We’ve crossed the Chasm and climbed out of the Trough.
Advances and advantages with iPSC-derived cell types in drug discovery.

Blake Anson Ph.D.
Oct 31, 2014
Where is stem cell technology as it applies to drug discovery?

*Particularly iCell® Products*

**CDI Overview**

**Sampling of iCell Product use in Industry**

*Toxicity and Drug Discovery*

**Population Diversity**

**Wrap-up**
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) to enable FTO

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
- **iCell Products**
  - iCell Cardiomyocytes
  - iCell Cardiac Progenitor Cells *(New)*
  - iCell Hematopoietic Progenitor Cells
  - iCell Endothelial Cells
  - iCell Hepatocytes
  - iCell Neurons
  - iCell Astrocytes
  - iCell DopaNeurons *(New)*
  - iCell Skeletal Myoblasts

- **MyCell Products**
  - iPS Cell Reprogramming
  - iPS Cell Genetic Engineering
  - iPS Cell Differentiation
  - MyCell Disease and Diversity Panel *(New)*

---

**Essential 8 Medium**
Episomal Reprogramming Kit
Vitronectin
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**
- Relevant & stable over time in culture

**Protein Expression**
- Recapitulates normal human cardiac function

**Metabolism**
- Appropriate for interrogating mitochondrial toxicity

**Electrophysiology, E-C Coupling, Contractility**
- Enables mechanistic toxicity testing

**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)


---

iCell Cardiomyocytes
Market Validation (8/2014)

~40 Peer-reviewed Publications (10/2014):
- Characterization
- Toxicity testing
- Disease modeling
Three main areas need to be considered for cardiotoxicity:

- **Electrical**
  - Electrical activity in the heart involves the conduction of impulses through the atria, AV node, and ventricles.
  - Different regions of the heart have distinct electrical potentials, as shown in the diagram.

- **Biochemical**
  - Biochemical processes are crucial for generating the electrical activity and maintaining cardiac function.
  - Key enzymes and pathways are involved in the regulation of ion channels and the generation of intracellular messengers.

- **Mechanical**
  - Mechanical activity is responsible for the force generation and contraction of cardiac muscle fibers.
  - The interaction between electrical and biochemical signals leads to the mechanical contraction of the heart.

These interconnected processes are essential for maintaining normal cardiac function and are critical to understand in the context of cardiotoxicity.
Predicting Proarrhythmia
Label Free Impedance Measurements

Label-free impedance measurements
Gold electrode

Arrhythmia Screening in 96-wells
Cardiomyocyte activity is detected as rhythmic deflections in the data tracing

Qualitative Assessment

0.1% DMSO
Aspirin
Aconitine
Alfuzosin
Astemizole

Guo et al., 2011

Relevant biology and metrics leads to greater predictivity

- Expanded dataset
  - ~120 compounds

- Fine tune metrics
  - Include beat rate, atypical beats, onset of IB20
  - Use concentration thresholds or IB20 rank ordering

Guo et al., 2013

Greater Predictivity
~120 Compounds
>90% -- QT prediction
>82% -- arrhy. prediction

iCell Cardiomyocytes provide a more predictive tool for detecting proarrhythmia
iCell Cardiomyocytes provide a predictive tool for detecting KI toxicity
Conventional Interrogation

IonOptix
- Good to excellent validation parameters
- Primary culture from dog heart
- Low throughput

1 AR Harmer. Tox App Pharm 2012

Screening with iCell Cardiomyocytes

xCelligence RTCA
- Good to excellent assay parameters
- Human cardiomyocytes
- Medium to high throughput

Parameter | IonOptix |
---|---|
sensitivity | 83% |
specificity | 84% |
accuracy | 82% |
pos predict | 90% |
eg neg predict | 76% |

Parameter | Impedance³ |
---|---|
sensitivity | 90% |
specificity | 74% |
accuracy | 84% |
pos predict | 85% |
eg neg predict | 82% |

49 compound validation set with actives and inactives

2 C. Scott (Tox Sci, 2014)

iCell Cardiomyocytes provide a predictive model for detecting contractility
iCell Cardiomyocytes and xCelligence RTCA: Predictive solutions for multi-modal cardiotoxicity
Case #1: Cardiac Hypertrophy
*iCell® Cardiomyocytes*

**Cell Size**

**Cytoskeletal Rearrangements**

**Fetal Gene Expression**

*iCell Cardiomyocytes exhibit classic hallmarks of cardiac hypertrophy*
iCell Cardiomyocyte Hypertrophy

Relevance

Aggarwal et al., Plos One 2014

iCell Cardiomyocytes are a relevant system for cardiac hypertrophy
Case #2: Diabetic Cardiac Myopathy
Environmental and Innate Induction

Application of a diabetic medium (ET-1, cortisol, glucose) to iCell CMs induces a hypertrophic phenotype.

Increases in:
- Cell and nuclear size
- Cytoskeletal disorganization
- Glycolysis
- Lipid accumulation
- ROS Accumulation

Diabetic patient iPSC-cardiomyocyte show a hypertrophic phenotype under basal conditions.

Compounds have been identified that revert the diabetic phenotype present in the iPSC-CMs.

Drawnel, et al., Cell Reports 2014

iCell Cardiomyocytes can be used for induced and innate disease modeling.
iCell Cardiomyocytes were used to verify a potential therapeutic target for human dilated cardiomyopathy

Over-expression and knockdown of target in iCell Cardiomyocytes affected related proteins

Over-expression of target increases function

Impedance measurements show rhythm and contractile phenotype

Phenotypic screens in iCell Cardiomyocytes were utilized to show functional effects of target modulation

ILK is in a protein scaffold from control, but not DCM cardiac tissue.

Does this represent a potential DCM targets?

iCell Cardiomyocytes provided a model for in-vitro target validation and subsequent functional screens
iCell Neurons; pure, functional, human neurons made at scale
iCell Neurons are a mixed population of cortical GABAergic and Glutamatergic neurons that enable new in-vitro research efforts.

- Alzheimer’s Research
- Autism Investigations
- Latent VZV Infection
- Botox batch release testing

5 ml Pellet of Pure Neurons = ~4 Billion Neurons
The Power of IPSC Technology

What about....

...populations?
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 (7 awardees)
- iPS Cell Derivation (CDI)
- iPS Cell Banking (Coriell; CDI primary subcontractor)

iPS Cell Derivation
- 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
● 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

Nature Reviews Drug Discovery (Aug, Sept 2013)
• Right species - *human*
• Essential functionality
• Active use in toxicity testing and drug discovery
• Scalable and applicable to populations
• Involved in the changing regulatory landscape