

# Using Electrical Field Stimulation for Maturation of hiPSC Cardiomyocytes, Assessment of Inotropic Compounds and Cardiac Safety Assessment

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*Yama A. Abassi, PhD*  
*ACEA Biosciences, Inc.*  
*Vice President*  
*Global Business Development*

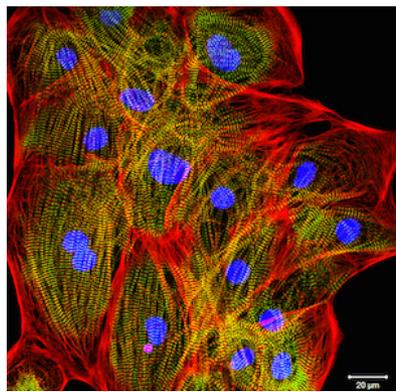
# Outline

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- Overview and utility of the hiPSC-CM as a model system for safety assessment, drug discovery and disease modeling
- Major gaps of the hiPSC-CM model and ramifications
- How to address the maturation gap?
- Introduction of the *ePacer* instrument for consistent and scalable maturation of hiPSC-CM
- Assessment of gene expression in paced hiPSC-CM
- Assessment of structural and functional features of paced hiPSC-CM
- The Applications of electrically paced and functionally matured hiPSC-CM
  - Drug Discovery
  - Safety Assessment
- Summary

# The Diverse Applications of hiPSC Cardiomyocytes

hiPSC-Cardiomyocytes



**Safety/Toxicity**

Electrophys    Contractile    Structural

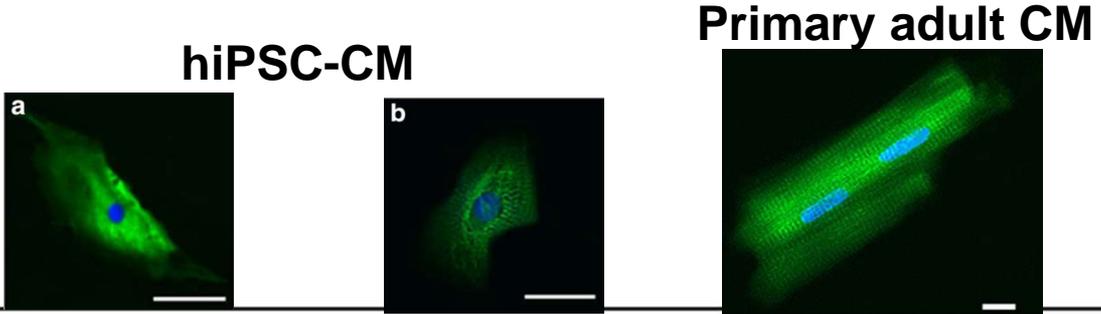
Acute vs Chronic  
Drug/Drug Interaction

**Drug discovery**

**Cardiac Disease  
Modeling**

**Regenerative  
Medicine**

# Comparison of hiPSC-CM with Adult Cardiomyocytes



Biophysical parameter	Early	Late	Adult
Force of contraction (mN/mm <sup>2</sup> ) <sup>a</sup>	0.3 [5]	0.5 [6]	51 [8]
Cell aspect ratio (length-to-width)	1:2 [9]	1:4 [10]	1:7 [10]
Sarcomeric organization	Disorganized [9]	Organized [9]	Highly organized [10]
Sarcomeric distance (μm)	1.65 [12]	1.81 [12]	2.15 [8]
Conduction velocity (cm/s)	<2 [13]	<20 [14]	100 [15]
Multinucleated cells (%)	5 [16]	20 [16]	26 [18]
Mitochondria-to-cell volume ratio	0.06 [19]	0.09 [20]	0.3 [20]
Resting membrane potential (mV)	-50 [21]	-73.5 [22]	-81.8 [23]
Voltage upstroke velocity (V/s)	<9 [21]	26.2 [22]	215 [23]
Calcium Transient Duration (ms)	Unknown	<370 (CaDT90) [24]	~300 (CaDT90) [15]
Calcium Transient Rise time (ms) (80%)	Unknown	>10 [26]	Unknown
Calcium Transient Decay (ms) (80%)	Unknown	>50 [26]	Unknown
ATP level	<2,000 <sup>c</sup> [27]	<3,000 <sup>c</sup> [27]	5.69 [28]
	(lum/#cell)	(lum/#cell)	(mmol/kg weight)

Van Meer, BJ et al (2016) Stem Cells, 34, 2008-2015

# Inappropriate Responses to Positive *Inotrope*

Several positive inotropic compounds have no/negative effect

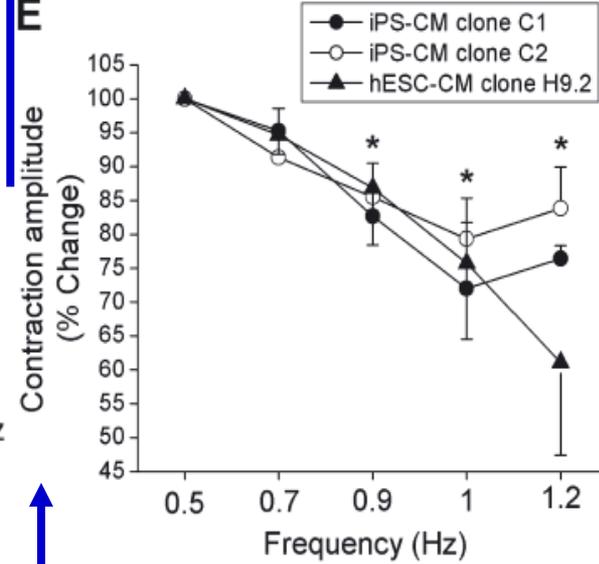
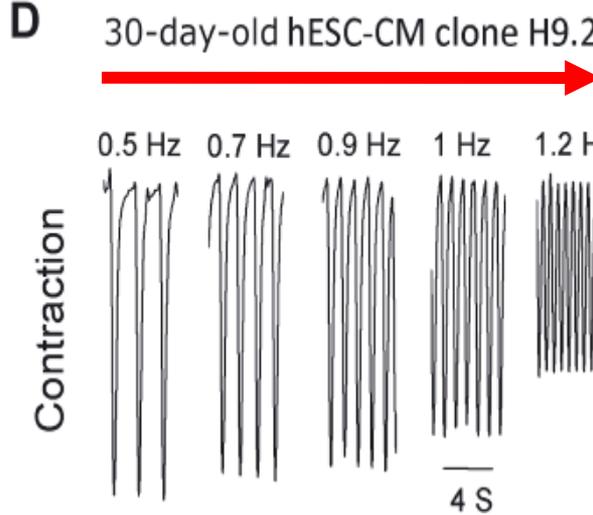
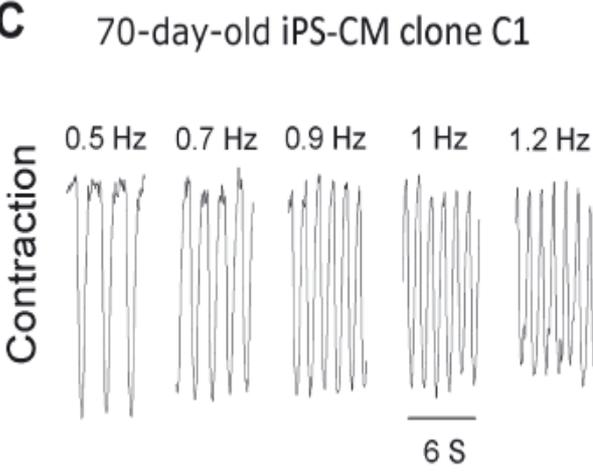
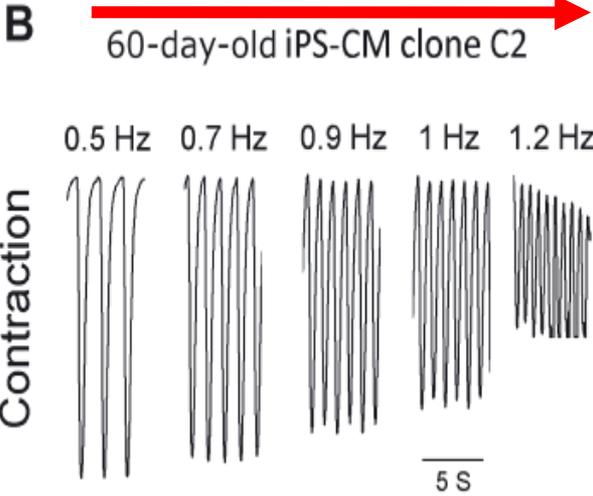
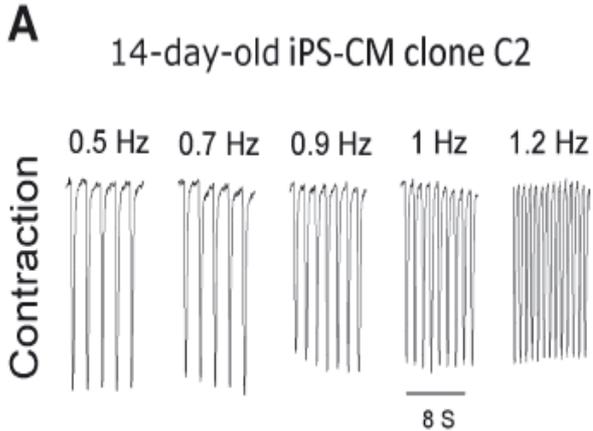
(*IonOptix*)

Compound	Drug Target	CM Microtissue IC <sub>50</sub> /EC <sub>50</sub> (μM)	In Vivo Effect
Amoxicillin	β-lactam antibiotic	—	NE
Paracetamol	Cyclooxygenase inhibitor	—	NE
Sildenafil	PDE-5 inhibitor	—	NE
Enalapril	Angiotensin-converting enzyme inhibitor	—	NE
Tolbutamide	ATP-sensitive potassium channel inhibitor	—	NE
Atenolol	β1-adrenergic receptor antagonist	NE	-ve inotrope
Verapamil	L-type Ca <sup>2+</sup> channel blocker	1↓	-ve inotrope
Diltiazem	L-type Ca <sup>2+</sup> channel blocker	2.47↓	-ve inotrope
Doxorubicin	Intercalates DNA	1.16↓	-ve inotrope
Lapatinib	EGFR-1/EGFR-2 (Her2) inhibitor	NE	-ve inotrope
Epinephrine	Nonselective adrenergic receptor agonist	0.0012↑	+ve inotrope
Glibenclamide	ATP-sensitive potassium channel inhibitor	NE	+ve inotrope
Levosimenden	Calcium sensitizer (binds cardiac troponin C)	NE	+ve inotrope
Digoxin	Inhibits Na <sup>+</sup> /K <sup>+</sup> ATPase membrane pump	0.4↓	+ve inotrope
Dobutamine	β1-adrenergic receptor agonist	0.03↓	+ve inotrope
Isoproterenol	β1-adrenergic receptor agonist	0.00009↓	+ve inotrope
Milrinone	PDE-3 inhibitor	NE	+ve inotrope

Positive  
Inotropes

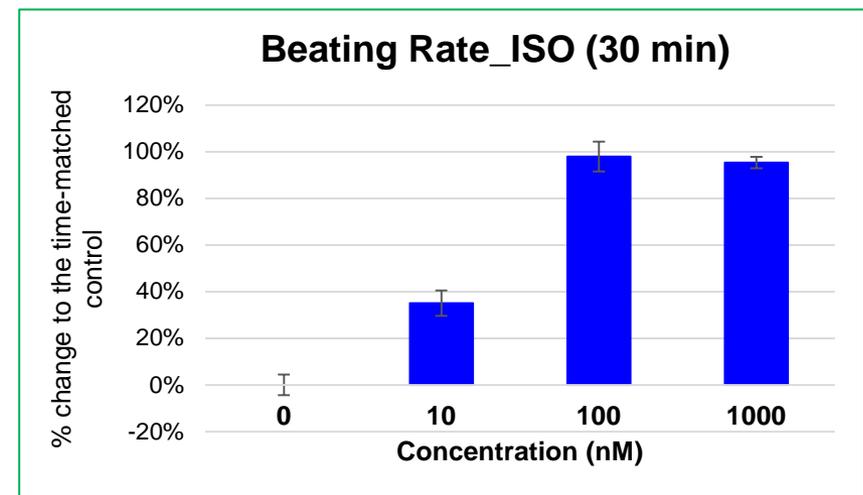
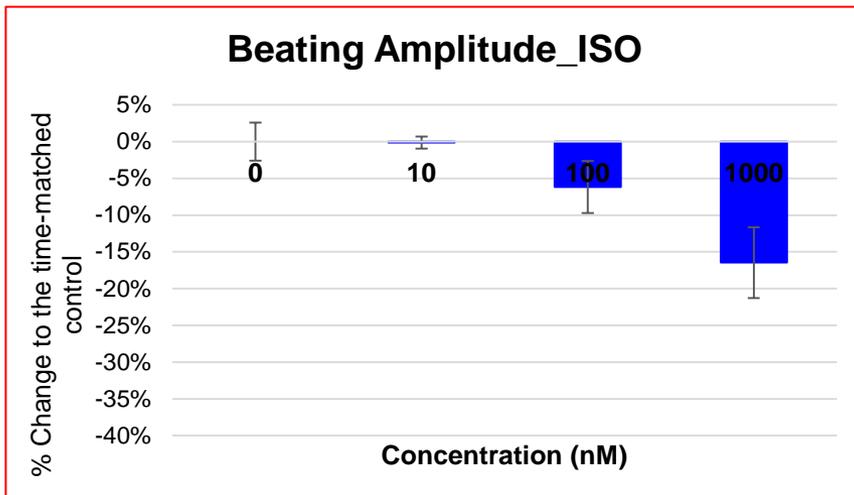
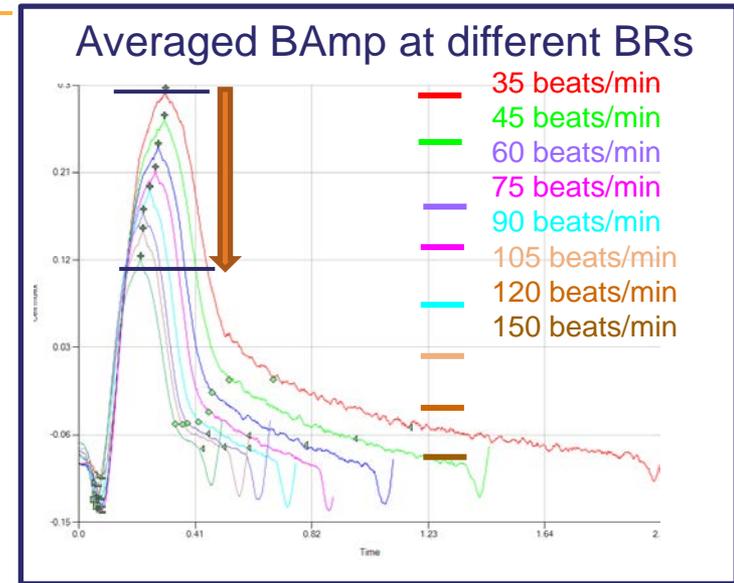
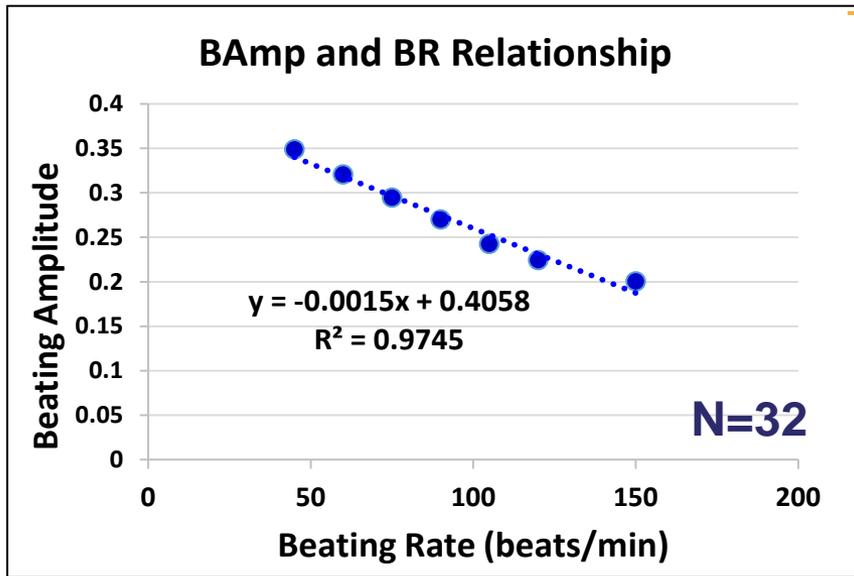
S.M. Ravenscroft et al. Toxicological sciences, 152:99-112 ( July, 2016)

# Functional Output of hiPSC-CM: Negative Force-frequency relationship



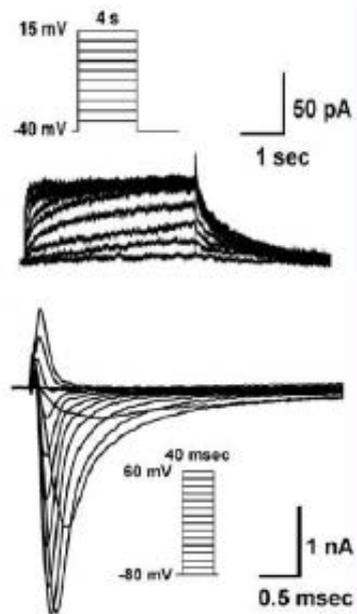
Germanguz I et al., *J. Cell. Mol. Med.* 2011

# Negative Beating Amplitude (BAmp) and Beating Rate (BR) Relationship of hiPSC-CMs



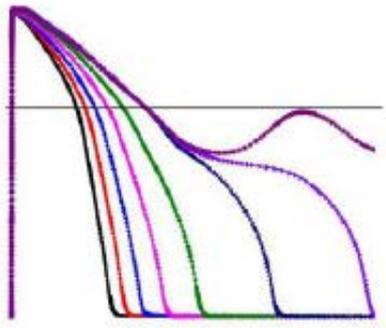
## the Comprehensive In Vitro Proarrhythmia Assay

**Drug Effects on Multiple Human Cardiac Currents**

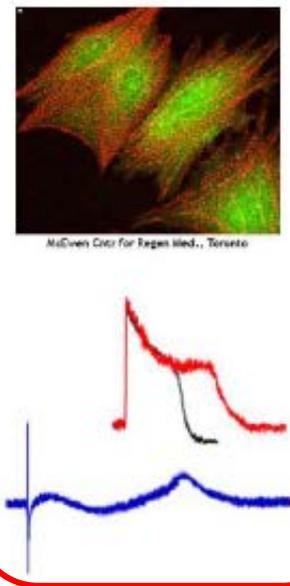


***In Silico* Reconstruction Cellular Human Ventricular Electrophysiology**

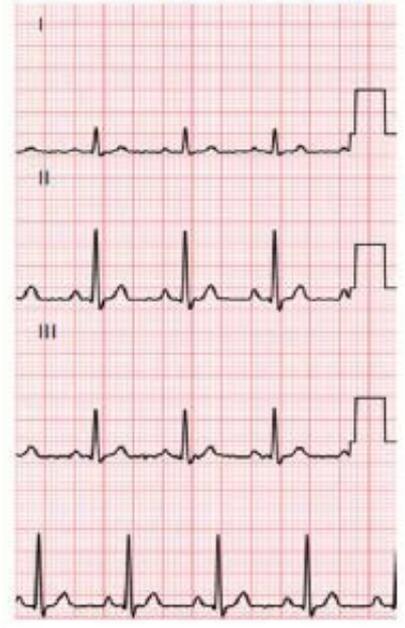
$$I_{stim} = C \frac{dV_m}{dt} + I_m$$



***In Vitro* Effects Human Stem-Cell Derived Ventricular Myocytes**



**Clinical Evaluation Unanticipated Electrophysiology**



**Characterize/Classify Effects**

**Check for Missed or Unanticipated Effects**

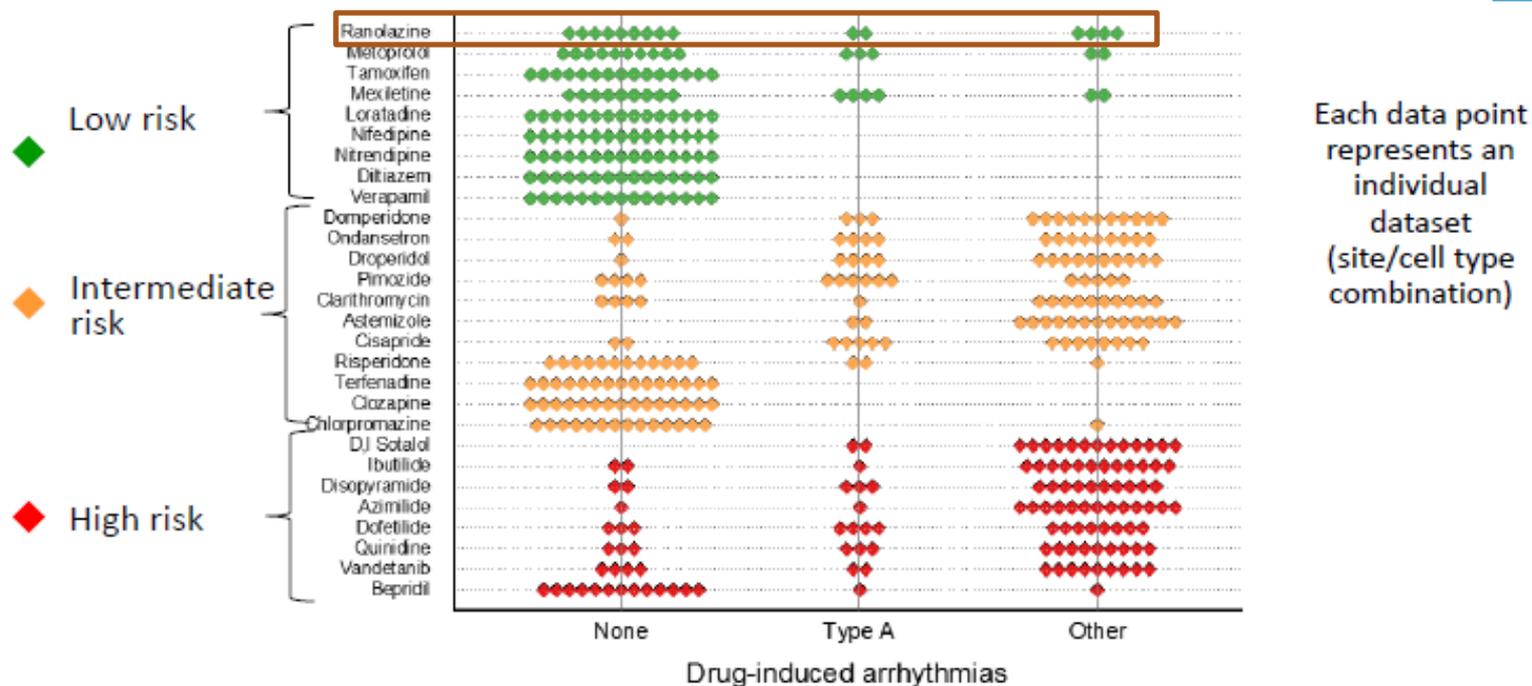
# Categorization of Reference Drugs Based on Risk of Arrhythmia

Table 1 | Set of 28 drugs ranked according to high, intermediate and low proarrhythmic risk

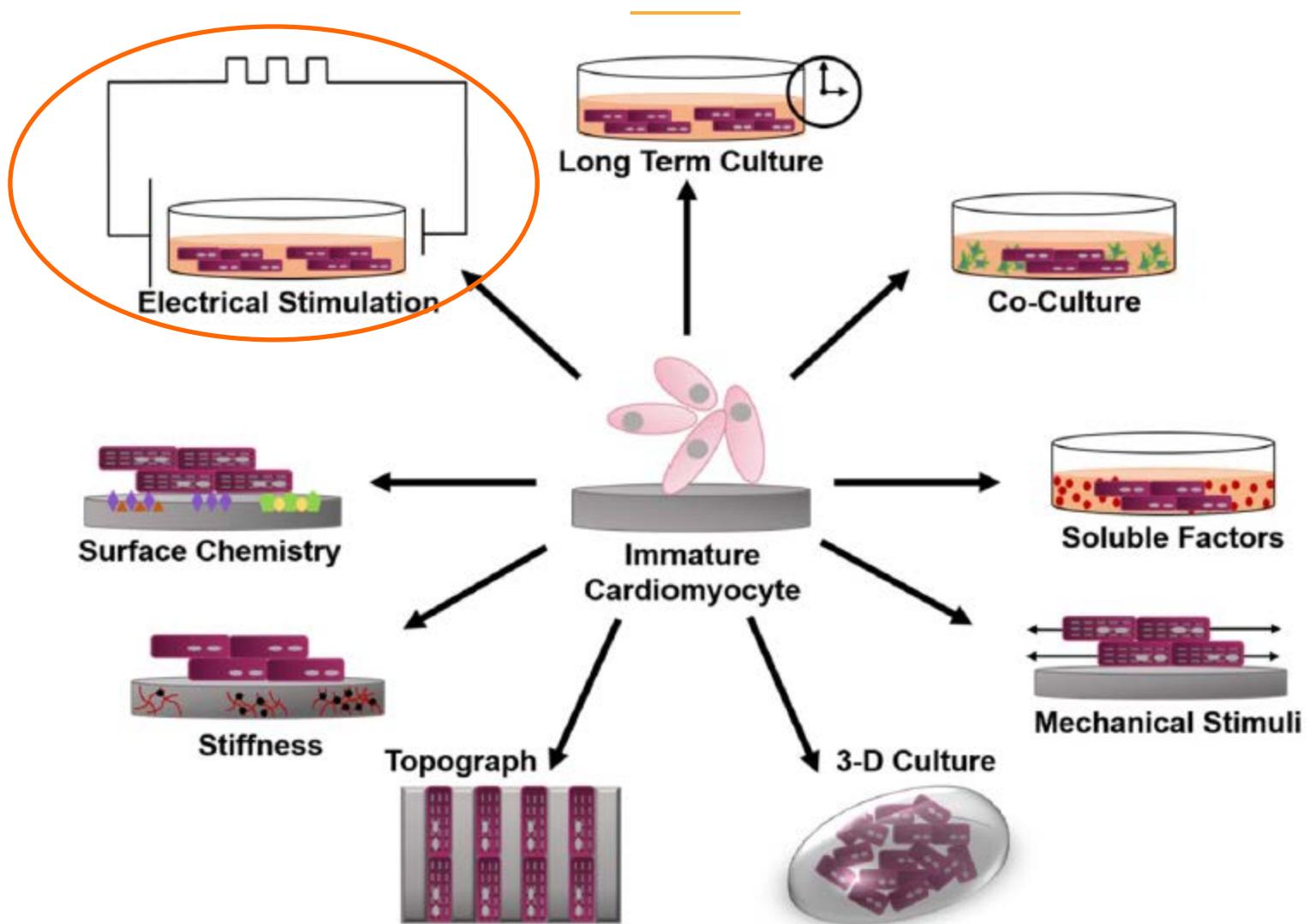
CiPA high risk	Redfern category*	CiPA intermediate risk	Redfern category	CiPA low or no risk	Redfern category
Azimilide	1	Astemizole	2	Diltiazem	5
Bepriidil	3	Chlorpromazine	–	Loratadine	5
Dofetilide	1	Cisapride	2	Metoprolol	–
Ibutilide	1	Clarithromycin	4	Mexiletine	–
Quinidine	1	Clozapine	–	Nifedipine	4
Vandetanib	–	Domperidone	4	Nitrendipine	5
Methadone	–	Droperidol	–	Ranolazine	–
D,L-sotalol	1	Terfenadine	2	Tamoxifen	5
		Pimozide	3	Verapamil	5
		Risperidone	5		
		Ondansetron	–		

# Categorization of the Compounds Based on Arrhythmic Events

## Predictor 1: Arrhythmia-like Events



# Published “Maturation” protocols for hiPSC-CMs

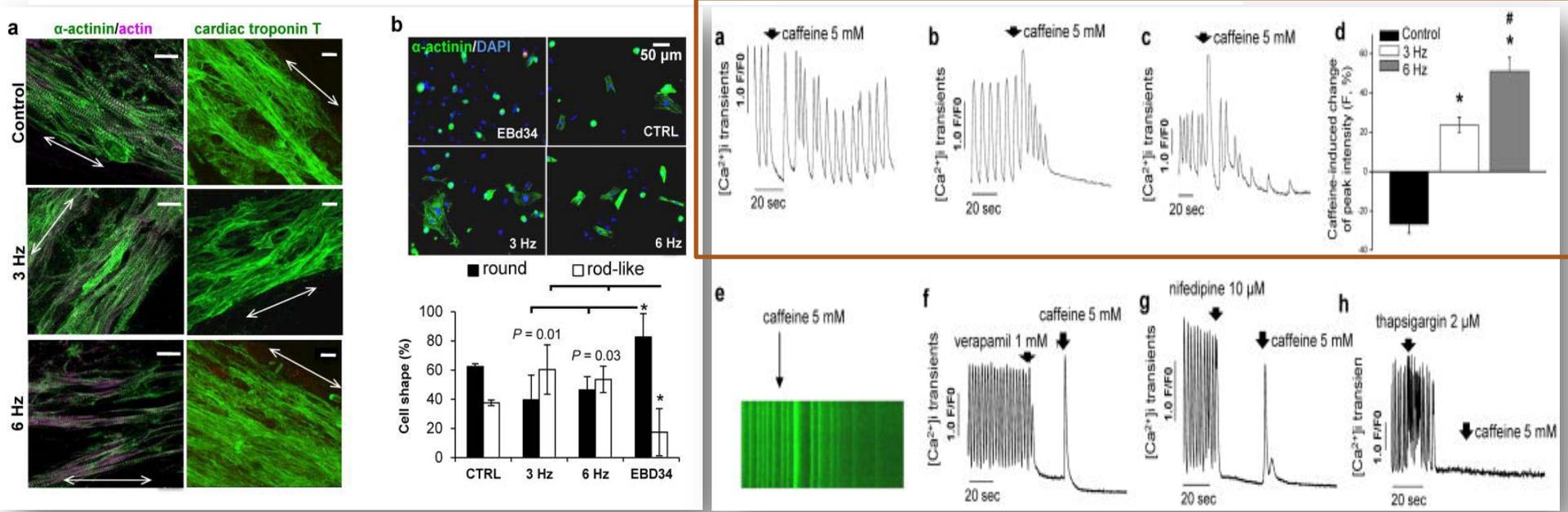


# Electrical Pacing of hiPSC-CM as a Method for Functional Maturation

## Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes

NATURE METHODS | VOL.10 NO.8 | AUGUST 2013

Sara S Nunes<sup>1,2</sup>, Jason W Miklas<sup>1,13</sup>, Jie Liu<sup>3,13</sup>, Roozbeh Aschar-Sobbi<sup>3</sup>, Yun Xiao<sup>1</sup>, Boyang Zhang<sup>1</sup>, Jiahua Jiang<sup>4</sup>, Stéphane Massé<sup>5</sup>, Mark Gagliardi<sup>6</sup>, Anne Hsieh<sup>1</sup>, Nimalan Thavandiran<sup>1</sup>, Michael A Laflamme<sup>7</sup>, Kumaraswamy Nanthakumar<sup>5</sup>, Gil J Gross<sup>4,8-10</sup>, Peter H Backx<sup>3,10,11</sup>, Gordon Keller<sup>6</sup> & Milica Radisic<sup>1,10,12</sup>



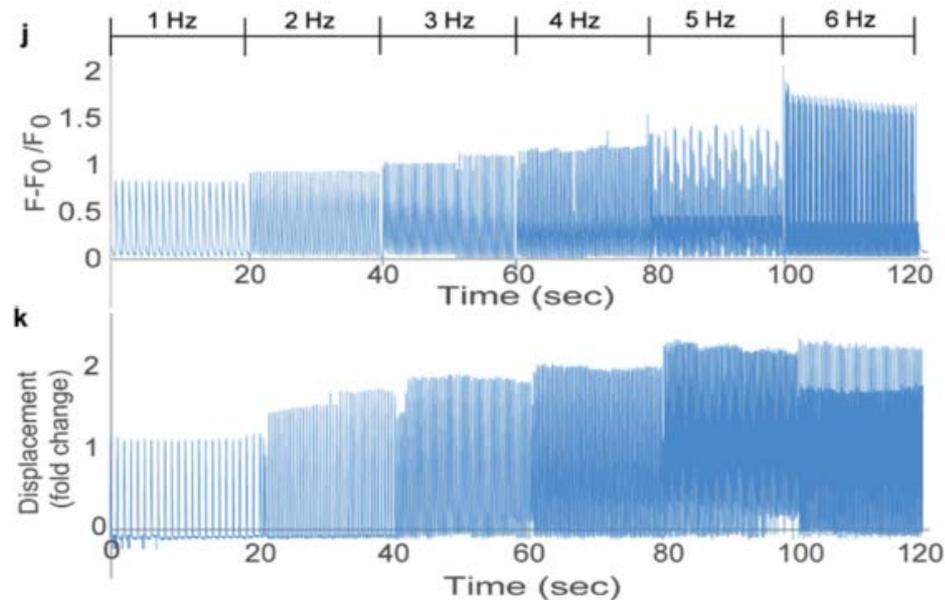
# Electrical Pacing of hiPSC-CM as a Method for Functional Maturation

## LETTER

<https://doi.org/10.1038/s41586-018-0016-3>

### Advanced maturation of human cardiac tissue grown from pluripotent stem cells

Kacey Ronaldson-Bouchard<sup>1</sup>, Stephen P. Ma<sup>1</sup>, Keith Yeager<sup>1</sup>, Timothy Chen<sup>1</sup>, LouJin Song<sup>2</sup>, Dario Sirabella<sup>1</sup>, Kumi Morikawa<sup>2</sup>, Diogo Teles<sup>1,3,4</sup>, Masayuki Yazawa<sup>2</sup> & Gordana Vunjak-Novakovic<sup>1,5\*</sup>



# The *ePacer* Instrument

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***ePacer***: generating better hiPSC-CM model as part of an integrated workflow solution

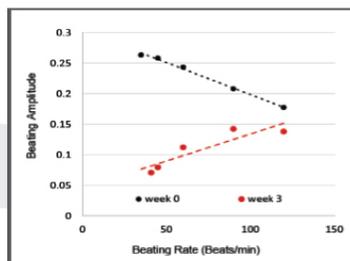
# ePacer is Designed to be Part of an Integrated Workflow

1 Plate your cells & initiate automated pacing protocol



BR Hz  
Time

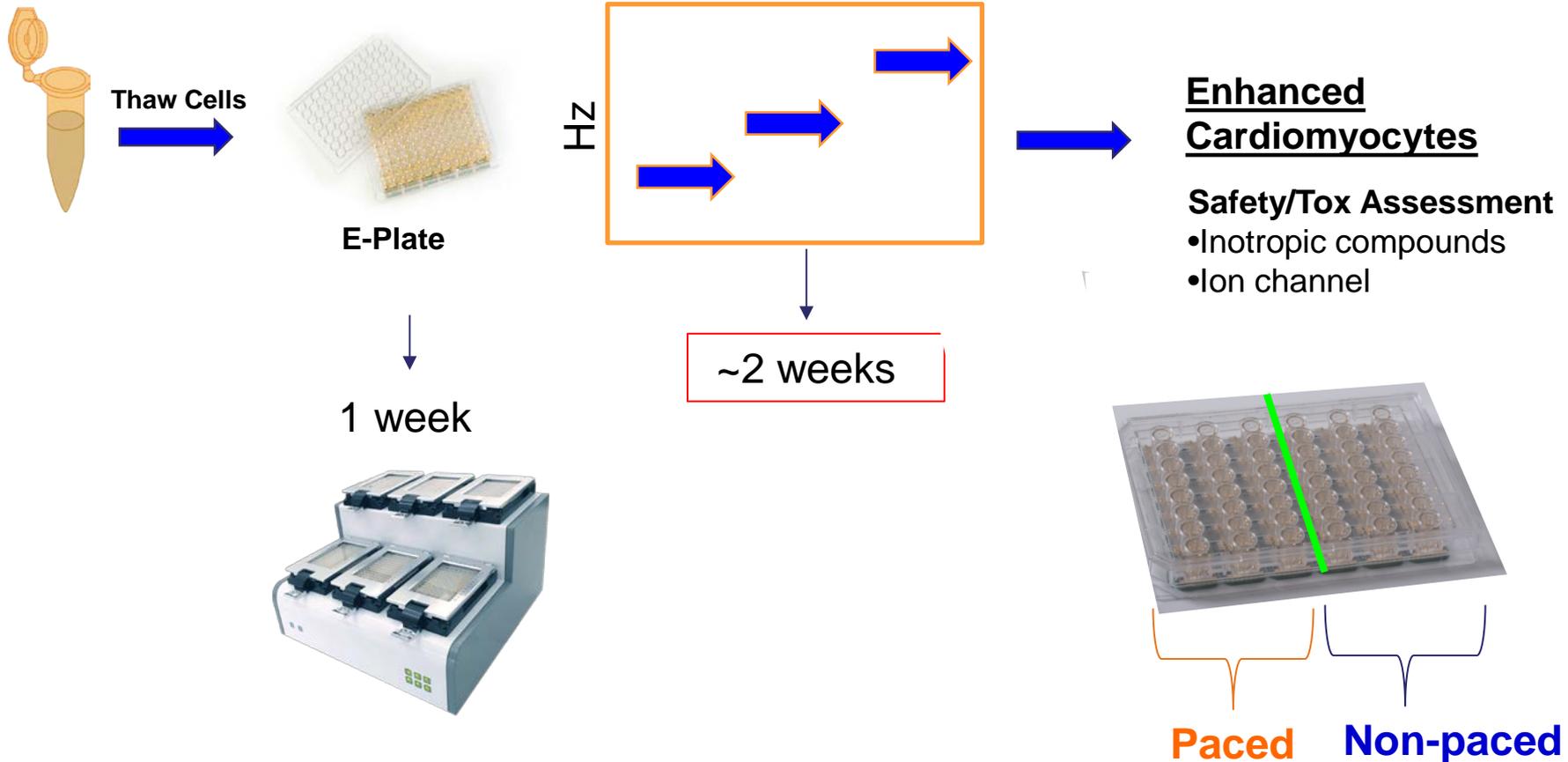
2 Assess maturity using amplitude/rate relationship with built-in algorithm



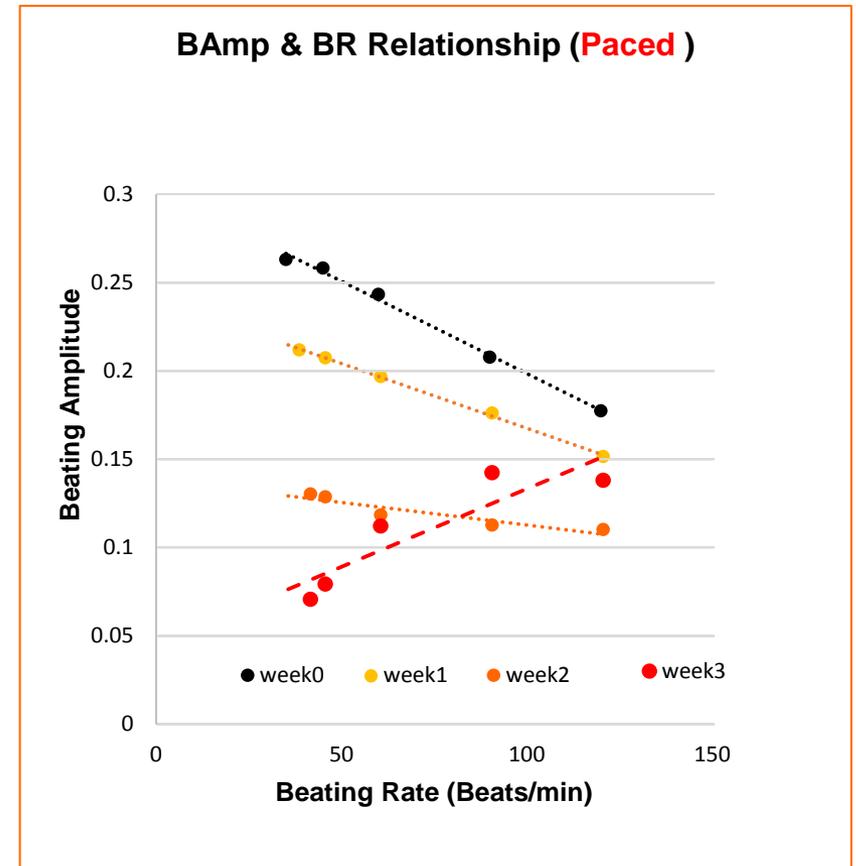
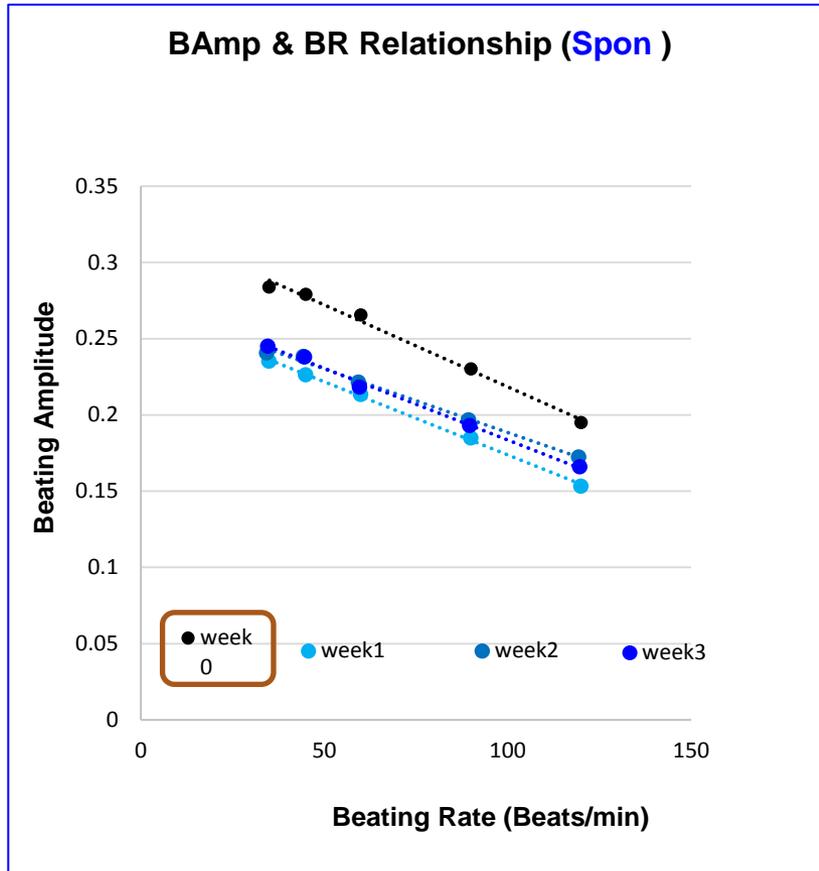
3 Integrate into workflow of choice



# Work Flow of Functional Maturation of hiPSC-CMs Using the ePacer



# Improved BAmp and BR Relationship During Chronic Pacing



	ISO (100 nM)	Bay K8644 (67 nM)	Pimobendan (10 μM)	Milrinone (30 μM)	OM (600 nM)	Digoxin (100 nM)	Isradipine (10 nM)
MOA	β adrenergic receptor agonist	L-type Ca <sup>2+</sup> ion channel activator	Ca <sup>2+</sup> sensitizer	phosphodiesterase-3 inhibitor	Myosin activator	Na-K ATP exchanger inhibitor	L-type Ca <sup>2+</sup> ion channel inhibitor
Inotropic effect	+	+	+	+	+	+	-
Impedance waveforms							
% change to the baseline (BAmp)	52 ± 14% ↑	32 ± 5% ↑	31 ± 6% ↑	30 ± 12% ↑	58 ± 15% ↑	175 ± 35% ↑	-47 ± 7% ↓
% change to the baseline (BR)	73 ± 7%	-23 ± 2%	3 ± 2%	14 ± 3%	-3 ± 4%	-49 ± 30%	20 ± 2%

# Gaps in Assessment of Ion Channel Modulators

## Categorization of Reference Drugs Based on Risk of Arrhythmia

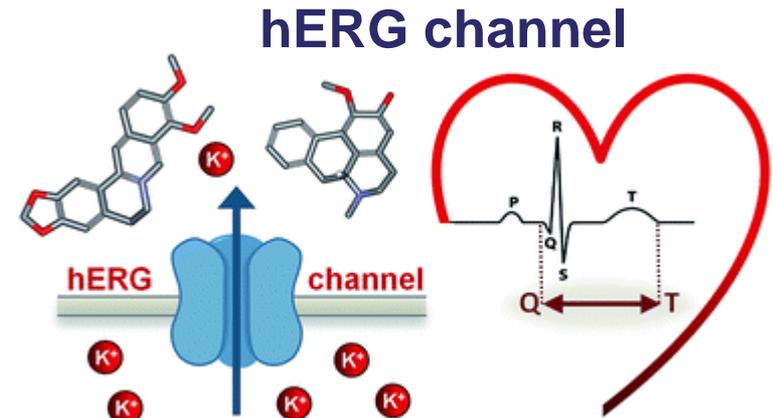


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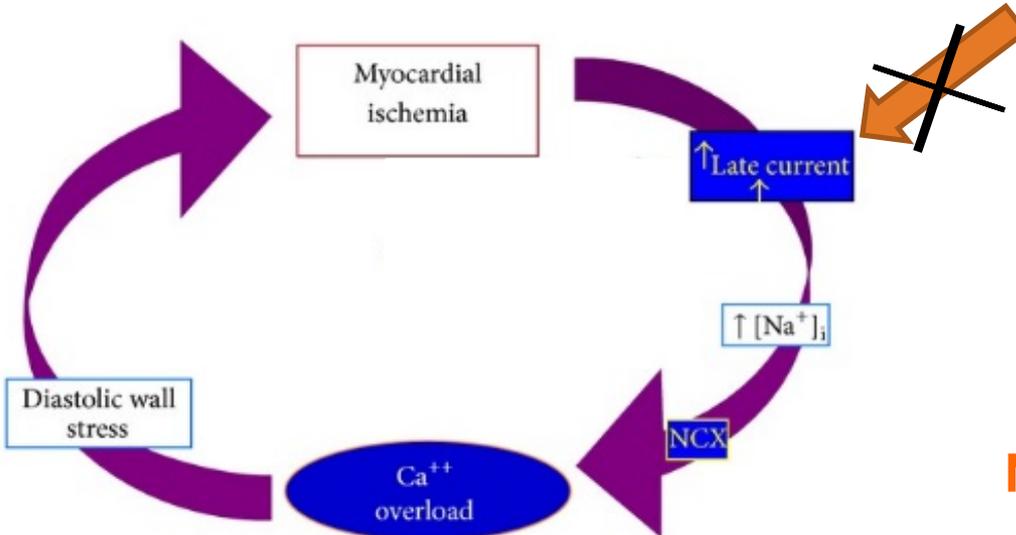
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D,L-sotalol	1	Terfenadine	2	Tamoxifen	5
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# Ranolazine

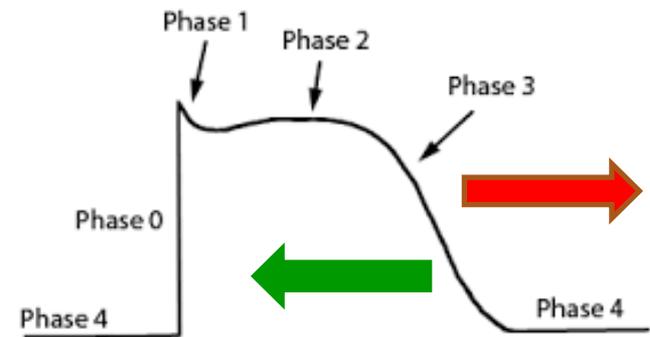
Ranolazine: Antianginal drug; inhibits **Late Na<sup>+</sup> current** and **hERG channel**



## Late Na<sup>+</sup> current



## Ranolazine

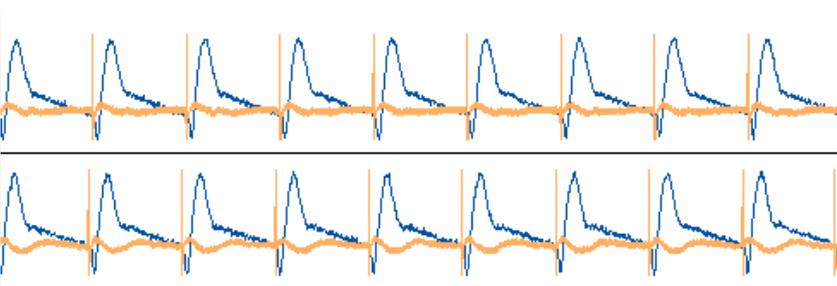


**No arrhythmia occurred in clinic**

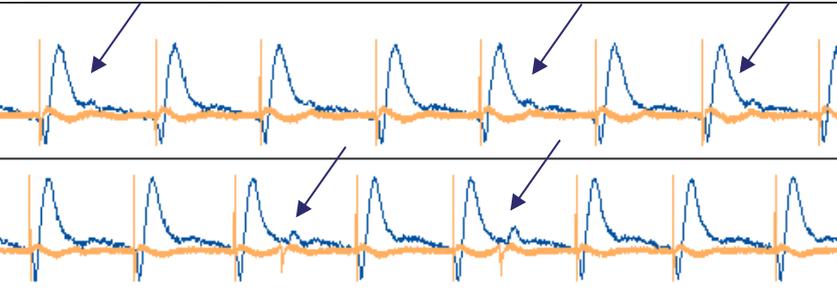
# Assessment of Ranolazine effects Using Functional Mature hiPSC-CMs

## Non-Paced

Pre-drug

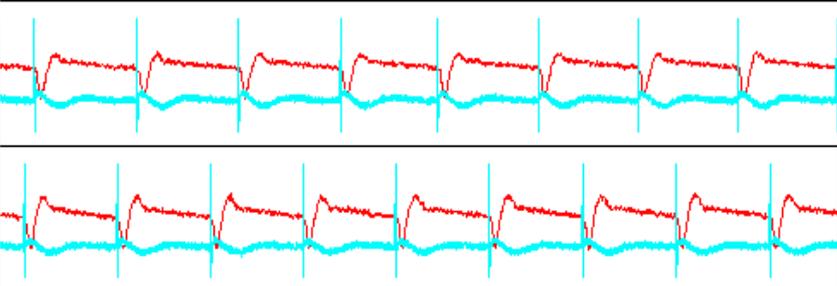


Post-drug

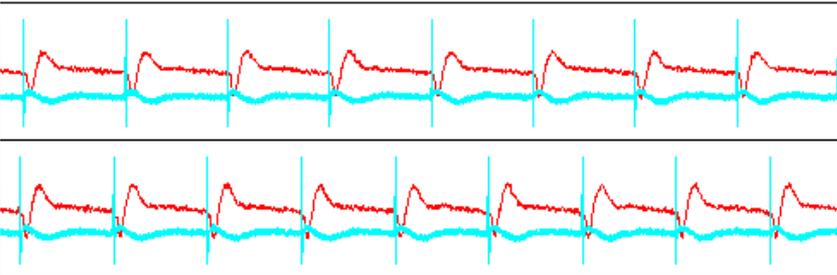


## Chronically Paced

Pre-drug



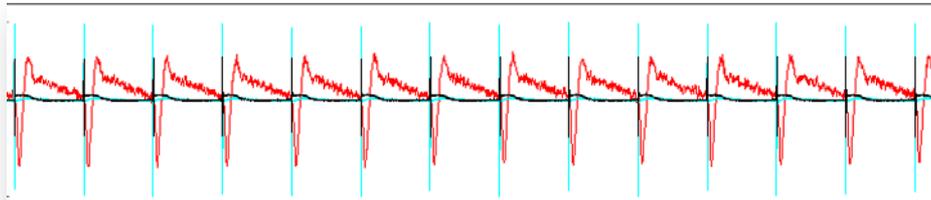
Post-drug



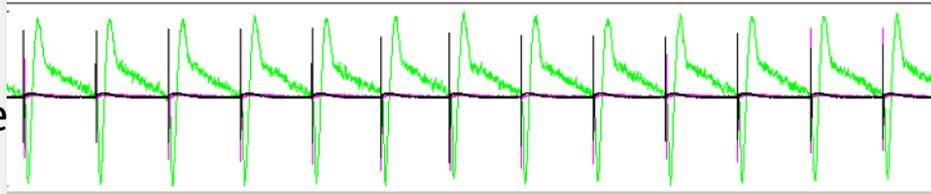
# Assessment of Ranolazine effects Using Functional Mature hiPSC-CMs

Paced iCell cardiomyocyte<sup>2</sup>s

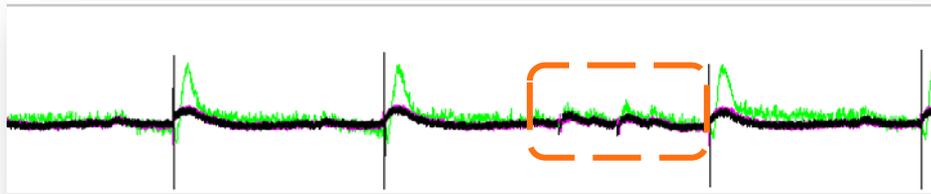
DMSO



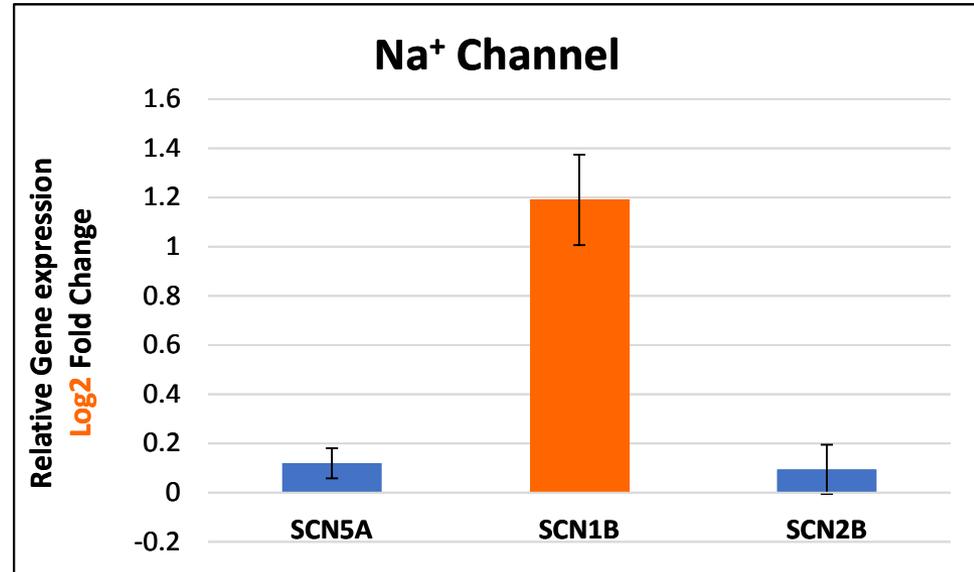
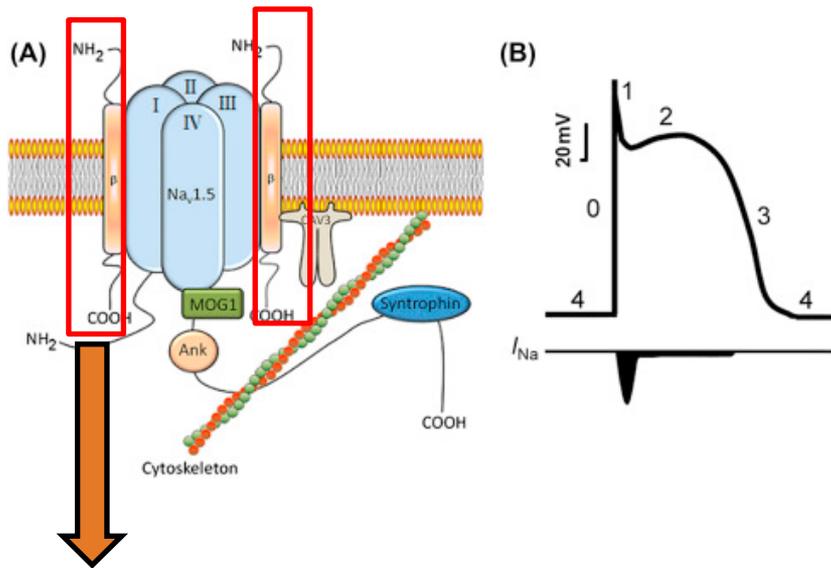
10  $\mu$ M Ranolazine



10  $\mu$ M Ranolazine  
+ 5 nM Dofetilide



# Gene Expression of Na<sup>+</sup> Channel



Late Na<sup>+</sup> current produced by Na<sup>+</sup> channel is regulated by its β1 subunit.

Maltsev V. et al., *J Physiol Sci.* 2009

# Summary

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- hiPSC-CM are typically less well developed compared to adult CM in terms of genetic, physical and functional attributes which limits their utility as a model system
- Long term electrical pacing has been shown to improve the maturation status of hiPSC-CM
- The ePacer system can be used to perform directed long term electrical pacing in a consistent, safe and high throughput manner which can improve the maturation status of hiPSC-CM
- These “functionally matured” hiPSC-CM have much more predictive response to certain ion-channel modulating compounds compared to standard hiPSC-CM; Show better responses to inotropic compounds; Better distinction between control and disease phenotypes

