Induced Pluripotent Stem Cells: *Predicting a Powerful Future*

Joseph C. Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Professor, Dept of Medicine/Cardiology & Radiology
Email: joewu@stanford.edu

Disclosure Statement of Financial Interest

Within the past 12 months, I or my spouse/partner have had a financial interest/arrangement or affiliation with the organization(s) listed below.

Affiliation/Financial Relationship	Company
• Grant/Research Support	• Gilead, IVIVI, Sanofi, MiRagen
• Consulting Fees/Honoraria	• Novartis, BMS, Merck
• Ownership/Founder	• Stem Cell Theranostics

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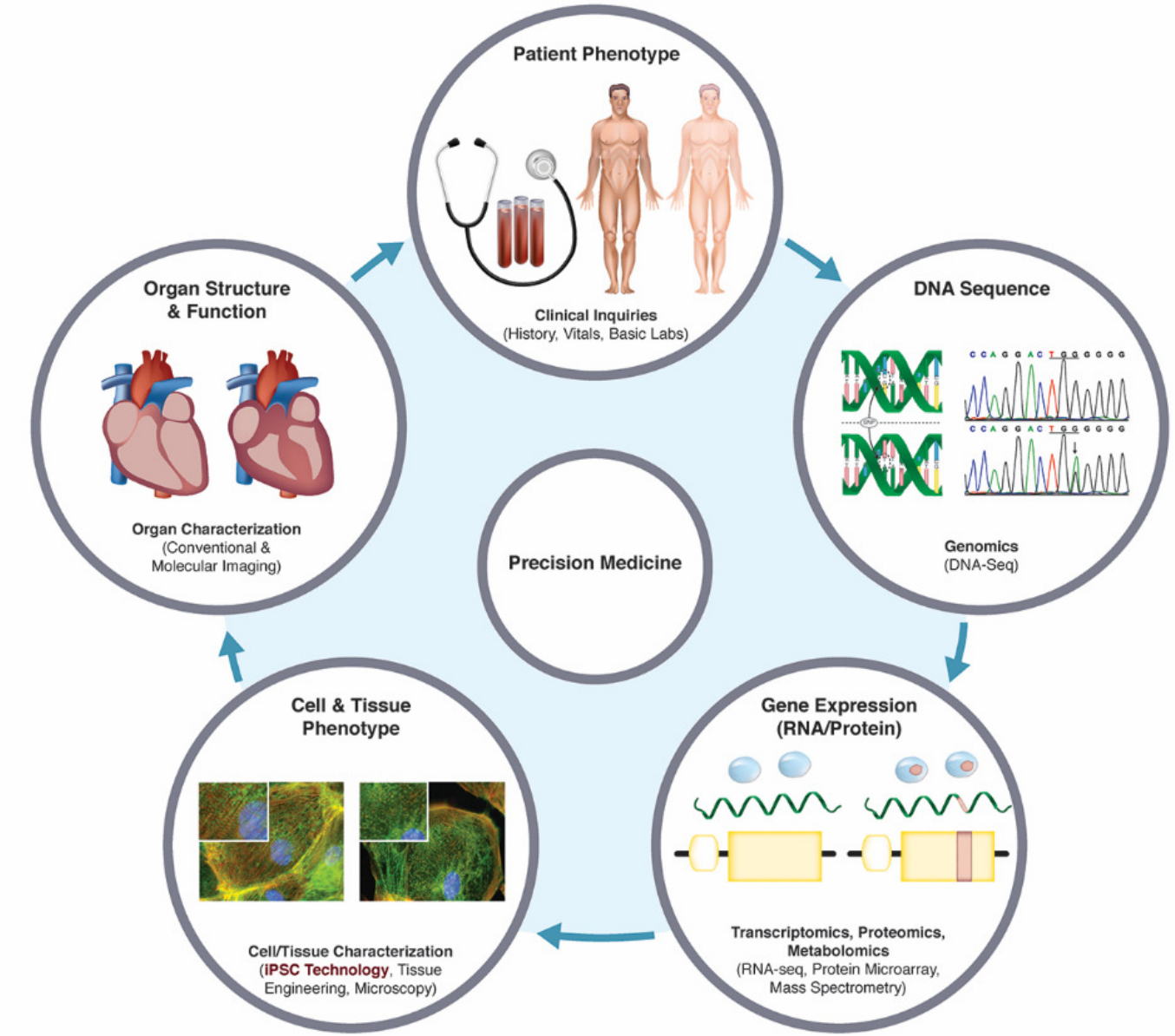
About the Precision Medicine Initiative

Far too many diseases do not have a proven means of prevention or effective treatments. We must gain better insights into the biology of these diseases to make a difference for the millions of Americans who suffer from them. Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. While significant advances in precision medicine have been made for select cancers, the practice is not currently in use for most diseases. Many efforts are underway to help make precision medicine the norm rather than the exception. To accelerate the pace, President Obama unveiled the Precision Medicine Initiative (PMI) — a bold new enterprise to revolutionize medicine and generate the scientific evidence needed to move the concept of precision medicine into every day clinical practice.

http://www.nih.gov/precisionmedicine/

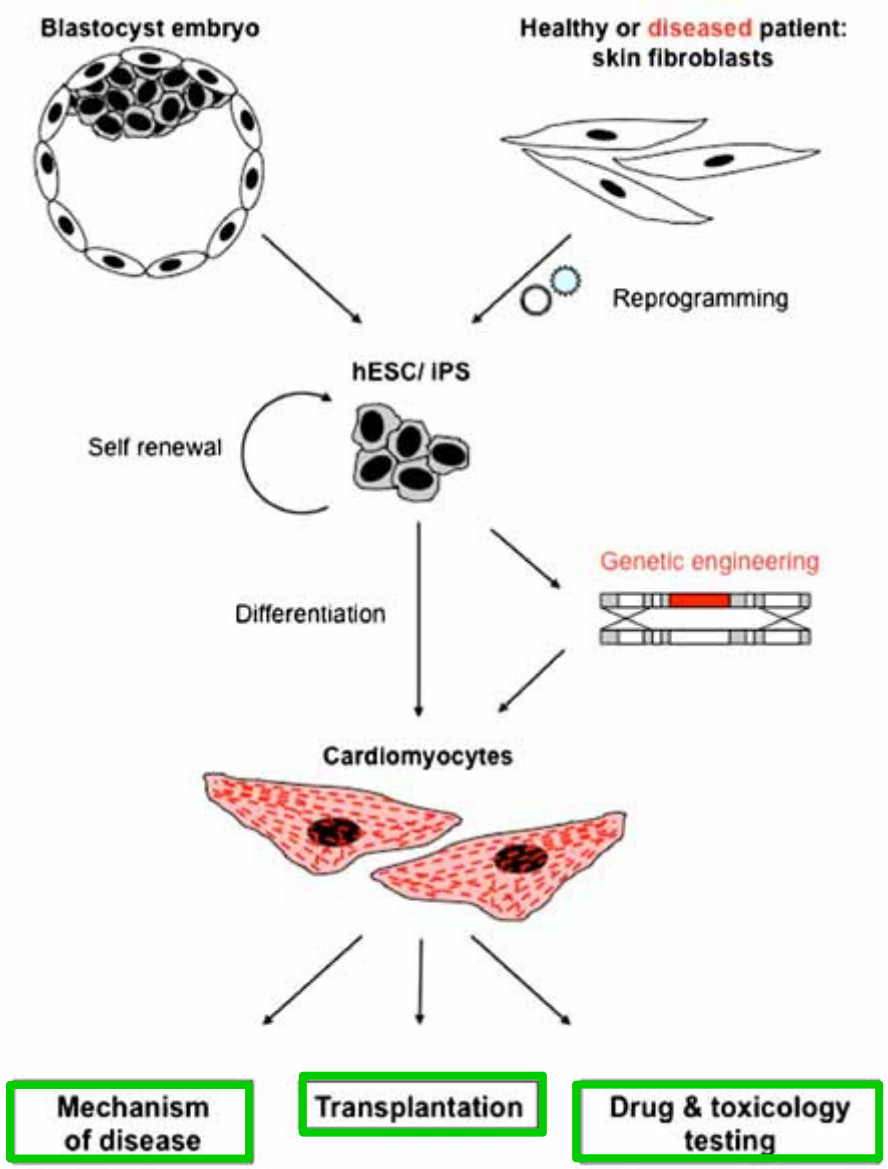
Integral Components of Precision *Cardiovascular* Medicine



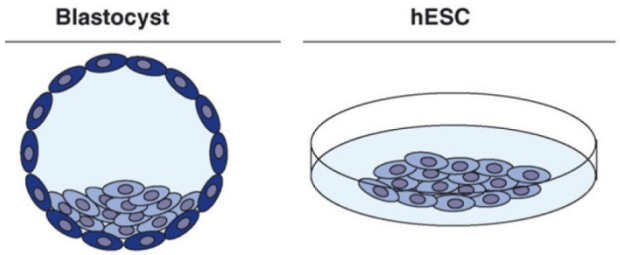
iPS Cells: *Game Changer*

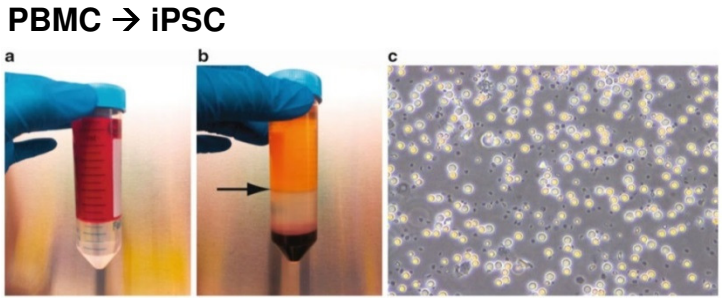
Shinya Yamanaka in Japan created the first induced pluripotent stem cells (iPS cells) from human in **2007**. He received the Nobel Prize in Medicine & Physiology in **2012** for his discovery.

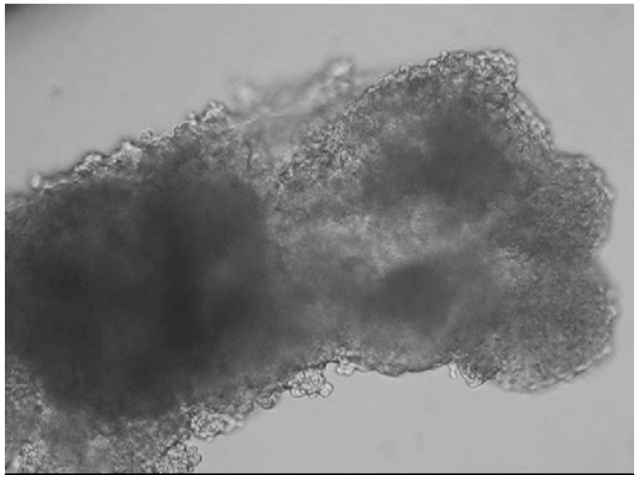
iPS cells can be generated from the patient's blood/skin/fat and then reprogrammed. Once reprogrammed, they are essentially the same as human embryonic stem cells (ie, **"self-renew"** and **"pluripotent"**).

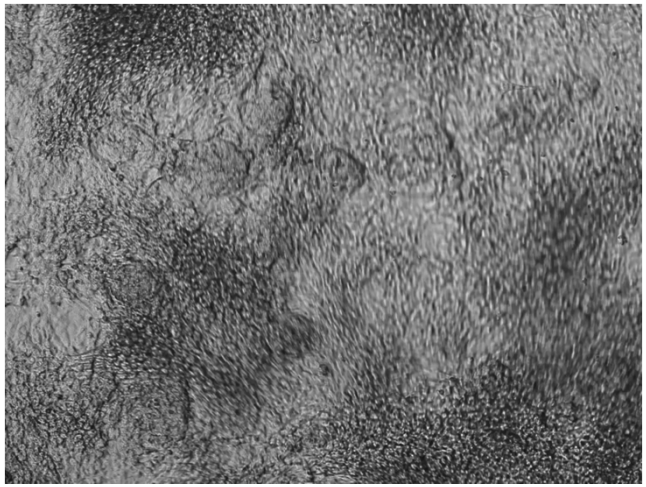


10 Years of Progress: Making Heart Cells *De Novo*







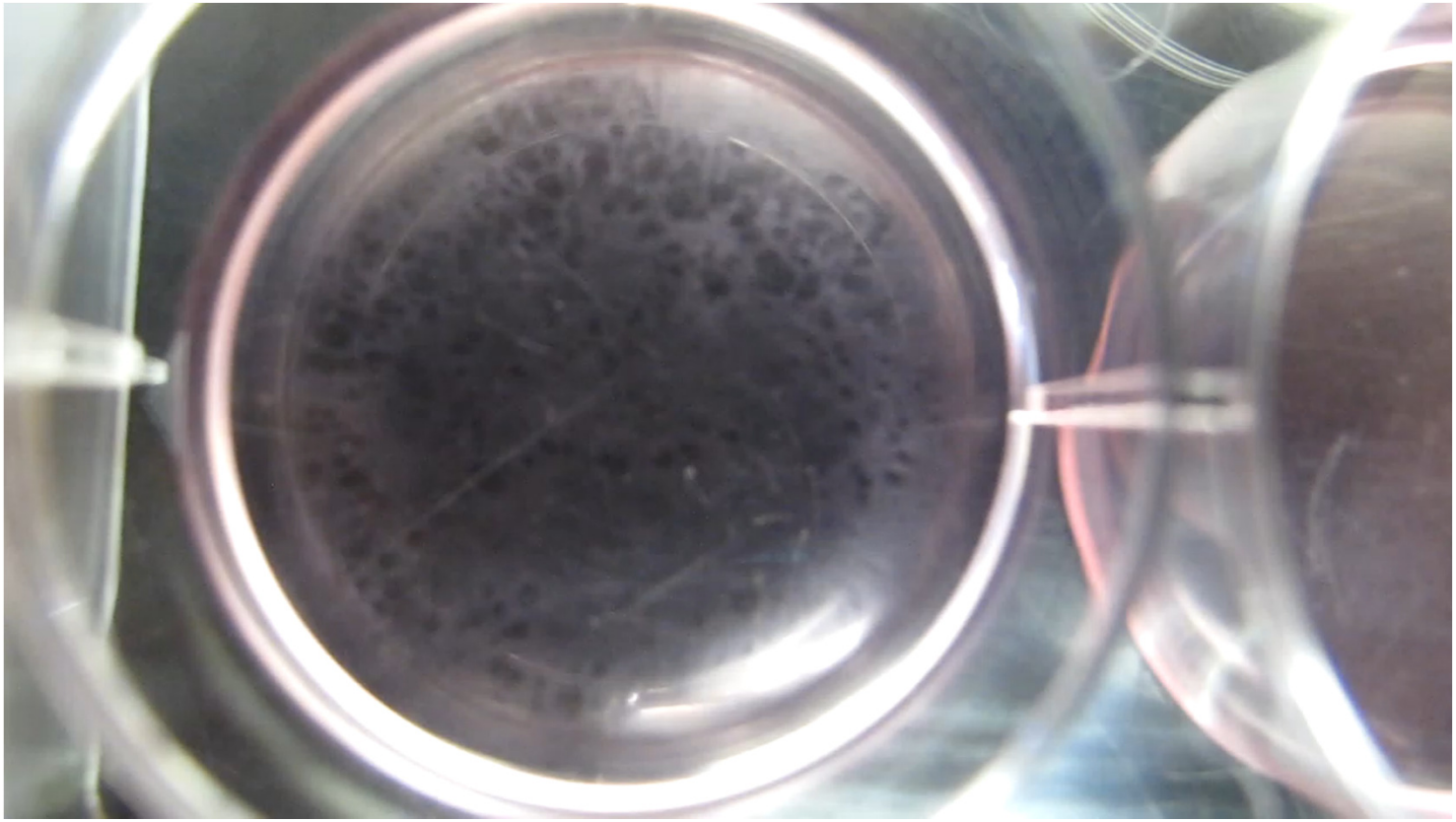


2005 (5% efficiency)

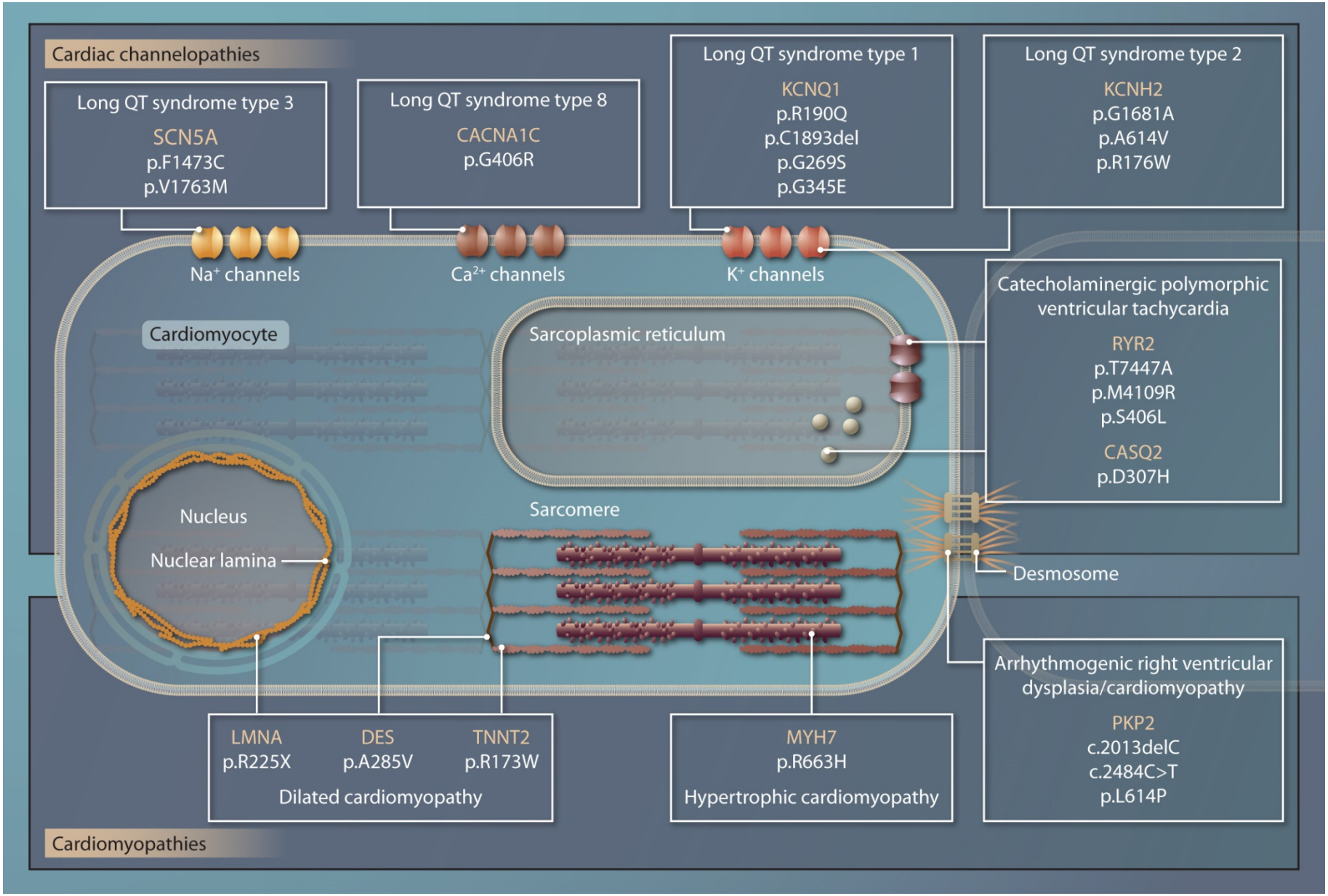
2015 (95% efficiency)

Circulation 2006, Circulation 2007, PNAS 2008, PNAS 2009, Nature Methods 2010, Cell Stem Cell 2011, JCI 2011, Nature Biotech 2011, Cell Stem Cell 2012, Science Transl Med 2012, Nature Medicine 2013, Science Transl Med 2013, Science 2013, JAMA 2013, Nature Comm 2014; Nature Materials 2014, Nature Methods 2014, Circ Res 2014, JACC 2014, Science Transl Med 2014, Circulation 2015, Circ Res 2015, JAMA 2015, Cell Stem Cell 2015

From 10 cc of Your Blood → Convert to Your iPS Cells → Make Millions of Your Beating Heart Cells



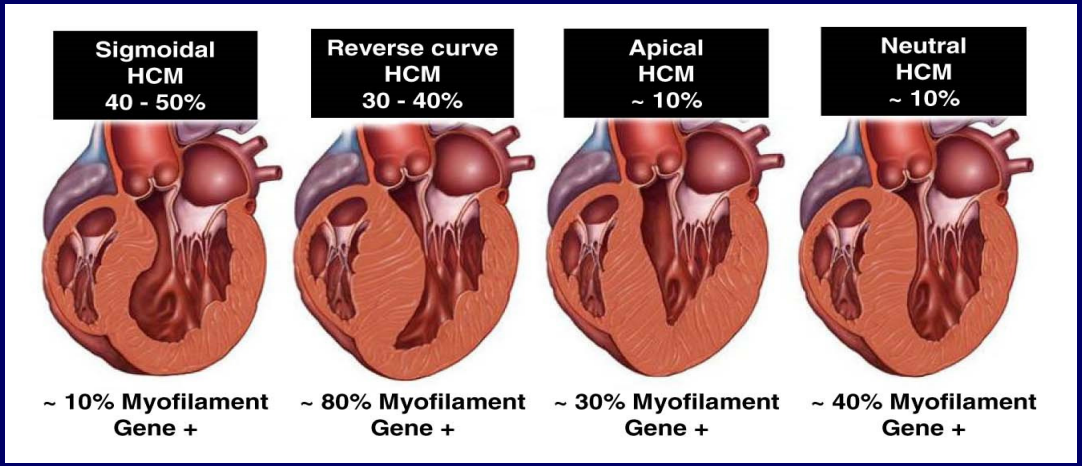
Circulation 2006, Circulation 2007, PNAS 2008, PNAS 2009, Nature Methods 2010, Cell Stem Cell 2011, JCI 2011, Nature Biotech 2011, Cell Stem Cell 2012, Science Transl Med 2012, Nature Medicine 2013, Science Transl Med 2013, Science 2013, JAMA 2013, Nature Comm 2014; Nature Materials 2014, Nature Methods 2014, Circ Res 2014, JACC 2014, Science Transl Med 2014, Circulation 2015, Circ Res 2015, JAMA 2015, Cell Stem Cell 2015

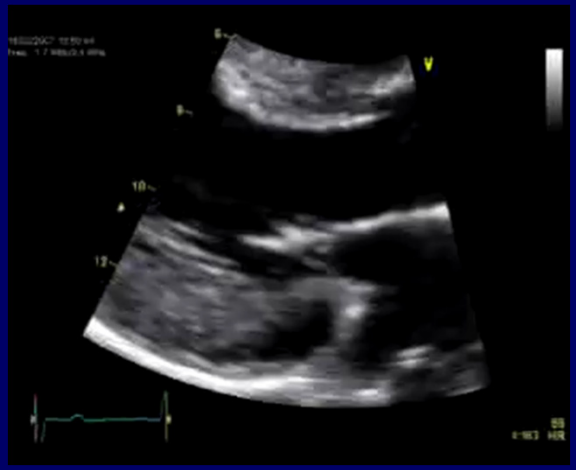


Matsa E, Wu JC. *Sci Transl Med* 2014

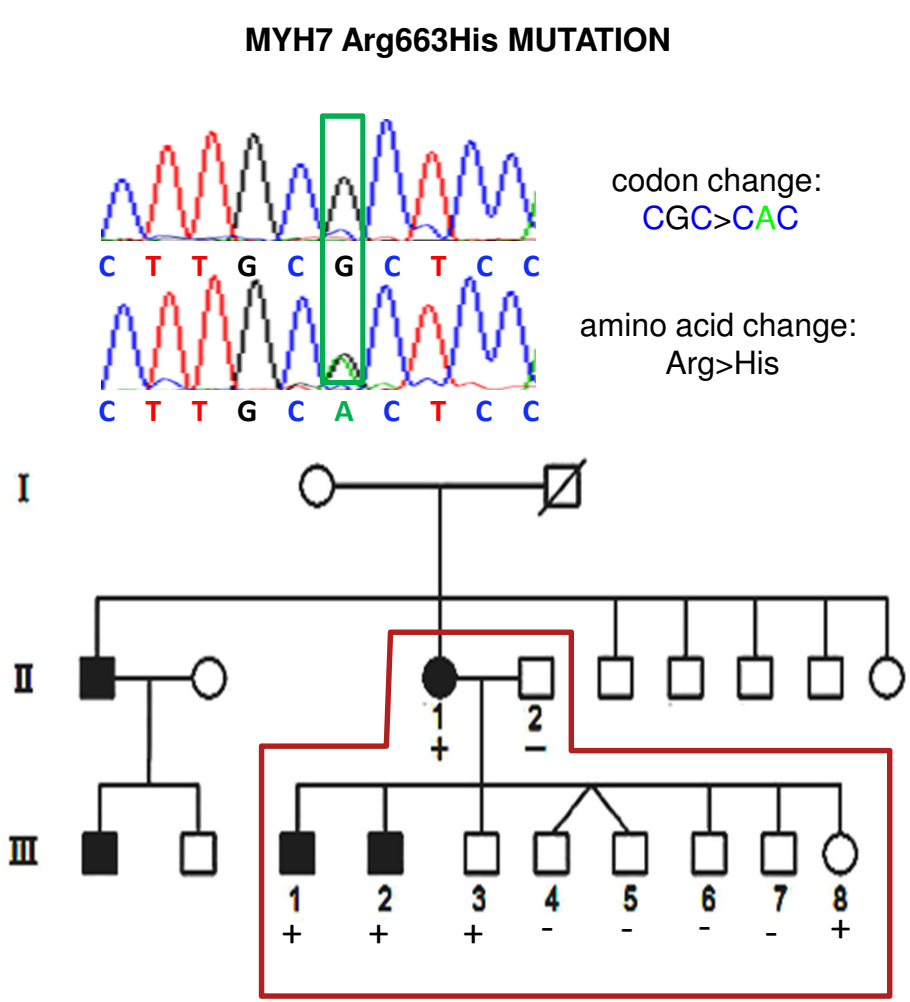
Familial Hypertrophic Cardiomyopathy

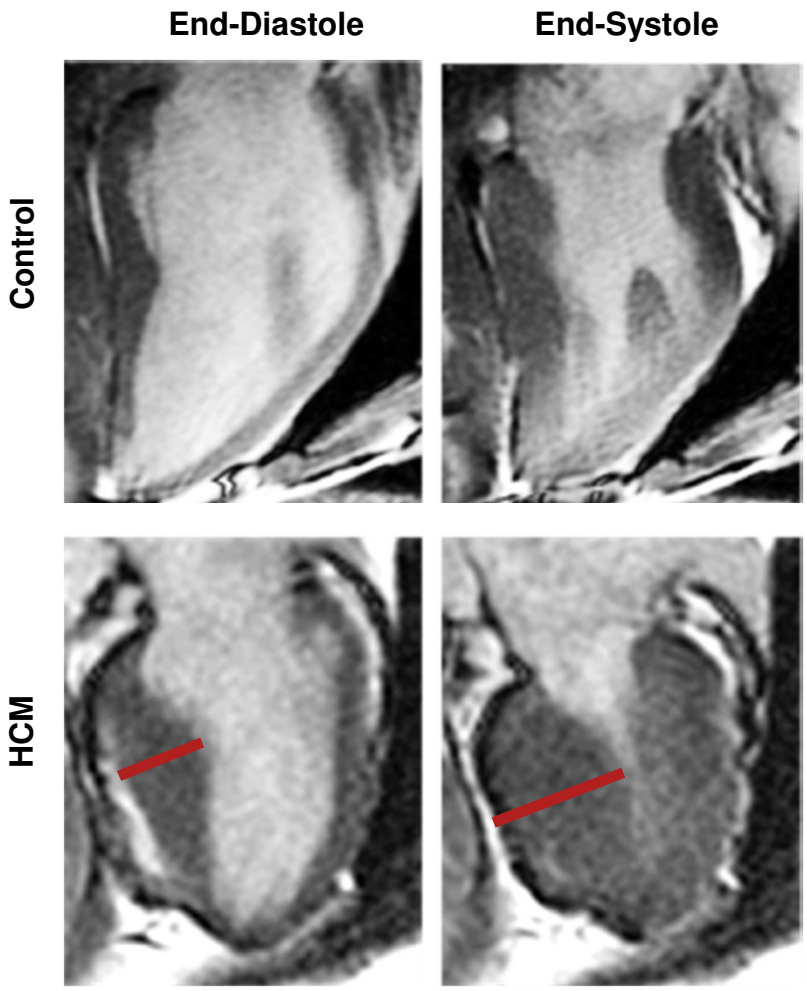
- Most prevalent inherited cardiovascular disease. Affects 1 in 500 people
- One of the most common causes of sudden cardiac death in young adults.
- First gene identified as mutation in MYH7 in 1989. Since then, >1000 mutations identified.



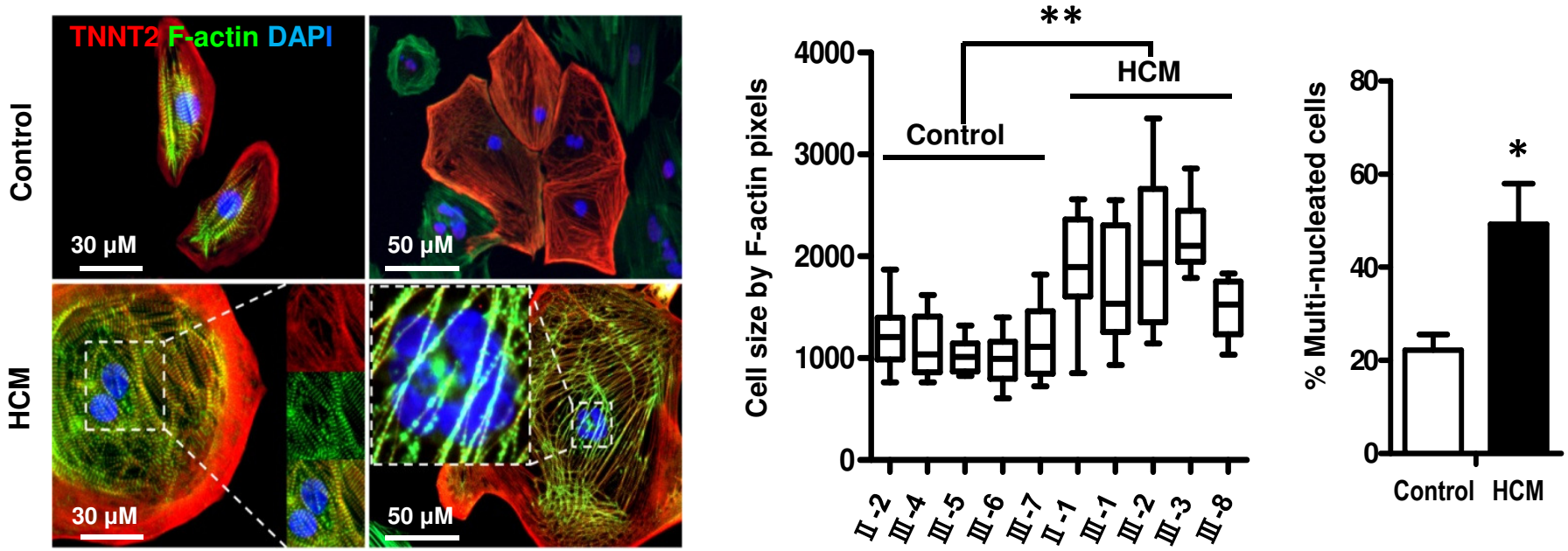


Generation of Disease-Specific iPSCs from Large Family Cohort with HCM Mutation



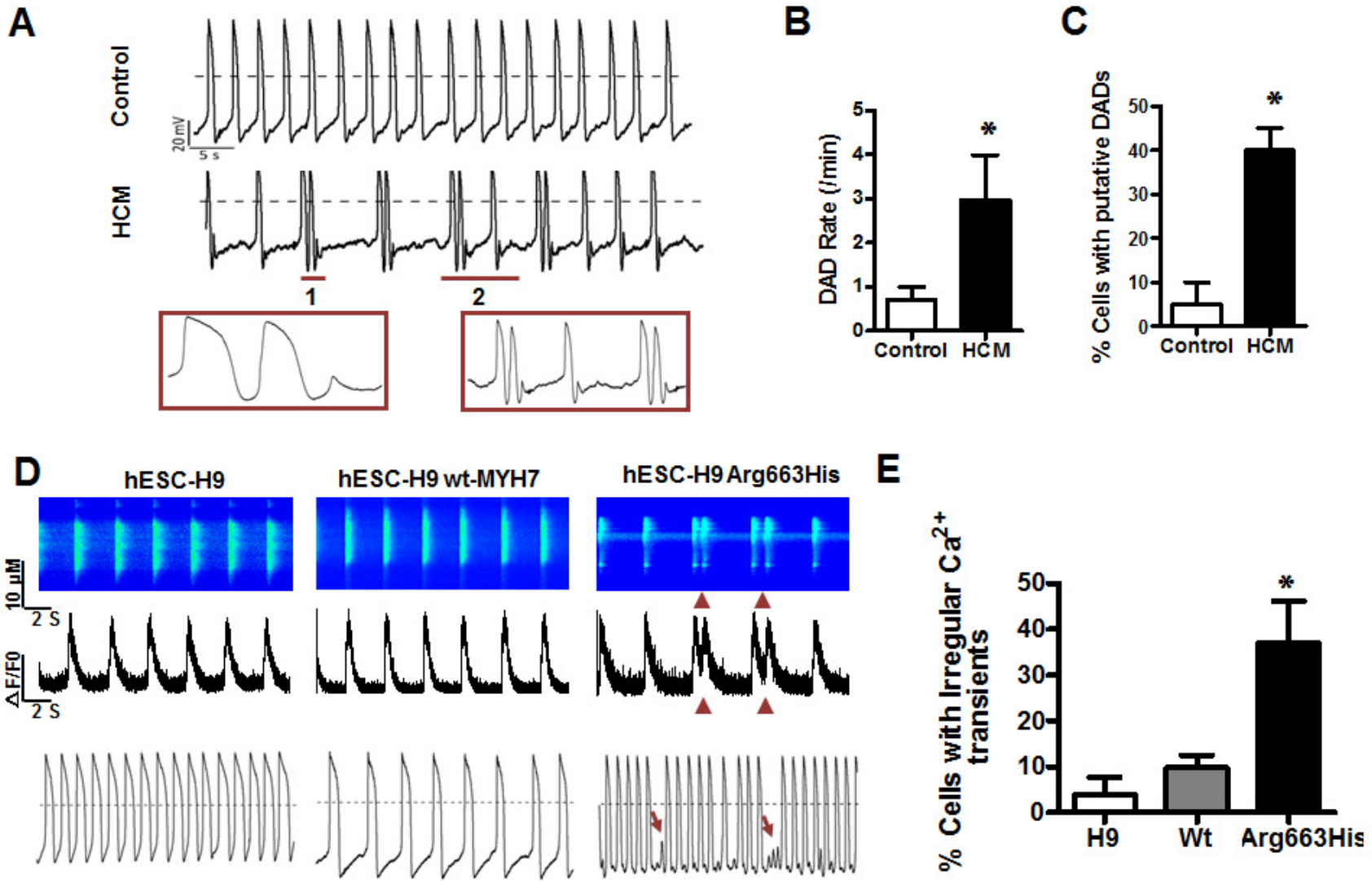


Patient Specific iPSC-CMs Recapitulate Hypertrophic Phenotype in HCM vs. Controls

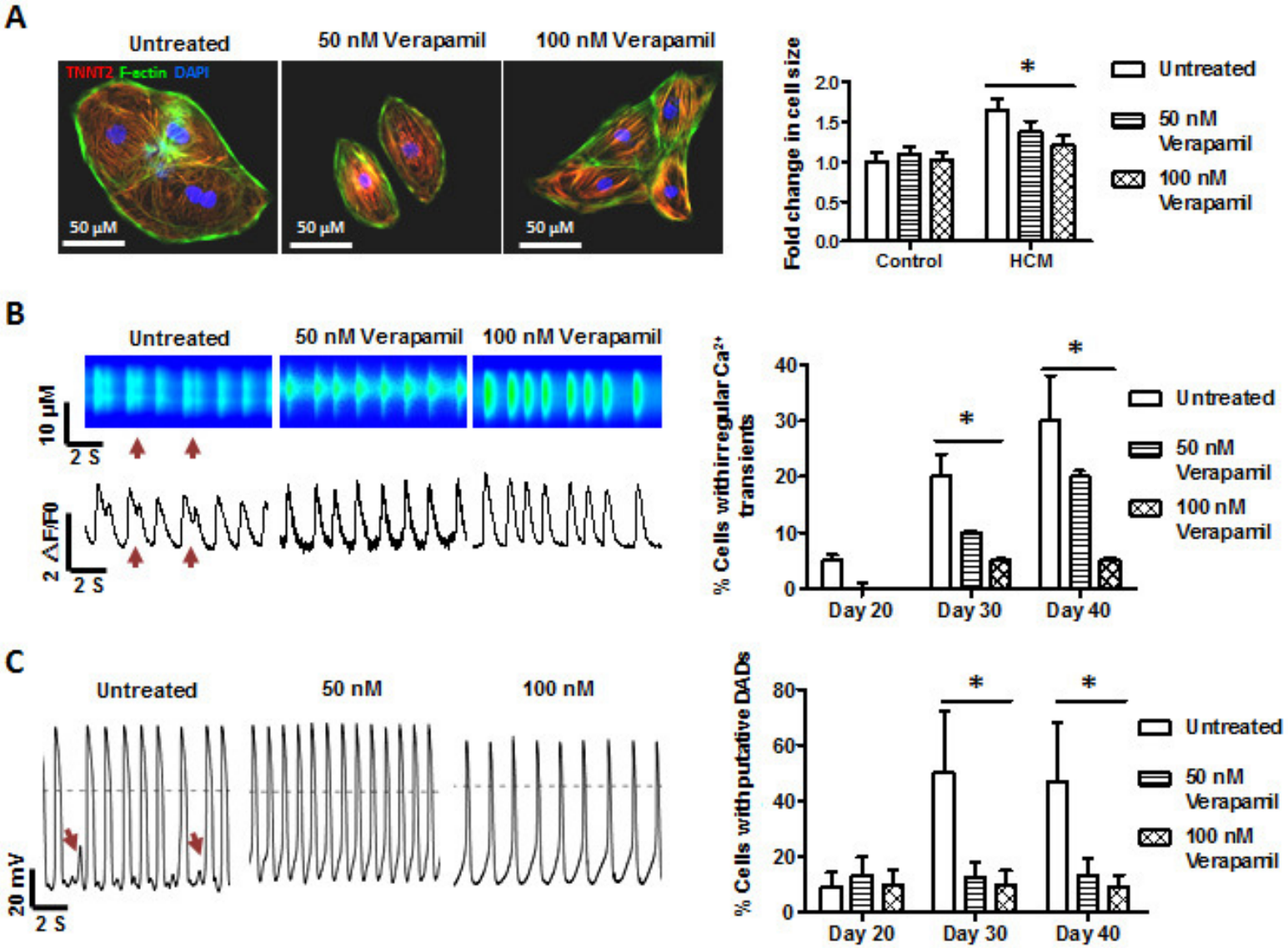


- HCM iPSC-CMs have *larger* cell size and *more* multi-nucleation.
- Diseased HCM iPSC-CMs showed *aggravated* hypertrophic phenotype in response to isoproterenol stimulation.
- HCM iPSC-CMs have activation of the calcineurin pathway. Calcineurin inhibitor Cs-A and FK506 can partially *reverse* the hypertrophic phenotype with or without isoproterenol stimulation.

Overexpression of Mutated MYH7 Causes More Arrhythmias and Irregular Calcium Transients



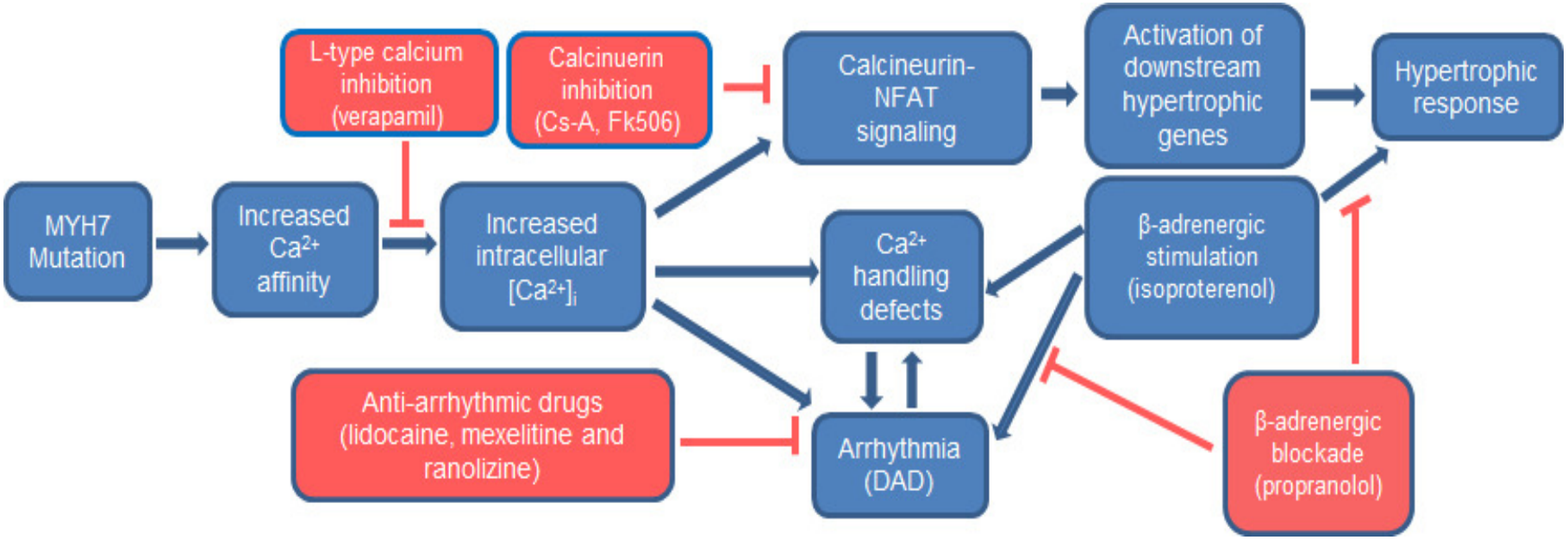
Treatment with CCB Verapamil Blocks Cellular Hypertrophy and Arrhythmias in HCM iPSC-CMs



Abnormal Calcium Handling Properties Underlie Familial Hypertrophic Cardiomyopathy Pathology in Patient-Specific Induced Pluripotent Stem Cells

Feng Lan,[1,2,3,12] Andrew S. Lee,[1,2,3,12] Ping Liang,[1,2,3,12] Veronica Sanchez-Freire,[1,2,3] Patricia K. Nguyen,[1] Li Wang,[1,2] Leng Han,[1,2] Michelle Yen,[4] Yongming Wang,[1,2,3] Ning Sun,[1,2] Oscar J. Abilez,[5] Shijun Hu,[1,2,3] Antje D. Ebert,[1,2,3] Enrique G. Navarrete,[2] Chelsey S. Simmons,[9] Matthew Wheeler,[1] Beth Pruitt,[9] Richard Lewis,[4] Yoshinori Yamaguchi,[10] Euan A. Ashley,[1] Donald M. Bers,[11] Robert C. Robbins,[2,6] Michael T. Longaker,[3,8] and Joseph C. Wu[1,2,3,7,*]

Cell Stem Cell 2013



Summary: We generated iPSC-CMs from a 10-member family cohort, half carry HCM missense mutation (Arg663His) in MYH7 gene. Patient-specific iPSC-CMs recapitulated numerous characteristics of HCM. Pharmacological treatment with calcium-channel blocker (verapamil), β-blocker (propranolol), and anti-arrhythmic drugs (ranolizine, mexelitine) prevented development of cellular hypertrophy and/or electrophysiological irregularities.

iPSCs to Study Genetic, Acquired and Multifactorial Causes of Heart Disease

Primary	Secondary
Genetic	Autoimmune (systemic lupus)
Arrhythmogenic right ventricular cardiomyopathy	Electrolyte imbalance
Hypertrophic cardiomyopathy	Endocrine (diabetes, hypothyroidism)
Mixed (genetic and nongenetic)	Endomyocardial (fibrosis)
Dilated cardiomyopathy	Infiltrative (amyloidosis, Gaucher disease)
Restrictive cardiomyopathy	Inflammatory (sarcoidosis)
Acquired	Neurologic (neurofibromatosis)
Myocarditis (inflammatory cardiomyopathy)	Nutritional (beriberi)
Peripartum (or postpartum) cardiomyopathy	Radiation
Stress cardiomyopathy	Storage (hemochromatosis)
	Toxic (medications)
	Velocardiofacial syndrome

Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy

Ning Sun,[1,2,3]* Masayuki Yazawa,[4]* Jianwei Liu,[5] Leng Han,[1,2] Veronica Sanchez-Freire,[1,2] Oscar J. Abilez,[6] Enrique G. Navarrete,[2] Shijun Hu,[1,2] Li Wang,[1,2,3] Andrew Lee,[1,2,3] Aleksandra Pavlovic,[1] Shin Lin,[1] Rui Chen,[7] Roger J. Hajjar,[8] Michael P. Snyder,[7] Ricardo E. Dolmetsch,[4] Manish J. Butte,[5] Euan A. Ashley,[1] Michael T. Longaker,[3,9] Robert C. Robbins,[10] Joseph C. Wu[1,2,3,10]†

Sci Transl Med 2012

Chemically defined generation of human cardiomyocytes

Paul W Burridge[1–3], Elena Matsa[1–3], Praveen Shukla[1–3], Ziliang C Lin[4], Jared M Churko[1–3], Antje D Ebert[1–3], Feng Lan[1–3], Sebastian Diecke[1–3], Bruno Huber[1–3], Nicholas M Mordwinkin[1–3], Jordan R Plews[1–3], Oscar J Abilez[1–3], Bianxiao Cui[5], Joseph D Gold[1] & Joseph C Wu[1–3]

Nature Methods 2014

Characterization of the molecular mechanisms underlying increased ischemic damage in the *aldehyde dehydrogenase 2* genetic polymorphism using a human induced pluripotent stem cell model system

Antje D. Ebert,[1,2,3] Kazuki Kodo,[1,2] Ping Liang,[1,2,3] Haodi Wu,[1,2,3] Bruno C. Huber,[1,2] Johannes Riegler,[1,2] Jared Churko,[1,2,4] Jaecheol Lee,[1,2,3] Patricia de Almeida,[1,2] Feng Lan,[1,2,3] Sebastian Diecke,[1,2,3] Paul W. Burridge,[1,2,3] Joseph D. Gold,[1] Daria Mochly-Rosen,[4]* Joseph C. Wu[1,2,3]*

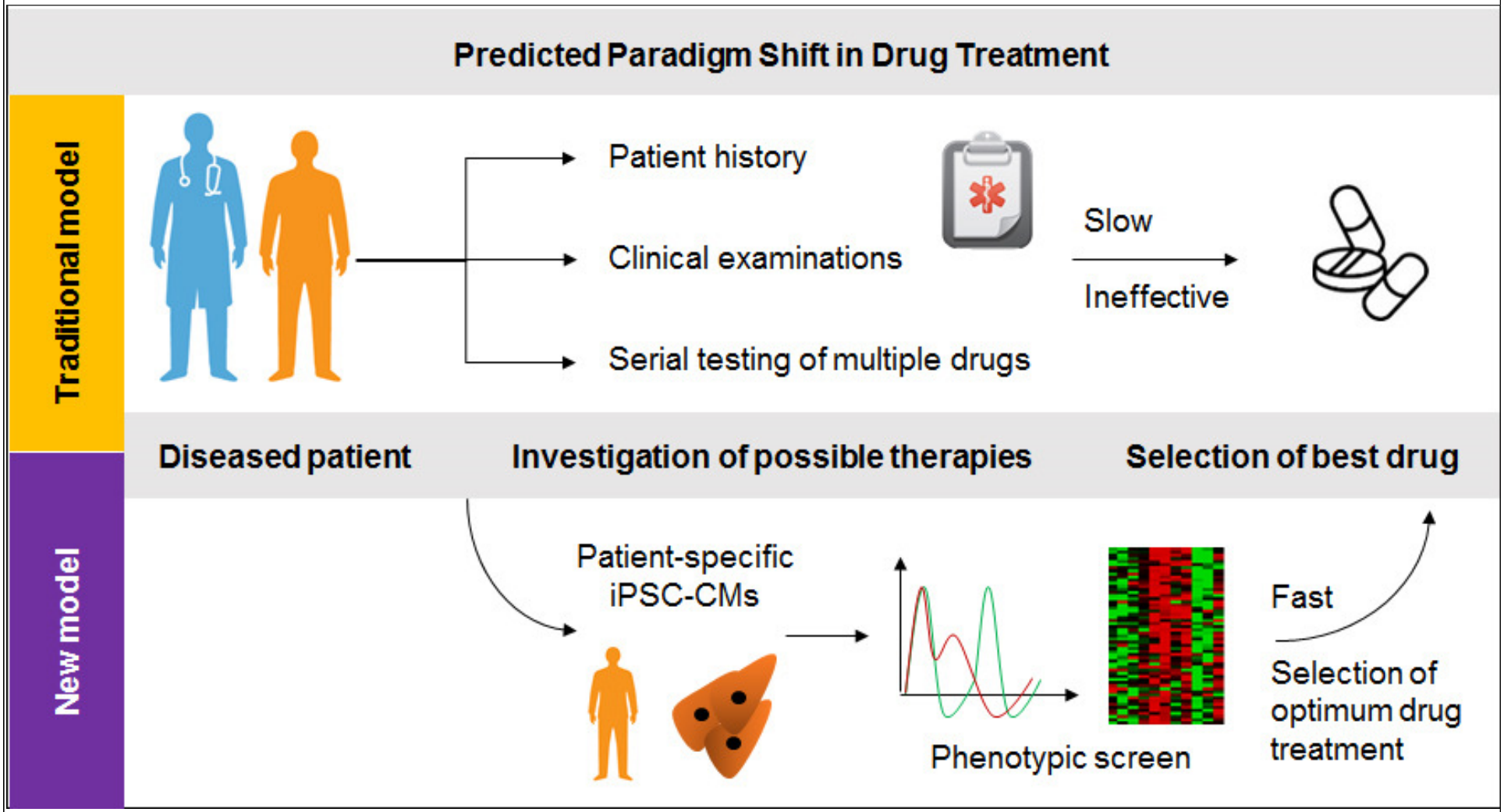
Sci Transl Med 2014

Epigenetic Regulation of Phosphodiesterases 2A and 3A Underlies Compromised β-Adrenergic Signaling in an iPSC Model of Dilated Cardiomyopathy

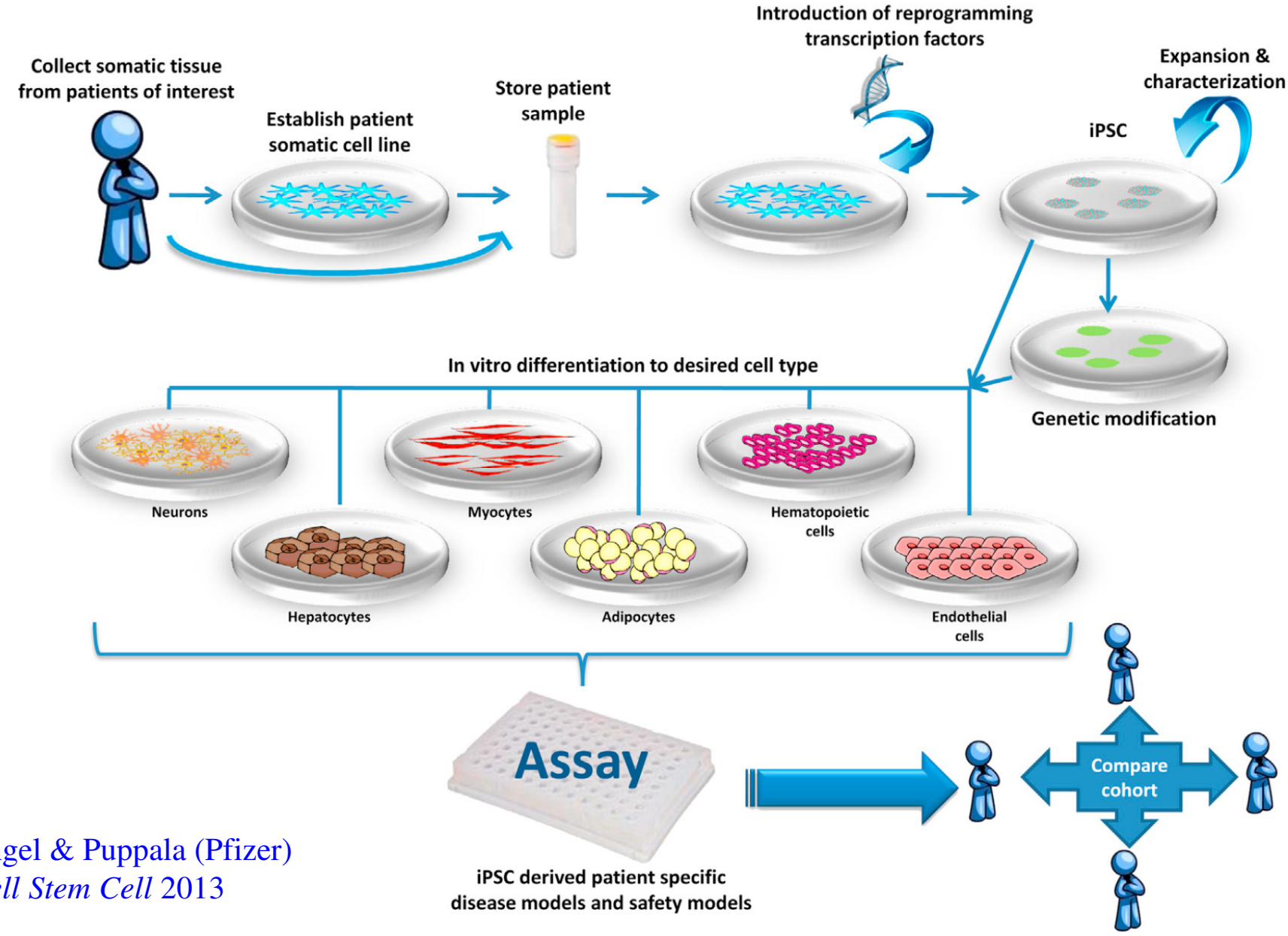
Haodi Wu,[1,2,3] Jaecheol Lee,[1,2,3] Ludovic G. Vincent,[4] Qingtong Wang,[5] Mingxia Gu,[1,2,3] Feng Lan,[1,2,3] Jared M. Churko,[1,2,3] Karim I. Sallam,[1,2,3] Elena Matsa,[1,2,3] Arun Sharma,[1,2,3] Joseph D. Gold,[1] Adam J. Engler,[4,6] Yang K. Xiang,[5] Donald M. Bers,[5] and Joseph C. Wu[1,2,3,*]

Cell Stem Cell 2015

Envisioning iPSC-Based Precision Medicine



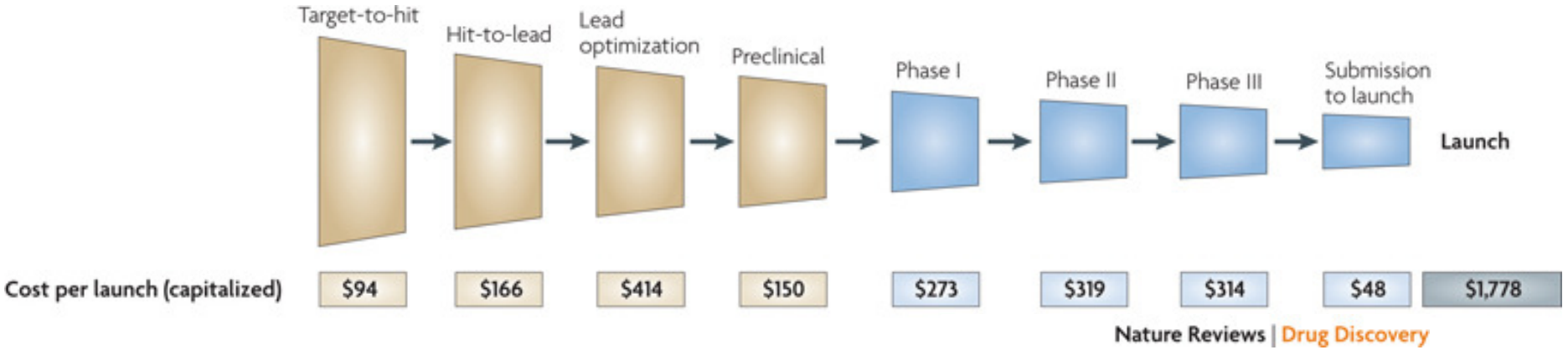
#2 iPSCs to Transform Drug Discovery



Engel & Puppala (Pfizer)
Cell Stem Cell 2013

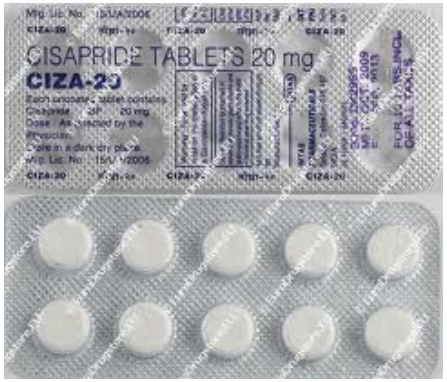
Pharmaceutical Innovation is Endangered

- Looming patent cliff for big pharma
- 5,000-10,000 compounds screened per new single drug
- New drugs require **$1.8 Billion** and **12 Years** on average
- Major reason for inefficiency is due to inaccuracy of preclinical drug discovery assays (ie, drug works in mice, but not in humans)
- Cardiotoxicity is the #1 cause of drug withdrawal post-marketing (~35%)



Paul SM. *Nat Rev Drug Discovery* 2010

Drugs Withdrawn Due to Cardiac Toxicity



Cisapride (Propulsid)

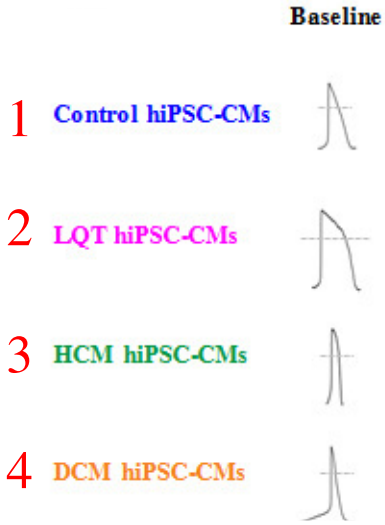
Sibutramine
Propoxyphene
Terfenadine
Rofecoxib
Tegaserod
Flosequinan
Valdecoxib
Encainide
1991
1993
1997
1998
1999
2000
2001
2004
2005
2007
2010
Terodiline
Mibefradil
Sertindole
Prenylamine
Astemizole
Grepafloxacin
Thioridazine
Cisapride
Dofetilide
Droperidol
Levomethadyl
Levacetylmethadol
Pro-arrhythmia
Heart Attack
QT Interval Prolongation and TdP

Drug Screening Using a Library of Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes Reveals Disease-Specific Patterns of Cardiotoxicity

Ping Liang, MD, PhD*; Feng Lan, PhD*; Andrew S. Lee, BS*; Tingyu Gong, MD; Veronica Sanchez-Freire, PhD; Yongming Wang, PhD; Sebastian Diecke, PhD; Karim Sallam, MD; Joshua W. Knowles, MD, PhD; Paul J. Wang, MD; Patricia K. Nguyen, MD; Donald M. Bers, PhD; Robert C. Robbins, MD; Joseph C. Wu, MD, PhD

Circulation 2013
(Best Basic Science Manuscript)

N=12 patients



"Clinical trial in a dish": **Cisapride** (Propulsid, Johnson & Johnson) was used to treat severe heartburn and diabetic gastroparesis. The FDA issued a warning on potential side effect of long QT (>400 reports with 80 deaths). Drug removed from U.S. market in 2000. **~$90M** awarded in class action lawsuits.

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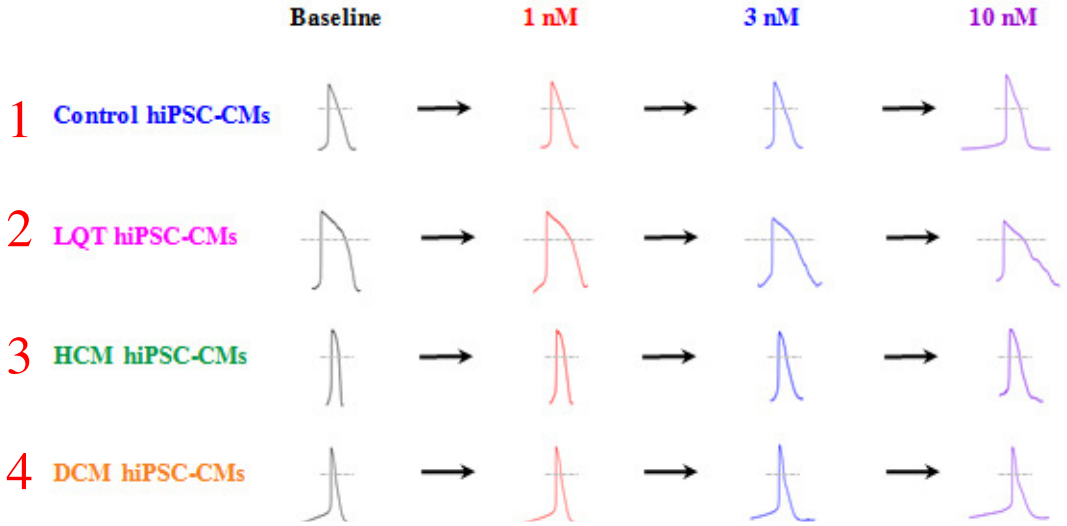

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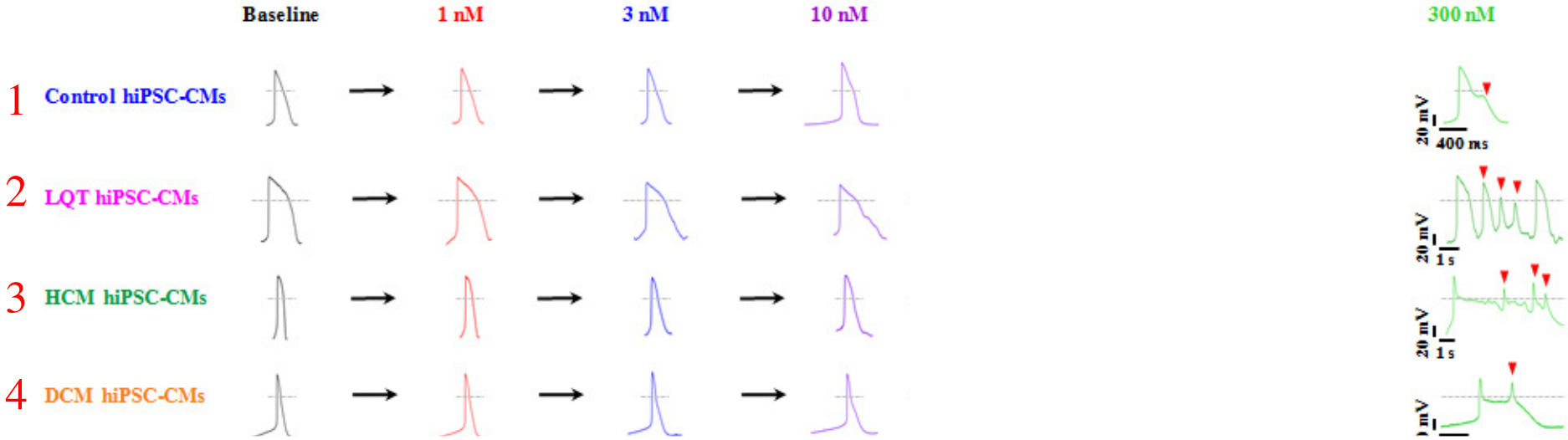

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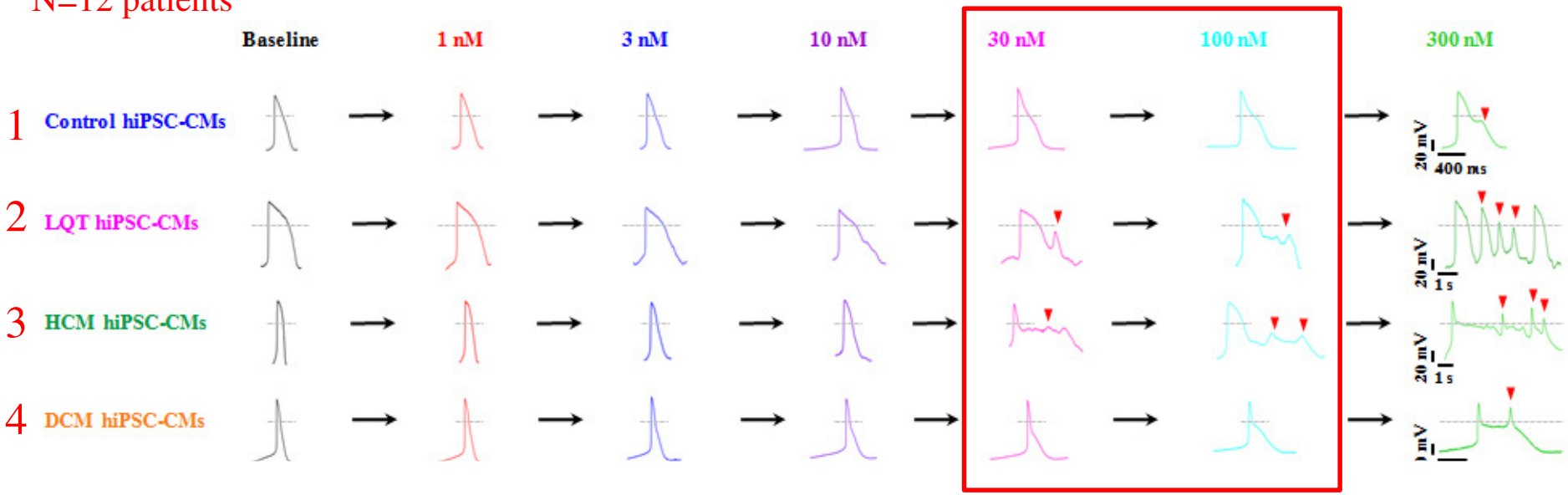
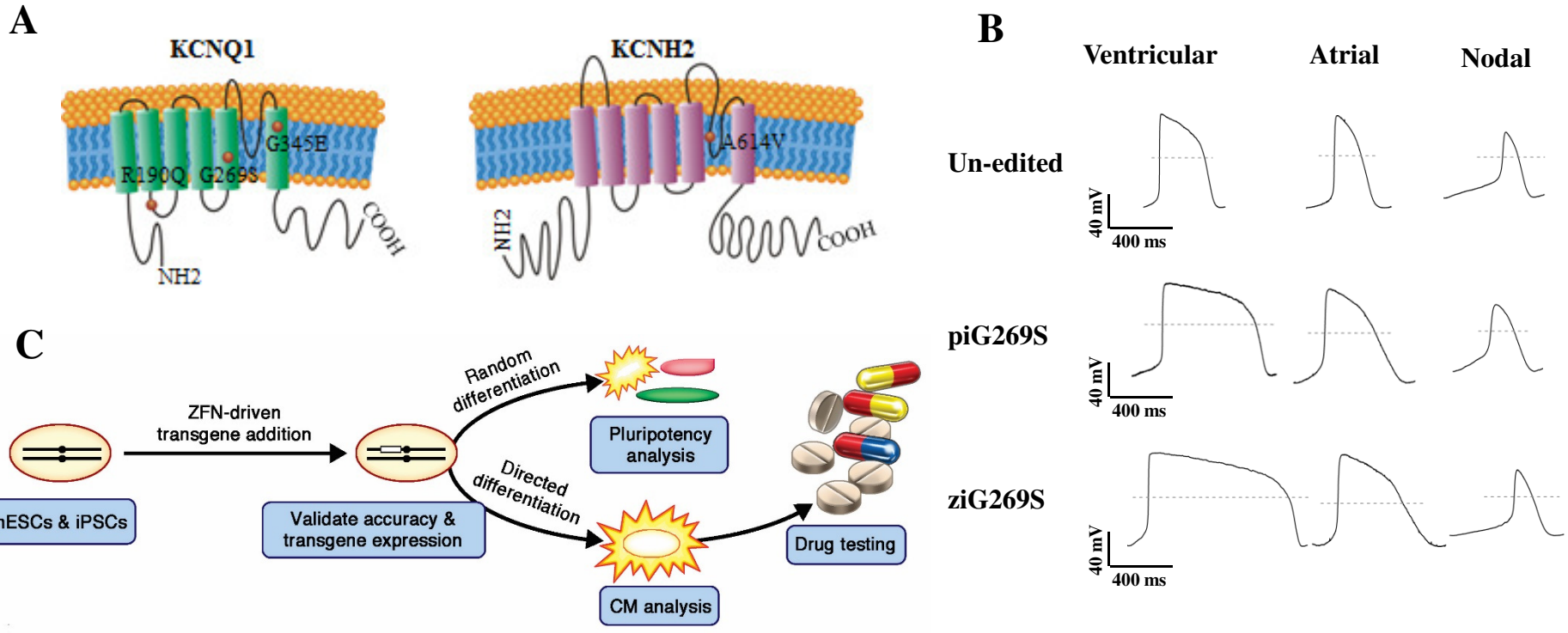


"Clinical trial in a dish": **Cisapride** (Propulsid, Johnson & Johnson) was used to treat severe heartburn and diabetic gastroparesis. The FDA issued a warning on potential side effect of long QT (>400 reports with 80 deaths). Drug removed from U.S. market in 2000. **~$90M** awarded in class action lawsuits. Note: drug *safe* in control & DCM, but *not safe* in LQT and HCM patients.

Genome Editing of Isogenic Human Induced Pluripotent Stem Cells Recapitulates Long QT Phenotype for Drug Testing

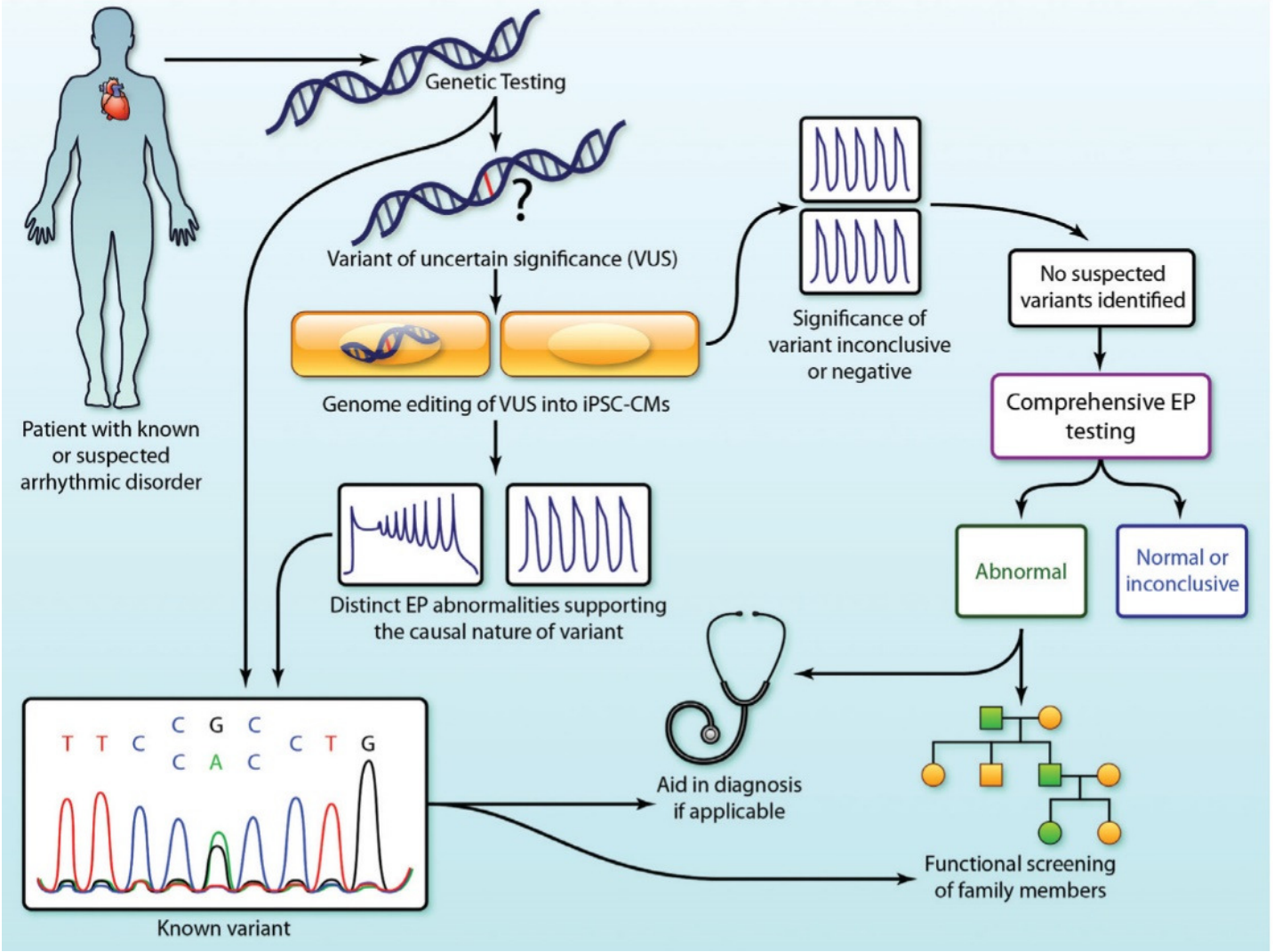
Yongming Wang[1,2,3†], Ping Liang[1,2,4†], Feng Lan[1,2,4†], Haodi Wu[1,2,4†], Leszek Lisowski[5], Mingxia Gu[1,2,4], Shijun Hu[1,2,4], Mark A. Kay[5], Fyodor D. Urnov[6], Rami Shinnawi[7], Joseph D. Gold[1,2], Lior Gepstein[7], Joseph C. Wu[1,2,4]

J Am Coll Cardiol 2014



KCNQ1 and KCNH2 with dominant negative mutations cause LQT1 and LQT2, respectively. Normal iPSCs ("un-edited") were genome edited to carry G269S mutation ("ziG269S") which then showed similar phenotype of prolonged action potential duration (APD) as compared to actual LQT1 patient ("piG269S").

Genome Editing of iPSCs to Determine Variant of Uncertain Significance (VUS) as Pathogenic or Benign



Sallam K et al. *Circ Res* 2015

Stanford CVI is Creating a Biobank of 1,000 Cardiovascular Disease Specific iPSC Lines

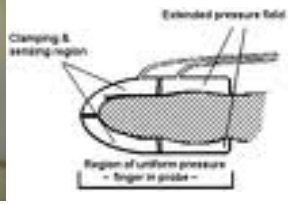
1) Create a biorepository of 1,000 cardiac specific iPSC lines from different ethnicity/sex/age, including isogenic lines using TALEN/CRISPR editing.

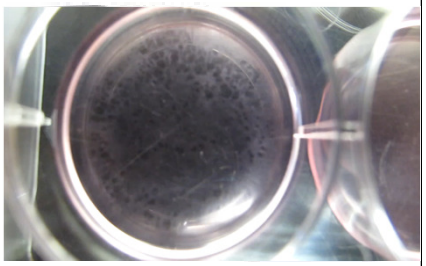
2) Perform DNA-seq of iPSCs and RNA-seq on iPSC-CMs

3) Use PharmGK (http://www.pharmgkb.org) to create a database on how human genetic variation impacts drug response phenotypes.

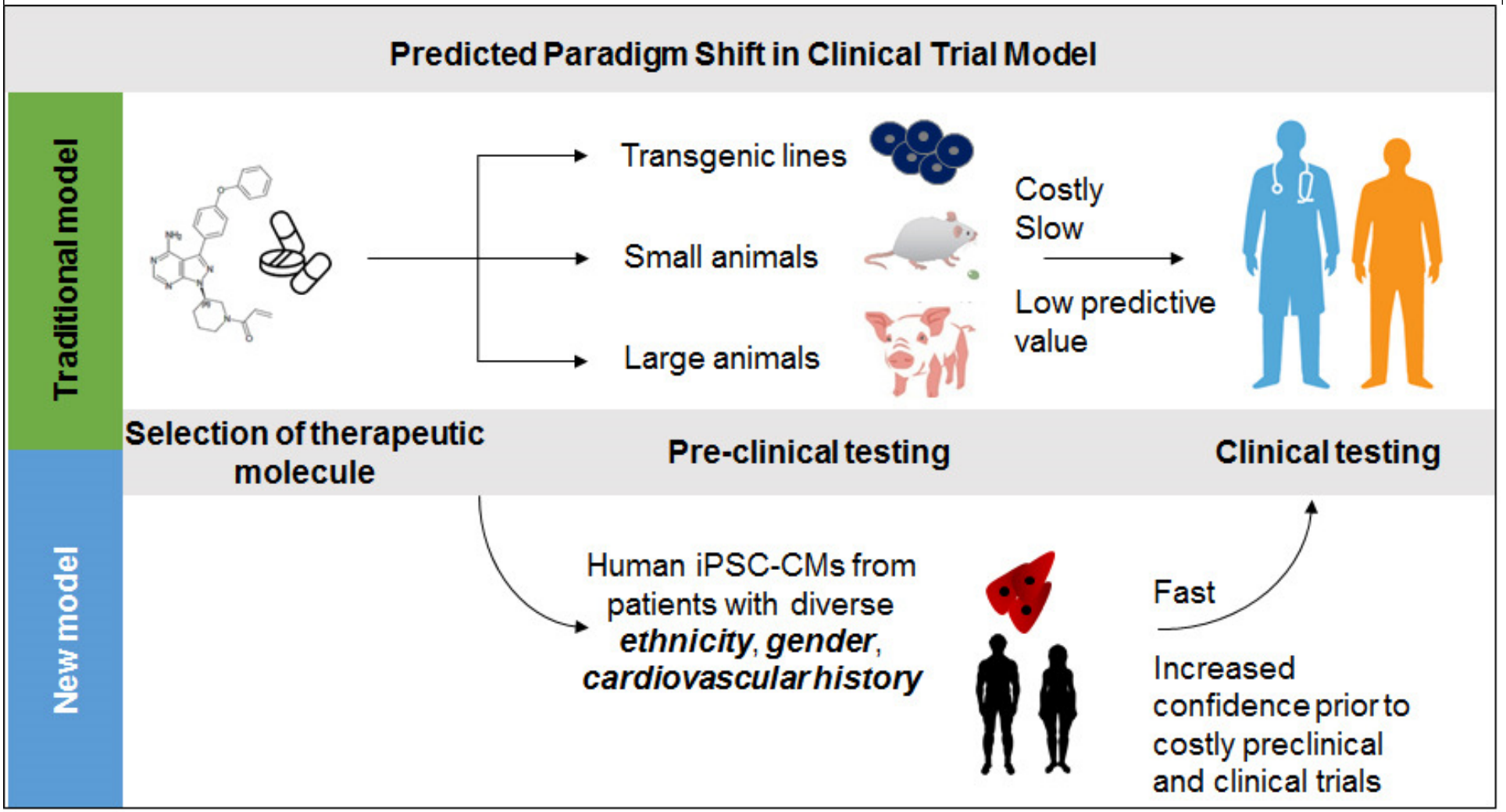
4) Link to medical information using clinical database *(STRIDE: Stanford Translational Research Integrated Database Environment)*

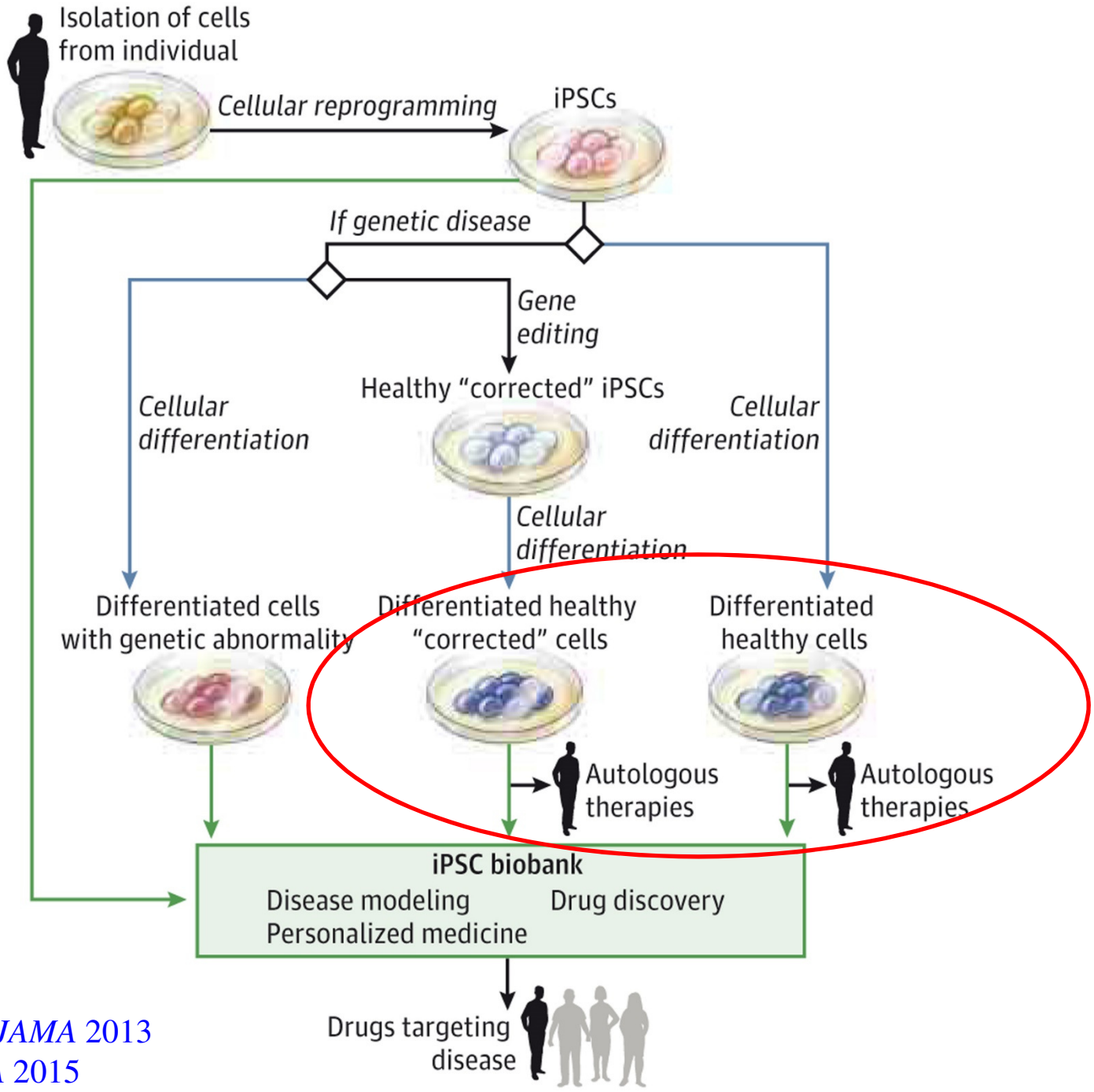
5) Working with NHLBI & CIRM on iPSC biobanking and FDA on drug safety testing. Established sharing resource plan with many investigators.



Summary: iPSC Based Clinical Trial in a Dish





Mordwinkin & Wu. *JAMA* 2013
Wilson & Wu. *JAMA* 2015

YOU'RE THE BIG PHARMA

2015: You're the CEO of company X. High costs of R&D is killing into your margins. CV clinical trials are costly and many show negative data. You're interested in adopting newer cellular assays that will allow you to study cardiac toxicity, contractility, channels, and metabolism.

YOU'RE THE DOCTOR

2020: Husband and wife have 5 kids. 2 already had heart transplant at age 15 and 12, the other 3 (age 10, 7, 5) are fine. DNA-seq shows no obvious genetic defect. They ask you what will happen to their 3 other kids? You say: "we don't know, but we'll check serial echo & MRI over time".

YOU'RE THE PATIENT

2030: You're now 65 yo and recently diagnosed with CHF/MI and need to be on medical Rx. What medication should you be taking? Instead of you being the guinea pig, can physicians in the future test drugs first on your "mini-me surrogates" using your iPSC-derived heart, brain, liver cells, etc?

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Postdoc Fellows
Antje Ebert
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Huaxiao Yang
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Elina Tzatzalos (T32)
Ilanit Itzhaki
Chun Liu
Priyanka Garg
Praveen Shukla

Instructors
Ioannis Karakikes (K99)
Oscar Abilez
Evgenios Neofytou (AHA)
Elena Matsa
Jared Churko (K99)
Nazish Sayed (AHA)

Joseph Gold

Cardiology Fellows
Ian Chen (T32)
Timon Seeger (DFG)
Kazuki Kodo (AHA)

Graduate Student
Arun Sharma (AHA)
John Stack (T32)

Biobank
Chunli Zhao
Mohammed Ameen
Rinkal Chauhan
Sekyung Oh

Lab Managers
Justin Vincent
Loan Nguyen

Trial Coordinators
Thu Vu

Collaborators
Michael Snyder
Michael Longaker
Marlene Rabinovitch
Irving Weissman
Sean Wu
Andrew Connolly
Matthew Porteus
Sam Gambhir
Kristy Red-Horse
Atul Butte
Phillip Yang
Beth Pruitt
Joseph Woo
Michele Calos
Dan Bernstein
Euan Ashley
Tom Quertermous
Russ Altman
Jay Rajadas
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Collaborators
Li Ping (FDA)
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Induced Pluripotent Stem Cells:
Predicting a Powerful Future

Joseph C. Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Professor, Dept of Medicine/Cardiology & Radiology
Email: joewu@stanford.edu