The UC San Diego Sanford Stem Cell Institute and
UC San Diego School of Medicine Present:

Sanford Stem Cell Institute Symposium

Thursday, October 20, 2022; and
Friday, October 21, 2022

DAY 1 SCHEDULE

Thursday, October 20, 2022	
Opening Remarks	
9:00AM – 9:05AM	Catriona Jamieson, MD, PhD
9:05AM – 9:10AM	Pradeep Khosla, PhD, MSEE Zea Borok, MD Patty Maysent, MPH, MBA
Session 1: *Regenerative Medicine* **Chaired by: Rob Signer, PhD**	
9:10AM – 9:55AM	**KEYNOTE SPEAKER** Elizabeth Blackburn, PhD
9:55AM – 10:30AM	Jeffrey Magee, MD, PhD
Session 2: *Stem Cells and Space Panel* **Chaired by: Alysson Muotri, PhD**	
10:30AM – 11:30AM	Twyman Clements, MSME
10:30AM – 11:30AM	Marc Giulianotti, PhD
10:30AM – 11:30AM	Michael Roberts, PhD
10:30AM – 11:30AM	Jana Stoudemire, MBio
10:30AM – 11:30AM	Peggy Whitson, PhD
Lunch Break: 11:30AM – 12:30PM	
Session 3: *Round Table Panel Discussion* ***Alpha Clinic Director's Panel*** **Chaired by: Maria Millan, MD**	
12:30PM – 12:35PM	Patient Story: Sandra Dillon
12:35PM – 1:30PM	Catriona Jamieson, MD, PhD
12:35PM – 1:30PM	Daniela Bota, MD, PhD
12:35PM – 1:30PM	Leo D. Wang, MD, PhD
12:35PM – 1:30PM	Mehrdad Abedi, MD
12:35PM – 1:30PM	Noah Federman, MD
12:35PM – 1:30PM	Sean Turbeville, PhD
12:35PM – 1:30PM	Sheila Chari, PhD
1:30PM – 1:40PM	Patient Stories/Patient Navigators

Eavesdropping on the Conversation Between the Lymphatic Niche and Tissue Stem Cells **Introduction by Catriona Jamieson, MD, PhD**	
1:40PM – 2:15PM	Shiri Gur-Cohen, PhD
Fellow Awards **Chaired by: Shiri Gur-Cohen, PhD**	
2:15PM – 2:30PM	Outstanding Graduate Student: Alyssa Holman, MEng
2:30PM – 2:45PM	Outstanding Postdoctoral Fellow: Helena Yu, MD
2:45PM – 2:50PM	Runner-Up Graduate Student: Karina Barbosa, BS
2:50PM – 2:55PM	Runner-Up Postdoctoral Fellow: Francesca Boscolo, PhD
Session 4: *Neural Stem Cells* **Chaired by: Catriona Jamieson, MD, PhD**	
2:55PM – 3:30PM	Viviane Tabar, MD
3:30PM – 4:05PM	Frank Furnari, PhD
Coffee Break: 4:05PM – 4:20PM	
Stem Cell Science and the Genesis of New Therapeutic Strategies for Patients	
4:20pm – 5:05pm	**KEYNOTE SPEAKER** Derrick Rossi, PhD
Day 1 Closing Remarks	
5:05PM – 5:15PM	Catriona Jamieson, MD, PhD
Network Cocktail Hour & Poster Presentations (In-Person) 5:30PM – 6:30PM	

----END OF DAY 1----

DAY 2 SCHEDULE

Friday, October 21, 2022	
Introductions	
8:00AM – 8:10AM	Alysson Muotri, PhD
8:10AM – 8:55AM	**KEYNOTE SPEAKER** Thomas C. Südhof, MD
Session 1: *Modeling Neurological Conditions for Children Using Stem Cells* **Chaired by: Evan Snyder, MD, PhD**	
8:55AM – 9:30AM	Alex Shcheglovitov, PhD
9:30AM – 10:05AM	Beth Stevens, PhD
10:05AM – 10:40AM	Alysson Muotri, PhD
10:40AM – 11:05AM	Panel Q&A
Lunch Break: 11:05AM – 12:05PM	
Session 2: *Stem Cells in the Early Embryo and Placenta* **Chaired by: Mana Parast, MD, PhD**	
12:05PM – 12:35PM	Myriam Hemberger, PhD
12:35PM – 1:05PM	Heidi Cook-Andersen, MD, PhD
1:05PM – 1:35PM	Shawn Chavez, PhD
1:35PM – 2:00PM	Panel Q&A

----END OF DAY 2----

Speakers

Mehrdad Abedi, MD

Professor, Department of Medicine, UC Davis
Panelist: Alpha Clinic Director's Panel

Dr. Mehrdad Abedi is a Professor of Medicine at University of California at Davis, a member of the Hematology/Oncology bone marrow transplant team, Director of the Alpha Stem Cell Therapy Clinic, GMP Facility, and Progenitor Lab. As a bone marrow transplant specialist, he spent the beginning of his professional carrier in laboratories focusing on hematopoietic stem cell transplant and approaches to reduce toxicity. He specifically worked on reduced intensity transplant regimens and the effect of co-stimulation inhibition in the outcome of transplant and graft-versus-host disease. Dr. Abedi also worked on stem cell plasticity and its potential on regenerative treatment of muscular and the skin injury. A major focus of his clinical research for the last 20 years has been on cellular therapy using CAR-T cells and bispecific antibody armed T-Cells for a treatment of cancer patients and more recently autoimmune disorders.

Karina Barbosa, BS

Graduate Student, Sanford Burnham Prebys
Fellow Awards: Runner-Up Graduate Student

Karina Barbosa is a PhD student in the laboratory of Ani Deshpande at the Sanford Burnham Prebys Medical Discovery Institute. Her research goal is to find novel ways to treat acute myeloid leukemia (AML) subsets that are resistant to conventional therapy, by studying what makes leukemia cells vulnerable at the molecular level. Karina is particularly focusing on ways to counteract the stem cell feature of self-renewal in AML cells, which may guide drug development for the AML cohorts in need of better therapy. Karina received the Horizon Award from the Department of Defense and holds a bachelor's degree in Biotechnology Engineering from Tec de Monterrey in Mexico.

Elizabeth H. Blackburn, PhD (Keynote Speaker)

Professor Emerita, UC San Francisco
Talk Title: "Telomere Maintenance in the Context of Human Aging Processes"

Dr. Blackburn is a leader in telomere and telomerase research including many current investigations into the role of telomere biology in human health and diseases. Dr. Blackburn earned her BSc and MSc degrees from the University of Melbourne, her PhD from the University of Cambridge in England and was a postdoctoral fellow at Yale University. Professor Blackburn has won many prestigious awards throughout her career including winning the Nobel Prize in Physiology or Medicine for discovering the molecular nature of telomeres.

Zea Borok, MD

Helen M. Ranney Professor and Chair, Department of Medicine, UC San Diego

Dr. Zea Borok is a Professor of Medicine and Chair of the Department of Medicine at UC San Diego School of Medicine. She previously served as Chief of the Division of Pulmonary, Critical Care and Sleep Medicine, and Director of the Hastings Center for Pulmonary Research at the University of Southern California. Her research focuses on differentiation and plasticity of the alveolar epithelial lining of the lung in the context of lung fibrosis, and mechanisms regulating repair and regeneration following injury. Dr. Borok has been continuously funded by the NIH for over 20 years and is the recipient of an R35 Outstanding Investigator Award from the National Heart, Lung and Blood Institute. She is an elected member of the Association for American Physicians and a recipient of a Recognition Award for Scientific Accomplishment from the American Thoracic Society. She is a graduate of the Executive Leadership in Academic Medicine (ELAM) program. She has mentored numerous trainees and junior faculty members over the course of her career and is committed to promoting greater gender equity in academic medicine.

Francesca Boscolo, PhD

Postdoctoral Fellow, UC San Diego

Fellow Awards: Runner-Up Post-Doctoral Fellow

Francesca Boscolo Sesillo is a postdoctoral fellow in Dr. Alperin lab where she is studying the changes that occur in muscle stem cell behavior during pregnancy. She started her work as a stem cell researcher in 2009 when she joined the lab of Dr. Loring at The Scripps Research Institute to work on gene expression regulation of embryonic stem cells. She then joined the PhD program at Sanford Burnham Prebys Medical Discovery Institute in the lab of Dr. Sacco where she developed her interest in muscle stem cells.

Daniela A. Bota, MD, PhD

Professor of Neurology

Vice Dean for Clinical Research

Director, Alpha Stem Cell Clinic

UC Irvine

Panelist: Alpha Clinic Director's Panel

Daniela Bota, MD, PhD is the Vice Dean for Clinical Research and Director of the UCI Alpha Stem Cell Clinic. Dr. Bota's academic research and clinical practice focuses on the innovative treatments for brain malignancies. She currently serves as a principal investigator for numerous studies including novel glioblastoma stem-cell targeted chemotherapy agents, cellular immunotherapy studies, and wearable devices.

Sheila Chari, PhD

Editor in Chief, Cell Stem Cell
Executive Editor, Cell Press
Panelist: Alpha Clinic Director's Panel

Sheila Chari, PhD, is Editor-in-Chief at Cell Stem Cell and Executive Editor at Cell Press. Her primary responsibilities are knowing and publishing the top stem cell discoveries, driving journal publishing strategy, and managing a global editorial staff. She travels to international scientific conferences and research institutions to be on top of the latest developments and meet with authors, reviewers, and readers. She is a proud member of the stem cell community and an ardent supporter of stem cell research.

Shawn L. Chavez, PhD

Associate Professor, Oregon National Primate Research Center
Associate Professor, Obstetrics and Gynecology
Associate Professor, Molecular and Medical Genetics
Oregon Health and Science University
Talk Title: "Chromosomal Instability in Preimplantation Embryos and the Potential Implications for Stem Cell Populations"

Shawn L. Chavez is an Associate Professor in the Division of Reproductive & Developmental Sciences at the Oregon National Primate Research Center (ONPRC) as well as in the Departments of Obstetrics & Gynecology and Molecular & Medical Genetics at Oregon Health & Science University (OHSU), where she has been a faculty member since September of 2013. Her research interests focus on the use of real-time imaging and low-input next-generation sequencing to investigate the genetic, epigenetic, and chromosomal requirements of early embryogenesis and placentation in humans, non-human primates (NHPs), and other mammals. In particular, her laboratory is investigating the underlying mechanisms mediating mitotic chromosome mis-segregation, embryonic micronuclei formation, and potential aneuploidy resolution during mammalian preimplantation development. Moreover, she is also examining the molecular connections between the formation of the placental-derived trophectoderm layer in embryos and subsequent placentation in normal versus abnormal pregnancies in both humans and NHPs. Collectively, the goals of this research are to enhance our understanding of embryogenesis and placentation across primate species, whilst improving assisted reproduction outcomes for infertile couples by preventing embryo or fetal loss during pregnancy.

Twyman Clements, MSME

Chief Executive Officer & Co-Founder, Space Tango
Panelist: Stem Cells and Space Panel

Twyman Clements, MS is the Chief Executive Officer and Co-Founder of Space Tango. Twyman leads a multidisciplinary team to deliver automated products that operate in the microgravity environment of low-Earth orbit (LEO). Under Twyman's leadership, Space Tango is maturing these research and hardware capabilities for sustainable manufacturing in space where microgravity is an asset to both the production process and their partners. Space Tango continues to excel in providing efficient and cost-effective product development cycles and rapid delivery of configurable systems to Fortune 500 companies, startups, research foundations, and academic institutions.

Heidi Cook-Andersen, MD, PhD

Assistant Professor, Department of Obstetrics, Gynecology and Reproductive Sciences
Assistant Professor, Department of Molecular Biology
UC San Diego
Talk title: "Molecular Determinants of Successful Implantation in the Human Blastocyst"

Heidi Cook-Andersen, MD, PhD, is an Assistant Professor at University of California, San Diego, with a joint appointment in the Departments of Obstetrics, Gynecology and Reproductive Sciences and Molecular Biology, and board certified in Reproductive Endocrinology and Infertility. Research in the laboratory is aimed at uncovering the molecular mechanisms that drive the transition from oocyte to embryo in mammals and the factors and pathways required for successful implantation of the human blastocyst. These efforts will advance our understanding of the molecular basis of oocyte and embryo competence with the long-term goal to improve the diagnosis and treatment of infertility.

Sandra Dillon

Cancer Survivor and Stem Cell Champion
Patient Advocate: Alpha Clinic Director's Panel

Sandra Dillon led a healthy, energetic life but at age 28 she was diagnosed with myelofibrosis, a rare blood cancer. Faced with a lack of treatment options her disease progressed and left her exhausted and in pain. Then she signed up for a clinical trial run by UC San Diego's Dr. Catriona Jamieson. The therapy involved use of a specific small molecule know to inhibit the mutant gene activity that causes myelofibrosis. This treatment was lifesaving for Sandra and was approved by the FDA, the first approval for a CIRM-supported therapy. Sandra works as Senior Manage of Brand and Marketing at Pattern Energy, utilizing her background in art and design.

Noah Federman, MD

Professor and Program Director, Department of Pediatrics and Orthopaedic Surgery
Director, Alpha Stem Cell Clinic
University of California, Los Angeles
Panelist: Alpha Clinic Director's Panel

Dr. Federman is a Pediatric Hematologist/Oncologist and clinical translational researcher at UCLA. His clinical interests are in bone and soft tissue cancers. In his role as Program Director of the UCLA CIRM ASCC he is focused on accelerating clinical translation of novel cellular and gene therapeutics and improving access to clinical trials in the community.

Frank Furnari, PhD

Professor, Department of Medicine, UC San Diego

Talk title: "iPSC-Derived Brain Cancer Avatars: Lessons learned and opportunities for therapeutic discovery"

Dr. Furnari earned his PhD in microbiology from the University of North Carolina-Chapel Hill where he studied cis-elements and trans-acting factors regulating expression of Epstein-Barr Virus (EBV) lytic replication genes. He subsequently joined the Ludwig Institute in San Diego as a postdoctoral fellow where he focused on the genetic alterations that drive the genesis of glioblastomas, notably the commonly amplified and truncated epidermal growth factor receptor gene (known as EGFRvIII) and mutation of the PTEN gene. During his postdoctoral studies, Dr. Furnari was credited with seminal work demonstrating the ability of PTEN to suppress glioma cell growth mediated through the enzyme's lipid phosphatase activity. Currently, Dr. Furnari is Member and Head of the Laboratory of Tumor Biology at the Ludwig Institute as well as Professor of Medicine at UCSD. His lab has made significant contributions to our fundamental understanding of mechanisms underlying therapeutic resistance in glioma, functionality of tumor heterogeneity, and the evolution of adult and pediatric brain tumors through genetic engineering of human pluripotent stem cell-derived avatar models. His recognition in the field of glioma biology is highlighted by scholar awards from the V, Kimmel and Goldhirsh Foundations, Awards for Basic and Translational Research from the Society for Neuro-Oncology, and by his service as associate editor for Neuro-Oncology, advisory board positions for the National Brain Tumor Society, the Society for Neuro-Oncology, and the Sontag Foundation. He is also co-founder and a scientific advisor for Trotana Therapeutics.

Marc Giulianotti, PhD

Sr. Manager In-Space Biomanufacturing, Sierra Space

Panelist: Stem Cells and Space Panel

Marc is Senior Manager, In Space Biomanufacturing at Sierra Space. His primary efforts are focused on positively impacting humankind by advancing transformative technologies in the area of in-space biomanufacturing. Previously Marc was the Director of Science and Technology with the International Space Station (ISS) U.S. National Laboratory. Part of his activities in this role include managing the Chips in Space programs cosponsored by the National Center for Advancing Translational Sciences and National Institute of Biomedical Imaging and Bioengineering as well as the Tissue Engineering and Mechanobiology in Space Programs cosponsored by the National Science Foundation. Prior to joining the ISS National Lab Marc spent 20+ years working in early drug discovery efforts at the Torrey Pines Institute for Molecular Studies. He received his BS in Chemistry/Biochemistry from UCSD, his MBA from SDSU and his PhD in Chemistry from USF.

Shiri Gur-Cohen, PhD

Assistant Professor, Department of Medicine, UC San Diego

Talk title: "Eavesdropping on the Conversation Between the Lymphatic Niche and Tissue Stem Cells"

Dr. Gur-Cohen is a stem cell biologist whose innovative work on lymphatic vascular niche for hair follicle stem cells opened the door to fundamental research areas to advance therapeutics for enhancing tissue regeneration. Dr. Gur-Cohen is currently an Assistant Professor of Medicine in the Division of Regenerative Medicine at the University of California San Diego. Dr. Gur-Cohen's work unearthed the lymphatic capillary network as a novel stem cell niche component, and her

multidisciplinary strategy has advanced our knowledge of how stem cells synchronize and coordinate tissue regeneration. Dr. Gur-Cohen has received several awards and prizes for her work, including the Helen and Martin Kimmel Stem Cell Award, the Revson-Weizmann Award, and the Tri-Institutional Breakout Award for Junior Investigators. Dr. Gur-Cohen seeks to perform innovative and fundamental research that would aid our understanding of 'stemness' identity and behavior.

Myriam Hemberger, PhD

Professor, Department of Biochemistry and Molecular Biology, University of Calgary
Program Director, Precision Medicine & Disease Mechanisms, Alberta Children's Hospital Research Institute
Talk title: "Placenta and Trophoblast Stem Cells"

Dr. Myriam Hemberger trained at the University of Freiburg and Max-Planck Institute for Molecular Genetics, Berlin, Germany, and as postdoctoral fellow in Toronto and at the University of Calgary, Canada. From 2004-2018, she held a Group Leader position at the Babraham Institute in Cambridge, UK. In October 2018, she joined the University of Calgary as Full Professor in the Department of Biochemistry and Molecular Biology; she is also Program Director of the Precision Medicine & Disease Mechanisms program at the Alberta Children's Hospital Research Institute (ACHRI). Her scientific expertise is centered on early developmental processes that lead to normal placentation and, consequently, healthy reproductive outcome. In 2019, Dr. Hemberger was awarded the March of Dimes and Richard B Johnson Prize in Developmental Biology for her contributions to the field.

Alyssa Holman, MEng

Graduate Student, UC San Diego
Fellow Awards: Outstanding Graduate Student

Alyssa Holman is a Biomedical Sciences PhD candidate in the labs of Drs. Neil Chi and Adam Engler. She received her Bachelor of Arts and Master of Engineering in Biology and Biomedical Engineering, respectively, from Cornell University. Alyssa's research examines the role of cell-cell interactions in generating ventricular cell types in a dish that better represent the cell types within the ventricles of the developing human heart. These fundamental studies may offer novel insights into the signals in the developing heart as well as provide possible treatment options for heart failure.

Catriona Jamieson, MD, PhD

Koman Family Presidential Endowed Chair in Cancer Research
Chief and Professor, Department of Medicine, Division of Regenerative Medicine
Director, Sanford Stem Cell Institute
Deputy Director, Moores Cancer Center
Director, Alpha Stem Cell Clinic
UC San Diego
Session Chair: Neural Stem Cells
Panelist: Alpha Clinic Director's Panel

Catriona Jamieson, MD, PhD is a leading physician-scientist who discovered missplicing, RNA hyper-editing, and splice isoform switching as mechanisms governing human cancer stem cell maintenance in selective niches. This pioneering cancer stem cell research has transformed therapies, including JAK2 and sonic hedgehog-inhibitor trials for myeloproliferative neoplasms and leukemia stem cell

targeting. Her research and efforts lead to the 2019 FDA approval of fedratinib for the treatment of adult patients with intermediate-2 or high-risk primary or secondary Myelofibrosis. She also sent the first bioreactors with cancer organoids that detect activation of cancer stem cell properties in real-time into space on April 8, 2022, as part of the Integrated Space Stem Cell Orbital Research (ISSCOR) Program. The purpose is to identify biomarkers for early detection, and interventional leads and lay the groundwork for future cancer stem cell research in space. She is a Professor of Medicine, Chief of the Division of Regenerative Medicine, the Koman Family Presidential Endowed Chair in Cancer Research, Deputy Director of the Moores Cancer Center, and the Director of the Sanford Stem Cell Institute at the University of California San Diego. Dr. Jaimeson received the 2017 MPN Hero's Award, the Moores Cancer Center Rell Sunn Award in 2020 (past awardees include Roger Tsien, Kary Mullis, Tony Hunter, Brian Druker, Carl June, J. Craig Venter), and the Top Doctor for the 10th consecutive year by Castle Connolly in 2021. Most recently, her visionary leadership resulted in the single largest gift in the history of UC San Diego, for $150 million from T. Denny Sanford, resulting in the creation of the Sanford Stem Cell Institute.

Pradeep K. Khosla, PhD, MSEE

Chancellor, UC San Diego

As UC San Diego's Chief Executive Officer, Chancellor Pradeep K. Khosla has positioned the institution to define the future of the public research university by activating the institution's first-ever strategic plan and launching the Campaign for UC San Diego, an ambitious 10-year, $2 billion endeavor aimed at transforming the university physically, intellectually, and culturally. Under Dr. Khosla's leadership, UC San Diego has expanded college access and affordability for underserved students, initiated campus-wide interdisciplinary research initiatives to foster collaboration and solve societal challenges, and strengthened university and community partnerships to drive regional impact.

Jeffrey Magee, MD, PhD

Associate Professor, Department of Pediatrics,
Washington University in St. Louis
Talk title: "Origins of Incurable Childhood Leukemias"

Dr. Magee obtained his MD and PhD degrees from Washington University School of Medicine. He completed residency and fellowship training at the University of Michigan and trained with Sean Morrison as a postdoctoral fellow. He returned to Washington University in 2013. His lab works on mechanisms that underlie pediatric leukemogenesis, and he directs the Pediatric Leukemia and Lymphoma Program at St. Louis Children's Hospital.

Patty Maysent, MPH, MBA

Chief Executive Officer, UC San Diego Health

Patty Maysent is Chief Executive Officer of UC San Diego Health, the region's only academic health system, comprising UC San Diego Medical Center, Jacobs Medical Center, Shiley Eye Institute, and Sulpizio Cardiovascular Center, as well as other primary and specialty practices located throughout Southern California. Appointed CEO in 2016, she oversees a $2 billion annual operating budget and more than 9,000 employees. Maysent has elevated UC San Diego Health services and programs to enhance the lives of patients and residents of the community. In 2018, UC San Diego Health provided $423.7 million in community benefits, including uncompensated and government-sponsored care, charity care and other health services.

Maria T. Millan, MD

President and Chief Executive Officer, California Institute for Regenerative Medicine

Session Chair: Alpha Clinic Director's Panel

Under Dr. Millan's leadership, CIRM has generated a robust and growing portfolio as a patient-centric funder, partner, accelerator, and de-risker for over 1,000 projects in basic, translational, and clinical research, as well as infrastructure and education programs. Of note, Dr. Millan led the implementation of CIRM's unique Alpha Stem Cell Clinic Network which supports clinical research in regenerative medicine to accelerate the delivery of treatments to patients through partnerships with patients, medical providers, and clinical trial sponsors.

Alysson Muotri, PhD

Professor, Department of Pediatrics

Professor, Department of Cellular and Molecular Medicine

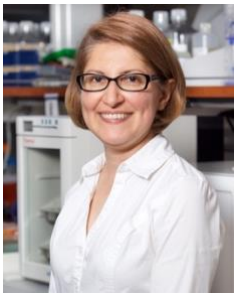
Director, Stem Cell Program and Archealization Center

UC San Diego

Session Chair: Stem Cells and Space Panel

Talk title: "Modeling Neurological Conditions with Functional Human Brain Organoids"

Dr. Muotri is a professor at the Departments of Pediatrics, and Cellular & Molecular Medicine at UC San Diego. He is also the Director of the UCSD Stem Cell Program and Archealization Center. Dr. Muotri earned a BSc in Biological Sciences from the State University of Campinas in 1995, and a PhD in Genetics in 2001 from University of Sao Paulo, in Brazil. He moved to the Salk Institute as Pew Latin America Fellow in 2002 for a postdoctoral training in the fields of neuroscience and stem cell biology. His research focuses on brain evolution and modeling neurological diseases using human induced pluripotent stem cells and brain organoids. He has received several awards, including the prestigious NIH Director's New Innovator Award, NARSAD, Emerald Foundation Young Investigator Award, Surugadai Award, Rock Star of Innovation, NIH EUREKA Award, two (2) Telly Awards for Excellence in Science Communication; among several others.



Mana Parast, MD, PhD

Professor, Department of Pathology

Director, Perinatal Pathology

Co-Director, Center for Perinatal Discovery

UC San Diego

Session Chair: Stem Cells in the Early Embryo and Placenta

Dr. Parast is a physician-scientist and Director of Perinatal Pathology Services at UC San Diego, while also leading a research program in stem cell and placental biology. Her laboratory has developed pluripotent stem cell-based models for probing human placental function across development and disease. As Co-Director of the Center for Perinatal Discovery, she collaborates extensively with colleagues in obstetrics and neonatology to understand the pathophysiology of adverse perinatal outcomes, including fetal growth restriction, preterm birth, and stillbirth.

Corinne Peek-Asa, PhD

Vice Chancellor for Research, UC San Diego

Session Chair: Blood and Cancer Stem Cells

Corinne Peek-Asa, PhD is the Vice Chancellor for Research, and Professor with Distinction of Epidemiology at the University of California, San Diego. Dr. Peek-Asa's research focuses on the epidemiology, implementation, and translation of programs and policies to prevent acute traumatic injuries and violence. She directs an NIH-funded International Trauma and Violence Research Training Program and was the Director of the CDC-funded Injury Prevention Research Center from 2004 to 2020. She is an elected member of the National Academy of Medicine (NAM) and serves on the NAM Accelerating Progress in Traumatic Brain Injury Forum. The impact of VCR Peek-Asa's work to reduce the burden of traumatic injury and violence led to numerous public health advancements, local and federal policies, and prevention programs.

Michael Roberts, PhD

Chief Scientific Officer, ISS National Labs

Panelist: Stem Cells and Space Panel

Michael Roberts, PhD is the Chief Scientist of the Center for the Advancement of Science in Space, Inc. (CASIS), the non-profit, non-governmental organization that manages the International Space Station National Laboratory (ISSNL). As a public service, the mission of the ISSNL is to advance fundamental and use-inspired science in space that advances knowledge, accelerates economic and technological innovation, and inspires STEM education and workforce development to benefit Earth.

Derrick Rossi, PhD (Keynote Speaker)

Chief Executive Officer, Convelo Therapeutics

Talk title: "Stem Cell Science and the Genesis of New Therapeutic Strategies for Patients"

Dr. Derrick Rossi is a stem cell scientist and biotechnology entrepreneur. He currently serves as the interim CEO of the New York Stem Cell Foundation, and CEO of Convelo Therapeutics where he leads a team that is developing regenerative therapies for demyelination diseases of the central nervous system. Before his retirement from academia, Dr. Rossi was an Associate Professor in the Department of Stem Cell and Regenerative Biology at Harvard University, an Investigator at Boston Children's Hospital, and a principal faculty member of the Harvard Stem Cell Institute. His efforts in the development of cutting-edge technologies and novel therapeutic strategies are at the forefront of regenerative medicine and biotechnology. Discoveries made in Dr. Rossi's lab have led to the formation of several biotechnology companies. In 2010, Derrick Rossi founded Moderna, a clinical-stage company focused on developing modified-mRNA therapeutics, and whose COVID-19 vaccine is being deployed around the world. In 2015, Dr. Rossi co-founded Intellia Therapeutics, a company focused on developing CRISPR/Cas9 based therapeutics. In 2016, he co-founded Magenta Therapeutics, which is focused on transplantation medicine.

Alex Shcheglovitov, PhD

Assistant Professor of Neurobiology, University of Utah
Talk title: "Modeling Human Brain Development and Disease Using Single Neural Rosette-Derived Organoids"

Alex Shcheglovitov is an Assistant Professor in the Department of Neurobiology at the University of Utah. He obtained his PhD in biophysics at the Bogomoletz Institute of Physiology, Kyiv, Ukraine and postdoctoral training in pharmacology and developmental neurobiology at the University of Virginia and Stanford University. His laboratory studies human brain development and neurodevelopmental diseases using induced pluripotent stem cells (iPSC) and iPSC-derived neurons and/or organoids. The main goal of Shcheglovitov's lab is to understand the cellular and molecular mechanisms disrupted in human neurodevelopmental disorders associated with disrupted brain connectivity.

Robert Signer, PhD

Associate Professor, Department of Medicine, UC San Diego
Session Chair: Regenerative Medicine

Dr. Robert A.J. Signer is a stem cell biologist whose trailblazing work on protein synthesis and homeostasis in hematopoietic stem cells opened the door to unchartered areas of cellular investigation. Dr. Signer is currently an Associate Professor of Medicine in the Division of Regenerative Medicine at the University of California, San Diego, and holds appointments in the Department of Bioengineering and at the La Jolla Institute for Immunology. Previously, Dr. Signer worked alongside Dr. Sean Morrison as a Postdoctoral Fellow at the University of Texas Southwestern Medical Center. He earned a PhD in Cellular and Molecular Pathology in Dr. Kenneth Dorshkind's lab at the University of California, Los Angeles, and a Bachelor of Applied Science in Engineering Science (Biomedical) from the University of Toronto. His discoveries have been recognized by numerous awards from the Leukemia and Lymphoma Society, the V Foundation for Cancer Research, and the California Institute for Regenerative Medicine, among others, and he was named a Distinguished International Young Investigator in Stem Cell Research.

Evan Snyder, MD, PhD

Professor & Founding Director of the Center for Stem Cells & Regenerative Medicine
Sanford Burnham Prebys Medical Discovery Institute & Sanford Child Health Research Center
Biomedical Sciences Graduate Program, Steering Committee; Medical Scientist Training Program, Physician
UC San Diego
Session Chair: Modeling Neurological Conditions Using Stem Cells

Evan Snyder earned his M.D. and Ph.D. jointly at the University of Pennsylvania and studied psychology, philosophy, and linguistics at the University of Oxford. He completed residencies in pediatrics and neurology and a clinical fellowship in Neonatology at Children's Hospital-Boston, Harvard Medical School, where he also served as Chief Resident in Medicine and then in Neurology. Concurrently, he was a postdoctoral research fellow in the Department of Genetics. In addition to going onto the clinical faculty there in both Neonatology and Neurology (becoming the country's first to be dual boarded in those specialties), he also started his independent lab where he helped define

the basic and translational properties of stem cells (particularly neural). He was recruited to the Sanford Burnham Prebys Institute in La Jolla to found a Center for Stem Cells & Regenerative Medicine and help build the stem cell program in California. Regarded as one of the "Fathers" of the stem cell field, he's been elected to the Association of American Physicians and to the American Institute of Medical & Biological Engineering. He served two terms as Chairman of the FDA's Cell, Tissue, & Gene Therapy Advisory Committee (after helping found the FDA/NIH Stem Cell Working Group) and presently chairs the SAB of NIH's Genetic Disease Biobank. He's a Diplomate of the Health Leadership Academy. His biography is included in Ashwal's *Child Neurology: Its Origins, Founders, Growth & Evolution*.

Beth Stevens, PhD

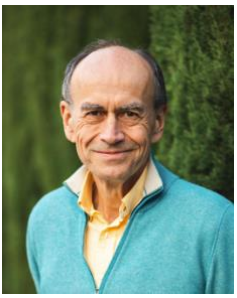
Investigator, Howard Hughes Medical Institute
F.M. Kirby Neurobiology Research Program
Boston Children's Hospital, Harvard Medical School
Broad Institute of Harvard and MIT
Talk title: "Modeling Human Microglia States and Function"

Stevens received her PhD in Neuroscience in 2003 from the University of Maryland and completed her postdoctoral fellowship at the Stanford University School of Medicine with Ben Barres in 2008. Since then, she has continued to perform seminal work elucidating the role of microglia and neuron-glia interactions in synapse and circuit plasticity and function. She continues to pursue the role that neuroimmune interactions play in development and disease, maintaining laboratories at Boston Children's Hospital and the Broad Institute as an HHMI Investigator.

Jana Stoudemire, MBio

Director of In-Space Manufacturing, Axiom Space
Panelist: Stem Cells and Space Panel

As part of the In-Space Solutions team for Axiom Space, Jana is helping to create new market sectors in the commercial space economy in low-Earth orbit for in-space manufacturing of biomedical and advanced material applications on the world's first commercial space station. She transitioned from pharma to lead life science research in microgravity as part of the team managing the International Space Station U.S. National Laboratory (ISS-NL). Jana then joined Space Tango where she successfully established the initial foundational partnerships that are helping to define an emerging biomedical market on orbit. Jana is a member of the National Academies of Sciences, Engineering and Medicine Committee on Biological and Physical Sciences in Space, Regenerative Medicine Manufacturing Society member, National Stem Cell Foundation International Space Station Program Advisor, United Mitochondrial Disease Foundation Board of Trustees Member, past New Organ Alliance Oversight Committee Member, and co-chair of the Microgravity Enabling Technology Committee. Jana also served as co-editor of the book entitled, In-Space Manufacturing and Resources: Earth and Planetary Exploration Applications published in 2022 (Wiley, ISBN: 978-3-527-83091-6).



Thomas C. Südhof, MD (Keynote Speaker)

Avram Goldstein Professor-Investigator,
Howard Hughes Medical Institute
Professor,
Department of Molecular & Cellular Physiology and of Neurosurgery; Department of Neurology & Neurological Sciences; and Department of Psychiatry & Behavioral Science
Stanford University
Talk title: "Using Human Neurons to Explore the Mechanisms of Alzheimer's Disease"

Dr. Thomas Christian Südhof is a neuroscientist whose work has described how neurons communicate with each other at synapses, and how such communication becomes impaired in brain disorders. He is known particularly for the discovery of how synapses rapidly release neurotransmitters, and how neurons form synapses via engagement of trans-synaptic adhesion molecules. Dr. Südhof was born in Göttingen, Germany, obtained his MD and doctoral degrees from the University of Göttingen. He performed his doctoral thesis work at the Max-Planck-Institut für biophysikalische Chemie in Göttingen with Professor Victor P. Whittaker on the biophysical structure of secretory granules. Dr. Südhof trained as a postdoctoral fellow with Drs. Mike Brown and Joe Goldstein at UT Southwestern in Dallas, TX, and elucidated the structure, expression, and cholesterol-dependent regulation of the LDL receptor gene. Subsequently, Dr. Südhof served on the faculty of UT Southwestern in Dallas among others as the founding chair of the Department of Neuroscience until 2008, when he assumed his current position as the Avram Goldstein Professor in the School of Medicine of Stanford University.

Viviane Tabar, MD

Professor and Chair, Memorial Sloan Kettering Cancer Center
Talk title: "Human Pluripotent Stem Cells: From the bench to the clinic in Parkinson's disease"

Dr. Viviane Tabar is the Chair of the Department of Neurosurgery and the Theresa C. Feng Professor in Neurosurgical Oncology at Memorial Sloan Kettering Cancer Center in New York. Her research is focused on harnessing the regenerative potential of human stem cells towards repairing the brain. Her lab portfolio includes the preclinical and clinical translation of human pluripotent stem cell-derived dopamine neurons for Parkinson's disease, which has now completed an early Phase clinical trial. Her lab has also pioneered the use of human embryonic stem cell-based models of brain tumors.

Sean Turbeville, PhD

Vice President of Medical Affairs and Policy, California Institute for Regenerative Medicine
Panelist: Alpha Clinic Director's Panel

As the Vice President of Medical Affairs and Policy at the California Institute for Regenerative Medicine (CIRM), Dr. Turbeville oversees the development of CIRM's infrastructure programs for clinical trials including the delivery of therapies, in particular the Alpha Clinics Network and the future Community Care Centers of Excellence. He works with the Accessibility and Affordability Working Group and the board to develop healthcare policy, reimbursement strategy, post-market activities and research.

Leo D. Wang, MD, PhD

Assistant Professor, Department of Pediatrics and Department of Immuno-Oncology,
Beckman Research Institute
Incoming Director, Alpha Stem Cell Clinic
City of Hope National Medical Center
Panelist: Alpha Clinic Director's Panel

Leo Wang is a pediatric oncologist and physician-scientist at City of Hope National Medical Center. His scientific background is in developmental immunology and stem cell biology, and his laboratory uses protein-focused techniques to evaluate how engineered immune cells make state and fate decisions. Dr. Wang's translational background is in therapeutic gene editing and early-phase clinical trials of gene and cellular therapies, and he currently leads pediatric CAR T cell trials for patients with brain tumors at City of Hope. He is looking forward to helping to expand patient access to cutting-edge cellular and stem cell therapies throughout Southern California.

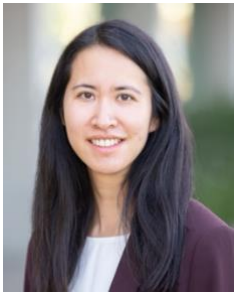
Peggy Whitson, PhD

Ax-2 Commander / Private Astronaut, Axiom Space
Panelist: Stem Cells and Space Panel

Dr. Peggy Whitson has over 35 years of space and science experience with NASA and as a consultant. During that time, she has held various positions, including Chief of the Astronaut Office, Commander of the International Space Station (twice), Chair of the Astronaut Selection Board, Science Officer on board ISS, Operations Branch Chief, Deputy Division Chief for both Medical Sciences and the Astronaut Office, and co-chair of the US/Russian Mission Science Working Group.

Having flown on three long duration missions to the International Space Station (ISS) (Expeditions 5, 16, 50/51/52), Peggy has more cumulative time in space than any US astronaut and more than any woman in the world (665 days). She has conducted 10 Extra-Vehicular Activities (EVA, spacewalks) with over 60 hours to her credit, and performed hundreds of research experiments on board the ISS.

Peggy is currently the Director of Human Space Flight at Axiom Space and the Commander of the second Axiom Space Private Astronaut mission to the ISS, expected to launch May of 2023.
Peggy received her Bachelor of Sciences degree, double majoring in Biology and Chemistry, from Iowa Wesleyan in 1981 and her doctoral degree in Biochemistry from Rice University in 1985. She has been honored with various NASA medals in Leadership, Outstanding Leadership, and Exceptional Service, as well as Glamour's Woman of the Year (2017), TIME 100 Most Influential People in the World (2018), and Women in Aviation Lifetime Achievement Award (2017).



Helena Yu, MD

Pediatric Hematology/Oncology Fellow, UC San Diego and Rady Children's Hospital

Fellow Awards Outstanding Post-Doctoral Fellow

Dr. Helena Yu is a third year Pediatric Hematology/Oncology fellow at the University of California San Diego/Rady Children's Hospital. In the Signer lab, she investigates hematopoietic stem cell protein homeostasis throughout development and in pediatric bone marrow failure syndromes. She completed her undergraduate degree at the University of Chicago, medical school at the Keck School of Medicine of USC, and pediatric residency at UCSF Benioff Children's Hospital Oakland.

Abstracts

1. **High-density CRISPR screens reveal chromatin regulation mechanisms of stemness networks in acute myeloid leukemia**

Karina Barbosa*, Ping Xiang, Anna Minkina, Fiorella Schischlik, Adam Brown, Neil Robertson, John Doench, Peter D Adams, R. Keith Humphries, Eytan Ruppin, Jay Shendure, Prashant Mali, and Anagha A. Deshpande*, Ani Deshpande. **equal contribution*

Several leukemia-associated oncoproteins activate transcriptional circuits that resemble a stem cell state in acute myeloid leukemia (AML). This activation of "stemness" genes is achieved by enlisting the activity of specialized components of the epigenetic machinery. We sought to comprehensively map the epigenetic regulators critical for perpetuating these stemness networks in AML, as they may represent new therapeutic targets. For this, we used a GFP reporter knocked into the endogenous locus of the key leukemia oncogene and self-renewal-associated gene *MEIS1* and conducted a pooled domain-focused CRISPR screen targeting >600 epigenetic modifiers. We identified and validated members of eight distinct chromatin-modifying complexes that were required for sustaining MEIS1 expression in diverse AML subtypes. These included several novel MEIS1 regulators such as TAF6, LDB1, KAT2A, AFF2, JADE3, casein kinase 2 (CK2), ENY2 and SGF29, in addition to known regulators such as DOT1L, AF10, ENL and HBO1.

A secondary pooled CRISPR screen, coupled with a single-cell transcriptome readout (CROPseq) revealed that the deletion of several of these MEIS1 activators not only reversed *MEIS1* activation but also reduced expression of stem-cell associated genes, including genes of the *HOXA* cluster, *BMI1*, *SATB1* and *Musashi 2*, and concomitantly activated expression of differentiation-associated genes.

Of particular interest to us was the TUDOR domain chromatin reader SGF29, a key component of the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex. CRISPR-ko of SGF29 significantly reduced the proliferation of cells with distinct self-renewal-activating mutations including KMT2A rearranged and AF10-rearranged AML cells. ChIP-seq studies showed that SGF29 occupied the promoters and enhancers of key leukemia-oncogenes in a TUDOR-domain-dependent manner and SGF29 deletion selectively attenuated their transcription. Importantly, chromatin proteomics showed that SGF29 deletion led to the eviction of the SAGA complex subunit KAT2A from chromatin and into the cytoplasm. Further, SGF29 knockout impaired blast colony formation and induced differentiation in the KMT2A-MLLLT3, KMT2A-AF10, and CALM-AF10 mouse AML models but did not affect normal hematopoietic colony formation. Lastly, SGF29 deletion delayed disease latency in two distinct human AML cell line models as well as a patient-derived xenograft model in vivo and resulted in striking antiproliferative effects.

Our study revealed several new attractive nodes for therapeutic targeting of leukemia stem cells in AML, including the chromatin reader SGF29, and provides a framework to identify vulnerabilities against recalcitrant oncogenic networks.

2. **The fate of muscle stem cells during pregnancy: characterization of autonomous cellular function in preclinical animal model**

Francesca Boscolo Sesillo, John Rudell, Michelle Wong, Marianna Alperin.

Maternal birth injury to pelvic soft tissues, including pelvic floor muscles (PFMs), constitutes the greatest modifiable risk factor for pelvic floor disorders, highly prevalent morbid conditions that predominantly impact women. The current paradigm is that parturition-associated strains exceed the physiologic limits of skeletal muscles. Thus, PFM trauma should occur in all vaginal deliveries, but many women don't sustain such injuries. This could be driven by the variability in the extent of protective antepartum adaptations, specifically longitudinal muscle growth via sarcomerogenesis, that alter PFMs' response to strains and protect against mechanical injury. Muscle stem cells (MuSCs) enable sarcomerogenesis in limb muscles, however, almost nothing is known about their role in female PFM plasticity. We aimed to determine the intrinsic pelvic MuSC properties to understand their role in PFMs' antepartum adaptations.

PFMs were harvested from non(NP)-, mid(MP)-, and late(LP)-pregnant 3-months old Sprague-Dawley rats, validated for the studies of the human PFMs. Isolated MuSCs were plated in growth media for 15h and subjected to timelapse miscroscopy for 48h. Time to first division was significantly reduced in MP compared to NP and LP groups. To validate this finding, cells were cultured for 24h and treated with EdU for 4h before fixation. Consistently, the proportion of proliferating cells was substantially greater in MP

compared to NP and LP states. These results indicate that pelvic MuSCs, quiescent in NP state, are activated in MP, returning to quiescence in LP. To confirm these novel results, we compared the expression of genes associated with MuSC quiescence between groups. The expression of CalcR and Notch3 was reduced in MuSCs in MP vs NP state, consistent with cellular activation. Surprisingly, reduction of these genes and Notch downstream effectors was also observed in LP, despite the quiescent MuSC phenotype observed in LP. These results confirmed that MP MuSCs are activated and highlighted major differences in the transcriptional profiles between NP and LP cells, suggesting the existence of distinct quiescence states.

Pelvic MuSC autonomous behavior changes across pregnancy continuum. Relative to MuSCs in NP and LP states, MP MuSCs exhibit an activated phenotype, characterized by increased proliferative ability and reduced time needed to enter cell cycle. These findings indicate that MP MuSCs have the intrinsic capacity to contribute to pregnancy-induced PFMs' adaptations. Secondly, despite no clear phenotypic differences between MuSCs in NP and LP states, the expression of genes associated with maintenance of quiescence is significantly reduced in LP. The above suggests that pelvic MuSCs might be differentially responsive to the external cues by the end of gestation, enabling a swift activation necessary for PFM postpartum regeneration.

3. Crosstalk of HIPPO, BMP, and WNT signaling pathways in VGLL1-dependent trophoblast lineage specification of early human development

Ruben Calderon and Francesca Soncin.

Tight spatio-temporal regulation of multiple pathways, including HIPPO, WNT, and BMP, determines cell lineage specification during early development. VGLL1 is a transcription coactivator that is specifically expressed in the proliferative compartment of the human placenta, where it competes with YAP1, a mediator of the HIPPO signaling pathway, for binding of TEAD4. Nuclear co-localization of TEAD4 and YAP1 is required for trophoblast lineage specification and TSC maintenance. Yet the functional role of VGLL1 in placenta development is unknown. BMP4 signaling is active in the trophectoderm, the outer layer of the blastocyst, during embryo development and, later, initiates gastrulation and mesoderm specification in the post-implantation embryo. *In vitro,* BMP4 differentiates human pluripotent stem cells (hPSC) into either trophoblast or mesoderm lineage, depending on the activation status of ß-catenin dependent WNT signaling. In this BMP4-dependent differentiation model, the WNT inhibitor small molecule IWP2 suppresses differentiation into the mesoderm lineage, thus directing the cells exclusively towards the trophoblast lineage. However, the establishment and maintenance of post-implantation hTSC requires activation of the WNT signaling pathway, suggesting a complex temporal relationship between BMP, HIPPO and WNT during placenta development. Using hPSC, we investigated the role of VGLL1 in early trophoblast specification. VGLL1 is detected in the mid-to-late stage of the BMP4-dependent differentiation of hPSC. Knockdown of VGLL1 in this *in vitro* model results in decreased transcription of early and late trophoblast lineage markers, including GATA3 and EGFR, for which YAP1 is unable to compensate. Overexpression of VGLL1 in hPSC, in absence of exogenous BMP4, upregulates the transcription of a subset of trophoblast markers, including GATA3 and EGFR, and increases cell surface EGFR to comparable levels as BMP4-derived trophoblast-like cells. Moreover, RNA-sequencing analysis confirms that over expression of VGLL1 in hPSC upregulates the transcription of a subset of placenta-specific genes, suggesting that VGLL1 regulates part of a trophoblast-specific transcriptional program that is distinct from YAP1. Interestingly, in this VGLL1 overexpression model, we also observe expression of WNT signaling-dependent mesoderm markers and RNA-seq analysis shows expression of several WNT pathway genes. Expression of VGLL1 is restricted to the placenta during development and is not known to regulate the mesoderm lineage *in vivo*, therefore we posit that BMP4-dependent mesoderm differentiation lies down-stream of VGLL1 and it is an unintended consequence of the activation of components of the WNT signaling pathway in hPSC that are predisposed to mesoderm differentiation.

4. Hematopoietic Stem Cells Depend Upon Aggrephagy to Maintain Protein Homeostasis and Self-Renewal Activity

Bernadette A. Chua, Connor J. Lennan, Mary Jean Sunshine, Ashu Chawla, Lorena Hidalgo San Jose, Daniela Dreifke, Eric J. Bennett, Robert A.J. Signer.

Hematopoietic stem cells (HSCs) regenerate blood and immune cells throughout life. To preserve their health and longevity, HSCs are particularly dependent on maintaining protein homeostasis (proteostasis). HSCs maintain proteostasis partly by sustaining low protein synthesis rates that limit the biogenesis of

misfolded proteins. Nevertheless, HSCs ultimately accumulate misfolded proteins that must be eliminated to preserve stem cell fitness. However, how HSCs purge misfolded proteins to maintain proteostasis is mostly unknown. We found that in contrast to most cell types that utilize the proteasome to degrade misfolded proteins, HSCs depend on aggrephagy, a selective form of autophagy, to maintain proteostasis in vivo. Young adult HSCs exhibit unusually high autophagic activity, and genetically disabling autophagy results in significant accumulation of protein aggregates and impaired HSC function. Furthermore, we determined that mouse and human HSCs preferentially express *Bag3*, a critical stress response gene that promotes aggrephagy and transport of misfolded proteins to aggresomes. Aggresomes are cytosolic inclusion bodies containing protein aggregates that typically form under stress conditions to help maintain proteostasis. Surprisingly, we found that the vast majority of HSCs contain aggresomes, even under steady state conditions in vivo. Conditional deletion of *Bag3* causes HSCs to accumulate protein aggregates and impairs their self-renewal activity in vivo. Finally, we determined that old adult HSCs exhibit diminished autophagic flux and become increasingly reliant on the proteasome to degrade misfolded proteins. Overall, these studies demonstrate that protein degradation pathways are uniquely configured in HSCs to preserve proteostasis and fitness, and that disruptions in proteostasis network activity contribute to cellular and molecular changes in HSCs during aging.

5. One Niche to Rule Them All: Lymphatics Instruct Regenerative Potential in Diverse Epithelial Stem Cells

Madison Conte and Shiri Gur-Cohen.

Adult stem cells (SCs) reside in all tissues, where they replenish dying cells and repair wounds. The human body is in a constant state of regeneration, either through fast regeneration, where SCs in the tissue are continually cycling, or by slow turnover, in which SCs spend prolonged periods in a resting quiescence state. It is increasingly evident that SCs are intimately associated with and functionally dependent on their local environment ('niche'), thereby preventing tissue overgrowth and SC exhaustion or insufficient tissue renewal. However, knowledge is still scant on how the environment changes to meet a tissue's regenerative demands and whether SCs can affect lineage fate decisions by remodeling their supportive niches.

Taking an interdisciplinary approach to address this challenge and by using the hair follicle as a model, we previously uncovered lymphatic vessels as a newly identified dynamic SC niche and demonstrated that SCs play a role in organizing and diversifying their lymphatic niches. Our studies revealed that SC-lymphatic connections are dynamic and determined by SC-driven secretome, thereby exposing an unrecognized need to balance the interstitial efflux of fluids and macromolecules to control SC behavior and regenerative potential.

However, whether the lymphatic niche architecture and regulation are tailored across diverse epithelial tissues remains unexplored. Here, we interrogate lymphatics in the intestinal crypt, which in contrast to the hair follicle SCs that undergo cycles of rest and activation, harbors SCs that are continuously active and regenerate tissue. By combining whole-tissue imaging and paired single-cell and spatial transcriptomics, we unearth cryptbase lymphatic capillaries as a new and critical intestinal SC niche component that functions as the major source of spatially-restricted factors essential to maintain SC regenerative potential.

In sum, our results identify the lymphatic capillaries as a hitherto under-appreciated SC niche element and illuminate the way that lymphatic fitness integrates the regenerative process. Our new findings highlight lymphatics as a main signaling hub that preserves SC identity and controls their behavior to fulfill the diverse regenerative needs in tissues. These findings may provide major implications for advancing tissue regeneration, wound repair and cancer therapeutics.

6. ELOVL2 is a molecular regulator for fatty acid metabolism and an epigenetic biomarker for aging in the liver

Elizabeth Diaz, Ashni A. Vora, Lara C. Avsharian, Fangyuan Gao, Dorota Skowronska-Krawczyk, Leslie A. Crews.

Human aging is associated with the prevalence of liver disorders such as non-alcoholic fatty liver disease. Together with inflammation-associated liver fibrosis and cirrhosis, these disorders can lead to malignancies such as hepatocarcinoma. Identifying molecular regulators of fatty acid metabolism and inflammation during liver aging may aid in the understanding of liver diseases and further development of

novel regeneration strategies. Aging has been associated with diverse lipid alterations, such as increased saturated fatty acid deposits and loss of omega-3 polyunsaturated fatty acid (PUFA) species, however the extent to which altered fatty acid metabolism contributes to liver aging and fatty liver disorders is less clear. To explore the effects of deficiency in a very long chain-PUFA elongation enzyme, *ELOVL2*, single-cell RNA-sequencing datasets from young and aged mouse livers were examined. Our results showed that *Elovl2* is abundantly expressed in liver compared with other tissues, with more variable but lower levels in aged wild-type mice. Gene pathway analyses revealed a broad deficit in cholesterol biosynthesis and amino acid metabolism pathways, including downregulation of a key tyrosine metabolism gene, fumarylacetoacetate hydrolase (*Fah*), during liver aging. Genetic Elovl2 deficiency was associated with hepatomegaly as well as increased inflammation-responsive gene expression, suggestive of potential accelerated aging-related functional deficits. The extent to which *ELOVL2* is involved in liver aging by regulating different biological pathways is still unknown. To explore the effects of *ELOVL2* downregulation, we used an antisense oligonucleotide (ASO) treatment and a small interfering RNA (siRNA) treatment to transfect an epithelial human liver cell line and an embryonic human kidney cell line. Our data for the embryonic kidney cell line showed to be inconclusive given that *ELOVL2* has a decreased overall expression in HEK 293 cells. Preliminary results for the levels of expression of *ELOVL2* in the human liver cell line are currently being analyzed to understand which is the most efficient method for knocking down the expression of *ELOVL2*. If any of these treatments prove to be successful, the next step would be to analyze how levels of expression of proteins involved in some of the major pathways involved in aging such as cholesterol biosynthesis are affected by *ELOVL2* downregulation. Overall, more research on the role of *ELOVL2* needs to be addressed as this could pioneer the development of new therapies for regulating aging-related diseases such as non-alcoholic fatty liver disease and hepatocarcinoma.

7. CD4+ and CD8+ T cells independently restrict medulloblastoma growth and dissemination

Tanja Eisemann, Alexander Wenzel, Theophilos Tzaridis, Jill Mesirov, Robert J. Wechsler-Reya.

The immune system serves as a powerful defense not only against pathogens but also against neoplastic cells. Emerging immunotherapies that enhance the patient's own immune system to destroy cancer cells have shown promising effects in certain cancers. However, the success of immunotherapy for brain tumors has been limited, highlighting the need for a better understanding of the immune microenvironment.

To study the immune microenvironment of the pediatric brain tumor medulloblastoma we use a mouse model that is derived from transformed murine neural stem cells. We show that depletion of either CD8+ or CD4+ T cells results in more aggressive growth and leptomeningeal metastasis, revealing a critical role of T cells in the control of medulloblastoma growth and dissemination. Our studies show that adoptive CD8+ T cell transfers in immune-compromised mice dramatically reduce tumor burden. Immune cell-derived interferon gamma induces MHC class I in medulloblastoma cells which normally suppress the presentation of MHC class I, making them a target for cytotoxic CD8+ T cell attack. Interestingly, we have identified an independent tumoricidal function of CD4+ T cells in medulloblastoma beyond their well-described CD8+ T cell helper function. In contrast to cytotoxic CD8+ T cells, which recognize and kill tumor cells directly via interaction of the T cell receptor and MHC class I, our experiments indicate no direct tumor cell killing by CD4+ T cells. Instead, we hypothesize that CD4+ T cells recruit and activate another effector immune cell type that eliminates tumor cells. Ongoing studies are aimed at identifying the effector immune cells type and elucidating the mechanisms by which CD4+ T cells and the effector immune cell type regulate medulloblastoma growth. These studies will advance our understanding of the immune microenvironment in medulloblastoma and allow us to design more effective therapies.

8. Investigation of cellular cues for differentiation of ventricular cardiomyocytes

Alyssa Holman, Elie Farah, Neil Chi, and Adam Engler.

Heart failure is a decline of cardiac function in part due to cardiomyocyte (CM) death. Since the heart has little if any ability to regenerate, *in vitro* differentiation of human pluripotent stem cells (hPSCs) into CMs is a key method for developing cell-replacement therapies for heart repair. To generate specific, mature CM sub-types for heart repair treatment, it is essential to understand the fate decisions and developmental cues that allow a cell to become a particular cell type. A cell's development is highly dependent on its environment within a tissue, particularly through cell-cell interactions between distinct cell types. To understand how CMs are influenced by their environment, we examined the differentiation states of CMs in a 2D (monolayer) versus 3D (embryoid body) cellular environment. Here, we observed

that ventricular CMs (vCMs) develop more efficiently and are more mature in the 3D environment, despite having a more heterogenous cellular environment and differing signaling cues versus cells from 2D environments. Based on these observations, **we hypothesized that non-cell autonomous effects of the surrounding cellular environment may influence vCM differentiation by generating more *in vivo*-like conditions**. To understand whether the supporting cell types generate cues that could influence the differentiation of vCMs, we mixed 2D cardiac differentiation cultures with either 3D-derived cells or conditioned media. We found that exposure of a 2D cardiac differentiation to either 3Dderived cells or conditioned media increased the percentage of vCMs (*MYL2*+ cells) as compared to a standard 2D cardiac differentiation, showing the importance of cellular cues in vCM generation. To discover which 3D cellular cues that are responsible for the increase in vCMs in the 2D environment, we computationally identified cell-cell interactions among the two differentiation methods using single cell analyses. Here, we discovered in CMs that BMP2 signaling is upregulated in the 2D environment, whereas BMP2 signaling is repressed by SMAD6 in the 3D environment. These studies identify the impact of all cardiac cell types on the generation of vCMs and the specific molecular cues that non-CM cell types produce to modify this process, thereby allowing us to generate pure populations of more in vivo-like cell types that can be used for heart failure treatment and therapeutic development.

9. Cellular stress activates an integrin αvβ3 Src/AMPK/PGC1α signaling axis to promote oxidative phosphorylation and stress tolerance in lung cancer

Shashi Jain, Hiromi Wettersten, Sara Weis, David A. Cheresh.

Therapeutic targeting of cancer energy metabolism has emerged as a compelling concept in oncology. Oxidative phosphorylation (OXPHOS) is known to play a crucial role during cancer progression, allowing cancer cells to rely on mitochondrial energy metabolism to support tumor initiation, stress tolerance, stemness, drug resistance and cancer progression. Thus, targeting OXPHOS could be a valuable approach to control a wide range of cancers. Here, we reveal that cellular stresses including nutrition deprivation, oxidative stress, and cancer therapeutics induce lung cancer cells to upregulate expression of integrin αvβ3, which reprograms cellular metabolism to depend on oxidative metabolism. Integrin αvβ3 is unique among integrins, as its ability to recruit and activate Src does not require ligand binding. Mechanistically, we show that αvβ3 is the only integrin capable of inducing this metabolic switch in the absence of cell-matrix adhesion and extracellular matrix ligand binding by virtue of its ability to trigger a Src dependent sustained activation of AMPK, driving nuclear location of the transcriptional coregulator PGC1α, which in turn increases expression of mitochondrial OXPHOS protein complexes. Our findings demonstrate for the first time how lung cancer cells mitigate stress by upregulating the expression of integrin αvβ3 to invoke a signaling cascade that no other integrin can trigger in the absence of ligand binding, leading to a metabolic shift to OXPHOS. Since stress-induced αvβ3 expression renders tumor xenografts addicted to mitochondrial respiration, blockade of the Src/AMPK/PGC1α axis can be exploited as a therapeutic vulnerability when tumors gain αvβ3 expression.

10. A Stress Response Pathway that Enhances Hematopoietic Stem Cell Longevity Promotes Acute Myeloid Leukemia Growth and Progression

Yoon Joon Kim, Kentson Lam, Fanny J. Zhou, Jeffrey A. Magee, Robert A.J. Signer.

Aging is the single biggest risk factor for the development of cancer. This association has historically been attributed to the fact that rate limiting genetic mutations accumulate over time. However, many other hallmarks of aging can convey selective advantages that may ultimately promote the development of cancer. We recently discovered that aging hematopoietic stem cells (HSCs) activate Heat shock factor 1 (HSF1), a key transcriptional regulator that dynamically remodels the protein homeostasis (proteostasis) network upon acute and chronic proteotoxic stress. HSF1 activation promotes HSC fitness and proteostasis maintenance during aging. Several high-risk leukemogenic mutations tend to arise disproportionately in older adults, but whether HSF1 confers fitness advantages to acute myeloid leukemia (AML) cells is untested. We found that HSF1 is highly expressed across multiple human AML cell lines harboring distinct mutational profiles, and is readily activated in response to stress. CRISPR-Cas9 mediated deletion of HSF1 significantly reduced AML cell growth and proliferation in vitro. Furthermore, HSF1-deficiency severely impaired AML progression and significantly extended survival within xenograft models in vivo. Finally, in line with its role in mitigating proteotoxic stress, we determined that HSF1 conferred human AML cells with significant therapeutic resistance to proteasome inhibitors. HSF1-deficient AML cells treated with the proteasome inhibitor carfilzomib exhibited severely reduced proliferation and increased cell death. This study reveals that age-associated stress response pathways

that promote HSC fitness can inadvertently support AML growth and confer therapeutic resistance. Identifying connections between molecular and physiological changes in aging HSCs with the emergence of leukemia holds potential for uncovering new therapeutic opportunities to prevent/treat leukemia by targeting underlying age-related changes in stem cell proteostasis.

11. ADAM-17 Deficient induced Pluripotent Stem Cell-Natural Killer cells Demonstrate Improved Antibody-Dependent Cellular Cytotoxicity and Enhanced Antitumor Activity

Meng-Wei Ko, Kenta Yamamoto, Robert Blum, Dan Kaufman.

Natural killer (NK) cell-based cancer immunotherapy has been gaining interest with multiple trials demonstrating that adoptive transfer of allogeneic NK cells can mediate effective anti-tumor activity and increase survival of diverse malignancies. One key mechanism of NK cell-mediated activity is through antibody-dependent cellular cytotoxicity (ADCC). NK cells mediate ADCC against antibody-opsonize tumor cells through binding to the Fc region of the antibodies with their CD16 receptor. Activation of CD16 induces the degranulation of NK cells and releases cytolytic granules and cytokines leading to the lysis of tumor cells. However, a disintegrin and metalloprotease 17 (ADAM17) cleaves CD16 on activated NK cells. This activity promotes CD16 shedding from NK cell surfaces and leads to decreased ADCC. Studies from our group have pioneered the use of human induced pluripotent stem cells (iPSCs) to derive NK cells as a standardized "off-the-shelf" immunotherapy. Here, we sought to utilize ADAM17-deficient (ADAM17-KO) iPSC-NK cells as a possible strategy to improve their anti-tumor activity. ADAM17- KO iPSC was generated using CRISPR-Cas9 and ADAM17-KO iPSC-NK cells were successfully produced using our standard hematopoietic organoid system, followed by our NK cell differentiation protocol. Throughout the differentiation process, ADAM17-KO iPSC-derived NK cells showed similar phenotypes to their wild-type (WT) counterparts. However, the CD16 expression on ADAM17-KO iPSC-NK cells surprisingly decreased compared to WT iPSC-NK cells. After sorting for CD16+ NK cells, the CD16+ADAM17-KO iPSC-NK cells continue to stably express the CD16 on the cell surface. Moreover, after stimulation, CD16+ADAM17-KO iPSC-NK cells maintained over 90% of CD16 while the CD16+WT iPSC-NK cells and peripheral blood NK cells (PB-NK) had diminished CD16 expression on their cell surfaces. Surface proteomic analysis confirmed that CD16+ADAM17-KO iPSC-NK cells maintained their CD16 expression, but CD16+WT iPSC-NK cells and PB-NK cells lost the CD16 on the cell surfaces (70% and 24%, respectively). Interestingly, no significant decrease in other ADAM17 substrate proteins was observed in all three cell types. In functional assays, CD16+ADAM17-KO iPSC-NK cells demonstrated increased anti-tumor activity against RAJI B-lymphoma cells combined with anti- CD20 Rituximab and MA148 ovarian carcinoma combined with anti-EGFR Cetuximab. Currently, the *in vivo* therapeutic efficacy of ADAM17-KO iPSC-NK cells compared to WT iPSC NK cells and PBNK is still under investigation. Taken together, these results demonstrate that ADAM17-KO iPSC-NK cells demonstrate improved ADCC and provide a promising cancer immunotherapy against various cancer types in conjunction with therapeutic antibodies

12. Combined Proteasome and Autophagy Inhibition Synergistically Impairs Human Acute Myeloid Leukemia Cell Growth by Disrupting Protein Homeostasis

Kentson Lam, Yoon Joon Kim, Carlo M. Ong, Andrea Liu, Bernadette A. Chua, Robert A.J. Signer.

Proteasome inhibitors are clinically approved and highly effective for treating multiple myeloma but exhibit modest efficacy for treatment of other hematological malignancies. In this study, we set out to determine why acute myeloid leukemia (AML) is largely refractory to proteasome inhibition and to determine how to sensitize human AML cells to this class of therapeutics. Efficacy of proteasome inhibitors in multiple myeloma partly depends on their ability to disrupt protein homeostasis (proteostasis) and activate a terminal unfolded protein response (UPR). Surprisingly, we found that proteasome inhibition failed to increase accumulation of unfolded proteins and induce UPR activation within hematopoietic stem cells (HSCs) in vivo and human AML cell lines. We determined that proteasome inhibition induced compensatory activation of autophagy within both HSCs and AML cells. This raised the possibility that activation of autophagy prevented the accumulation of unfolded proteins and preserved proteostasis in response to proteasome inhibition. Indeed, we determined that combined pharmacological inhibition of the proteasome and autophagy synergistically increased unfolded proteins across multiple human AML cell lines harboring distinct mutational profiles. Furthermore, combined proteasome and autophagy inhibition synergistically and dramatically reduced AML cell viability and proliferation by more than 85%. In contrast, HSCs tolerated acute proteasome and autophagy disruption in vivo suggesting the presence of a therapeutic window to preferentially eradicate AML cells by disrupting proteostasis. RNA-sequencing studies revealed that proteasome or autophagy inhibition had

minimal effects on AML cell transcriptional profiles individually, but together they synergistically altered gene expression and significantly activated EIF2 signaling, which is indicative of UPR activation. Consistent with this, we found that combined proteasome and autophagy inhibition severely reduced protein synthesis within human AML cells. Overall, this study reveals that remodeling of the proteostasis network protects human AML cells against proteasome inhibition by enhancing autophagy, and that combined proteasome and autophagy inhibition synergistically impairs AML cell growth and survival by disrupting proteostasis.

13. Generating a prefusable vascularized brain organoid model using microfluidics and photodegradable polymer scaffolds

Eric LaMontagne, Laura J. Macdougall, Fabio Papes, Evan L. Teng, Jaimie Mayner, Bryan P. Sutherland, Kristi S. Anseth, April M. Kloxin, Alysson R. Muotri, Adam J. Engler.

Although brain organoids have advanced our understanding of the human brain, their growth, cellular complexity, and functionality are limited by the absence of vasculature. Current organoid vascularization efforts rely on either: (1) the *in situ* formation of neo-vessels in endothelial co-cultures, or (2) microfluidics channels that flow adjacent to organoids. Neither produces vasculature that resembles native cerebral vessels. We hypothesize that a system in which patent vessels form throughout the organoid as it matures will enable organoid growth beyond the diffusion limit of oxygen and promote the formation of more complex cerebral structures. To fabricate this system, we first developed a microfluidic device containing posts onto which a fiber scaffold is woven in a lattice resembling the cerebral vascular network. Photodegradable fibers are formed by extruding and crosslinking a solution of two multi-arm polyethylene glycol (PEG) components that contain allyl sulfide to facilitate degradation via radical addition. Brain organoids that we have generated from pluripotent stem cells (PSCs) are to be placed within the woven fiber network when at the neuroprogenitor cell (NPC) stage so that they grow around fibers as they enlarge. Upon radical addition, the polymer scaffold will solubilize and provide the framework for a continuous, hollow lumen that will be seeded with PSC-derived endothelial cells to generate vasculature. The microfluidic channels are perfusable at high flow rates and produce low wall shear stresses, confirmed using computational physics models. Photodegradable PEG fibers can be extruded, woven into a scaffold, and degraded via 365nm, 5mW/cm2 light in under 5 minutes. All components of the sacrificial scaffold are biocompatible with brain organoids. Future experiments include the fabrication of multielectrode arrays to stimulate and read electrical activity of the organoids, long-term perfusion at varying shear stresses, and single-cell RNA sequencing analysis to evaluate maturation markers. To our knowledge, this will be the first vascularized brain organoid model with highly integrated vasculature that can be perfused over long periods of time. This will generate opportunities to study neurovascular development, brain cancers, or other neurological conditions affecting vasculature using a completely human and *in vitro* system.

14. Neuronal timescales across the lifespan: using human cortical organoids to study neurodevelopment and aging

Blanca Martin and Bradley Voytek.

In order to support complex cognition, neuronal circuits must integrate information across multiple temporal scales. The neuronal timescale—the duration over which the activity of a neuronal population typically persists—has the potential to explain how information might be maintained by spike trains over several orders of temporal magnitude. In recent work from our lab, we have demonstrated that neuronal timescales exhibit hierarchical spatial organization, mapping onto genetic and anatomical gradients in humans. Timescales are also functionally dynamic, and compress with age, highlighting the importance of studying how timescales change with development and aging. Despite recent progress in the study of timescales, little is known about the underlying circuits that give rise to them, nor to how they develop or change in aging. In order to understand the development of circuit and cellular level functional dynamics, we need invasive recordings sampled across the lifespan. Although long-term invasive data acquisition cannot be performed in humans, recently, cortical organoids generated from human induced pluripotent stem cells (hiPSCs) have emerged as a promising 3D model of human circuits. These cortical organoids allow us to study the long-term functional dynamics over development via electrophysiological recordings. In recent work from our lab, we used a novel spectral parameterization method to estimate timescales from multi-electrode array (MEA) recordings of human cortical organoids over development. We found that neuronal timescales follow an increasing and nonlinear developmental trajectory in neurotypical cortical organoids. To explore changes in timescales with aging, we used organoids with

altered telomere length, where telomere shortening has been shown to relate to aging and life span across species. These organoids with shorter telomeres exhibited shorter, more compressed neuronal timescales, suggesting reduced memory capacity. This combined approach using novel timescale estimation methods on human cortical organoid models allows us to delineate how timescales emerge and change over healthy and disordered human lifespan compared to neurotypical development.

15. Investigating BPIFA1 and Its Role in Regulating Epithelium Homeostasis in the Developing Human Neonatal Airway

Rachael McVicar, Sandra Leibel, and Evan Snyder.

The human airway epithelium serves to protect the host from foreign pathogens and environmental insults. However, infants who are born prematurely have underdeveloped lungs with weakened barrier function, and insult is added to injury after the rapid transition from hypoxia in-utero to normoxia postnatal. Consequently, premature infants are at greater risk of severe respiratory syncytial virus (RSV) infections and developing chronic airway inflammation. One protein of interest, BPIFA1, has been shown to regulate airway epithelium homeostasis and prevent pathogen induced airway disease severity in adult mice models. While this protein has been rigorously studied for its role in as an antimicrobial peptide during respiratory infections in mice, little research has been done investigating BPIFA1 in a developmentally compromised human neonatal airway. Therefore, we wish to investigate if endogenous BPIFA1 expression is tied to gestational lung maturity, influenced by changes in oxygen tensions, and if supplemental BPIFA1 decreases the severity of RSV infection in the preterm lung epithelium. First, the level of BPIFA1 expression was assessed in human stem cell (iPSC) derived and human fetal lung tissue derived air-liquid interface (ALI) epithelial cultures representing the preterm airway. Next, the influence of oxygen tension on BPIFA1 expression was investigated by culturing airway epithelial cells in either hypoxic (5% oxygen) or normoxic (21% oxygen) conditions; where an increase in BPIFA1 expression was observed in normoxic airway cultures. Lastly, the antiviral potential of BPIFA1 is being investigated by infecting airway epithelial cultures with RSV to determine if supplementing recombinant BPIFA1 protein can decrease overall RSV infection. Our findings will help elucidate how premature birth impacts airway development and BPIFA1 expression and explore the therapeutic potential of exogenous BPIFA1 in the premature infant population.

16. A human pluripotent stem cell model of HNF4/MODY1 provides mechanistic insight into disease phenotypes

Kim-Vy Nguyen-Ngoc, Medhavi Mallick, Vivian Lin, Winnie Gong, Han Zhu, Maike Sander.

Background: Heterozygous mutations in the transcription factor HNF4 cause maturity onset diabetes of the young (MODY). Clinically, HNF4/MODY1 mutations are associated with postnatal hyperinsulinemic hypoglycemia (HI), evolving into diabetes later in life.

Methods and Results: To gain insight into disease mechanisms of HNF4/MODY1, we introduced the heterozygous *HNF4*R141X point mutation into human pluripotent stem cells (hPSC) and differentiated *HNF4*$^{R141X/+}$ and control cells into pancreatic islet cells (SC-islet). We observed no impact of the *HNF4*R141X mutation on islet cell differentiation. However, *HNF4*$^{R141X/+}$ SC-islets exhibited insulin hypersecretion in basal and high glucose, which resolved during SC-islet maturation. These findings are consistent with the transient HI phenotype in the MODY1 patients. To understand *HNF4*-dependent gene regulatory programs in beta cells, we compared transcriptomes and chromatin accessibility in *HNF4*$^{R141X/+}$ and control SC-islets at single cell level. Beta cell-specific analyses revealed reduced expression of *NEUROD1* and ion channels, consistent with reported insulin hypersecretion in loss-of-function models for these genes. In addition, *HNF4*$^{R141X/+}$ SC-beta cells exhibited reduced expression of pro-survival genes (*BCL2L1*, *SERPINA1*, *HSPA5*, *ANKS4B*), which are known to compensate for ER stress. Through gene regulatory network analyses, we identified *BCL2L1* and *SERPINA1* as direct target genes of HNF4 in beta cells. In agreement with these molecular findings, *HNF4*$^{R141X/+}$ SC-beta cells were more prone to undergo apoptosis in response to thapsigargin-induced ER stress, a phenotype that was rescued by supplementing SERPINA1 protein. These results suggest that beta cells in HNF4/MODY1 patients are more susceptible to stress-induced cell death, providing a possible mechanism for progression to diabetes.

Conclusions: Our hPSC-based HNF4/MODY1 model recapitulates key aspects of the HNF4/MODY1 phenotype. By providing mechanistic insight into *HNF4*-regulated cellular processes in beta cells, our work identifies opportunities for therapeutic intervention for MODY1 patients.

17. Exploring the role of RNA editing on cell fate specification

Sami Nourreddine and Prashant Mali.

In development, cell identity is determined by specific gene expression programs triggered by transcription factors and modulated by epigenetic modifiers at the chromatin level. With the improvement of genomic technologies, more than 150 types of post-transcriptional RNA modifications have been identified. In eukaryotes, adenosine-to-inosine (A-to-I) editing is one of the most prevalent RNA modifications and is performed by a family of enzymes named ADARs. ADAR proteins can impact diverse cellular processes such alternative splicing, microRNAs, the innate immune system, and protein recoding which in turn could impact cell specification. However, the role of A-to-I editing remains relatively uncharted in the context of development. Thus, we propose to examine the role of A-to-I editing on cell fate specification by systematically mapping the A-to-I landscape during stem cell differentiation and dissecting the role of ADARs in this process. To explore the role of ADARs on stem cell differentiation we used human pluripotent stem cells (hPSCs) derived teratomas as an experimental model. When coupled to single-cell RNAseq (scRNAseq) this approach allows to simultaneously track and study hPSC differentiation across more than 20 cell types from all 3 germ layers. Using CRISPR/Cas9 system, we have generated hPSC knockout lines for each ADAR isoforms (ADAR1,2 and 3), transplanted into mice to form teratomas, and processed the tissues for scRNAseq. Using this dataset, we are currently developing an analysis pipeline to determine genome-wide and cluster-specific A-to-I editing profiles on the different cell types found in teratomas. Furthermore, this loss of function study in teratomas will allow us to decipher which ADAR isoforms is required for a given cell type. Finally, our study will provide for the first time the landscape of A-to-I editing across differentiation to the 3 germ layers and could reveal relevant RNA edited sites as potential biomarkers to track lineage specification from hPSCs and develop new tools for stem cell reprogramming.

18. Single-cell mapping predicts a common origin of multiple subtypes in a new mouse model of pancreatic cancer

Nirakar Rajbhandari, Michael Hamilton, and Tannishtha Reya.

Understanding the molecular basis of tumor initiation and progression is essential for the effective management and treatment of this lethal disease. To determine if the stem and progenitor cells can serve as cells of origin for pancreatic cancer, we have created a new mouse strain in which tamoxifen-inducible Cr*e* (*CreERT2*) is knocked into the endogenous locus encoding the stem cell regulator Musashi2 (Msi2). When crossed to Cre-regulatable Myc transgenic (*CAG-LSL-Myc^{T58A})* strain*, Msi2-Cre^{ERT2}* mice develop multiple pancreatic cancer subtypes: pancreatic ductal adenocarcinoma (PDAC), adenosquamous carcinoma of the pancreas (ASCP), and acinar cell carcinoma (ACC) following Myc activation in Msi+ cells in the adult stage. A combination of single-cell genomics with computational analysis of developmental states and lineage trajectories of tumors in our model suggests that oncogenic Myc preferentially triggers the transformation of the most immature subset of Msi2+ cells, leading to the rise of a common pool of pre-cancer cells with multi-lineage potential. These pre-cancer cells subsequently take distinct fates within the same mice by activation of distinct transcriptional programs and large-scale genomic changes. Additionally, combining transcriptomic and functional genomic approaches, we have identified *Ifne, Tspan4, Hmmr* and A*tf3* as potential novel dependencies of ASCPs, providing a framework to develop interception strategies for this lethal disease. In summary, our study shows that multiple pancreatic cancer subtypes can arise from a common pool of Msi2+ cells and provides a powerful framework to understand and control the programs that shape divergent fates in pancreatic cancer.

19. Acute deletion of TET enzymes results in aneuploidy in mouse embryonic stem cells through decreased expression of *Khdc3*

Hugo Sepulveda, Romain O. Georges, eric Johnson, Susan Palomino, Roberta Nowak, Arshad Desai, Isaac F. Lopez-Moyado, Anjana Rao.

TET (Ten-Eleven Translocation) dioxygenases effect DNA demethylation through successive oxidation of the methyl group of 5-methylcytosine (5mC) in DNA. In humans and in mouse models, TET loss-of-function has been linked to DNA damage, genome instability and oncogenesis. Here we show that acute

deletion of all three *Tet* genes, by brief exposure of triple-floxed, Cre-ERT2-expressing mouse embryonic stem cell (mESC) to 4-hydroxytamoxifen, results in chromosome mis-segregation and aneuploidy; moreover, embryos lacking all three TET proteins showed striking variation in blastomere numbers and nuclear morphology at the 8-cell stage. Transcriptional profiling revealed that mRNAs encoding a KH-domain protein, KHDC3 (Filia), was downregulated in triple Tet-deficient mESC, concomitantly with increased methylation of CpG dinucleotides in the vicinity of the *Khdc3* gene. Restoring KHDC3 levels in triple Tet-deficient mESC prevented aneuploidy. Our data imply that TET proteins, which have been tied to DNA damage responses in other cell types, prevent mitotic abnormalities and maintain chromosome stability in mESC.

20. The Role of BMP Signaling in the Specification of Trophectoderm in Mouse and Human

Jasmine Temple and Mana Parast.

Introduction: BMP signaling is essential for mouse peri-implantation development. *In vitro*, BMP4 induces trophoblast-associated gene expression in mouse embryonic stem cells (mESC); however, the identity of these cells has not been functionally tested. Multiple groups have used BMP4-based protocols for the derivation of trophoblast stem cells (TSC) from primed and naïve human pluripotent stem cells (hPSC). We applied the conditions used to derive TSC from primed hPSC to the equivalent mouse epiblast-like cells (mEpiLC), as this model allows for functional assays. We also compared trophoblast induction from primed and naïve hESC using BMP4-based protocols. ***We hypothesize that BMP signaling is key in the induction of the trophoblast lineage in mouse and human embryogenesis.***

Methods: We treated mEpiLC with BMP4 and IWP2 for four days, following a protocol that induces a trophectoderm (TE)-like state in primed hESC. We evaluated these cells by qPCR, immunofluorescence (IF), and single-cell RNA-sequencing. We established an extended culture mouse embryo protocol, a pre-implantation blastoid model, and a post-implantation STEMbryo model, to evaluate BMP signaling patterns and the functionality of the BMP4/IWP2-treated mEpiLC. Finally, we compared methods of TSC derivation from naive and primed hESC using BMP4.

Results: By qPCR, mEpiLC expressed markers of pluripotency and 2-cell (2C) stage embryos, while trophoblast and 2C markers were induced in BMP4/IWP2-treated cells. Phospho-SMAD1, a marker of active BMP signaling, was noted in the outer (TE) cells of the developing mouse embryo, and BMP4 treatment of 2C-stage mouse embryos caused them to stall at the morula stage. We confirmed lineage-specific gene expression in the proper compartment of both the blastoid and STEMbryo models using IF and will use them to test the ability of BMP4/IWP2-treated mEpiLC to functionally replace mTSC. We were able to derive TE-like cells from both naïve and primed hPSC using BMP4-based protocols.

Conclusions: Our previous research has confirmed the ability to convert primed hESC to TSC following induction with BMP4/IWP2. We recapitulated these findings in the mouse, inducing a population of trophoblast-like cells following BMP4/IWP2 treatment of mEpiLCs. We are analyzing single-cell RNAseq data from these cells, comparing them to embryonic and extraembryonic stem cells, and will test the functionality of these cells in the blastoid/STEMbryo models. We confirmed that BMP signaling is particularly active in mouse TE and that excess BMP4 interferes with the development of mouse embryos. We are in the process of comparing TE-like cells induced from primed and naïve hESC using BMP4 by single-cell RNAseq. Our findings suggest a role for BMP signaling in pre-implantation embryo development, specifically in the TE. The comparison of cells at each step of BMP4 treatment to *in vivo* cell types may point to the mechanism through which this signaling pathway enhances stem cell plasticity.

21. IRF4 Mediated Reprogramming of Myeloma-Associated Macrophages to M1 Anti-Tumoral Macrophages

Trung Tran and Leslie Crews.

Multiple myeloma (MM) is a fatal plasma cell neoplasm that is characterized by the malignant expansion of immature antibody-producing $CD138^{+}$ plasma cells. Disease relapse is common and can occur due to the uncontrolled regeneration of myeloma progenitor cells or cancer stem-like cells in inflammatory microenvironments. In MM, aberrant expression of interferon-regulatory factor-4 (IRF4), a key B cell fate determinant, has been shown to promote MM progression through enhanced survival of malignant plasma cells. In addition to the well-described role of IRF4 in myeloma cells, IRF4 also regulates macrophage polarization, where its expression drives alternative activation of macrophages primarily to M2-like phenotypes. Within the MM tumor microenvironment, myeloma-associated macrophages

promote disease progression by exhibiting M2-like (pro-tumor) properties including chemoresistance, tumor proliferation and survival, angiogenesis, immunosuppression, and metastasis. However, the extent to which IRF4 modulation governs the polarization status and pro-tumor activity of macrophages in the myeloma niche has not been fully elucidated. To address this, we first characterized macrophage polarization markers in *in vitro* macrophage polarization models using THP-1 monocytes and $CD14^+$ peripheral blood-derived monocytes (PBMCs) that were exposed to M1-like (pro-inflammatory/anti-tumoral) or M2-like (anti-inflammatory/pro-tumor) macrophage induction conditions using defined cocktails of growth factors and cytokines. Macrophage polarization status was assessed by quantitative RT-PCR. These analyses verified that M1 macrophage polarization conditions upregulated M1 markers (CD80, TNFα, and IL-1β) and downregulated M2 genes (MRC1, CCL22, and FN1), while the converse was observed under M2 macrophage polarization conditions. To investigate the extent to which IRF4 gene activation or inhibition governs macrophage polarization status, *in vitro* polarized M2 macrophages were transfected with IRF4 siRNA. Gene expression analyses revealed that IRF4 knockdown in M2-induced macrophages increased CD80 expression and decreased MRC1 gene expression compared to non-targeting siRNA control-treated M2 macrophages. These findings suggest that IRF4 inhibition in M2-like macrophages shifts the polarization balance towards a more M1-like (anti-tumoral) phenotype. Future co-culture experiments with IRF4 siRNA-treated M2 macrophages and MM cells will be performed to evaluate the functional anti-tumoral activity of IRF4-depleted macrophages. These results provide further insights into the role of IRF4 in macrophage polarization and highlights the potential of IRF4-targeting regenerative medicine therapies in reprogramming the MM tumor niche. The findings also have potential applications to a variety of other human cancers with microenvironments rich in anti-inflammatory macrophages.

22. Disrupted lipid homeostasis and loss of IRF4 impairs lymphoid progenitor maintenance in a murine model of accelerated aging

Silvia Vicenzi, Lara C. Avsharian, Fangyuan Gao, Qianlan Xu, Kseniya Malukhina, Dorota Skowronska-Krawczyk, and Leslie A. Crews.

The aging immune system is characterized by increased bone marrow (BM) adiposity, myeloid skewing, impaired lymphocyte function and response to infection, and loss of hematopoietic stem and progenitor cell (HSPC) regenerative capacity. Human systemic aging studies have found alterations in lipid and fatty acid metabolism, such as increased saturated fatty acid accumulation and decays in n-3 polyunsaturated fatty acid (PUFA) species leading to cellular membrane rigidity. However, the extent to which impaired fatty acid metabolism contributes to immune system aging and altered HSPC development is largely unexplored.

Therefore, we characterized BM aging phenotypes in a robust aging model that expresses a mutant inactive form of the very long chain (VLC) PUFA elongation enzyme, Elongation of very long chain fatty acids protein 2 (ELOVL2), which is responsible for the elongation of 22-carbon PUFAs to 24-carbon PUFAs. To investigate the extent to which impaired VLC-PUFA production in the setting of ELOVL2 enzymatic deficiency might also impair immune cell regeneration associated with aging phenotypes, we performed multi-omics studies in BM samples from young (3 months old) and aged (18 months old) wild-type mice, compared with age-matched *Elovl2* mutant mice. Total BM samples were subjected to lipidomics analyses, whole transcriptome RNA-sequencing (RNA-seq), and ATAC-seq analyses.

Lipidomic profiling showed an almost complete depletion of direct and secondary products of ELOVL2 in BM isolated from mutant animals, suggesting significant changes in the biophysical properties of membranes in the cells. Gene set enrichment analyses (Reactome) of RNA-seq data revealed that pathways involving B cell receptor (BCR) signalling and immunoregulatory interactions between lymphoid and non-lymphoid cells were among the most differentially regulated in *Elovl2* mutant mouse BM compared to age-matched controls. Key lymphoid lineage maturation markers (Cd19, Cd22, and Slamf7, among others) were downregulated in the BM of aged versus young wild-type mice, and to a greater extent in aged *Elovl2* mutant animals. This was accompanied by a significant downregulation of the lymphoid and plasma cell transcription factor, interferon-regulatory factor-4 (Irf4), in aged and *Elovl2* mutant groups compared with young mice. Finally, ATAC-seq data revealed changes in DNA accessibility related to *Elovl2* mutation, suggesting significant epigenetic changes in nuclear programs.

Together, these results provide insights into a functional link between disrupted lipid homeostasis and aging-related defects in hematopoietic stem cell development towards the lymphoid lineage, leading to impaired maturation of B cell and plasma cell populations. Together, lipid catabolism pathways may

provide a novel regenerative medicine target for modeling and reversing a variety of age-related immune deficits.

23. A phenotypic brain organoids atlas for neurodevelopmental disorders

Lu Wang, David Sievert, Arzoo Patel, Joseph Gleeson.

Human brain organoids (hBOs) are self-assembled from human-induced pluripotent stem cells (iPSC) and mimic both the structural and cellular complexity of the developing human brain. Their ability to reproducibly model early brain development and molecular logistics highlights their advantage in investigating the pathogenesis of neurodevelopmental disorders (NDDs). The genetic causes for NDDs vary and the convergent pathologies for NDDs treatments are largely missing. To fill this gap, we developed a "phenotypic atlas" of hBOs for recessive NDDs. We generated a total number of 5182 hBOs derived from 46 independent iPSC lines, including over 1000 control lines, ~2000 non-structural mental disability (NS) lines, ~2000 microcephaly (MIC) and polymicrogyria (PMG) lines, and half of them carry known disease-causing mutations. Using 2D/3D immunostainings, we found that hBOs robustly model NDDs with 0% of shown a phenotype, showing identical morphologic phenotypes within the same disease category. By analyzing 663749 cells from 27 libraries of single-cell RNA-seq, we observed uniform abnormality in the radial glia cells for MIC and intermediate progenitor cells (IPCs) for PMG. Particularly, PMG hBOs has shown significant DLX5+ IPCs but few EOMES+ IPCs, this observation has been confirmed by a lineage tracing DNA-barcoding system. Whereas for NS hBOs, no convergent cellular types were observed. Our study, leveraging thousands of NDD hBOs, represents a comprehensive phenotypic and transcriptomic atlas, demonstrating the structural NDDs convergent in the dysregulation of progenitor cells and linking the imbalanced DLX5+ and EOMES+ IPCs to PMG pathogenesis.

24. Pancreatic cancer cell upregulation of LPA receptor 4 in response to stress creates a niche to support tumor initiation

Chengsheng Wu and David Cheresh.

Defining drivers of cancer stemness can provide opportunities to control cancer progression. Here, we report that a receptor for bioactive lipid lysophosphatidic acid (LPA), LPAR4/GPR23/P2Y9, becomes upregulated on pancreatic cancer cells exposed to environmental stress or chemotherapy in vitro/vivo where it promotes stress tolerance, self-renewal, and tumor initiation. Pancreatic cancer cells gain LPAR4 expression in response to stress by downregulating a tumor suppressor, miR-139-5p. Even in the absence of exogenous LPA, the canonical LPAR4 ligand, LPAR4-expressing tumors display an enrichment of key extracellular matrix (ECM)-related genes that are established drivers of cancer progression and stemness. Mechanistically, upregulation of fibronectin via a LPAR4/AKT/CREB axis is indispensable for LPAR4-induced tumor initiation, and fibronectin deposited by LPAR4-expressing cells signals through integrins α5β1 or αVβ3 to transfer stress tolerance to LPAR4-negative cells. Therefore, stress- or drug-induced LPAR4 enhances cell-autonomous production of a fibronectin-rich ECM, allowing cells to survive “isolation stress” and compensate for the absence of stromal-derived factors by creating their own tumor-initiating niche.

25. Protein Homeostasis Is Dynamically Regulated Throughout Hematopoietic Stem Cell Ontogeny

Helena Yu and Robert A.J. Signer.

Hematopoietic stem cells (HSCs) are required for establishing hematopoiesis during development and for maintaining lifelong regeneration of blood cells. As demands for blood cell production change throughout ontogeny, there is a commensurate change in HSC biology. Fetal, neonatal, and adult HSCs exhibit differences in gene expression, developmental potential, and self-renewal activity. Thus, fetal, neonatal, and adult HSCs are, at least functionally, distinct populations. We recently found that fetal HSCs exhibit substantially higher rates of protein synthesis as compared to adult HSCs, which depend on unusually low protein synthesis rates to limit the biogenesis of misfolded and unfolded proteins to preserve protein homeostasis (proteostasis) and fitness. This raised questions of whether fetal HSCs experience significant proteotoxic stress in vivo and how proteostasis is regulated throughout HSC development. Remarkably, we found that fetal HSCs restrict accumulation of unfolded proteins to levels similar to those observed in adult HSCs, despite having nearly 7-fold higher protein synthesis rates. Surprisingly, however, we determined that unfolded protein abundance spikes dramatically within HSCs at birth. This transient burst of proteotoxic stress is followed by rapid restoration of proteostasis in neonates, as unfolded protein

abundance declines precipitously, ultimately reaching unusually low levels by P14 before moderately rising again in adulthood. In contrast to the dynamic oscillation of unfolded protein abundance, protein synthesis rates progressively decline throughout HSC ontogeny. Fetal HSCs synthesize significantly more protein per hour than HSCs from newborn and postnatal mice, which in turn have nearly 3-fold and 2-fold higher protein synthesis rates than adult HSCs, respectively. Strikingly, however, postnatal declines in HSC protein synthesis occur gradually and non-uniformly. While fetal and adult HSCs have narrow distributions of protein synthesis rates, neonatal HSCs exhibit significant heterogeneity, suggesting an uncoordinated transition to achieving low protein synthesis rates and proteostasis in adulthood. Taken together, these findings indicate that HSC ontogeny is associated with rapid, dynamic, and robust changes in proteostasis activity, and that fetal, neonatal, and adult HSCs utilize distinct mechanisms to regulate proteostasis in vivo. The regulation of proteostasis is thus a key distinguishing feature of HSC ontogeny that could provide a framework to uncover how pathogenic changes disrupt hematopoietic development and function in an age-specific manner. Furthermore, harnessing developmental proteostasis mechanisms could open new avenues to restore HSC fitness in response to various stressors and blood disorders that emerge throughout life.

Thank you!

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We invite you to join us next year here in San Diego for the annual Sanford Stem Cell Institute Symposium. For more information, please contact Michelle Ghani at: mghani@ucsd.edu.

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