Lessons Learned from the Comprehensive In Vitro Proarrhythmia Assay (CiPA) Initiative for Safety/Toxicology Testing with Microphysiological Systems

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Microphysiological Systems: Bridging Human and Animal Research
National Academy of Sciences ILAR Virtual Workshop
Jan. 19, 2021

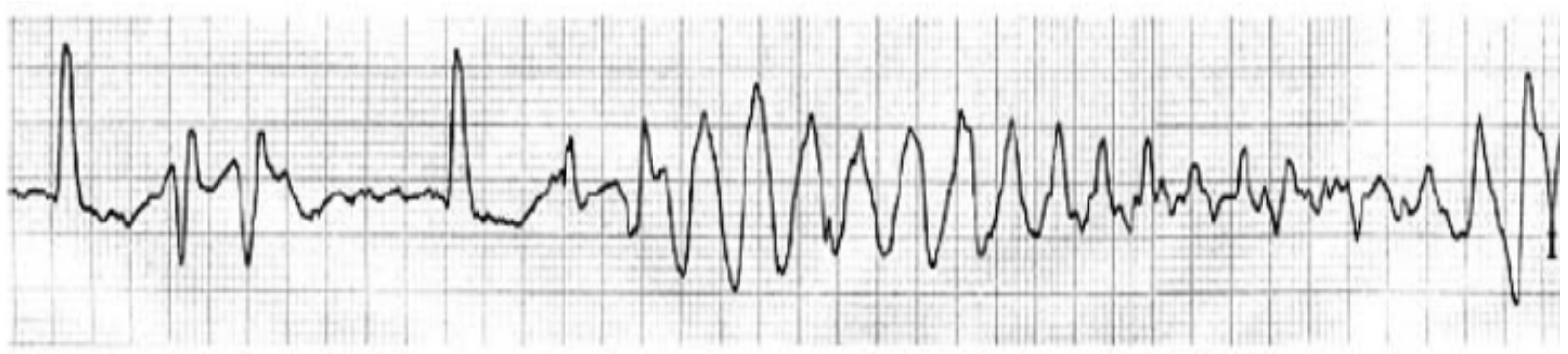
Topics

Background on CiPA (Comprehensive In Vitro Proarrhythmia Assay) Initiative

Lessons learned from CiPA for testing/screening drugs (and chemicals)
that apply to evolving MPS systems

Perspectives:
Role of Human-derived Cardiac Preparations in Safety/Toxicity testing

CiPA:
Comprehensive *In Vitro* Proarrhythmia Assay

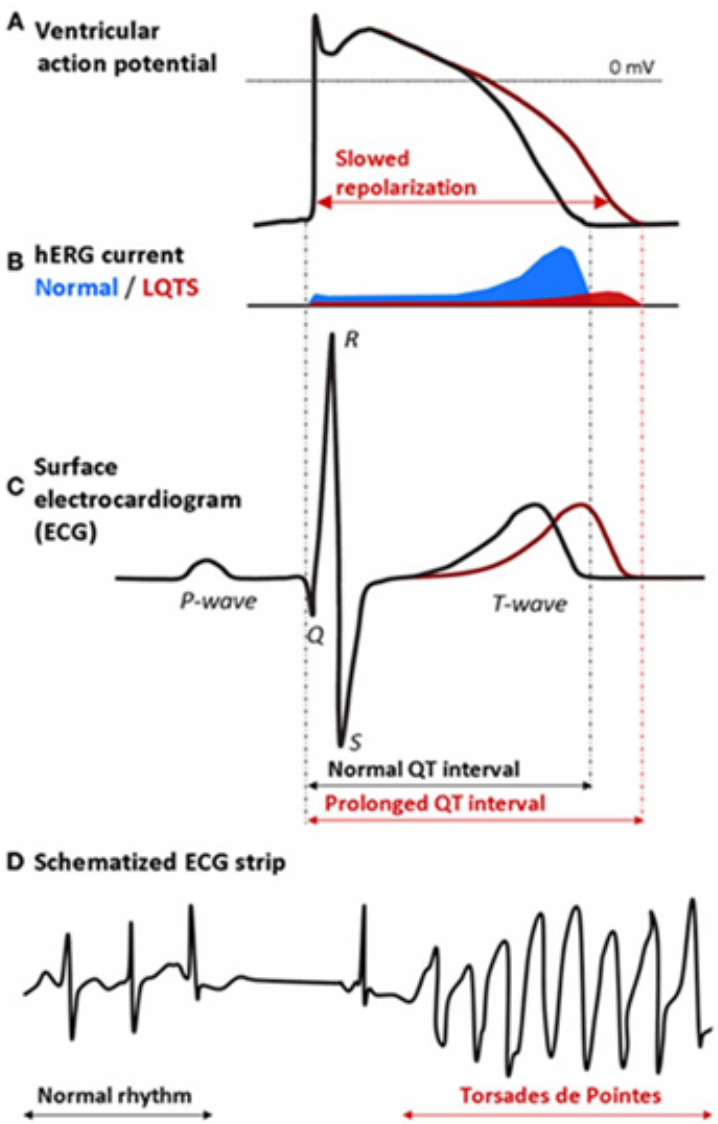


Yap and Camm, 2003

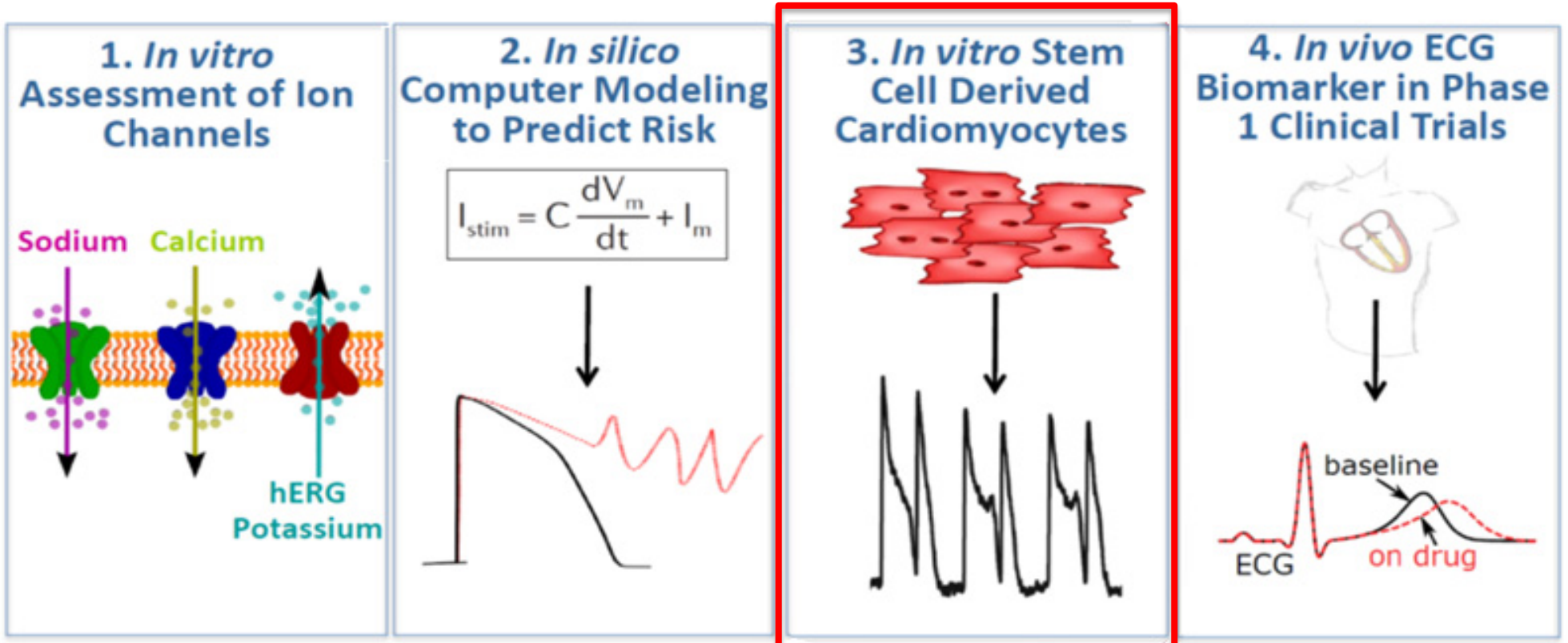
- **Goal**: Define the risk of drug-induced Torsades-de-Pointes (TdP)
  - a rare and potentially fatal arrhythmia – an “electrical toxicity”
  - Preclinical surrogate marker: delayed repolarization (manifest as prolongation QTc interval on ECG of animals, humans)
    - Time-consuming, expensive (cost and animal use)
    - Careful nonclinical studies needed to ensure adequately sensitivity
    - Do not provide mechanistic insights for arrhythmic risk assessment

Defining Proarrhythmic Risk: Mechanistic Insights

- We understand the cellular mechanisms that cause delayed repolarization & predispose to TdP proarrhythmia
- The QT interval represents integrated effects of multiple cardiac ionic currents across both ventricles
  - Inward (depolarizing) and outward (repolarizing currents)active with each heartbeat -> QT interval
- Outward current inhibition leads delayed repolarization (QT prolongation)
- *Excessive* outward current inhibition leads to
  a) dangerous QT prolongation &
  b) greater heterogeneity of repolarization that promotes Torsades-de-Pointes



CiPA:
Four Components Provide Mechanistic Assessment of a Drug's Proarrhythmic Risk



Define Proarrhythmic Risk based on In silico Integration of Human Ionic Current Effects

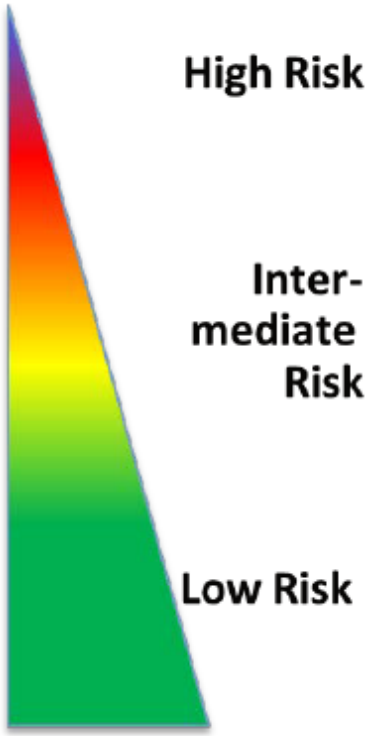
Check for Missed or Unanticipated Effects with Human-Derived Myocytes

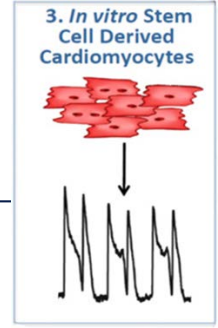
Increase Confidence in Risk Assessment

CiPA: 28 Clinical References / 3 Categories of Proarrhythmic Risk

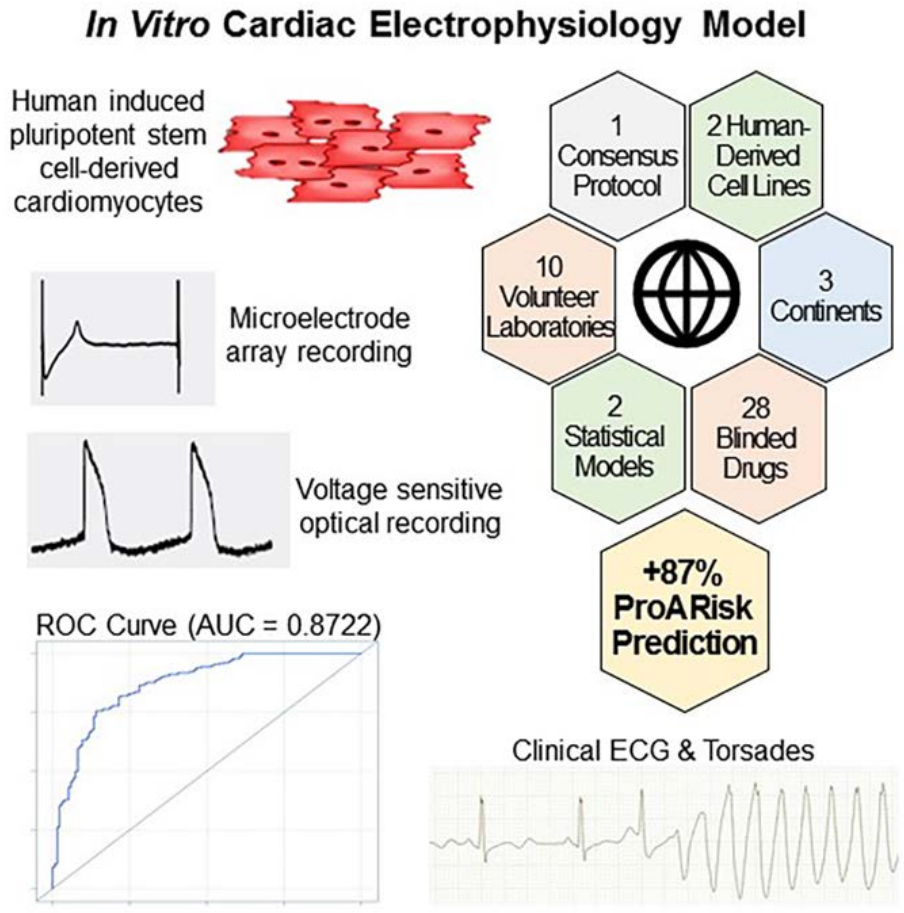
High TdP Risk	Intermediate TdP Risk	Low TdP Risk
Calibration:	**Calibration:**	**Calibration:**
Bepridil	Chlorpromazine	Diltiazem
Dofetilide	Cisapride	Mexiletine
Quinidine	Terfenadine	Ranolazine
D,l Sotalol	Ondansetron	Verapamil
Validation:	**Validation:**	**Validation:**
Azimilide	Astemizole	Loratadine
Ibutilide	Clarithromycin	Metoprolol
Vandetanib	Clozapine	Nifedipine
Disopyramide	Domperidone	Nitrendipine
	Droperidol	Tamoxifen
	Pimozide	
	Risperidone	

Clinical Translational Working Group





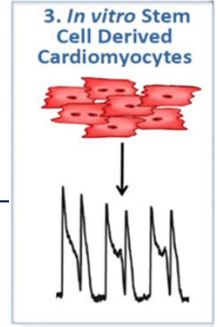
CiPA Validation Study: *In Vitro Human Stem Cell-Derived* Cardiomyocytes



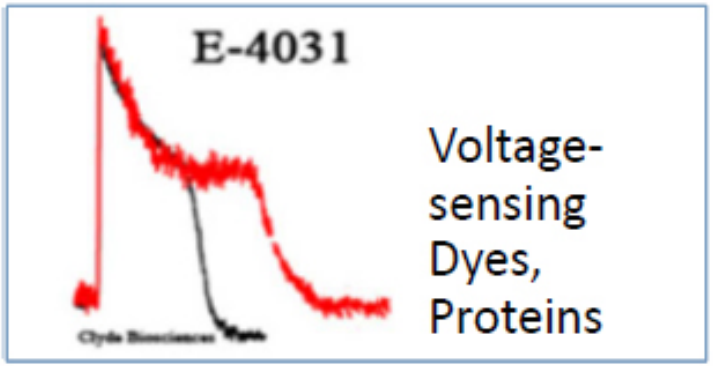
Blinova et al., Cell Reports, 2018

- Overview
  - using human induced pluripotent stem cell-derived cardiomyocytes (2D cultures)
  - Test ability of myocyte/ test platforms (multielectrode arrays, voltage sensing dyes) to categorize proarrhythmic risk of 28 drug dataset based on predicted risk probability using statistical models
  - Good in vitro prediction of TdP risk

Examples: Electrophysiologic Changes Measured Using MEA (Multi-Electrode Array) and VSO (Voltage-Sensing Optical) Techniques

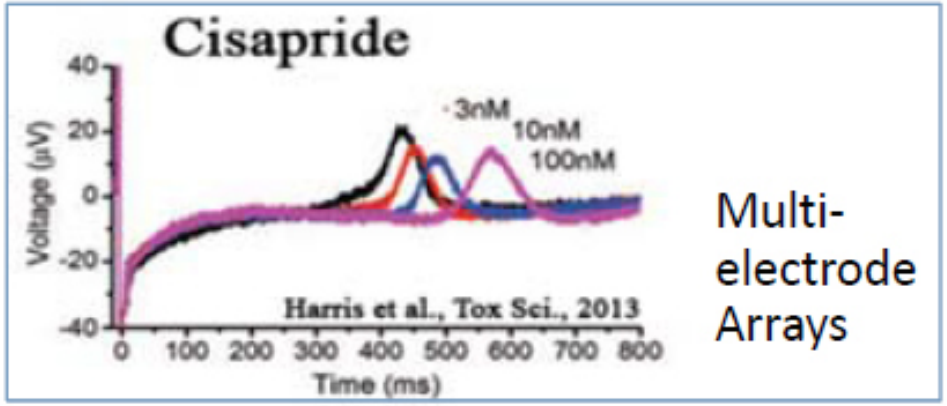


Transmembrane Recordings (Electrical, Optical)



Voltage-sensing Dyes, Proteins

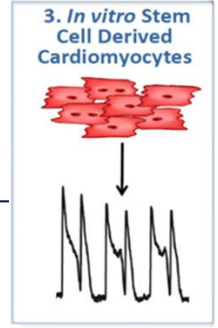
Extracellular (Field Potential) Recordings



Multi-electrode Arrays

- Effects on cardiomyocyte repolarization to be compared to *in silico* reconstructions (delays & cellular proarrhythmia)

FDA-HESI Blinded Validation Study with Standardized Protocol: Do Drugs Affect Human-Derived Cardiomyocyte Repolarization?



Two commercial cell lines prepared according to vendor specifications

Five commercial instruments/platforms, 10 experimental sites

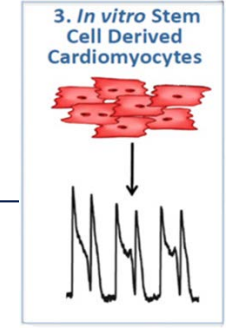
Blinded drugs ("CiPA 28") with instructions for preparing

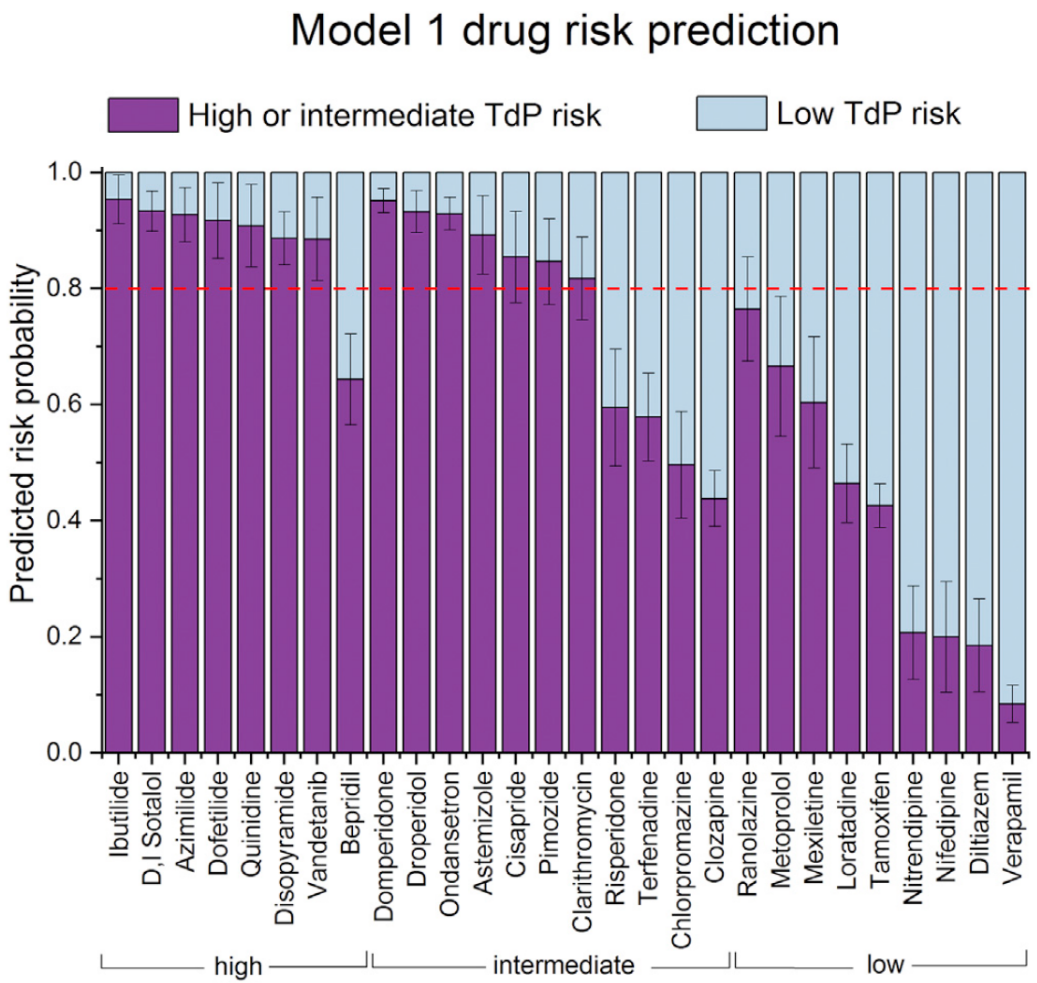
Individual dosing per well, 30 min. exposure
- 4 pt conc.-response curve (Log units)
- 5/6 replicates per point, 2 min recordings

Focus on delayed repolarization, cellular proarrhythmia ("cellrhythmia")
- Delayed repolarization and incidence of EAD's;

Testing & Defining Limits of Stem Cell Myocyte Model
- Translation with clinical proarrhythmic risk?

CiPA Validation Study Results: Categorization of Proarrhythmic Risk of 28 Drug Dataset





Combined average across 10 sites, 15 Myocyte Type/Platform Combinations
Bars: 95% Confidence Intervals

Blinova et al., Cell Reports, 2018

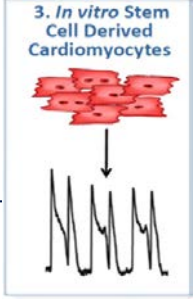
Three Predictors in logistic regression model used to categorize proarrhythmic risk:

-Cellrhythmia (EAD's), any conc.
- Max Prolongation @ all conc's.
- Prolongation @Clinical Cmax

Good agreement: High/Interm vs Low Risk Categories

Outliers:
- Bepridil: High risk categorized as Low risk
- Ranolazine: Low risk categorized as High risk (possibly due to minimal InaLate current)
- Four intermediate drugs labeled as low risk (Clarithromycin, Risperidone,Chlorpromazine, Clozapine); possible original misclassification of clinical risk debated

- Receiver-Operator Curve: (Model 1): **AUC=0.872,** consistent with Good to Excellent results



Lessons Learned with CiPA

Model-Utility

Convenient, simple 2D human-derived cardiomyocyte model (readily available) reasonably predicted the proarrhythmic risk of 28 clinical drugs based on cellrhythmias & prolongation

- ROC curve values (0.872) equal or better than ex vivo (non-human) tissue studies

Assay-based Considerations:

Controlled culture conditions critical to reduce biological variability, promote reproducibility

Standardization of protocols essential to minimize variability, enhance statistical power, increase reproducibility, promote assay use/adoption, enhance communication with end users/stakeholders

- "Best Practices" derived from studies published (Gintant et al., Regul Toxicol Pharm., 2020), and contained in regulatory documents (Intn'tl Conf. on Harmonization, E14-S7B Q&A 2.2-2.5) at Step 2.

Beyond CiPA: Utility of Human-Derived Cardiomyocytes for Safety/Toxicity/Efficacy Testing

Other investigations with human-derived cardiomyocytes

- Cardiac Contractility
- Heart Failure/Hypertrophy
- Cardiac Injury/Regenerative models
- Disease Models – personalized medicine, individual/combination drug tox testing

All potential opportunities to bypass or supplement traditional in vivo animal models

Beyond CiPA: Considerations for Evolving MPS Platforms (besides engineering)

"Simple Chocolate"

"Complex Chocolate"

Goal of the assay?

-Early (hazard i.d.) or later (risk assessment) screening

-Consideration of false positive or false negative rates

-Assay throughput

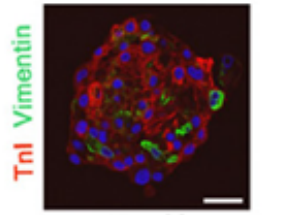
-What level of biological maturation or structural complexity is needed to reproduce the intended (integrated)? effects

- More complex preparations may be advantageous if adverse effects are "to be discovered" or require an integrated multicellular response

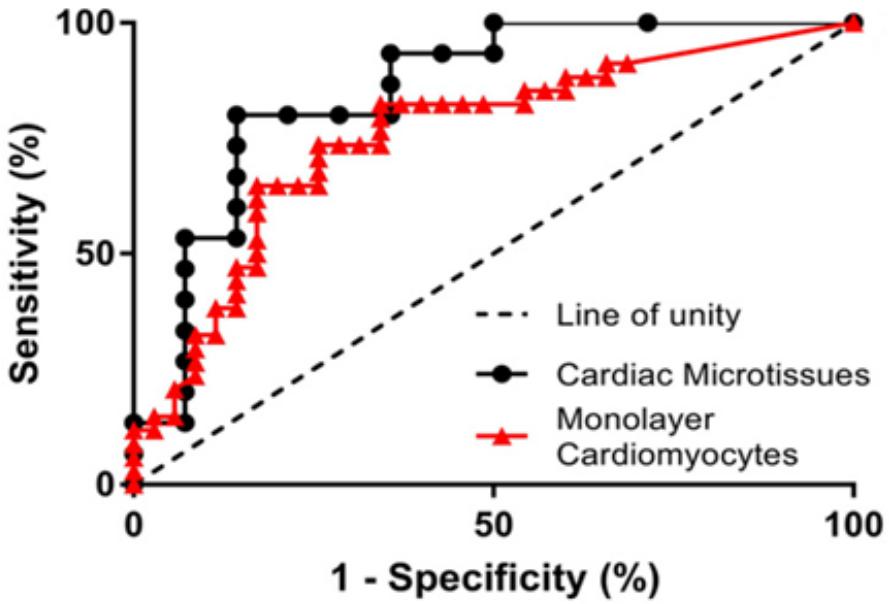
- What are tradeoffs/disadvantages of greater complexity?
  - Increase cost
  - Increased variability, stability over time
  - Reduced accessibility
  - Reduced reproducibility

MPS- Cardiac Safety Assays: Do More Complex Microtissue Preparations Ensure Superior Results?

A



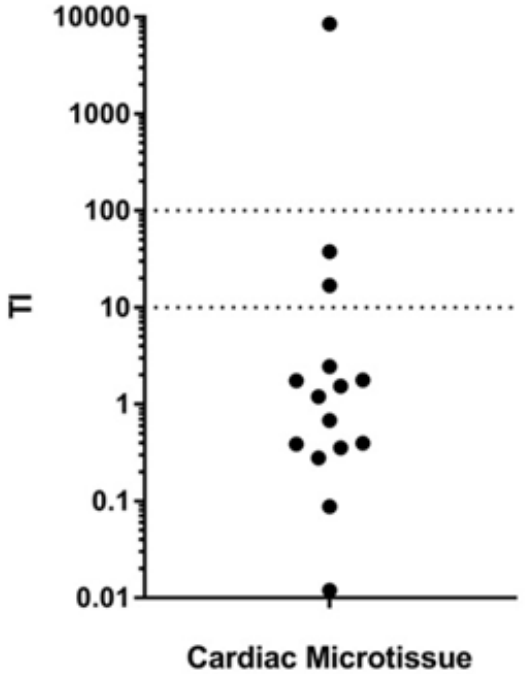
Cardiac Microtissue



Structural Cardiotoxicity with Microtissues
(hiPSC-CMs, cardiac endothelial cells & fibroblasts)

- Structural toxicity assessed using cell viability, ER integrity, MMP
- ROC analysis shows slight improvement: microtissues vs monolayers
- “Fit for Purpose” question

B



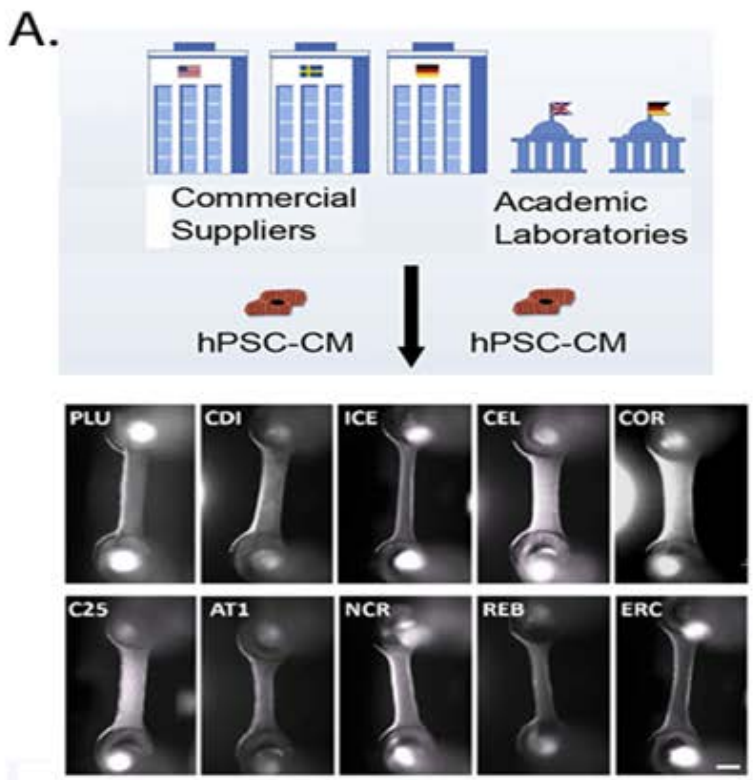
Archer et al., Nature Sci. Reports, 2018

Therapeutic Index (TI) for In vitro Structural Cardiotoxicity

- Potency over/under - estimation vs clinical exposures
- A general issue for in vitro MPS assays

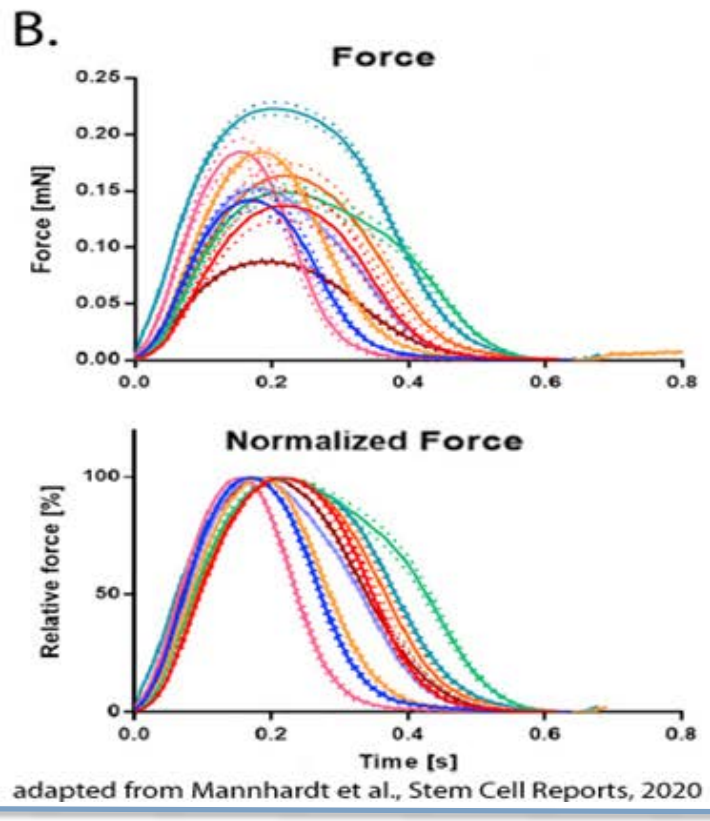
Variability in Engineered Heart Tissues: Baseline Contractility and Electrophysiological Measures

Engineering



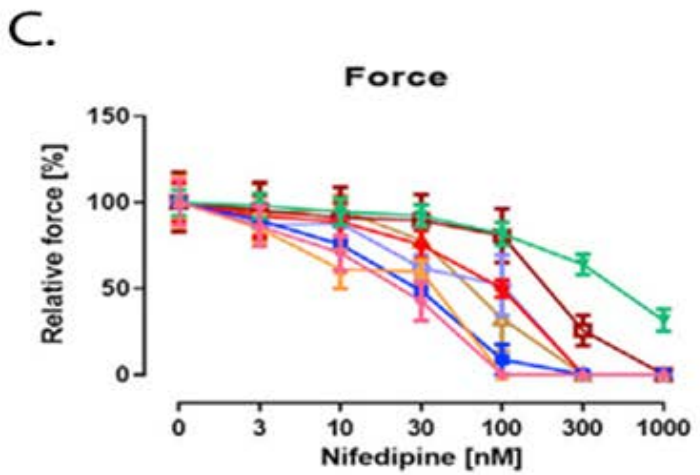
10 cell Lines

Contraction



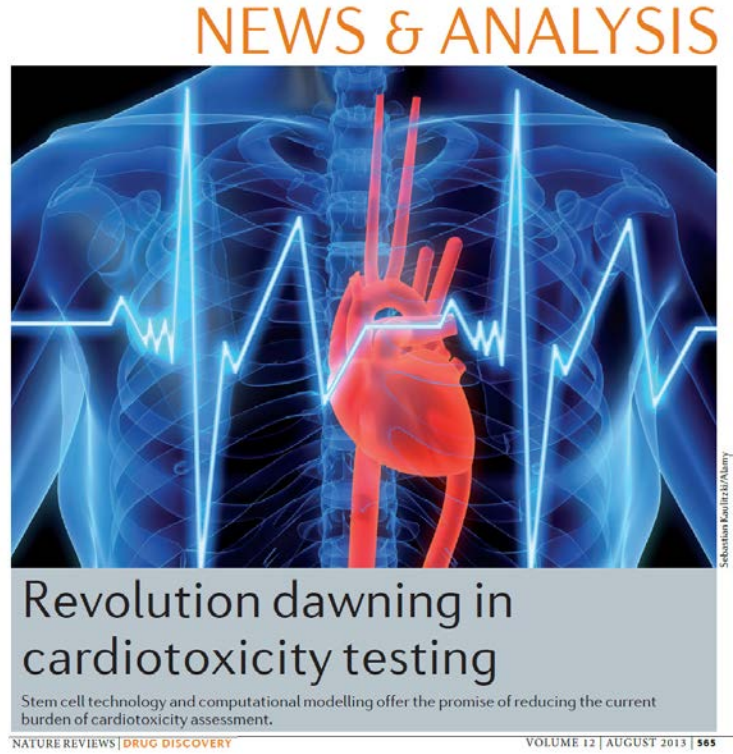
adapted from Mannhardt et al., Stem Cell Reports, 2020

Cell-Line Specific Drug Responses



- Heterogeneity of baseline characteristics and inotropic responses, 10 EHT constructs
- Similar directionality of responses to inotropic drugs suggests variability across lines less relevant for early drug screening, but possibly misleading for risk assessments in later risk assessments
- Supports use of isogenic controls in disease modeling

Conclusions: General Lessons for CiPA for MPS Testing



- As with all in vitro studies, pay attention to details
  - Variability, signal/noise, experimental reproducibility… all matter
- One assay may not be sufficient for *in vitro* to clinic translation
  - Single or multiple mechanisms (level of complexity) involved?
    - Define “fit for purpose” models
- Tools/Approaches need to be readily available and accessible
  - Cost, familiarity and complexity will affect implementation/adaption
  - Commercialization plays a prominent role in adaption of new approaches
  - Standardization extends beyond technologies/platforms used to protocol details
- Strengths of new models/approaches should be apparent and appreciable
  - Difficult to change established procedures, perspectives and habits
- Choose your model wisely!

Acknowledgments-CiPA Initiative Volunteers/Participants

- Nonprofits- Public Private Partnerships
  - Health and Environmental Sciences Institute (HESI)
  - Cardiac Safety Research consortium (CSRC)
  - Safety Pharmacology Society (SPS)
- Global Regulatory Agencies
  - US Food and Drug Administration
  - European Medicines Agency
  - Japan Pharmaceuticals & Medical Devices Agency /NIHS
  - Health Canada
  - ICH
- Industry / Academia
  - JiCSA
  - Numerous Pharma & Laboratory Device Co's., CRO's, Stem Cell Providers
  - Multiple Academic Groups