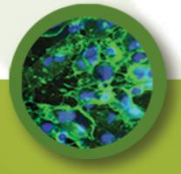
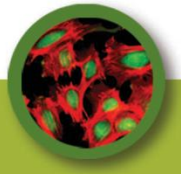
Cellular Dynamics international

# Induced Pluripotent Stem Cell-Derived Tissues and their Role in Developing Novel Assays for Drug Discovery

*Kyle Kolajaj*

*March 14th 2013*

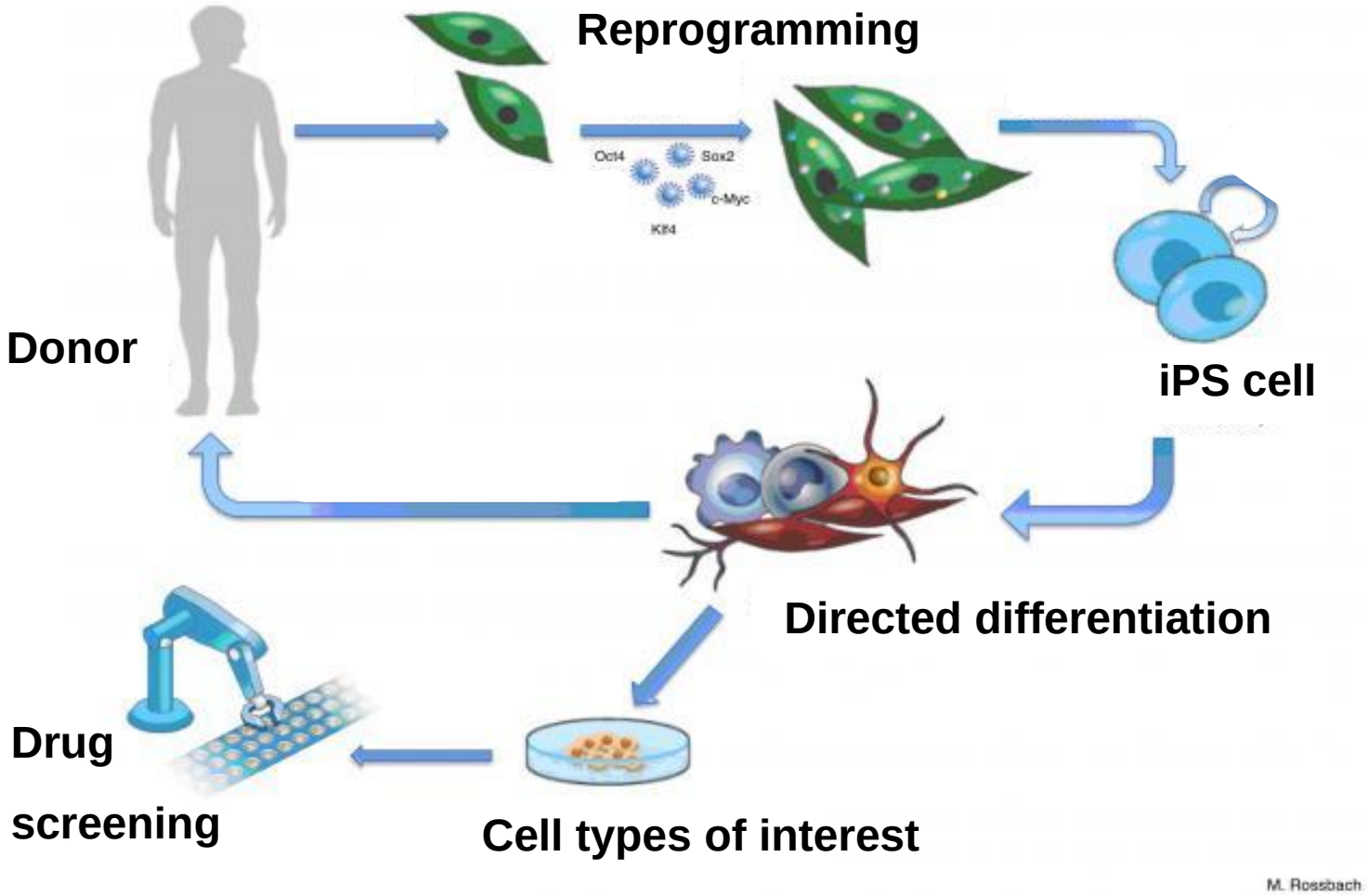




# Overview

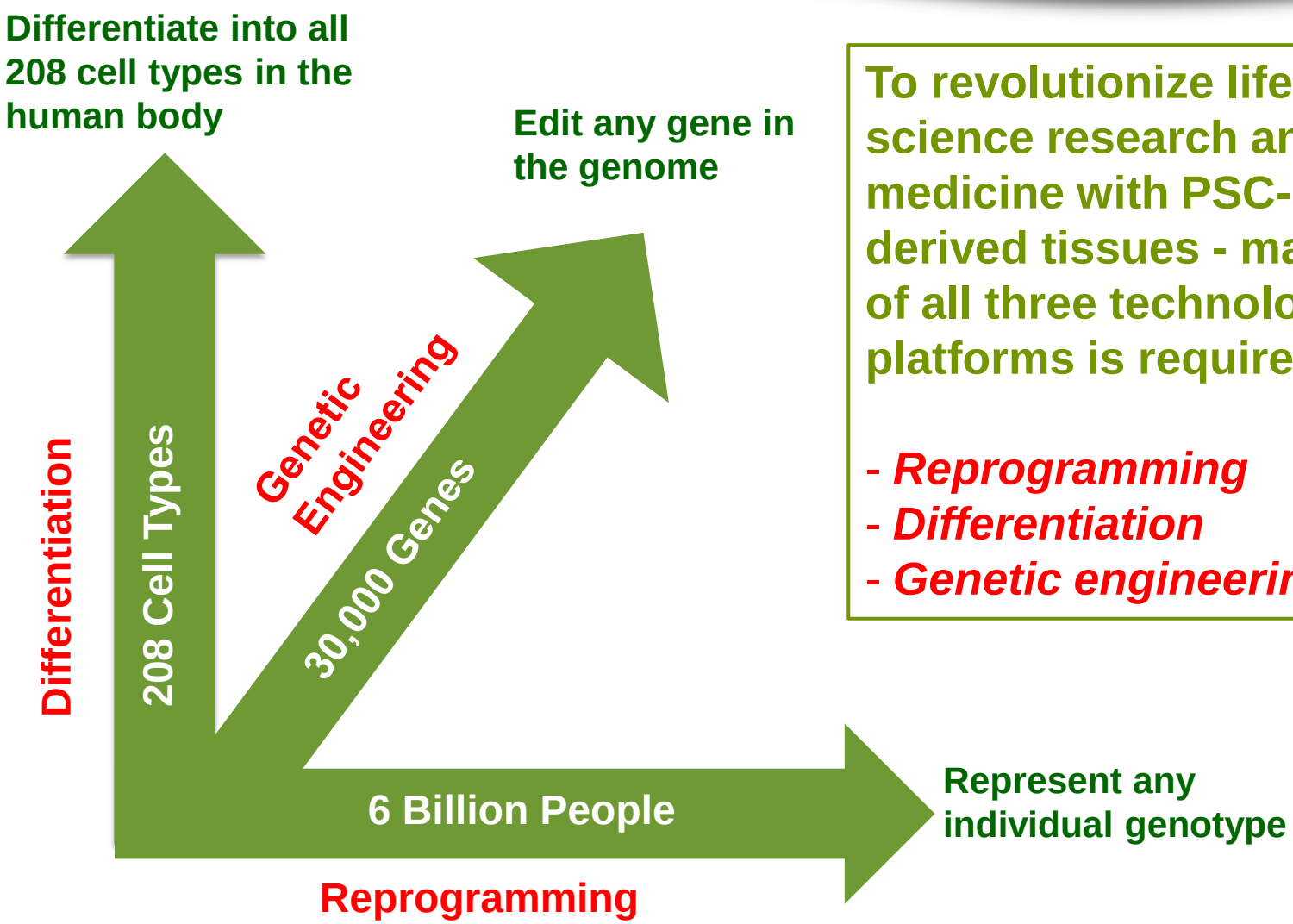
- **Company Overview**
  - Core competencies
  - Cell types
  - iPS cells
- **iCell Cardiomyocytes**
  - Manufacture/Characterization
  - Arrhythmia model
  - Hypertrophy model
  - NHLBI grant with MCW
- **iCell Neurons**
  - Manufacture
  - Botulinum Toxin
  - Discovery Applications
- **iCell Hepatocytes**
  - Applications
- **Conclusions**

# The Potential of iPS Cells



# iPS Cell Technology

*Revolutionary Access to Human Biology*




**To revolutionize life-science research and medicine with PSC-derived tissues - mastery of all three technology platforms is required:**

- ***Reprogramming***
- ***Differentiation***
- ***Genetic engineering***

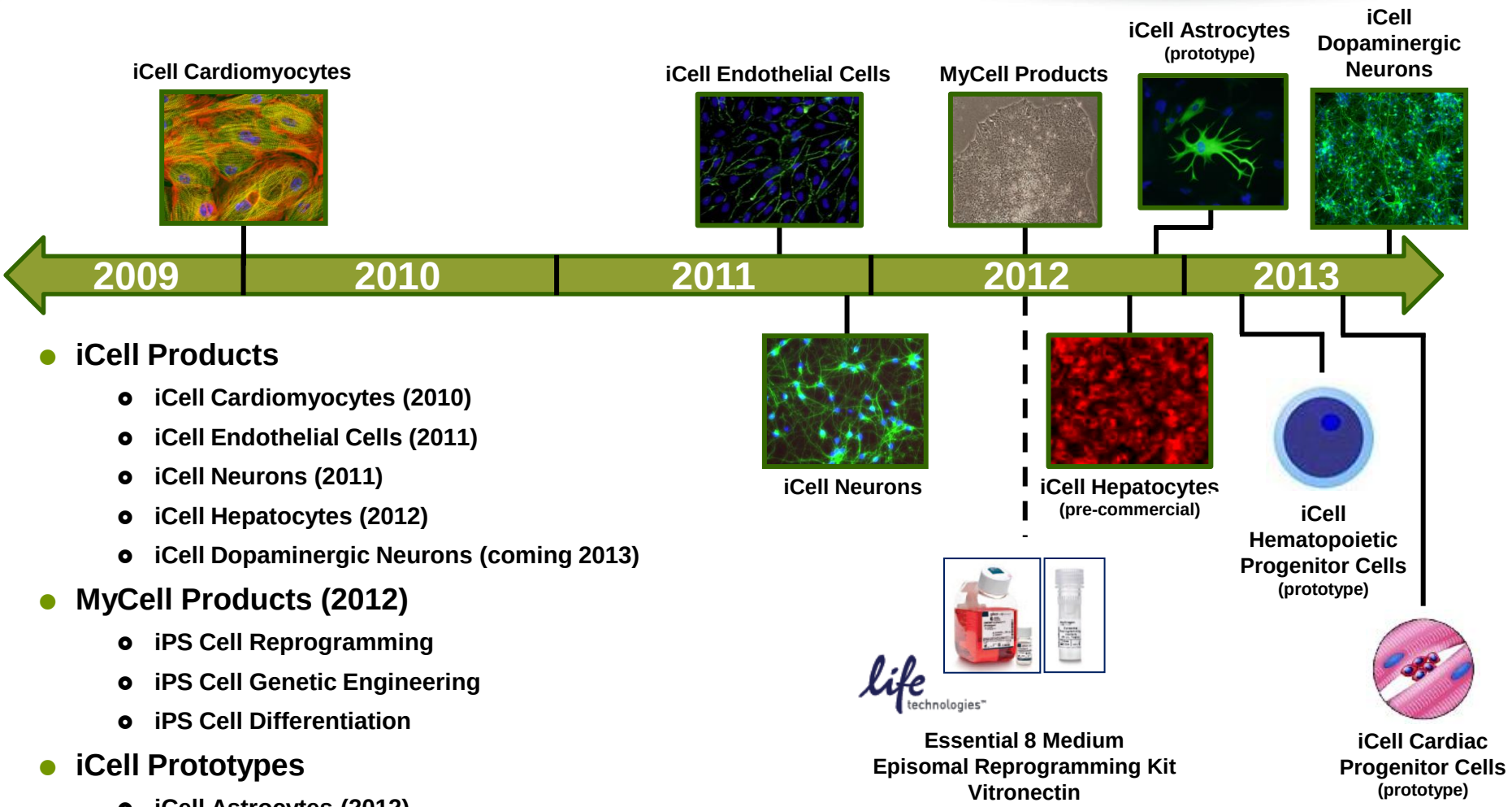
# Company Overview

- **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 ~116 total staff (mostly scientists)**
- **~550 yrs human stem cell experience (in a field ~15 yrs old)**
- **>700 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**

**The Wall Street Journal**
**Gold Winner: CDI**
**Technology Innovation Awards 2011**

# Product Portfolio

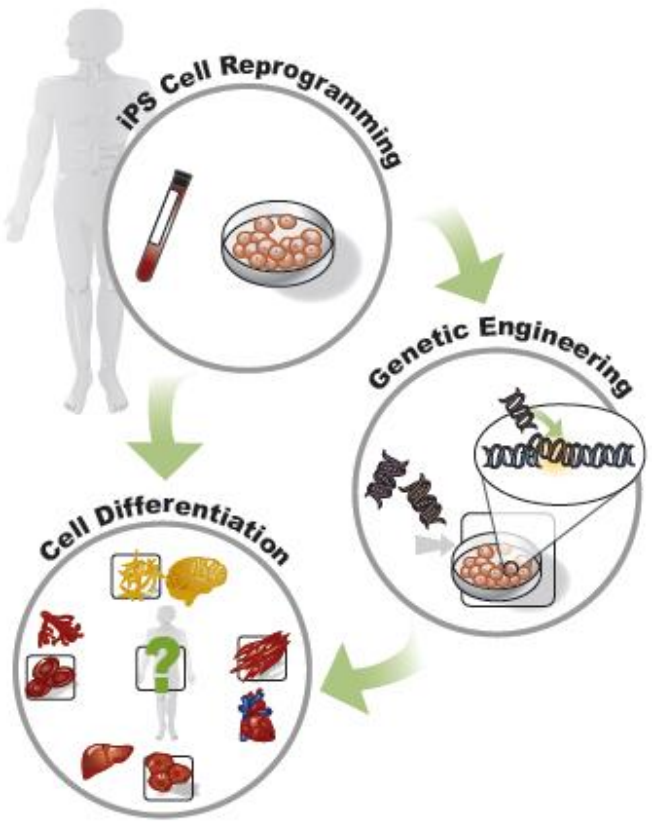
*2010 -2013*




- **iCell Products**
  - iCell Cardiomyocytes (2010)
  - iCell Endothelial Cells (2011)
  - iCell Neurons (2011)
  - iCell Hepatocytes (2012)
  - iCell Dopaminergic Neurons (coming 2013)
- **MyCell Products (2012)**
  - iPS Cell Reprogramming
  - iPS Cell Genetic Engineering
  - iPS Cell Differentiation
- **iCell Prototypes**
  - iCell Astrocytes (2012)
  - iCell Hematopoietic Progenitor Cells ( coming 2013)
  - iCell Cardiac Progenitor Cells (coming 2013)

# Induced Pluripotent Stem Cells

- **iPS Cell Reprogramming**
  - *Episomal "footprint-free" method*
  - *Only small amounts of peripheral blood or other tissues*
  - *Feeder-free, defined conditions*
- **iPS Cell Genetic Engineering**
  - *Endogenous & safe harbor loci*
  - *HR, ZFN and Talens*
- **iPS Cell Differentiation -> tissues of interest**
- **Uses**
  - Stem cell line banking
  - Ethnically diverse panels
  - Disease models (engineered)
  - Genetically predisposed samples
  - Drug Discovery
  - Retrospective study of Hypo-, Hyper responders, Idiosyncratic toxicities, etc



# Sample Consent and Freedom to Operate

- **"Proper" Consent has matured since Henrietta Lacks (→ HeLa Cells)**
  - Consent
  - Ownership
  - Donor health history and patient identifiabilty
  - Commercialization

- **Intellectual Property and Freedom To Operate**
  - IPS cell patents issued
  - Differentiation and purification methods
  - Engineering methods
  - Fluorescent proteins
  - Applications/Uses
  - Downstream rights

*I hereby give consent to the staff of The Johns Hopkins Hospital to perform any operative procedures and under any anaesthetic either local or general that they may deem necessary in the proper surgical care and treatment of:*

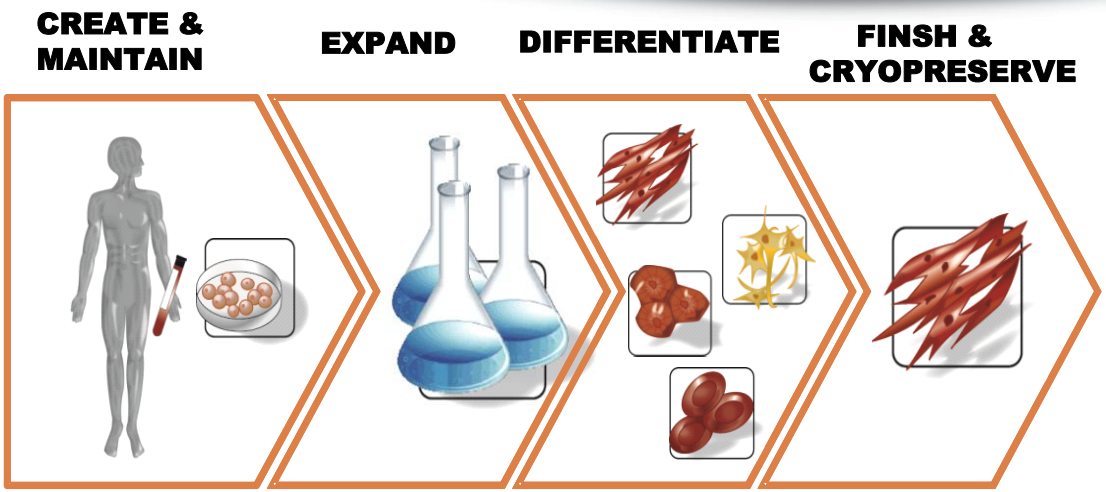
_____.

# Cardiomyocyte Manufacturing Process

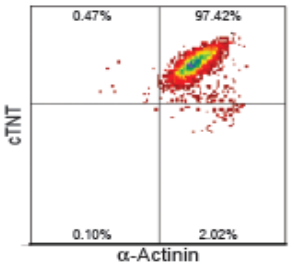
**Cellular Dynamics international**

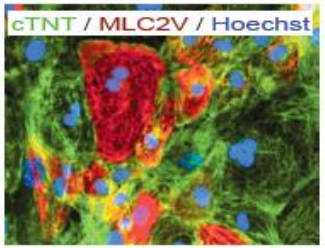
**Cardiomyocyte Production**

- Quality
- Quantity
- Purity

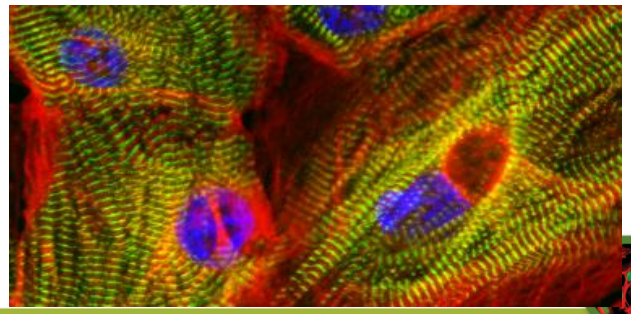



**Purity and Morphology**

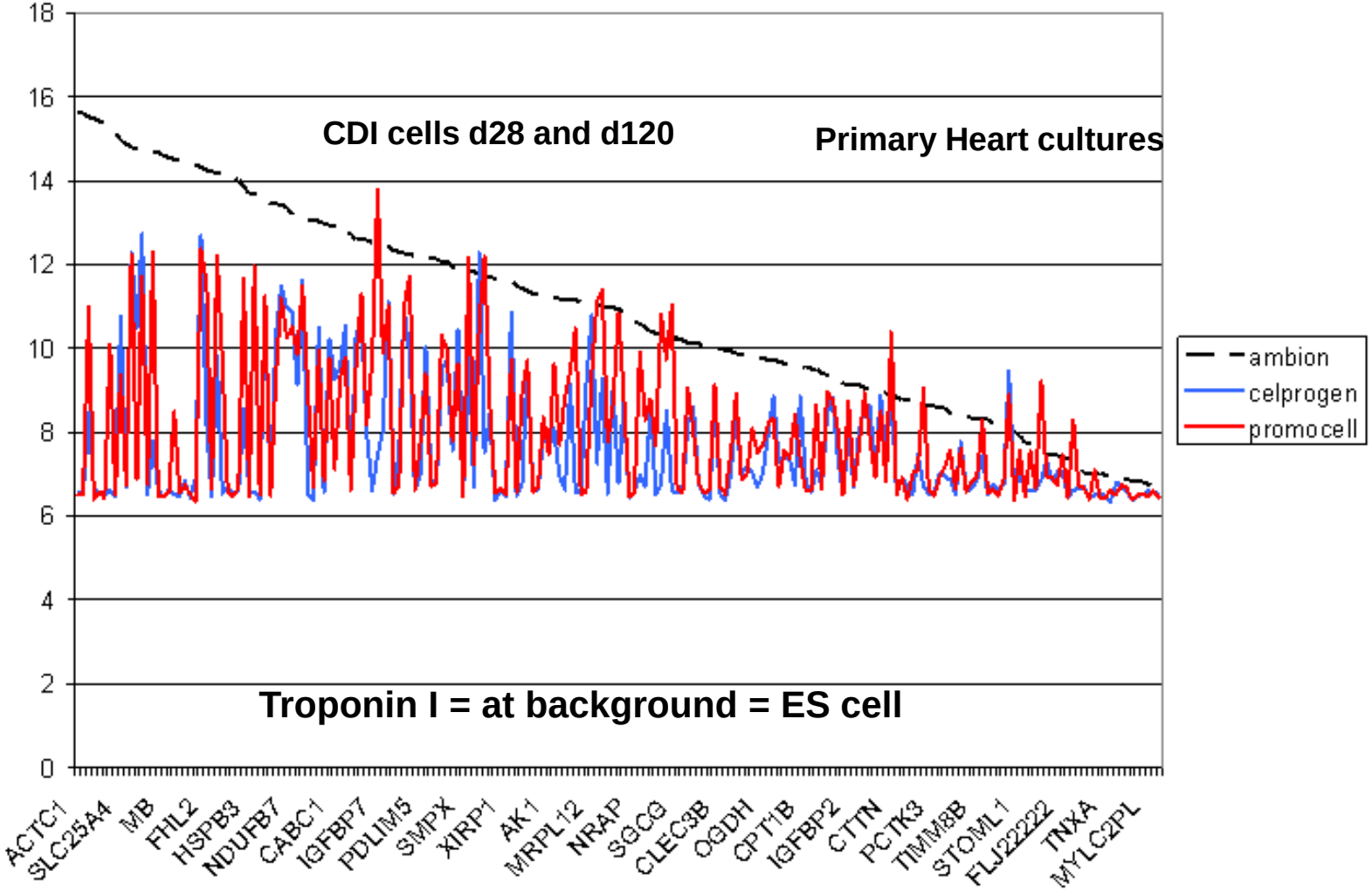



- ‘Spontaneous’ differentiation yields ~2 - 10% purity
- Directed differentiation can yield > 40% purity
- Differentiation with expression-based selection can yield >98% purity
- Cryopreservation makes easier for everyone
- Morphology with sarcomeres & striated myofibers

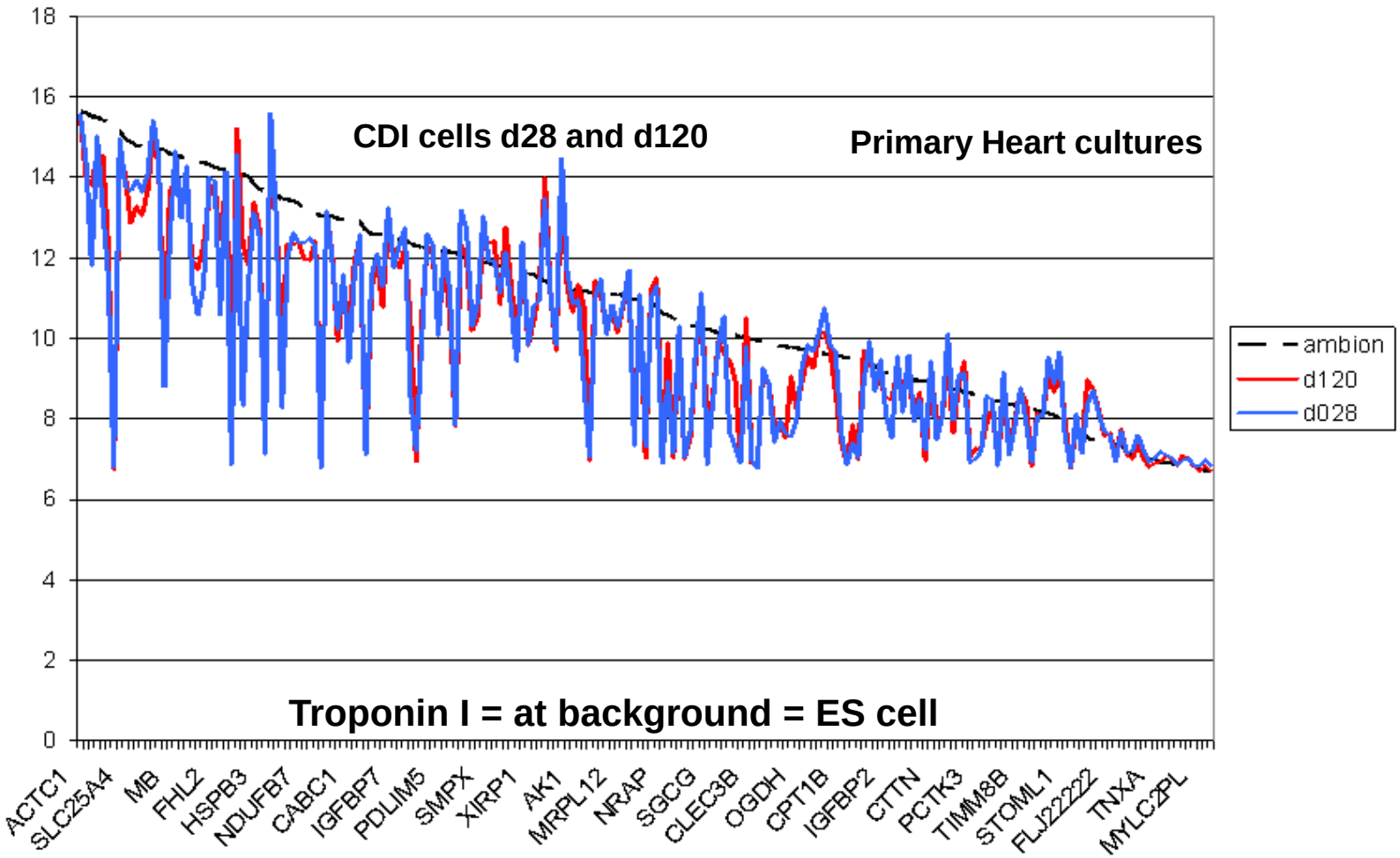


# iCell Cardiomyocytes are more similar to Adult Human Heart Samples than Primary Cultures




Babiarz et al Stem Cells Dev. Jan 2012

# iCell Cardiomyocytes are more similar to Adult Human Heart Samples than Primary Cultures

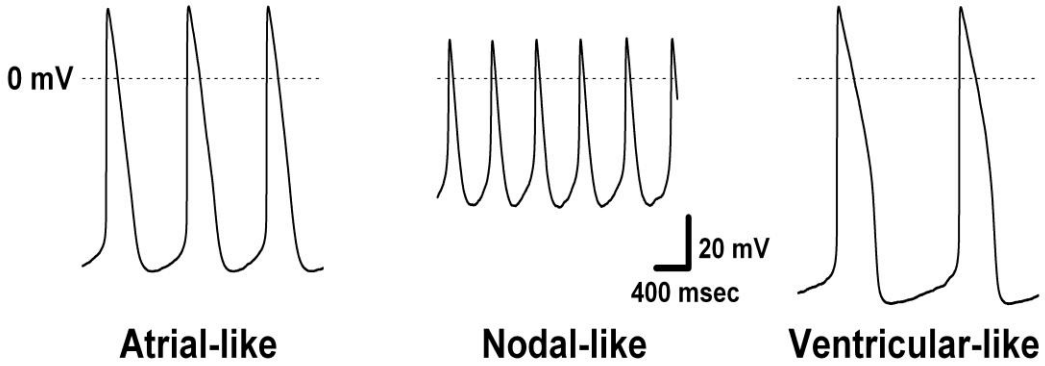



Babiarz et al Stem Cells Dev. Jan 2012

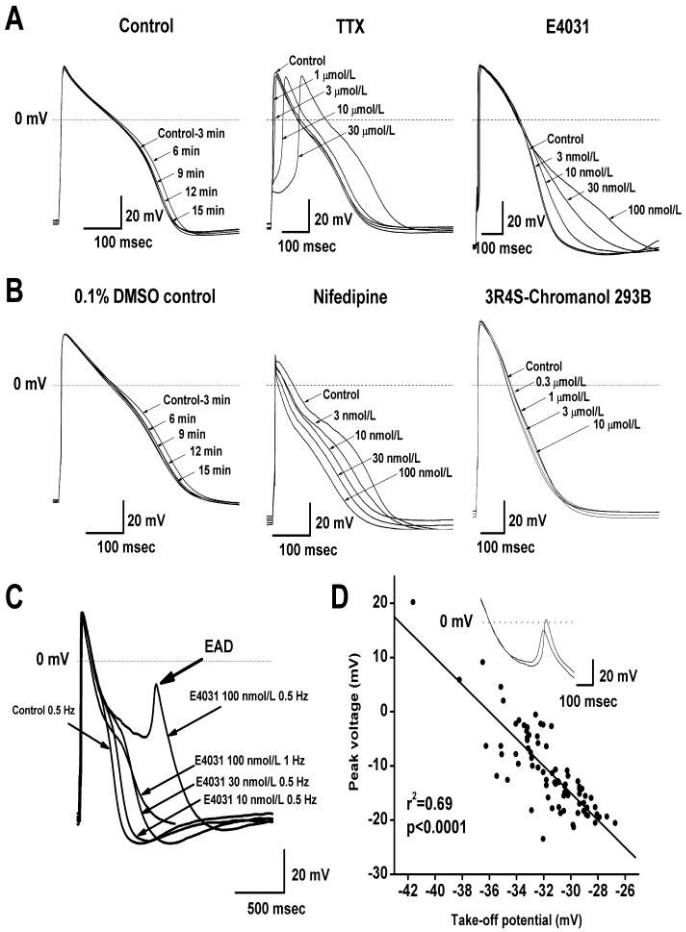
# Confirmation of Cardiomyocyte Nature

## iCell Cardiomyocytes

- Express major cardiac proteins
- Exhibit cardiac action potential wave forms
- Functionally active cardiac channels
- Respond as expected to channel blockers

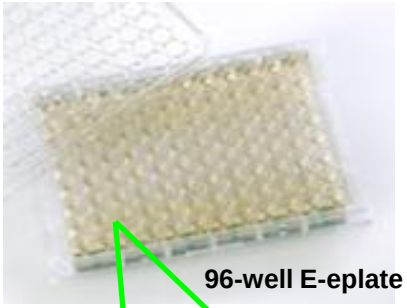


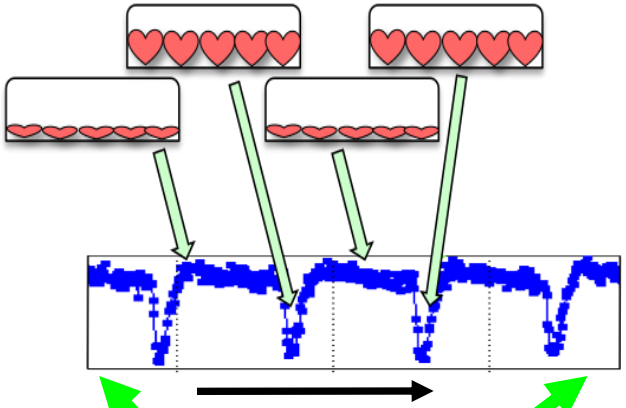

| APs (n=59) | Interval (sec) | MDP (mV) | Peak (mV) | Amp (mV) | $dV/dt_{max}$ (V/sec) | APD10 (msec) | APD30 (msec) | APD90 (msec) | APD30-40 /APD70-80 |
|---|---|---|---|---|---|---|---|---|---|
| Atrial-like (n=13) | 1.2±0.2 | -73.5±1.5 | 26.7±1.4 | 100.2±2.1 | 26.2±3.9 | 60.8±5.5 | 123.1±10.3 | 286.2±21.2 | 1.1±0.1 |
| Nodal-like (n=14) | 1.0±0.1 | -60.1±2.4 | 19.5±2.0 | 79.6±2.9 | 5.5±0.4 | 65.3±18.4 | 111.8±15.9 | 254.7±20.5 | 1.0±0.1 |
| Ventricular-like (n=32) | 1.7±0.1 | -75.6±1.2 | 28.3±1.0 | 104.0±1.1 | 27.8±4.8 | 74.1±4.8 | 180.0±10.7 | 414.7±21.8 | 2.5±0.2 |

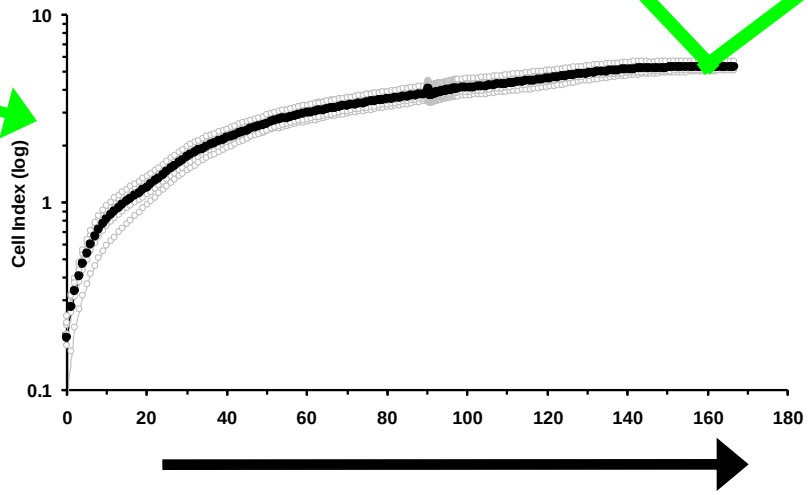



**Ma et. al Am J Physiol Heart Circ Physiol**. (2011) High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents

# Impedence Detection of Cardiomyocyte Beating





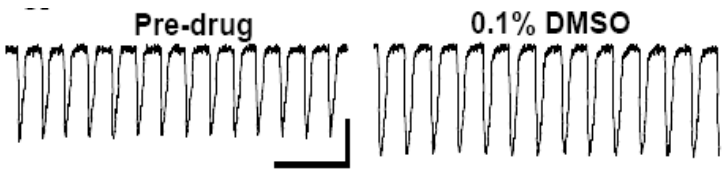



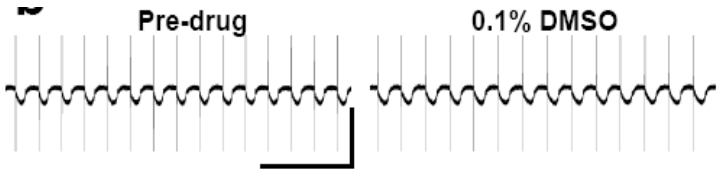
# Drug-induced changes in beat rate

*Indication of functional cardiac ion channels*

**xCELLigence: Impedance** | **MEA: Electrical Field**

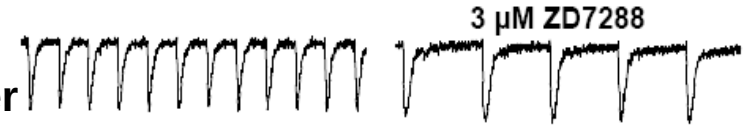
**Control**

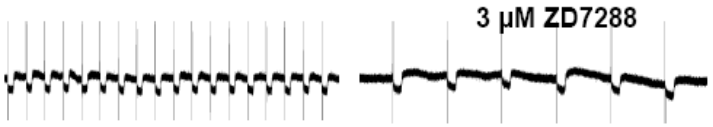


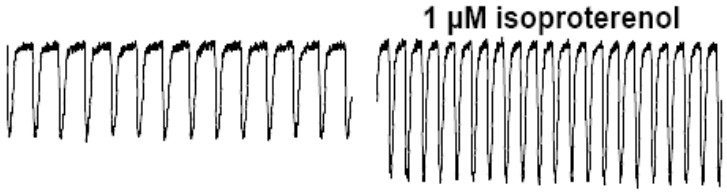

**Na+ Channel Blocker**

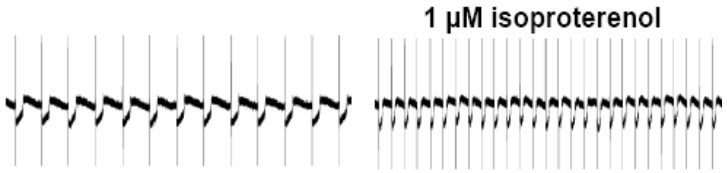
**Pace-maker Current Blocker**



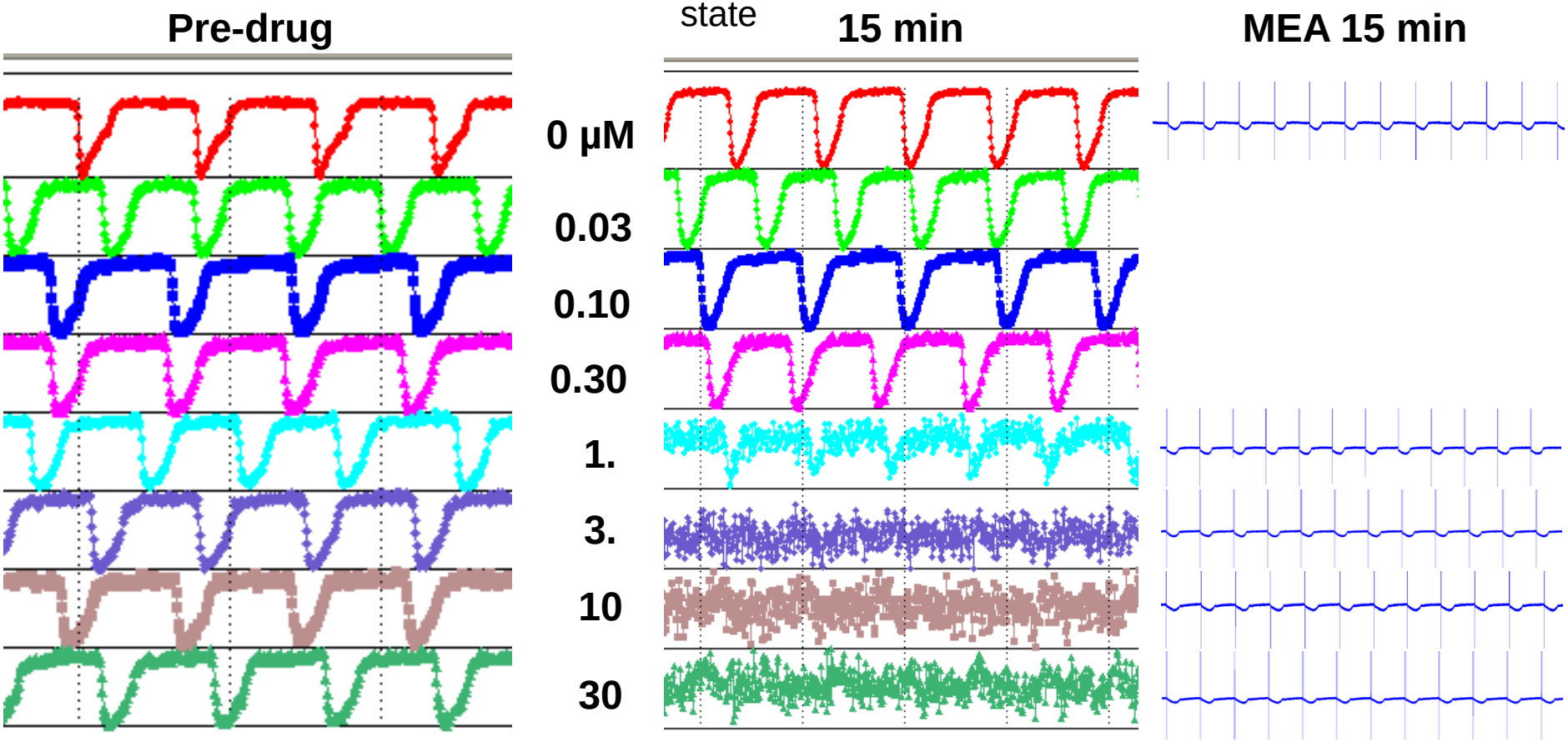

**Agonist of β-Adrenergic Receptor**



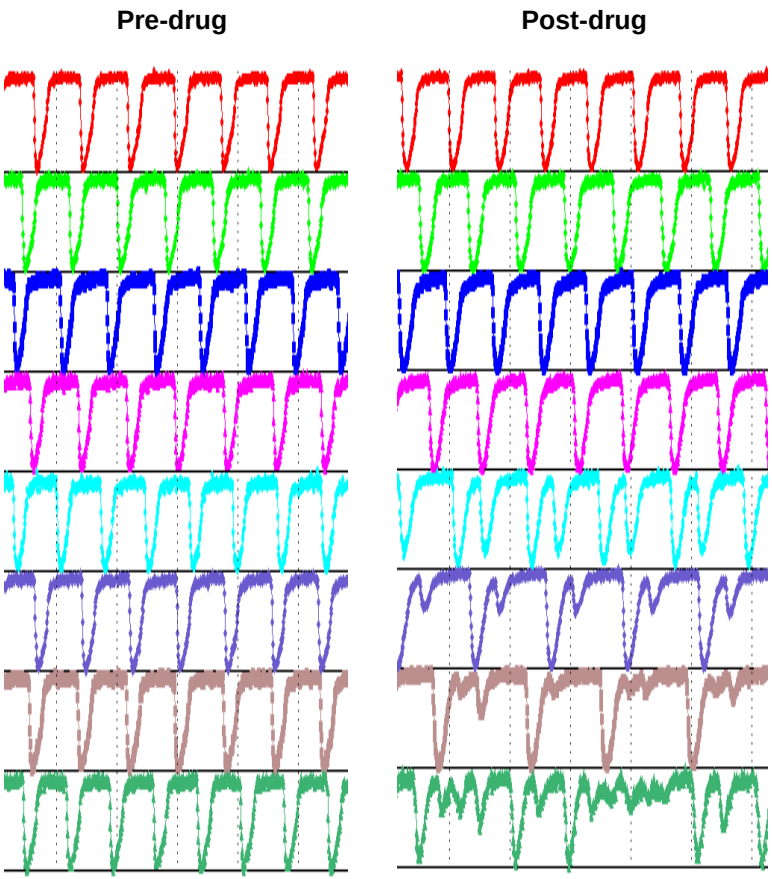



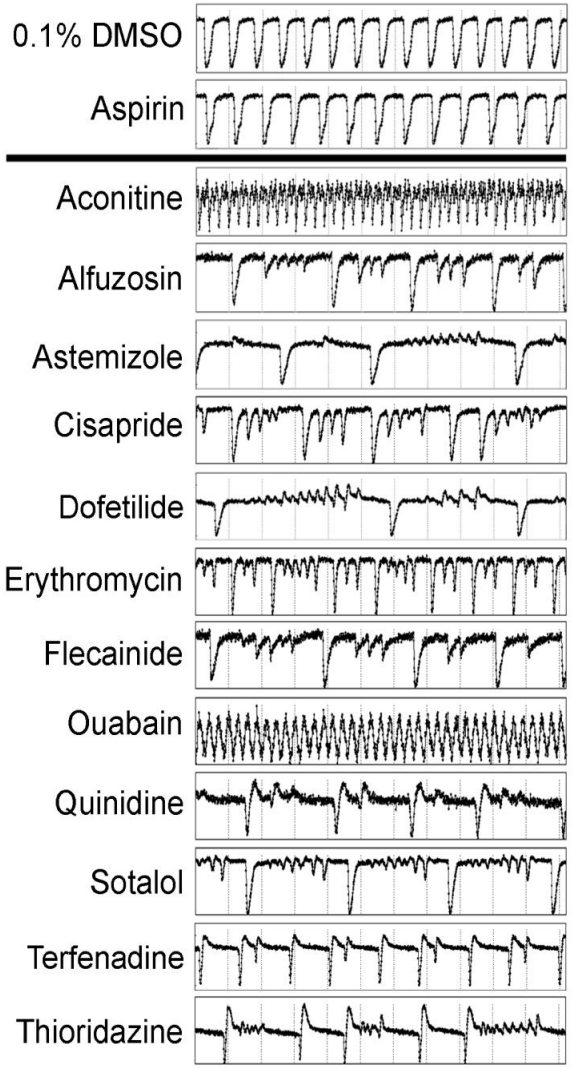
# Does impedance detect the physical beating or the electrical changes?

**Blebbistatin:**
Blocks myosin II in an actin-detached state



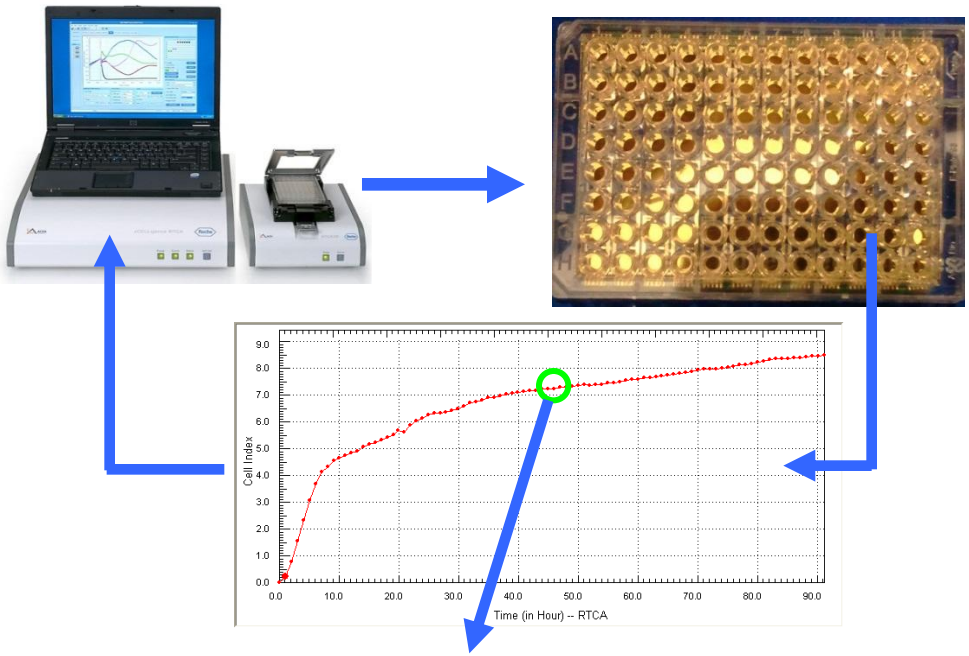
# *In vitro* Detection of Arrhythmias with Human iCell Cardiomyocytes

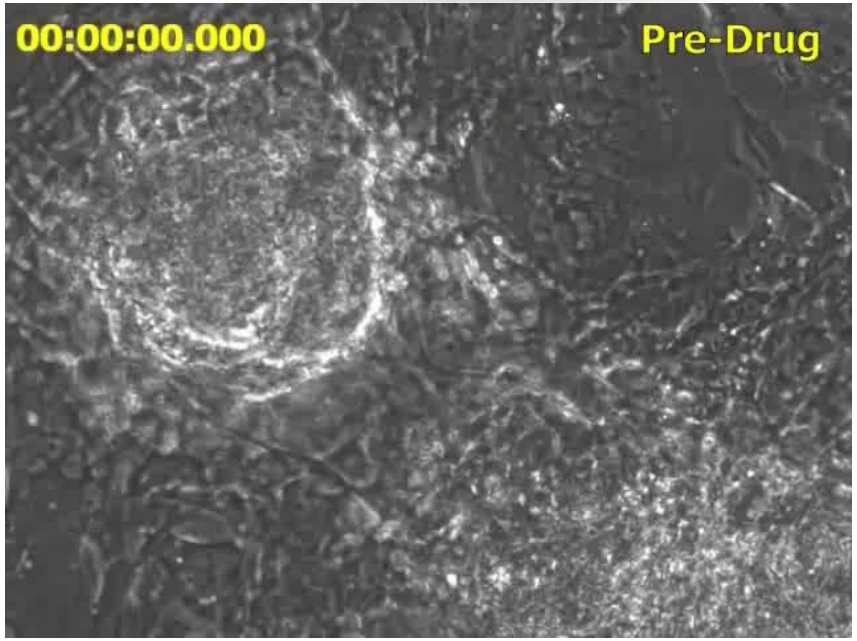


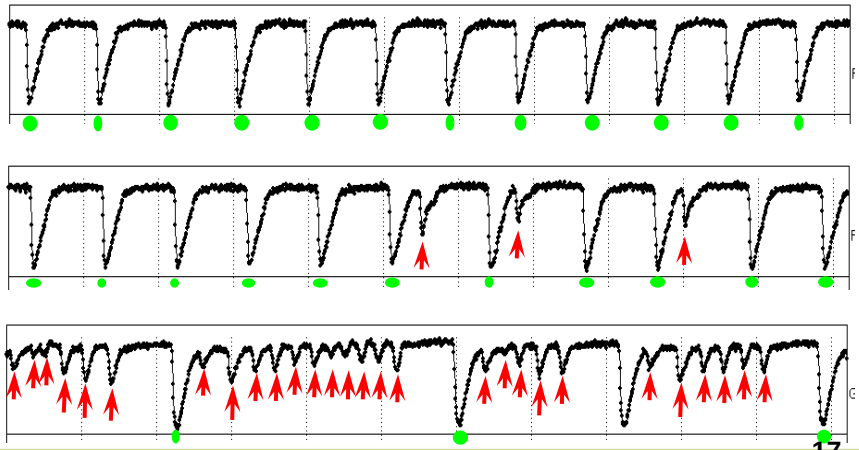



# Human Cardiomyocytes (hCAR) Assay

*iPS cell-derived human cardiomyocytes*



**IB20**= lowest tested concentration resulting in 20% irregular beats

Cellular Dynamics international

# hCAR Assay Validation:

## *23 Commercially available compounds with known in vivo effect*

- 12 Pro-arrhythmic
- 11 Non-arrhythmic
- **IB20 30 uM**
  - **One False Positive**
  - **No False Negatives**

- **IB20= lowest tested concentration resulting in 20% irregular beats**

| Drug | IB20 (µM) | hERG | QT | Clinical arrhythmia |
|---|---|---|---|---|
| Dofetilide | 0.003 | (+) | (+) | (+) |
| Ouabain | 0.03 | (-) | (-) | (+) |
| Aconitine | 0.03 | (-) | (-) | (+) |
| Cisapride | 0.03 | (+) | (+) | (+) |
| E-4031 | 0.03 | (+) | (+) | (+) |
| Astemizole | 0.03 | (+) | (+) | (+) |
| Terfenadine | 0.3 | (+) | (+) | (+) |
| Flecainide | 1 | (+) | (+) | (+) |
| *Alfuzosin* | 1 | (-) | (+) | (-) |
| Thioridazine | 3 | (+) | (+) | (+) |
| Quinidine | 10 | (+) | (+) | (+) |
| Erythromycin | 30 | (+) | (+) | (+) |
| Sotalol | 30 | (+) | (+) | (+) |
| Fluoxetine | >30 | (+) | (+) | (-) |
| Verapamil | >30 | (+) | (±) | (-) |
| Moxifloxacin | >100 | (+) | (+) | (±) |
| Amiodarone | >100 | (+) | (+) | (±) |
| Ranolazine | >100 | (+) | (+) | (-) |
| Captopril | >100 | (-) | (-) | (-) |
| Rofecoxib | >100 | (-) | (-) | (-) |
| Amoxicillin | >1000 | (-) | (-) | (-) |
| Aspirin | >1000 | (-) | (-) | (-) |
| Nifedipine | >3 | (-) | (-) | (-) |

Guo et al. Toxicol Sci. 2011 Sep;123(1):281-9.

Cellular Dynamics international

# Human Cardiomyocytes (hCAR) Assay

*Next Round of Validation*

| Drug | $C_{eff}$ (nM) | $IB_{50}$ (μM) | ↓hERG | ↑QT | TdP |
|---|---|---|---|---|---|
| Digitoxin | 33 | 0.003 | (-)[f] | (-) | (+)[r] |
| Dofetilide | 6 | 0.003 | (+) | (+) | (+) |
| Digoxin | 3 | 0.01 | (-)[f] | (-) | (+)[r] |
| Ouabain | 170 | 0.01 | (-)[f] | (-) | (+)[r] |
| Aconitine | 77 | 0.03 | (-) | (-) | (+)[r] |
| Astemizole | 8 | 0.03 | (+) | (+) | (+) |
| E-4031 | 13 | 0.03 | (+) | (+) | (+) |
| Pimozide | 217 | 0.1 | (+) | (+) | (+) |
| Sertindole | 318 | 0.1 | (+) | (+) | (+) |
| Cisapride | 129 | 0.3 | (+) | (+) | (+) |
| Geldanamycin | 16,300 | 0.3 | (-)[f] | (-) | (+)[r] |
| Idarubicin | 123 | 0.3 | (-) | (+) | (+)[r] |
| Terfenadine | 300 | 0.3 | (+) | (+) | (+) |
| Alfuzosin | 56 | 1 | (-) | (+) | (-) |
| Dobutamine | 3,819 | 1 | (+) | (-) | (-) |
| Doxorubicin | 15,344 | 1 | (-) | (+) | (+)[r] |
| Flecainide | 1,931 | 1 | (+) | (+) | (+) |
| Pentamidine | 2,181 | 1 | (-)[f] | (+) | (+) |
| Tacrine | 100 | 1 | (-) | (-) | (-) |
| Amphotericin B | 89,818 | 3 | (-) | (+) | (+)[r] |
| Arsenic Trioxide | 12,132 | 3 | (-)[f] | (+) | (+) |
| Clozapine | 1 | 3 | (-) | (+) | (±) |
| Mitoxantrone | 3,311 | 3 | (-) | (+) | (+)[r] |
| Prenylamine | 70 | 3 | (-) | (+) | (+) |
| Sunitinib | 253 | 3 | (+) | (+) | (+) |
| Thioridazine | 1,781 | 3 | (+) | (+) | (+) |
| Zimeldine | 328 | 3 | (+) | (±) | (±) |
| Ajmaline (i.v.) | 105 | 10 | (+) | (+) | (+) |
| Chlorpromazine | 2,630 | 10 | (+) | (+) | (+) |
| Clarithromycin | 6,025 | 10 | (+) | (+) | (+) |
| Dantrolene | 7,900 | 10 | (-) | (-) | (-) |
| Desipramine | 601 | 10 | (+) | (+) | (+) |
| Epirubicin | 16,036 | 10 | (-) | (+) | (+)[r] |
| Nefazodone | 4,898 | 10 | (+) | (+) | (-) |
| Phentolamine | 100 | 10 | (-) | (-) | (-) |
| Quinidine | 21,578 | 10 | (+) | (+) | (+) |
| Erythromycin (i.v.) | 34,064 | 30 | (+) | (+) | (+) |
| Fluvoxamine | 1,257 | 30 | (+) | (-) | (-) |
| Imatinib | 3,541 | 30 | (-) | (+) | (-) |
| Mesilatine | 11,161 | 30 | (+) | (-) | (-) |
| Propafenone | 4,827 | 30 | (+) | (+) | (+) |
| Propranolol (i.v.) | 193 | 30 | (-) | (+) | (-) |
| Sotalol | 14,733 | 30 | (+) | (+) | (+) |

| | | | | | |
|---|---|---|---|---|---|
| 27 | >100 | >100 | - | - | - |
| 28 | >100 | >100 | - | - | - |
| 29 | >100 | >100 | - | - | - |
| 30 | >100 | >100 | - | - | - |

| | *in vivo* ECG | | hERG | | |
|---|---|---|---|---|---|
| Cyclosporin A | 1,498 | 100 | (-) | (-) | (-) |
| Lidocaine (i.v.) | 36,000 | 100 | (+) | (-) | (-) |
| Pimobendan | 164 | 100 | (-) | (-) | (-) |
| Ranolazine | 6,009 | 100 | (+) | (+) | (-) |
| Nifedipine | 194 | >3 | (-) | (-) | (-) |
| Amitriptyline | 793 | >30 | (-) | (+) | (±) |
| Bepridil | 3,298 | >30 | (+) | (+) | (+) |
| Cetirizine | 300 | >30 | (-) | (-) | (-) |
| Cibenzoline | 2,168 | >30 | (+) | (+) | (-) |
| Dofetamprídine | 493 | >30 | (-) | (-) | (-) |
| Diltiazem | 552 | >30 | (-) | (-) | (-) |
| Diphenhydramine | 157 | >30 | (-) | (+) | (+) |
| Fluorouracil | 4,613 | >30 | (-) | (-) | (+)[r] |
| Fluoxetine | 485 | >30 | (+) | (+) | (+) |
| Imipramine | 1,070 | >30 | (+) | (+) | (±) |
| Ketoconazole | 17,689 | >30 | (+) | (+) | (±) |
| Loratadine | 23 | >30 | (-) | (-) | (-) |
| Nitrendipine | 150 | >30 | (-) | (-) | (-) |
| Olanzapine | 74 | >30 | (-) | (±) | (-) |
| Rosiglitazone | 1,673 | >30 | (-) | (-) | (-) |
| Troglitazone | 6,387 | >30 | (-) | (-) | (-) |
| Verapamil | 315 | >30 | (+) | (-) | (-) |
| Acetamidophenol | 130,000 | >100 | (-) | (-) | (-) |
| Alpidem | 284 | >100 | (-) | (-) | (-) |
| Amiodarone | 3,874 | >100 | (+) | (+) | (±) |
| Atenolol | 1,284 | >100 | (-) | (-) | (-) |
| Captopril | 2,466 | >100 | (-) | (-) | (-) |
| Colchicine | 16 | >100 | (-) | (-) | (-) |
| Cyclophosphamide | 153,200 | >100 | (-) | (±) | (-) |
| Dexrazoxane | 136,052 | >100 | (-) | (-) | (-) |
| Levosimendan | 136 | >100 | (-) | (-) | (-) |
| Mechlorethamine | ? | >100 | (-) | (-) | (-) |
| Moxifloxacin | 10,276 | >100 | (+) | (+) | (+) |
| Nimesulide | 15,000 | >100 | (-) | (-) | (-) |
| Pemoline | 6,925 | >100 | (-) | (-) | (-) |
| Rofecoxib | 1,021 | >100 | (-) | (-) | (-) |
| Tolcapone | 21,959 | >100 | (-) | (-) | (-) |
| Zalcitabine | 119 | >100 | (-) | (-) | (-) |
| Amoxicillin | 17,036 | >1000 | (-) | (-) | (-) |
| Aspirin | 10,000 | >1000 | (-) | (-) | (-) |

**83 Compounds**

**~82% -- arrhy. prediction**
**>90% -- QT prediction**

**30 Internal Compounds**

**80% -- arrhy. prediction**
**95% -- QT prediction**

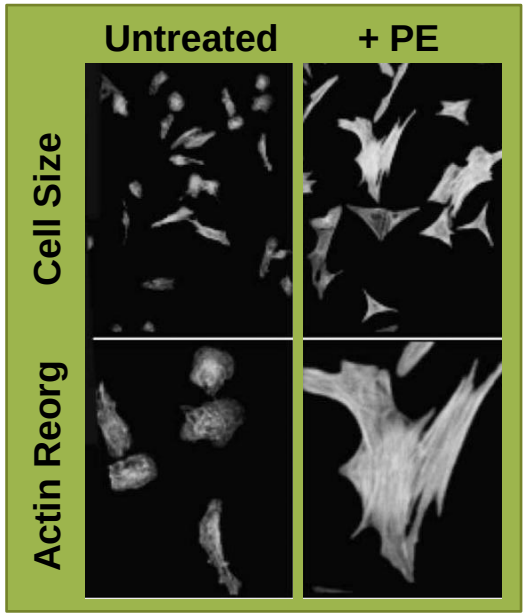
**HESI?**

# Hallmarks of Cardiac Hypertrophy

Normal

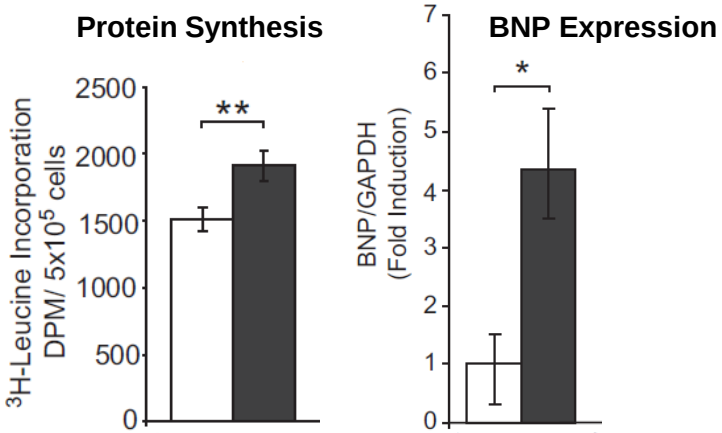
Diseased

- Increased cell size
- Enhanced protein synthesis
- Higher organization of the sarcomere
- Re-activation of the fetal gene program
- Expression of B-type natriuretic protein (BNP)



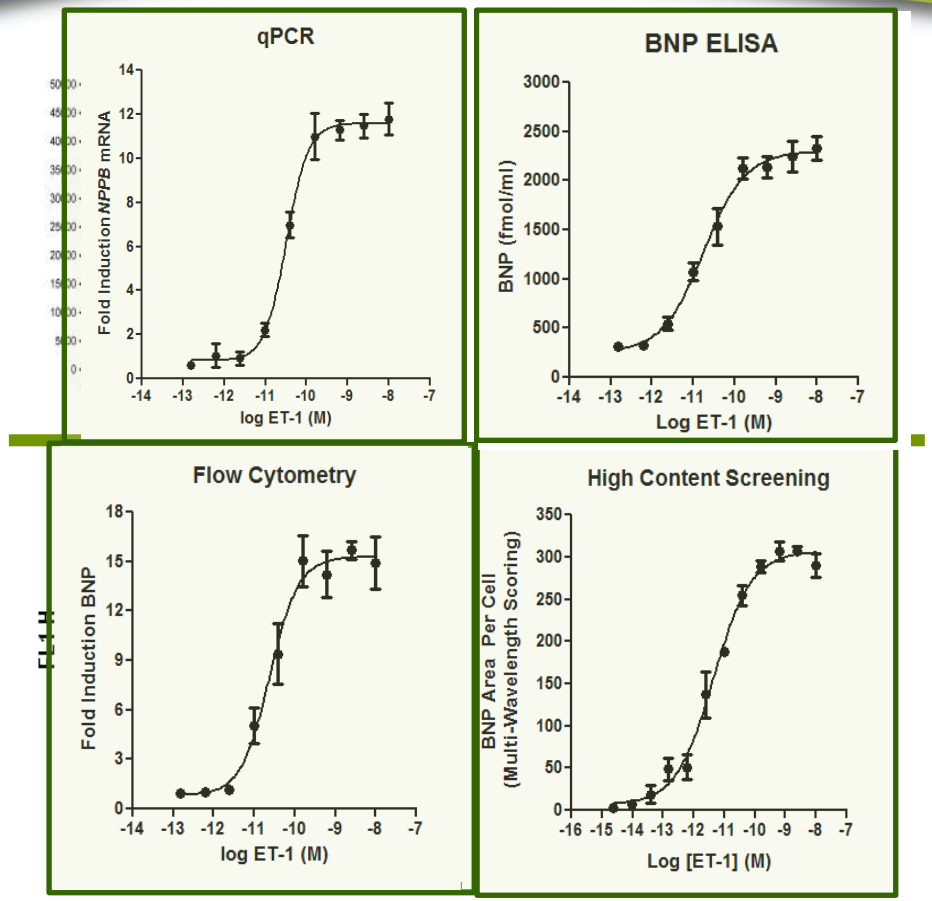

Lister K et al. Cardiovasc Res 2006;70:555-565

Kuwahara K et al. J Pharmacol Sci 2012;119:198-203




Glenn D et al. Hypertension 2009;53:549-555

# Hypertrophy Application Suite




## qPCR

- Measure activation of BNP gene (*NPPB*)

## ELISA

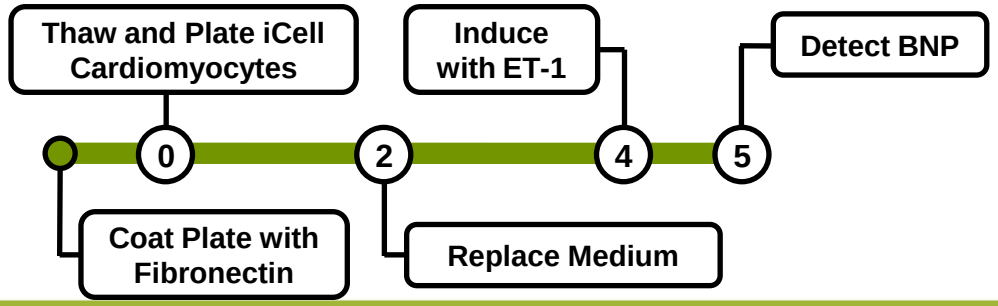
- Quantify levels of secreted BNP

## Flow Cytometry

- Detect BNP expression

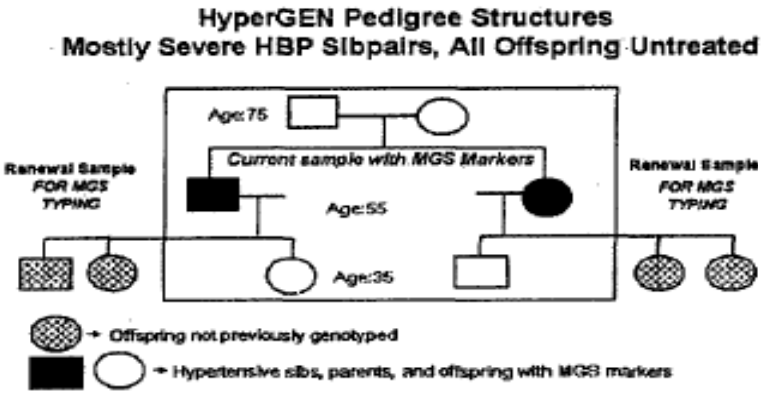
## High Content Analysis

- Image BNP expression, Cell Size, Cell



# NHLBI Family Blood Pressure Program – GWAS now iCell Cardiomyocytes

- GWAS identification of associated SNPs with left Ventricular Hypertrophy
- Phenotyping through cardiovascular phenotypes and risk factors
- Family-based ascertainment




**Genetic Variation in NCAM1 Contributes to Left Ventricular Wall Thickness in Hypertensive Families**

Donna K. Arnett, Kristin J. Meyers, Richard B. Devereux, Hemant K. Tiwari, Charles C. Gu, Laura K. Vaughan, Rodney T. Perry, Amit Patki, Steven A. Claas, Yan V. Sun, Ulrich Broeckel, Sharon L. Kardia

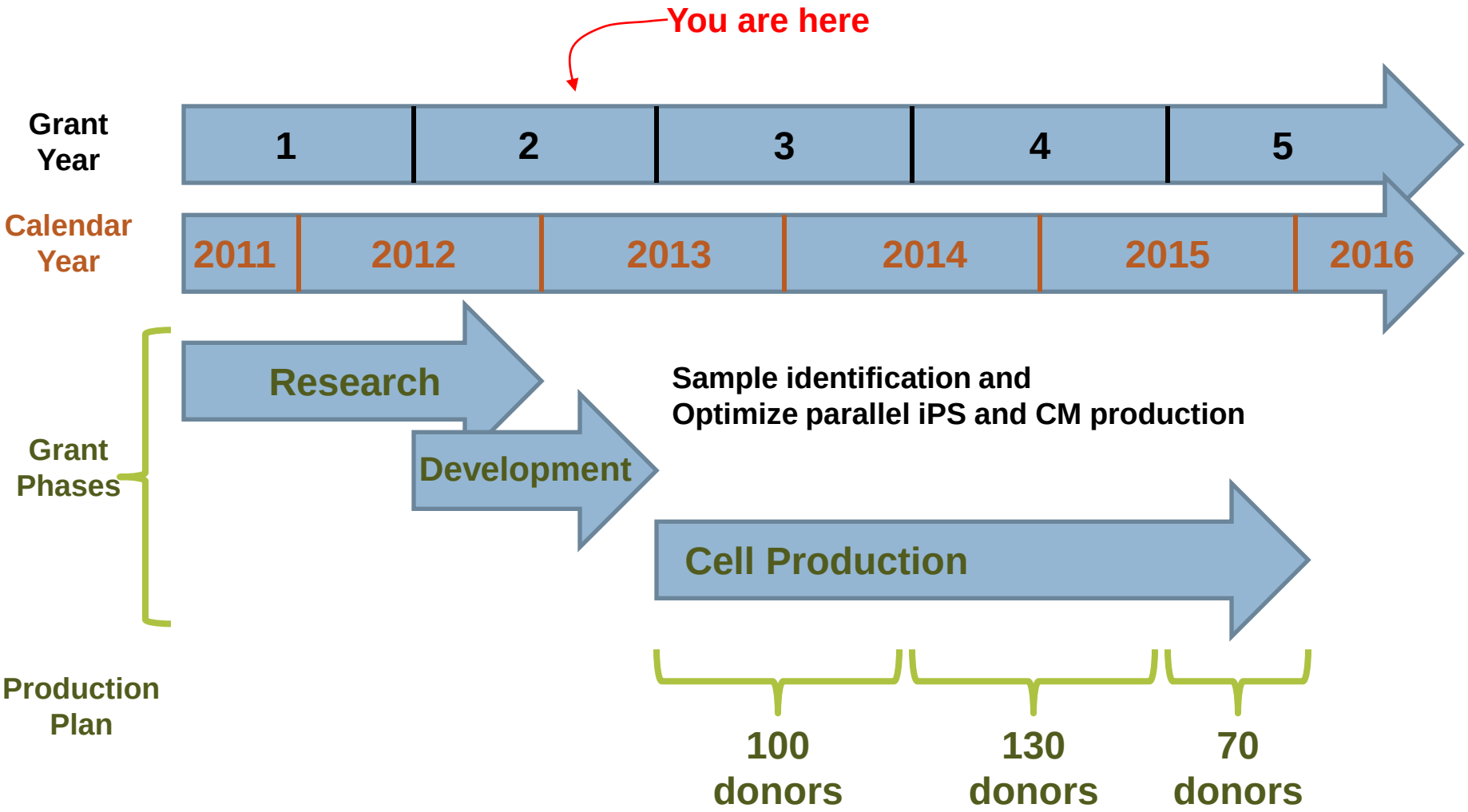
Table 2. Probability Values for SNP rs1436109 in HyperGEN and GENOA Samples

| Phenotype* | Stage I: HyperGEN Black | Stage II: GENOA Black | Stages I+II Metaanalysis† | Stage III: HyperGEN White | Stages I+II+III Metaanalysis† |
|---|---|---|---|---|---|
| LVM | $2.92\times10^{-3}$ | $1.56\times10^{-1}$ | $3.96\times10^{-3}$ | $8.88\times10^{-2}$ | $2.52\times10^{-3}$ |
| RWT | $6.22\times10^{-7}$ | $3.24\times10^{-2}$ | $3.77\times10^{-7}$ | $5.47\times10^{-4}$ | $3.80\times10^{-9}$ |
| PWT | $3.60\times10^{-7}$ | $2.52\times10^{-2}$ | $1.77\times10^{-7}$ | $1.49\times10^{-1}$ | $3.12\times10^{-7}$ |
| LVID | $4.90\times10^{-1}$ | $9.83\times10^{-1}$ | $8.34\times10^{-1}$ | $1.86\times10^{-4}$ | $4.80\times10^{-3}$ |
| IVST | $1.78\times10^{-7}$ | $5.29\times10^{-2}$ | $1.83\times10^{-7}$ | $4.47\times10^{-1}$ | $8.69\times10^{-7}$ |

*Phenotypes adjusted for age, age$^2$, sex, center, and principal components 1–30. †Fisher's combined probability.

- **NHLBI, Next Generation Genetic Association Studies, RFA-HL-11-066**
- CDI will make pure ventricular cardiomyocytes from 250 different patients
- 100 most informative families derived from HyperGEN cohort
- Induce hypertrophy phenotype in vitro, perform molecular analyses
- Attempt to correlate GWAS findings with in vitro phenotype

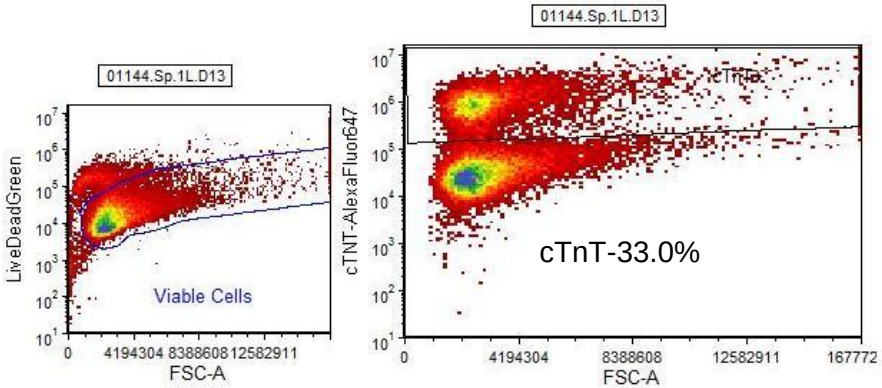
# CDI Grant Milestones




**25 donors completed or in progress**

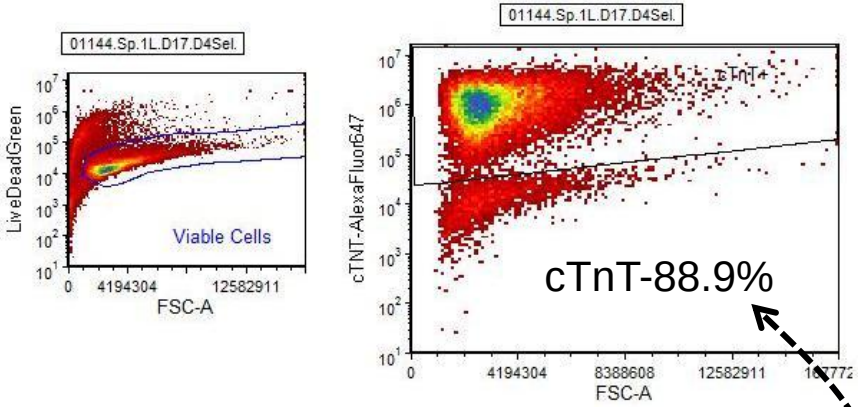
# Example Pre and Post Selection Purity and Yield Sample ID O1144

## Start of Selection (day 12)




CM yield (start of selection): 256M

## End of Selection (day 16)




CM yield (end of selection): 280M

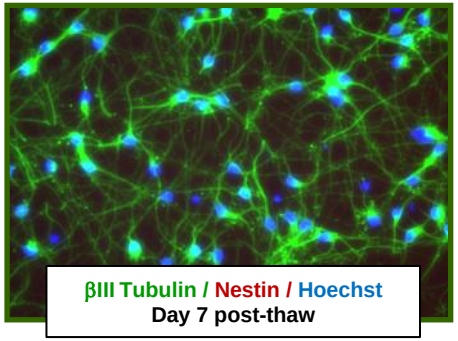
This will drift up to ~97% by day 30

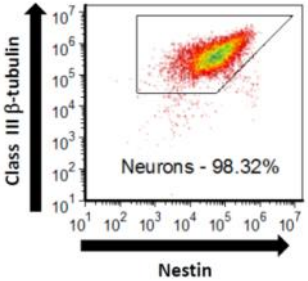
- >90% purity (cTnT+)
- > 4.3 million viable cells per cryovial
- >50 million total yield (200M target)
- Mycoplasma negative
- Identity screen (STR) match
- Observed beating rate

# iCell Neurons

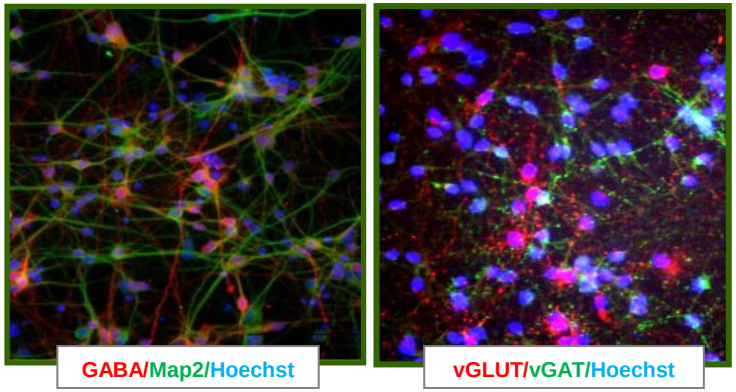
| Key Neuron Characteristics | |
|---|---|
| **Morphology** | Bipolar or multi-polar neurite outgrowths |
| **Molecular Markers** | βIII-Tubulin, Map2, Synaptophysin, Gephyrin, PSD-95, vGAT (GABAergic), vGLUT (Glutamatergic), Tau, A-Beta |
| **Functional Characteristics** | - Neurotransmission: receptor and channel activation<br>- Electrophysiology: patch clamp and MEA<br>- Neurite outgrowth/sprouting |

## Purity and Morphology

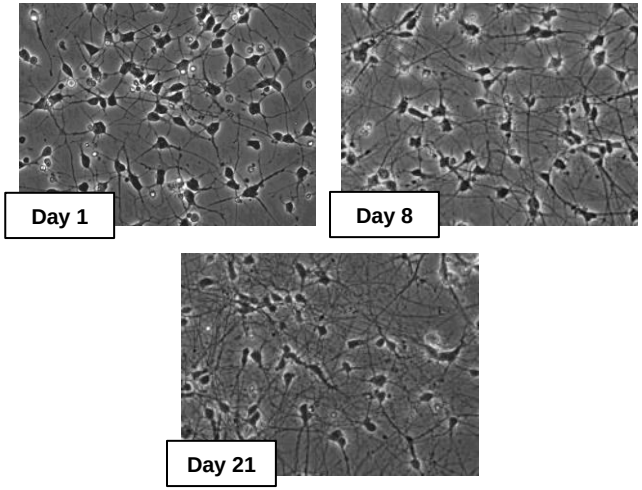



## Molecular Markers




**iCell Neurons are a mixed population of primarily GABAergic and Glutamatergic neurons**



**5 ml Pellet of Pure Neurons = ~4 Billion Neurons**

# iCell Neurons
*Disease modeling*

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

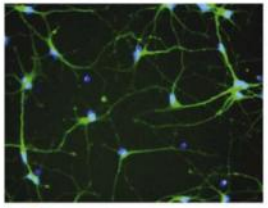
ELSEVIER

Constitutive secretion of tau protein by an unconventional mechanism

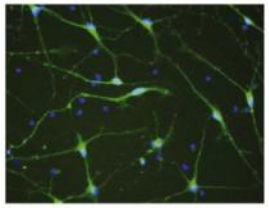
Xiyun Chai, Jeffrey L. Dage, Martin Citron *

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

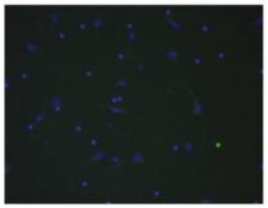
## Expression of Tau Isoforms



**Total Tau, Hoechst**



**3R Tau, Hoechst**



**4R Tau, Hoechst**

*iCell Neurons demonstrate endogenous expression of the 3R tau isoform at 8 days post-thaw*

## Tau Protein Secretion

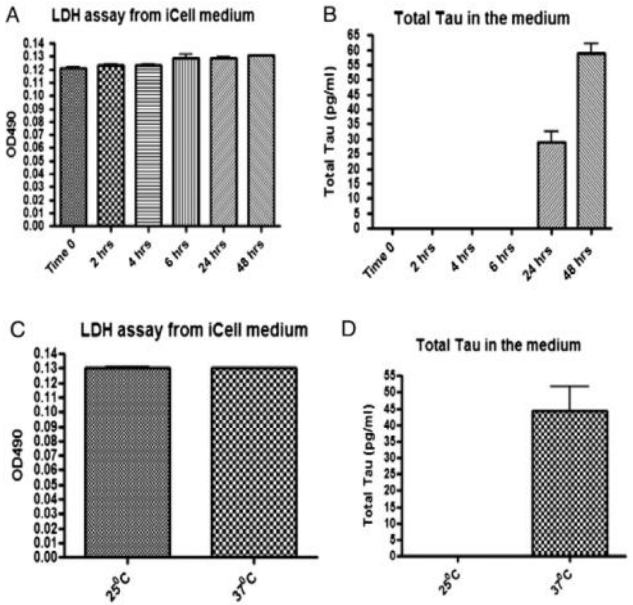



**Table 1**
Percentage of tau released from cells.

| | Tau in medium (pg/ml) | Intracellular tau (ng/ml) | % Release |
|---|---|---|---|
| HEK 293 Trex tau wt | 134 ± 19 | 102 ± 18 | 0.1 |
| HEK 293 Trex tau ΔK280 | 261 ± 56 | 267 ± 86 | 0.1 |
| iCell® Neurons | 59 ± 6 | 20 ± 2 | 0.3 |

*Above tau concentrations determined by ELISA*

iCell Neurons secrete tau at levels that translate to the in vivo situation;
Comparable to tau release occurring under normal physiological conditions in vivo.

**Prevention of β-amyloid induced toxicity in human iPS cell-dervied neurons by inhibition of cyclin-dependent kinases and associated cell cycle events**

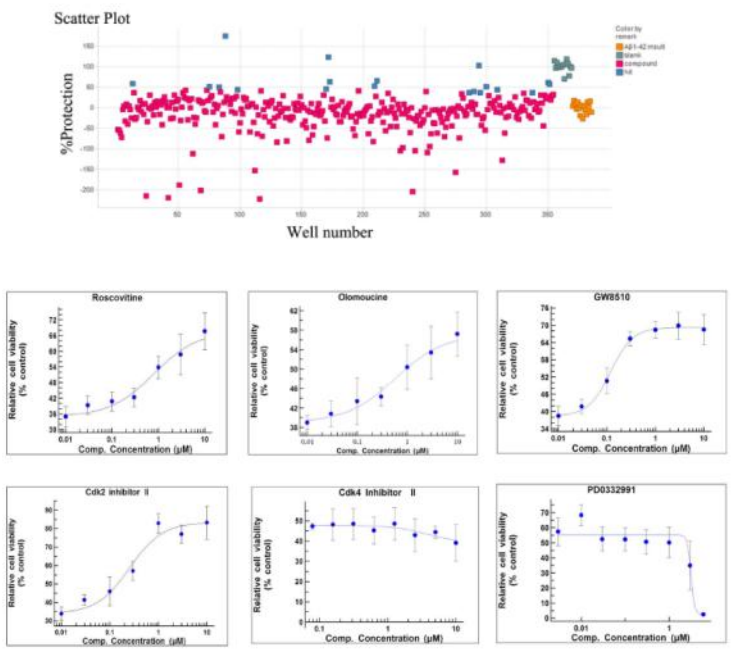
Xiaohong Xu[a,b], Ying Lei[b], Jie Luo[b], Shu Zhang[b], Jamie Wang[b], Emile Nuwaysir[c], Guohuang Fan[b], Jing Zhao[b], Lei Lei[b], Zhong Zhong[b]

[a]Department of Histology and Embryology, Harbin Medical University, Harbin 150081, China; [b]Regenerative Medicine DPU, GlaxoSmithKline (China) R&D Co., Ltd. Shanghai 201203, China; [c]Cellular Dynamics International, Inc. 525 Science Drive, Suite 200 Madison, WI 53711, U.S.A.

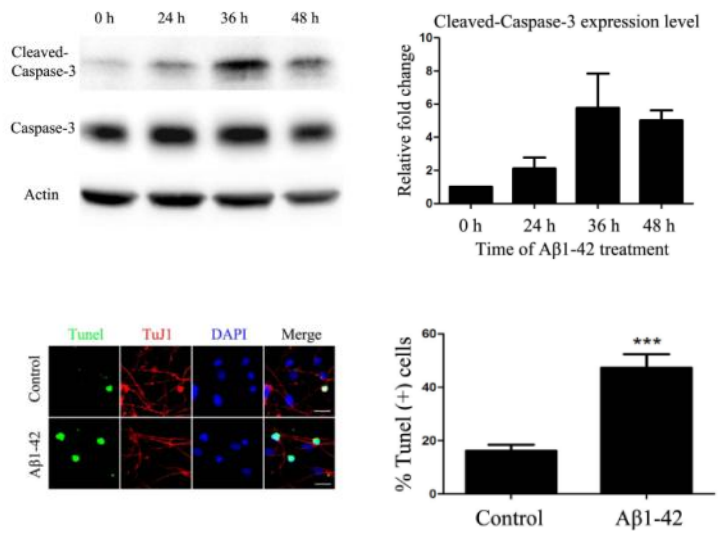
**Stem Cell Research, in press,** DOI (10.1016/j.scr.2012.11.005)

GlaxoSmithKline

**Screen for inhibitors of Aβ1-42 toxicity in iCell Neurons**




**Aβ1-42 induced toxicity to iCell Neurons**




- **Cellular toxicity was elicited by Aβ1-42.**
- **Compound screen identified small molecule Cdk2 inhibitors as blockers of Aβ1-42 – induced cellular toxicity.**
- **Target validation was performed via shRNAs against Cdk2 and Cdk4.**

# iCell Neurons - Utility

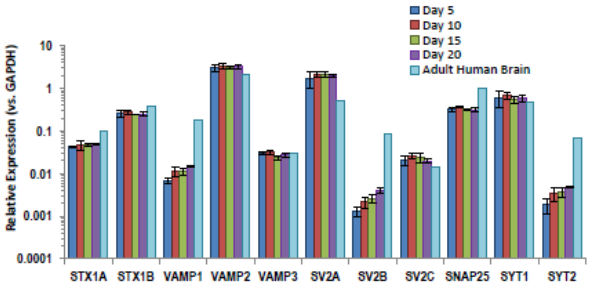
## *Toxicity - Botulinum Neurotoxin Detection*

Novel Application of Human Neurons Derived from Induced Pluripotent Stem Cells for Highly Sensitive Botulinum Neurotoxin Detection

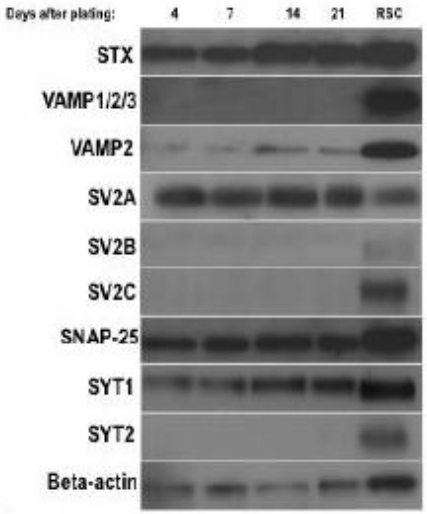
Regina C. M. Whitemarsh,* Monica J. Strathman,† Lucas G. Chase,* Casey Stankewicz,† William H. Tepp,* Eric A. Johnson,* and Sabine Pellett*,[1]

*Department of Bacteriology, University of Wisconsin, Madison, Madison, Wisconsin 53706 and †Cell Biology Group, Cellular Dynamics International, Inc., Madison, Wisconsin 53711

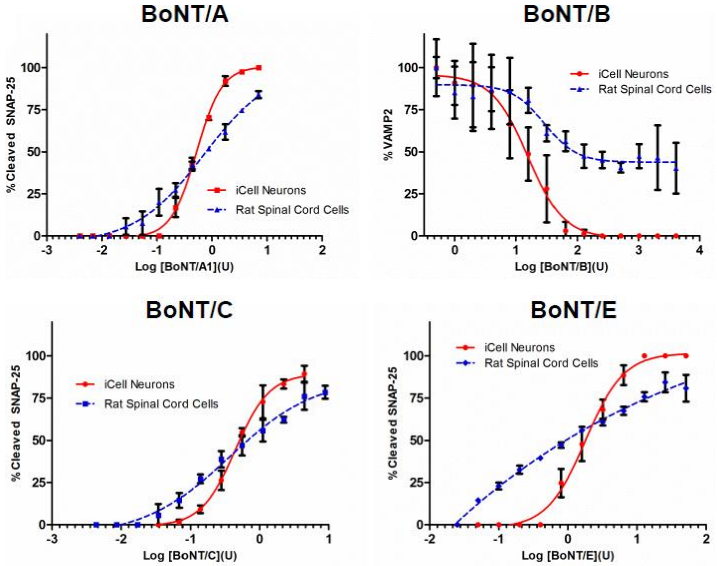
### BoNT Receptor Gene Expression




### BoNT Receptor Protein Expression




### BoNT Receptor Cleavage




iCell Neurons express the receptors and enzymatic targets necessary for BoNT cell entry and catalytic activity. They reproducibly show equivalent or greater sensitivity to BoNT activity than rat spinal cord cells.

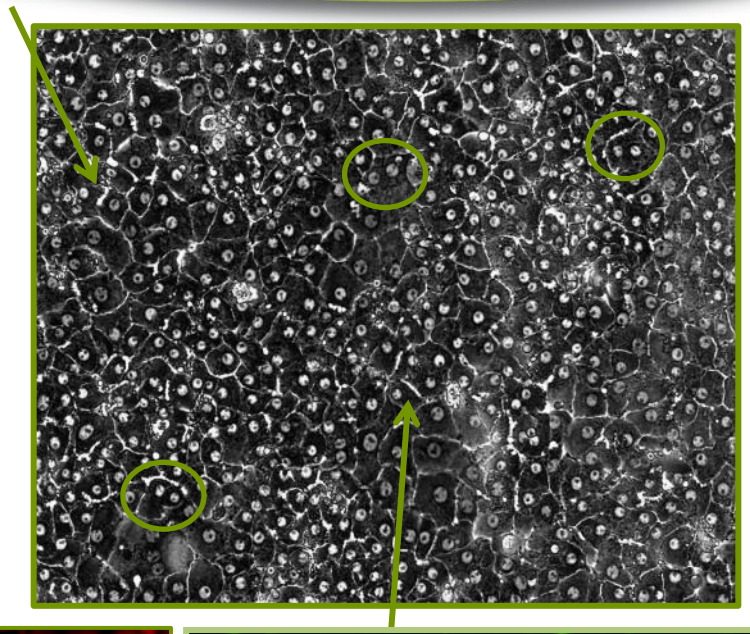
# iCell Neurons

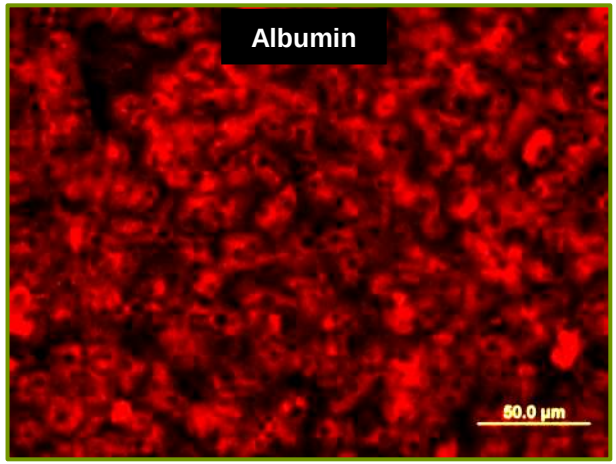
## *Recapitulated Disease Phenotypes*

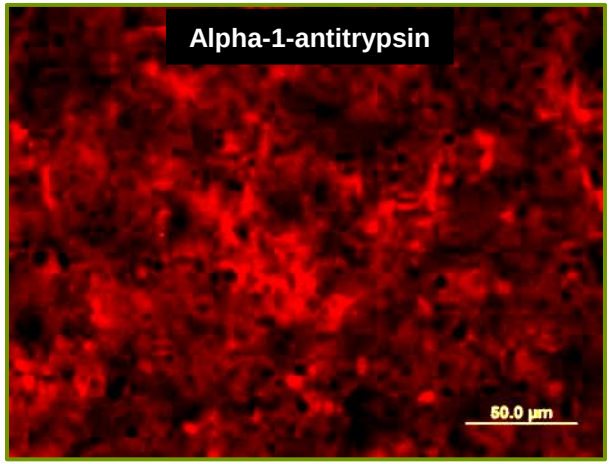
| Neuronal | | |
|---|---|---|
| **Disease** | **Cell Phenotype** | **Citation** |
| Familial Dysautonomia | Decreased neurogenic differentiation; loss of neural crest cells | Lee, et al. (2009) |
| Spinal Muscular Atrophy | Selective motor neuron death; loss of SMN gene expression | Ebert, et al. (2009) |
| Rett Syndrome | Reduced synapses & spine density, smaller soma size, altered calcium signaling, electrophysiological defects | Marchetto, et al. (2010) |
| | Smaller soma size | Cheung, et al. (2011) |
| Schizophrenia | Decreased synapse density | Brennand, et. al. (2011) |
| Alzheimer's Disease | Increased levels of amyloid-β (1-40), p-tau/total tau, and aGSK-3b levels; early endosome phenotype | Israel, et. al. (2012) |
| | Increased amyloid β42 secretion; sharply responds to γ-secretase inhibitors and modulators. | Yagi, et al. (2011) |
| Parkinson's Disease | Elevated a-synuclein expression, increased sensitivity to cellular stressors | Nguyen, et al. (2011) |
| | Less recruitment of Parkin to the mitochondria | Seibler, et al. (2011) |
| Amyotrophic lateral sclerosis | Reduced VAPB levels in ALS8 patients | Mitne-Neto, et al. (2011) |
| Prader-Willi Syndrome | Imprint disorder | Chamberlain, et al. (2010) |
| Angelmans' Syndrome | | |
| Timothy Syndrome | Decreased expression of genes in lower cortical layers and callosal projection neurons; abnormal expression of TH and increased production of norepinephrine and dopamine. | Pasca, et al. (2011) |

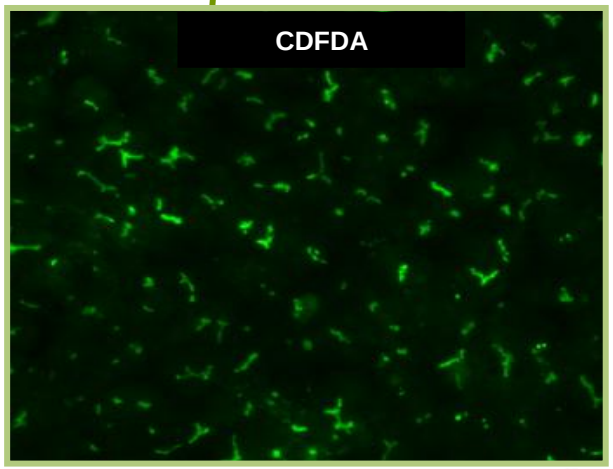
# iCell Hepatocytes

| Key Hepatocyte Characteristics | |
|---|---|
| **Morphology** | Adherent monolayer; polarized phenotype; functional bile canalicular network; polynucleation |
| **Molecular Markers** | Albumin; α-1-antitrypsin; HNF family transcription factors |
| **Intrinsic Metabolism** | Glycogen storage; lipid metabolism; insulin responsiveness; urea synthesis |
| **Phase I & II Metabolism** | - CYP3A4, 2C9, 2C19, 2D6, 1A2, 2B6, 2C8<br>- UGT, ST, GSTα activity |
| **Transporter Function** | Transport via uptake (e.g. OATP, NTCP) and efflux transporters (e.g. MDR-1/P-gp, BCRP, BSEP, MRP2) |



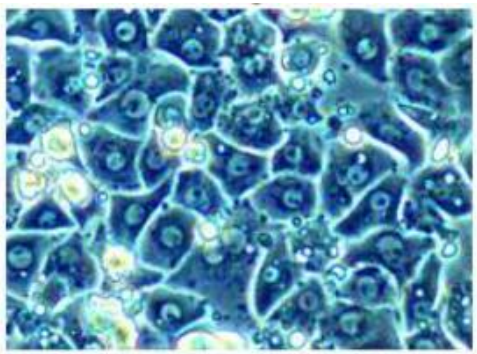
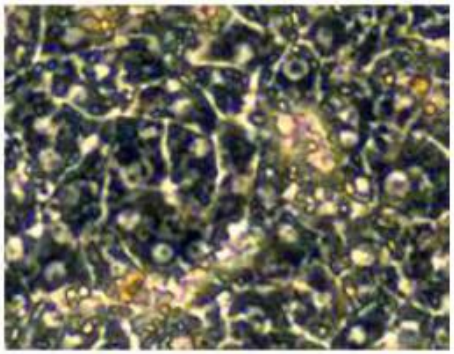
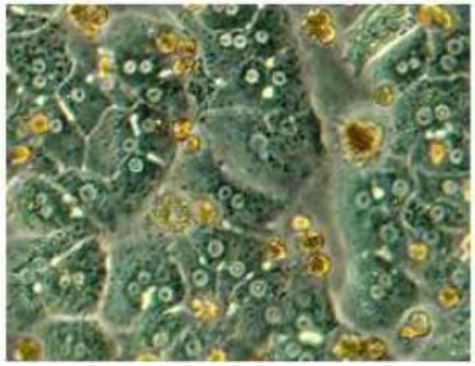
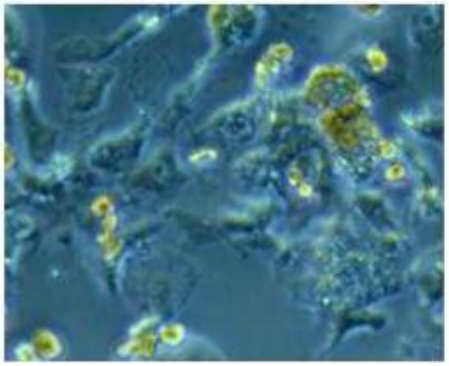


# iCell Hepatocytes

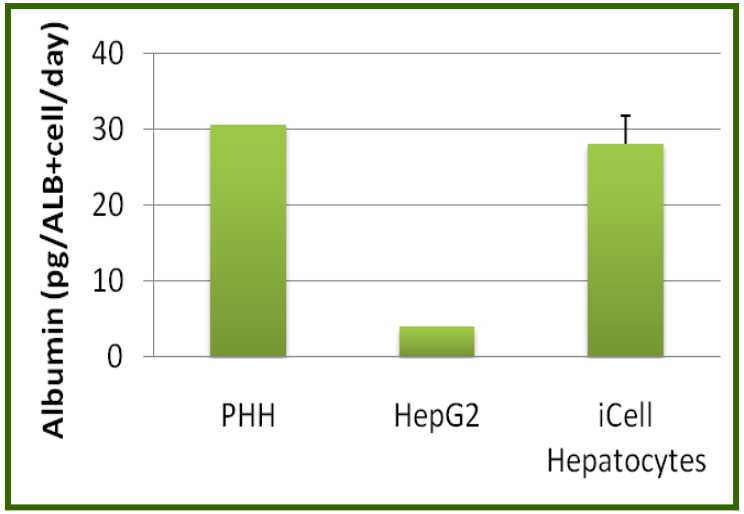
*Long-term culture vs. PHH*

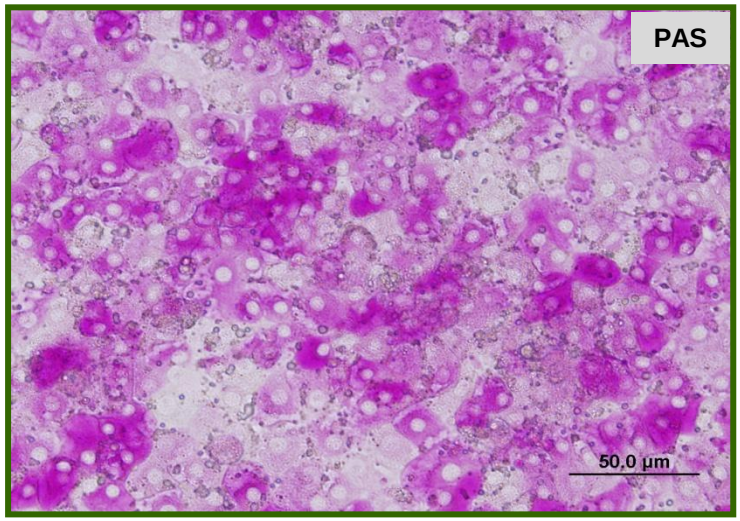
| | Day 1 | Day 21 |
|---|---|---|
| **iCell Hepatocytes** |  |  |
| **Primary Human Hepatocytes** |  |  |

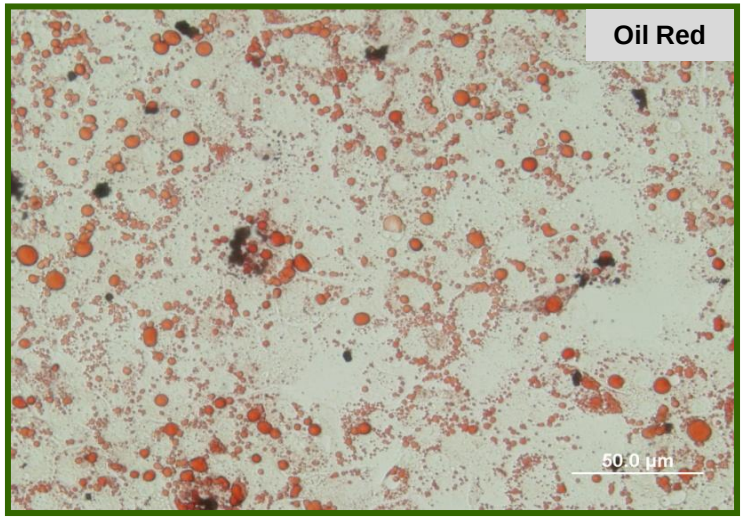
**Data presented at SOT 2012**

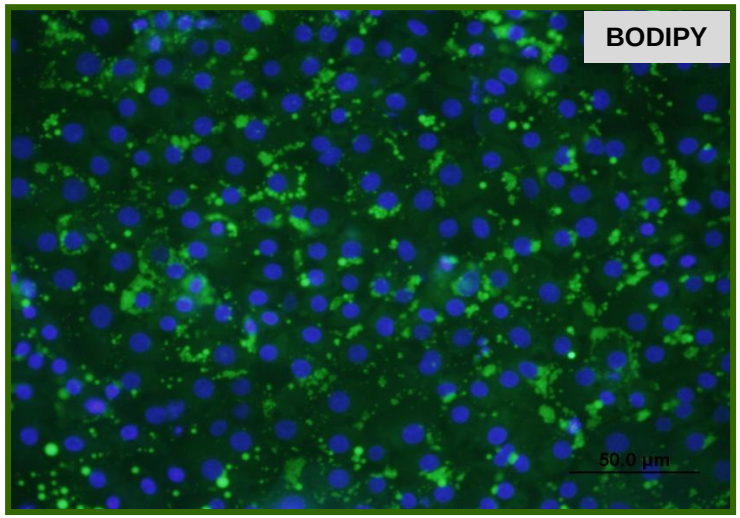
# iCell Hepatocytes

*Intrinsic Function*



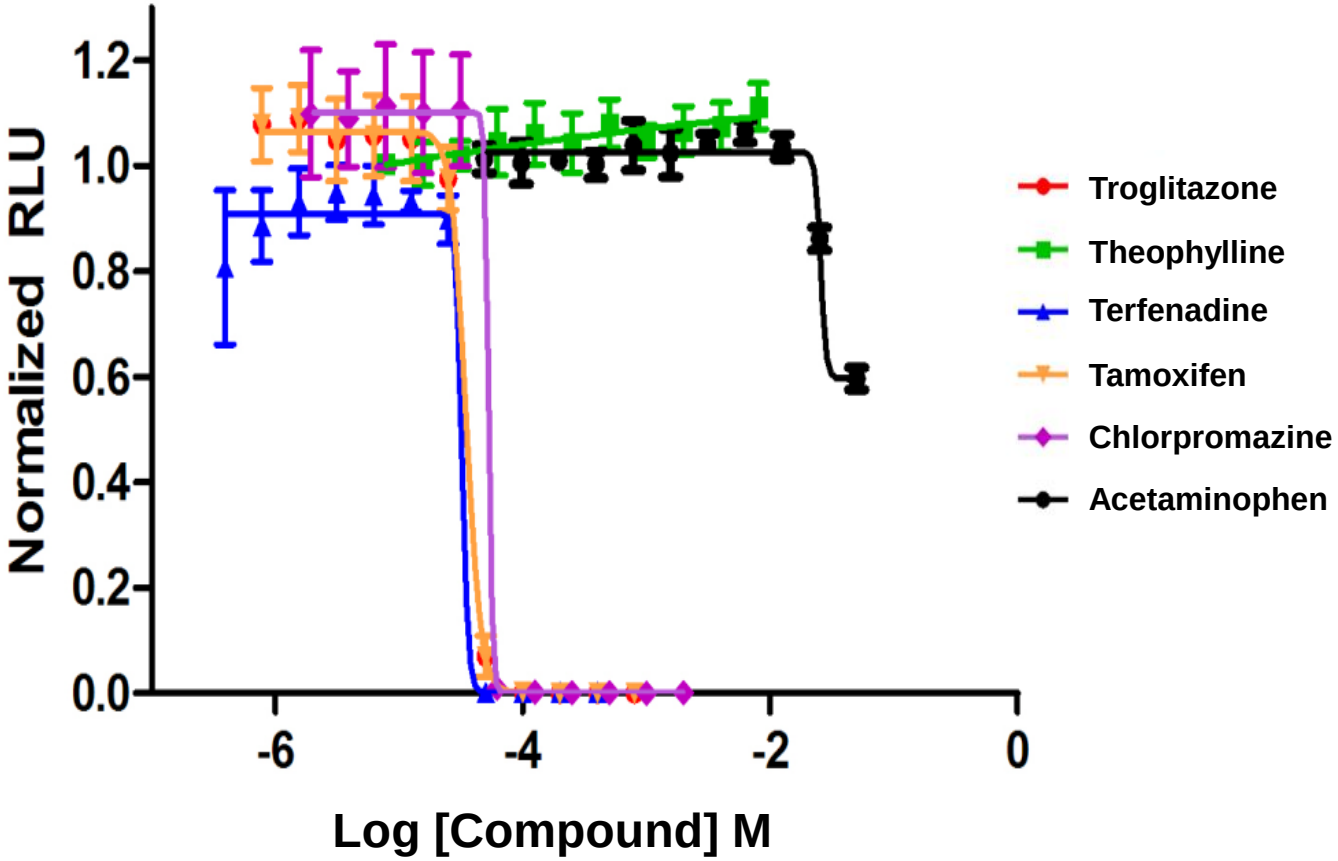



# iCell Hepatocytes

## *Cell Viability - Hepatotoxicity*

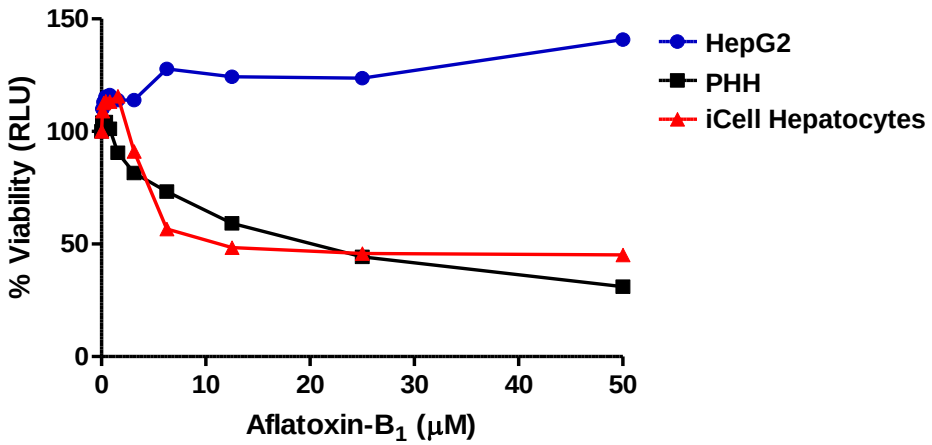


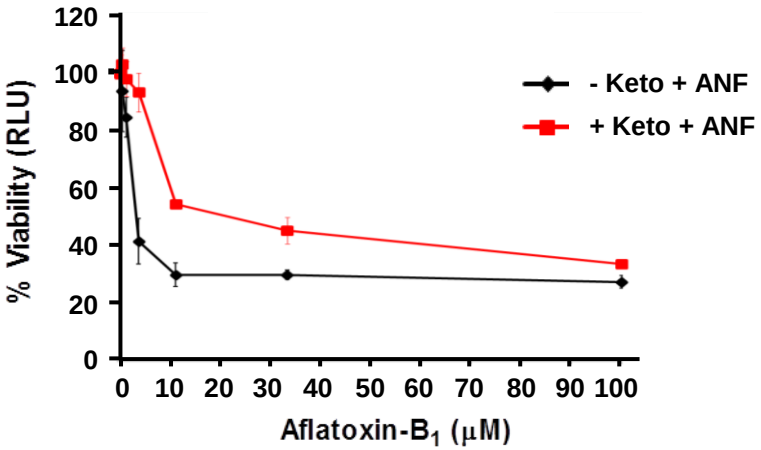
# iCell Hepatocytes

## *Aflatoxin-Mediated Hepatotoxicity*

**Aflatoxins**:

- Naturally occurring mycotoxins produced by the fungus *Aspergillus*
- Metabolized by the liver to a reactive intermediate



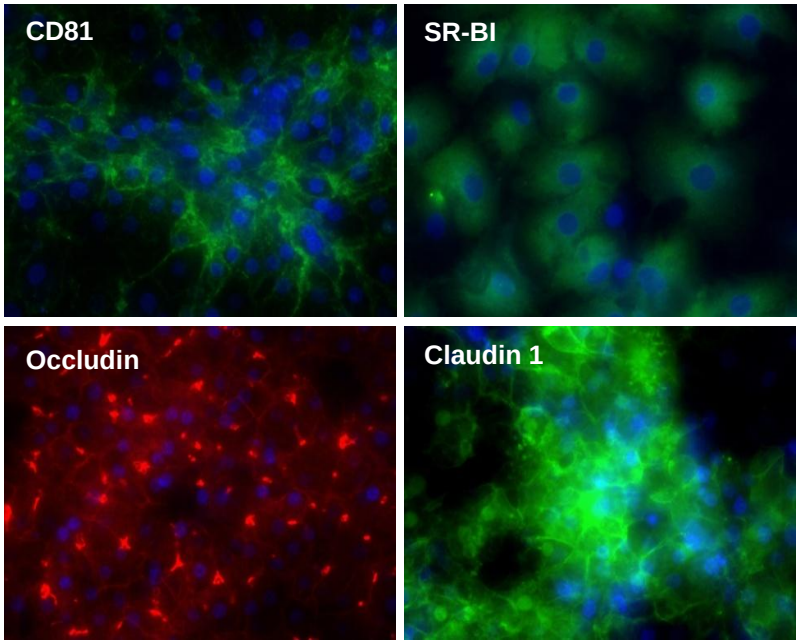

## Conclusions:

- **iCell Hepatocytes exhibit a hepatotoxic response to Aflatoxin-$B_1$, similar to primary human hepatocytes**
- **Aflatoxin-$B_1$ induced hepatotoxicity is inhibited by ketoconazole and alpha-naphthoflavone, inhibitors of P450 enzymes**

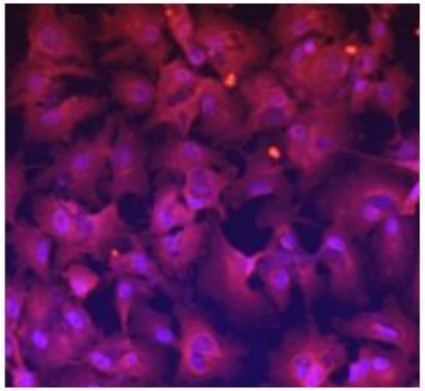
# iCell Hepatocytes
## *Viral Infectivity*

Roche

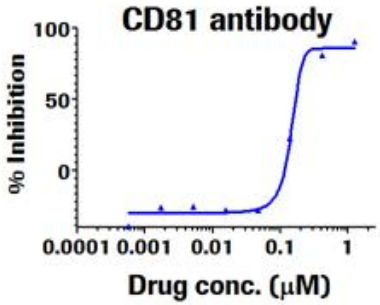
**A**

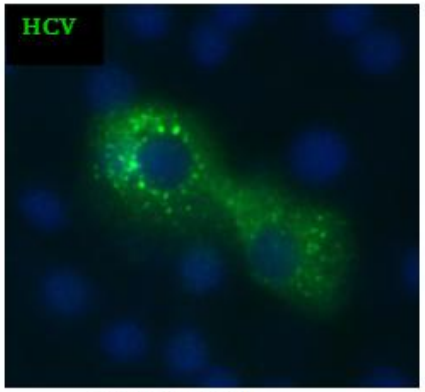



**B**

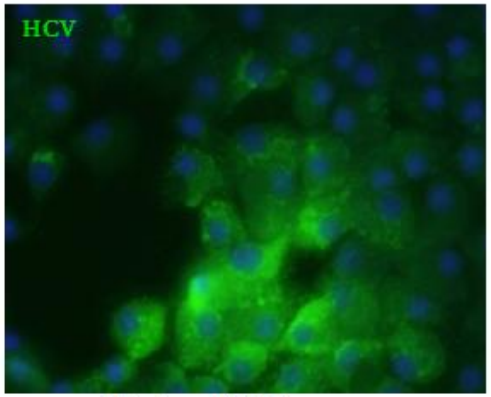


HCVpp encoding Firefly luciferase




HCVcc (GT1a/2a)




Patient serum (GT 1a)

**(Data courtesy of Miriam Triyatani, Roche)**

# iPS cell-derived Tissues and Potential

- **Three critical areas to leverage iPS cell-derived tissues in research**
  - Creation and culture of human iPS cells
  - Genetic engineering of iPS cells
  - Manufacture of human iPS cell-derived cell types
  - Cardiomyocyte, Hepatocyte, Neuronal iPS cell-derived cells

- **Stem cell derived tissues improvement over existing models and assays improve over existing**

- **Integration of human diversity offers renewed potential**
  - Connect disease patient to in vitro models – improved safety
  - Clinical trial design – enrollment and interpretation
  - Potential for in vitro “personalized health care”