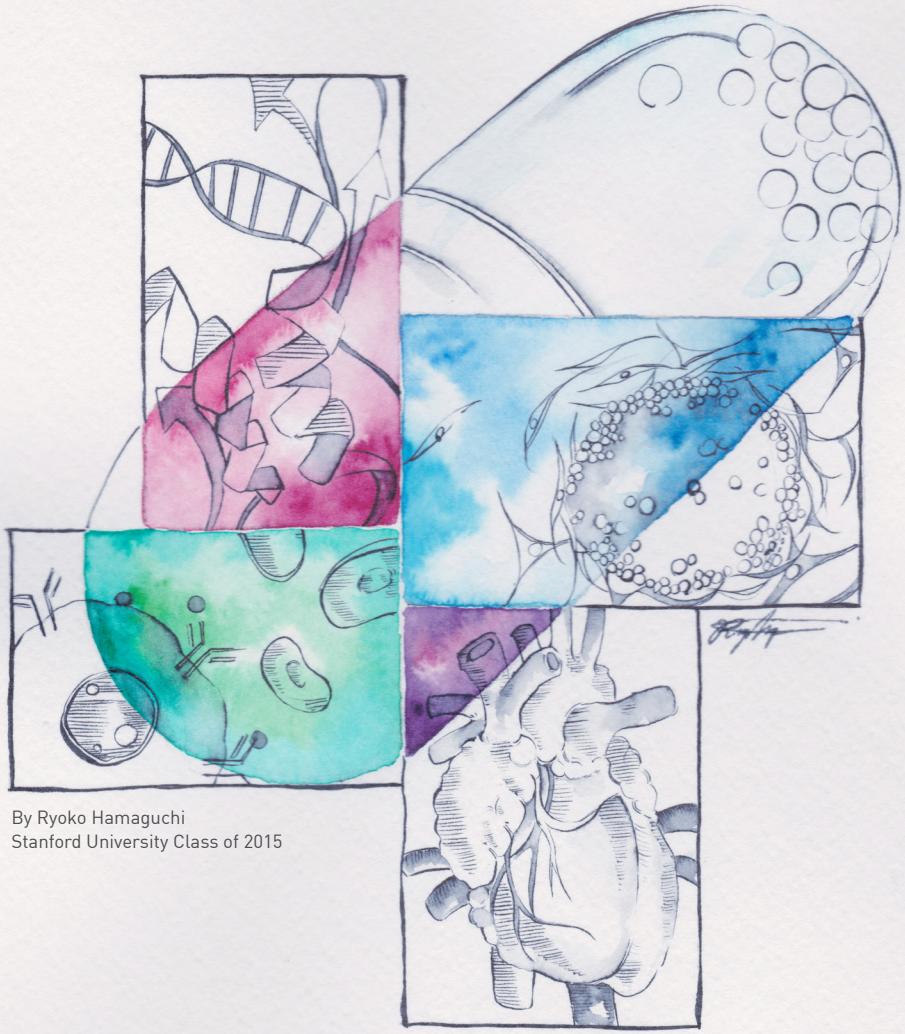
2016

LI KA SHING CENTER

Stanford Drug Discovery Conference



By Ryoko Hamaguchi
Stanford University Class of 2015

March 29, 2016

Stanford Cardiovascular Institute

CIRM
CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

Stanford Cancer Institute

SCHEDULE

REGULATORY POLICY AND CLINICAL TRIALS

9:00am **Norman L. Stockbridge, MD, PhD**
Director, Division of Cardiovascular and Renal Products, FDA/CDER
What Level of Risk Aversion is Sensible in Drug Development?

9:15am **Robert A. Harrington, MD**
Arthur L. Bloomfield Professor of Medicine
Chair, Stanford Department of Medicine
Re-thinking Randomized Control Trials

9:30am **Kelly A. Shepard, PhD**
Senior Science Officer, Discovery and Translation
California Institute for Regenerative Medicine (CIRM)
CIRM 2.0 - A New Funding Paradigm for Stem Cell R&D

9:40am **Christopher P. Austin, MD** *(Keynote)*
Director, National Center for Advancing Translational Sciences
National Institutes of Health
Catalyzing Translational Innovation

10:05am Group Q&A and Panel Discussion
Moderator: **Kevin V. Grimes, MD, MBA**
Associate Professor (Teaching) of Chemical and Systems Biology

10:25am Coffee Break, Networking Session

INDUSTRY LEADERS

10:45am **Aarif Y. Khakoo, MD, MBA**
Vice President, Research, and Site Head, Amgen South San Francisco
Translating Insights From Human Genetics to Drug Development

11:00am **Bradley P. Morgan, PhD**
Vice President, Drug Discovery & Early Development
Cytokinetics, Inc.
Modulation of Muscle Contractility

11:15am **Charles Homcy, MD**
Venture Partner, Third Rock Ventures
Third Rock Ventures' Approach to Building Therapeutic Companies

11:30am **Peter S. Kim, PhD**
Virginia and D. K. Ludwig Professor of Biochemistry
Stanford University
Preventing Diseases with Vaccines

11:45am Group Q&A and Panel Discussion
Moderator: **Jonathan C. Fox, MD, PhD**, Chief Medical Officer
MyoKardia

12:05pm Buffet Lunch

ONCOLOGY RESEARCH

12:40pm **Jennifer R. Grandis, MD**
Director, Clinical and Translational Science Institute
UCSF School of Medicine
STAT3 as a Cancer Drug Target

12:55pm **Chaitan Khosla, PhD**
Wells H. Rauser and Harold M. Petiprin Professor
Director, Stanford ChEM-H
Discovery and Engineering Novel Immunomodulatory Natural Products from the Microbiome

1:10pm **Amato Giaccia, PhD**
Jack, Lulu and Sam Willson Professor
Stanford Department of Radiation Oncology
Targeting HIF pathway to inhibit Metastasis and Increase the Efficacy of Radiotherapy

1:25pm **Sanjiv Sam Gambhir, MD, PhD**
Virginia & D.K. Ludwig Professor
Chair, Department of Radiology
Molecular Imaging of the Immune System in Humans

1:40pm Group Q&A and Panel Discussion
Moderator: **Sanjay Malhotra, PhD**
Associate Professor (Research) of Radiation Oncology

2:00pm Networking Break

CARDIOVASCULAR RESEARCH

2:20pm **Russ B. Altman, MD**
Kenneth Fong Professor, Bioengineering, Genetics, and Medicine
Informatics Methods to Understand the Effects (Good and Bad) of Small Molecule Drugs

2:35pm **Colleen E. Clancy, PhD**
Professor, Department of Pharmacology
University of California, Davis
Cardio-pharmacology - From Molecule to Cardiac Rhythm

2:50pm **Mark Mercola, PhD**
Professor of Medicine - Cardiovascular Medicine
Physiological Screening for Heart Failure Drug Discovery

3:05pm **Joseph C. Wu, MD, PhD**
Simon H. Stertzer, MD, Professor
Director, Stanford Cardiovascular Institute
Patient-Specific iPSCs for Precision Cardiovascular Medicine

3:20pm Group Q&A and Panel Discussion
Moderator: **Philip Sager, MD**
Chair, FDA Cardio-Renal Advisory Committee

BLUE SKY TALKS

3:40pm **Jessica L. Mega, MD, MPH** *(Keynote)*
Chief Medical Officer, Verily
Using New Technologies and Analytic Tools to Enhance Drug Discovery and Development

4:05pm **Brian K. Kobilka, MD** *(Keynote)*
Helene Irwin Fagan Chair and Professor
Stanford Department of Molecular and Cellular Biology
2012 Nobel Laureate
Challenges in GPCR Drug Discovery

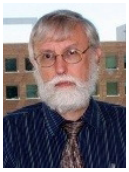
4:35pm Research Reception:
*Wine & Cheese
*Piano by Sheun Aluko
***$750 research awards**

SPEAKER BIOS

Christopher P. Austin, MD *(Keynote)*
Dr. Austin leads the National Center for Advancing Translational Sciences. Under his direction, NCATS researchers and collaborators are developing new technologies, resources and collaborative research models; demonstrating their usefulness; and disseminating the data, analysis and methodologies for use by the worldwide research community.

Robert A. Harrington, MD
Dr. Harrington leads the Department of Medicine at Stanford with 450 faculty members in 15 divisions. "My science progressed from the focused study of thrombosis to using more broadly the tools of clinical science to answer clinical questions while finding new and innovative ways to design clinical trials and use clinical data to improve the care of patients."

Kelly A. Shepard, PhD
Dr. Shepard has served as a Scientific Program Officer at the California Institute for Regenerative Medicine for seven years, contributing at multiple levels to the development and implementation of programs that support stem cell research. More recently, she is focusing her efforts in the Discovery and Translation group, creating new strategies for advancing novel stem cell technologies towards improved patient care.



Norman L. Stockbridge, MD, PhD
Dr. Stockbridge has served as the Director of the Division of Cardiovascular and Renal Products (DCaRP) Division since 2004. The division regulates and reviews Investigational New Drug (IND) applications and marketing applications for drug and biologic products for the treatment of cardiovascular conditions and diseases.

Kevin V. Grimes, MD, MBA
Dr. Grimes co-directs the SPARK Translational Research Program at Stanford. SPARK's two-fold mission is to advance promising research discoveries into the clinic as new therapeutics and diagnostics, and to educate faculty, post-doctoral fellows and students regarding the translational process. Nearly 60% of projects completing the program have advanced to commercial partnership and/or clinical study.

Aarif Y. Khakoo, MD, MBA

Dr. Khakoo is responsible for the discovery and advancement of Amgen's Metabolic Disorders pipeline including cardiovascular disease, obesity, diabetes, bone disease, and chronic kidney disease. His research organization is focused on leveraging findings from common and rare variant human genetics in an effort to increase the speed and success rate of drug development in complex cardiovascular and metabolic diseases.

Bradley P. Morgan, PhD

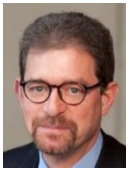
Dr. Morgan received his PhD in Chemistry from the University of California, Berkeley and completed his post-doctoral fellowship at the California Institute of Technology. Today he is the Senior Vice President of Drug Discovery and Early Development at Cytokinetics.

Charles Homcy, MD

Dr. Homcy joined Third Rock Ventures in 2010 and focuses on the formation of companies discovering and developing novel therapeutic approaches. He has played an integral role in launching several portfolio companies. He is a Board member and co-founder of Global Blood Therapeutics and Board member and former interim CEO of MyoKardia.

Peter S. Kim, PhD

Dr. Kim was the former President of Merck Research Laboratories (2003-2013). He is currently a Professor of Biochemistry at Stanford University. Some of his studies are aimed at creating an HIV vaccine. The Kim group studies the mechanism of viral membrane fusion and its inhibition by drugs and antibodies using the HIV envelope protein (gp120/gp41) as a model system.



Jonathan C. Fox, MD, PhD *(Moderator)*

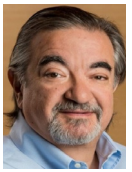
Dr. Fox joined MyoKardia in January 2013. He has extensive drug development experience spanning all phases of clinical development, from clinical pharmacology to large cardiovascular outcomes trials. He served as Vice President of clinical development at AstraZeneca (2004 to 2012).

Jennifer R. Grandis, MD

Dr. Grandis is a Professor of Otolaryngology-Head and Neck Surgery and Associate Vice Chancellor for Clinical and Translational Research at UCSF, where she also directs the Clinical and Translational Science Institute (CTSI). Her research efforts are focused on elucidating and targeting key signaling pathways in head and neck cancer. She is a member of the Developmental Therapeutics Program at the HDFCCC and facilitates interactions between the cancer center and the CTSI.

Chaitan Khosla, PhD

Dr. Khosla is the Director of Stanford ChEM-H and his group focuses on the interface of enzyme chemistry and medicine. Over the next decade he envisions that the predictive power of polyketide biosynthetic engineering will mature analogous to current protein engineering capabilities.

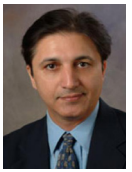


Amato Giaccia, PhD

During the last five years, Dr. Giaccia has identified several small molecules that act to kill VHL deficient renal cancer cells through a synthetic lethal screening approach. He is the Director of Basic Science at the Stanford Cancer Institute.

Sanjiv Sam Gambhir, MD, PhD

Dr. Gambhir's laboratory is pioneering imaging assays to monitor fundamental cellular and molecular events. Technologies such as micro positron emission tomography (microPET), bioluminescence optical imaging, fluorescence optical imaging, micro computerized axial tomography (microCAT), ultrasound, photoacoustics, Raman imaging are all being actively investigated.



Sanjay Malhotra, PhD *(Moderator)*

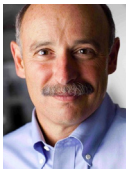
Dr. Malhotra's research interests focus on the design and discovery of synthetic, and natural product inspired small molecules which can be used as probes for developing understanding of biological phenomena, including protein-protein interactions and modulation of signal transduction pathways. His laboratory employs the tools of synthetic medicinal chemistry, molecular modeling and chemical biology for translational research in drug discovery, development, imaging and radiation.

Russ B. Altman, MD, PhD

Dr. Altman is a Professor of Bioengineering, Genetics, & Medicine (and of Computer Science, by courtesy) and past chairman of the Bioengineering Department at Stanford University. His research interests are in the application of computing and informatics technologies to problems relevant to medicine. He is particularly interested in methods for understanding drug action at molecular, cellular, organism and population levels.

Colleen E. Clancy, PhD

Dr. Clancy's research aims to develop and implement computational approaches to understand mechanisms of excitable disease in the heart and brain. The general approach used in the laboratory is to design detailed models of ion channel activity that can be used to study perturbations, such as those caused by effects of drugs, mutations or phosphorylation.



Mark Mercola, PhD

Dr. Mercola is a Professor of Medicine at Stanford. Prior to arriving at Stanford, he co-founded the screening center at Sanford-Burnham-Prebys Medical Discovery Institute. He is known for developing tools and instrumentation for high throughput physiological screening, and has pioneered the use of iPSC-derived cardiomyocytes and patient cells to discover basic disease mechanisms and candidate therapeutic targets, as well as guide the development of small molecule drugs.

Joseph C. Wu, MD, PhD

Dr. Wu is the Director of the Stanford Cardiovascular Institute. His group studies the biological mechanisms of adult stem cells, embryonic stem cells, and induced pluripotent stem cells. Using a combination of approaches including, next generation sequencing, tissue engineering and molecular imaging technologies his research aims to uncover novel treatments for cardiovascular diseases.

Philip Sager, MD *(Moderator)*

Dr. Sager is a voting member of the FDA Cardio-Renal Advisory Committee and is actively involved in developing collaborations between regulators, industry, and academia on new approaches to innovative drug development as well as CV safety assessment of pharmaceuticals and devices.

Jessica L. Mega, MD, MPH *(Keynote)*
Dr. Mega is a faculty member at Harvard Medical School (on leave), a senior investigator with the TIMI Study Group, and a cardiologist at Brigham and Women's Hospital. She has led large, international, randomized trials evaluating novel cardiovascular therapies. Her findings are published in the New England Journal of Medicine, Lancet and JAMA.

Brian K. Kobilka, MD *(Keynote)*
Dr. Kobilka won the 2012 Nobel Prize for Chemistry for describing the structure and function of the receptors through which cells sense and respond to chemical signals. His group studies adrenergic receptor biology, which forms the interface between the sympathetic nervous system and the cardiovascular system.

ABSTRACTS

1. **Stanford Fragment Library to Enable the Discovery of Chemical Probes or Drugs**

Bruce Koch, Senior Director, Scientific Service Centers, Stanford University School of Medicine

Screening of large (hundreds of thousands to millions) of drug-like chemical compounds is often used as a starting point to discover new chemical probes or to find drug leads. However, drug-like chemical space is vast (est. 1040 to 1060) and so these libraries represent only a tiny fraction of that space. An alternative approach is to screen compounds of smaller size and complexity ("fragments", often ← 250MW), which, as the result of their reduced complexity, have a much smaller possible number of combinations (est. 1010). Thus even a small compound fragment collection can represent a larger fraction of the available chemical space. Here we describe the design, purchase, and availability to Stanford researchers of a 5000 compound fragment library.

2. **Hipk4-Dependent Activation of the Hedgehog Signaling Pathway**

J. Aaron Crapster, Paul G. Rack, Zane Hellmann, Michael Eisenberg, Gary Flynn, and James K. Chen

Department of Chemical & Systems Biology, Stanford University
Spacefill Enterprises, LLC

Regulation of Gli transcription factors by the Hedgehog signaling pathway is essential during embryonic development to maintain adult tissue homeostasis. Aberrant pathway activation can lead to developmental defects and/or a number of human cancers. We discovered that exogenous homeodomain interacting protein kinase 4 (Hipk4) induces Gli-dependent transcription. In collaboration with Spacefill Enterprises, LLC we have developed a potent and specific small molecule inhibitor of Hipk4 activity. We are exploring links between Hipk4 function and Hh pathway regulation of spermatogenesis, as Hipk4 may represent a new target for non-hormonal male contraception.

3. **Development of a New Antiarrhythmic Therapeutic for Long QT Syndrome Type 3**

Wesley L. McKeithan[1,2], Daniel A. Ryan, Karl J. Okolotowicz, Alex Savtchenko, Robert S. Kass, John R. Cashman[3] and Mark Mercola[1]

[1]Cardiovascular Institute, Stanford University
[2]Graduate School of Biomedical Sciences, Sanford Burnham Prebys Medical

We developed an iPSC disease-in-a-dish model of congenital LQT3 and used the model to guide the structural optimization of the class 1b antiarrhythmic, Mexiletine. Using the Kinetic Imaging Cytometer (Vala Sciences) and a novel small molecule fluorescent voltage sensitive probe, we acquired and analyzed iPSC-derived cardiomyocyte action potential kinetics and calculated physiological parameters using Cyteseer software. In iterative cycles of syntheses and testing, we evaluated 93 Mexiletine analogs in both normal donor and LQT3 patient iPSC-cardiomyocytes for modulation of action potential duration (APD) and pro-arrhythmic induction of early after depolarizations (EADs).

4. **RNS Promotes Transdifferentiation through RING1A S-Nitrosylation**

Shu Meng, Gang Zhou, John P. Cooke

Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX

Activation of innate immune signaling is required for epigenetic plasticity in nuclear reprogramming such as transdifferentiation of fibroblasts into endothelial cells. Prominent effectors of innate immunity are reactive nitrite species (RNS). We hypothesized that the action of RNS may be required for epigenetic plasticity.

Using a previously established protocol of PolyI:C (a TLR3 agonist) followed with endothelial growth factors, 2% of fibroblasts could be fully transdifferentiated into endothelial cells over four weeks and assessed by immunohistochemistry, functional assays and by RNASeq. PolyI:C stimulated human fibroblasts and increased intracellular levels of NO. RNS mediates the effect of innate immune activation in chromatin remodeling through S-nitrosylation of RING1A and decreased H3K27me3 binding to chromatin.

5. **Small Molecule Cocktail Enhancement of in Vitro and in Vivo Direct Cardiac Reprogramming of Postnatal Cardiac Fibroblasts**

Tamer M. A. Mohamed, Nicole Stone, Ethan Radzinsky, Pengzhi Yu, Yu Huang, Haixia Wang, Sheng Ding, Deepak Srivastava

Gladstone Institute of Cardiovascular Disease and University of California, San Francisco

The ability to directly trans-differentiate fibroblasts into cardiomyocytes through overexpression of three core transcription factors, Gata4, Mef2C and TBX5 (GMT), has been demonstrated by our group and others. Using a chemical approach we aimed to increase the efficiency, speed and maturation of these directly reprogrammed cardiomyocytes. We screened 5500 compounds and identified a combination of a TGF-β inhibitor and WNT inhibitor enhanced the efficiency and maturation of the reprogrammed cells, as we observed beating cells after only 1 week of reprogramming.

6. **High-Throughput Screening of Human Induced Pluripotent Stem Cell Cardiomyocytes Predicts Tyrosine Kinase Inhibitor Cardiotoxicity**

Arun Sharma[1], Paul W. Burridge, Wesley L. McKeithan, Ricardo Serrano, Alexandra Holmström, Jared M. Churko, , Elena Matsa, Yuan Zhang, Anusha Kumar, Juan C. del Alamo, Mark Mercola, Sean M. Wu, MD, Joseph C. WC

[1]Stanford Cardiovascular Institute, Stanford University School of Medicine; Institute for Stem Cell Biology and Regenerative Medicine, Stanford University

Tyrosine kinase inhibitors (TKIs) represent a large portion of current chemotherapeutic agents, but many exhibit cardiotoxic side effects. We utilized human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to screen FDA-approved TKIs in vitro for cardiotoxicities by observing CM viability, contractility, and signaling and to develop a "TKI safety index". We also derived endothelial cells (hiPSC-ECs) and cardiac fibroblasts (hiPSC-CFs) to examine cell-type specific cardiotoxicities. From high-throughput screening, we determined that treatment with a subset of VEGFR2/PDGFR-inhibiting TKIs—sorafenib (Nexavar), regorafenib (Stivarga), and ponatinib (Iclusig)—led to significant cytotoxicity in hiPSC-CMs, hiPSC-ECs, and hiPSC-CFs alike. Our results suggest that hiPSC-CMs can be utilized to screen for cardiotoxic side effects associated with chemotherapeutic compounds.

7. **Combination Therapy that Limits Pyrimidine Availability for a Broad-Range Antiviral Effect**

*Ayse Ökesli[1], Richard M. Deans, David W. Morgens, Chaitan Khosla and Michael C. Bassik[2,3]**

[1]Department of Chemistry, Stanford University
[2]Department of Genetics, Stanford University
[3] Stanford University Chemistry, Engineering, and Medicine for Human Health (ChEM-H), Stanford

Our laboratory has developed a new broad spectrum antiviral therapeutic that targets host cell processes, rather than viral proteins. Such broad spectrum antivirals have the potential to treat a wide range of viruses, and therefore are particularly useful given the rapid rise in the number of emerging pathogens across the globe. By targeting host biology, they also minimize the chances of developing drug resistant strains of viral pathogens. To achieve this goal, we developed a new antiviral drug that limits pyrimidine (a key component for viral replication) availability in cells thereby disrupting viral replication.

8. **Higher Levels of Asymmetric Dimethylarginine are Associated with Lower Fractional Flow Reserve After Orthotopic Heart Transplantation**

Rushi Parikh[1], Kiran Khush, Helen Luikart, Vedant Pargaonkar, Yuhei Kobayashi, Janghoon Lee, Seema Sinha, Garrett Cohen, Hannah Valantine, Alan Yeung, and William Fearon[1]

[1]Division of Cardiovascular Medicine, Stanford University School of Medicine

Cardiac allograft vasculopathy (CAV) is the leading cause of late mortality after orthotopic heart transplantation (OHT). In patients with native coronary artery disease, elevated asymmetric dimethylarginine (ADMA) levels correlate with greater ischemia as assessed by fractional flow reserve (FFR). The relationship between ADMA and FFR in the OHT population is unknown. Intracoronary ADMA and FFR of the left anterior descending artery were measured within 1 month of OHT (baseline) and at 1 year. In 47 OHT recipients, higher ADMA levels were significantly associated with lower FFR values at baseline and 1 year (R=0.33, p=0.024 and R=0.39, p=0.0085, respectively). Those with high-risk ADMA levels (→0.70 μM) had significantly lower FFR values than those with low-risk ADMA levels at these time points. Elevated ADMA levels early after OHT are significantly associated with lower FFR values. This novel finding suggests that ADMA predicts the epicardial ischemic potential of CAV.

9. **Canonical Wnt Signaling Activation Enhances Cardiac Tissue Repair and Regeneration by Arteriole Formation, Cardiogenesis and Attenuation of Fibrosis**

David T. Paik[1], Meena Rai, Sergey Ryzhov, Lehanna N. Sanders, Omonigho Aisagbonhi, Mitchell J. Funke, Igor Feoktistov, Antonis K. Hatzopoulos

[1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine,

Myocardial infarction (MI) causes irreversible tissue damage, leading to heart failure. We found canonical Wnt signaling pathway and the Wnt10b ligand are strongly induced in mouse and human hearts after MI. Wnt10b regulates cell fate in various organs, yet its role in the heart is unknown. To investigate the effects of Wnt10b gain-of-function on cardiac repair mechanisms and functional outcomes after injury, we generated αMHC-Wnt10b transgenic (TG) mouse line that overexpresses Wnt10b in adult cardiomyocytes. Following acute myocardial injury, the TG mice displayed improved recovery of cardiac function, accompanied by enhanced neovascularization and attenuated scar fibrosis. Together, these effects led to stabilized blood vessel formation

10. Inhibition of One Substrate Phosphorylation of a Protein Kinase Out of Many Substrates by a Selective Peptide Inhibitor of Kinase-Substrate Interaction

N. Qvit[1], D. Mochly Rosen, M. Disatnik

[1]Chemical and Systems Biology and Stanford Cardiovascular Institute

Pleiotropic protein kinases phosphorylate multiple protein substrates, thus leading to diverse and at times opposing functions. Identifying the particular substrate that mediates a given function involves extensive mutagenesis, which is time-consuming and expensive. We hypothesized that unique protein-protein interactions (PPIs) exist between pleiotropic kinases and at least some of their substrates. In addition to the catalytic site on the enzyme, such substrate docking-sites increases selectivity and affinity of the kinase for a given substrate. We identified a selective inhibitor of pyruvate dehydrogenase kinase (PDK) docking to delta protein kinase C (δPKC), using a rational approach. With an IC_{50} of ~5 nM, and without affecting the phosphorylation of other δPKC substrates even at 1 micromolar, this inhibitor demonstrated that PDK phosphorylation alone is critical for δPKC-mediated injury by heart attack. This illustrates the power of our approach to identify a new class of highly selective pharmacological tools, which we termed separation-of-function inhibitors.

11. Treatment of Barth Syndrome iPSC-Derived Cardiomyocytes Using a Mitochondrially Targeted, Cardiolipin-Binding Peptide

Gang Wang[1], Ling Xiao, Lillian L. Ye, David J. Milan, and William T. Pu[1]

[1]Department of Cardiology, Boston Children's Hospital, 300 Longwood Ave, Boston, MA

Barth syndrome is an X-Linked cardiomyopathy with no targeted treatment. Recently we described an isogenic human iPSC model for this disease, in which we used Cas9 genome editing to introduce a patient mutation into the Tafazzin gene. Using this model, we showed that excessive mitochondria reactive oxygen species (ROS) was an important contributor to muscle weakness in this disorders and this led us to test therapies designed to reduce mitochondrial ROS. Mitochondrial targeted aromatic-cationic peptides are small water soluble synthetic peptides (less than 10 amino acids) that gather together in the inner mitochondrial membrane. In the present study we tested Bendavia on our human iPSC-CM model. We found that Bendavia decreased ROS in the Barth syndrome iPSC-CMs.

12. **The Activation of Wnt5a-Ror2 Axis Enhances Endothelial-Pericyte Interaction During Pulmonary Angiogenesis**

Ke Yuan[1], Mark Orcholski, Marielle Discipulo, Divya Sriram and Vinicio de Jesus Perez

[1]Divisions of Pulmonary and Critical Care Medicine and Stanford Cardiovascular Institute, School of Medicine, Stanford University

Pulmonary arterial hypertension (PAH) is a life-threatening disorder characterized by impaired angiogenesis, progressive small vessel loss and occlusive vasculopathy. Pericyte recruitment and establishment of endothelial-pericyte interactions is a critical event to ensure structural integrity and proper function of new vessels and at the center of this is Wnt/PCP. We demonstrate that the existence of a novel Wnt5a/ROR2 signaling axis that regulates communication of EC and pericytes during pulmonary angiogenesis which appears disrupted in PAH and correlates with small vessel loss. This study will also produce a blueprint for how we should approach the development of novel therapeutics that target Wnt5a/ROR2 signaling in the pulmonary circulation.

13. **A Novel "Trigger and Release" Strategy for Imaging Tumor Hypoxia In Vivo**

Samuel D. Banister[1], Bin Shen, Marjan Rafat, Marta Vilalta, Edward E. Graves, J. Martin Brown, Sanjay Malhotra, Ananth Srinivasan, and Frederick T. Chin

[1]Departments of Radiology and Radiation Oncology, Molecular Imaging Program at Stanford (MIPS), Stanford University

Tumor cells exhibiting reduced oxygen concentration (hypoxia) are associated with increased aggressiveness, ability to metastasize, and resistance to therapeutic intervention. For all current hypoxia positron emission tomography (PET) imaging agents, such as [18F]FMISO reactive nitroimidazole intermediates remain covalently bound to [18F]fluorine and form conjugates with nucleophilic biomolecules. Diffusion and circulation of these radioactive metabolites causes significant non-tumor tissue accumulation and delays clearance, resulting in low signal-to-noise ratios and limited clinical utility. We propose a novel "trigger and release" strategy for imaging tumor hypoxia in vivo.

14. Molecular Graph Convolutions: Moving Beyond Fingerprints

Steven Kearnes[1], Kevin McCloskey, Marc Berndl, Vijay Pande, Patrick Riley

[1]Department of Chemistry and Structural Biology, Stanford University

Molecular "fingerprints" encoding structural information are the workhorse of cheminformatics and machine learning in drug discovery applications. However, fingerprint representations necessarily emphasize particular aspects of the molecular structure while ignoring others, rather than allowing the model to make data-driven decisions. We describe molecular graph convolutions, a novel machine learning architecture for learning from undirected graphs, specifically small molecules. Graph convolutions use a simple encoding of the molecular graph (atoms, bonds, distances, etc.), allowing the model to take greater advantage of information in the graph structure.

15. Notch1 Promotes Group 3 Medulloblastoma Metastasis

Suzana A. Kahn, Xin Wang, Ryan T. Nitta, Sharareh Gholamin, Gregor Hutter, Vijay Ramaswamy, Tej D. Azad, Debashis Sahoo, Sara Bolin, Rogelio Esparza, Pauline Chu, Michael Edwards, Paul G. Fisher, Hannes Vogel, Gordon Li, Yoon-Jae Cho, Michael D. Taylor, Irving L. Weissman, Siddhartha S. Mitra, Samuel H. Cheshier.

Division of Pediatric Neurosurgery, Lucile Packard Children's Hospital, Stanford University School of Medicine
Departments of Neurology and Neurological Sciences, Stanford University School of Medicine

Medulloblastoma is the most common pediatric malignant brain tumor originating in the central nervous system. Group 3, the most aggressive molecular subgroup of medulloblastoma, arises in the cerebellum and/or floor of the 4th ventricle and frequently disseminates through the cerebrospinal fluid (CSF) in the leptomeningeal space to different regions of the brain and spinal cord. The presence and stage of metastasis are inversely related to the overall survival and progression-free survival of medulloblastoma patients. Here we show that the key neurodevelopmental Notch1 signaling pathway regulates both the initiation of metastasis and the self-renewal of Group 3 medulloblastoma. We identify a mechanism in which Notch1 activates BMI1 through the activation of Twist1. These findings identify Notch1 as a pivotal driver of Group 3 medulloblastoma metastasis and maintenance through self-renewal, supporting the development of therapies targeting this pathway.

16. **Identification of Significantly Mutated Regions Across Cancer Types Highlights a Rich Landscape of Functional Molecular Alterations**

Carlos L. Araya, Can Cenik, Jason A. Reuter, Gert Kiss, Vijay S. Pande, Michael P. Snyder, William J. Greenleaf

Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA
Department of Chemistry, Stanford University, Stanford, CA 94305, USA

Cancer sequencing studies have primarily identified cancer driver genes by the accumulation of protein-altering mutations. An improved method would be annotation independent, sensitive to unknown distributions of functions within proteins and inclusive of noncoding drivers. We employed density-based clustering methods in 21 tumor types to detect variably sized significantly mutated regions (SMRs). SMRs reveal recurrent alterations across a spectrum of coding and noncoding elements, including transcription factor binding sites and untranslated regions mutated in up to ~15% of specific tumor types. SMRs demonstrate spatial clustering of alterations in molecular domains and at interfaces, often with associated changes in signaling. Mutation frequencies in SMRs demonstrate that distinct protein regions are differentially mutated across tumor types, as exemplified by a linker region of PIK3CA in which biophysical simulations suggest that mutations affect regulatory interactions. The functional diversity of SMRs underscores both the varied mechanisms of oncogenic misregulation and the advantage of functionally agnostic driver identification.

17. **Novel Nrf2 Inhibitors to Combat Chemoresistance in Cancer Therapy**

Vineet Kumar[1], Ramasamy Paulmurugan and Sanjay V. Malhotra

[1]Department of Radiation Oncology and Radiology, Stanford University, CA

Nuclear factor E2-related factor 2 (Nrf2) is a master regulator of oxidative stress and plays an important role in reducing tumorigenic events in normal cells. However, growing evidences have suggested that several human cancers, including lung, breast, colon, ovary and pancreas, have developed mutations, which promote the stability and activity of Nrf2 in these tumors. We screened 150 flavonoid-based compounds from our rationally designed library of polyphenolic compounds, using a novel intact cell-based bioluminescence assay that measures the downstream effect of Nrf2, and identified several Nrf2 inhibitors.

18. **Chemical Genetic Approach Identifies Role of Proton Sensing Gpr68 in Modulation of Migration in Melanoma**

Charles H. Williams; H. Russel day; Charles C. Hong

Department of Cell and Developmental Biology
Department of Medicine, Division of Cardiovascular Medicine, Vanderbilt University Medical Center

Increased glycolysis resulting in local acidification is a hallmark of cancer. However, the mechanisms by which this acidification affects cellular behaviors such as migration are not understood. We report the discovery of Ogremorphin (OGM) a first in class inhibitor of GPR68 in a phenotypic zebrafish screen. The target of OGM was identified through a cheminformatics and receptor profiling, and validated genetically with knock down technology. GPR68 plays a critical role in neural crest development during zebrafish development. GPR68 is proton sensitive GPCR that is maximally active at ~pH6.6, and is upregulated in melanoma cell lines. We show that melanoma are more motile in acidic media. Furthermore, the increased migratory capacity is attenuated by OGM, which attenuates the formation of focal adhesions complexes. This data suggests that GPR68 represents a possible novel pharmacological target for melanoma metastasis.

19. **Human Induced Pluripotent Stem Cell Derived Cardiomyocytes Enable Disease Modelling and Interrogation of Cardiac Diseases**

Malini Pearce, Cellular Dynamics International, Inc., Madison, WI

A major hurdle for cardiovascular disease researchers has been the lack of robust and physiologically relevant cell-based assays for disease modeling and drug discovery. Derivation of hIPSC-cardiomyocytes from healthy and disease populations at high purity, quality, and quantity overcomes this hurdle. Disease modeling with iPSC-tissue cells can be accomplished through induction of a disease phenotype through altered culture conditions, derivation of iPSC tissue cells from disease backgrounds, or the introduction (or removal) of genetic elements associated with the disease. Here we demonstrate the utility of human iPS cell-derived cardiomyocytes as an in vitro model of a cardiac conditions including hypertrophy, diabetic cardiomyopathy, and hypoxia via induced and innate techniques.

20. TRPV1 Regulates Mitochondrial Membrane Potential and Myocardial Reperfusion Injury

Honit Piplani[1,2], Yao Lu, Creed Stary, Carl M. Hurt, Bryce A. Small, Travis J. Urban, Nir Qvit, Garrett J. Gross, Daria Mochly-Rosen, Eric R. Gross

[1]Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University
[2]Department of Chemical and Systems Biology, School of Medicine, Stanford University

The transient receptor potential vanilloid 1 (TRPV1) mediates cellular responses to pain, heat, or noxious stimuli by calcium influx. The cellular localization and function of TRPV1 in the cardiomyocyte is largely unknown. In primary cardiomyocytes, confocal and electron microscopy demonstrate TRPV1 is localized to the mitochondria. Capsaicin, the specific TRPV1 agonist, dose-dependently reduced mitochondrial membrane potential and was blocked by the TRPV1 antagonist, capsazepine, or the calcineurin inhibitor, cyclosporine. We synthesized a peptide, V1-cal, to inhibit the interaction between TRPV1 and calcineurin. These data suggest that TRPV1 is an end-effector of cardioprotection and modulating the TRPV1 protein interaction with calcineurin limits reperfusion injury.

21. Identification of New Drug Candidates Against Borrelia burgdorferi by High-Throughput Screening using BacTiter-Glo Assay

Venkata Raveendra Pothineni[1], Dhananjay Wagh, Mohammed Inayathullah, David Solow-Cordero, Lobat Tayebi, Jayakumar Rajadas[1]

[1]Biomaterials and advanced drug delivery laboratory, Stanford Cardiovascular Pharmacology Division, Cardiovascular Institute, Stanford University School of Medicine, CA

Using a High-Throughput Screening of FDA approved molecular libraries containing 7450 compounds; we identified ~300 unique compounds that inhibit → 90% of Borrelial growth at concentrations below 20μM. These 300 unique compounds comprise many safe antibiotics, non-antibiotics and small molecules from plant sources. Based on these encouraging results, we measured the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for 50 select, FDA approved molecules. The candidates showing the lowest MIC and MBC values were chosen as lead molecules for further tests.

22. Live Cell RNA Labeling Using Spherical Nucleic Acid Nanoparticles (Nanoflares)

Mitch Biermann, Annie Shao, Tianxiao Han, Gary Lyons, Timothy Kamp

University of Wisconsin-Madison School of Medicine

Characterization of specific living cell populations differentiating from human pluripotent stem cells, such as cardiac progenitors, often relies on genetic reporters. Methods to label live cells expressing any RNA will empower progenitor cell biology and drug discovery research. Therefore we pursued nanoflare technology as a method to characterize live progenitor RNA without genetic manipulation. We first developed a nanoflare to Xist, a long non-coding RNA present only in female cells that labeled female H9 hPSCs but not male H1 hPSCs. We then developed a probe for the ventricular progenitor transcription factor Irx4. The synthesized nanoflare had greater than 90% uptake, was nontoxic by Annexin V/7AAD and did not affect expression of cardiac genes, including Irx4. After spontaneous differentiation, nanoflare-separated Irx4+ cells gave rise to cardiomyocytes with greater organization, and sensitive droplet digital RT-PCR detected early upregulation of ventricular lineage-specific mlc2v and Irx4 but not atrial genes (Hey1, CoupTF2, SLN) relative to Irx4Low cells.

23. A New Therapeutic Strategy for GLI-Dependent Cancers

Marisa Hom, Alison Ondrus, Zach Rosenthal, Tomoyo Kato, James Chen

[1]Department of Chemical and Systems Biology, Stanford University School of Medicine
[2]Department of Developmental Biology, Stanford University School of Medicine

While GLI transcription factors (GLI1, GLI2, and GLI3) within the Hedgehog signaling pathway are essential in embryonic and post-natal development, their uncontrolled activation can result in cancers such as basal cell carcinoma and medulloblastoma. Current small-molecule therapies for GLI-dependent cancers target SMOOTHENED, an upstream component of Hedgehog pathway. However, these therapeutics are effective against only a subset of GLI-dependent tumors and are highly susceptible to chemoresistance. By conducting a high-throughput screen, we recently identified a new class of compounds ("glimidazoles") that act downstream of SMOOTHENED to selectively suppress GLI1 activity with nanomolar potencies. Using photoaffinity labeling, we have determined that glimidazoles directly interact with specific members of the aldehyde dehydrogenase (ALDH) family, including ALDH1 enzymes associated with cancer stem cell function.

24. Covalent Inhibitors of Caspase-6 Derived from Disulfide Trapping (Tethering)

Kurt S. Van Horn, Dongju Wang, Priya Jaishankar, Yinyan Tang, Michelle Arkin, Adam Renslo

Small Molecule Discovery Center, University of California-San Francisco

Tauopathies are neurodegenerative diseases characterized by the formation of neurofibrillary tangles that consist of tau protein in the brain. Tau protein is a substrate for the protease caspase-6, which is found associated with tau tangles in post mortem brains of people with Alzheimer's disease. Inhibition of caspase-6 may suppress disease progression, but selective caspase inhibitors are not known. Utilizing tethering technology, we have discovered caspase-6 specific inhibitors by targeting a non-active site cysteine that is not present in other caspases. By improving the electrophile and linker for reactivity and selectivity, we are moving towards covalent small molecule inhibitors that selectively bind to C264 of caspase-6. Crystal structures show that we are opening a new pocket near the active site and further optimization of the small molecule is being pursued to enhance the binding affinity

25. Novel Small Molecules Facilitate Angiogenesis and Vessel Formation

Luqia Hou[1,2], Zak Strassberg, Prajakta Joshi, MS, Shibing Tang, Sheng Ding, Ngan F. Huang

[1]Stanford Cardiovascular Institute, Stanford University
[2]Veterans Affairs Palo Alto Health Care System

ω-(2-carboxyethyl)pyrrole (CEP) is an end product of lipid oxidation during inflammation and wound healing processes. To develop rationale chemical entities that may have further enhanced angiogenic effects, we synthesized a family of ω-(2-carboxyethyl) pyrrole related compounds and investigated their angiogenic effect. The CEPs were designated as CEP 1-6, and they were synthesized using the Grignard reaction and Paal-Knorr pyrrole synthesis. To evaluate the effects of these CEPs on angiogenesis, we compared proliferation and tube-like formation of primary human microvascular endothelial cells in the presence of each CEP. Plugs containing CEP3 demonstrated a 6-fold increase in newly formed capillaries (n=2), relative to matrigel plugs without CEP3. Together, these data suggest that CEP3 has a profound effect in angiogenesis and may have therapeutic potential to enhance vascular formation in ischemic tissues.

26. Sirolimus for the Treatment of Vascular Anomalies in Children: Volumetric Analysis

Joanna H Tu[1,2], Heather I Cohn[1], Huy M Do[1], Kristen Yeom[1], Anne Marqueling[1], Marc Sofilos[1,3], Michael Jeng[1], Joyce M Teng[1]

[1]Stanford University Vascular Anomalies Clinic
[2]Columbia College of Physicians and Surgeons
[3]Stanford University 3DQ Lab

This study evaluated the clinical and radiological outcomes of vascular anomalies in children treated with systemic sirolimus. Retrospective chart review was performed on 9 children treated with sirolimus for venolymphatic malformation (VLM), lymphatic malformation (LM), or arteriovenous malformation (AVM) in our institution for the past three years. Volumetric analysis was completed using pre- and post-treatment MRI data. All 9 subjects were refractory to previous therapies prior to systemic sirolimus treatment. Subjects' ages were 3 months to 17 years. Median treatment duration was 16 (7-35) months. Eight of nine patients had clinical response evident by decreased swelling and pain, but all patients had a greater than 10% decrease in volume. Average volume change was -19.4%. There is no clear correlation between duration of treatment and extent of response. This study concludes that sirolimus can be beneficial for both the symptomatic and volumetric improvement of VLMs, LMs, and AVMs, especially in children.

Note: All authors' affiliations are listed on posters.

NOTES:

cvi.stanford.edu