contraction/duration via internal calcium transients
  o Cell viability

• This science and services add significant value with reduced costs to the drug development process with a relevant tissue.

• We provide "manual” and HTS services to clients.

• We strive to increase laboratory capacity, service offerings, and client base with new customers and assistance from investors.

• As we work on the plan in 2014, we build for future growth.
Thank You


6. Available from: http://folk.ntnu.no/stoylen/strainrate
Backup
Drug Discovery Continuum

Drug Discovery
- $77 MM
- 5,000–10,000 compounds
- 3–6 years

Preclinical
- $86 MM
- 250

Clinical Trials
- $423 MM
- 5
- Phase 1: 20–100
- Phase 2: 100–500
- Phase 3: 1,000–5,000
- 6–7 years

FDA Review

Scale-up to MFG.
- $234 MM
- 0.5–2 years

Ionic Transport added value