

Regulatory Research at FDA

**FDA Small Business
Regulatory Education for Industry (REdI)
May 9, 2017**

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Induced Pluripotent Stem Cells for Cardiac Safety Assessment

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Technology for Assessing the Risk of Drugs



1930s



2017



Division of Applied Regulatory Science



Vision

- To move new science into the CDER review process and close the gap between scientific innovation and product review

What does DARS do?

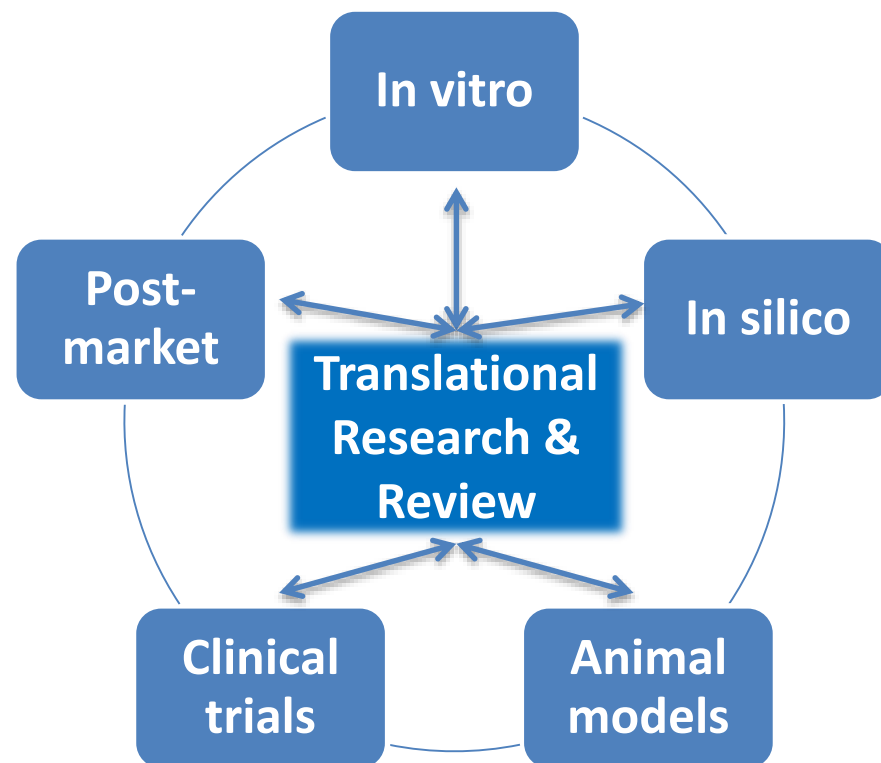
- Performs mission-critical applied research to develop and evaluate tools, standards and approaches to assess the safety, efficacy, quality and performance of drugs
- Performs expert regulatory review consultations for mechanistic safety evaluation for immediate regulatory needs combining
 - Critical review of existing knowledge
 - Computational analyses with informatics tools and disease-pharmacology models
 - In vitro and in vivo laboratory studies
 - Translational analysis of preclinical studies, clinical trials and postmarket data

Division of Applied Regulatory Science

Priority Areas

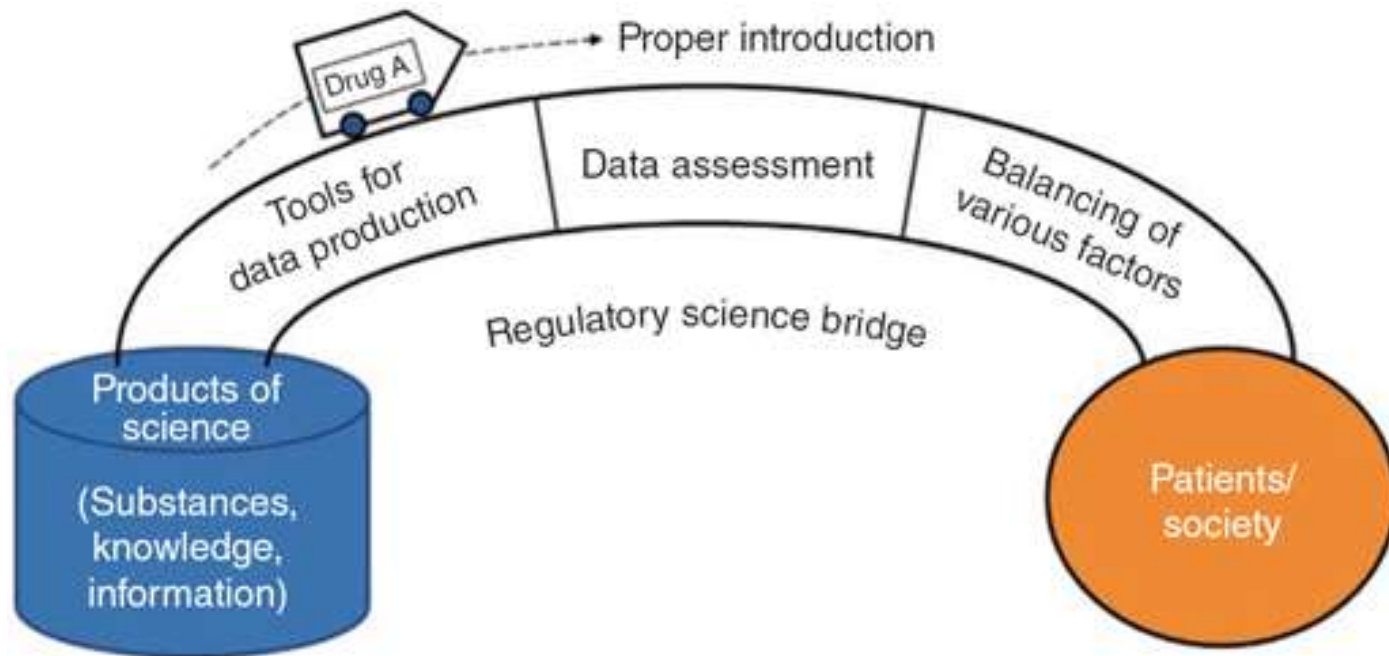


- Translational research
- Collaboration and interdisciplinary team approaches
- Implementation of new regulatory review methods and programs



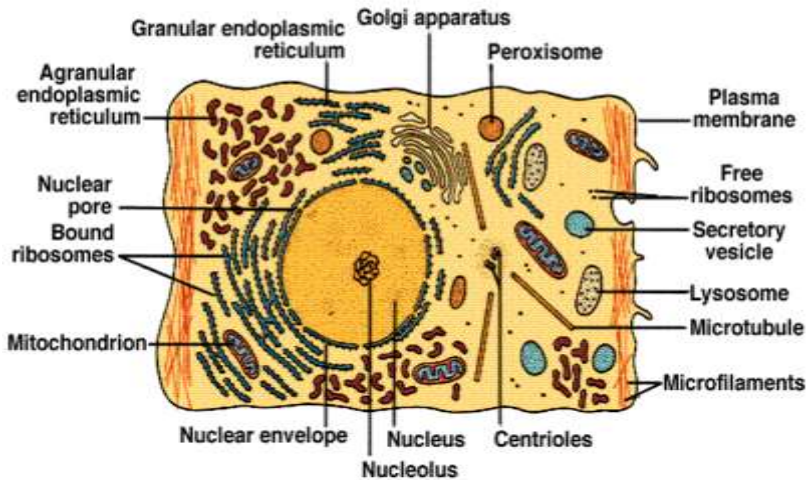
Collaboration: Office of New Drugs, Office of Surveillance and Epidemiology, Office of Generic Drugs, Center for Devices and Radiological Products, Center for Biologics Evaluation and Research, National Center for Toxicological Research, National Institute of Health, Industry, Universities/Academia

Use science to improve the drug regulatory process



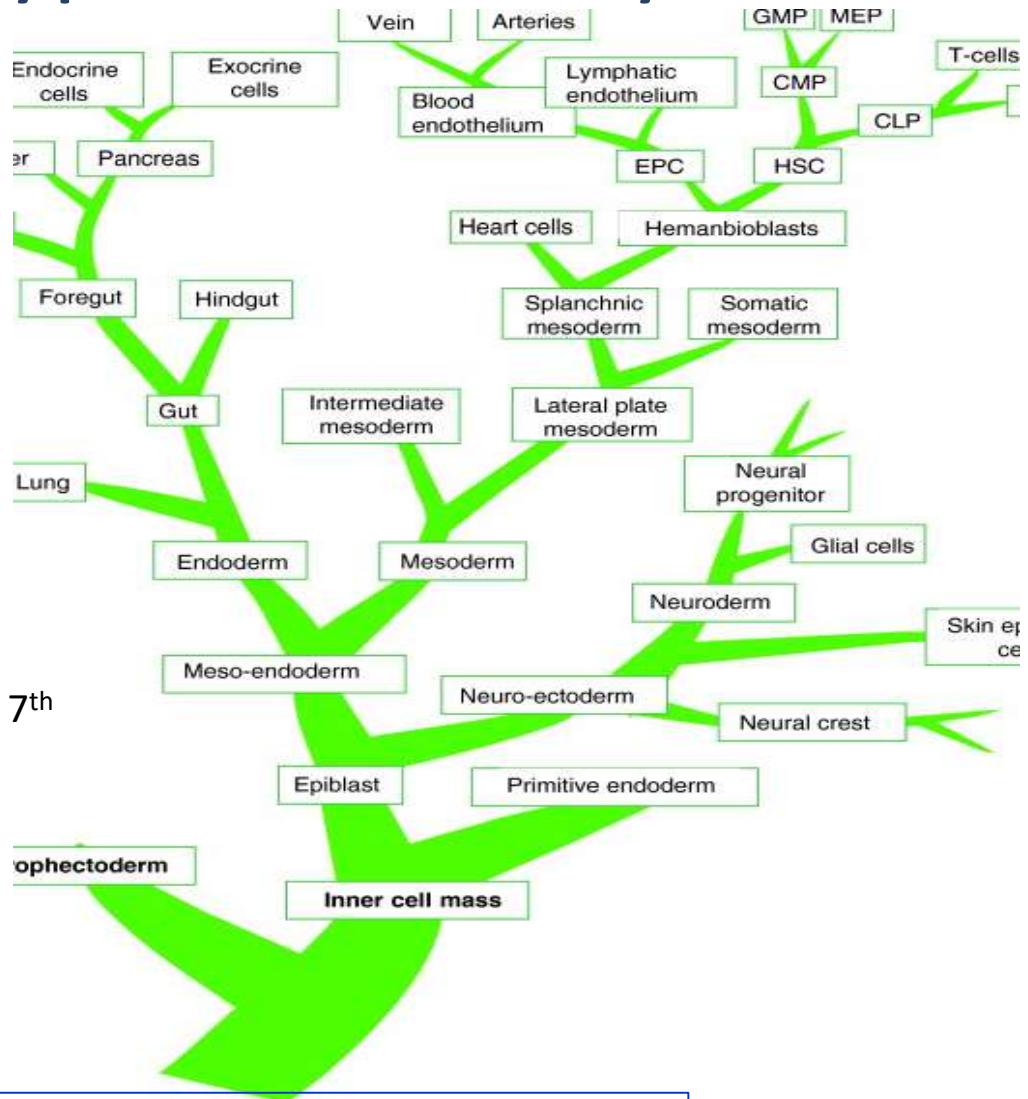
We predict the clinical toxicity of drugs with pluripotent stem cells

Pluripotent stem cells can differentiate into any cell type in the body



Vander/Sherman/Luciano Human Physiology. 7th Edition. 1998 McGraw-Hill

Pluri = several
Potent = being able



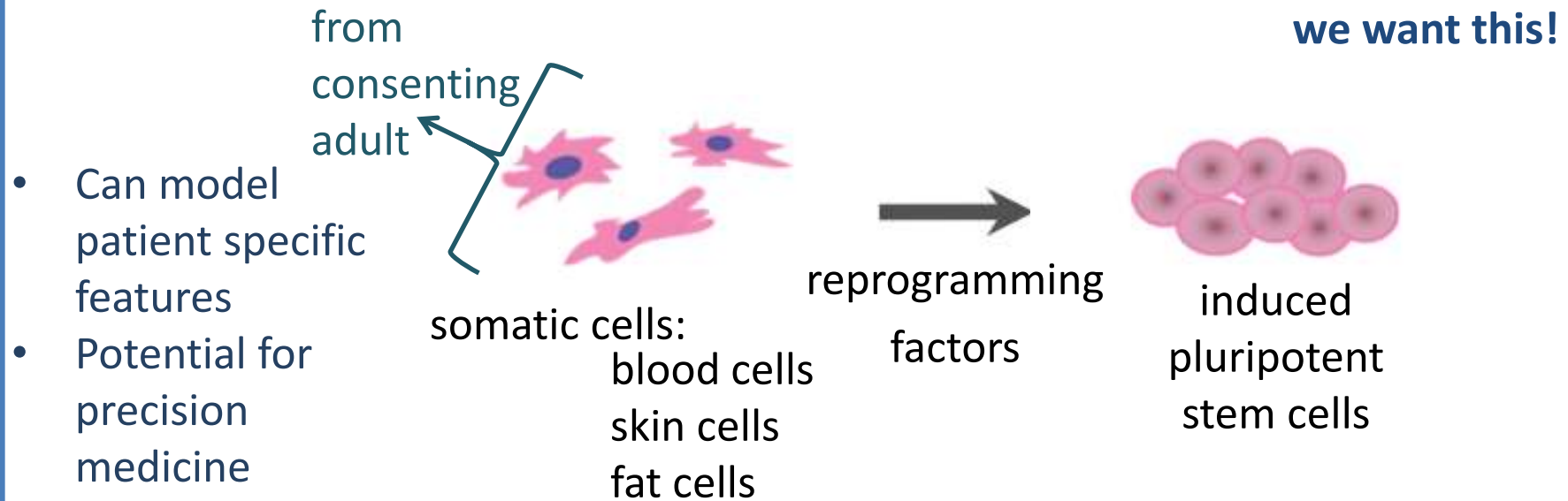
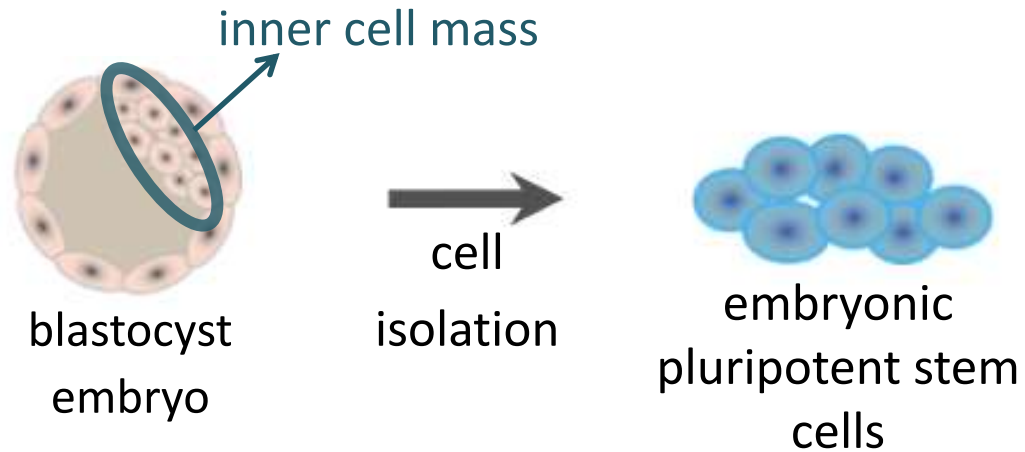
Key takeaways today

1. Pluripotent stem cells have high potential for predicting clinical drug toxicity
2. Engineered microfabricated systems can address the main problems of using pluripotent stem cells:
 - a) Physiological immaturity
 - b) Variability

Main focus of presentation:

- Structural maturity
- Genetic variability

Two types of pluripotent stem cells: embryonic cells and induced pluripotent stem cells

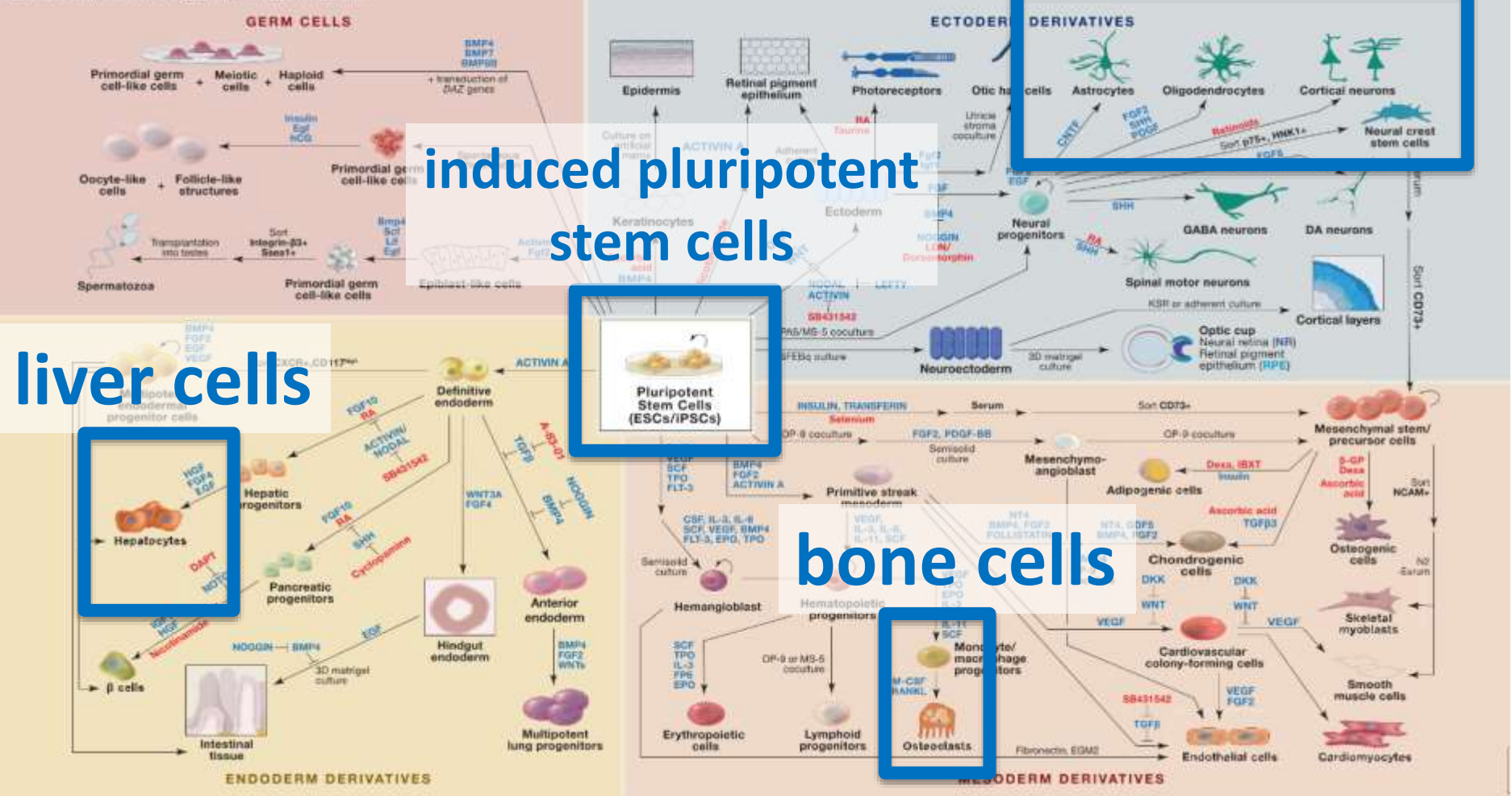


Protocols to differentiate into different tissues



Snapshot: Directed Differentiation of ESCs and iPSCs

Luis A. Williams, Brandi N. Davis-Dusenbery, and Kevin C. Eggen
HIML, Harvard University, Cambridge, MA 02138, USA
First Snapshot was previously published in Cell 149, May 20, 2012 (http://dx.doi.org/10.1016/j.cell.2012.04.017)



neural cells

liver cells

bone cells

Poll Question

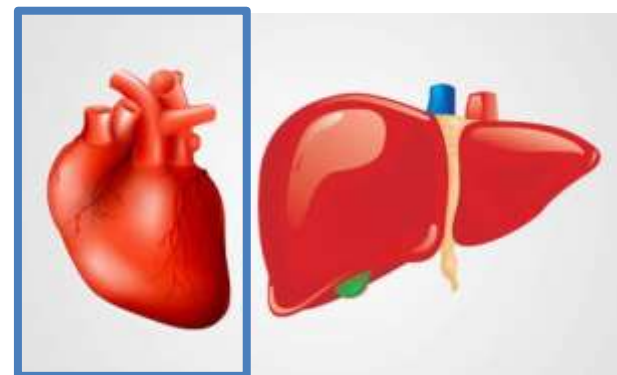
What is the type of tissue more affected by drug adverse effects?

(Pick three choices)

Poll Question

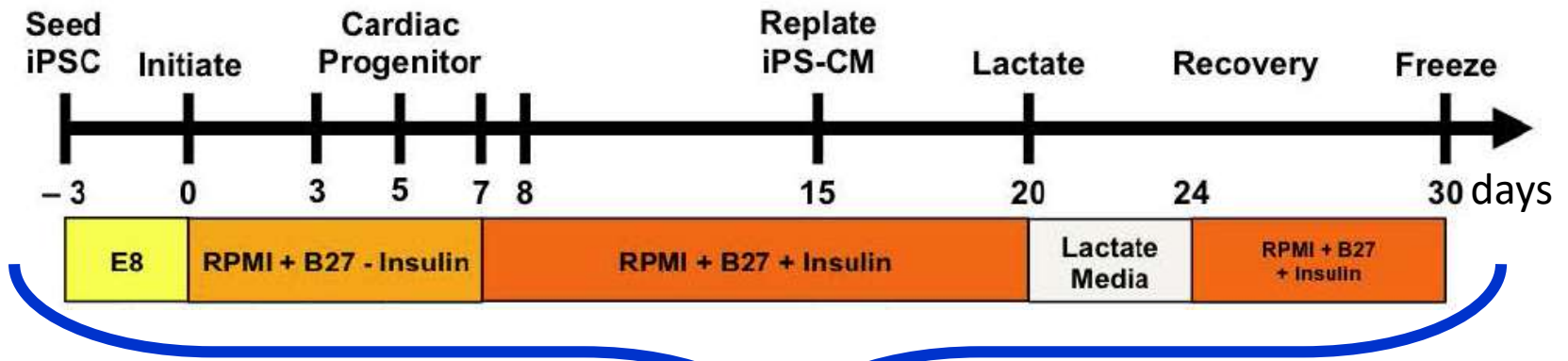
What is the type of tissue more affected by drug adverse effects?

- | | |
|----------|-------------|
| 1. Brain | 5. Kidney |
| 2. Liver | 6. Pancreas |
| 3. Heart | 7. Lung |
| 4. Bone | 8. Blood |



I focus my research in heart cells

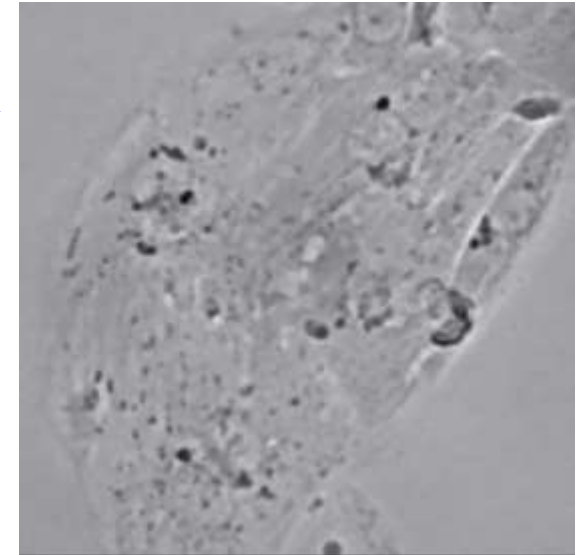
We differentiate heart muscle cells: cardiomyocytes



Pluripotent stem cell colony
(iPSCs)

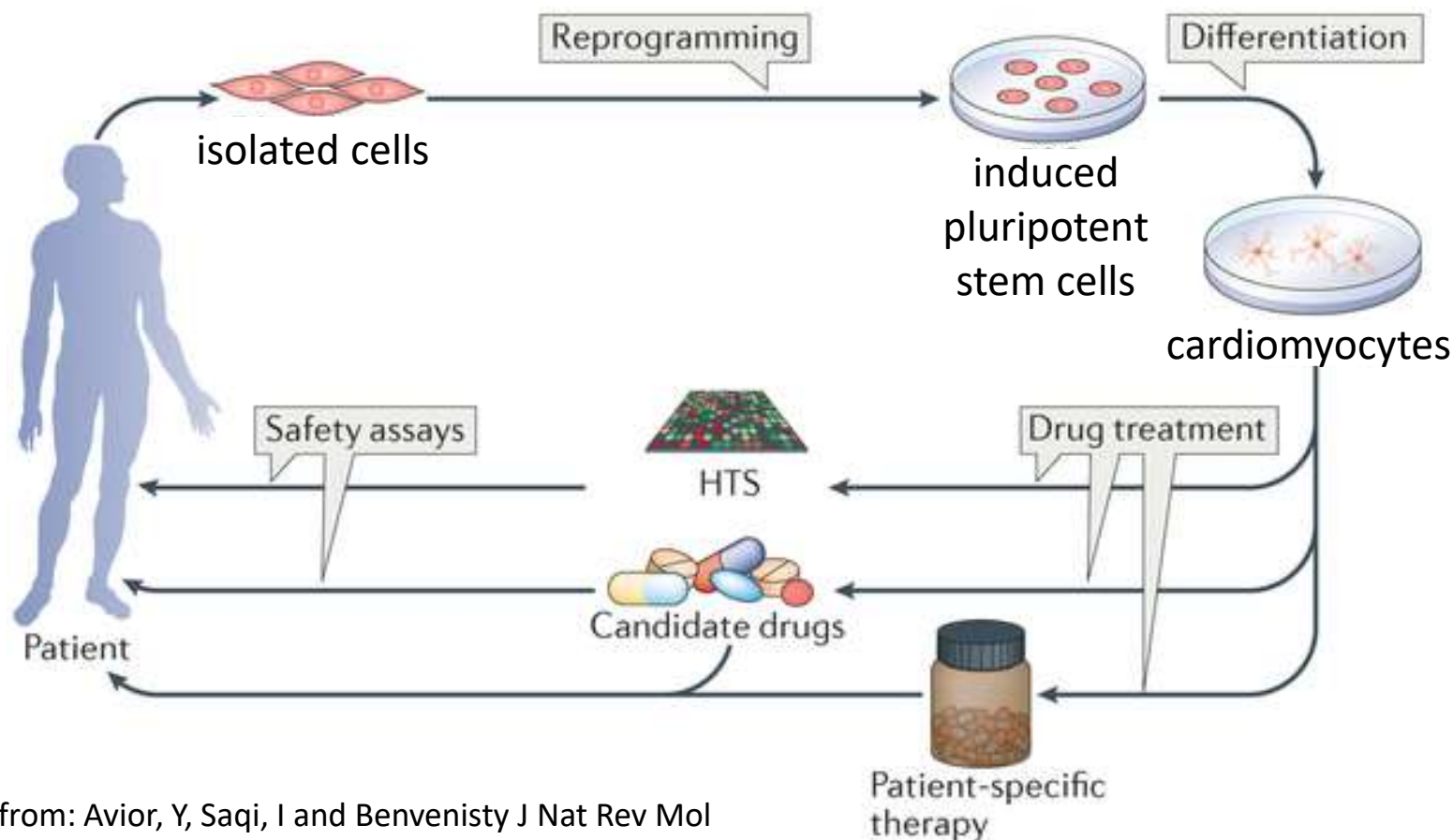
Involves:

- Changes in culture media
- Different small molecules
- Purification steps



Differentiated cardiomyocytes
(iPS-CMs)

Ultimate goal: assay human heart function in a dish



Adapted from: Avior, Y, Saqi, I and Benvenisty J Nat Rev Mol Cell Biol. 2016

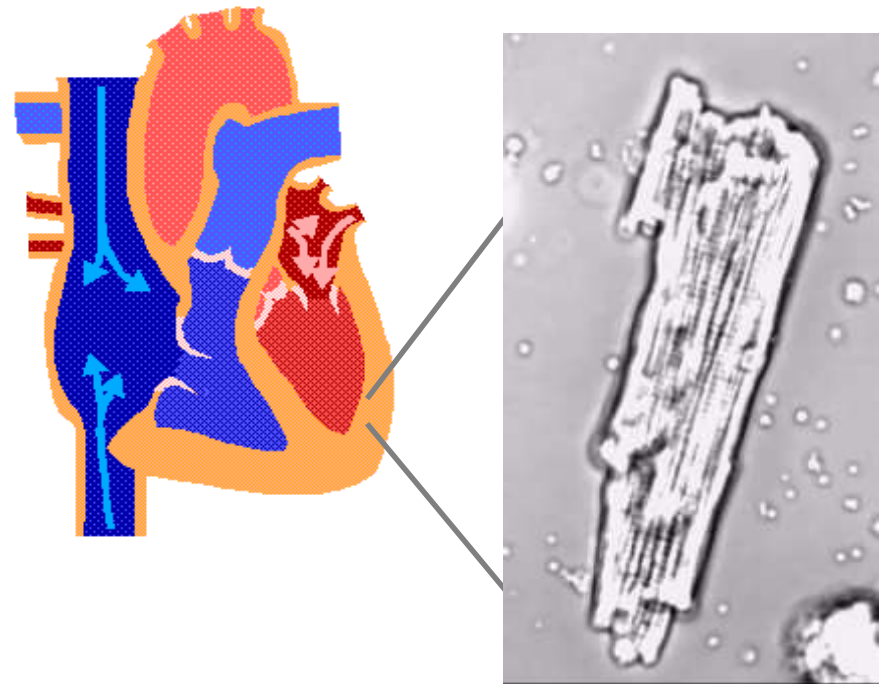
Nature Reviews | Molecular Cell Biology

How do we assay heart function in a dish?



- Physiological function
 - beating activity: contractility
 - electrophysiology
 - calcium flow
 - metabolism
- Expression of genes that encode for proteins that regulate function

Cardiomyocyte
Isolated from a Mouse

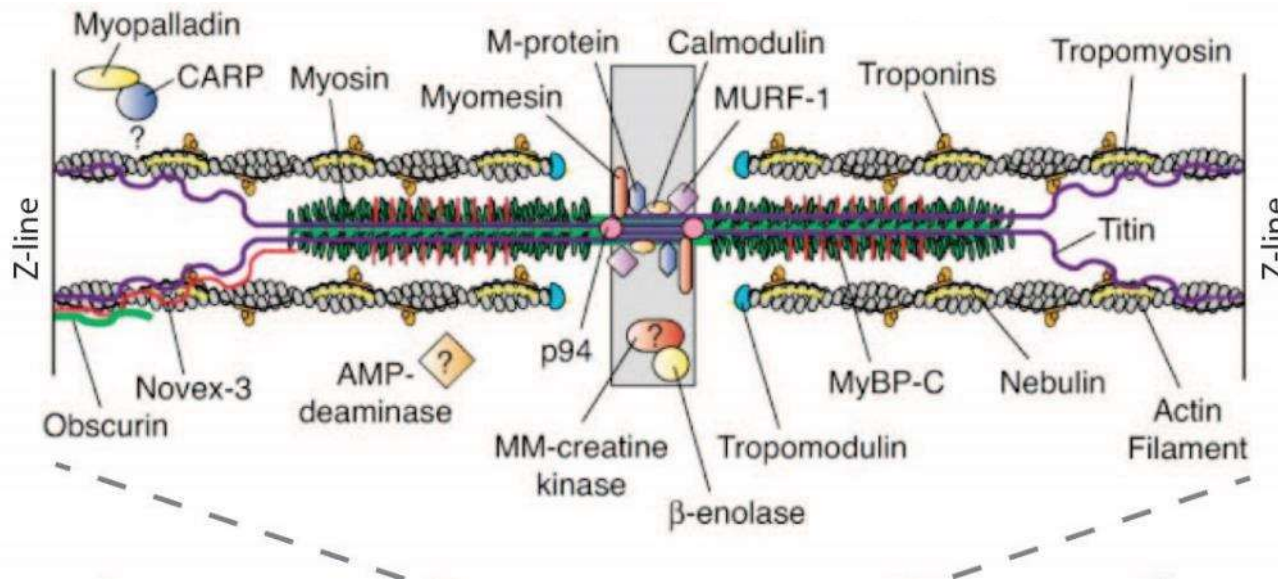


Braunwald's Heart Disease - A Textbook of Cardiovascular Medicine, 9th ed. - 2011

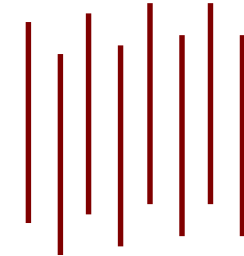
Mature cardiomyocytes have aligned contractile fibers and well registered Z-lines



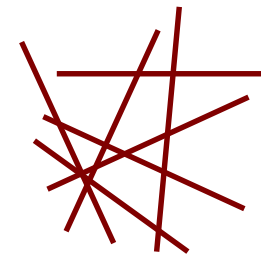
Between Z-lines



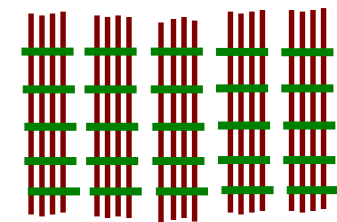
aligned



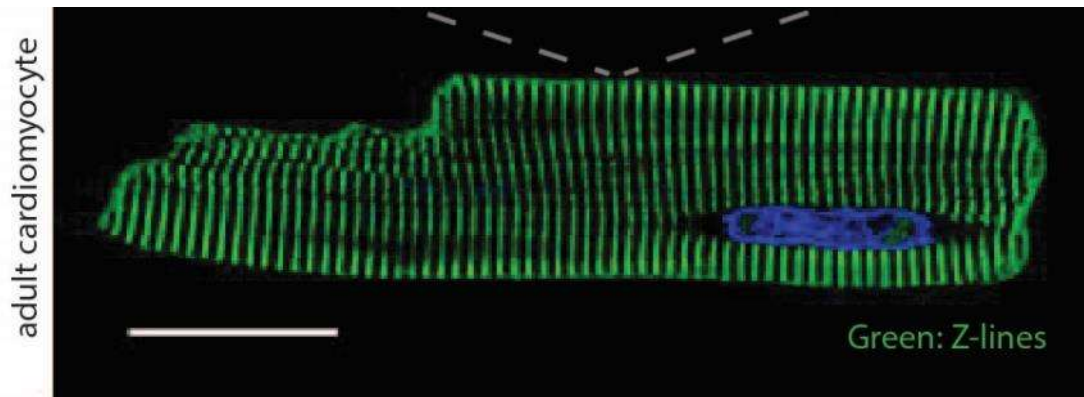
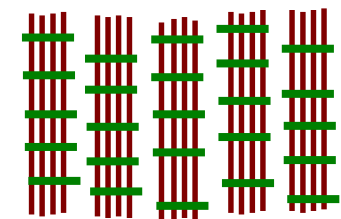
non-aligned



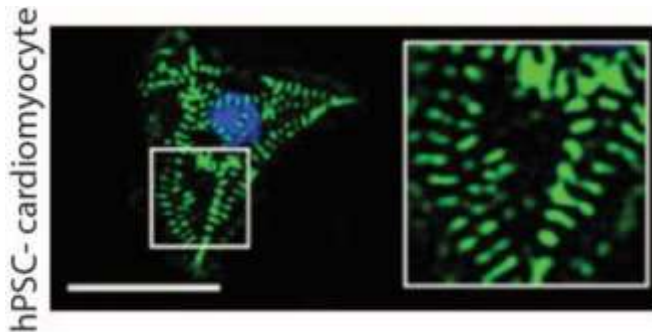
high registry



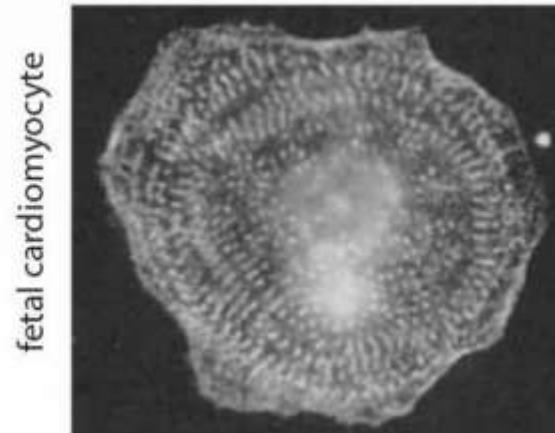
low registry



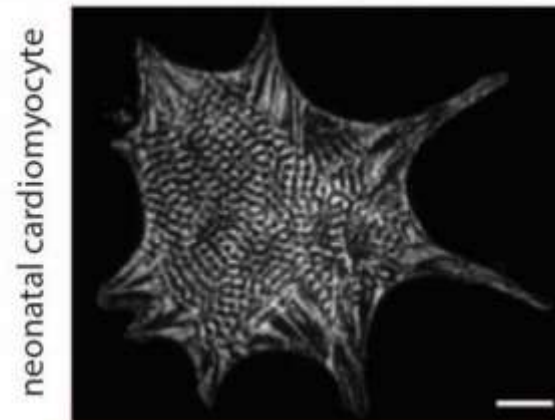
1st problem: immature physiological function in stem cell derived cardiomyocytes



Yang, X et al. Circ Res 2014



Rhee, D et al.
Cell Motil
Cytoskeleton
1994



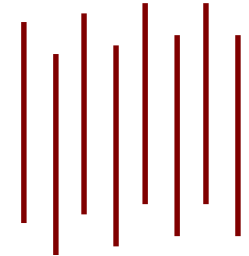
Bray, MA et al.
Cell Motil
Cytoskeleton
2008

Stem cell derived cardiomyocytes have:

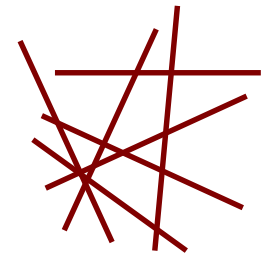
- Non-aligned fibers
- Low registry of Z-lines

immature

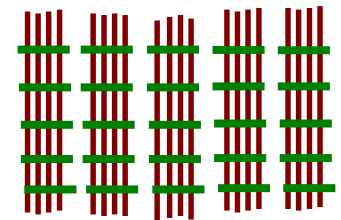
aligned



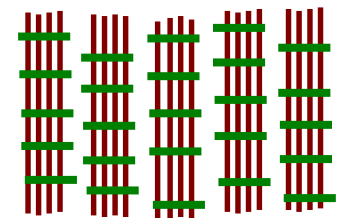
non-aligned



high registry



low registry



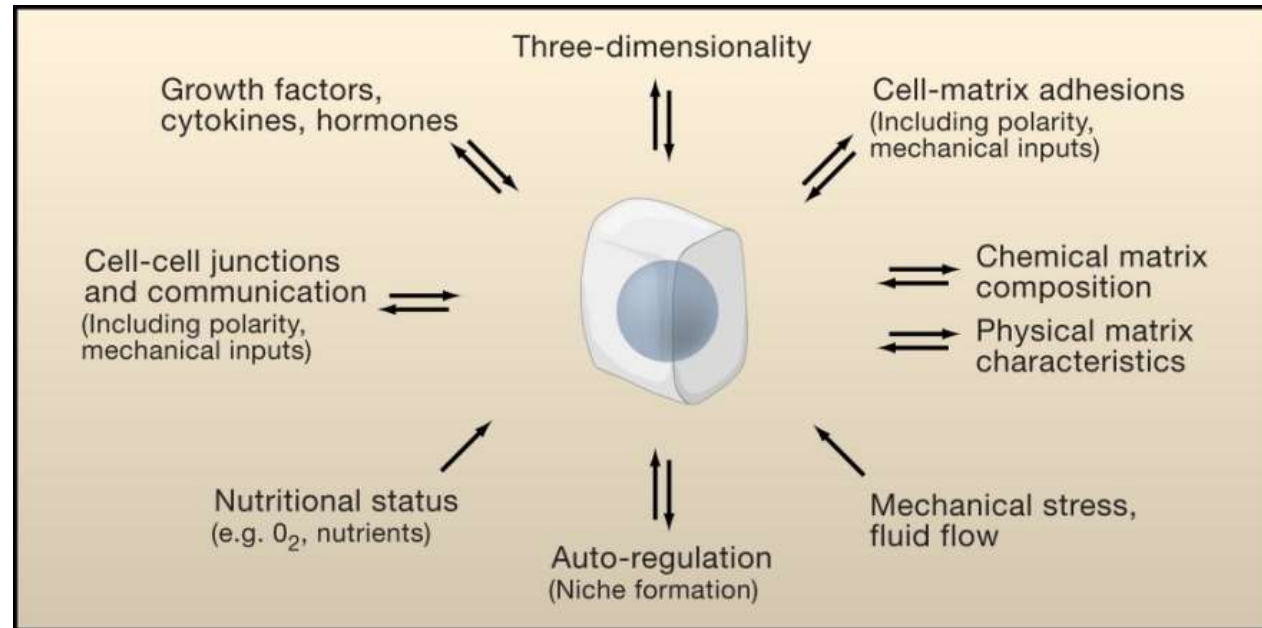
Microfabricated devices can mature cardiomyocytes differentiated from stem cells



Microfabrication of cell culture platforms:

- Polymer chemistry
- Material sciences
- Surface chemistry
- Lithography
- Etching
- Microforming
- Photochemistry
- Patterning
- Microinjection
- Microelectronics

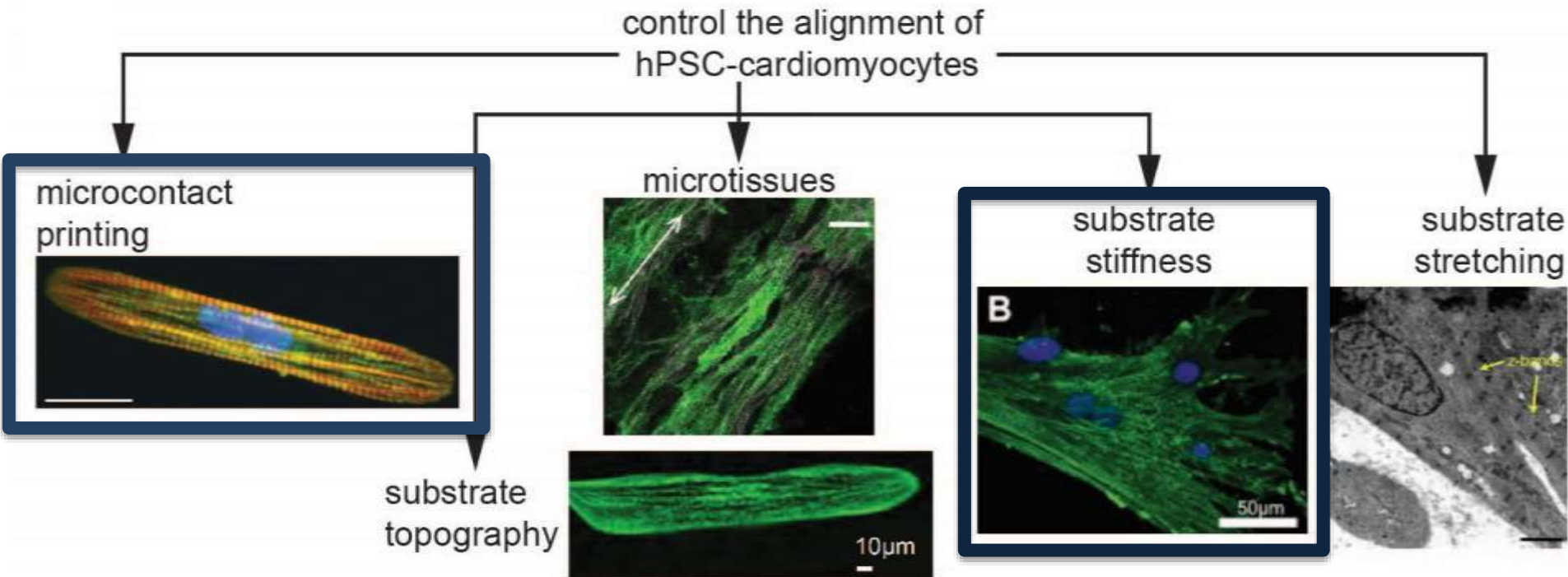
Cell culture platforms that replicate physiological cues *in vitro*



Santos, E et al, Cell-Biomaterial Interactions: Strategies to Mimic the Extracellular Matrix, 2011

Build physiological settings in a dish to engineer cell maturity

Cell and fiber alignment is central for enhancing the physiology of hiPSC-cardiomyocytes



Aligned cells:

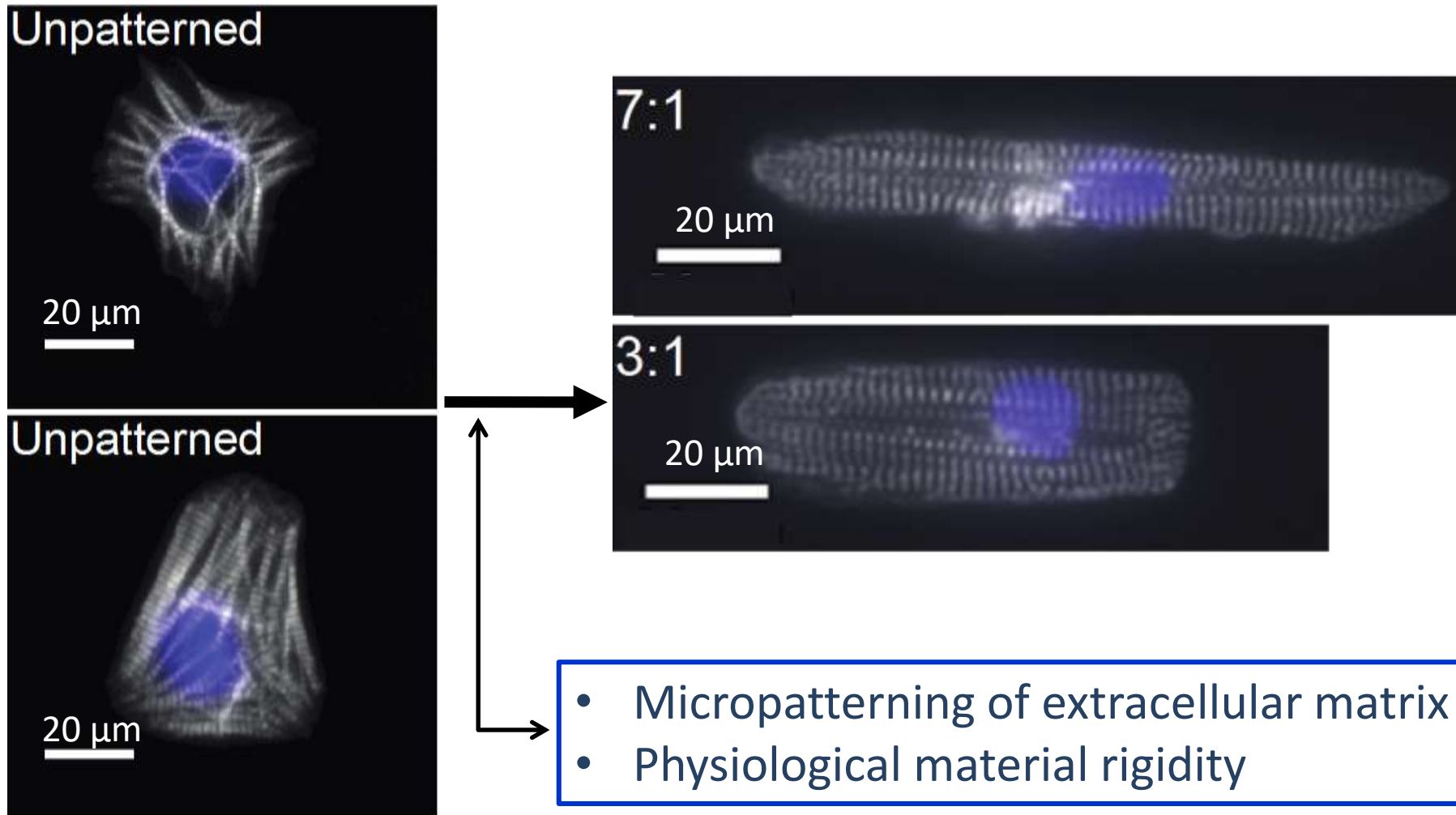
- have a more physiological structural organization,
- have a more mature function,
- are more suitable for toxicity assays.

Nunes SS et al. Nat Methods 2013

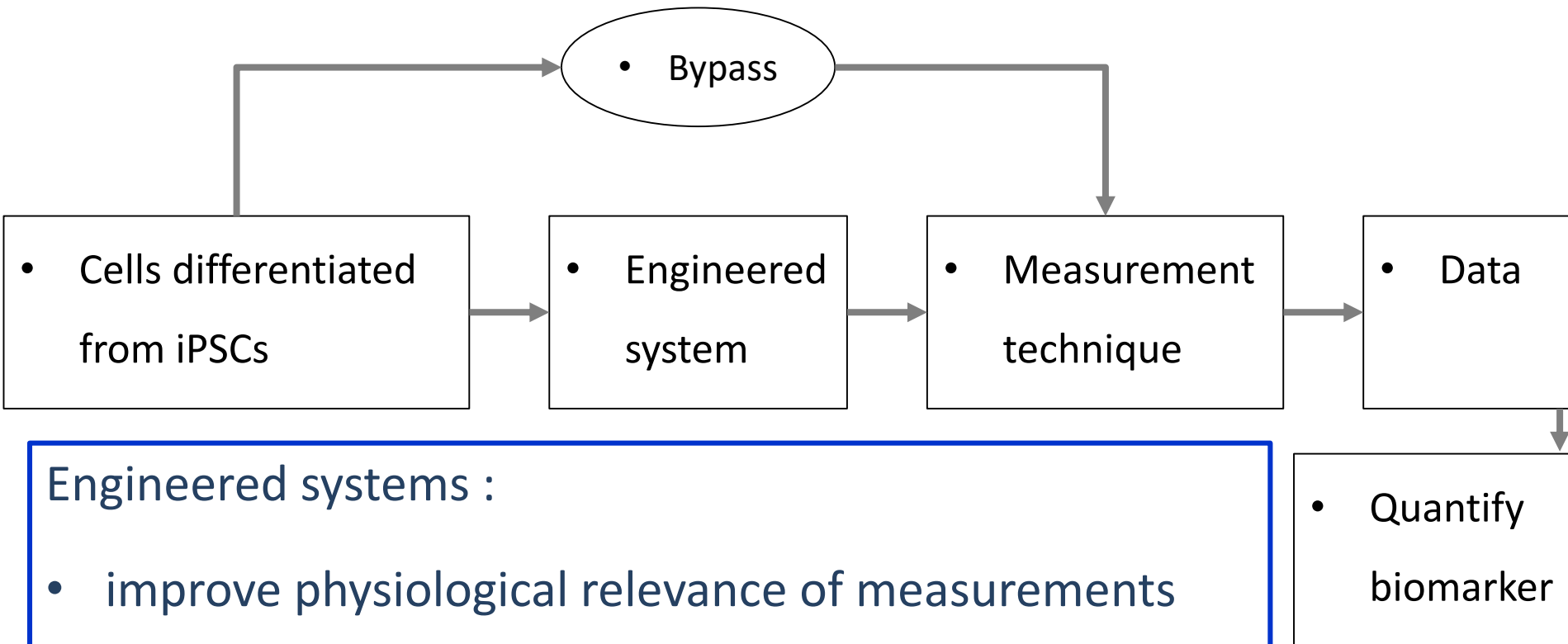
Jung J et al. FASEB J 2015

Ribeiro AJS et al. Proc Natl Acad Sci USA 2015

Disorganized cell becomes more mature with combined micropatterning and physiological rigidity



Engineered microfabricated systems for designing stem cell assays



Engineered systems :

- improve physiological relevance of measurements
- mature cells by delivering different types of stimuli:
 - chemical
 - electrical
 - mechanical
 - biological

Problem number 2: Genetic Variability



Type of variability	Occurrence			
	Among ESC	Among iPSC	Between ESC, iPSC	Heterogeneity within PSC line
Functional • in vitro differentiation	+	+	+	+
RNA • mRNA	-	+	+	+
Epigenetic • DNA methylation	?	+	+	?
Genetic • Germline (background) • Result of derivation • selection • induced	+ ?	+ +	+ na	? ?

+ high

- low

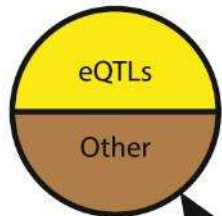
? unclear

Cahan, P, Daley, GQ J Nat Rev Mol Cell Biol. 2013

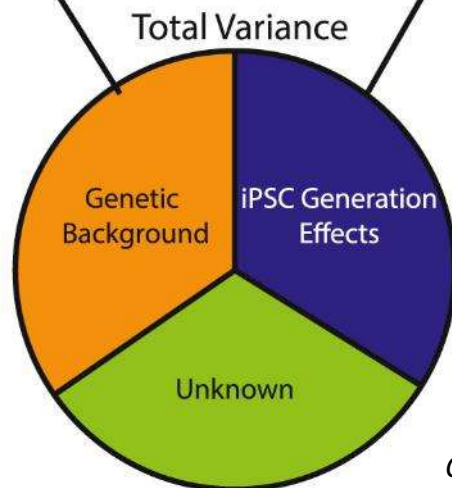
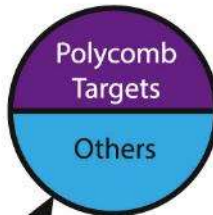
- Gene expression is highly variable
- Function is affected by gene expression

Only 50% of genetic variability is explained by variation across individuals

Contribution to Variance



Magnitude of Variance



- A set of genes regulates variance
- Another set of genes regulates magnitude of variance
- Differentiation is affected by the variability of pluripotent stem cells

Cell Stem Cell 2017 20, 518-532.e9DOI: (10.1016/j.stem.2016.11.005)

Populations of differentiated cardiomyocytes are highly variable

day 25 differentiated cardiomyocytes

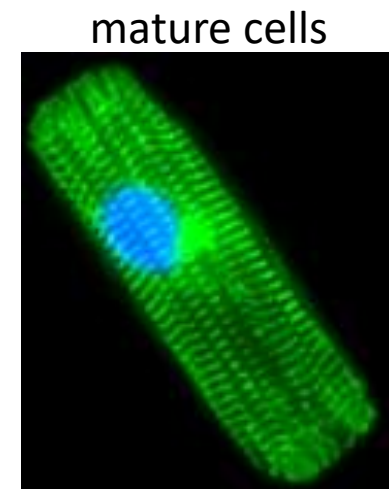
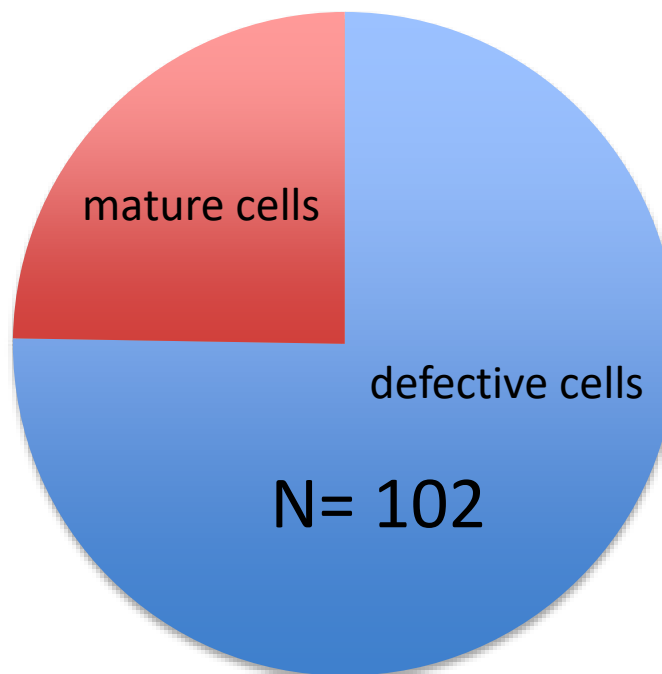
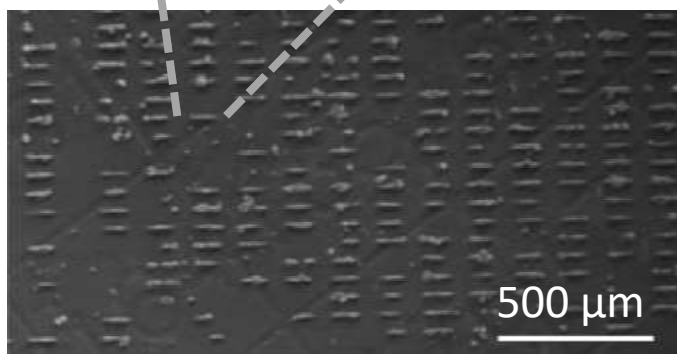
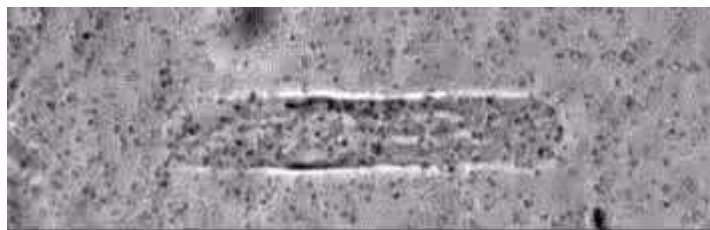


green: Z-lines
white: DNA in nucleus

20 μ m

- Some cells have well developed Z-lines
- Some cells have poorly developed Z-lines
- Some cells have no Z-lines

How can microfabrication help study variability?



The way of the future:

single cell studies

- function (contractility, electrophysiology, calcium)
- omics (genomics, proteomics, metabolomics, etc)

Goal: Learn how to select for more mature cells

Key Takeaways

1. Induced pluripotent stem cells have high potential for modeling heart function *in vitro*
2. Engineered microfabricated systems can solve the main limitations of these cells:
 1. Lack of Structural Maturity
 2. High Genetic Variability

Case Study:

Prevention of drug-induced cardiac arrhythmias

Ksenia Blinova, PhD

Staff Fellow

Division of Biomedical Physics
Office of Science and Engineering Laboratories
Center for Devices and Radiological Products
U.S. Food and Drug Administration

Regulatory research at CDRH



Center for Devices and Radiological Health (CDRH)

- oversees **175,000 medical devices** on US market
- **22,000 premarket submissions** each year
- **1.4 M adverse events** and **malfunctions** reports each year

Office of Science and Engineering Laboratories

- Ensures readiness for **emerging and innovative** medical technologies
- **Develops** appropriate evaluation **strategies and testing standards**

Division of Biomedical Physics (DBP)

- 38 full time employees (71% Ph.D.)
- 34 fellows, 2 contractors, 12 volunteers (March 2017)

Division of Biomedical Physics

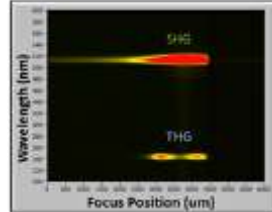
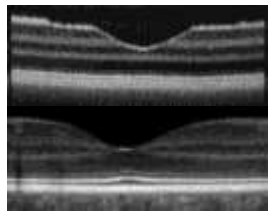
Optical Physics



Retina image

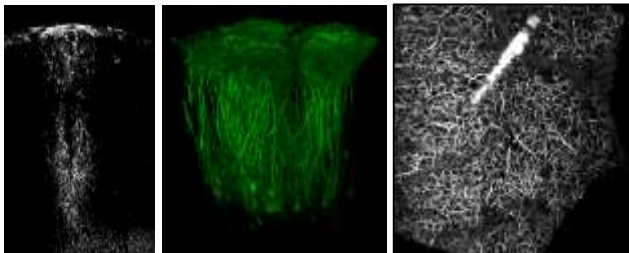
Phantom w/Hb

HbO₂ map

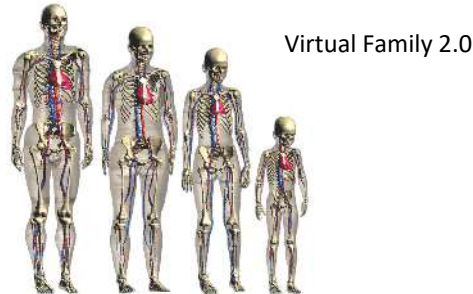


Biomimetic retina phantom SHG and THG in ex-vivo corneas

Neuroscience

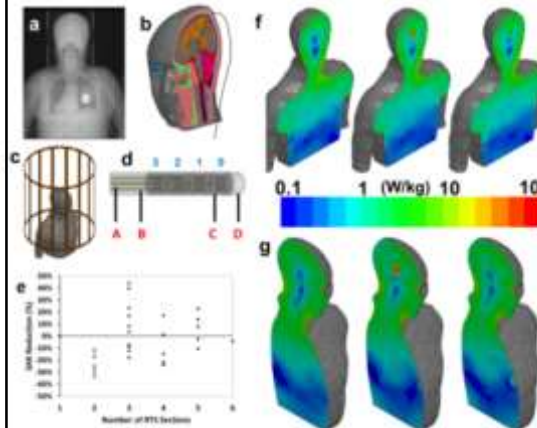


Electromagnetics and MRI



Virtual Family 2.0

MIDA head model



Modeling of heating from DBS leads during MRI

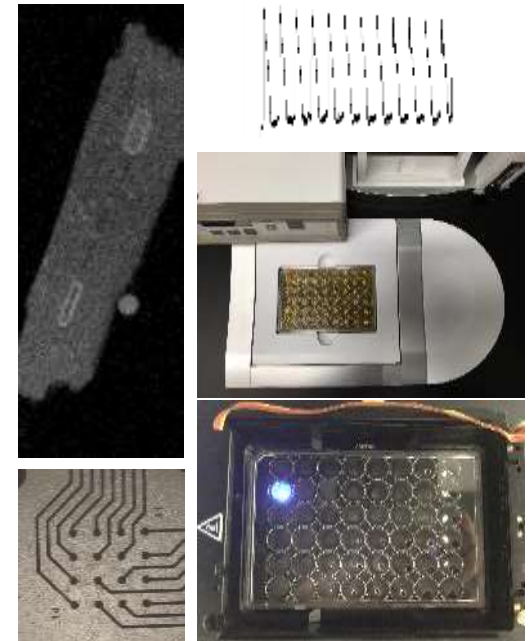
3-D Printing



3D-printed skull

Patient matched cutting guide

Cardiac Modeling



High-throughput devices for cardiac electrophysiology testing

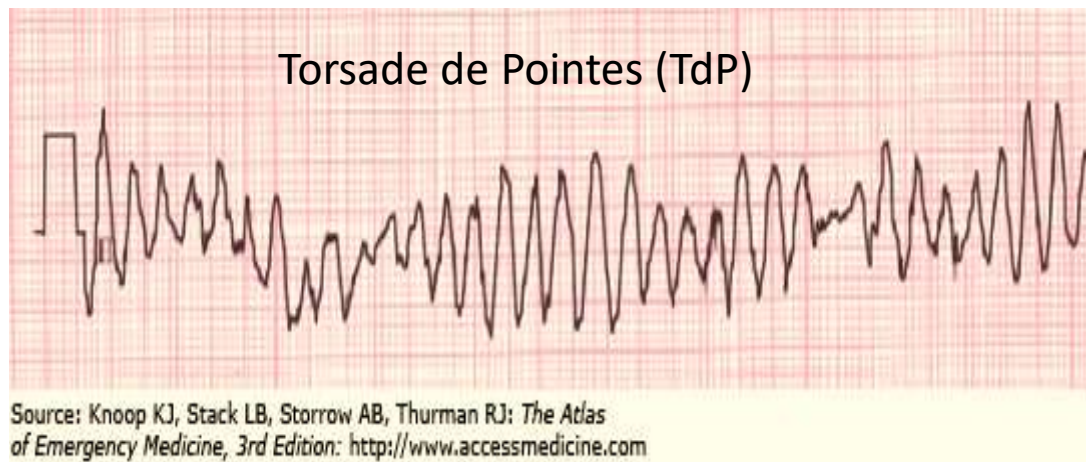
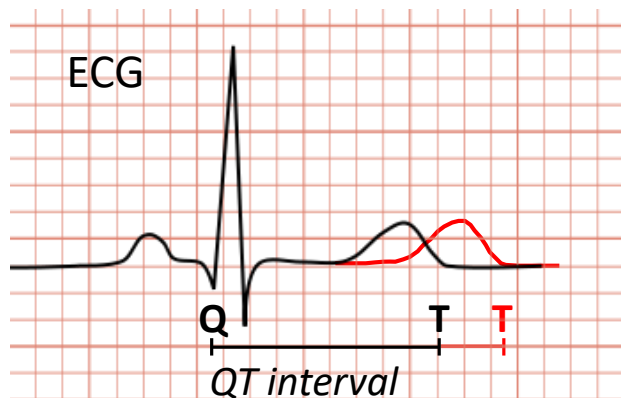
Learning Objectives

- Review current regulatory approach to prevent drug-induced arrhythmias
- Why and how it can be improved
 - Comprehensive *In vitro* Proarrhythmia Assay (CiPA)
- Role of induced pluripotent stem cells in CiPA
- Stem cells for individual risk assessment

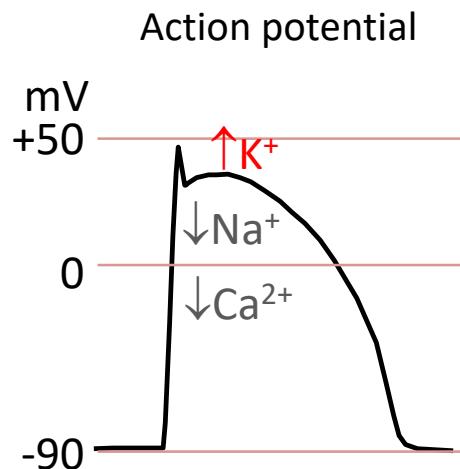
Drug-induced Arrhythmia



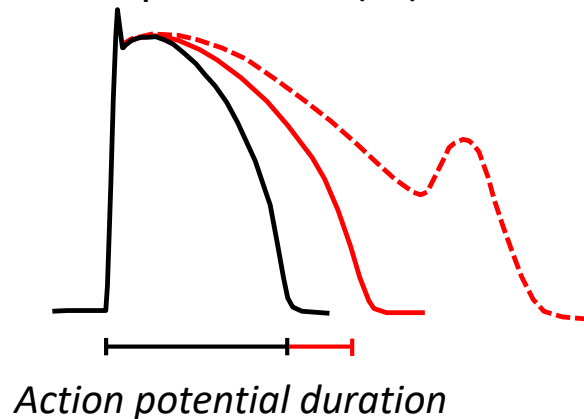
Whole heart



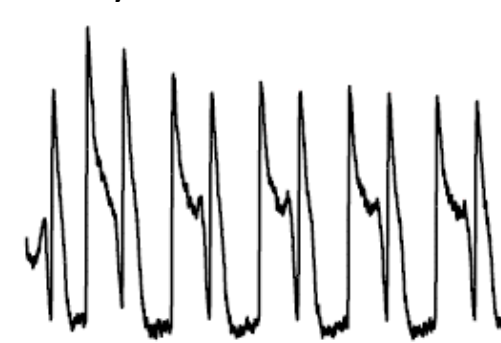
Single cell



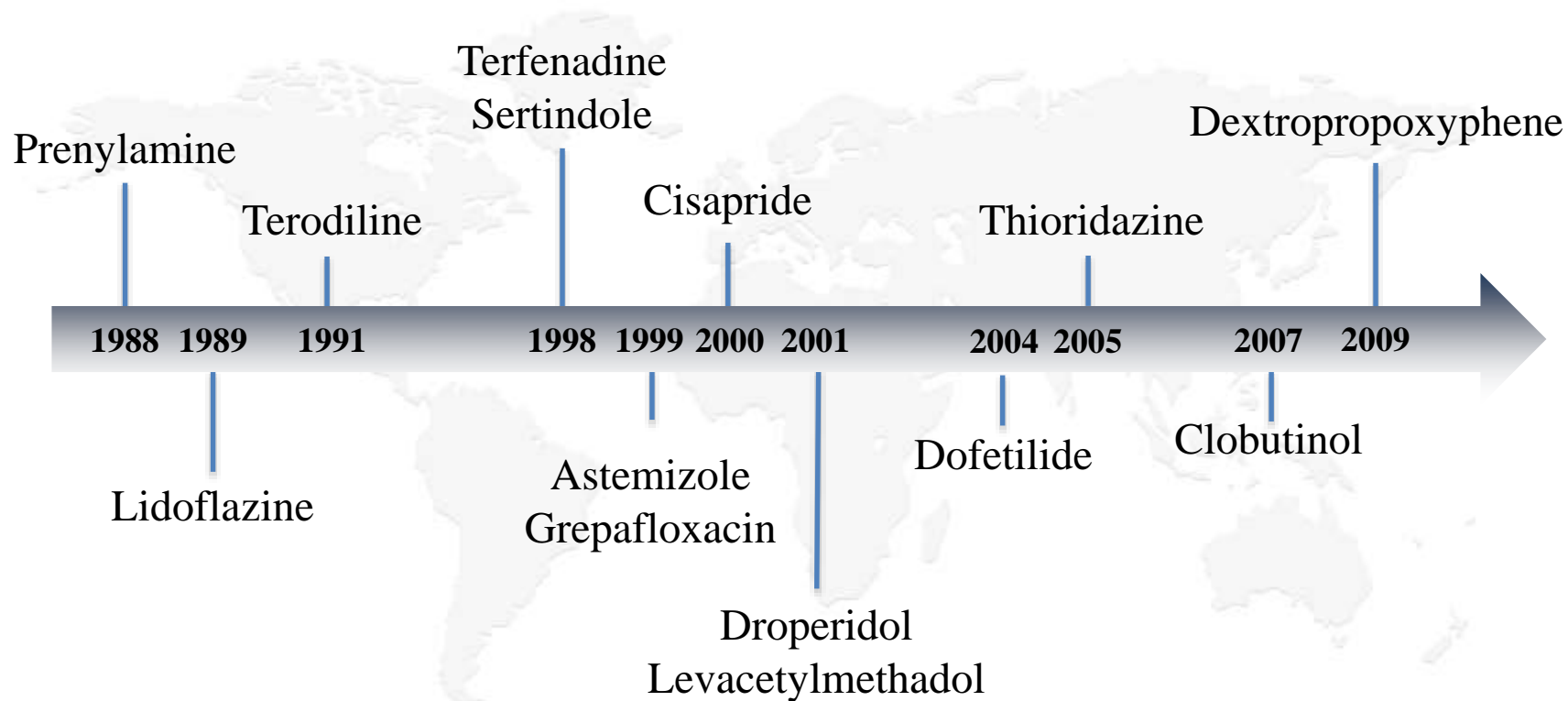
hERG potassium (K⁺) block



Arrhythmia in iPSC-CM



Drugs withdrawn from market



Adapted from Table 1
N. Stockbridge et al
Drug Saf 2013

Regulatory Guidelines



- International Conference on Harmonization (ICH)
- [ICH S7B](#): The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals (focus on hERG)
- [ICH E14](#): The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs

Why to improve current approach?



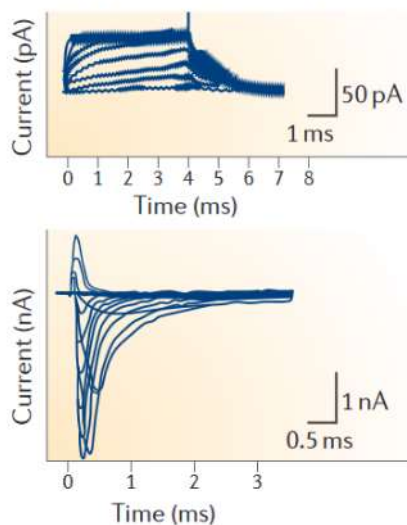
- ICH S7B/E14 have resulted in **no drugs** with unrecognized TdP risk being approved or removed from the market
- However,
 - hERG block and QT prolongation are not perfect predictors of Torsade
 - » Verapamil, ranolazine
 - Many sponsors chose to terminate drug development if hERG or QT “signal” observed
 - Potentially useful drugs never get evaluated in humans due to hERG effect

How to improve current approach?

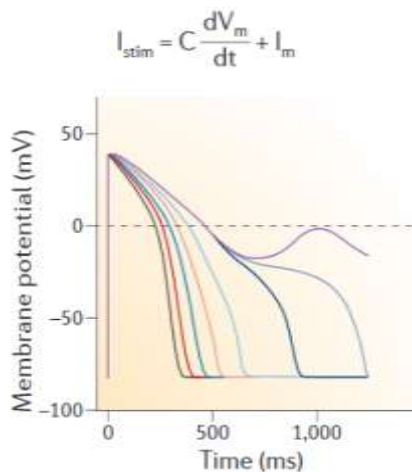


Comprehensive *in Vitro* Proarrhythmia Assay (CiPA)

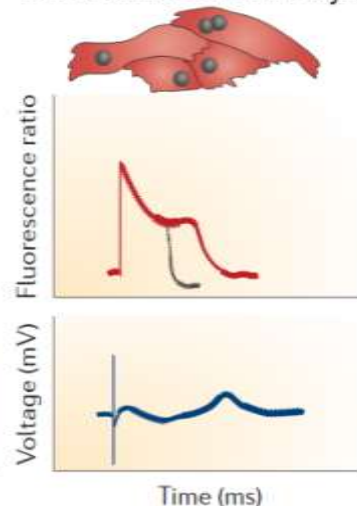
a Drug effects on multiple human cardiac currents



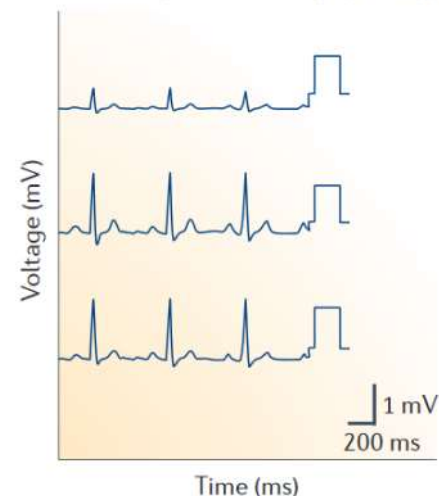
b *In silico* reconstruction of cellular human ventricular electrophysiology



c *In vitro* effects on human stem cell-derived ventricular myocytes



d Clinical evaluation of unanticipated electrophysiology



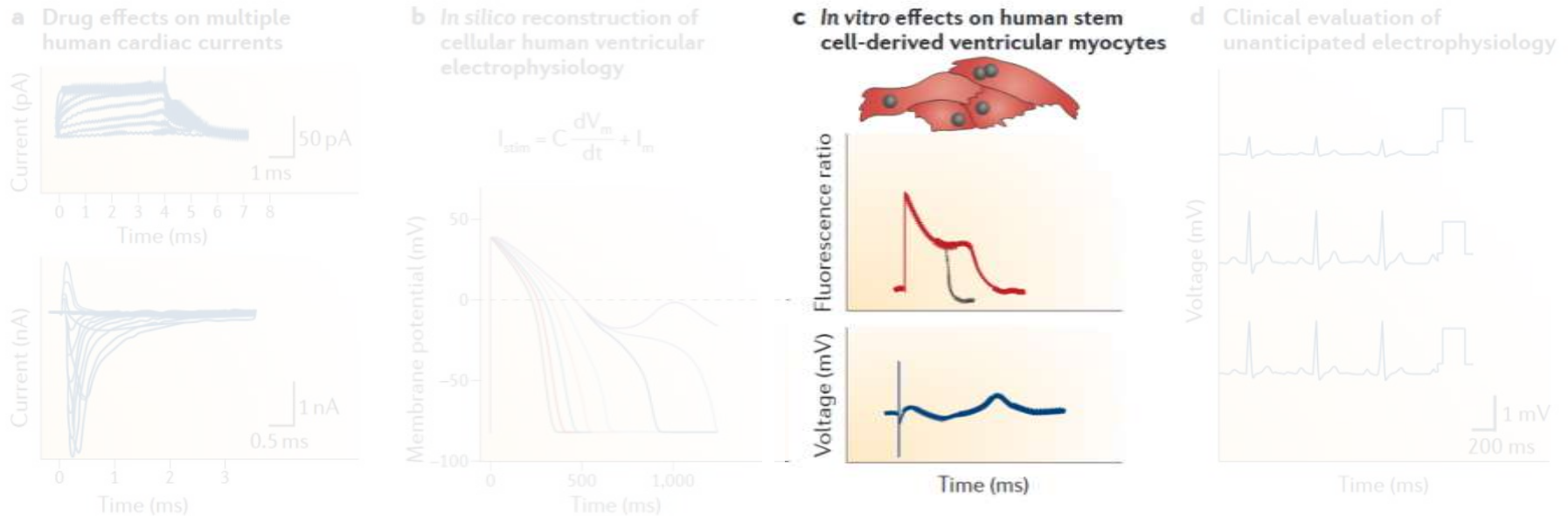
Gintant et al. *Nat Rev Drug Discov.* 2016 Jul;15(7):457-71

Goal: Develop a new *in vitro* paradigm for cardiac safety evaluation of new drugs that provides a more accurate and comprehensive mechanistic-based assessment of proarrhythmic potential

Induced Pluripotent Stem Cells (iPSC)



Comprehensive *in Vitro* Proarrhythmia Assessment (CiPA)



Role in CiPA: to identify potential gaps in cellular electrophysiologic effects, not detected from ionic current/*in silico* reconstructions that may impact TdP risk assessment

Proof of Concept Study at FDA

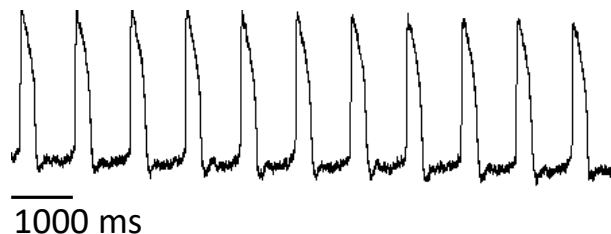


- 26 drugs (blinded)
- 2 commercially-available stem cell lines
 - iCell cardiomyocytes (Cellular Dynamics)
 - Cor.4U cardiomyocytes (Axiogenesis)
- 2 plate-based platforms selected for CiPA myocyte studies:
 - Microelectrode arrays (MEA)
 - Voltage-sensitive dyes (VSD)
- Acute (30 min) and chronic (72 h) drug effects
- 4 ion currents effects with manual patch clamp
 - hERG potassium, sodium and calcium

Optical imaging of stem cell derived cardiomyocytes

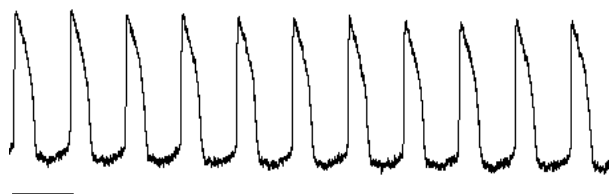


Baseline action potential

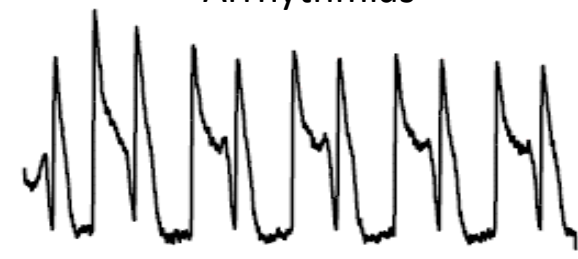


Drug addition

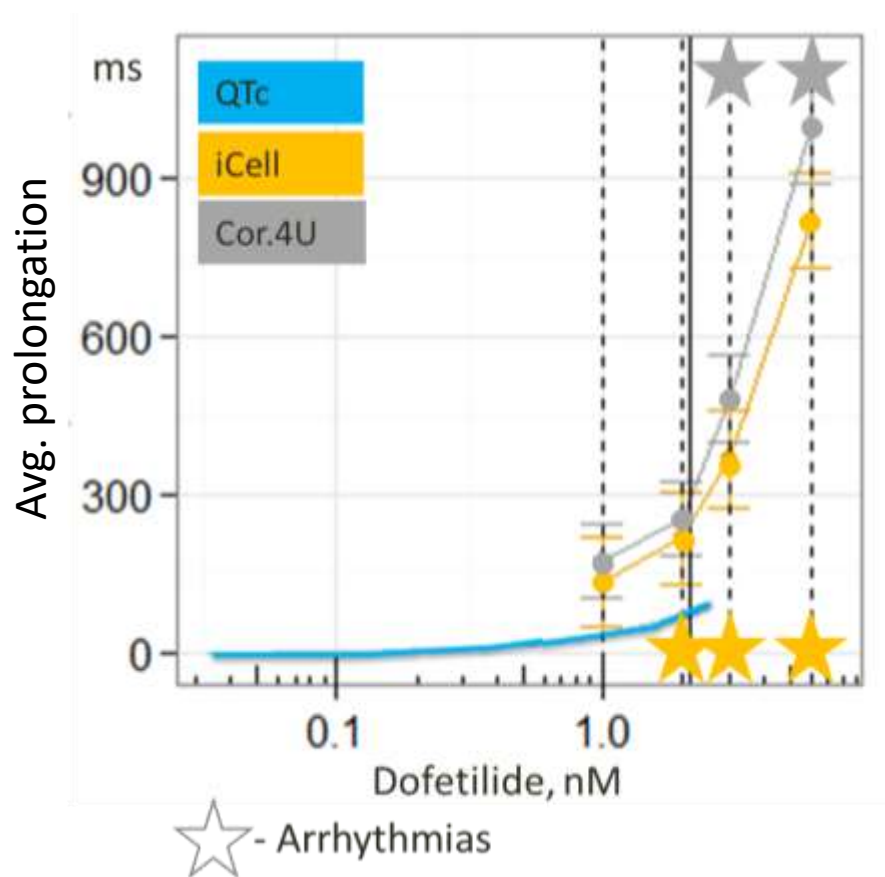
Action potential prolongation



Arrhythmias



Comparison to clinical data



FDA Study Drugs



QT prolongation (20)

Quinidine	Amiodarone
Dofetilide	Azithromycin
Quinine	Sertindole
Propafenone	Pentamidine
Moxifloxacin	Ranolazine
Chloroquine	Ritonavir
Bepridil	Amitriptyline
Chlorpromazine	Nilotinib
Terfenadine	Toremifene
Cisapride	Cibenzoline

TdP
risk
(14)

No arrhythmia risk (6)

Lidocaine
Mexiletine
Licarbazepine
Diltiazem
Verapamil
Mibefradil

iPSC-CMs Results in FDA Study



QT prolongation

<i>Quinidine</i>	<i>Amiodarone</i>
<i>Dofetilide</i>	Azithromycin
<i>Quinine</i>	<i>Sertindole</i>
<i>Propafenone</i>	<i>Pentamidine</i>
<i>Moxifloxacin</i>	<i>Ranolazine</i>
<i>Chloroquine</i>	<i>Ritonavir</i>
<i>Bepridil</i>	Amitriptyline
<i>Chlorpromazine</i>	<i>Nilotinib</i>
<i>Terfenadine</i>	Toremifene
<i>Cisapride</i>	<i>Cibenzoline</i>

TdP
risk

No arrhythmia risk (6)

Lidocaine
Mexiletine
Licarbazepine
Diltiazem
Verapamil
Mibefradil

Of 20 drugs associated with QT↑ in iPSC-CMs induced repolarization↑
- **17 in VSD platform (bold)** and - *16 in MEA platform (italic)*

iPSC-CMs Results in FDA Study



QT prolongation

<i>Quinidine</i>	<i>Amiodarone</i>
<i>Dofetilide</i>	Azithromycin
<i>Quinine</i>	<i>Sertindole</i>
<i>Propafenone</i>	<i>Pentamidine</i>
<i>Moxifloxacin</i>	<i>Ranolazine</i>
<i>Chloroquine</i>	<i>Ritonavir</i>
<i>Bepridil</i>	Amitriptyline
<i>Chlorpromazine</i>	<i>Nilotinib</i>
<i>Terfenadine</i>	Toremifene
<i>Cisapride</i>	<i>Cibenzoline</i>

TdP
risk

No arrhythmia risk (6)

Lidocaine
Mexiletine
Licarbazepine
Diltiazem
Verapamil
Mibefradil

Of 14 drugs associated with TdP, induced arrhythmias in iPSC-CMs
(any device) 10 (**red**)

iPSC-CMs Results in FDA Study



QT prolongation

<i>Quinidine</i>	<i>Amiodarone</i>
<i>Dofetilide</i>	Azithromycin
<i>Quinine</i>	<i>Sertindole</i>
<i>Propafenone</i>	<i>Pentamidine</i>
<i>Moxifloxacin</i>	<i>Ranolazine</i>
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TdP
risk

No arrhythmia risk (6)

Lidocaine
Mexiletine
Licarbazepine
Diltiazem
Verapamil
Mibefradil

No repolarization prolongation was observed in iPSC-CMs with any of the 6 drugs with no arrhythmia risk on the label

FDA Study Results



SOT | Society of
Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 155(1), 2017, 234–247

doi: 10.1093/toxsci/kfw200

Advance Access Publication Date: October 3, 2016

Research article

Comprehensive Translational Assessment of Human-Induced Pluripotent Stem Cell Derived Cardiomyocytes for Evaluating Drug-Induced Arrhythmias

Ksenia Blinova,^{*,1} Jayna Stohlman,^{*} Jose Vicente,^{*,†,‡} Dulciana Chan,^{*} Lars Johannesen,^{*} Maria P. Hortigon-Vinagre,^{§,¶} Victor Zamora,^{§,¶} Godfrey Smith,^{§,¶} William J. Crumb,^{||} Li Pang,^{|||} Beverly Lyn-Cook,^{|||} James Ross,^{|||} Mathew Brock,^{|||} Stacie Chvatal,^{|||} Daniel Millard,^{|||} Lorian Galeotti,^{*} Norman Stockbridge,[†] and David G. Strauss^{*,#}

Overall, iPSC-CMs assay demonstrated 100% specificity, 79% sensitivity for Cor.4U cardiomyocytes on both platforms, 63% for iCells on VSD, and 47% for iCells on MEA platform

This is one site study – need to assay site-to-site variability

CiPA myocyte working group



- Led by health and environmental sciences institute (HESI)
- 10 core sites, 20+ non-core from US, Europe and Japan
- 5 devices (MEA, VSO) ; 2 iPSC-CMs providers



LSI Medience Corporation



CiPA Validation Study Drugs



High TdP Risk	Intermediate Risk	Low/No risk
Dofetilide Quinidine D,l Sotalol Bepridil Ibutilide Vandetanib Azimilide Disopyramide	Ondansetron Chlorpromazine Cisapride Terfenadine Droperidol Domperidone Pimozide Clozapine Clarithromycin Risperidone Astemizole	Mexiletine Diltiazem Ranolazine Verapamil Loratadine Metoprolol Nifedipine Nitrendipine Tamoxifen
8	11	9

CiPA myocyte studies



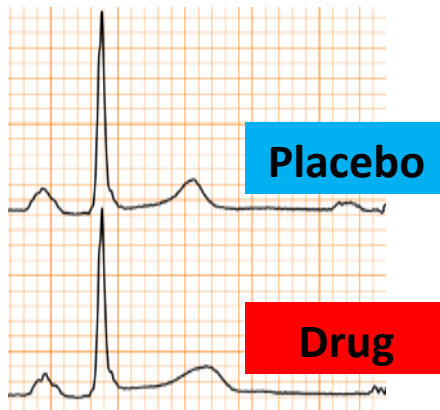
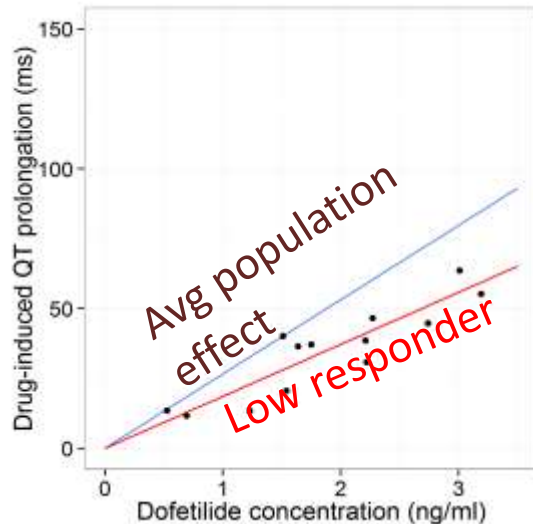
- Pilot multisite study with 8 drugs completed, manuscript in preparation
- Phase II validation study with all 28 CiPA drugs is completed by core sites, data submitted and unblinded (Apr 2017)
- Statistical analysis of the data/model development is ongoing at FDA
- Results will be compared with prospective *in silico* reconstructions and categories of high-, intermediate- and low-risk of torsade de points
- Role of myocytes in regulatory pro-arrhythmia risk assessment will be defined

Beyond CiPA: stem cells in personalized medicine

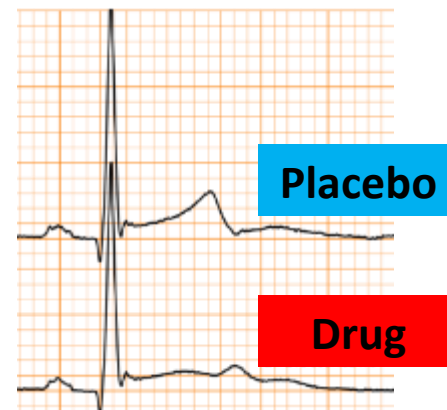
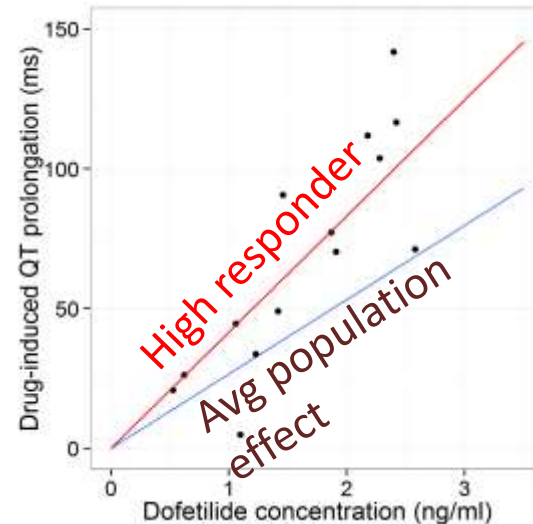
Personalized Drug Response in FDA clinical trial



Subject 1 – “Low responder”



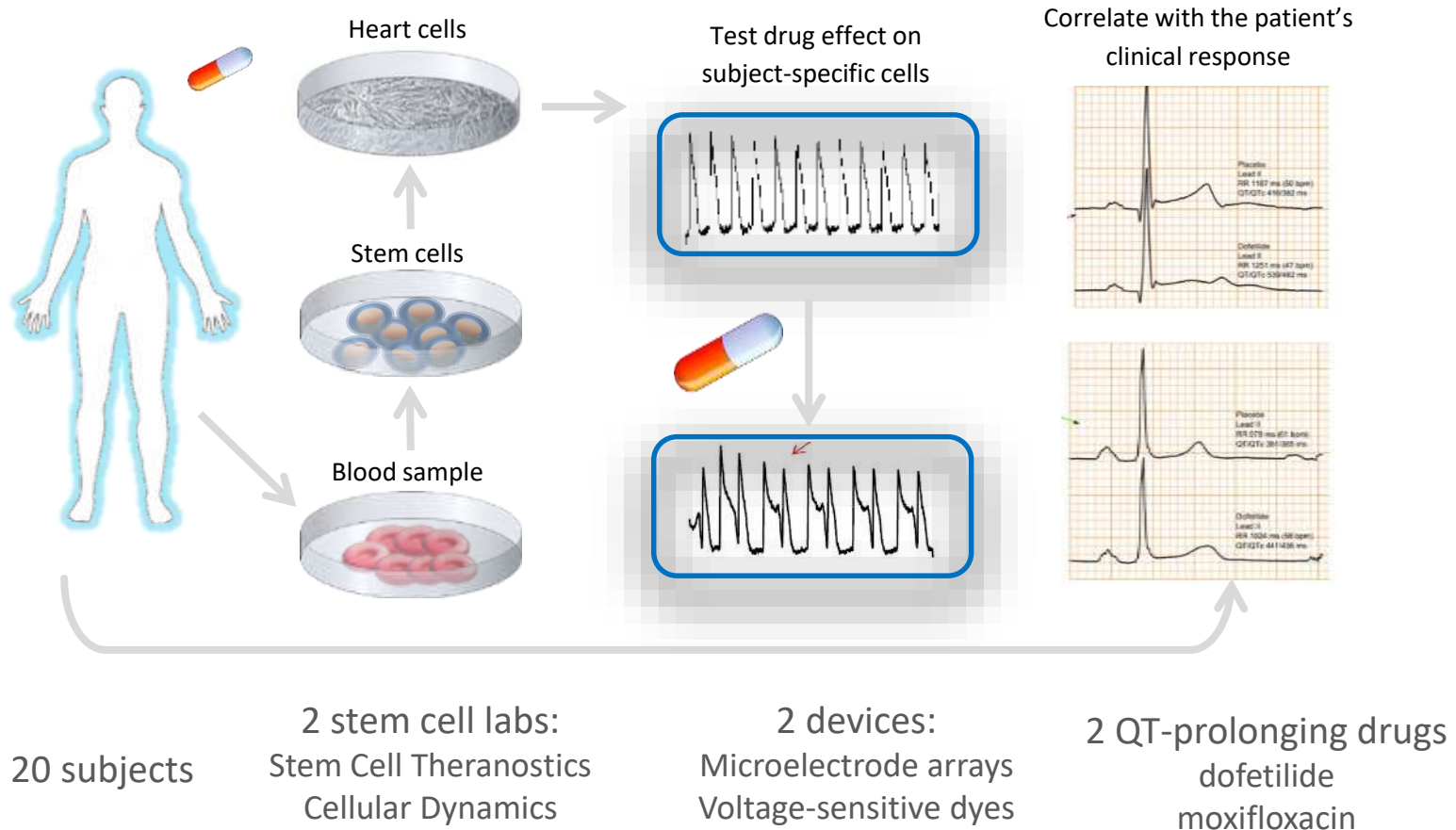
Subject 2 – “High responder”



Ongoing FDA study



Can individual drug response be predicted using subject-specific stem cells?



Published studies on subject-specific iPSC-CMs



1. Stillitano et al. eLife 2017;6:e19406.

Modeling susceptibility to drug-induced long QT with a panel of subject-specific induced pluripotent stem cells

2. Burrige et al. Nat Med. 2016 May;22(5):547-56

Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity.

Results from FDA study on subject-specific iPSC-CMs will be published soon

Key Takeaways

- FDA regulatory research supports review of emerging and innovative products, including induced pluripotent stem cells
- FDA develops new alternative approaches to measuring electrophysiology in these cells
- Pro-arrhythmic assessment of new drugs may soon become more accurate under CiPA
- Stem cell potential to predict individual clinical response is currently being assessed at FDA

Acknowledgements



CDRH / CDER / NCTR

- David Strauss
- Victor Krauthamer
- Jayna Stohlman
- Derek Schocken
- Dakshesh Patel
- Lars Johannesen
- Jose Vicente
- Li Pang
- Matt Whites
- Zhihua Li
- Wendy Wu
- Alexandre Ribeiro
- Christine Garnett
- Norman Stockbridge

Axion Biosystems

- James Ross
- Matt Brock
- Daniel Millard
- Stacie Chvatal
- Tom O'Brian

Clyde Biosciences

- Godfrey Smith
- Maria Hortigon
- Victor Zamora
- Margaret Craig

Zenas Technologies

- William Crumb

Stanford University

- Joseph Wu

Stem Cell Theranostics

- Chris Armstrong
- Nirupama Pike
- Jason Lam

Axiogenesis

- Greg Luerman
- Ralf Kettenhofen
- Brian Murphy

Cellular Dynamics

- Blake Anson
- Kyle Kolaja
- Steve Fiene

CiPA myocyte team

- Gary Gintant
- Jennifer Pierson

Funding Support

FDA Chief Scientist
Challenge Grant
FDA Critical Path Initiative
FDA Office of Women's
Health Grant
FDA CDER
FDA CDRH



2017 BMES/FDA

Frontiers in Medical Devices Conference

**Innovations in Modeling and Simulation:
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The College Park Marriott Hotel and
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The Biomedical Engineering Society and the US Food and Drug Administration have formed a partnership to co-host the BMES/FDA Frontiers in Medical Devices Conference, a meeting for researchers, engineers, clinicians and other professionals in the fields of designing, building and using medical devices.

Questions

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