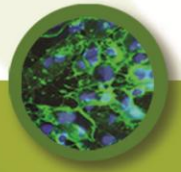
Cellular Dynamics international

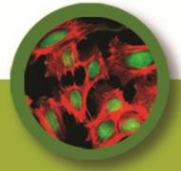
Humanizing the Tissue Chip: Use of Stem Cell Derived Tissues to Develop Biological Platforms

Kyle Kolaja, Ph.D., DABT, Fellow, ATS

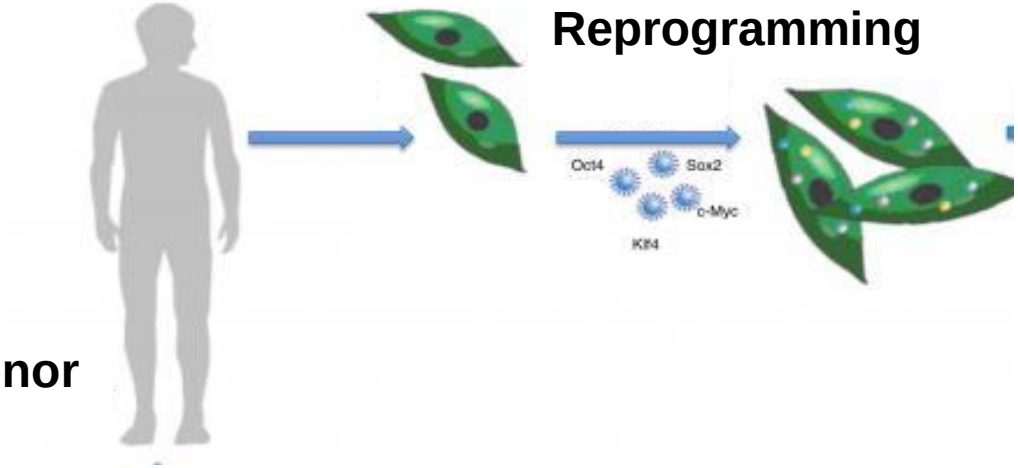
July 2014

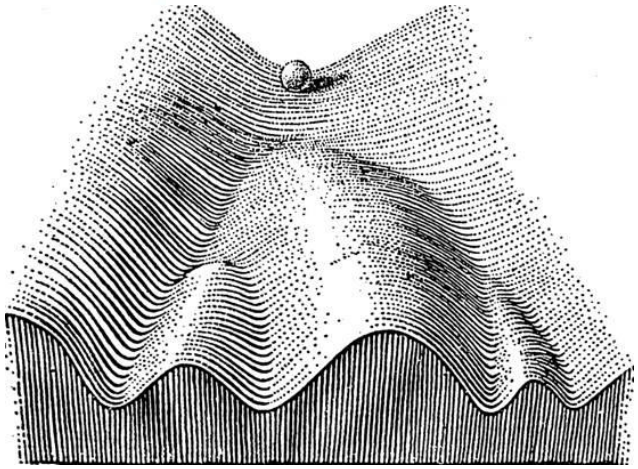


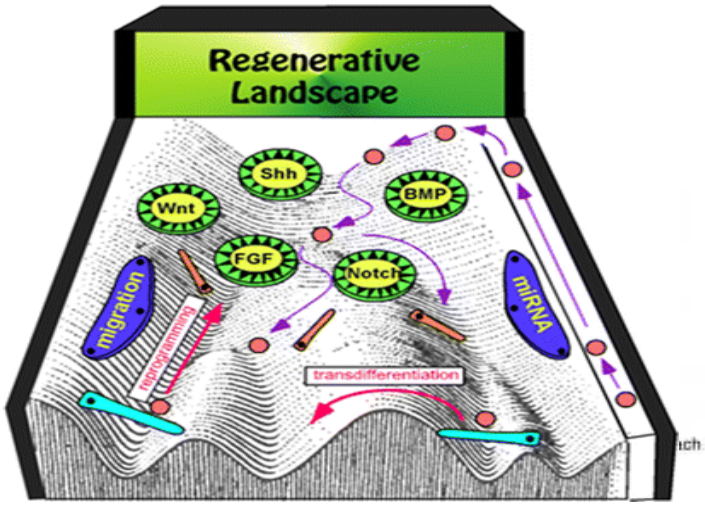




The Potential of iPS Cells: Genetic Diversity







Stem Cell Tissues >Primary Culture

Primary isolation of organs/tissues/cells

- **>100 years since Harrison first cultured frog neurons**
- **>60 years since Gey first immortalized human cell line (HeLa)**
- **Immeasurable innovations, advancements, and knowledge**

Primary Human Cells

Yet, cell culture limitations havent changed much and prevented the ultimate potential of replacing animal and human experiments

- **Variability of isolation, timing, etc**
- **Degeneration of phenotype with time**

Transformed Cell Lines

IPS cell derived tissues have a number of advantages & improvements

- **Footprint free method**
- **Human**
- **Gene editing/engineering**
- **Made from anyone**

Stem Cell derived tissues show a maturing phenotype in vitro

Primary cultured cells dedifferentiate and/or readily lose their phenotype in culture

- Primary hepatocytes
- Primary cardiomyocytes

Main driver for 3D culture models

- Ischemia-reperfusion stress induced during the isolation process
- Disruption of the tissue architecture and surviving in the new one

Maturation of stem cell derived tissues occurs in vitro

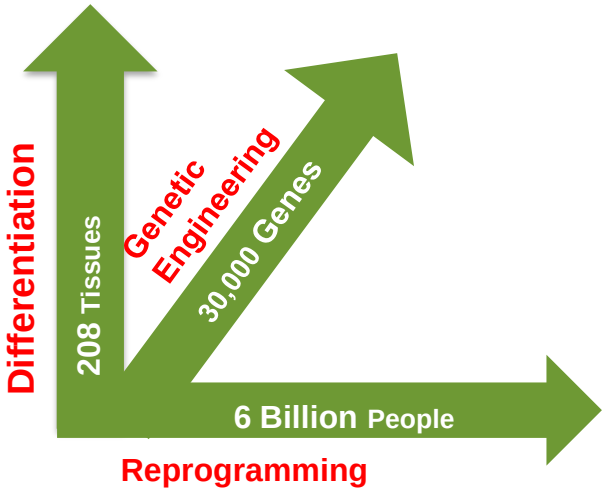
- Recapitulate embryonic development
- Incubate extended periods
- Electrical stimulation (cardiomyocytes)
- Small molecules
- Microphysiological systems

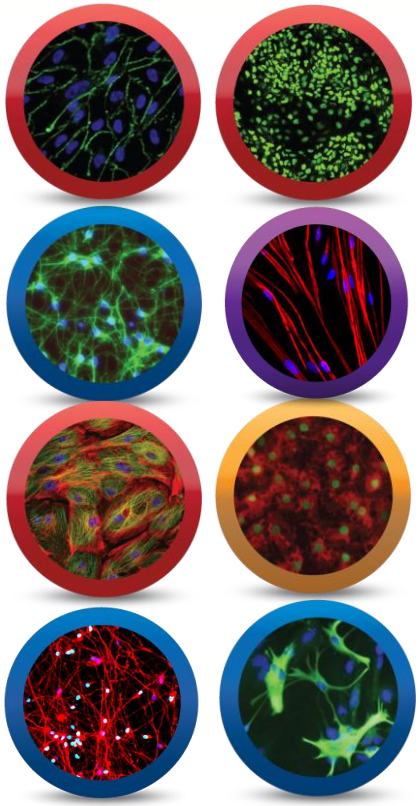
Make stem cell derived tissues mature faster!

- “fit for purpose”

Cellular Dynamics Overview

- **Largest Producer of hiPS and Derived Products**
- **Madison, WI, site Novato, CA (NASDAQ, ICEL)**
- **150 employees (>600 FTE yrs of stem cell expertise)**
- **>800 Patents (Owned or Licensed)**



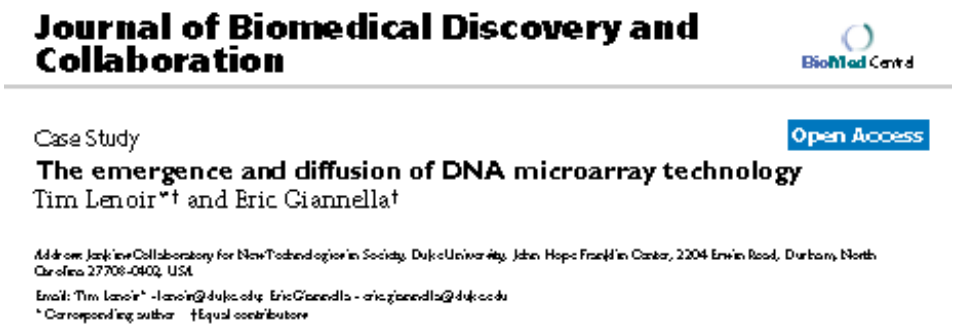


- **MesoDerm**
  - iCell Cardiomyocytes
  - iCell Skeletal Myoblasts
  - iCell Cardiac Progenitor
  - iCell Hematopoietic Progenitor
- **EctoDerm**
  - iCell Neurons
  - iCell Dopa Neurons
  - iCell Astrocytes
- **EndoDerm**
  - iCell Hepatocytes
  - iCell Endothelial Cells
- **Custom iPS Cells (MyCell)**

Stem Cell Derived Tissues – State of the Art

- **Robust manufacturing = enterprise wide quality management system**
  - **Defined media and control of components**
  - **Substrate shift from feeder layers to recombinant proteins (e.g., laminin, vitronectin, etc)**
  - **Control of reagents from start to finish**
  - **Automation**

- **Successful, broadly used items become commercialized → ACCESS**
  - **Media and substrates above**
  - **Micro-arrays great example**
    - Academia – Govt – Industry
    - Homemade to QC product



Journal of Biomedical Discovery and Collaboration

BioMed Central

Case Study

Open Access

The emergence and diffusion of DNA microarray technology

Tim Lenoir*† and Eric Giannella†

Address: Kenan Collaboratory for New Technologies in Society, Duke University, John Hope Franklin Center, 2204 Erwin Road, Durham, North Carolina 27708-0402, USA

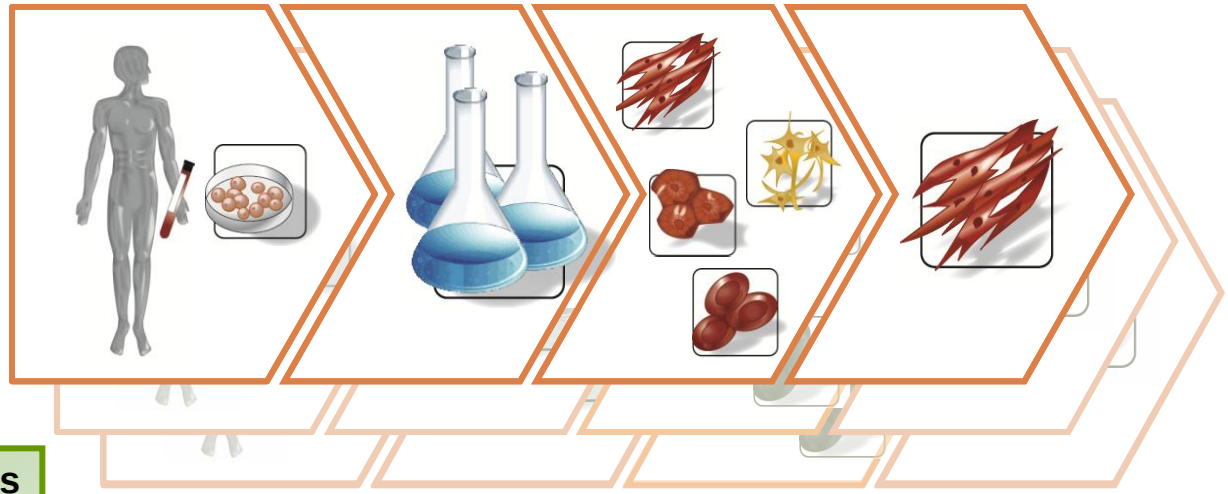
Email: Tim Lenoir* - lenoir@duke.edu; Eric Giannella - eric.giannella@duke.edu

* Corresponding author †Equal contributors

Manufacturing Benchmarks

Scale-Up Manufacturing

- Quality
- Quantity
- Purity



CDI Manufacturing Benchmarks
(cells per day, >95% purity)

- 2 billion iPS cells
- 1 billion iCell Cardiomyocytes
- 1 billion iCell Neurons
- 0.5 billion iCell Endothelial cells
- 0.4 billion iCell 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

iPS Cell Manufacture Scale Out

- **California Institute for Regenerative Medicine (CIRM)**

- **Human iPS Cell Initiative – 3 Awards (Total $32M)**
  - Sample Collection (7 awardees - $0.5M - $1.5M)
  - iPS Cell Derivation (CDI - $16M)
  - iPS Cell Banking (Coriell - $10M; 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**
  - Brings together expertise in electronic record-keeping, sample tracking, iPS cell derivation & characterization, cell banking & distribution
  - Joint facility located within the Buck Institute of Aging, Novato, CA

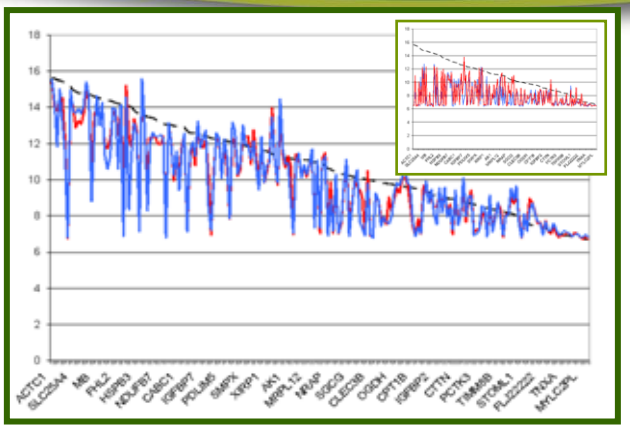
Stem Cell Derived Cardiomyocytes – State of Art

- **First stem cell derived cell type**
  - ~4-5 years of publications now
  - Used in regulatory filings to support claims

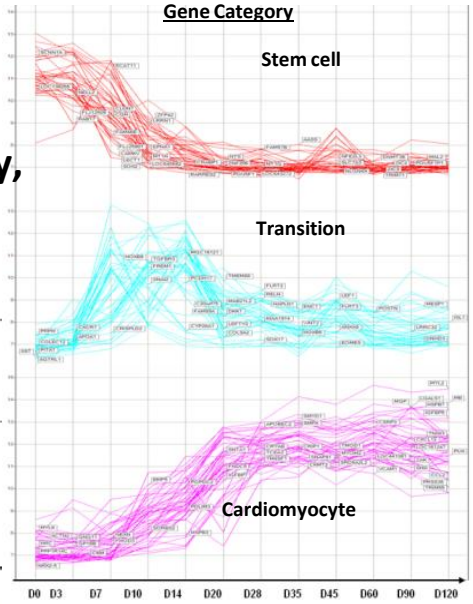
- **Improvements in purity and quantity sped the growth and adoption**

- **Proof of comparability (+) established**
  - Gene expression
  - Morphology
  - Electrophysiology and contractility
  - Biochemical properties
  - Functional (pharm and tox)

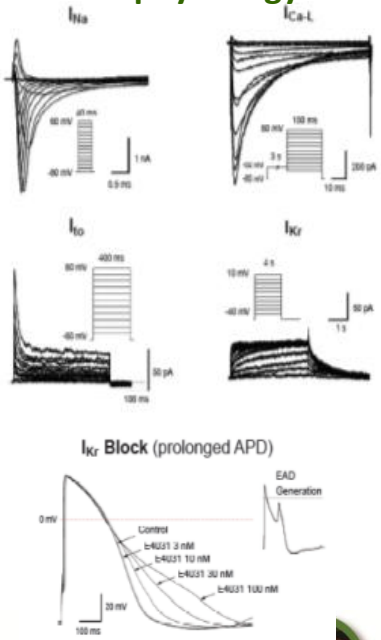
- **Rapidly emerging opportunity in arrhythmia detection, but ample applications in pharmacology, toxicology, and disease biology research**

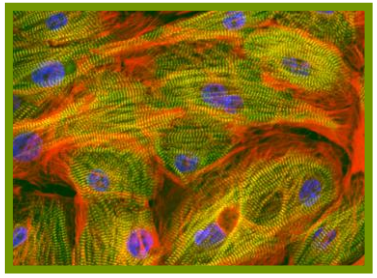


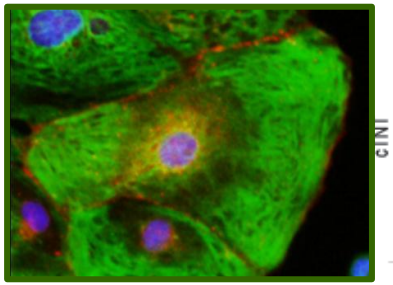
Gene Expression

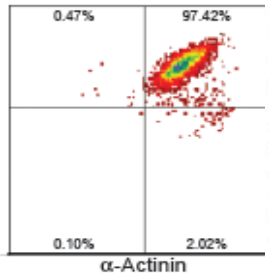


Electrophysiology



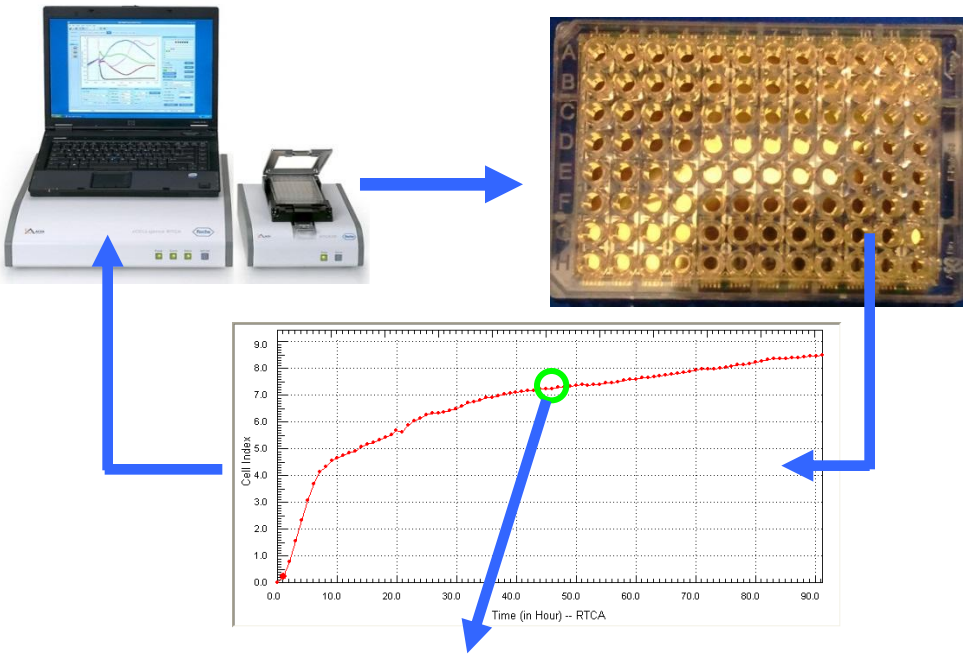


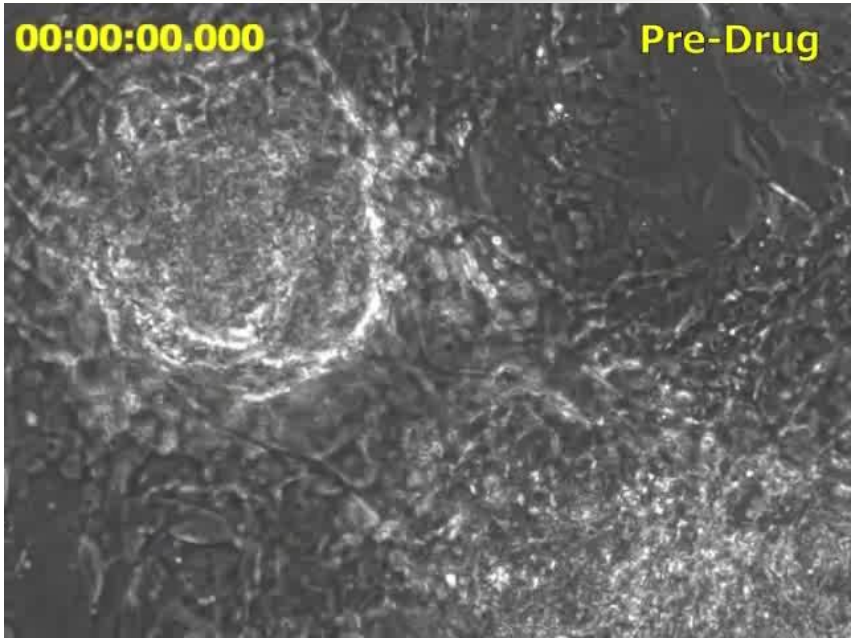


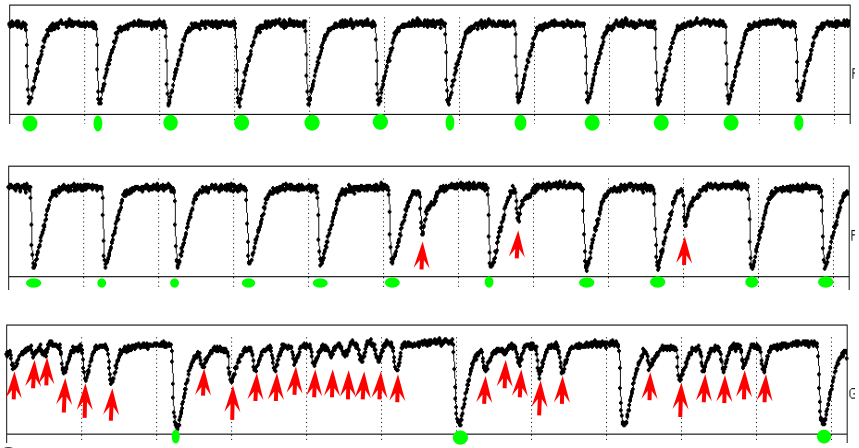


Ma, et al. (2011) Am J Phys. Heart Circ. Physiol.

Human Cardiomyocytes (hCAR) Assay







IB20= lowest tested concentration resulting in 20% irregular beats

Human Cardiomyocyte Arrhythmia Assay Validation: *23 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.

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Human Cardiomyocyte Arrhythmia (hCAR) Assay

2nd Set of Validation and Model Refinement

Drug	C_{max} (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]
Artemisole	8	0.03	(+)	(+)	(+)
E-4031	13	0.03	(+)	(+)	(+)
Pimozide	217	0.1	(+)	(+)	(+)
Sertindole	318	0.1	(+)	(+)	(+)
Cisapride	129	0.3	(+)	(+)	(+)
Geldanamycin	16,800	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	(+)	(+)	(+)
Zimelidine	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	4,858	10	(+)	(+)	(-)
Phentolamine	100	10	(-)	(-)	(-)
Quinidine	21,578	10	(+)	(+)	(+)
Erythromycin (i.v.)	34,064	30	(+)	(+)	(+)
Fluvoxamine	1,257	30	(+)	(-)	(-)
Imatinib	3,541	30	(-)	(+)	(-)
Mesoridine	11,161	30	(+)	(-)	(-)
Propafenone	4,827	30	(+)	(+)	(+)
Propranolol (i.v.)	193	30	(-)	(+)	(-)
Sotalol	14,733	30	(+)	(+)	(+)

	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	800	>30	(-)	(-)	(-)	
Cibenzoline	2,168	>30	(+)	(+)	(-)	
Delta mpridine	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	(-)	(-)	(-)	
Olanzipine	74	>30	(-)	(±)	(-)	
Rosiglitazone	1,673	>30	(-)	(-)	(-)	
Troglitazone	6,387	>30	(-)	(-)	(-)	
Verapamil	815	>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	(-)	(±)	(-)	
Dexamethasone	136,052	>100	(-)	(-)	(-)	
Levamisole	136	>100	(-)	(-)	(-)	
Mechlorethamine	?	>100	(-)	(-)	(-)	
Moxifloxacin	10,276	>100	(+)	(+)	(+)	
Nimesulide	15,000	>100	(-)	(-)	(-)	
Pemoline	6,525	>100	(-)	(-)	(-)	
Rofecoxib	1,021	>100	(-)	(-)	(-)	
Tolcapone	21,959	>100	(-)	(-)	(-)	
Zalcitabine	115	>100	(-)	(-)	(-)	
Amoxicillin	17,036	>1000	(-)	(-)	(-)	
Aspirin	10,000	>1000	(-)	(-)	(-)	

30	-	-	-	4.9		
00	-	-	-	30		
27	>100	>100	-	-	-	4.0
28	>100	>100	-	-	-	4.3
29	>100	>100	-	-	-	30
30	>100	>100	-	-	-	>300

83 Compounds

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

30 Internal Compounds

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

Refining the Human iPSC-Cardiomyocyte Arrhythmic Risk Assessment Model

Liang Guo,[1] Luke Coyle, Rory M.C. Abrams,[2] Raymond Kemper,[3] Eric T. Chiao,[4] and Kyle L. Kolaja[5,6]

TOXICOLOGICAL SCIENCES
doi:10.1093/toxsci/kft205

iCell Cardiomyocytes

Development →Regulatory Guidance

- **Product launch → regulatory evaluation in <u>3 years</u>**
- **iPS cell-derived cardiomyocytes are being evaluated for use in arrhythmia assessment & as a <u>replacement for thorough QT studies</u>**

Cellular Dynamics international

Product Ideation (2008)

Roche

Product release (2010)

Cellular Dynamics international

First Publication (2011)

Roche

MERRIMACK PHARMACEUTICALS

First IND including iCell Cardiomyocytes (2012)

FDA

FDA interactions begin (2012/13)

HESI

HESI meetings (2013)

BIOBUSINESS BRIEFS

REGULATORY WATCH

Speedy validation sought for new cardiotoxicity testing strategy

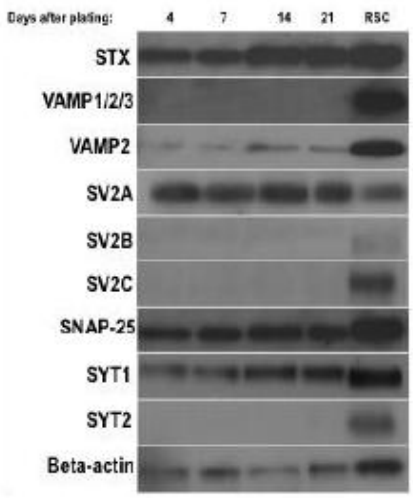
NEWS & ANALYSIS

Revolution dawning in cardiotoxicity testing

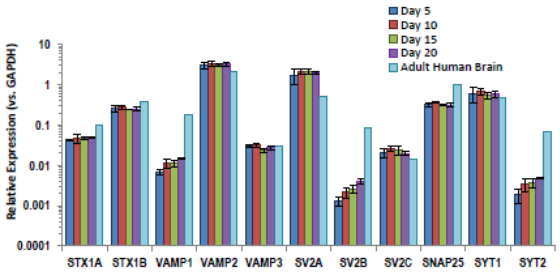
Nature Reviews Drug Discovery (Aug, Sept 2013)

Stem Cell Derived Tissues and Toxicology

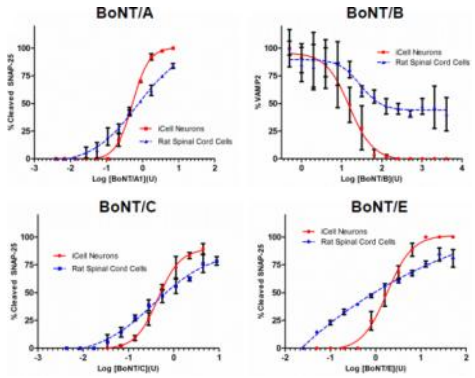
BoNT Receptor Protein Expression



BoNT Receptor Gene Expression



BoNT Receptor Cleavage



- Assess the potency of botulinum neurotoxin (BoNT) better than rat spinal cord neurons or mouse LD50.
- a consortium of BoNT manufacturers is in the process of validating the use of Stem Cell derived Neurons to replace the current industry "gold" standard, a high-cost and labor-intensive in vivo bioassay.

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

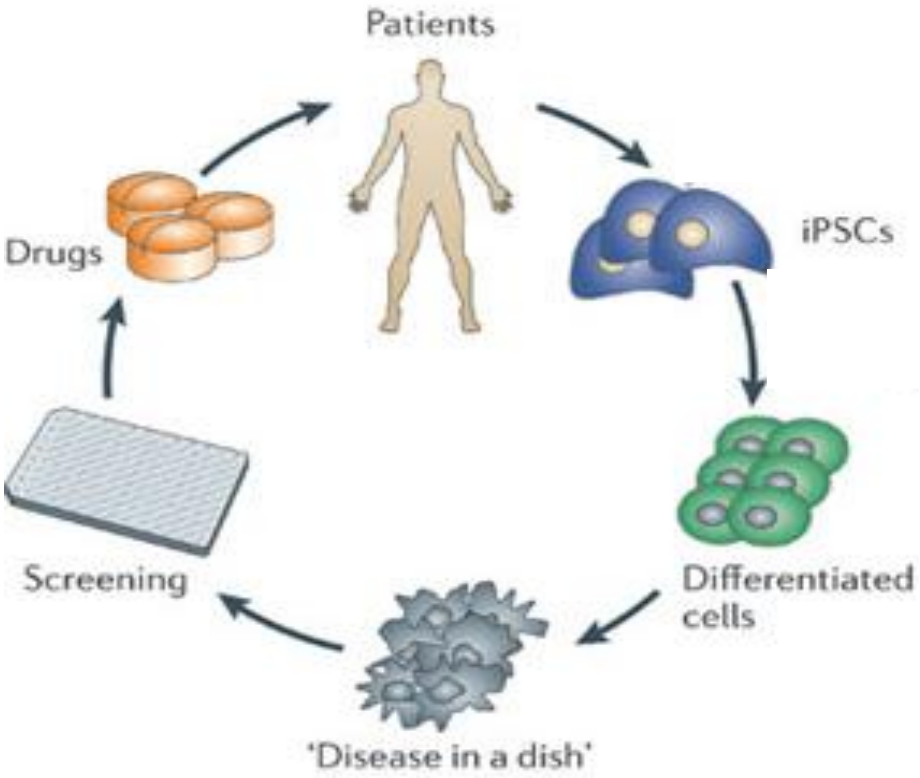
Novel Application of Human Neurons Derived from Induced Pluripotent Stem Cells for Highly Sensitive Botulinum Neurotoxin Detection

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

‘Disease in a Dish”

Modeling Human Disease using iPSCs



Adapted from Grskovic, et al. (2011)

iPSC technology can be used to model human **Innate**, **Induced** and **Infectious** diseases that cannot be interrogated using conventional cell lines, primary cells or animal models

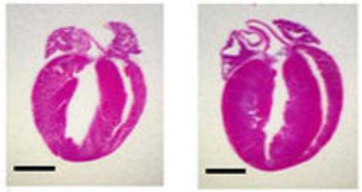
Reviews & summaries of disease-specific iPSCs created:

- Grskovic, et al. (2011) *Nature Reviews Drug Discovery*
- Rajamohan, et al. (2012) *Bioessays*
- Trounson, et al. (2012) *Current Opinion Genetics & Development*

Induced Disease Modeling

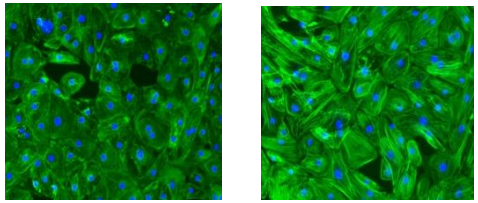
Cardiac Hypertrophy

Mouse Phenotype
(increased cell size)

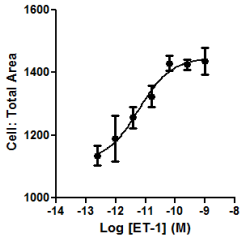


Normal Disease

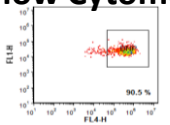
Endothelin Induced iCell CM



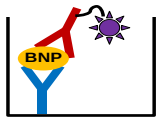
Control ET-1 (10 nM)



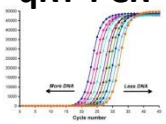
Flow Cytometry



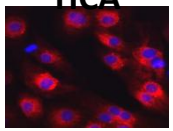
ELISA



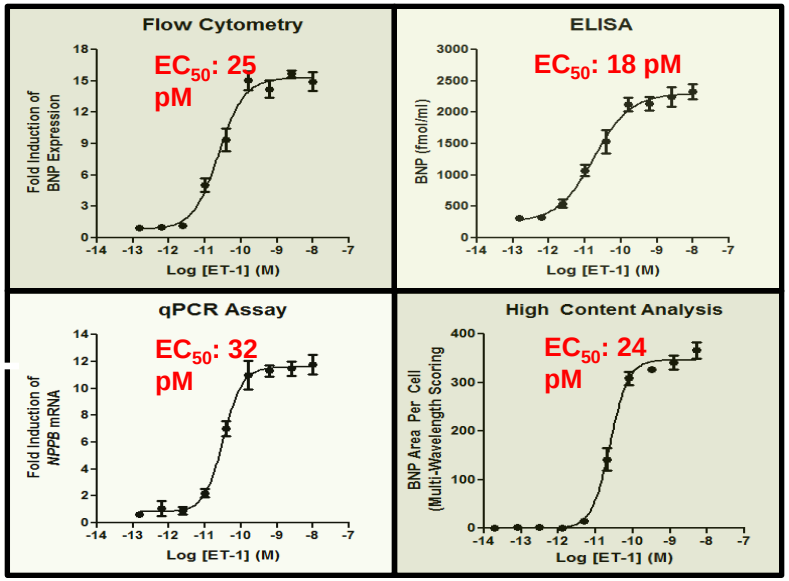
qRT-PCR



HCA

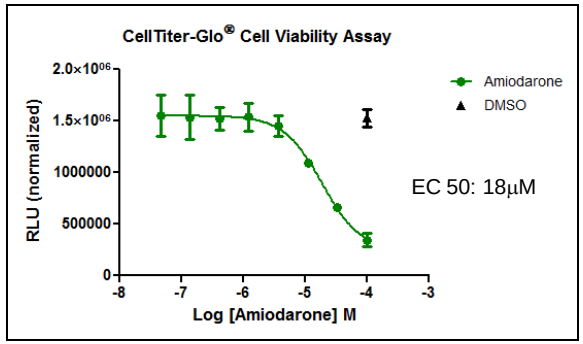


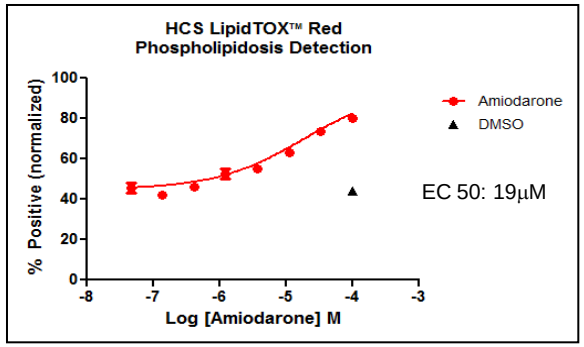
Robust and Reproducible in-vitro assays
(BNP-based readouts)

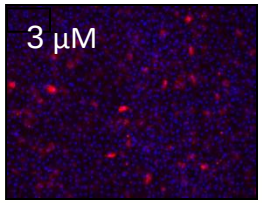


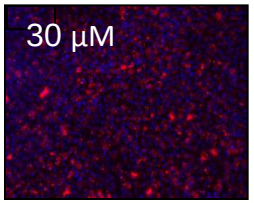
Steatohepatitis

Amiodarone Induced iCell HC





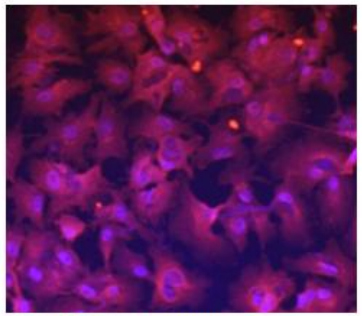




Infectious Disease Modeling

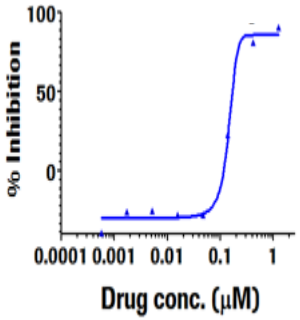
iCell Hepatocytes
HCV Infection (Clinical Genotypes)

Luc Expressing HCV pseudoparticle (HCVpp) uptake

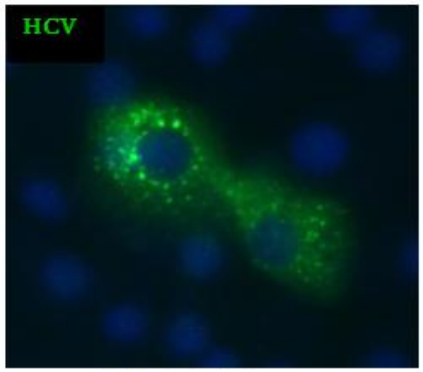


HCVpp encoding Firefly luciferase

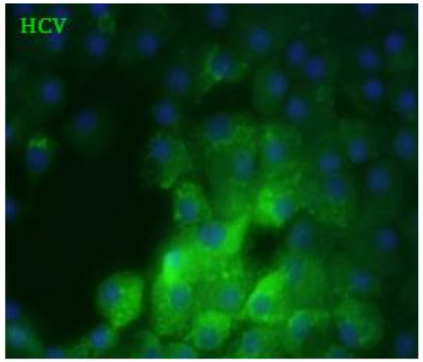
Inhibition of HCVpp Uptake by anti-CD81 Ab



iCell Hepatocytes are Susceptible to Multiple HCV Genotypes

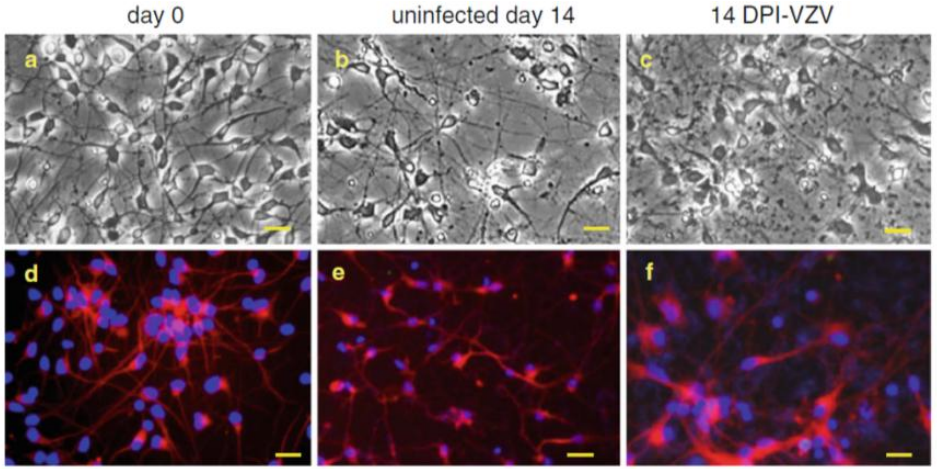


HCVcc - Cell Culture Passaged Virus (Genotype 1a/2a)

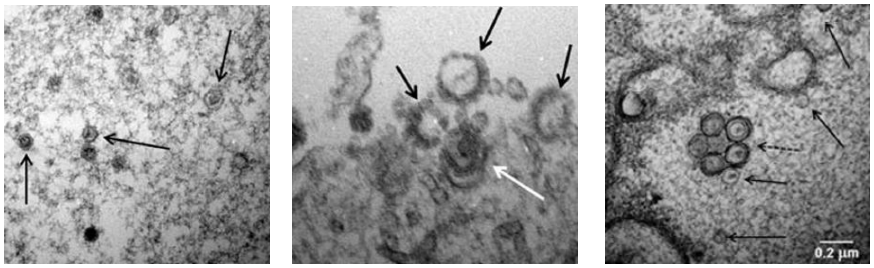


Patient Serum HCV (Genotype 1a)

iCell Neurons
Physiologic VZV Latent Infection



VZV infection did not produce a cytopathic effect



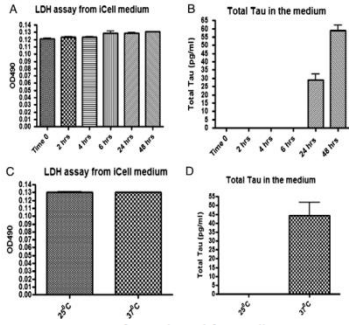
Viral Particles and Capsids in iCell Neurons

Yu, et al. (2013) J Neurovirology

iCell Neurons:
Novel Alzheimer's Disease Biology & Use in Phenotypic Screens

Novel Alzheimer's Disease Biology

Tau Secretion



◄ *Constitutive extracellular secretion of tau protein by an unconventional mechanism. Secretion was influence by both time and temperature.*

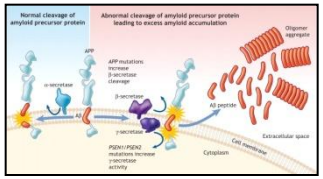
Percentage of tau released from cells.

	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 ± 36	267 ± 86	0.1
iCell® Neurons	59 ± 6	20 ± 2	0.3

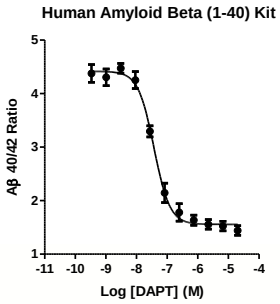
Chai X, et al. (2012) Neurobiol Dis.

β-Amyloid Processing

CDI (2013)

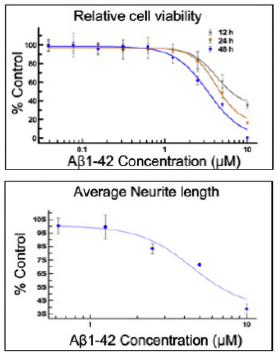


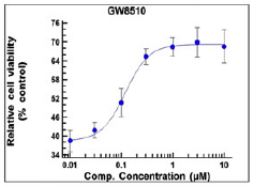
► *Endogenous levels of Aβ40 was modulated upon treatment with a secretase inhibitor .*



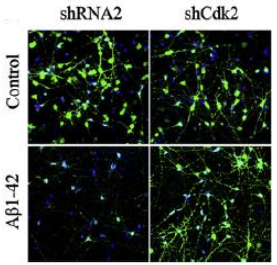
Relevant Neuronal Model for Alzheimer's Disease

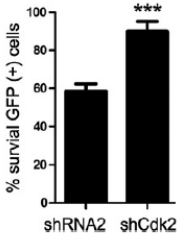
▼ *iCell Neurons showed sensitivity to Aβ1–42.*

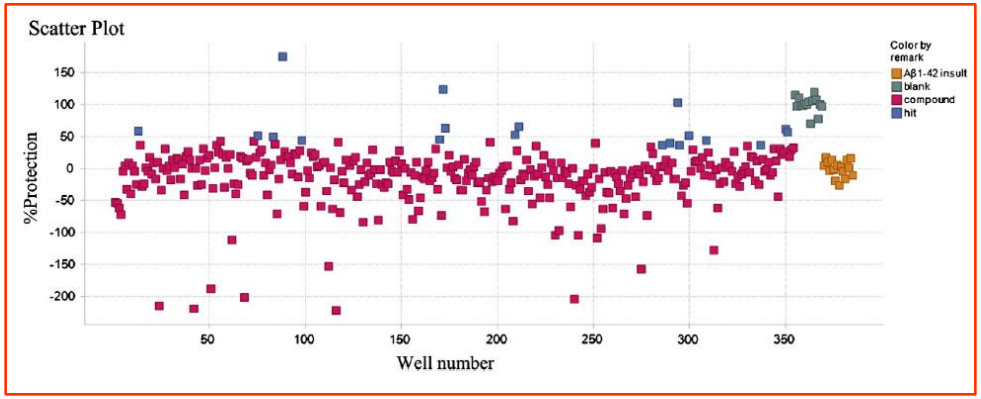




◄ ▼ *Neuroprotection from induced Aβ1–42 toxicity was demonstrated by small-molecule inhibition and shRNA knockdown of CDK2.*







▲ *HT phenotypic screen identified 19 hits, including a CDK2 inhibitor confirming reliability and sensitivity of the platform.*

Xu X, et al. (2013) Stem Cell Res

iPS Cell Disease Lines with Phenotypes

Neuronal Diseases
Amyotrophic lateral sclerosis
Spinal muscular atrophy
Olivopontocerebellar atrophy
Parkinson's disease
Huntington's disease
Down's syndrome
Fragile X syndrome
Friedrichs Ataxia
Familial dysautonomia
Rett's syndrome
Mucopolysaccharidosis type IIIB
Schizophrenia
X-linked adrenoleukodystrophy
childhood cerebral ALD
Adrenomyeloneuropathy
Autism spectrum disorders
Angelman syndrome
Pradder-Willi

Skin
Recessive dystrophic
epidermolysisbullosa

Eye
Retinitis pigmentosa
Age-related cataract
Gyrate atrophy

Multi-organ
Down syndrome - Trisomy 21
Shwachman-Bodian-Diamond syndrome
Dyskeratosiscongenita

Current status of drug screening and disease modelling in human pluripotent stem cells

*Divya Rajamohan, Elena Matsa, Spandan Kalra, James Crutchley, Asha Patel, Vinoj George and Chris Denning**

Bioessays 35: 281–298, © 2012 WILEY Periodicals, Inc.

Muscle
Duchene Muscular Dystroph
Becker muscular dystrophy
Hutchinson-Gilford progeria syndrome

Metabolic
Gaucher disease type III
Lesch-Nyhan syndrome
Juvenile Diabetes
Type 2 diabetes
Familial hypercholesterolemia
Alpha1-antitrypsin deficiency
Glycogen storage disease type 1a

Immune
Adenosine deaminase deficiencyassociated severe combined immunodeficiency (ADA-SCID)
Multiple Sclerosis

Cardiovascular Diseases
Flavors of long QT syndrome
CPTV
LEOPARD syndrome
Timothy Syndrome

Haematological
Sickle cell anaemia b-Globin alleles
Fanconi anaemia
Acquired myeloproliferativedisordes
b-Thalassaemia major (Cooley's anaemia)

Cellular Dynamics international

Discovery

Phenotypic vs Target-based Screens

Historical Phenotypic Screens

- MMOA knowledge not required
- Target ID difficult
- Lack of relevant human biology

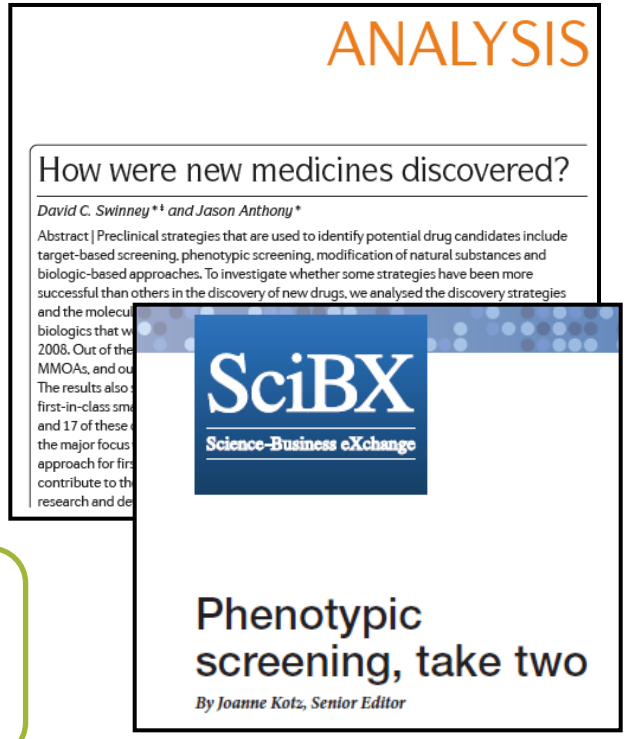
1980-2000 Target-based Screens

- Driven by genomics & informatics
- Limited new validated targets under study
- Lack of translatability in vivo
- ↑ **R&D investments; ↓first-in-class drugs**

2000-Present Phenotypic Screens

- Rapid discovery of disease modifying molecules
- Improved Target ID (genetic and chemical proteomics)
- **Improved relevance of human biology**

Swinney & Anthony (2011) Nat Rev Drug Discovery


ANALYSIS

How were new medicines discovered?

David C. Swinney‡ and Jason Anthony**

Abstract | Preclinical strategies that are used to identify potential drug candidates include target-based screening, phenotypic screening, modification of natural substances and biologic-based approaches. To investigate whether some strategies have been more successful than others in the discovery of new drugs, we analysed the discovery strategies

SciBX
Science-Business eXchange

Phenotypic screening, take two

By Joanne Kotz, Senior Editor

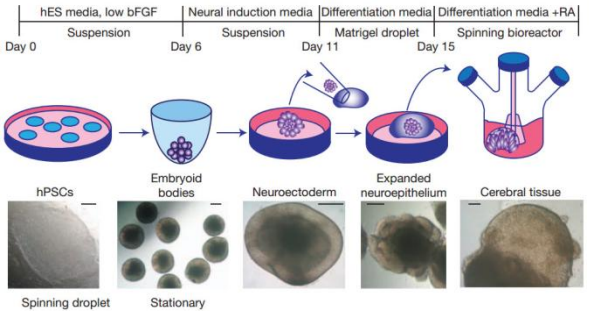
Kotz (2012) SciBx – April 12, 2012

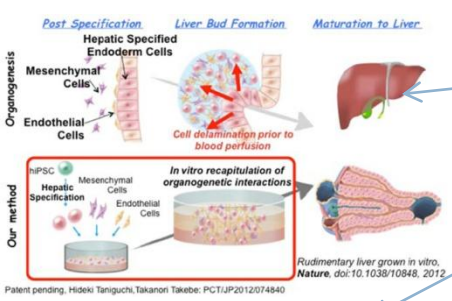
iPSC technology can be used to model diseases with known MMOA, as well as in phenotypic-based screens for complex diseases with unknown genetic mechanisms

Regenerative Medicine and the Future of iPSC: Cell, Tissue and Organ Creation

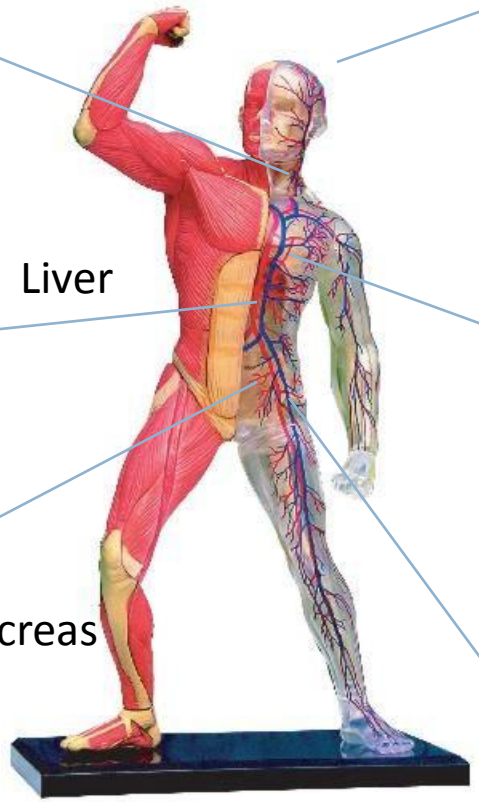
Trachea

Brain

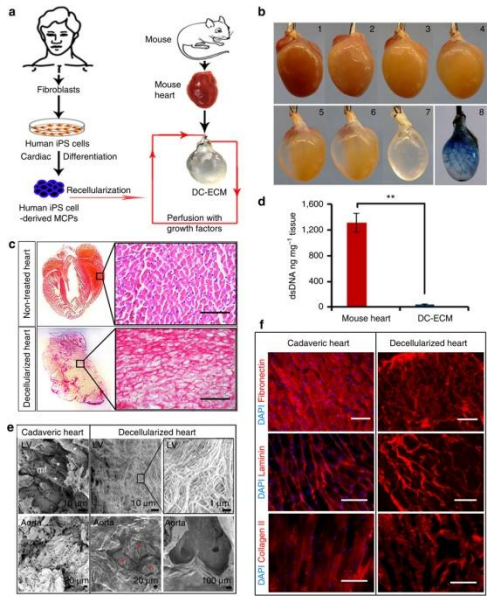




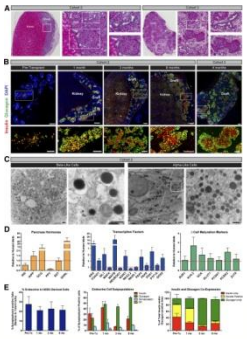
Liver



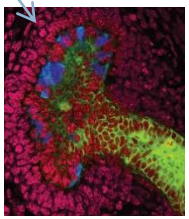
Heart



Pancreas



Kidney



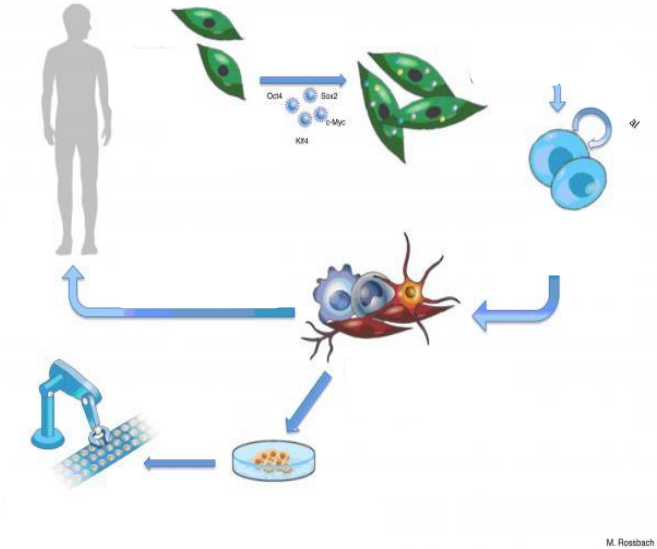
iPS cell-derived Tissues and Potential

IPS-derived cells improvement over primary culture

- Amenable to genetic engineering
- Maturing phenotype
- Relevant disease models can be induced or derived

Improved functionality → Ask better questions

Robust manufacturing a necessity



iPS cells allow direct control over genetic diversity

- Patient disease phenotype recapitulation in vitro
- Retrospective clinical trials
- Prospective clinical trials ?

- **Clinical applications have potential to completely change medicine**