New Techniques for MEA-Based Interrogation of Human Stem Cell Derived Cardiomyocytes to Support CiPA and In-vitro Safety Pharmacology Assays

Blake Anson, Ph.D. Cellular Dynamics International
Jim Ross, Ph.D. Axion Biosystems

Oct 21, 2014
● Proarrhythmia Testing
  - moving from single channel to holistic assessments

● iCell Cardiomyocytes

● Population Diversity

● Key manufacturing components
In-vitro detection of proarrhythmia

The road to in-vitro proarrhythmia testing….

…. started in a fly
Drug–induced Electrophysiological Aberrations

not a new phenomenon

Quinidine Syncope and Delayed Repolarization Syndromes.
Reynolds E and Vander Ark C. M

Cardiotoxic effect with convulsions in terfenadine overdose

Drs Anthony J Davies, V Harindra, A McElmasta, and R R Ghose (Singleton Hospital, Swansea, SA2 8QA) write: Terfenadine (Triludan) is a spasmolytic, histamine H₁ receptor antagonist.

Davies et al., BMJ 1989:298

….but took on a new meaning when caused by non-cardiac compounds

Cisapride and Fatal Arrhythmia

To the Editor: From September 1993, the month in which the marketing of cisapride (Propulsid, Janssen Pharmaceutica, Titusville, N.J.) began, to April 1996, the Food and Drug Administration's MedWatch reporting program (telephone number, 1-800-FDA-1088) received reports of 34 patients in whom torsade de pointes developed and 23 in whom prolonged QT intervals developed.

Wyosowski and Bacsanyi NEJM 1995:335

Astemizole-induced Arrhythmia.
From Vorperian et al., JACC 1996:15
Fruit flies provided insight to arrhythmia

**EAG Gene: Ether-sedated Drosophila (Fruit Flies)**

Leg shaking EAG mutant (*ether-a-go-go*)

Wild type

B. Ganetzky
hERG is a member of the EAG superfamily of $K^+$ channels

The **hERG** gene is linked to Long QT Syndrome

**Summary**

The hERG gene encodes IKr
Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole

H. Suessbrich, S. Waldegger, F. Lang, A.E. Busch*

Physiologisches Institut 2, Albert-Ludwigs-Universität, Zülpicher Str. 4, 74076 Tübingen, Germany.

Received 18 March 1996, revised version received 22 March 1996.

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**Arrhythmogenic drugs block hERG channels and prolong the cardiac AP**

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**Zhou and January, 1997**
Guidance for Industry

S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

Contains Nonbinding Recommendations

currently available information. The figure illustrates the component elements of the testing strategy, but not specific test systems or their designs.

1. **In Vitro I_{Kr} Assay (2.3.1)**

An in vitro I_{Kr} assay evaluates the effects on the ionic current through a native or expressed I_{Kr} channel protein, such as that encoded by hERG (see section III.B (3.1.2)).

2. **In Vivo QT Assay (2.3.2)**

Highly sensitive with questionable specificity
Comprehensive in-vitro Proarrhythmia Testing

The Future

Comprehensive in-vitro Proarrhythmia Assessment (CiPA)

1. Assess effects on multiple individual ion channels
2. Model effects (if any) on the ventricular action potential and proarrhythmia
3. Verify conclusions with cardiomyocyte recordings

http://www.ilsieextra.org/hesi/science/cardiac/cipa
• Product launch → regulatory evaluation in 3 years

• iPS cell-derived cardiomyocytes are being evaluated for use in arrhythmia assessment & as a replacement for thorough QT studies
Cellular Dynamics International (CDI) is the world’s largest producer of human iPS cells and iPS cell-derived cell types.

- Headquartered in Madison, WI
- Currently employs ~138 total staff
- ~650 yrs human stem cell experience
- >800 patents (owned or licensed)

Core competencies:
- Creation and culture of human iPS cells
- Normal and disease phenotypes
- Genetic engineering of iPS cells
- Lineage and pathway-specific markers can be introduced
- Development of new differentiation protocols
- Differentiated cells from all three germ layers
- Manufacture of human iPS cell-derived cell types
- Scalable production of highly purified cells

Partnership with iPS Academia Japan enables access and support for CDI’s products in Japan.
Human Cardiomyocytes

- >95% pure, cryopreserved, ready to use
- >4x10^6 cardiomyocytes per unit
- Normal human biology
- Broad platform utility for life science research, drug discovery and toxicity testing
- Improved workflow with greater predictivity
- Full product solution; unlimited quantities
iCell Cardiomyocytes
Characterization

Whole-Genome Gene Expression

Protein Expression

Metabolism

Relevant & stable over time in culture
Recapitulates normal human cardiac function
Appropriate for interrogating mitochondrial toxicity

Electrophysiology, E-C Coupling, Contractility

iCell Cardiomyocytes native human biology enables:

- Mechanistic interrogation of cardiac function
- Toxicity testing; disruption of normal processes
- Disease modeling; corruption of normal processes
- Well represented in the peer reviewed literature

~40 iCell Cardiomyocytes pubs to-date
More than all other commercial iPSC-CMs combined

Babiarz et al., 2012, Kattman et al., 2011, Rana et al., 2012, Ma et al., 2011
(For a complete list of iCell Cardiomyocytes publications go to www.cellulardynamics.com)
Quality

- Exhibit key cellular characteristics
- Recapitulate normal human biology
- Reproducible
- Known and relevant genotype

Quantity

- Sufficient to support HTP drug screening and safety testing
- Currently 1Bn iCell Cardiomyocytes/day

Purity

Days in Culture

Cell Purity

Target Cell (non proliferating)

Non-Target Cell (proliferating)
Standardization
Manufacturing Benchmarks

Scale-Up Manufacturing
- Quality
- Quantity
- Purity

CDI Manufacturing Benchmarks (cells per day, >95% purity)
- 2 billion iPS cells
- 1 billion cardiomyocytes
- 1 billion neurons
- 0.5 billion endothelial cells
- 0.4 billion hepatocytes

Scale-Out Manufacturing
- 1000’s of individuals
- Billions of cells

NHLBI Next Generation Genetic Association Studies (RFA-HL-11-066)
- 250 patient samples - HyperGEN cohort
- GWAS – Left Ventricular Hypertrophy (LVH)
- Derive iPS cells and cardiomyocytes from all 250 individuals
- Induce hypertrophy phenotype, perform molecular analyses
- Correlate GWAS findings with in vitro phenotype
NHLBI Next Generation Genetic Association Studies (RFA-HL-11-066)

- 250 patient samples – HyperGEN cohort
- GWAS – Left Ventricular Hypertrophy (LVH)
- Derive iPS cells and cardiomyocytes
- Induce hypertrophy, perform molecular analyses
- Correlate GWAS findings with in vitro phenotype

Progress as of July 2014:

- 250 donors reprogrammed
- Differentiation protocol optimized to work robustly across all lines
- 128 iPS cell lines (1 per donor) are differentiated or in progress
- Cardiomyocytes from 89 donors cryopreserved & all pass QC
- 20 batches of cardiomyocytes are in currently being tested in hypertrophy assays

Initial data show Et-1 EC50 correlation with progression of disease (Uli Broeckel, MCOW)

CDI’s iPSC technology is enabling population studies
California Institute for Regenerative Medicine (CIRM)

Human iPS Cell Initiative – 3 Awards
- Sample Collection, iPSC Derivation (CDI), iPS Cell Banking

iPS Cell Derivation (CDI)
- 3000 donors (healthy & disease phenotypes)
- 3 iPS cell clones per donor
- Disease categories: epilepsy, autism, cerebral palsy, cardiomyopathy, Alzheimer’s disease, eye diseases, hepatitis (HCV), non-alcoholic steatohepatitis (NASH), pulmonary fibrosis
- Derived from peripheral blood (preferred) or skin fibroblasts
- Episomal “footprint-free” method

CDI – Coriell Partnership
- Extensive collaboration to bring together expertise in electronic record-keeping, sample tracking, iPS cell derivation & characterization, cell banking & distribution
- Joint facility located within the Buck Institute, Novato, CA

Demonstrated success in generating high quality iPSC ‘populations’
Well poised to enable population diversity in toxicity and safety assessments
### QMS Framework Overview

<table>
<thead>
<tr>
<th>Key Systems</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>QA/QC</td>
<td>Compliance and product consistency</td>
</tr>
<tr>
<td>Standard Operating Procedures</td>
<td>Consistent procedures</td>
</tr>
<tr>
<td>Calibration/Qual/Val</td>
<td>Equipment/facilities/processes fit for intended use</td>
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<tr>
<td>Change Management</td>
<td>Changes are documented, assessed for risk, and tested</td>
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<tr>
<td>CAPA</td>
<td>Report, correct, and prevent product quality issues</td>
</tr>
<tr>
<td>Supplier Qual &amp; Mgmt</td>
<td>Quality and reliability of raw materials</td>
</tr>
<tr>
<td>Materials Management</td>
<td>Control, trace, and monitor stock inventory</td>
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<tr>
<td>Training</td>
<td>Education and proficiency</td>
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<tr>
<td>Complaint Handling</td>
<td>Customer satisfaction and continuous improvement</td>
</tr>
<tr>
<td>New Product Introduction</td>
<td>Improve likelihood that product meets market need</td>
</tr>
</tbody>
</table>

- An ISO / GMP hybrid QMS system ensures customer safety and satisfaction


iCell Cardiomyocytes provide a human system for measuring all aspects of EC functionality.
“MEA assays using iPSC-CMs offer a reliable, cost effective, surrogate to preclinical in vitro testing, in addition to the 3Rs (refine, reduce, and replace animals in research) benefit”

Harris et al, Toxicol Sci 2013

<table>
<thead>
<tr>
<th>Compound</th>
<th>iPSC within ½ log of wedge, in some cases more sensitive</th>
<th>MEA/wedge fold difference (Log units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine repolarization</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>Cisapride repolarization</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Terfenadine repolarization</td>
<td>1</td>
<td>−1*</td>
</tr>
<tr>
<td>Terfenadine conduction</td>
<td>1</td>
<td>−1*</td>
</tr>
<tr>
<td>Verapamil repolarization</td>
<td>0.03</td>
<td>−1.52*</td>
</tr>
<tr>
<td>Pleoainide conduction</td>
<td>1</td>
<td>−0.47*</td>
</tr>
<tr>
<td>Pleoainide repolarization</td>
<td>3</td>
<td>−1*</td>
</tr>
<tr>
<td>Quinidine repolarization</td>
<td>0.1</td>
<td>0.47*</td>
</tr>
<tr>
<td>GSK A repolarization</td>
<td>10</td>
<td>−1*</td>
</tr>
<tr>
<td>GSK B repolarization</td>
<td>0.6</td>
<td>n/a due to beat rate changes</td>
</tr>
</tbody>
</table>
summary

- **Proarrhythmia Testing**
  - moving toward a cellular, mechanistic approach that may take advantage of stem cell cardiomyocytes

- **iCell Cardiomyocytes**
  - Human biology validated in the peer-reviewed literature

- **Population Diversity**
  - Well poised to enable population testing in toxicity and safety assessments

- **Key manufacturing components**
  - Quality is king!
New techniques for MEA-based assays to support CiPA and in-vitro safety pharmacology assays

October 21, 2014

Jim Ross, PhD
CTO, Axion Biosystems
Agenda

1. Introduction to Axion
2. Brief MEA Technology Review
3. Role of MEAs in CiPA
4. Arrhythmia assessment
5. Cardiomyocyte Pacing
6. New developments
Axion’s Objective: Enable Great Science
Complicated Network Electrophysiology Made Accessible

- Questions
- Experiments
- Quality Control
- Analysis
- Answers
Applications Driven Innovation
High Performance, Scalable Technologies that are Simple to Use

Engineering

Electronics

Sensors

Software

Applications
CiPA Objective

- Ion Channel Panel
- In Silico Simulations
  
  → Proarrhythmia Score
  
  Mechanism Based,
  Continuous Scale,
  Rank Ordered,
  Contextual Data
  
  ← Integrated Human
  Cellular Studies

↓

Clinical Assessment
Human ECG
Myocyte Objective

1. Repolarization
2. Arrhythmia
3. Scalability/Reproducibility
4. Risk Assessment

- Ion Channel Panel
- In Silico Simulations
- Integrated Human Cellular Studies
- Clinical Assessment Human ECG
CM-MEA bridges the gap between AP and ECG
Criteria for monitoring cardiac networks

(1) Real-time measurement of the phenotypic signal: voltage
(2) Sufficient resolution to capture field potentials
(3) Multiple recording sites to improve reliability and assess field potential propagation
(4) Label-free, non-invasive operation to observe natural cell function
(5) Preservation of cellular interconnectivity
A grid of microelectrodes interfaces with electro-active tissue, modeling complex, human systems in a dish.
CM-MEA Assay

Measures: Field potential duration ("QT"), Conduction velocity, Beat rate, Field potential metrics (Amplitude, Slope, etc.)

Applications: Cardiac Safety Screening, Drug Discovery, Functional Disease Models, Stem Cells, Patient-specific Therapies
MEAs in CiPA: Current Status
Characterizing cross-site reliability of CM Assays

**MEA Pilot Studies beginning in October 2014:**

12 Pilot Sites, including Cyprotex, Bristol-Myers Squibb, Chantest, NCI, Janssen (JNJ), Merck, NMI, Sanofi, and others

11 of 12 test sites are independent from the MEA supplier
Tracking Repolarization
Direct Measures of Depolarization & Repolarization

Action Potential

Field Potential

max Slope

max ΔSlope

FPD
Tracking Repolarization
Direct Measures of Depolarization & Repolarization

Action Potential

Field Potential

max Slope

max ΔSlope

FPD

ΔFPD
Continuous monitoring enables evaluation of stable spontaneous beating. Red data points highlight the most stable region of spontaneous beating between 15 – 20 minutes post dose, avoiding a drift in beat rate at 18 minutes (red square).
Detecting Arrhythmia
Beat Irregularity

Beat irregularities can emerge and disappear over time.

Dofetilide

Quinidinate
Detecting Arrhythmia
Arrhythmic Event Classification

Beat Irregularity
Notch/EAD
Alternans & Triggered Activity

MEA classifies aberrant events

Quinidine 1µM
Navarrete 2013

Nakamura 2014

Harris 2013

1000 nM
Detecting Arrhythmia
Advantages for Label-Free Assessment

1 minute dye-load; Serum-containing media
Label-free assessment minimizes perturbations to cardiomyocytes
Detecting Arrhythmia
Advantages for Label-Free Assessment

Persistent dye; Serum-containing media
Label-free assessment minimizes perturbations to cardiomyocytes
Pacing with AxIS 2.0

Evoked field potentials in a high throughput instrument
Pacing: Entraining Cultures

Cardiomyocytes rapidly entrain to electrical stimuli
Pacing: Specifying Beat Period

FPD shortens as pacing rate increases.
Changes in FPD occur over the timescale of minutes...
Pacing: Practical Considerations

...but are reliable across beats and across wells
Pacing: Improving Accuracy

Control of beat rate reduces well-to-well variability.

Pacing enables accurate beat rate correction.
Pacing: Adding Depth

Reverse Use-Dependence

Pacing uncovers reverse use-dependence of candidate compounds.
Pacing: Controlling Propagation

Pacing establishes consistent propagation patterns
Pacing: Controlling Conduction

Pacing establishes consistent conduction measures
Questions?

The Maestro