Use of Human-induced Pluripotent Stem Cell-derived Cardiomyocytes As a Screen for Drug-induced Cardiotoxicity

Kyle Kolaja PhD, DABT Fellow, AST

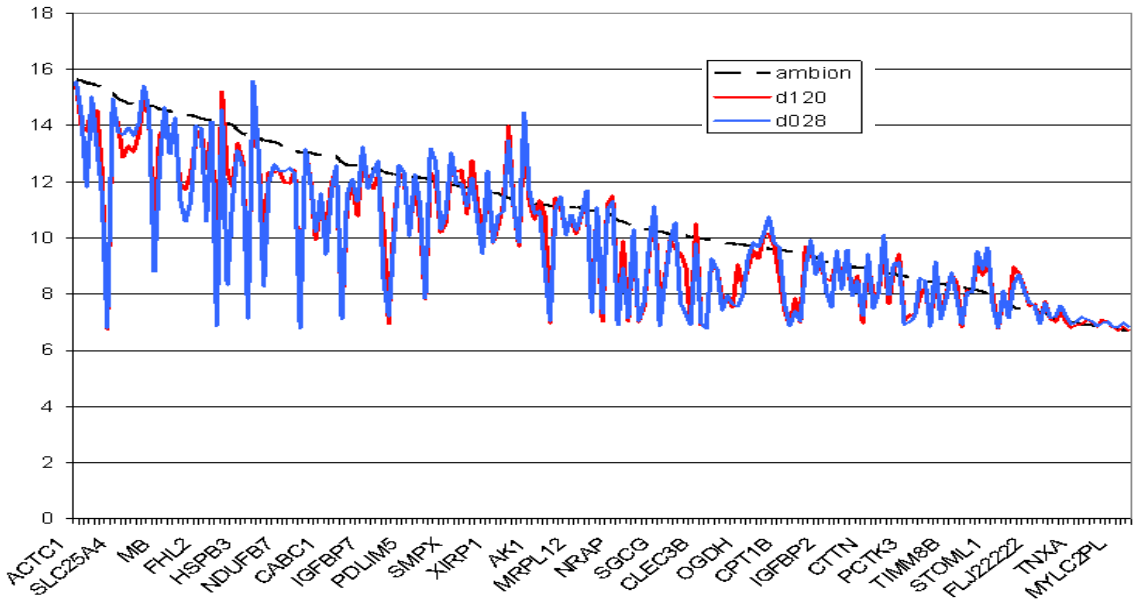
SOT Annual Meeting

March, 2015

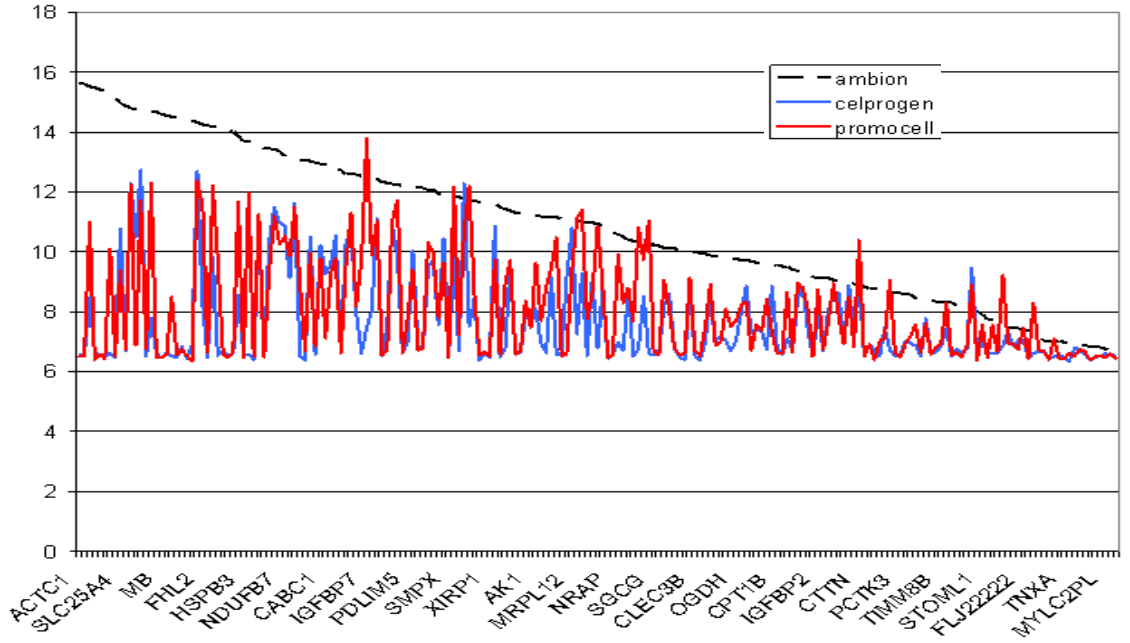
iCell Cardiomyocytes are more similar to Adult Human Heart Samples than Primary Cultures

Cardiac gene expression profiles from

Adult cardiac tissue (ambion) and iCell Cardiomyocytes (d028 and d120)



Adult cardiac tissue (ambion) and commercially available primary cardiomyocytes samples

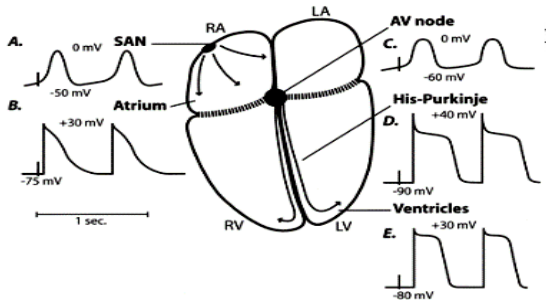


iCell Cardiomyocytes provide a stable expression profile that correlates with adult cardiac tissue

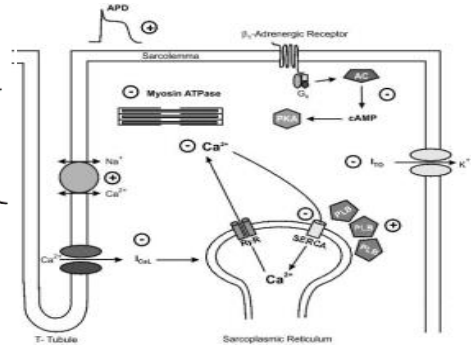
Babiarz et al Stem Cells Dev. Jan 2012

Ability to Predict In Vitro Cardiotoxicity Signals Improved with iPSC derived Cardiomyocytes

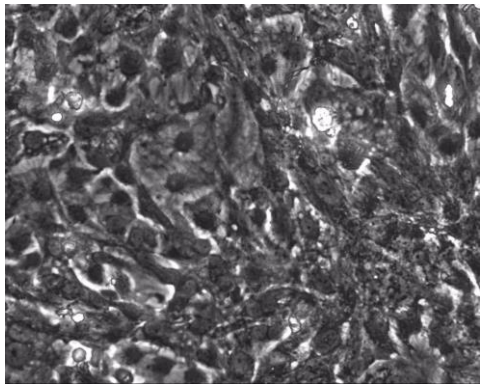
Electrical



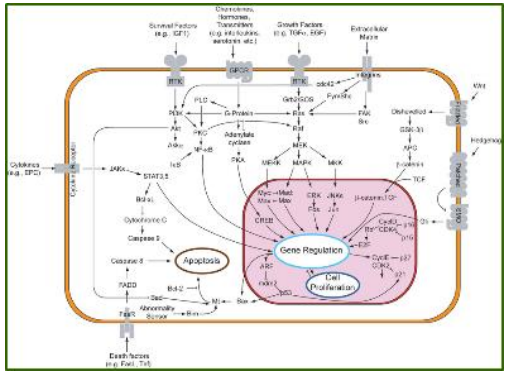
Ca^{2+} Signaling



Mechanical



Structural Toxicity



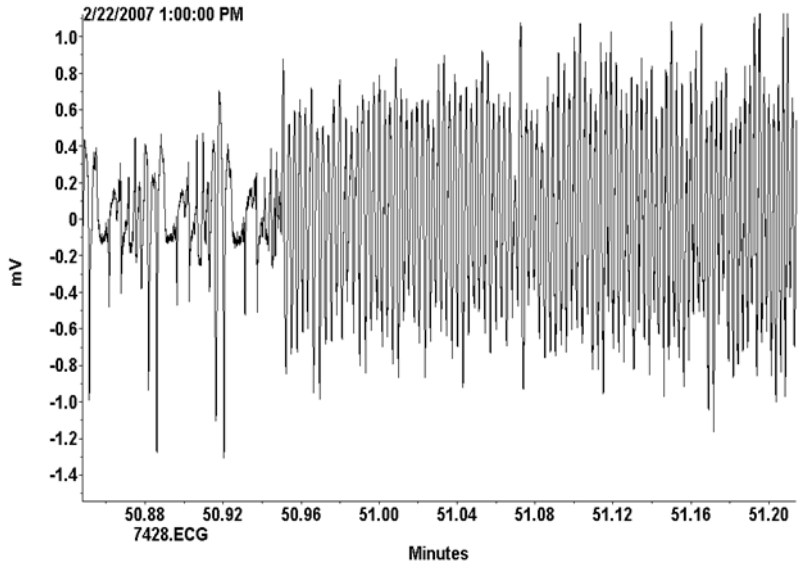
Measurement of Cardiomyocyte Electrical and Mechanical Activity					
Process	**Electrical Activity**	**Ca^{2+} cycling / signaling**	**Contraction**	**Other**	**Platform**
Measurement (Direct = text Indirect = arrow)	Transmembrane Im or Vm				Patch Clamp
	Extracellular Vm				MEA
	→	Intracellular Ca2+			FliPR (MoDev)
	→		Physical movement		IonOptix ImageXpress Kinetic Image Cytometer
	→			Cell shape attachment	xCELLigence (Roche/ACEA)

Endpoint	Platform(s)
Viability	Cell-based assays, HCI
Mitochondrial health	Cell-based assays, HCI
Oxidative stress	Cell-based assays
Bioenergetics	Seahorse XF-Analyzer

iPSC Cardiomyocytes enable mechanistic and phenotypic assays across multiple functional endpoints

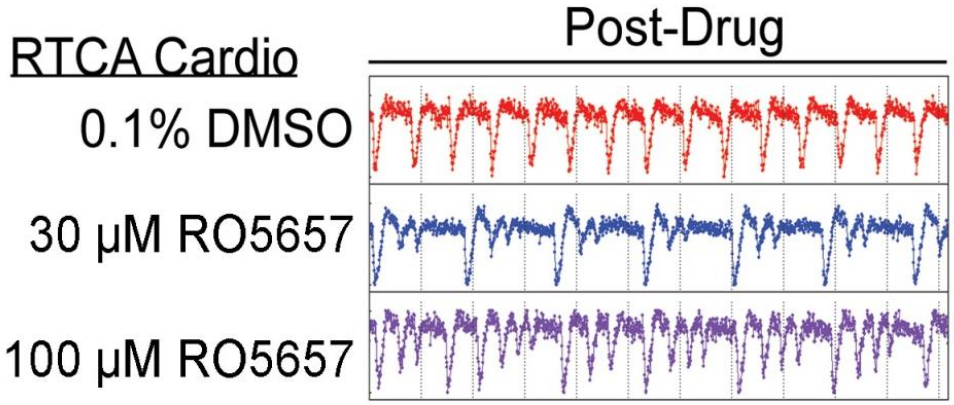
Torsade Induction in Cynomolgus Monkey Reproduced in Vitro

Arrhythmia induction in cynomolgus monkey via administration of the CCR5 antagonist RO5657



Misner et al Br J Pharmacol. 2012 Apr;165(8):2771-86.

Arrhythmia induction in iPSC cardiomyocytes (iCell Cardiomyocytes) via administration of the CCR5 antagonist R5067z



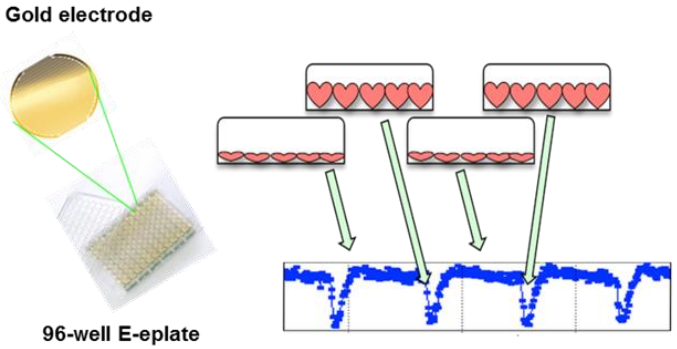
Guo et al. Toxicol Sci. 2011 Sep;123(1):281-9.

iPSC-Cardiomyocytes recapitulate proarrhythmic behavior

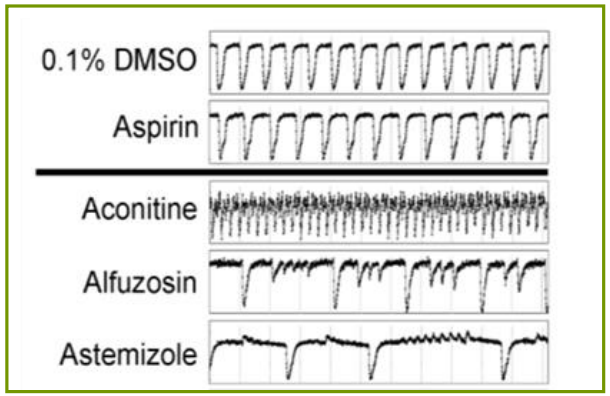
Measuring Electrical Activity - Predictivity Screens

Label Free Impedance Measurements

Proarrhythmia screening in 96 wells



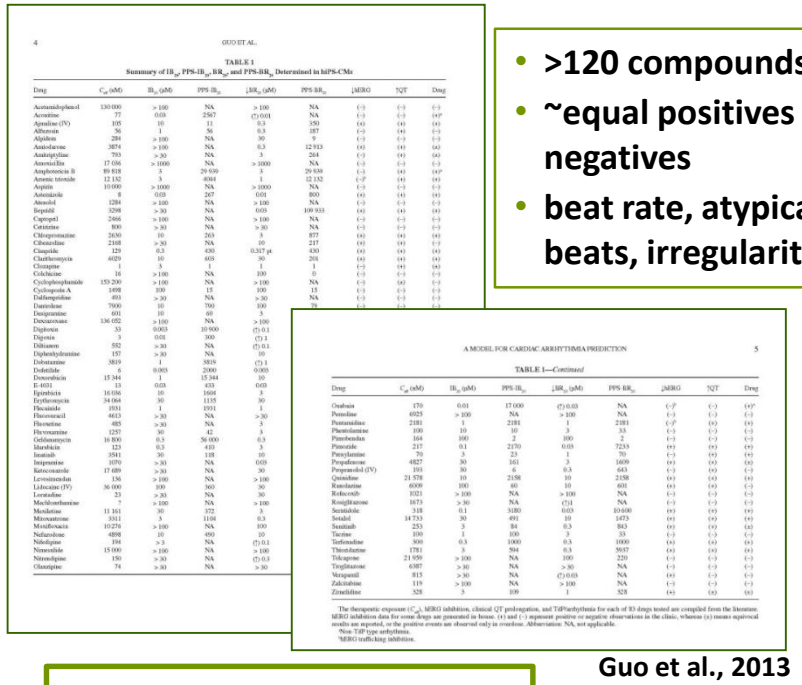
Cardiomyocyte activity generates rhythmic deflections of the impedance baseline



Guo et al., 2011

Easily implemented higher throughput proarrhythmia screening

Larger screens with quantitative analytics provides greater predictivity



- >120 compounds
- ~equal positives and negatives
- beat rate, atypical beats, irregularity

Guo et al., 2013

> 90% -- QT prolongation
> 80% -- Proarrhythmia

iPSC cardiomyocytes provide a more predictive tool for early proarrhythmia screening

Comprehensive In Vitro Proarrhymia Assay

Objective:

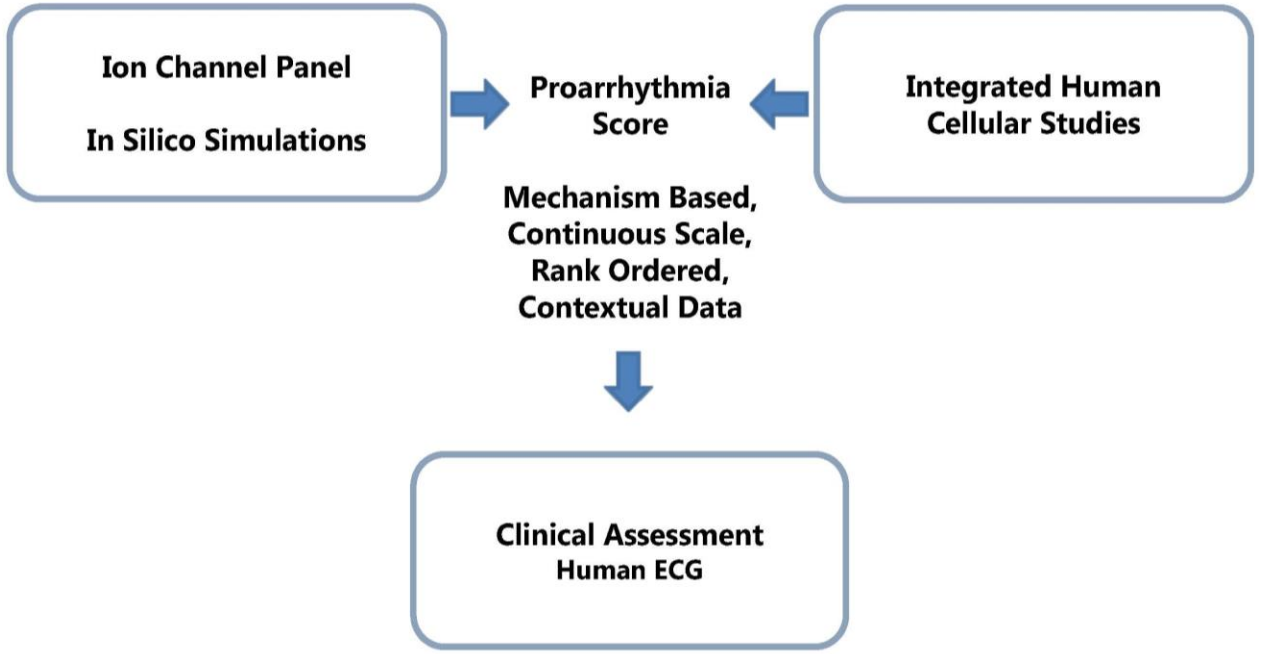
- Facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation.
- CIPA is envisioned to ultimately require modification or replacement of the existing ICH S7a/b guidelines and elimination of E14 guidelines.

Anticipated Final Outcome:

- Eliminate the need for a TQT study for compounds entering clinical development with a negative dataset based on the newly proposed in vitro and in silico paradigm

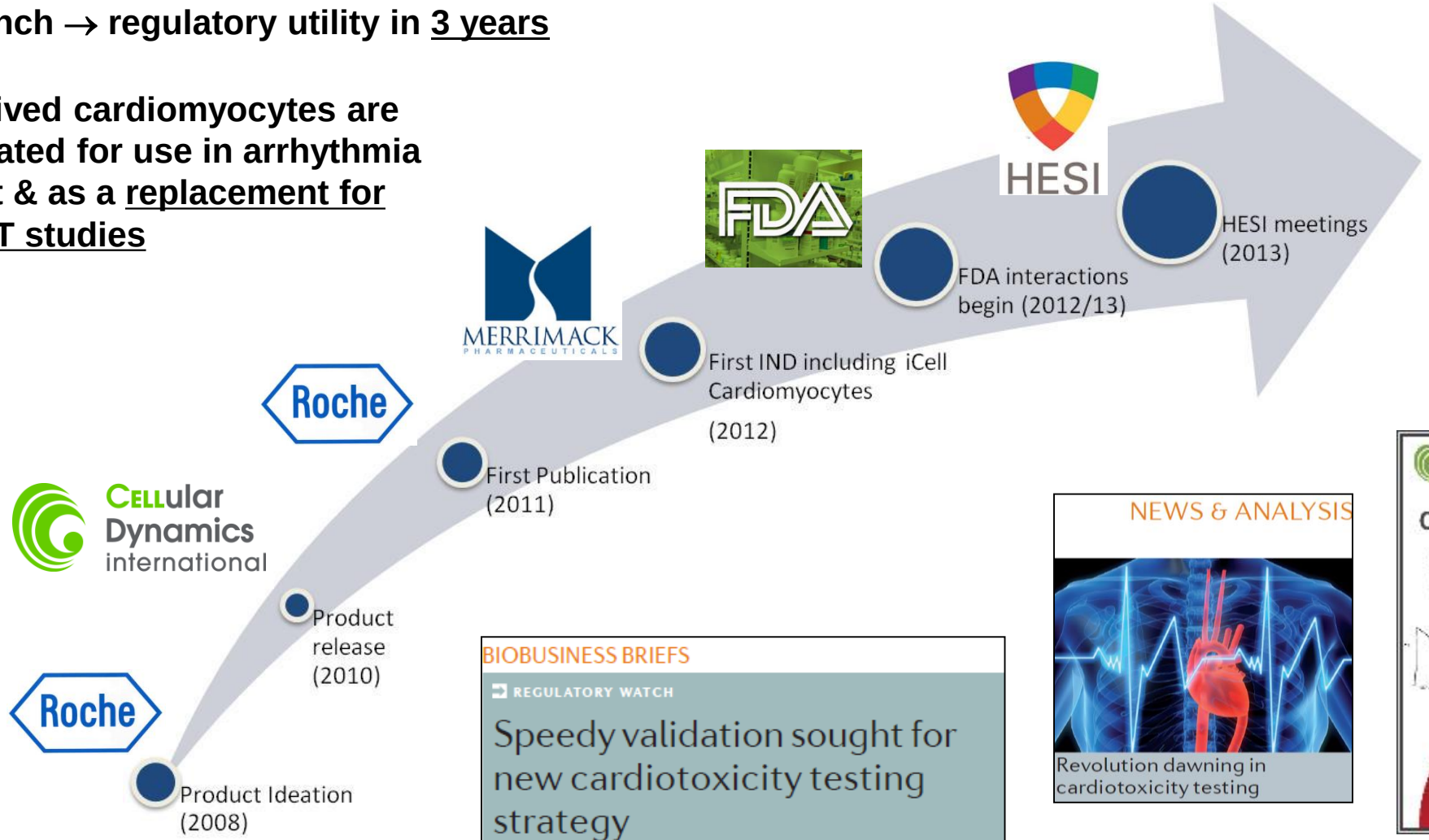
CIPA Partners:

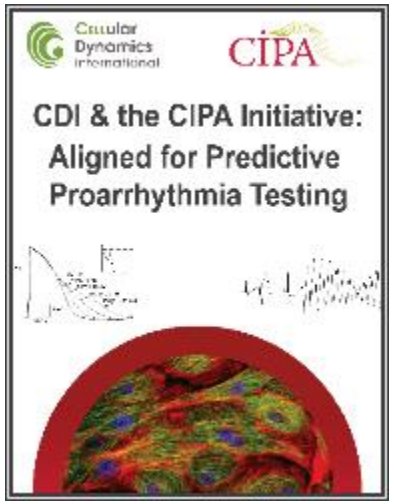
- USU FDA, HESI, CSRC, SPS, EMA, Health Canada, Japan NIHS, PMDA



iCell Cardiomyocytes:
Development → Regulatory Guidance

- **Product launch → regulatory utility 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>**





Nature Reviews Drug Discovery (Aug, Sept 2013)

Nature Reviews Drug Discovery (Aug, Sept 2013)

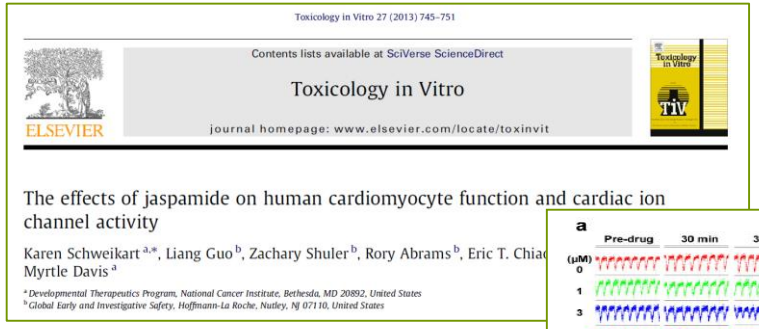
Cardiotoxicity – Channel Effects Can Contribute to Injury

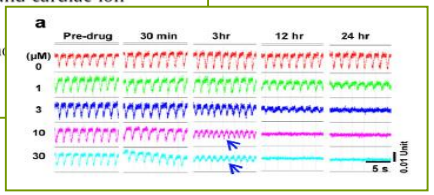
- **Jaspamide** - potential cancer therapeutic agent
  - a cyclodepsipeptide (marine sponge Jaspis johnstoni)
  - Affects actin binding to cytoskeleton
  - Pulmonary edema /cardiac hemorrhage /congestion in tox species
  - inhibited Kv1.5 activity by 98.5%. inhibited Cav1.2, Cav3.2, and HCN2;
  - but not a hERG blocker
  - Induced arrhythmic beats in vitro in stem cell derived CMs

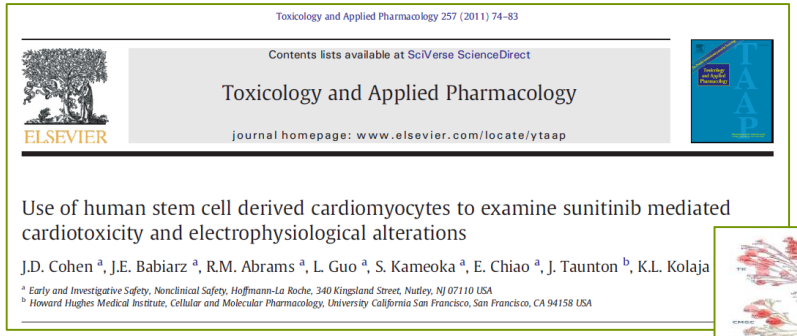
- **Sunitinib -** multi-targeted inhibitor – oncology
  - cardiac dysfunction and cardiotoxicity (CHF)
  - potently cardiotoxic in stem cell derived cardiomyocytes
  - **AMPK inhibited but no attenuation**
  - Inhibit hERG, Ca++ cycling and NaV1.5 -> arrhythmia
  - arrhythmia and cytotoxicity in stem cell dervied CMs

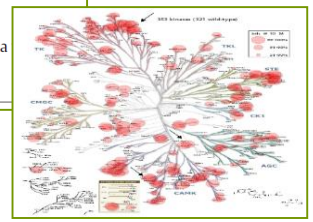
- **RO5657**
  - CCR5 antagonist, mild hERG inhibitor (IC 50 = 12 uM)
  - Myofiber loss and morbundity in 2 monkeys
  - Expanded telemetry study showed only torsades, no cardiac tox.
  - Stem cell derived CMs show arrhythmia and no cytotoxicity

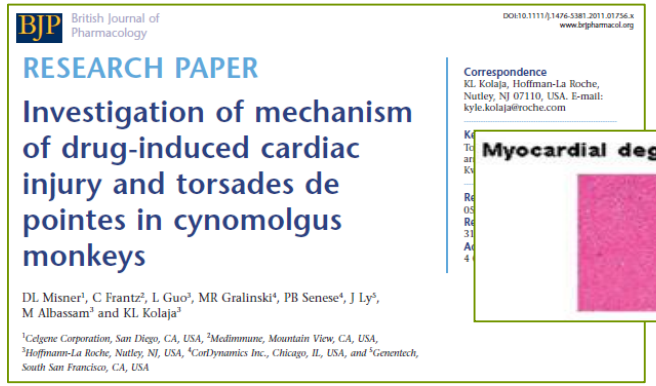
- **Multi-factorial combination of cytotoxicity, cardiac conduction abnormalities, hypoxia, suppressed response/accommodation mechanisms**











iPSC-CMs ideal model to assess cardiotoxicity, electrophysiology and contractility effects in parallel

Off Target Cellular Gene Therapy Targeting MAGE A3

Toxicity due to Titin-cross reactivity

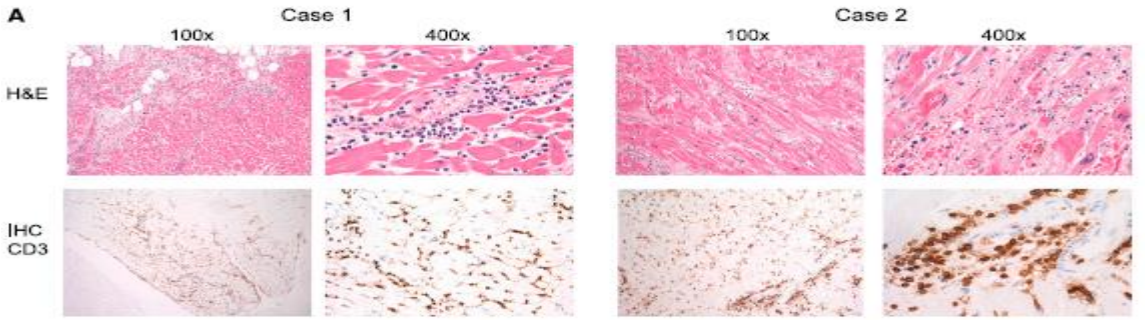
- **Modified T cell to increase affinity to MAGE 3A receptor, a putative tumor antigen**

- **Phase I trial – 2 patients died of cardiogenic shock and fever**
  - Ventricular myofiber loss with infiltrate
  - MAGE 3A not expressed on heart samples
  - No toxicity in preclinical toxicity studies

- **Bioinformatic modeling to detect - off target recognition of titin, a protein that is a component of striated muscle**
  - only expressed in beating cells
  - not expressed in static primaries
  - 1um long, largest protein in body, 3rd most abundant, 0.5 kg/person

- **T cells expressing the affinity-enhanced TCR but not wild type were toxic to IPSC-CMs**

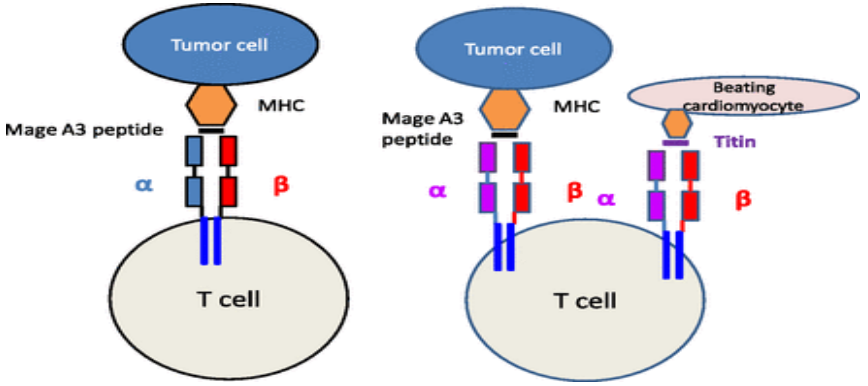
- **2 Main points**
  - Affinity enhancing T cells may create unintended targets
  - Complex development programs need to test toxicity in human models



Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma

Gerald P. Linette,[1] Edward A. Stadtmauer,[2] Marcela V. Maus,[2] Aaron P. Rapoport,[3] Bruce L. Levine,[2] Lyndsey Emery,[2] Leslie Litzky,[2] Adam Bagg,[2] Beatriz M. Carreno,[1] Patrick J. Cimino,[1] Gwendolyn K. Binder-Scholl,[4] Dominic P. Smethurst,[4] Andrew B. Gerry,[4] Nick J. Pumphrey,[4] Alan D. Bennett,[4] Joanna E. Brewer,[4] Joseph Dukes,[5] Jane Harper,[5] Helen K. Tayton-Martin,[4] Bent K. Jakobsen,[4,5] Namir J. Hassan,[5] Michael Kalos,[2] and Carl H. June[2]

[1]Siteman Cancer Center and Departments of Medicine and Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; [2]Abramson Cancer Center, Department of Medicine, and Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; [3]The Greenebaum Cancer Center, University of Maryland, Baltimore, MD; [4]Adaptimmune Ltd, Philadelphia and Abingdon, United Kingdom; and [5]Immunocore Ltd, Abingdon, United Kingdom



iPSC-CMs provided relevant biology not found in other pre-clinical models to enable detection of off-target toxicity

Determining if Mechanism is Electrical or Structural is Most Important

Screening assays to delineate mechanism

Biomarkers, backup selection, and mechanistic understanding

Pushing the limits further with a better understanding of genetic diversity

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

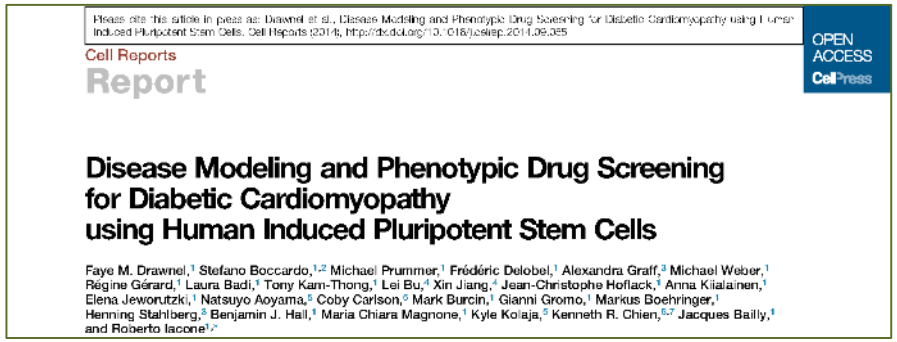
Immune
Adenosine deaminase deficiencyassociated severe combined immunodeficiency (ADA-SCID)
Multiple Sclerosis

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)

iCell and Patient-derived Cardiomyocytes in Drug Discovery

Drawnel et al, 2014 Cell Reports


Cell Reports
Report

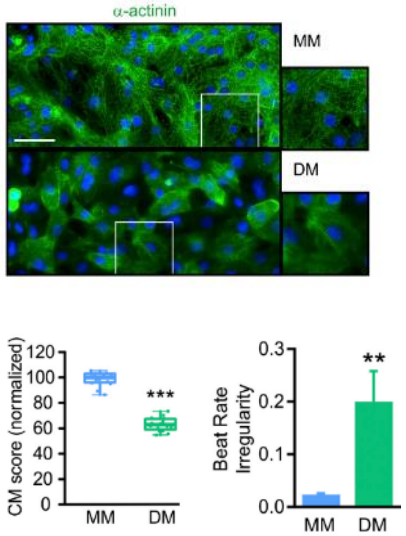
OPEN ACCESS

Disease Modeling and Phenotypic Drug Screening for Diabetic Cardiomyopathy using Human Induced Pluripotent Stem Cells

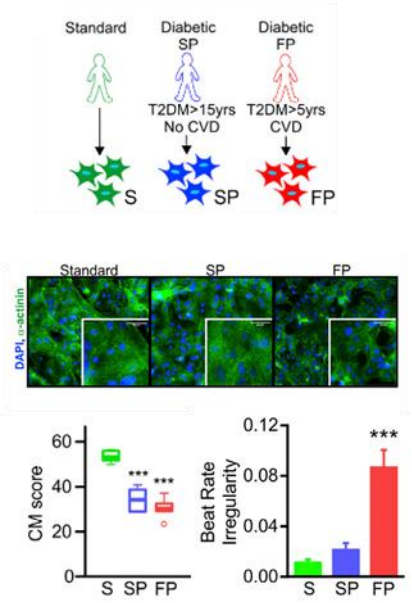
Faye M. Drawnel,[1] Stefano Boccardo,[1,2] Michael Prummer,[1] Frédéric Delobel,[1] Alexandra Graff,[3] Michael Weber,[1] Régine Gérard,[1] Laura Badi,[1] Tony Kam-Thong,[1] Lei Bu,[4] Xin Jiang,[4] Jean-Christophe Hoflack,[1] Anna Kiialainen,[1] Elena Jeworutzki,[1] Natsuyo Aoyama,[5] Coby Carlson,[6] Mark Burcin,[1] Gianni Gromo,[1] Markus Boehringer,[1] Henning Stahlberg,[3] Benjamin J. Hall,[1] Maria Chiara Magnone,[1] Kyle Kolaja,[5] Kenneth R. Chien,[6,7] Jacques Bailly,[1] and Roberto Iacone[1,*]

- **Diabetes causes pathological remodeling of cardiac muscle, which impairs heart function**
- **Diabetic media induces hallmarks of in vivo diabetic cardiomyopathy**
  - **Sarcomeric disorganization, altered Ca^{2+} transients, cellular hypertrophy, lipid accumulation, oxidative stress, BNP release, gene expression**
- **MyCell diabetic patient-specific CMs mimic diabetic phenotype**
  - **Severity dependent on their original clinical status**

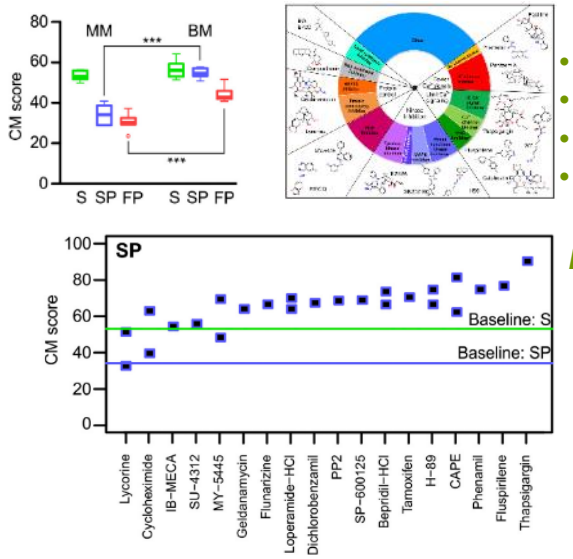
Diabetic media induces disease phenotype



Patient-derived CMs show baseline pathology



Tiered phenotypic screens identified functionally diverse hits



- ***480 compounds***
- ***47 hits***
- ***28 confirmed DR***
- ***Across a wide MOA***

Phenotypic screen enabled large diversity of hits

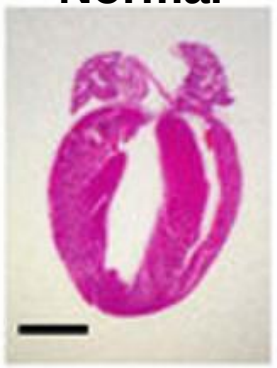
Drawnel et al, Cell Reports, 2014

"Induced" diabetes model in normal iPSC CMs = Innate" diabetes patient iPSC CMs

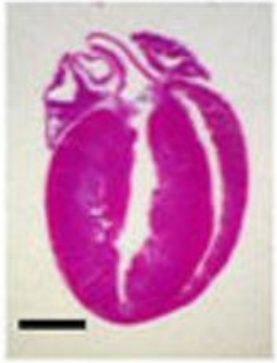
Induced Disease Model of Cardiac Hypertrophy

Abnormal Gross
Cardiac Phenotype (Rat)

Normal

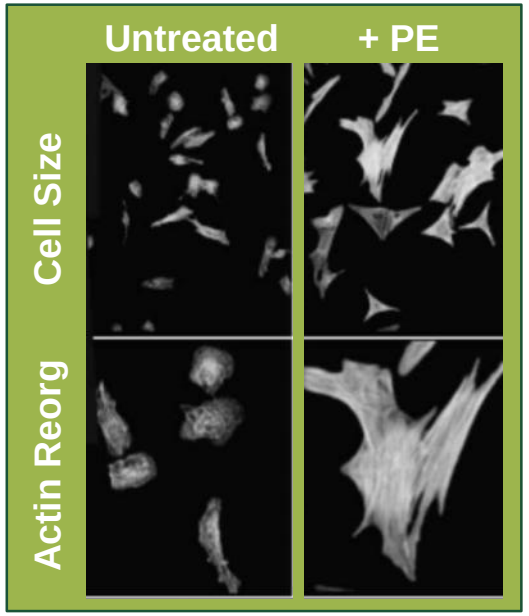


Diseased

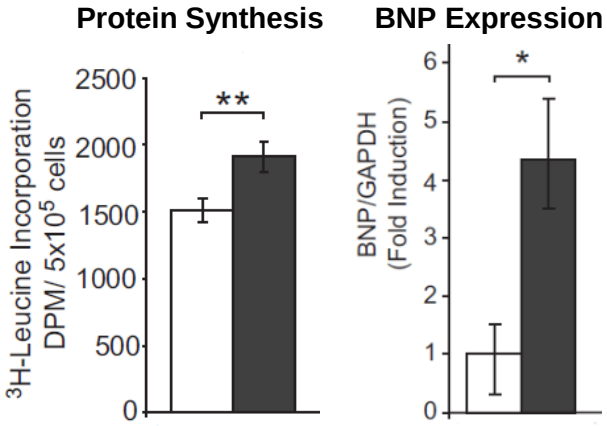


Cellular and Molecular Hallmarks

- Increased cell size
- Enhanced protein synthesis/ sarcomeric organization
- Re-activation of the fetal gene program (BNP, ANP, etc)



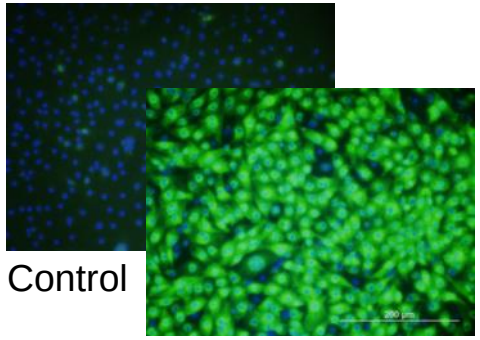
Lister K et al. Cardiovasc Res 2006;70:555-565

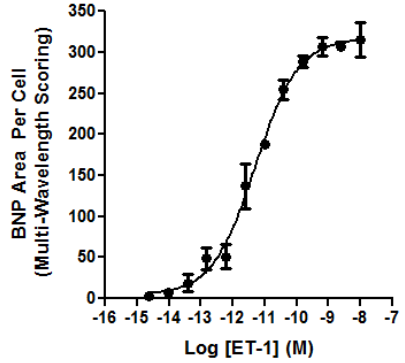


Glenn D et al. Hypertension 2009;53:549-555

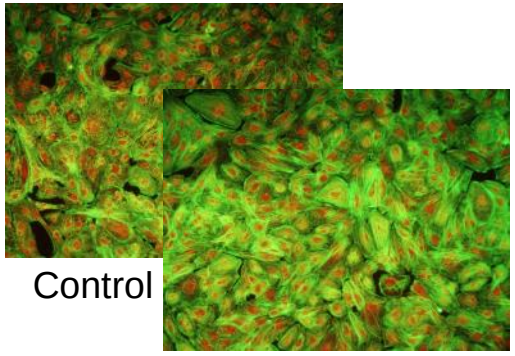
In Vitro Induction in Cardiac Hypertrophy:

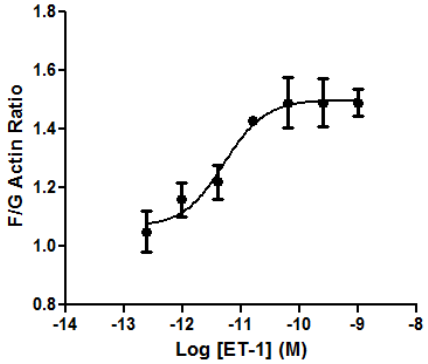
Fetal Gene Expression



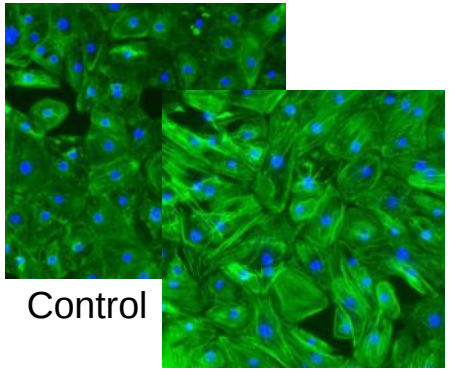


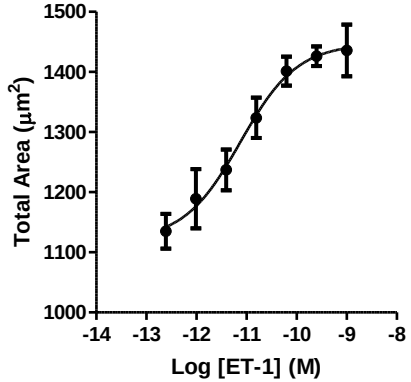
Cytoskeletal Rearrangements





Cell Size



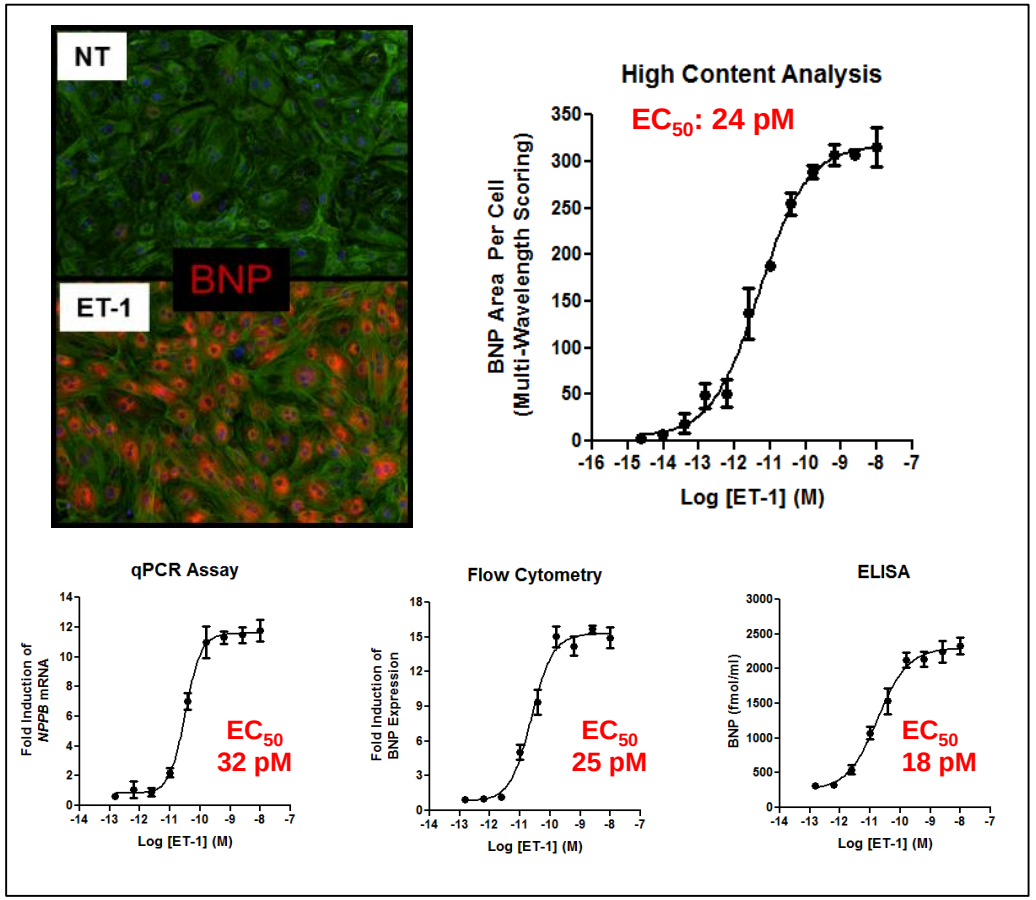


Carlson et al., J Biomol Screen 2013

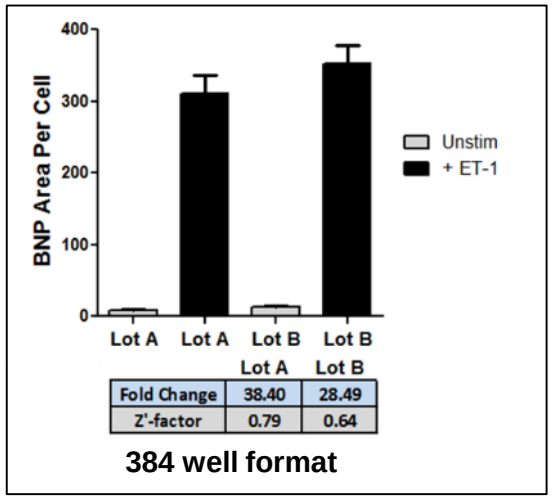
ET-1 induced iCell Cardiomyocytes exhibit classic hallmarks of cardiac hypertrophy

Development of Hypertrophy Model :
Screening Assay Validation

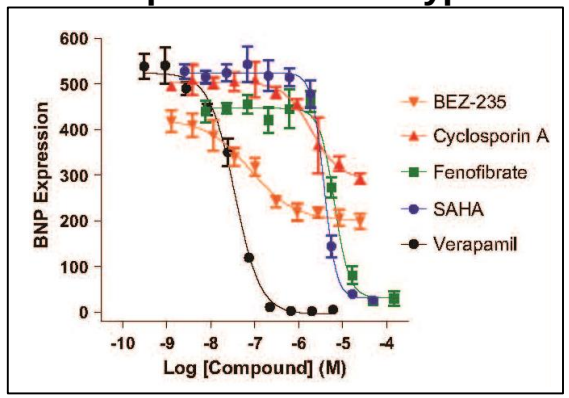
Consistent ET-1 induction across assay readouts



High Z'



Responsive Phenotype

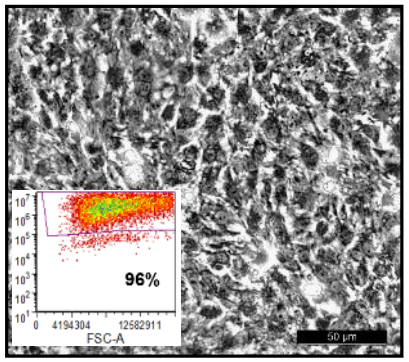


iCell Cardiomyocytes provide a robust and implementable screening system

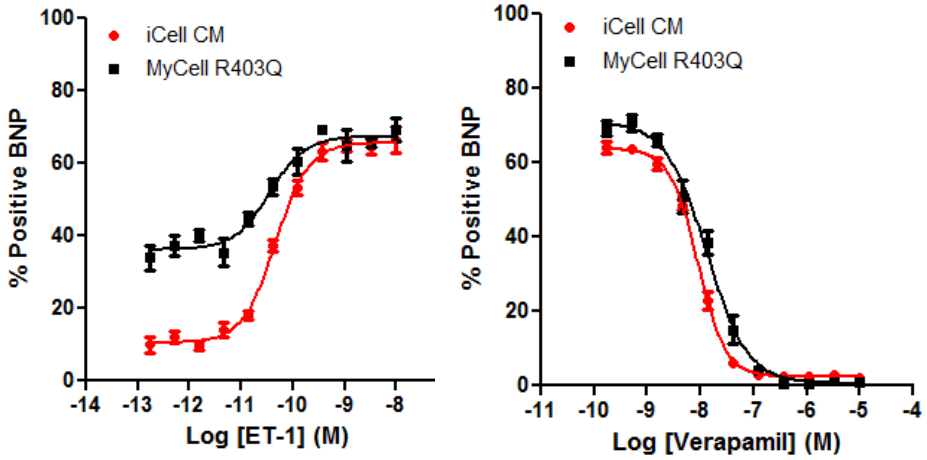
Carlson et al, J Biomol Screen, 2013

Innate Cardiac Disease Models:
MYH7-R403Q linked hypertrophic cardiomyopathy

MyCell MYH7 R403Q CM
Familial Cardiac Hypertrophy



Phenotype induction and rescue

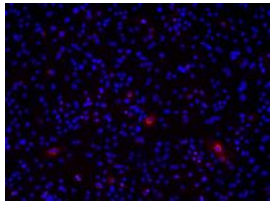


Baseline Pathology

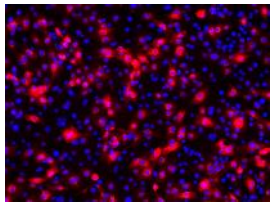
	ET-1 induced iCell CM	MyCell MYH7 R403Q CM
NPPB		
ACTA1		
DUSP4		
ACTC1		
ACTN1		
CREB5		
MYH7		
NPPA		
MYH6		
TRIM63		
ADM		
FBXO32		
PDCD4		

Expression levels normalized to uninduced iCell CMs

iCell CM



MYH7 R403Q CM



***BNP** / **DAPI** 384-well plate.*

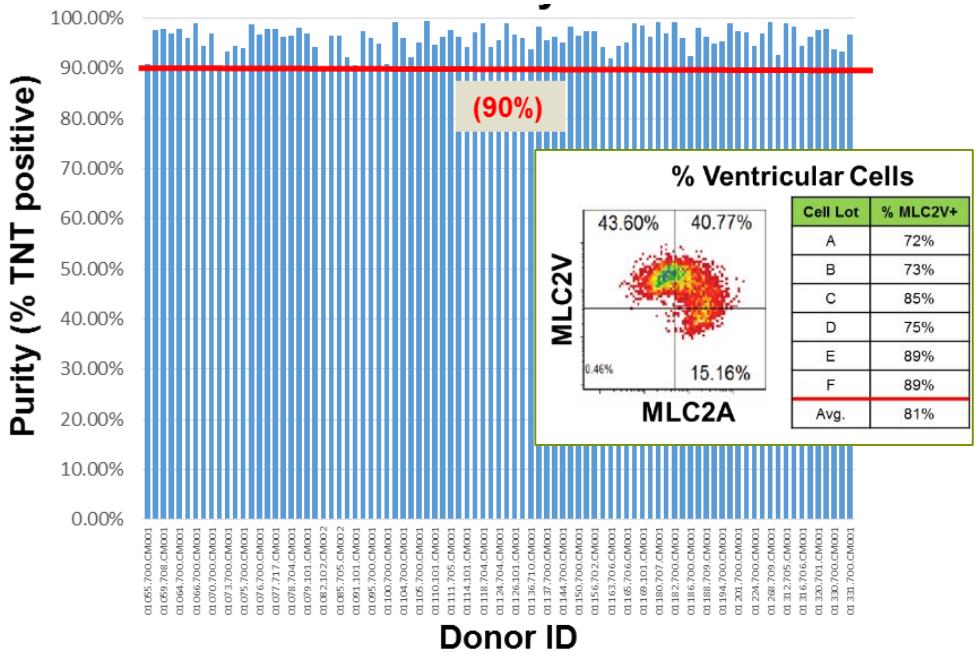
MYH7 R403Q MyCell Cardiomyocytes

- *Show innate and induced signs of cardiac hypertrophy*
- *Hypertrophic phenotype can be rescued*

Provide another model/example of innate disease models suitable for discovery and screening

Cardiac Hypertrophy "Next Generation" GWAS Study : Studying Population Genetics across *250 different iPSC-CMs*

CMs from hundreds of lines provided at high quality and purity



NHLBI Next Gen Genetic Association Studies (Uli Broeckel, MCOW)

- GWAS ID'd Left Ventricular Hypertrophy (LVH) from HyperGEN cohort
- 250 patient samples reprogrammed
- Cardiomyocytes from >90 donors cryopreserved to date - all pass QC
- Cardiomyocytes in hypertrophy assay: correlating ET-1 sensitivity with disease progression
- Drug screening for tailored therapy ID (personalized medicine) ongoing

Preliminary findings include

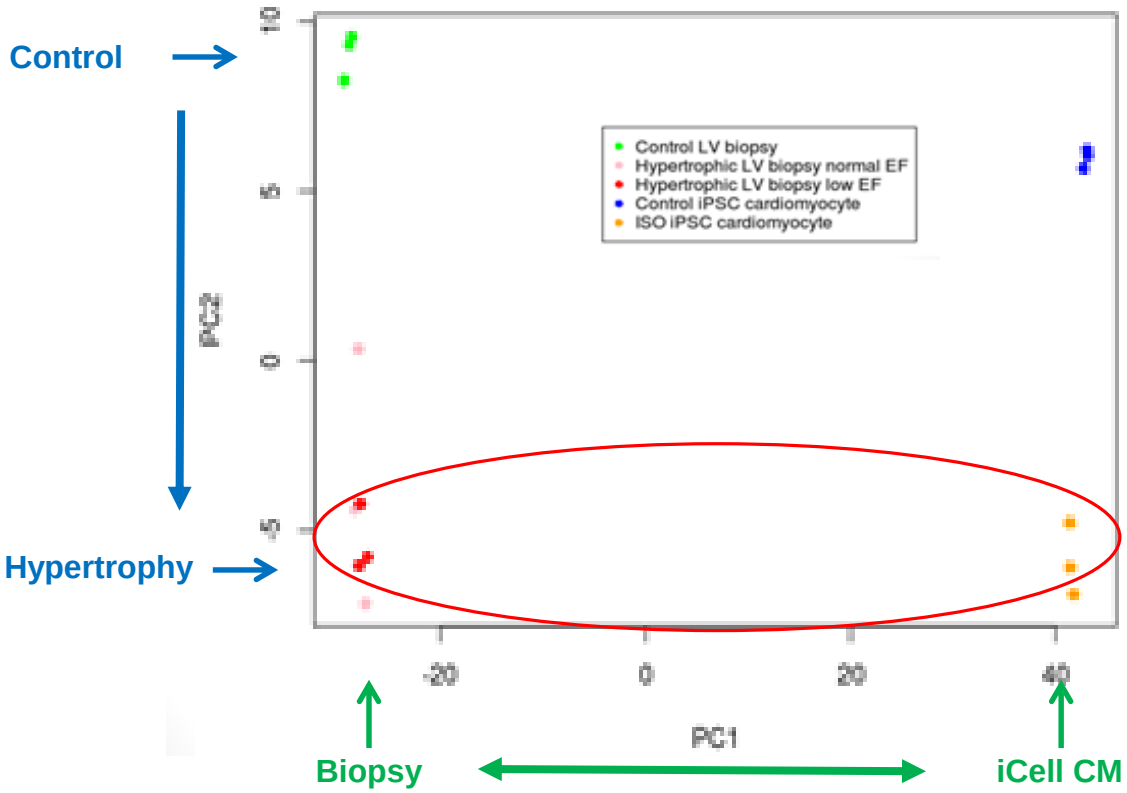
- Unique and common phenotypes across disease CMs
- Correlation between in-vitro phenotype and disease progression *(Uli Broeckel, MCoW)*

CDI technology and capability enables functional population studies from relevant cohorts
laying the foundation for precision medicine and patient stratification

Induced Cardiac Hypertrophy

In-vitro condition reflects native phenotype

Principal Component Analysis (Expression Array) of Human LV Biopsy vs iCell Cardiomvocytes



Comparing normal and LVH tissue samples with normal and hypertrophic iCell Cardiomyocytes

PC1; Biopsy vs iCell Cardiomyocytes

- Difference attributed to heterogeneous tissue sample vs. pure cardiomyocytes

PC2; control vs hypertrophy

- Shift along the axis indicative of pathology

Similar location of hypertrophic samples along PC2 indicates common pathology components

iCell Cardiomyocytes provide a <u>relevant</u> inducible model of cardiac hypertrophy

Zhi et al, Front in Genetics, 2012
Aggarwal et al., Plos One 2014

Conclusions

Stem cell derived cardiomyocytes have changed what‘s possible for in vitro biology, toxicology, and pharmacology

Tools are there to predict and understand mechanism in vitro

Future of in vitro leverages genetic diversity through ipsc-derived tissues