Advances in Safety Pharmacology: Utilizing iPSC-derived cardiomyocytes in early stage safety pharmacology investigations

Blake Anson, PhD.
I. Safety Pharmacology and in-vitro models
   Need for human cardiac model
   Potential for stem cell technology to provide a solution

II. Functional characterization of stem cell derived cardiomyocytes
   Genomic, protein, metabolic, electrophysiological

III. Methods for interrogating cardiomyocyte function
   Electrophysiology, Ca2+ handling, Contractility
   Direct and Indirect (screening) methodologies

IV. Case studies
   Retrospective Analysis

V. Summary
   Mechanistic and Phenotypic Approaches
Current common in-vitro models include cell lines and non-human cellular preparations.
• Your heart is an electrically driven pump.
• It usually beats 60-80 times a minute, or about 100,000 times a day, or about 35 million times a year, or about 3 billion times in a normal life span.
• If the normal pumping rhythm is severely disrupted for more than a few minutes, irreversible multi-organ damage and death occur.
iPSC-derived cardiomyocytes – Utility
Cardiotoxicity: Two basic mechanisms

Drug induced Arrhythmias

Biochemical induced events
- Cell Permeability
- Cell energetics
- Oxidative stress
- Mitochondrial dysfunction
- Necrosis
- Apoptosis

The two toxicities can be species specific and may not be causal or linked

The ideal test system is species specific and biologically relevant.

Human iPSC-derived cardiomyocytes
• Larger mammals have slower rates
• Longer APD
• Greater endo to epi dispersion of repolarization

Why Human?:

Very Different Ionic Mechanisms Between Large Mammals and Rodents

**Human Cardiomyocytes**

- ES or iPSC source material
- Recapitulate cardiac cellular behavior
- Unlimited Quantity
- High Purity
- Relevant Biology
- Broad End-use Platform Utility
INDUCED PLURIPOTENT STEM CELLS

EMBRYONIC STEM CELLS

NEURONAL CELLS
HEMATOPOIETIC CELLS
CARDIOMYOCYTES
RENAL CELLS
HEPATOCYTES

Source: CDI website; www.stemcelltechniques.blogspot.com
**Key Characteristics for iPSC use in Safety Pharmacology**

**Purity**
- Highly pure cells yield accurate results

**Quantity**
- Must be able to support large scale investigations

**Quality**
- Exhibit key cellular characteristics
- Recapitulate normal human biology
- Reproducible
- Known and relevant genotype
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~200 Cardiac genes were identified from the Novartis GNF expression atlas
  • Expression levels of these genes were obtained from adult human heart mRNA library (ambion)
  • Expression levels of these genes were obtained from pure populations of iPSC-cardiomyocytes over a 3 month period (d028 to d120)
  • Relative levels compared with each other

Purified iPSC-Cardiomyocytes (iPSC-cardiomyocytes) show a stable cardiac expression profile that matches adult human tissue

Adapted from Babiarz et al., 2011
Human iPSC Cardiomyocytes
Protein Expression

From Kattman et al., 2011
Human iPSC Cardiomyocytes
Metabolic Characterization

iPSC-cardiomyocytes utilize mitochondrial oxidative phosphorylation

Adapted from Rana et al., 2012

iPSC-cardiomyocyte mitochondria are functional

Glycolysis

Shift in Energy Metabolism

Mitochondrial Respiration

iPSC-cardiomyocytes utilize non-glycolytic substrates
Human iPSC-Cardiomyocytes
Contractility / Ca\textsuperscript{2+} handling Characterization

A. Contraction

B. Ca\textsuperscript{2+} Transient

Contraction IC\textsubscript{50} = 87nM
Ca\textsuperscript{2+} Transient IC\textsubscript{50} = 437nM (extrapolated)
Human iPSC-cardiomyocytes
Depolarizing Currents

**Human iPSC-cardiomyocytes Repolarizing Currents**

**A**

- **Control**
- **E4031 500 nmol/L**
- **Subtraction**

**I_Kr**

- Current traces for different conditions are shown.
- Graph: Normalized tail current vs. voltage.
- Data: 
  - $V_{1/2} = -22.7 \text{ mV}$
  - $k = 4.9 \text{ mV}$/e-fold change
  - $n = 8$

**B**

- **Control**
- **3R4S-Chromanol 10 µmol/L**
- **Subtraction**

**I_Ks**

- Current traces for different conditions are shown.
- Graph: Step current (pA/pF) vs. voltage.

Other Currents

Spontaneous Action Potential Beating Rate (MEA): 

$G_{\alpha s} - \beta 1$  
Isoproterenol

$G_{\alpha q} - \alpha 1$  
Phenylephrine

$G_{\alpha i} - m2$  
Carbachol

iPSC Cardiomyocytes have the appropriate GPCR pathways
Stem cell derived CMs exhibit cardiac like action potentials

### Stem Cell - Cardiomyocytes Action Potentials

#### iPSC-CMS

![Graph showing action potentials for iPSC-CMS](image)

**Ma, et al., Am. J. Physiol., 2011**

#### hES-CMS

![Graph showing action potentials for hES-CMS](image)

**Peng et al., 2010**

<table>
<thead>
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<th>Atrial-like</th>
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<th>Ventricular-like</th>
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</table>
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Basic functional electrical unit
Drives contraction by initiating increases in intracellular Ca\(^{2+}\) levels

How are these processes interrogated in-vitro?
**iPSC Cardiomyocytes**

**Safety Pharmacology - APD Assay**

**I Na Block** – Decreased $dv/dt$

**I Ca Block** – shortened APD

**I Kr Block** – prolonged APD

**EAD Generation**

<table>
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<tr>
<th>% of control</th>
<th>Dose</th>
<th>Peak</th>
<th>MDP</th>
<th>APD10</th>
<th>APD50</th>
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<td>16.8±2.0*</td>
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<td>87.2±6.9</td>
<td>97.3±1.8</td>
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<td>65.7±3.0*</td>
<td>74.0±2.3*</td>
<td>84.8±11.2</td>
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</tbody>
</table>


**iPSC-Cardiomyocytes show expected pharmacology**
Arrhythmic Triggers

Response Across Ion Channel Families

Quantifiable Responses

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<tr>
<th>Compound</th>
<th>Rabbit PF</th>
<th>Statistical significance</th>
<th>Canine PF</th>
<th>Statistical significance</th>
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<td>266</td>
<td>0.004</td>
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<td>&gt; 10 micromolar</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.004</td>
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</tbody>
</table>

heSC-Cardiomyocytes show expected pharmacology

Peng et al., 2010
Stem cell Cardiomyocytes recapitulate pro-arrhythmia triggers
EAD amplitude varies inversely with take off potential

Adult Canine Purkinje Fiber APs

Bay k 8644


iPSC CM APs

Human iPSC Cardiomyocytes
Organotypic Recordings MultiElectrode Array (MEA)

Compounds induce expected effects
Human iPSC Cardiomyocytes
Multi-electrode Array – Measuring Conduction Velocity

iPSC Human Cardiomyocyte Field Potentials
48-well MEA plate

iPSC Human Cardiomyocyte FPD Initiation
depends on well position within the plate

iPSC Conduction velocity
can be measured

Drug-induced effects on conduction velocity
can be measured

Organotypic preparations provide additional/alternative endpoints
- Calcium 5 Assay Kit (Molecular Devices)
- Fluorescence intensity of Ca\(^{2+}\) transients from autonomously beating cells.
- The entire experiment was finished in 100 seconds.

![Beats/min vs Concentration, uM](image)

**96w format**

- Positive Chronotropes:
  - Isoproterenol
  - Epinephrine
  - Dopamine

- Negative Chronotropes:
  - Doxazosine
  - Verapamil
  - Propranolol

Modified from Sirenko et al., 2013
- Calcium 5 Assay Kit (Molecular Devices)
- Fluorescence intensity of Ca\(^{2+}\) transients from autonomously beating cells.
- The entire experiment was finished in 100 seconds.

New algorithms are being developed for analysis.
Human iPSC Cardiomyocytes
Functional Utility - Contractility

A. Contraction

Contraction Amplitude Change (%)

N = 3 or 4

Contraction

- Nifedipine
- Verapamil
- Diltiazem
- Isoproterenol
- Bay K8644

Determined by edge detection

Puppala et al, 2012
Human iPSC Cardiomyocytes
Relevant Mitochondrial Toxicity Screens

Assess viability while inhibiting mitochondrial function (rotenone, antimycin, and oligomycin)

Decreased viability is masked by glucose

Adapted from Rana et al., 2012
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Calculus 5 Assay Kit (Molecular Devices)
Fluorescence intensity of Ca\textsuperscript{2+} transients from autonomously beating cells.
The entire experiment was finished in 100 seconds.

Electrical activity at the membrane is reflected in the Ca\textsuperscript{2+} transient

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**iPSC Cardiomyocytes – Screening**

\( \text{Ca}^{2+} \) Imaging reflects electrical activity
iPSC-cardiomyocytes – Screening
FLIPR® Tetra System - Quantitation

Intracellular Ca\(^{2+}\) oscillations can be used to measure membrane ion channel activity in 96-384 well plates.
A surrogate measurement platform for electrical activity, Ca\textsuperscript{2+} handling, contractility
### iPSC-cardiomyocytes - Utility
Label-Free Measurements – Arrhythmogenesis

**Arrhythmia Screening in 96-wells**

<table>
<thead>
<tr>
<th>Drug</th>
<th>PPS</th>
<th>hERG</th>
<th>QT</th>
<th>Clinical Arrhythmia</th>
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</table>

*Guo et al., 2011*

**PPS - Predicted ProArhythmia Score**

- Identifies False Negatives
- Identifies False Positives
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Compound B: Cardiovascular outcome (bradycardia) in humans not predicted by dog ECGs

A: Dog

A. In a dog TK study, compound B induced sinus arrest and junctional rhythm. Cmax was \(~100 \mu M\).

B: Human

B. In a healthy human volunteer compound B induced bradycardia (43 bpm) with a junctional escape rhythm noted on all ECGs from 4 (~Tmax) to 9 hours. Cmax was \(~0.11 \mu M\) (1000-fold less than in the dog).

- Compound B, an inhibitor of sinoatrial node automaticity, caused bradycardia in humans at a much lower plasma concentration than in dog or rabbit. This cannot be explained by similar moderate protein bindings in human, dog and rabbit.
Compound B decreased FP beat rate in rE and hiPSC CMs

% inhibition of beat rate in hiPSC and rE CMs

- Compound B reduced FP beating rate in hiPSC CMs at similar concentrations caused bradycardia in humans.
- Rat embryonic CMs were less sensitive than hiPSC CMs.
- The human iPSC CM MEA assay was a sensitive predictor of the human heart rate effect.
Compound C: clinical QT effect not predicted by standard non-clinical cardiovascular safety assays

In vitro non-clinical
- hERG channel: 10% inhibition at 10 μM, free concentration
- rabbit Purkinje-fiber action potential: 10% prolongation of APD$_{90}$ at 30 μM (free concentration)

In-vivo non-clinical
- cynomolgus monkey: QT effect observed at 33 μM total, 6 μM free

* hERG and cyno QT effect correlated reasonably well.

Clinical
- human Holter study: QT effect observed at 2.25 μM total, 0.5 μM free

* Humans appeared more sensitive, with QT increase occurring at about 15x lower exposure than in cyno.
Compound C increased field potential duration (FPD) in hiPSC and rE CMs

- Compound C dose-dependently increased FP duration in hiPSC CMs, consistent with the clinical findings of QT prolongation (≥0.5 μM, free).
- rE CMs (rat) were less sensitive.
- The hiPSC CM MEA assay sensitively predicted human QT prolongation.
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## Example subset

<table>
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## Functional Endpoints

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Human Stem Cell Derived Cardiomyocytes

Human cardiomyocytes

Provide an in-vitro recapitulation of native cellular biology and physiology

Can be used across a variety of interrogation platforms

Have, in some cases, shown greater utility than current models

Can be used for phenotypic and mechanistic screening investigations
Thank-you