Ncardia
Stem cell experts

MEA assays using human iPSC-derived cardiomyocytes; challenges and opportunities

Event	EMA Workshop 2017
Presenter	Tessa de Korte, MSc
Date	2017, October 5

Ncardia at a glance

✓	Foundation	Ncardia was established in September 2017 following a merger of Pluriomics and Axiogenesis
✓	Based in	Leiden (NL), Gosselies (BE), Cologne (DE)
✓	Team	~65 people
✓	Technology	Human iPSC-derived technology
✓	Business	Products – Human iPSC-derived cardiac and neural products Services – *In vitro* assay services for safety screening and drug efficacy screening
✓	Active in geography	Worldwide, direct presence in EU and US, distributors in Japan
✓	Clients	Pharma, Biotech, Academics labs

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Stem cell experts

Chapter 1

hiPSC-derived cardiomyocytes

- Current use, challenges and opportunities

Chapter 2

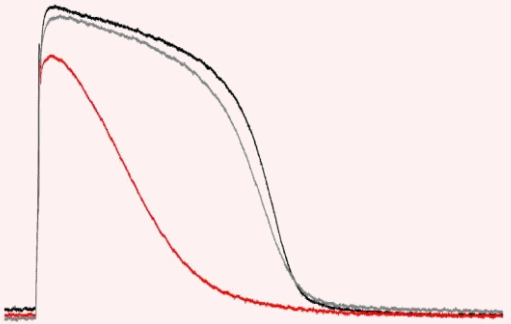
CiPA

- An opportunity for hiPSC-derived cardiomyocytes: regulatory guidance for novel cardiac safety assessment

Chapter 3

Future prospects

- Moving forward with additional hiPSC-derived cell types for safety assessment



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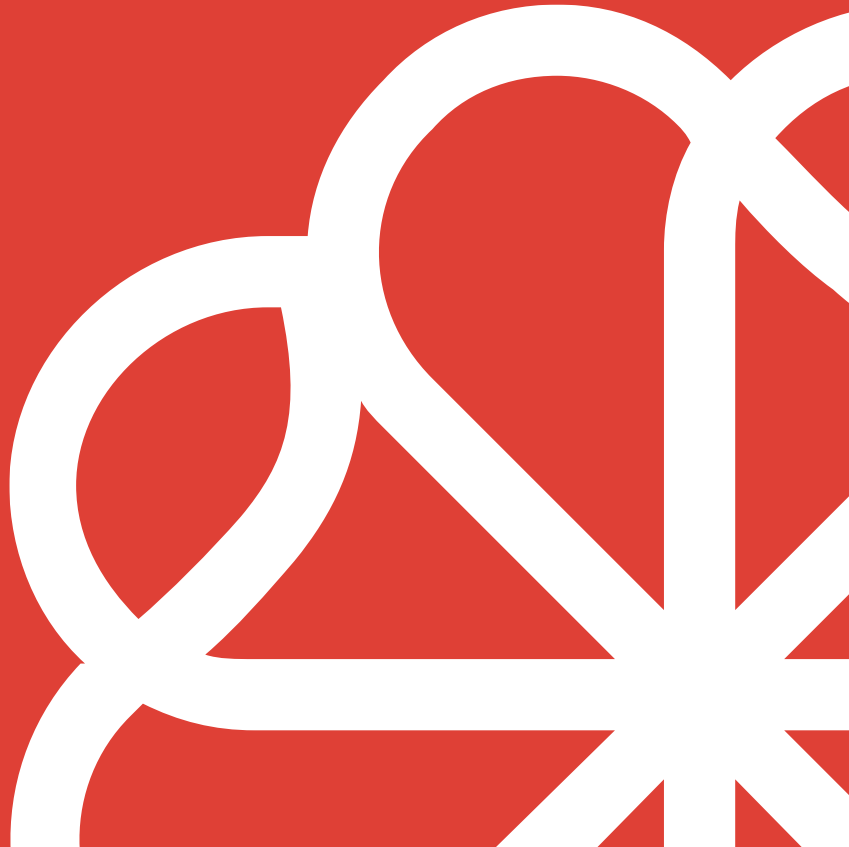
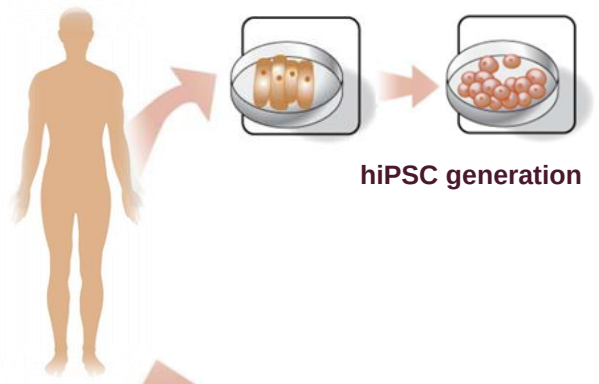
hiPSC-derived cardiomyocytes

Current use, challenges and opportunities



Human cellular models for drug discovery and development



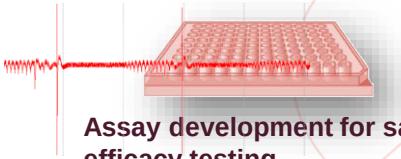
hiPSC generation

Differentiation & manufacture

Neurons

Cardiomyocytes

Endothelial cells



Assay development for safety & drug efficacy testing

- Electrophysiology
- Biochemistry
- Contraction

Drug development Pharmacological research

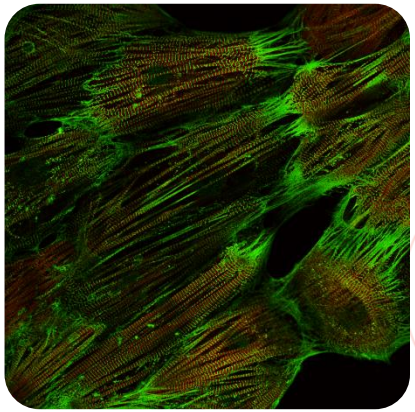
Human iPSC-derived cardiomyocytes

Fully functional human iPSC-derived cardiomyocytes

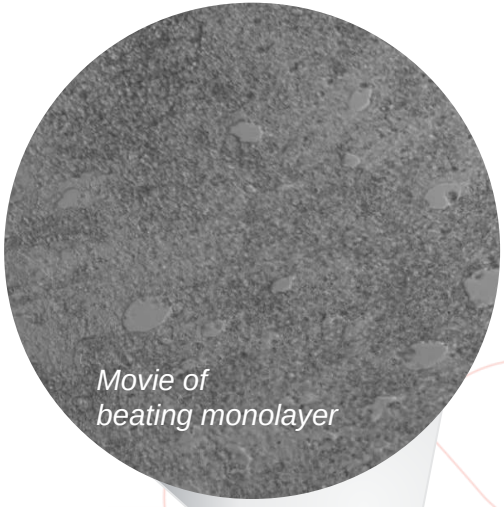
- obtained through *in vitro* differentiation of transgenic hiPSCs and puromycin selection technology
- obtained without any genetically modification or purification/selection procedures

✓ Showing the essential human physiological conditions and pharmacological responses (3R's benefit)

Pluricyte® Cardiomyocyte Kit
Cor.4U® Cardiomyocyte Kit
CardioPlate™

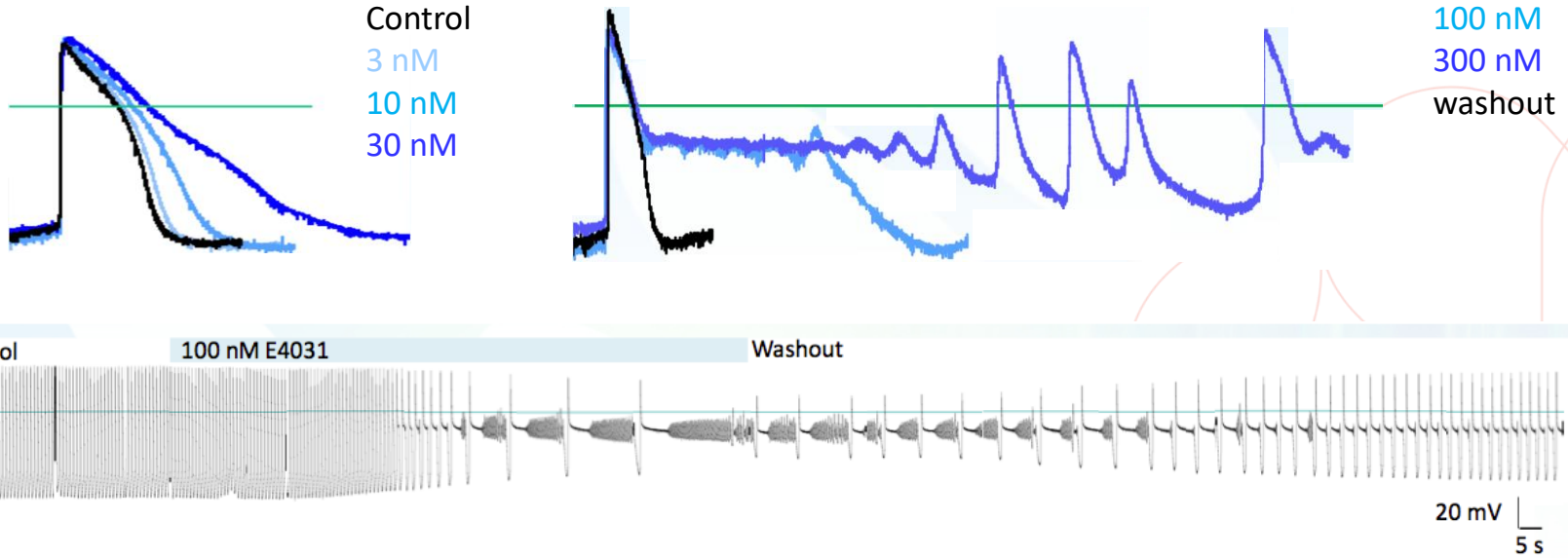


Alpha actin (green)
Myosin heavy chain 7 (red)



Acute pharmacology: gold standard compound E-4031 induces arrhythmia as expected

Current clamp mode - hERG blocker E-4031



Challenges of human iPSC-derived cardiomyocytes for cardiac safety assessment

- Passive acceptance of implementation
- Large data sets preferred to better understand the benefits

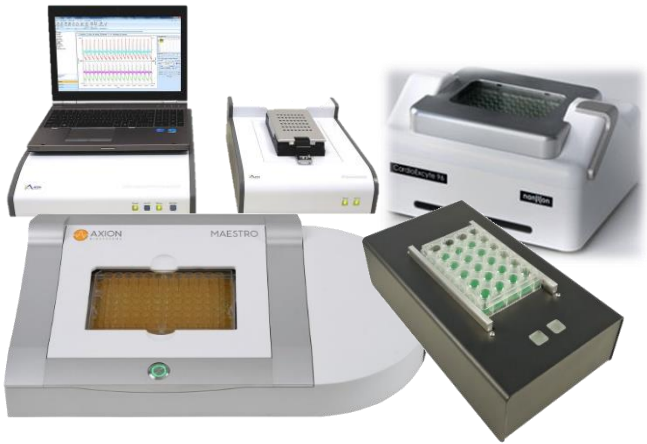
- Not a multi-organ system
- Resembles the celullar electrophysiology more than human heart
- Maturation stage

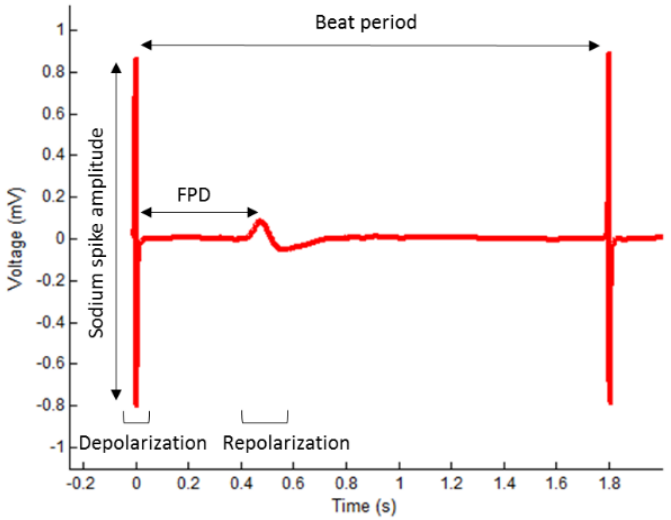
- ✓ Modulating form/stiffness, investigating various chemical compounds → efficient way to provide "adult" **maturation**
- ✓ Find out the relevant parameters that could accurately **predict** different forms of cardiotoxicity (e.g. FPD ←→ QT using MEA analysis)
- ✓ Early stage drug screening
- ✓ Personalized safety testing (investigate potential disease-causing genetic variants)

Multielectrode array (MEA) assays for predicting drug-induced ion-channel blockade

Robust de- and repolarization peaks, enabling easy analysis of electrophysiological parameters:

- Field potential duration (FPD)
- Beat period
- Depolarization peak amplitude



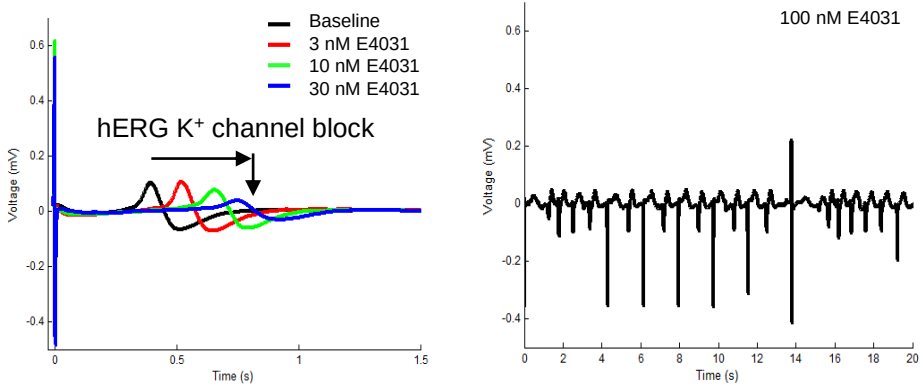


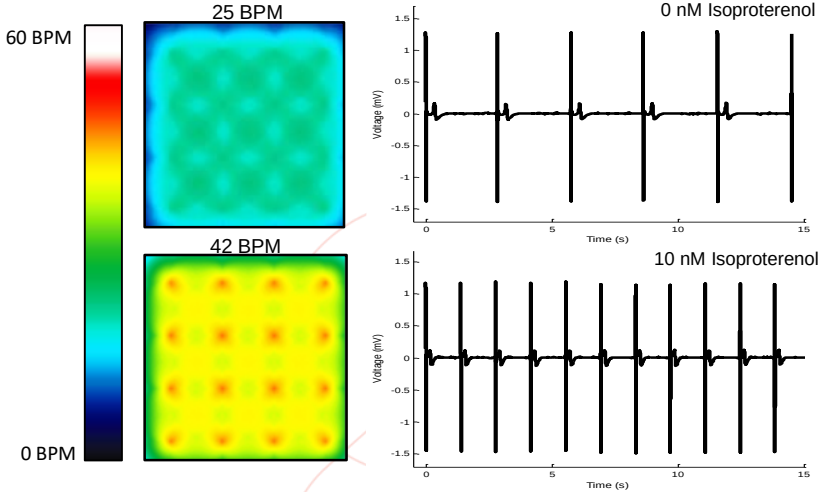
Data generated using Axion BioSystems' Maestro MEA system

Multielectrode array (MEA) assays for predicting drug-induced ion-channel blockade

Robust de- and repolarization peaks, enabling easy analysis of electrophysiological parameters:

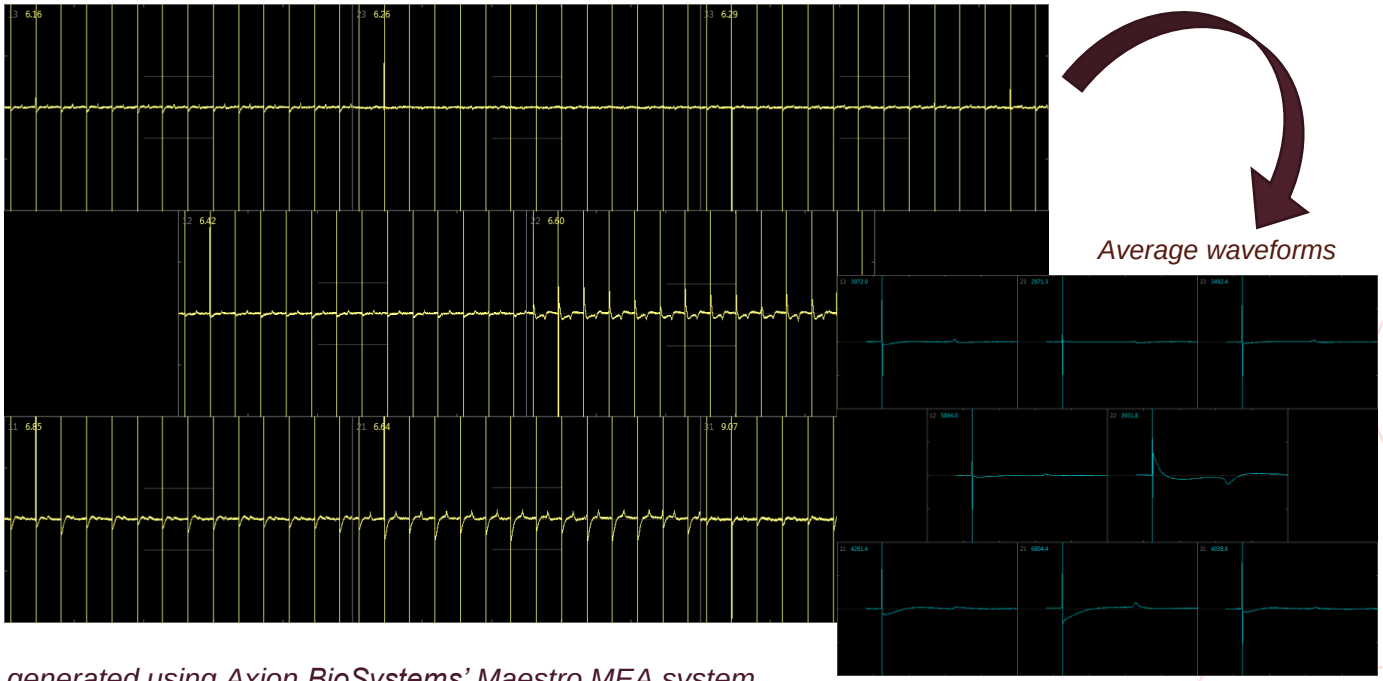
- Field potential duration (FPD) >> hERG channel blockade, TdP-like arrhythmia
- Beat period >> chronotropic effects
- Depolarization peak amplitude >> Na^+ channel blockade



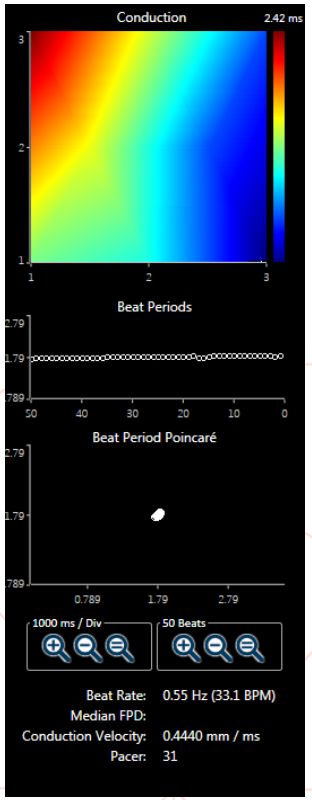


Data generated using Axion BioSystems' Maestro MEA system

Reproducible Field Potential Waveforms from 8 Individual Electrodes in 1 Well



Average waveforms



Data generated using Axion BioSystems' Maestro MEA system

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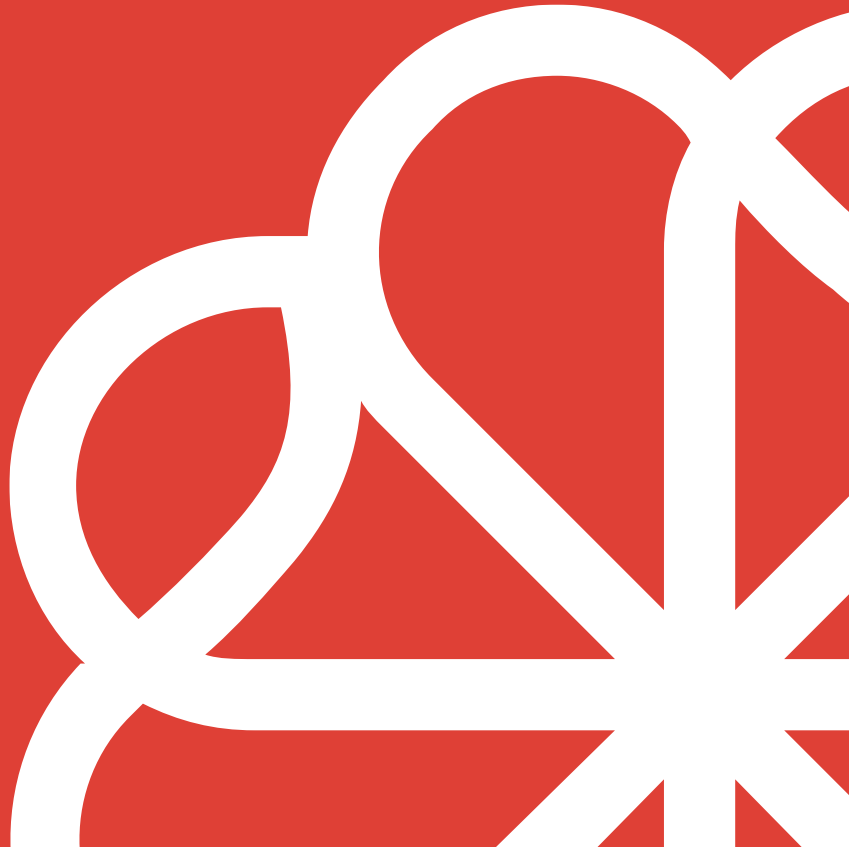
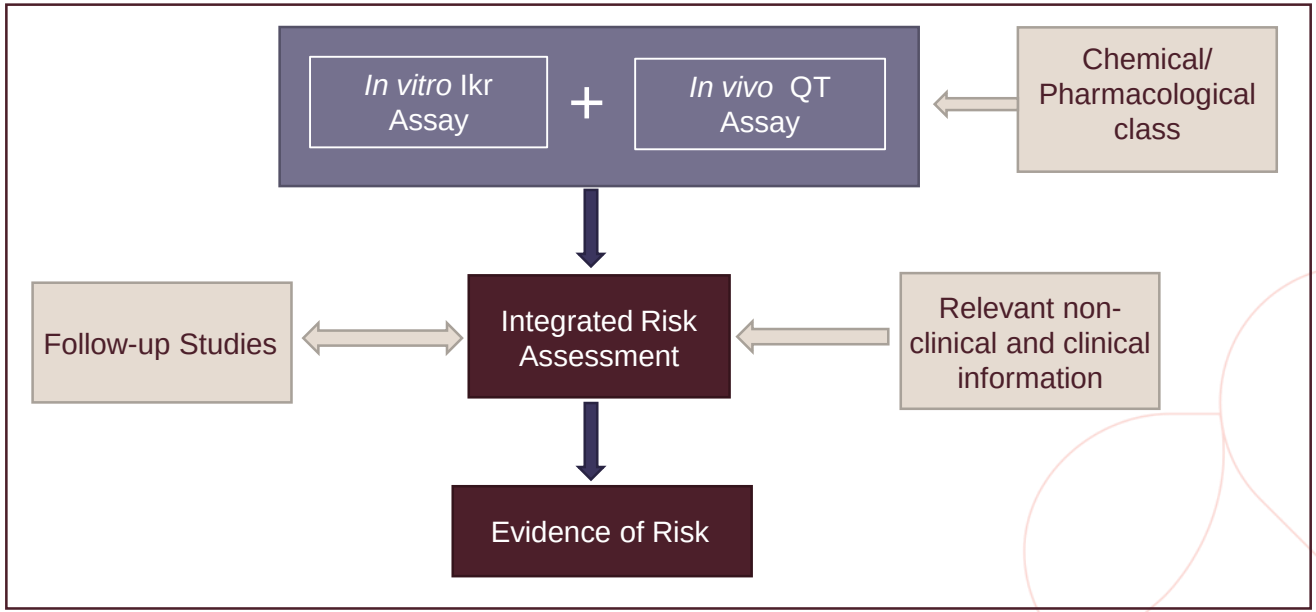
CiPA

An opportunity for hiPSC-derived cardiomyocytes: regulatory guidance for novel cardiac safety assessment



Non-clinical testing strategy, ICH S7B



ICH S7B, step 4, May 2005

ICH S7B Approach

ICH S7B Approach “Successful” → No drugs removed from market due to TdP since 2003

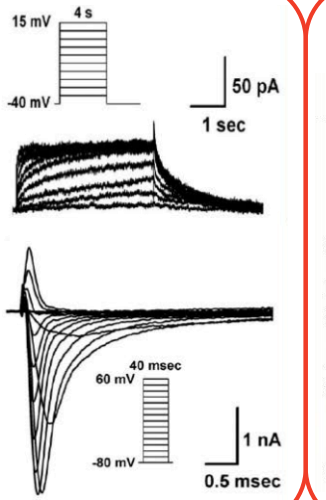
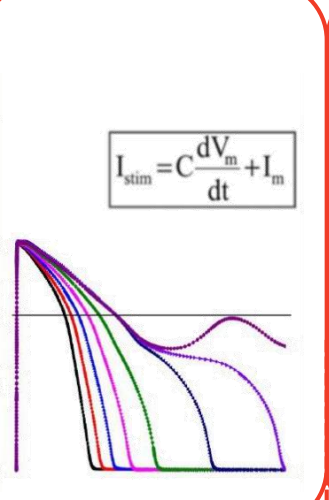
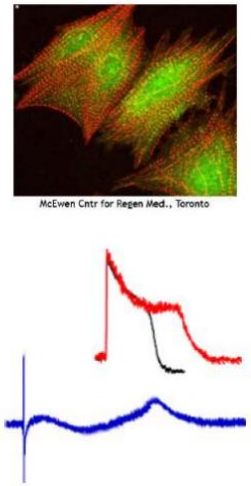
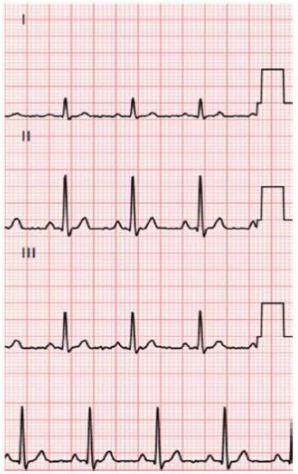
HOWEVER:

- hERG not very predictive of QTc effects
- Findings of hERG/iKr block in early screening of drug candidates often discourages compound progression, “throwing the baby out with the bathwater”
- Some drugs might not be available today if hERG assay results “enforced” (**pentobarbital, verapamil, ranolazine**)

FINALLY:

- Translation of current *in vitro* assays to human QT findings imperfect, QT only accounts for a minor form of cardiotoxicity

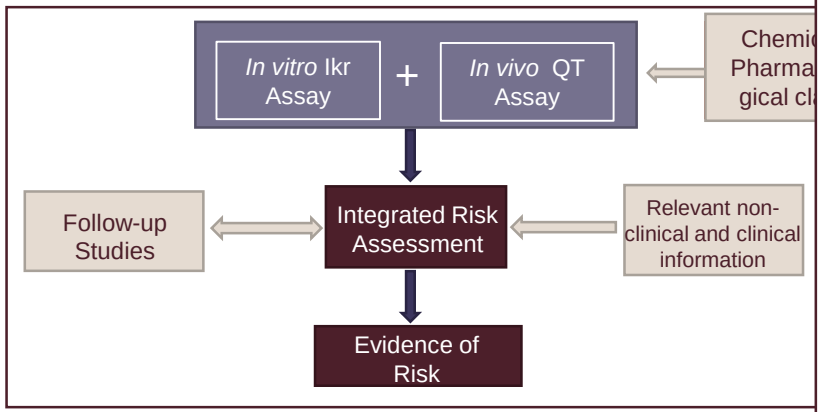
CiPA: Comprehensive *in vitro* Proarrhythmia Assay, the four components

Drug effects on multiple human ion-channels	*In silico* reconstruction celular human ventricular electrophysiology	*In vitro* Effects human iPSC-derived cardiomyocytes	Clinical evaluation unanticipated electrophysiology
			

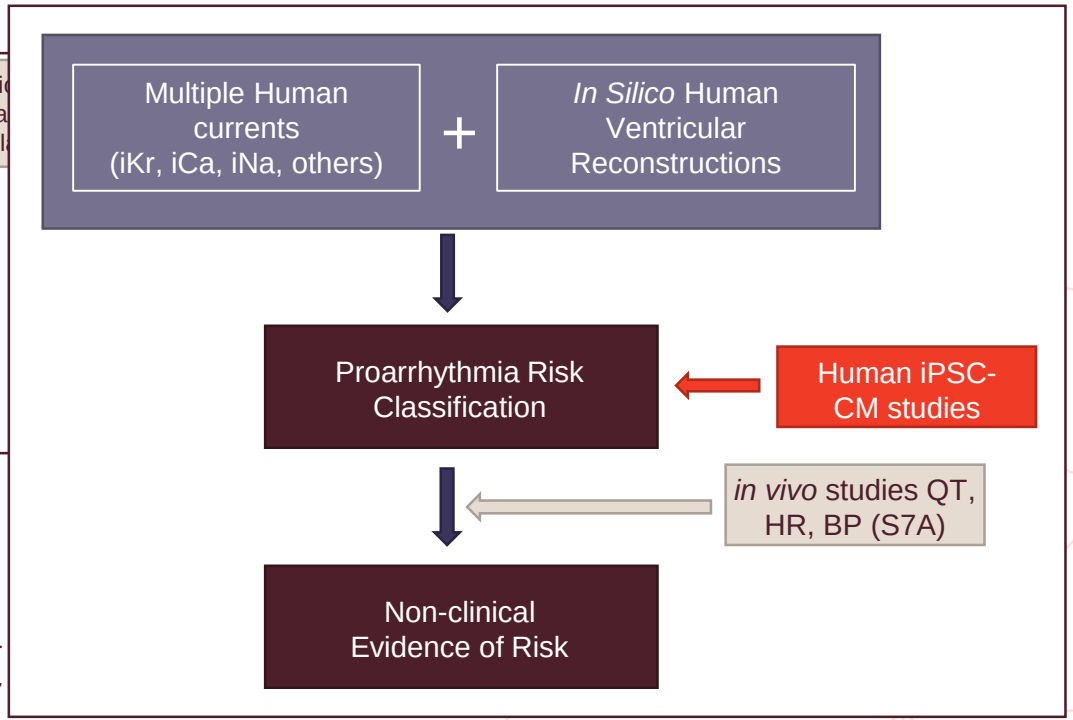
Characterize/Classify Effects

Check for Missed or Unanticipated Effects

FDA drives: *in vitro* cell based tests for the prediction of cardiac safety



ICH S7B, step 4, May 2005



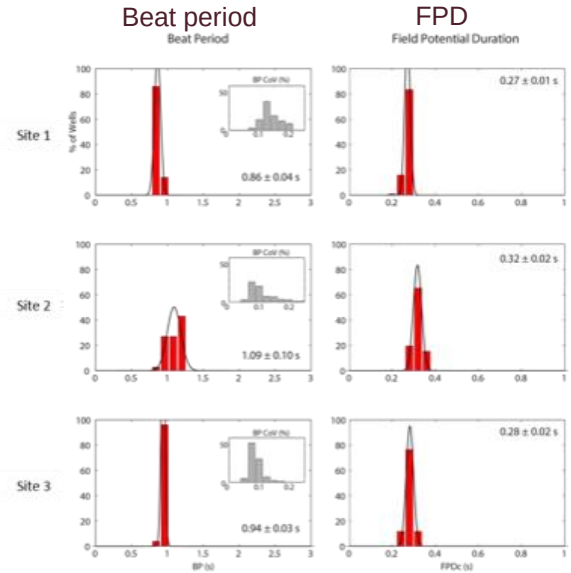
Proposed modified scheme, presented by Dr. Gary Gintant SPS Meeting 2017

3 independent testing sites confirmed the predictivity of Ncardia's hiPSC-derived Cardiomyocytes as a human cellular model

Ncardia
Stem cell experts

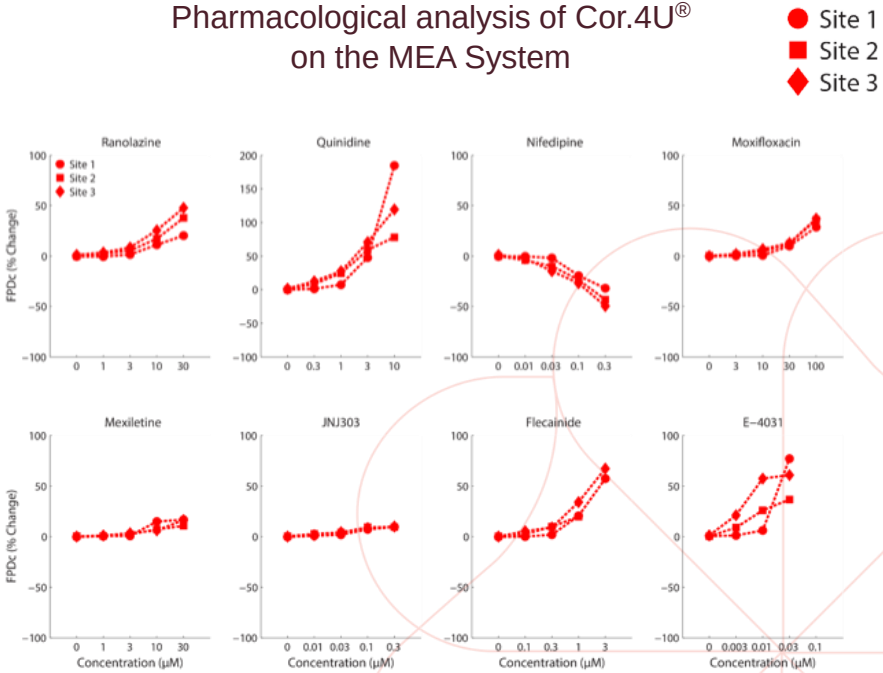
CiPA

Electrophysiological analysis of Cor.4U®
on the MEA System



Analysis of different parameters by testing site

Pharmacological analysis of Cor.4U®
on the MEA System



Comparison of different testing sites per compound

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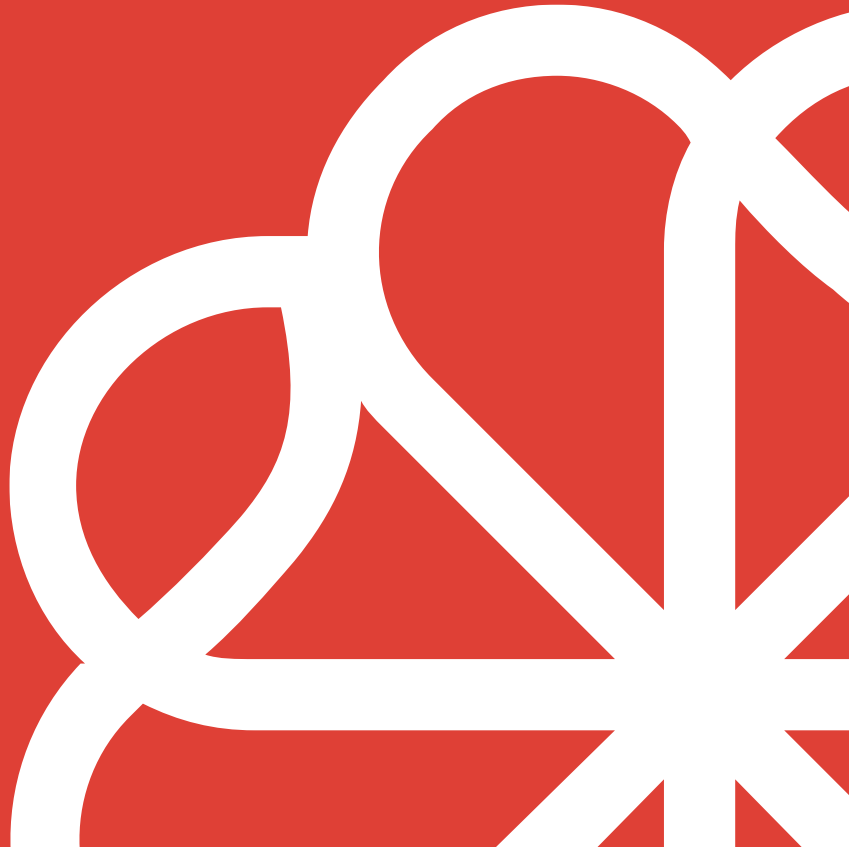
Future prospects

Moving forward with additional hiPSC-derived cell types for safety assessment



HESI Contractility consortium using human iPSC-derived cardiomyocytes

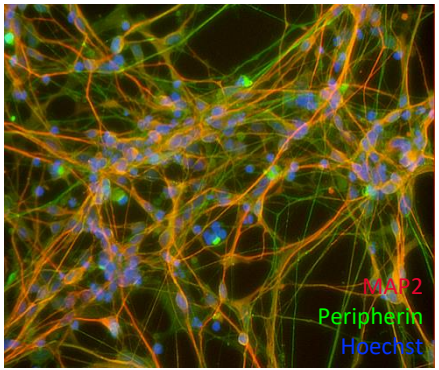
- **GOAL**
  Develop a pilot program that utilizes cellular systems for the prediction of drug-induced depression of cardiac contractility that translates to clinical cardiotoxicity.

HESI

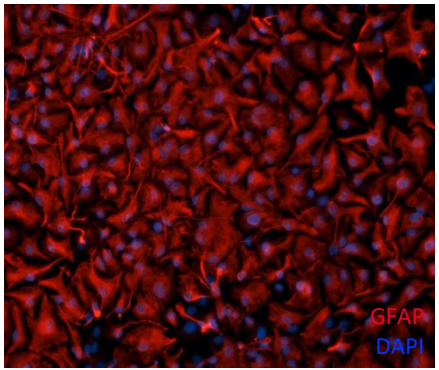
*Human iPSC-derived CMs are a **rapidly evolving technology** that is already being evaluated for cardiac electrophysiological toxicity in the **CiPA intitiative**. Their use for evaluation of **cardiac contractility** is somewhat more preliminary in development, however this methodology is also moving forward in a very promising manner, and an effort to advance that evaluation is beginning in the **Division of Applied Regulatory Science in the Office of Clinical Pharmacology at FDA**.*

Neuronal cell products follow the Cardiac products and are now considered for regulatory purposes

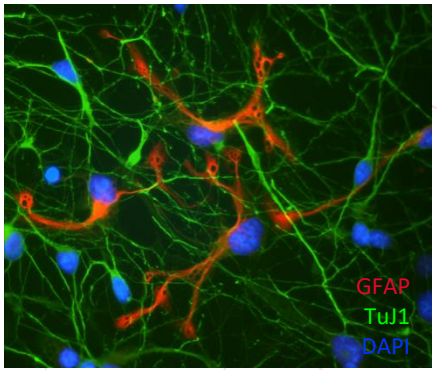
Peri.4U™ - Peripheral neurons



Astro.4U™ - Astrocytes



CNS.4U™ - CNS neurons



To be used for seizure liability testing (initiated by the FDA)

BOTOX safety testing

Contact us!

www.ncardia.com

Ncardia
Stem cell experts

We are shaping the future of drug discovery & development

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Thank you!

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