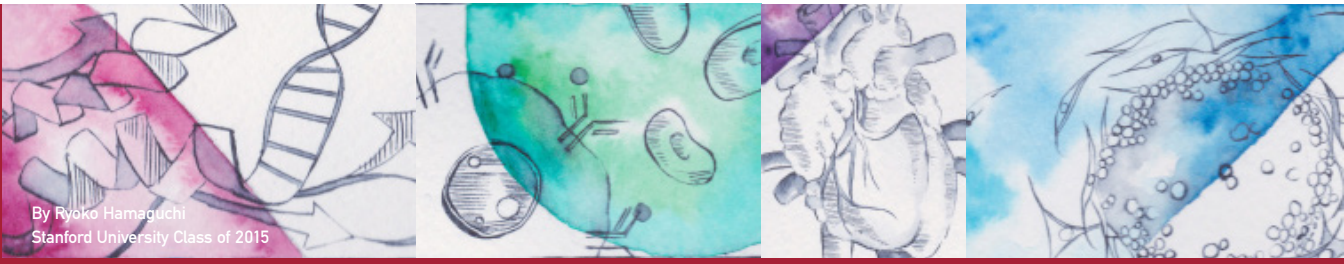


Stanford Drug Discovery Conference

April 24, 2017

Li Ka Shing Center, Stanford University

Stanford Cancer Institute

Stanford Cardiovascular Institute

CIRM
CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

SCHEDULE

8:30 am Registration & Continental Breakfast

9:00 am Introduction: **Joseph C. Wu, MD, PhD**
Director, Stanford Cardiovascular Institute
Simon H. Stertzer, MD, Professor of Medicine (Cardiology) and Radiology

9:05 am Welcome Remarks:
Christopher G. Dawes, MBA
President and Chief Executive Officer
Lucille Packard Children's Hospital

David Entwistle, MHSA
President and Chief Executive Officer
Stanford Health Care

9:15 am *Why Understanding and Treating Neuropsychiatric Disorders is So Difficult*
Keynote: **Thomas Südhof, MD, PhD**
Avram Goldstein Professor, Stanford School of Medicine and Professor, by courtesy, of Neurology and of Psychiatry and Behavioral Sciences
2013 Nobel Laureate

9:55 am Break

BENCH TO BEDSIDE - CARDIOVASCULAR MEDICINE

10:00 am *Protease-Activated Receptors: Bench to Bedside to Bench*
Shaun R. Coughlin, MD, PhD
Director, Cardiovascular Research Institute
Professor in Cardiovascular Biology and Medicine, UCSF

10:20 am *Muscle Making and Muscle Breaking: From Developmental Mechanisms to Therapeutics for Heart and Muscle Disease*
Eric Olson, PhD
Annie and Willie Nelson Professor in Stem Cell Research
Robert A. Welch Chair in Science, Department of Molecular Biology, UT Southwestern

10:40 am *Single Cell Genomics*
Stephen Quake, PhD
Lee Otterson Professor, Stanford School of Engineering and Professor of Bioengineering of Applied Physics and, by courtesy, of Physics
Co-President of the Chan Zuckerberg Biohub

11:00 am Panel Discussion
Chair: **Helen M. Blau, PhD**
Donald and Delia Baxter Foundation Professor, Stanford
Director, Baxter Laboratory for Stem Cell Biology

11:20 am *Research Program Overview: iPSC Biobank Genomics Initiative*
Stephen Lin, PhD
Senior Science Officer, Strategic Infrastructure
California Institute for Regenerative Medicine

11:40 am Networking Lunch & Poster Viewing

INDUSTRY - PROMISES & CHALLENGES

1:00 pm *The New Science of Therapeutics*
James (Jay) E. Bradner, MD
President, Novartis Institutes for BioMedical Research

1:20 pm *Realizing the Promise of Human Genetics in Drug Discovery and Development*
Sean E. Harper, MD
Executive Vice President, Research and Development, Amgen

1:40 pm *Innovation Driven Research and Development*
Andrew Plump, MD, PhD
Chief Medical and Scientific Officer, Takeda Pharmaceuticals

2:00 pm *Humanizing Drug Discovery*
David Altshuler, MD, PhD
Executive Vice President, Global Research and Chief Scientific Officer
Vertex Pharmaceuticals

2:20 pm Panel Discussion
Chair: **Robert A. Harrington, MD**
Arthur L. Bloomfield Professor of Medicine
Chair, Stanford Department of Medicine

2:40 pm Break

BENCH TO BEDSIDE - CANCER THERAPIES

2:50 pm *Synthetic Lethal Approaches to Cancer Therapy*
Alan Ashworth, PhD, FRS
Director, UCSF Helen Diller Family Comprehensive Cancer Center
Senior Vice President, Cancer Services, UCSF Health

3:10 pm *Structure Guided Strategies to Optimize Cancer Therapeutics*
Gideon Bollag, PhD
Chief Executive Officer, Plexxikon

3:30 pm *Translating Disoveries to Cures - Designing Early Phase Trials of Anticancer Agents*
Shivaani Kummar, MD
Professor of Medicine (Oncology) and of Radiology (Molecular Imaging), Stanford
Director, Phase I Clinical Research Program, Division of Oncology

3:50 pm Panel Discussion
Chair: **Beverly S. Mitchell, MD**
Director, Stanford Cancer Institute
George E. Becker Professor in Medicine and Professor, by courtesy, of Chemical and Systems Biology

4:10 pm *Key Elements of the Drug Development Ecosystem*
Keynote: **Robert Califf, MD, MACC**
Donald F. Fortin Professor of Cardiology, Duke School of Medicine
Former Commissioner of U.S. Food and Drug Administration

4:50 pm Closing

5:00 pm Reception and Research Poster Awards

SPEAKER BIOS

WELCOME REMARKS

David Entwistle, MHSA

Mr. Entwistle is the President and Chief Executive Officer of Stanford Health Care (SHC). As a recognized healthcare thought leader, he is leading the way to innovate across the continuum of care and the 2018 expansion of the new Stanford Hospital.

Christopher G. Dawes, MBA

Mr. Dawes is the President and Chief Executive Officer at the Lucile Packard Children's Hospital (LPCH). A long advocate for children's health, he has led LPCH and Stanford Children's Health towards tremendous growth and success in clinical, research, education and community benefit programs.

KEYNOTE SPEAKERS

Thomas Südhof, MD, PhD

Dr. Südhof is the 2013 Noble Laureate for discoveries of key machinery regulating vesicle traffic, a major transport system in our cells. He is the Avram Goldstein Professor in the School of Medicine and Professor, by courtesy, of Neurology and of Psychiatry and Behavioral Sciences. He is also an Investigator of the Howard Hughes Medical Institute. His group studies how synapses form in the brain and the molecular basis for synaptic information transfer.

Robert M. Califf, MD

Dr. Califf is the Donald F. Fortin, MD, Professor of Cardiology at the Duke University School of Medicine and the Chair of the People-Centered Research Foundation, a new organization formed by PCORnet investigators. He is also the former Commissioner of U.S. Food and Drug Administration (FDA).

SPEAKERS

Shaun R. Coughlin, MD, PhD

Dr. Coughlin is the Director of Cardiovascular Research Institute and Professor in Cardiovascular Biology and Medicine at the University of California, San Francisco (UCSF). His lab focuses on mechanisms and roles of protease signaling and other signaling mechanisms in cardiovascular homeostasis and development.

Eric Olson, PhD

Dr. Olson is the Annie and Willie Nelson Professor in Stem Cell Research, Pogue Distinguished Chair in Research on Cardiac Birth Defects, and The Robert A. Welch Distinguished Chair in the Department of Molecular Biology at UT Southwestern Medical Center. His lab focuses on the mechanisms of muscle development and disease.

Stephen Quake, PhD

Professor Quake is the Lee Otterson Professor in the Stanford School of Engineering and Professor of Bioengineering, of Applied Physics and, by courtesy, of Physics. He is also the Co-President of the Chan Zuckerberg Biohub. His research focuses on developing new approaches to biological measurement and applying these approaches to problems of both fundamental and medical interest. Areas of interest include genomic diagnostics, systems biology, microbial ecology, and single cell genomics.

Helen M. Blau, PhD (Panel Chair)

Dr. Blau is the Donald E. and Delia B. Baxter Foundation Professor and Director of the Baxter Laboratory for Stem Cell Biology at Stanford. Her lab studies muscular dystrophies and cardiomyopathies using patient-derived induced pluripotent cells (iPS), deciphering dynamic regulatory networks controlling nuclear reprogramming, and developing molecular approaches to rejuvenate cells to combat aging.

James (Jay) E. Bradner, MD

Dr. Bradner is the President of the Novartis Institutes for BioMedical Research and a member of the Executive Committee of Novartis. Prior to joining Novartis, Dr. Bradner was a member of the research faculty at Harvard Medical School and an attending physician in stem cell transplantation within the Department of Medical Oncology at the Dana-Farber Cancer Institute. The research focus of the Bradner laboratory has been the study of BET bromodomain proteins and their function in gene control, innovating chemical probes and investigational drugs to study and treat cancer.

Sean E. Harper, MD

Dr. Harper is the Executive Vice President of Research and Development at Amgen. He has also held several leadership roles, including heading the establishment of Amgen's Medical Sciences capability, a translational medicine group focused on biomarker discovery, development, and integration into early phase clinical studies. Dr. Harper served as the Vice President of Global Regulatory Affairs and Safety before assuming the role of Senior Vice President of Global Development and Corporate Chief Medical Officer. Prior to Amgen, Dr. Harper was the Senior Director of Clinical Genomics at Merck Research Laboratories.

Andrew Plump, MD, PhD

Dr. Plump is the Chief Medical and Scientific Officer of Takeda Pharmaceuticals and is a member of the company's Board of Directors. He leads Takeda's global Research & Development organization, where he provides strategic direction and oversight for all Takeda research and development activities globally. Prior to Takeda, Dr. Plump served as Senior Vice President of Research and Translational Medicine, Deputy to the President of R&D at Sanofi, and as Vice President, Worldwide Cardiovascular Research Head of Merck.

David Altshuler, MD, PhD

Dr. Altshuler is the Executive Vice President of Global Research and Chief Scientific Officer at Vertex Pharmaceuticals. Dr. Altshuler leads Vertex's research efforts aimed at discovering new medicines for the treatment of CF and other diseases. Previously, he was one of the founding members, Deputy Director and Chief Academic Officer at the Broad Institute of Harvard and MIT and professor at Harvard and MIT, and a physician at Massachusetts General Hospital. He was a leader of the SNP Consortium, HapMap and 1,000 Genome Projects, and discovered over 100 gene variants associated with type 2 diabetes and other common diseases. A member of the National Academy of Medicine and the American Academy of Arts and Sciences, Dr. Altshuler was named a Champion of Change by the White House for his leadership in creating and leading the Global Alliance for Genomics and Health.

Robert A. Harrington, MD (Panel Chair)

Dr. Harrington is the Arthur L. Bloomfield Professor of Medicine and Chair of the Department of Medicine at Stanford University with 530 faculty members in 15 divisions. His research interests include evaluating antithrombotic therapies to treat acute ischemic heart disease and to minimize the acute complications of percutaneous coronary procedures, studying the mechanism of disease of the acute coronary syndromes, understanding the issue of risk stratification in the care of patients with acute ischemic coronary syndromes, building local, national and international collaborations for the efficient conduct of innovative clinical research and trying to better understand and improve upon the methodology of clinical trials. In 2016, he was inducted into the National Academy of Medicine/Institute of Medicine.

Alan Ashworth, PhD

Dr. Ashworth is the President of the UCSF Helen Diller Family Comprehensive Cancer Center and the Senior Vice President for Cancer Services of UCSF Health. He was part of the team that discovered BRCA2, and identified a way exploit genetic weaknesses in cancer cells including mutated BRCA1 and BRCA2 using PARP inhibitors, leading to a new approach to cancer treatment.

Gideon Bollag, PhD

Dr. Bollag is the Chief Executive Officers of Plexxikon, a member of the Daiichi Sankyo group based in Berkeley, CA. He leads the drug discovery and development efforts, and management of internal and external resources necessary to build a pipeline of compounds for cancer and other diseases. Among other achievements, the Plexxikon team discovered the RAF inhibitor Zelboraf, an FDA-approved product that has significantly impacted not only the treatment of metastatic melanoma but also the understanding of basic RAF biology. More recently, the CSF1R inhibitor pexidartinib has advanced to phase 3 clinical evaluation, achieving Breakthrough Therapy designation from the FDA. Prior to Plexxikon, he was at Onyx Pharmaceuticals and led the team that discovered Nexavar and Ibrance, both now approved cancer treatments.

Shivaani Kummar, MD

Dr. Kummar is Professor of Medicine (Oncology) and of Radiology (Molecular Imaging Program) and Director of Phase I Clinical Research Program at Stanford Medical Center. She also serves as Director of the Translational Oncology Program at the Stanford Cancer Institute. Her research interests focus on integrating laboratory correlates into early phase trials to inform and expedite drug development.

Beverly S. Mitchell, MD (Panel Chair)

Dr. Mitchell is the Director of the Stanford Cancer Institute and is the George E. Becker Professor in Medicine and Professor, and by courtesy, of Chemical and Systems Biology. Dr. Mitchell's research relates to the development of new therapies for hematologic malignancies. She is also interested in preclinical proof of principle studies on mechanisms inducing cell death and on metabolic targets involving nucleic acid biosynthesis in malignant cells.

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

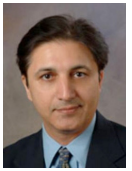
Stephen Lin, PhD

Dr. Lin is a Senior Science Officer at the California Institute for Regenerative Medicine (CIRM). He oversees its $32M initiative to create a repository of iPSCs covering both genetically complex and rare diseases, as well as the $40M genomic initiatives that applied cutting edge genomics and bioinformatics approaches to stem cell research and therapeutic development.

ORGANIZING COMMITTEE

Joseph C. Wu, MD, PhD

Dr. Wu is the Director of the Stanford Cardiovascular Institute and the Simon H. Stertzer, MD, Professor of Medicine (Cardiology) and Radiology. His group studies the biological mechanisms of adult stem cells, embryonic stem cells, and pluripotent stem cells. Using a combination of approaches, including next generation sequencing, tissue engineering, and molecular imaging technologies, his research aims to uncover novel treatment for cardiovascular diseases.



Sanjay Malholtra, PhD

Dr. Malhotra is the Associate Professor (Research) of Radiation Oncology (Radiation and Cancer Biology) and of Radiology (Molecular Imaging Program) at Stanford. His laboratory employs the tools of synthetic medicinal chemistry, molecular modeling and chemical biology for translational research in drug discovery, development, imaging and radiation.

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 the development of small molecule drugs.

Hana Lee, MPH

Hana Lee is the Associate Director of the Stanford Cardiovascular Institute and leads program planning and operations of the Institute. Prior to this role, she was the Director of Strategic Initiatives at the Stanford Center for Population Health Sciences and an epidmiologist at the University of California, San Francisco.

ABSTRACTS

1. Exosomes from damaged human iPSC-derived cardiomyocytes are cardioprotective against myocardial injury

Ji-Hye Jung, Michelle R. Santoso, Phillip Yang

Stanford Cardiovascular Institute, Division of Cardiovascular Medicine, Stanford University School of Medicine

Despite the tremendous worldwide effort on research for over decades, myocardial infarction (MI) is still one of the leading causes of death with a high rate of morbidity and mortality. In recent years, rapid emergence of induced pluripotent stem cells (iPSCs) and iPSC-derived cardiomyocytes (iCMs) has presented a valuable opportunity to replace the functional cells to the heart. Although the therapeutic effects of iPSC-derived cells have been attempted in many preclinical studies, the successful engraftment has been limited and the underlying mechanisms of iPSC-derivatives are still unclear. Recent evidence indicates that the stem cells exert their therapeutic action via paracrine mechanisms through exosomes. They contain unique cytoplasmic microRNAs (miRNAs) and proteins that function as intercellular messengers and effectors, controlling a wide spectrum of genetic regulation. However, the mechanism of endogenous cardiac regeneration and enhanced cardioprotection is poorly understood. Our study found significantly improved survival rate in the exosome treated hypoxic-injured iCM group than the control group. We have identified altered expression patterns of miR-106a-363 cluster in the exosome from iCMs in the hypoxic condition compared to the normoxic condition. Our data showed that exosomal miR-106a-363 cluster has anti-apoptotic effect and resistance to oxidative stress on hypoxic-injured iCMs. Taken together, these results indicate that the exosomes and their miR-106a-363 cluster released from damaged iCMs exert a protective role against oxidative stress to prevent apoptosis of cardiac cell types under ischemic injury.

2. Toward small-molecule inhibitors against human immune checkpoint PD-1

Shaogeng "Steven" Tang, Lindsay N. Deis, Peter S. Kim

Department of Biochemistry, Institute of ChEM-H, Stanford University, Stanford, CA

Immune checkpoints that normally suppress the immune system are often co-opted by cancer cells for evasion. FDA-approved monoclonal-antibody (mAb) therapies against immune-checkpoint proteins, including programmed cell death protein 1 (PD-1), have shown dramatic clinical responses. However, these therapies may have side effects, including a life-threatening runaway immune response, which can be exacerbated by the long half-lives of mAbs. Small-molecule drugs would offer safety advantages due to their shorter half-lives and efficacy advantages resulting from increased penetration and distribution within the tumor microenvironment. To date, no small molecule PD-1 inhibitors have been identified, likely

due to the flatness of the PD-1 surface. Strikingly, upon binding its ligands PD-L1 or PD-L2, PD-1 undergoes structural rearrangements, forming a large cavity in the receptor-ligand interface to accommodate ligand binding. In this proposal, I hypothesize that the PD-1 cavity in the ligand-binding interface is a druggable pharmacophore. I aim to characterize the ligand-induced structural rearrangement of human PD-1 and to isolate antibody fragments that stabilize a surface-exposed PD-1 cavityMy work would enhance the understandings of the conformational dynamics of PD-1 to enable small-molecule drug discovery. My work would also have broad implications for the development of small molecules against traditionally "non-druggable" protein-protein interactions.

3. A novel nanoformulation for the targeted delivery of shikonin at the tumor microenvironment

Efthymia-Iliana Matthaiou,[1,2] Jaleh Barar,[1,3] Yadollah Omidi, [1,3] Raphael Sandaltzopoulos,[2] Chunsheng Li,[1] George Coukos[1,4]

[1]Ovarian Cancer Research Center, School of Medicine, University of Pennsylvania,[2] Department of Molecular Biology and Genetics, School of Medicine, Democritus University of Thrace, [3]Tabriz University of Medical Sciences, [4]Ludwig Cancer Research Center, School of Medicine, University of Lausanne

Shikonin (SHK) belongs to a class of necroptotic inducers that bypasses cancer drug resistance. Despite having immunomodulatory effects, SHK has been toxic to all cancer cell lines studied so far. Tumor Endothelial Marker-1 (TEM1) is expressed by tumor stromal cells and tumor vascular endothelial cells in various cancers and is promoting tumor angiogenesis, proliferation, migration, and metastasis. Combining the above with nanodelivery could be an important strategy against cancer. To deliver SHK solely to tumor microenvironment, biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were formulated and modified with polyethylene glycol and anti-TEM1 antibody fragment (78Fc). Electron microscopy/ dynamic light scattering revealed smooth spherical shape, size of ~120nm and ζ potential of –30mV. Drug-entrapment and 78Fc-bioconjugation reached ~92% and ~90% respectively. SHK showed sustained-release profile which fitted various kinetics models. Targeting efficacy in vivo was studied using the MS1 mouse model. SHK-loaded, 78Fc-armed NPs showed higher accumulation and toxicity at the TEM1-positive tumors compared to free drug/unarmed-NPs. TC1 subcutaneous and intravenous/metastatic mouse models were selected to evaluate the therapeutic efficacy. In both models, 78Fc-armed NPs improved SHK efficacy dramatically compared to unarmed-NPs/free drug. Furthermore, the 78Fc-armed NPs had better therapeutic effect to C57/BL6 mice compared to nu/nu strain, suggesting immunostimulation. 78Fc-armed NPs-treated mice that were tumor free after treatment were subjected to tumor rechallenge. Tumors were eliminated in few days after rechallenge, while our ELISPOT results indicated specific immune response to tumor cells' antigens. These findings suggest that our nanodelivery platform not only ameliorates SHK therapeutic efficacy but also triggers an immune response.

4. Bioactive lipids stimulate human induced pluripotent stem cell-derived dardiomyocyte cell cycle activity and differentiation

Yuan Zhang[1], Arun Sharma[1,2*], Nina Kosaric[2], Paul W. Burridge[1+], Jan Willem Buikema[1], Jared M. Churko[1], Elda Dzilic[1,3], Alice Shieh1, Joseph C. Wu[1,2,4], Sean M. Wu[1,2,4]*
**These authors contributed equally to this work.*

[1]Stanford Cardiovascular Institute, Stanford University School of Medicine, [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, [3]Department of Cardiovascular Surgery, German Heart Centre Munich, Technical University Munich, [4]Department of Medicine, Division of Cardiology, Stanford University School of Medicine

+Current address: Department of Pharmacology and Center for Pharmacogenomics, Northwestern University Feinberg School of Medicine

Bioactive lipids such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) regulate cellular processes including proliferation, differentiation, and migration. However, their roles in human cardiomyocyte function are poorly understood. We employed human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as a surrogate for human cardiomyocytes and examined the ability of biolipids to stimulate CM proliferation and differentiation. We discovered that S1P/LPA initiated cell cycling in cardiomyocytes, as demonstrated by increased ERK signaling and ki67 expression. However, the overall cardiomyocyte number was unchanged, suggesting no cell division. We also established a hiPSC-CM differentiation platform and showed that S1P/LPA can enhance cardiomyocyte formation. Furthermore, we showed that combined S1P/LPA treatment increased nuclear accumulation of Wnt pathway mediator β-catenin. In summary, we demonstrate a biphasic role of bioactive lipids to enhance hiPSC mesodermal differentiation and hiPSC-CM cell cycle reentry. These findings will help improve hiPSC-CM generation for cardiac disease modeling, precision medicine, and regenerative therapies.

5. Novel Small molecule facilitate angiogenesis and vessel formation through activating endothelial progenitor cells

Luqia Hou, PhD[1,2], Shibing Tang*, PhD[3], Cynthia Alcazar, BS[1,2], Zak Strassberg, BS[1,2], Prajakta Joshi, MS[1,2], Masashi Kawamura, MD[1,5], Y. Joseph Woo, MD[1,5], Sheng Ding, PhD[3,4], Ngan F. Huang, PhD[1,2,5]*
(Submitted by Shibing Tang. To be presenter.)

[1] Stanford Cardiovascular Institute, Stanford University, Stanford, CA[2] Veterans Affairs Palo Alto Health Care System, Palo Alto, CA [3] Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA [4] Department of Pharmaceutical Chemistry, University of California, San Francisco, CA [5] Department of Cardiothoracic Surgery, Stanford University, Stanford, CA

ω-(2-carboxyethyl)pyrrole (CEP) protein adducts are end products of lipid oxidation during inflammation and wound healing processes. CEP protein adducts are associated with enhanced angiogenesis through activation of toll-like receptor 2 (TLR2) signaling pathway. To develop rationale chemical entities with enhanced angiogenic effects and druglikeness, we synthesized a family of small-molecule CEP compounds, designated as CEP 1-6 that were fully characterized to ensure their structures and purity. Next, we compared proliferation and tube-like formation of primary human microvascular endothelial cells in the presence of each CEP. The results showed that CEP3 has profound effects in angiogenesis over the rest of the CEPs. After 24 hour incubation, cell proliferation in the presence of CEP3 increased by 26% and 27% under normoxic and hypoxia (1% O2) conditions, respectively, relative to vehicle control. Besides, primary endothelial cells treated with CEP3 demonstrated a 72% and 76% increase reagarding to tube-formation ability under normoxic and hypoxic conditions, respectively. Furthermore, we co-injected CEP3 with matrigel into subcutaneous pockets of mice to validate its activity in vivo. The matrigel plugs containing CEP3 demonstrated a 6-fold increase in newly formed capillaries, relative to matrigel plugs without CEP3. More importantly, injection of matrigel plugs containing CEP3 in mouse hind limb ischemia models not only significantly enhanced micro vessel formation but also improved blood perfusion by more than 100%. Together, these data suggest that CEP3 has a profound effect in angiogenesis and vessel formation and may have therapeutic potential to enhance vascular formation in ischemic tissues.

6. ApoE2, ApoE3 and ApoE4 differentially stimulate amyloid precursor protein transcription and amyloid-beta secretion in human neurons

Yu-Wen Alvin Huang,[1] Marius Wernig,[2] and Thomas C. Südhof[1]

[1]Department of Molecular and Cellular Physiology and [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University Medical School

Human apolipoprotein E (ApoE) apolipoprotein is primarily expressed in three isoforms (ApoE2, ApoE3, and ApoE4) that differ only by two residues. ApoE4 constitutes the most important genetic risk factor for Alzheimer's disease (AD), ApoE3 is neutral, and ApoE2 is protective. How ApoE isoforms influence AD pathogenesis, however, remains unclear. Central to AD pathology is oligomerization and deposition of peptide amyloid-beta (Abeta) in brain. Using ES-cell-derived human neurons, we show that ApoE secreted by glia stimulates neuronal A-beta production with an ApoE4 > ApoE3 > ApoE2 potency rank order. We demonstrate that ApoE binding to ApoE receptors activates dual leucine-zipper kinase (DLK), a MAP-kinase kinase kinase that then activates MKK7 and ERK1/2 MAP kinases. Activated ERK1/2 induces cFos phosphorylation, stimulating the transcription factor AP-1, which in turn enhances transcription of A-beta precursor protein (APP) and thereby increases A-beta levels. This molecular mechanism also regulates APP transcription in mouse brain. Our data describe a novel signal transduction pathway in neurons whereby ApoE activates a non-canonical MAP kinase cascade that enhances APP transcription and A-beta synthesis.

7. Disrupting the Aurora Kinase A interactome in pediatric cancer

Mukherjee, S., Tu, C., Meyerowitz, J. G., Nekritz, E. K., Chen, J., Benes, C., Charron, E.A., Simonds, E., Benes, C., Eilers, M., Matthay, K. K., Seeger, R., Hertz, N. T., Shokat, K.M., Weiss, W. A., and Gustafson, W. C.

University of California, San Francisco, San Francisco, CA

Neuroblastoma is the most common extra-cranial solid tumor of childhood. Amplification of the MYCN proto-oncogene occurs in 50% of the high-risk neuroblastoma and indicates an aggressive and lethal form of the disease. As a transcription factor with no apparent small-molecule surfaces, direct inhibition of MYCN has proven challenging. Proteolytic cleavage of MYCN is regulated in part by an activity-independent scaffold function of Aurora Kinase A (AURKA). The dynamic expression and activation of AURKA allows for precise cell cycle regulation of several substrates identified by protein-protein interaction studies that are also viable anti-cancer targets, such as the aforementioned MYCN oncoprotein. Utilizing co-immunopreciptation, flow cytometry, and other pertinent cell biology techniques, we aim to delineate a novel mechanism of targeting the AURKA interactome by conformation disruption of AURKA. We have recently described a neoteric class of confirmation disrupting “amphosteric” inhibitors of AURKA (CD-AURKAi) that orthosterically inhibit the ATP-binding pocket to dramatically disrupt the active conformation of AURKA to dissociate and degrade MYCN, a critical oncogenic driver of the pediatric cancer neuroblastoma. In addition to MYCN, our preliminary results show CD532 will also dissociate other oncogenic proteins that interact with AURKA. We hypothesize that conformation disruption of AURKA and subsequent blockade of an array of protein-protein interactions will delineate the roles of AURKA, MYC, and MYCN in the cell cycle and expand the clinical applications for CD-AURKAi in cancer to include non-MYCN driven diseases.

8. First-in-class neuroprotective nanodrug: selective antagonist of extrasynaptic NMDA receptors

Alex Savtchenko[1,2], Gary B. Braun[3], Audrey S. Dickey[2], Albert La Spada[2], Elena Molokanova[4]

[1]Stanford University, Stanford, CA 94305; [2]Department of Pediatrics, University of California, San Diego, CA; [3]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; [4]NTBS, Encinitas, CA

Glutamatergic cytotoxicity mediated by overactivation of NMDA receptors (NMDARs) is implicated in numerous neurological disorders, from acute hypoxic-ischemic brain injury to chronic neurodegenerative diseases such as Alzheimer’s, Huntington’s, and Parkinson’s diseases, and amyotrophic lateral sclerosis. Traditional NMDAR antagonists are not well-suited for therapeutic intervention, because they can adversely interfere with a critical role that synaptic NMDARs (sNMDARs) play in numerous physiological processes, including developmental plasticity, learning, and memory. To be therapeutically viable, NMDAR

antagonists must preserve physiological role of sNMDARs in neurotransmission, and block only excessive pathological activation of NMDARs. Here we present a novel NMDAR antagonist that satisfies this two-fold requirement by exploiting spatial differences in NMDAR subcellular locations. Specifically, we designed a hybrid nanodrug (AuM) to be larger than the synaptic cleft by attaching memantine, NMDAR antagonist, via polymer linkers to a gold nanoparticle. We show that AuM efficiently and selectively inhibited extrasynaptic NMDARs (eNMDARs), while having no effect on sNMDARs and synaptic transmission. AuM exhibited neuroprotective properties both in vitro and ex vivo during such neurotoxic insults as NMDAR-mediated cytotoxicity in cerebrocortical cell culture, and oxygen-glucose deprivation in acute hippocampal slices. AuM also prevented dendritic spine loss triggered by Aβ oligomers in organotypic hippocampal slices, and was more effective than free memantine. Furthermore, in the quinolinic acid-based model of Huntington's disease (HD) model, AuM decreased the cell death and mHtt aggregates in in hippocampal neurons from wild-type and transgenic HD mice. These findings validate a novel rational drug-design strategy for a new class of neuroprotective drugs.

9. Human iPSC-derived ECs as a platform for drug discovery in LMNA-related dilated cardiomyopathy

Nazish Sayed[1], Chun Liu[1], Farhan Himmati[1], Joe Zhang[1], Vittavat Termglinchan[1], Jan-Ranier Moonen[2], Jon Stack[1], Haodong Chen[1], Elena Matsa[1], Karim Sallam[1], Marlene Rabinovitch[2], Joseph C. Wu[1]

[1]Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA [2]Department of Pediatrics, Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA

Mutations in the gene that encodes the nuclear envelope proteins lamin A and C (LMNA) are now considered to be the most common cause of DCM. However, the molecular mechanisms that underlie "cardiolaminopathy" remain elusive, and it is unknown why mutations in this ubiquitously expressed gene have such a disproportionate effect on the heart. Despite the fact that LMNA is abundantly expressed in endothelial cells (ECs) and mutations in LMNA are known to induce EC dysfunction, little is known about the EC-specific phenotype of LMNA-related DCM. As EC dysfunction has been known to contribute to DCM, we hypothesize that EC dysfunction due to LMNA mutation has a significant impact on the pathogenesis and disease progression of DCM. Intriguingly, our preliminary data showed that iPSC-ECs derived from patients harboring the LMNA-mutation exhibit decrease functionality as seen by impaired angiogenesis and decreased NO production. Similarly, genome editing of isogenic iPSC lines enabled us to recapitulate the EC disease phenotype further allowing us to dissect the effects of LMNA mutations on EC function. Furthermore, whole genome RNA-sequencing identified Krüppel-like Factor 2 (KLF2) as a potential transcript responsible for EC dysfunction in LMNA-mutated patients. Importantly, treatment of LMNA-mutated ECs with KLF2 agonists rescued the EC dysfunction. This study is a first step towards understanding the molecular mechanisms of cardiolaminopathy by modeling endothelial dysfunction using patient-specific iPSCs. Results from this work could potentially lead to new strategies that could improve the management of DCM patients.

10. A first-in-class inhibitor of parasite FtsH disrupts plastid biogenesis in human pathogens

Katherine Amberg-Johnson[1,3], Suresh M. Ganesan[4], Hernan A. Lorenzi[5], Jacquin C. Niles[4], Ellen Yeh[1,2,3,]*
*Corresponding author

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There is an urgent need for antimalarials with distinct mechanisms-of-action to combat resistance to frontline drugs. The malaria parasite Plasmodium falciparum and related apicomplexan pathogens contain an essential, non-photosynthetic plastid organelle, the apicoplast, which is a key antiparasitic target. Despite its biomedical potential, broadly effective antimalarials targeting the apicoplast have been elusive due to the slow onset-of-action of drugs that inhibit apicoplast translation and the apicoplast's limited metabolic function in the symptomatic stage of Plasmodium. Apicoplast biogenesis depends on novel, but largely cryptic, mechanisms for protein/lipid import and organelle inheritance during parasite replication. These critical pathways present untapped opportunities to discover new parasite-specific drug targets. We used an innovative chemical rescue screen to identify the natural product antibiotic, actinonin, as a first-in-class antimalarial compound inhibiting apicoplast biogenesis. Both chemical-genetic interaction and resistant mutation indicated that the unexpected target of actinonin in P. falciparum and Toxoplasma gondii is FtsH1, a homolog of a bacterial membrane AAA metalloprotease. We show that PfFtsH1 is essential for apicoplast biogenesis and parasite replication, making it the first apicomplexan-specific regulator of organelle biogenesis to be identified in a forward screen. Taken together, our findings demonstrate that FtsH1 is a novel and, importantly, druggable antimalarial target. Development of actinonin derivatives as FtsH1 inhibitors will have significant advantages over existing apicoplast-targeting compounds with improved drug kinetics, lower potential for clinical resistance, and multistage efficacy against multiple human parasites.

11. Discovery of a new class of anesthetic agents

Ed Bertaccini, M. Frances Davies (Submitted by Frances Davies)

Stanford University School of Medicine

All currently used intravenous anesthetic agents are associated with an entire spectrum of undesirable side effects, most notably cardiovascular instabilities. These side effects are poorly tolerated in all surgical patients without our intervention, but especially in very young children who possess immature physiologic compensatory mechanisms, as well as in the elderly with confounding comorbidities and otherwise

exhausted compensatory mechanisms. In light of this, we have pursued the development of new lead compounds to produce the next generation of safer anesthetic agents. Our methodologies of in silico screening and prediction of compounds which bind to our validated model of the gamma amino butyric acid receptor (GABAR) have now identified a class of lead compounds which demonstrate overt anesthetic activity in both tadpoles and rats with a potency greater than that of propofol, the current intravenous anesthetic standard. Of even greater importance is the fact that our new class of compounds shows minimal to no suppression of blood pressure, in stark contrast to the deleterious hemodynamic effects of propofol. Electrophysiologic analyses of our compounds are consistent with a GABAR mechanism. These compounds are derived from novel chemical structures, and are the subject of patent filing through the Stanford Office of Technology and Licensing. We are now refining these lead compounds via our current state-of-the-art techniques in molecular modeling, in vitro ion channel and brain slice electrophysiology, as well as extended behavioral and physiologic analyses in mammals so as to design the next generation of safer anesthetic agents.

12. Machine learning harnesses molecular dynamics to enrich opiate efficacy prediction

Evan N. Feinberg, Amir Barati Farimani, Vijay S. Pande

Stanford University

Computational chemists typically assay drug candidates by virtually screening compounds against crystal structures of a protein despite the fact that some targets, like GPCRs, traverse many non-crystallographic states. The μ Opioid Receptor (μOR) – the primary target for clinically-used opioid analgesics – exemplifies such conformational diversity. We discover new conformational states of μOR with molecular dynamics simulation and then machine learn ligand–structure relationships to substantially improve prediction of opioid ligand function.

13. Restoration of impaired diastolic function in hypertrophic cardiomyopathy induced pluripotent stem cell-derived cardiomyocytes by re-balancing the calcium homeostasis

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Diastolic dysfunction is commonly seen in hypertrophic cardiomyopathy (HCM). However, the cellular mechanism is not fully understood, and no effective treatment so far has been developed. We hypothesize

here that HCM patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) can recapitulate the cellular mechanism, and provide us a platform for mechanistic study and for drug screening of diastolic dysfunctions in HCM. We generated beating iPSC-CMs from healthy individuals and HCM patients carrying familial mutations (MYH7 R663H (n=2 lines) and MYBPC3 R943ter (n=2 lines)). Sarcomere shortening measurement in patterned iPSC-CMs with live cell confocal imaging showed significantly prolonged diastolic phase and slower relaxation velocity in HCM iPSC-CMs compared to WT cells. To elucidate the cellular mechanism, Fura-2 AM ratiometric calcium imaging showed marked elevation of resting calcium level and increased abnormal calcium handlings in HCM iPSC-CMs, which were exaggerated by β-adrenergic activation with isoproterenol. By applying calcium transient and contractile force simultaneous recording, we defined a "risk index of diastolic dysfunction" (measured as transient-contraction gain factor), which was significantly increased in HCM iPSC-CMs. Thus, both elevated basal calcium level and increased calcium sensitivity of myofilament contribute to the abnormal diastolic function in HCM iPSC-CMs. Gene expression profiling of HCM and WT iPSC-CMs indicated that increased calcium channels may underlie the increased basal calcium concentration in HCM cells. Indeed, partially blocking the calcium influx by calcium blockers reset the basal calcium level, attenuated calcium mishandling, and restored the diastolic function in HCM iPSC-CMs. Moreover, re-balancing calcium homeostasis significantly improved long-term survival rate of HCM iPSC-CMs at both basal level and under β-adrenergic stress. The iPSC-CM models carrying patient-specific HCM mutations recapitulated diastolic dysfunction on single cell level. Future studies using these platform may reveal additional novel cellular mechanisms and therapeutic targets of diastolic dysfunction in HCM disease.

14. Non-genetic purification of ventricular cardiomyocytes from differentiating embryonic stem cells through molecular beacons targeting IRX-4

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Isolation of ventricular cardiomyocytes (vCMs) has been challenging due to the lack of specific surface markers. Here we show that vCMs can be purified from differentiating mouse embryonic stem cells (mESCs) using molecular beacons (MBs) targeting specific intracellular mRNAs. We designed MBs (IRX4 MBs) to target mRNA encoding Iroquois homeobox protein 4 (Irx4), a transcription factor specific for vCMs. To purify mESC vCMs, IRX4 MBs were delivered into cardiomyogenically differentiating mESCs, and IRX4 MBs-positive

cells were FACS-sorted. We found that, of the cells isolated, ~98% displayed vCM-like action potentials by electrophysiological analyses. These MB-purified vCMs continuously maintained their CM characteristics as verified by spontaneous beating, Ca2+ transient, and expression of vCM-specific proteins. Our study shows the feasibility of isolating pure vCMs via cell sorting without modifying host genes. The homogeneous and functional ventricular CMs generated via the MB-based method can be useful for disease investigation, drug discovery, and cell-based therapies.

15. Telomere shortening as a hallmark of lethal dilated cardiomyopathy

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Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive disease that results from mutations in the dystrophin gene and is the most common myopathic disease in humans with a prevalence of one in every 3500 males. Dystrophin is crucial for the formation of a dystrophin-glycoprotein complex (DGC), which connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix in both skeletal and cardiac muscles. In the heart, loss of dystrophin leads to increased fibrosis and death in the third decade of life due to dilated cardiomyopathy. A conundrum in studying and developing therapies for DMD has been the lack of a mouse model that fully recapitulates the clinical phenotype. When we 'humanized' the mdx mouse by creating a novel mouse model with shortened telomere lengths (similar to humans), both the skeletal muscle and cardiac muscle phenotype of DMD culminating in lethal dilated cardiomyopathy (DCM) were fully recapitulated (Cell. 2010; Nat Cell Biol. 2013; PNAS 2016). Moreover, we found that the cardiomyocytes in four hearts of patients with DMD had telomeres 50% the length of unaffected controls. Notably, telomere lengths in smooth muscle cells within the same cardiac tissues are unaffected, serving as robust internal controls for the specificity of telomere shortening to those cells requiring dystrophin for function. To study telomere shortening in the course of disease progression, we generated three DMD human induced pluripotent stem cell lines and differentiated them into beating cardiomyocytes (hiPSC-CMs). Here we present new evidence of progressive telomere shortening in non-dividing hiPSC-CMs that leads to a marked DNA damage response culminating in mitochondrial dysfunction. These cells recapitulate in 30 days the telomere shortening that occurs in 30 years of life in DMD patients, enabling studies of cause and effect and tests of interventions.

16. Cell surface GRP78 activation by anti-GRP78 autoantibodies confers prostatetumour growth via tissue factor activation

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(Submitted by Richard Austin. Will be presenter.)

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Prostate cancer (PC) is characterized by increased prothrombotic state due to enhanced tissue factor (TF) expression/procoagulant activity (PCA). We and others observed that GRP78, a molecular chaperone, is expressed on the surface and stimulates the production of anti-GRP78 autoantibodies in PC patients. We report the binding of these autoantibodies to cell surface GRP78 enhances TF PCA. We hypothesize that disruption of this autoantibody/cell surface GRP78 would represent a viable target for the treatment of advanced PC. Wild-type, TF knockdown DU145 cells, and NOD/SCID mouse model system was used to investigate the effect of anti-GRP78 autoantibodies on tumor growth. Protein expression was determined using western blotting and qRT-PCR. TF activity was determined using the TF PCA continuous assay. Blood samples from patients diagnosed with PC were obtained from the Ontario Tumour Bank and St. Joseph's Hamilton. Pre-prostatectomy patients demonstrated high levels of anti-GRP78 autoantibodies (60-100µg/ml) vs. healthy individuals (5-10µg/ml). These titers were significantly reduced 24-weeks post prostatectomy. We show here that anti-GRP78 autoantibodies upregulate TF and its PCA. Furthermore, these autoantibodies were shown to accelerate tumor growth in a NOD/SCID mouse model. Finally, heparin and low molecular weight heparin were shown to interfere with the binding of these antibodies to cancer cells and prevent PC cell activation. Anti-GRP78 autoantibodies, that correlate with PC stage in patients and increase TF PCA and promote PC progression in mice. The effect of this autoantibody can be reversed using heparin, thereby suggesting a novel therapeutic target for PC.

17. High throughput single cell manipulation and genome editing using nanoneedle microrobotic actuators

Steven Banerjee, Mekonos Inc., Anil Narasimha, Stanford University Department of Genetics

Collaborators: Irving Weissman, Stanford University Stem Cell Institute, Bianxiao Cui, Stanford University Department of Chemistry, Allister Francis McGuire, Stanford University Department of Chemistry

Gene therapy is beginning to show potential to treat a disease at its genetic roots, being implemented by viral vector mediated transfer of genes of interest into the cells affected by the disease, but a safer and more efficient means of delivery will be necessary. The persistent adverse effects, such as insertional mutagenesis

caused by the vector employed and the nature of the transgene, highlight the potential benefits of our non-viral based single-cell technological platform that can safely edit genes of interest using the CRISPR system in individual target cells. Most devices for cell manipulation are limited in the number of single cells that can be automatically captured and processed in parallel. We are developing a new nanoneedle-based Z-axis actuation technology for manipulating and analyzing single cells on a massively parallel scale. Our objective is to develop a platform that will increase the throughput of single-cell manipulation and analysis by at least 1000-fold while significantly decreasing costs. Here we are establishing this technology for performing non-viral-based CRISPR-Cas9 genome editing of single T cells with high transduction efficiency and minimal invasiveness. The silicon-based microrobotic actuator is designed to accurately track and target desired positions within single cells under an open loop control without a position feedback sensor, thus avoiding complicated control system electronics. Overall this technology will significantly improve our ability to deliver foreign biomolecules into single cells at a high throughput in a targeted and localized manner, and leverage this ability by enabling rapid, multiplexed single-cell genome editing.

18. Cell-in-Gel system to simulate physiological and pathological mechanical loading for accurate cardiotoxicity screening

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In recent decade, 33% of marketed drugs had to be withdrawn or restricted due to cardiotoxicity, which incurs huge financial cost and unacceptable human cost. Cardiac cell-based functional assays report drug effects on the basic unit of heart function; and millions of cells also provide for high-throughput cardiotoxicity screening of a large number of drug candidates. However, previous and current cell-based cardiotoxicity screen has been using 'load-free' cardiomyocytes, despite that the cells are subjected to mechanical load in beating heart. Recently we developed innovative Cell-in-Gel technology to impose controlled mechanical loading on cells. Studies show that cardiomyocytes behave very differently under mechanical load versus load-free: the action potential is longer, the Ca2+ handling is altered, and the contraction is slower. Hence, mechanically loaded cells provide more sensitive assay to report the CardioToxic effects of drug candidates on causing electrical arrhythmia, Ca2+ dysregulation and contractile impairment. We develop Cell-in-Gel systems with various load levels: Physiological-Load Model uses softer gel to impose physiological load on cells in mimicking healthy heart under normal blood pressure. Pathological-Load Model uses stiffer gel to impose higher load in mimicking pathological conditions such as high blood pressure, dilated cardiomyopathy, fibrosis and stiff myocardium. Our gel system can be used to embed various types of cardiomyocytes (i.e. adult ventricular and atrial, neonatal, stem cell and iPSC-CM). The goal is to simulate the mechanical load in the heart to conduct accurate cardiotoxicity screening of all drug candidates (for various diseases) and to discover new drugs for treating heart diseases.

19. Interrogation of contractility of pluripotent stem cell cardiomyocytes by means of Atomic Force Microscopy

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Single cell "beating" measurements with Atomic Force Microscopy (AFM) have been shown to provide information on cell mechanobiology properties similar to those provided by cell patch clamping experiments, while at the same time being much less stressful for the cells. Three lines of dilated cardiomyopathy (DCM) cells, derived from induced pluripotent stem cells (iPSCs) from a patient with a defective gene leading to DCM, have been studied in comparison to two lines of healthy control cells. Their beating force and rate histograms have been compared, along with measures of arrhythmia. Acute and extended-term drug effects from isoprotenol and norepinephrine treatment on the healthy cells have been monitored with the technique. The changes in beat shape versus increasing mechanical loading of single cardiomyocytes on their contractility have been identified. Finally, Young's moduli of DCM cells, healthy cells and negative controls have been calculated using the same instrumentation with DCM and negative control cells being stiffer on average, although the difference was not statistically significant.

20. An integrative bioinformatics approach identifies in vivo validated drug candidates with novel mechanisms of action in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an area of active drug development, with over 100 candidates in clinical trials. However, most act on a small number of immunomodulatory targets. Drug candidates that act through new targets or mechanisms could expand treatment options for RA. We applied a data-driven bioinformatics approach and in vivo screen to identify and test new drug candidates and targets that could form the basis of future drug development in RA. A computational model of RA was constructed by integrating patient gene expression data, molecular interactions, chemical structures, and clinical drug-disease associations. Candidates were scored based on their predicted efficacy in the computational model. FDA-approved treatments for RA were significantly enriched among the top-ranked candidates. Ten high scoring novel candidates were then screened in the collagen-induced arthritis model of RA in rats. Therapeutic treatment

with three candidates significantly reduced ankle size, alleviated limb inflammation, improved joint histopathology, and reduced mobility impairments tracked by a novel digital motion endpoint. These candidates are currently approved for metabolic, allergic, and psychiatric indications, and do not act on common RA therapeutic targets. However, links between known candidate pharmacology and pathological processes in RA suggest hypothetical mechanisms that could contribute to efficacy. Future studies will inform the druggable targets, pathways, and mechanisms that could contribute to each candidate's efficacy in RA. The candidates could themselves be modified and optimized to increase efficacy in RA. Novel targets identified in these studies could also be the basis of new drug discovery initiatives.

21. Laminin 521 enhances the engraftment potential of mouse derived myogenic pogenitors during long-term culture expansion

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Large-scale expansion of myogenic progenitors is necessary to support the development of high throughput, bioengineered cellular assays in vitro and to develop cellular therapies for rare muscle diseases. A significant challenge exists to significantly expand myogenic progenitors since they progressively lose their ability to differentiate when cultured and passaged long term in vitro. In order to overcome this challenge, we evaluated the consequence of propagating mouse and human myogenic stem cell progenitors on various extracellular matrices to determine if they could enhance long-term myogenic potential. We comprehensively examine the effect of physiologically relevant laminins, laminin 211 and laminin 521, compared to traditionally utilized ECMs (e.g. laminin 111, FN, and matrigel) to assess their capacity to preserve myogenic stem cell potential. Laminin 521 supported the increase of myogenic proliferation in early phases of expansion and was the only substrate facilitating high-level fusion following more than 8 passages in mouse cultures. In human cultures, laminin 521 also supported increased proliferation during expansion and superior differentiation with myotube hypertrophy and increased nuclear spacing. In addition we performed engraftment studies in NOD/SCID mice using these ECMs and established that laminin-521 was superior to LN-111 and matrigel expanded cells. These results suggest that laminin-521 could be employed to facilitate the development of future cellular therapy approaches that employ satellite cells.

22. Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions

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Identification of effective combination therapies is critical to address the emergence of drug-resistant cancers, but direct screening of all possible drug combinations is infeasible. Here we introduce a CRISPR-based double knockout (CDKO) system that improves the efficiency of combinatorial genetic screening using an effective strategy for cloning and sequencing paired single-guide RNA libraries and a robust statistical scoring method for calculating genetic interactions (GIs) from CRISPR-deleted gene pairs. We applied CDKO to generate a large-scale human GI map, comprising 490,000 double-sgRNAs directed against 21,321 pairs of drug targets in K562 leukemia cells and identified synthetic lethal drug target pairs for which corresponding drugs exhibit synergistic killing. These included the BCL2L1 and MCL1 combination, which was also effective in imatinib-resistant cells. We further validated this system by identifying known and previously unidentified GIs between modifiers of ricin toxicity. This work provides an effective strategy to screen synergistic drug combinations at high-throughput and a CRISPR-based tool to dissect functional GI networks.

23. Novel CMKLR1 inhibitors and structure activity relationship studies for application in demyelinating disease

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Multiple sclerosis (MS) is a devastating demyelinating disease of the central nervous system (CNS) that affects approximately 2.5 million people worldwide. Although a number of MS treatments are available, there remains a substantial unmet need for improved therapeutics. Therapies that target white blood cells can reduce disease activity and improve clinical outcomes in MS. We identified a small molecule inhibitor of a white blood cell receptor important in guiding inflammatory cell migration into the CNS. The new inhibitor, 2-(α-naphthoyl) ethyltrimethylammonium iodide (α-NETA), significantly suppresses MS-like symptoms in preclinical mouse models. Despite having multiple favorable features, α-NETA possesses certain liabilities that limit its potential for clinical development. To study preliminary structure-activity-relationship (SAR) experiments, we generated a variety of α-NETA analogs by modifying its key structural features and determined IC50 values for inhibition of chemerin-stimulated CMKLR1 signaling in vitro. This data provides proof-of-concept that we can generate analogs with similar or improved potency (compared with α-NETA), with potential to improve plasma stability,

pharmacokinetics and in vivo efficacy. Improved α-NETA analogs have the potential to impact the clinical management of MS and potentially other autoimmune or inflammatory disorders.

24. Use Of patient-derived long QT syndrome type 3 hiPSC cardiomyocytes to develop new anti-arrhythmic therapeutics

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hiPSC-derived cardiomyocytes (hiPSC-CMs) coupled to high throughput screening is an exciting new paradigm for personalized medicine and drug development, but there are substantial challenges to implementation and no new drugs have yet been developed based on hiPSC disease-in-dish models. We have developed a hiPSC-based model of congenital Long QT syndrome type 3 (LQTS3) to guide the chemical optimization of the class 1b antiarrhythmic, mexiletine. Congenital LQTS3 is a prolongation of the QT interval in the electrocardiogram due to a mutation in Nav1.5 that impairs fast inactivation. Failure of fast inactivation results in an increased sodium current during the plateau phase of the action potential (AP) (late sodium current, INaL) that can cause potentially life-threatening arrhythmia and sudden death. Mexiletine is a weakly selective inhibitor of INaL used to treat LQTS3, but carries liabilities that limit dosing and effectiveness. Thus, we developed a high throughput physiological screen for AP kinetics using an automated microscope (Kinetic Imaging Cytometer, Vala Sciences) and a fluorescent voltage sensitive dye (E. Miller and R. Tsien) to evaluate 150 mexiletine analogs in iterative cycles of synthesis and physiological screening. Our study yielded distinct structure activity relationships for the desired INaL inhibition and undesired pro-arrhythmic effects. Electrophysiological studies confirmed the substantially increased potency and selectivity of our new compounds for INaL. Lead molecules will now advance to large animal pre-clinical studies to verify that they do not cause undesirable side effects (nausea, seizures, proarrhythmia) at therapeutic doses for treatment of LQTS3 and acquired cardiac arrhythmia.

25. The Undiagnosed Disease Network: Opportunities for advancing research on rare diseases

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The Undiagnosed Disease Network (UDN) is a collaborative scientific effort funded through the NIH Common fund, designed to improve diagnoses and treatment options for patients with previously undiagnosed diseases. Patients receive rigorous case review and access to the entire diverse resources of the network. While clinically relevant genomic variants are often found through genome sequencing, often only variants of unknown significance or no clear genomic findings are uncovered. The UDN then initiates research into the molecular or biochemical etiology of the undiagnosed disease. Here we will describe one example of research done by the Stanford UDN. A developmentally normal 7-year old patient presented with unspecified mitochondrial disorder including episodic metabolic decompensation. Through exome analysis, we discovered a homozygous mutation in a mitochondrial ATP synthase subunit gene. To validate this finding, we embarked on a series of functional assays to research this variant. In-vitro assays such as Blue Native Page gel and Seahorse Mitochondrial Respiration Stress tests demonstrated defects in mitochondrial output from the diseased cells as compared to wildtype. The UDN Model Organism Core generated fruit flies carrying this specific mutation, in which host of functional assays were carried out. We are also carrying out RNA-sequencing, metabolomics analysis, and iPSC cells have been differentiated into cardiomyocytes for further mitochondrial analysis. Patient derived cells are being submitted to drug screens to seek potential therapies. This case report demonstrates the breadth and depth of research tactics employed to truly uncover the molecular pathology of predicted deleterious mutations observed in UDN patients.

26. Thrombin cleavage of osteopontin plays an important role in melanoma growth and progression

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Osteopontin (OPN) is a matricellular protein with a highly conserved integrin binding RGD domain. Thrombin cleavage at Arg153 results in OPN-R and OPN-CTF (C-terminal fragment). OPN-R, with SVVYGLR at its C-terminus, binds to new subset of integrins ($\alpha4\beta1$and $\alpha9\beta1$). OPN expression is increased in inflammatory conditions. The growth of B16 melanoma is suppressed in OPN knock-out (KO) mice. However, despite extensive studies on the importance of OPN in cancer progression, the role of thrombin cleavage of OPN is unknown. To investigate the role of thrombin cleavage of OPN in B16 melanoma growth. Mice resistant to thrombin cleavage were generated by replacing Arg153 with Ala (OPN knock in [KI]). B16 cells were inoculated subcutaneously in mice. B16 cell adhesion and migration in presence of various rOPN were performed in vitro. Lung metastasis experiment was performed by jugular vein injection. Robust tumor growth was observed

in wild-type (WT) mice while being suppressed to the same extent in both OPN KO and OPN KI mice. B16 cell adhesion assays showed a 4-fold increase in adhesion to OPN-R compared to OPN-full length (OPN-FL). Migration assays showed ~4-fold increase with OPN-R compared to OPN-FL. Preliminary histology showed a trend to more necrosis in OPN KO and KI compared to WT not related to tumor weight. Preliminary metastasis experiments show ~4-5 fold higher metastasis in WT compared to KO and KI mice. Our data shows for the first time role of thrombin cleavage of the OPN in melanoma growth in vivo.

27. Anisotropic microfibrous scaffolds promote the contraction and maturity of cardiomyocytes derived from pluripotent stem cells

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In the myocardium, cardiomyoctyes (CMs) have highly organized physiological structure with ordered cellular alignments and gap junctions in order to direct efficient electromechanical coupling and contractility. We hypothesize that the microtopograhic cues coupled with the cell-cell interactions between CMs and endothelial cells (ECs) facilitate integration with the host vasculature, as well as by enhancing cardiac function through chemical factors released by ECs. Here we demonstrated that the microtopograhic cues provided by electrospun anisotropic scaffolds coupled with the cell-cell interactions between CMs and endothelial cells (ECs) derived from human pluripotent stem cells (hiPSC) promote CM maturation and organization. Our results reveal that the benefit of scaffold anisotropy was evident in maintaining maximum contraction velocity at 15 days after cell seeding. The co-seeding of human pluripotent stem cell-derived ECs (iECs) did not appear to influence human pluripotent stem cell-derived CMs (iCMs) iCM alignment but negatively influenced iCM contractility. These findings demonstrate an important role of scaffold anisotropy, but not intercellular interactions with iECs, in engineering cardiovascular tissues that maintain iCM organization and contractile function.

28. Molecular and functional resemblance of terminally differentiated cells derived from isogenic human iPSCs and somatic cell nuclear transfer derived ESCs

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Patient- and disease-specific pluripotent stem cells (PSCs) can be generated experimentally via Yamanaka factors (iPSCs) or by somatic cell nuclear transfer (SCNT). However, abnormalities and clinical application of differentiated cells made by different reprogramming mechanisms have yet to be evaluated. Here we assessed the molecular and functional features, and drug response of differentiated cells derived from human isogenic iPSCs and nt-ESCs, as well as genetically matched in vitro fertilization ESCs (IVF-ESCs). We found that differentiated cells derived from isogenic iPSCs and nt-ESCs showed comparable lineage marker gene expression, heterogeneity, nitric oxide production, and in vitro angiogenesis. Genome-wide transcriptome and DNA methylome analysis indicated that iPSC derivatives were closer to isogenic nt-ESC counterparts than those from IVF-ESCs. In addition, cardiomyocytes derived from iPSC and nt-ESCs could both recapitulate doxorubicin-induced cardiotoxicity. Therefore, human iPSCs can replace nt-ESCs as alternatives for generating patient-specific differentiated cells for drug testing and personalized medicine.

29. The cronos titin is involved in myoblast differentiation

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Previously we discovered a titin isoform: Cronos, which span the C-terminal two-thirds of titin. However, the function of this isoform and its regulation is not clear.Cronos knock out myoblast (C2C12) lines were generated with CRISPR/Cas9 method and their differentiation capabilities were characterized. Primary myoblasts from cronos knock out and FLAG-tag knock-in mouse lines were generated to characterize its expression pattern and function. Immunostaining, Western blot, FACS, qPCR and reporter assays were used to interrogate the expression of cronos in myoblast differentiation.In vitro, the expression of Cronos is rapidly induced upon differentiation in the myoblast lines. In vivo, the Cronos protein is early expressed and incorporated into the sarcomere during myofibrillogenesis. The cronos promoter was regulated by transcriptional factors including myoD, Mef2. The differentiation and fusion abilities were strikingly inhibited in the mutant C2C12 and myoblasts lines. Cronos is an early expressed sarcomere protein and it is involved in myoblast differentiation.

30. Assessing the arrhythmogenic risk of drugs using computer simulations

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Early detection of pro-arrhythmic risk of drugs has gathered substantial interest among pharmaceutical companies, regulatory agencies, and researchers. This risk has been linked to prolongation of both action potential duration (APD) at the cellular level and QT interval on electrocardiogram clinical observations. Computer models can be used to understand the relationship between ion channel mechanisms and these macro quantities. In this work, we use single cell and one-dimensional simulations to identify what are the most relevant ion channels that affect the APD and QT interval. To represent the electrophysiology of cardiac myocytes, we used the O'Hara Rudy model. For single cells simulations, we considered endocardial, endocardial, and mid cells. For the simulations, we considered a 10 mm cable with an equal proportion of epicardial, M, and endocardial cells and we computed pseudo-electrograms to obtain the QT interval. We included the effect of drugs by blocking seven ion channels in a range recently reported for 30 drugs. To quantify the sensitivity of APD and QT interval with respect to block in the different ion channels, we used the elementary effects method. Our results agree with previous findings, showing that the hERG channel is the most relevant to our quantities of interest. We also found that the L-type Ca current Cav1.2 has a significant influence. The effect of this current was exacerbated when we increased the heart rate both in single cell and one-dimensional simulations. We now plan to perform whole heart simulations to understand the spatial characteristics of drug induced arrhythmias.

31. Molecular target identification of a novel, neuroprotective, and anti-inflammatory drug for FTD treatment using genome-wide CRISPR screening

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We recently developed a novel small molecule compound (SRI-011381) that exhibits dose-dependent anti-inflammatory bioactivity in a mouse model of FTD. This, in addition to other neuroprotective activity, suggests that SRI-011381 or a derivative compound can be used to treat FTD. SRI-011381 activates Smad-regulated genes, suggesting a TGF-β-like Smad-dependent signaling mechanism. We have produced in vitro and in vivo ADMET data for the drug showing no major concerns. However, the molecular target of SRI-011381 is yet to be identified. In collaboration with Michael Bassik's lab, we are using state of the art CRISPR-Cas9 mediated whole-genome knockout screens to identify the molecular target. SRI-011381 has a protective effect on H2O2 toxicity in multiple cell lines, and based on this effect we have developed a screen using a full genome knockout library of K562 cells. After several rounds of treatment, the surviving SRI-011381 and H2O2 treated cells were sequenced and analyzed in comparison with cells exposed to H2O2 alone. By looking at the relative representation of knockouts in each population, genes responsible for the protective effect of SRI-011381 can be identified. We will also perform a more focused CRISPR knockout screen using an exogenous expression plasmid containing a Smad-driven toxin. SRI-011381 treatment will kill responsive cells, and cause an enrichment of cells with knockouts of genes responsible for the drug's activity. Hits identified by both screens

will be validated in single knockout cell lines followed by protein binding experiments. Once the target is identified, SRI-011381 will be optimized and developed for clinical testing.

32. Repurposing drugs for use against the protozoan parasite Entamoeba

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Entamoeba histolytica is an important human pathogen causing invasive disease in 50 million people annually. Current standard of treatment includes metronidazole to treat invasive disease followed by paromomycin to treat cysts in the colonic lumen. However, adverse side effects/resistance to metronidazole, complicated drug regimen, and unsafe use in pregnant/nursing women reiterates the need for new drugs. Our primary goal is to identify/repurpose drugs that overcome the paucity of drugs against Entamoeba. We will also test the hits against other parasites including Cryptosporidium, Giardia and Naegleria with the goal of identifying 'one drug against multiple bugs'. Entamoeba growth was optimized in 384-well plates for both the trophozoite and cyst stages. This first report of encystation in 384-well plate opens the door to high-throughput analyses for this life cycle stage. To identify compounds that inhibit both stages, we screened libraries with 3,444 known bioactives. The screen led to 1.5% hits with compounds with activity against trophozoites, cysts, and some compounds with activity against both trophozoites and cysts. A secondary assay confirmed the activity of 27 compounds including 8 that had IC50 lower than Metronidazole (< 5μM). We are pursuing 3 candidates that are highly active against both trophozoites and cysts for lead optimization with help from the MedChem group at SPARK. Future work entails testing compounds against mature cysts, drug resistant strains and other pathogenic protozoan parasites. We are optimistic that this drug repurposing approach can be successfully used to develop new treatments for globally important, but understudied parasites.

33. Cardioprotection by targeted growth factors after acute myocardial infarction

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(Submitted by Timothy Stowe, Silver Creek Pharmaceuticals)

There are no therapies to protect the heart after an acute myocardial infarction (AMI). Silver Creek Pharmaceuticals is developing Smart Growth Factors (SGFs) that are first-in-class protein therapeutics

engineered to overcome the short half-lives and off-target effects of naive growth factors. We engineered a targeted version of insulin-like growth factor I (IGF-I) using computational modeling to design a molecule that drives AKT phosphorylation (pAKT) selectively in apoptotic cardiomyocytes. We generated bispecific fusion proteins in which the signaling arm and targeting arm are composed of variants of IGF-1 or Annexin V (AnxV), respectively. These were screened for stimulation of pAKT selectively in apoptotic iPSC-derived cardiomyocytes. A selectively potent lead candidate, SCP-776, was identified that also protected cardiomyocytes from apoptosis in vitro ($p \leq 0.0001$). In a rat AMI model, SCP-776 administered as an IV bolus at reperfusion significantly reduced infarct size ($p \leq 0.05$; n=12/15 animals/group) and serum troponin levels ($p \leq 0.01$ for all dose levels). Interestingly, we also observed dose-dependent reduction in area-at-risk ($p \leq 0.05$; 0.43 and 2.47 mg/kg, n=12/15 animals/group). In a pilot pig AMI study, SCP-776 rapidly accumulated in the infarct/border zone ($p \leq 0.0001$, n=3/group) and significantly increased pAKT levels compared to vehicle ($p \leq 0.001$; n=3/group). Treatment with SCP-776 significantly reduced serum levels of the cardiac damage-specific biomarkers CK-MB and AST, compared to vehicle ($p \leq 0.001$, n=3 animals/group). Ongoing studies will continue to assess the efficacy of SCP-776 in the pig model of AMI.

34. Class effects of statins on transcriptome and functional properties of donor-derived iPSC-cardiomyocytes

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Statins impair cholesterol biosynthesis and mediate various pleiotropic effects leading to prevention of cardiovascular diseases. Although statins are well-tolerated drugs, patient response to treatment is highly variable. A detailed characterization of inter-patient variability to statin treatment might lead to identification of loci associated with statin response in iPSC-CM. Healthy control-derived iPSCs (N=5) were differentiated into cardiomyocytes (CM). iPSC-CM were treated with atorvastatin, fluvastatin, simvastatin, and lovastatin for 7 days. Each statin was applied at its Cmax, corresponding to the clinically administered dosages of 20mg, 40mg and 80mg. iPSC-CM cytotoxicity was carried out in all lines following dose-dependent statin treatment. Functional properties of iPSC-CM were determined by contractility assays. Gene expression examination was performed by whole genome transcriptomic analysis. Statin treatment (48hrs and 7d) did not induce cytotoxicity in iPSC-CMs as measured by Calcein-AM assays. Additionally, statin treatment did not alter the contraction and relaxation velocity of all iPSC-CMs. Whole genome transcriptomic analysis revealed statin-specific gene expression signatures in iPSC-CM. Fluvastatin mediated the strongest effects on gene expression followed by simvastatin, atorvastatin, and lovastatin respectively. Signaling pathway analysis showed that all statins affect mainly metabolic properties of iPSC-CM. Furthermore, statins influence the expression of various statin-response associated loci in iPSC-CM. Finally, qRT-PCR gene expression analysis revealed inter-patient variability in statin-induced HMGCR expression. Our data show that statins distinctly affect the

transcriptome of iPSC-CM expression while modulating statin-response associated loci in iPSC-CM. Thus, iPSC-CM might represent a valid model to assess inter-patient variability in response to drug treatment.

35. First-in-class treatment for both acute and chronic heart failure by tunable [Pyr1]-apelin-13 delivery via novel poloxamer-based encapsulation

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Apelin belongs to a class of peptides that are endogenous ligands to G protein-coupled receptors known as APJ. They are promising therapeutic molecules for the treatment of heart failure, hypertension and other cardiovascular diseases. However, because of its short half-life, the therapeutic application of [Pyr1]-Apelin-13 has been very limited to date. Herein we investigated in mice whether [Pyr1]-apelin-13 lipid / poloxamer complex (poloxome) encapsulated nanocarriers could prolong apelin stability in the blood stream and potentiate beneficial effects of apelin to restore and improve cardiac function. Controlled delivery of [Pyr1]-apelin-13 was achieved by engineering FDA approved poloxamer / lipid complex with specific size distributions. Rapid release of [Pyr1]-apelin-13 for few hours was designed to address acute decompensated heart failure (ADHF) while extended release up to 1 week was designed to address chronic heart failure (CHF). Apelin-loaded poloxomes with various apelin / lipid ratios were prepared using ethanol-based eco-friendly methods. Poloxomes were sized with bulk nano extruders and characterized by atomic force microscopy (AFM) and dynamic light scattering (DLS). [Pyr1]-apelin-13 encapsulation in poloxome nanocarriers resulted in sustained and extended apelin release both in vitro and in vivo. Moreover, intraperitoneal injection of [Pyr1]-apelin-13 poloxome in a mouse model of pressure-overload induced heart failure demonstrated a sustainable long-term effect of [Pyr1]-apelin-13 in preventing cardiac dysfunction and remodeling. We concluded that this engineered nanocarrier system could serve as a delivery platform for treating heart disease through increased bioavailability of the apelin cardioprotective therapeutic.

36. Pericyte fate mapping in mice with hypoxia-induced PAH

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Pericytes are specialized perivascular cells with mesenchymal stem cell-like properties that can give rise to different cell lineages. Little is known regarding the contribution of pericytes to pulmonary vascular disorder, such as pulmonary arterial hypertension (PAH), with major pathological features of muscularized distal arterioles due to excessive proliferation of PASMCs. Whether pericytes can differentiate into PASMCs and contribute to muscularization of distal vessels in PAH remain poorly understood. We utilized fate mapping and develop an approach for generating a timeline of key cellular events during hypoxia-induced PAH using the NG2-Cre-ER+R26-TdTomato-red murine line. After mice were exposed to hypoxia at various time points (0, 7, 14 and 21 days), lungs were extracted, vibratome sliced and stained by different cell markers. Individual red fluorescent cells after hypoxia day 21 were sorted and tested with 48 specific cell markers using Fluidigm single cell Q-PCR.Under normoxia, red cells were found in the lung parenchyma associated with alveolar capillaries. In hypoxia day 7, red cells were accumulated at distal and continued to approach larger vessels at hypoxia day 14. In hypoxia day 21, red cells attached on the vessels and coexpressing mature SMC markers. Single cell qPCR from cells at hypoxia day 21 demonstrated that 65% expressed SMC markers while the remainder expressed a mixture of pericyte, EC and fibroblast markers. Our results suggest that pericytes contribute to muscularization of distal precapillary vessels in response to hypoxia by differentiating into SMCs. Our findings provide insight into the pathobiology of hypoxia induced PAH.

37. Double layer PEG/Dextran influences cell viability, colloidal stability, and in vivo fate of iron oxide nanoparticles

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Biosafety of superparamagnetic iron oxide nanoparticles (SPIONs) has been compromised recently, and initial clinical approvals for some of SPIONs are now withdrawn. Exposure of enhanced reactive surface area of nanoparticles (NPs) to cells could intensify their cytotoxic potential. To overcome this shortcoming, this study proposes a double layer polymeric corona comprising of dextran as an interior layer, and polyethylene glycol (PEG) as an exterior layer. Our obtained results showed when compared to only-dextran coated-SPIONs, a double layer of PEG/dextran coated-SPIONs significantly enhanced cell viability by preventing the release of Fe2+ from the core SPIONs. Pondering the current issues to control the NPs-based toxicity and biodistribution of NPs, the double layer corona we developed will serve as a useful approach in the field of targeted drug delivery and functional tissue imaging using magnetic resonance imaging (MRI).

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