Induced Pluripotent Stem Cells: 
*Predicting a Powerful Future*

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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
- Grant/Research Support
- Consulting Fees/Honoraria
- Ownership/Founder

Company
- Gilead, IVIVI, Sanofi, MiRagen
- Novartis, BMS, Merck
- Stem Cell Theranostics
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
**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)
From 10 cc of Your Blood → Convert to Your iPS Cells → Make Millions of Your Beating Heart Cells

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

MYH7 Arg663His MUTATION

codon change: CGC>CAC

amino acid change: Arg>His

End-Diastole
End-Systole

Control

HCM
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
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

<table>
<thead>
<tr>
<th>Primary</th>
<th>Secondary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>Autoimmune (systemic lupus)</td>
</tr>
<tr>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
<td>Electrolyte imbalance</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>Endocrine (diabetes, hypothyroidism)</td>
</tr>
<tr>
<td>Mixed (genetic and nongenetic)</td>
<td>Endomyocardial (fibrosis)</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>Infiltrative (amyloidosis, Gaucher disease)</td>
</tr>
<tr>
<td>Restrictive cardiomyopathy</td>
<td>Inflammatory (sarcoidosis)</td>
</tr>
<tr>
<td>Acquired</td>
<td>Neurologic (neurofibromatosis)</td>
</tr>
<tr>
<td>Myocarditis (inflammatory cardiomyopathy)</td>
<td>Nutritional (beriberi)</td>
</tr>
<tr>
<td>Peripartum (or postpartum)</td>
<td>Radiation</td>
</tr>
<tr>
<td>cardiomyopathy</td>
<td>Storage (hemochromatosis)</td>
</tr>
<tr>
<td>Stress cardiomyopathy</td>
<td>Toxic (medications)</td>
</tr>
<tr>
<td></td>
<td>Velocardiofacial syndrome</td>
</tr>
</tbody>
</table>
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. Dalmetsch,4 Manish J. Butte,5 Euan A. Ashley,7 Michael T. Longaker,3,9 Robert C. Robbins,10 Joseph C. Wu1,2,3,10*

Sci Transl Med 2012

Chemically defined generation of human cardiomyocytes


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. Wu1,2,3*

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,2 Donald M. Bers,5 and Joseph C. Wu1,2,3,*

Cell Stem Cell 2015
Envisioning iPSC-Based Precision Medicine

Predicted Paradigm Shift in Drug Treatment

Traditional model

Diseased patient

Investigation of possible therapies

Selection of best drug

New model

Patient-specific iPSC-CMs

Phenotypic screen

Fast
Selection of optimum drug treatment

Patient history
Clinical examinations
Serial testing of multiple drugs

Slow
Ineffective

Fast
Selection of optimum drug treatment
#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 \textbf{Billion} and \textbf{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%)
Drugs Withdrawn Due to Cardiac Toxicity

- Cisapride (Propulsid)
- Flosequinar
- Sibutramine Propoxyphene
- Rofecoxib
- Tegaserod
- Valdecoxib
- Encainide
- Terodiline
- Terfenadine
- Mibefradil Sertindole Prenylamine
- Astemizole Grepafloxacin
- Thioridazine
- Cisapride
- Droperidol Levomethadyl Levacetlymethadol
- Dofetilide
- 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

Baseline

1 Control hiPSC-CMs

2 LQT hiPSC-CMs

3 HCM hiPSC-CMs

4 DCM hiPSC-CMs

“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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Circulation 2013
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<th>Baseline</th>
<th>1 nM</th>
<th>3 nM</th>
<th>10 nM</th>
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<tbody>
<tr>
<td>1</td>
<td>Control hiPSC-CMs</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>2</td>
<td>LQT hiPSC-CMs</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>3</td>
<td>HCM hiPSC-CMs</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>4</td>
<td>DCM hiPSC-CMs</td>
<td>→</td>
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<th></th>
<th>Baseline</th>
<th>1 nM</th>
<th>3 nM</th>
<th>10 nM</th>
<th>300 nM</th>
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<tr>
<td><strong>1 Control hiPSC-CMs</strong></td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
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<tr>
<td><strong>2 LQT hiPSC-CMs</strong></td>
<td><img src="image6" alt="Graph" /></td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
<td><img src="image9" alt="Graph" /></td>
<td><img src="image10" alt="Graph" /></td>
</tr>
<tr>
<td><strong>3 HCM hiPSC-CMs</strong></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
<td><img src="image15" alt="Graph" /></td>
</tr>
<tr>
<td><strong>4 DCM hiPSC-CMs</strong></td>
<td><img src="image16" alt="Graph" /></td>
<td><img src="image17" alt="Graph" /></td>
<td><img src="image18" alt="Graph" /></td>
<td><img src="image19" alt="Graph" /></td>
<td><img src="image20" alt="Graph" /></td>
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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

<table>
<thead>
<tr>
<th>Predicted Paradigm Shift in Clinical Trial Model</th>
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<tbody>
<tr>
<td>Traditional model</td>
</tr>
<tr>
<td>Transgenic lines</td>
</tr>
<tr>
<td>Small animals</td>
</tr>
<tr>
<td>Large animals</td>
</tr>
<tr>
<td>Costly</td>
</tr>
<tr>
<td>Slow</td>
</tr>
<tr>
<td>Low predictive value</td>
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<tr>
<th>Selection of therapeutic molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>New model</td>
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<tr>
<td>Human iPSC-CMs from patients with diverse</td>
</tr>
<tr>
<td>ethnicity, gender, cardiovascular history</td>
</tr>
<tr>
<td>Fast</td>
</tr>
<tr>
<td>Increased confidence prior to costly preclinical and clinical trials</td>
</tr>
</tbody>
</table>
Isolation of cells from individual

Cellular reprogramming

iPSCs

If genetic disease

Gene editing

Healthy “corrected” iPSCs

Cellular differentiation

Differentiated cells with genetic abnormality

Differentiated healthy “corrected” cells

Differentiated healthy cells

Cellular differentiation

Autologous therapies

iPSC biobank

Disease modeling

Personalized medicine

Drug discovery

Autologous therapies

Drugs targeting disease

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?
Acknowledgment

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Nazish Sayed (AHA)
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Andre Terzic (Mayo)
Puget (INSERM)
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Shoukhrat Mitalipov (OHSU)
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Kevin Healy (UCB)
Donald Bers (UCD)
Kevin Xiang (UCD)
Wolfram Zimmermann (Ger)
John Solaro (UIC)
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Loan Nguyen

**Trial Coordinators**
Thu Vu

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Induced Pluripotent Stem Cells: Predicting a Powerful Future

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