DRIVING DISCOVERIES BEYOND BOUNDARIES

2021 INTERNATIONAL TSC & LAM RESEARCH CONFERENCE

OCTOBER 28—30, 2021

PRESENTED BY:

THE ROTHBERG INSTITUTE For childhood diseases

GREENWICH BIOSCIENCES

CO-HOSTED BY:

TSC Alliance

THE LAM FOUNDATION
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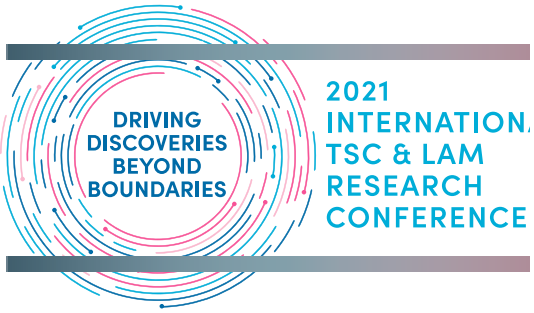
2021 International
TSC Research Conference

Driving Discoveries
Beyond Boundaries

Welcome



Dear Conference Participants,

On behalf of TSC Alliance® and The LAM Foundation, welcome to the virtual 2021 International TSC & LAM Research Conference: Driving Discoveries Beyond Boundaries, presented by Greenwich Biosciences and The Rothberg Institute for Childhood Diseases.

Both organizations are thrilled our conference collaboration has attracted researchers and clinicians from around the world. This is the second research conference we have co-hosted together. It builds upon a solid foundation of partnership, which includes hosting 13 Regional Conferences for individuals with tuberous sclerosis complex (TSC) and lymphangioleiomyomatosis (LAM), co-funding research grants, co-nominating clinicians and researchers for American Thoracic Society Public Advisory Roundtable awards, and holding a joint board meeting to align our goals. By uniting once again to stage this conference, we believe this meeting will ensure a more hopeful future for people living with LAM and/or TSC.

We deeply thank conference co-chairs Drs. Nishant Gupta and Rebecca A. Ihrie and the Conference Steering Committee, who worked hard over the past 10 months to create an extensive agenda with exciting oral presentations, focused panel discussions, and breakout sessions to foster collaboration even in this virtual setting. Poster sessions and exhibits will provide opportunities to share with and learn from other attendees. Our virtual conference platform provider has created a welcoming environment that will enable attendees to easily engage across oceans and time zones to forge new relationships and connect with colleagues.

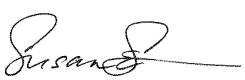
Our conference features a keynote presentation titled, "Transforming Drug Discovery Using Digital Biology," by Daphne Koller, PhD, Founder and CEO of Insitro, Inc. The proceedings will close with an engaging discussion panel led by the Conference Co-Chairs, Drs. Nishant Gupta and Rebecca A. Ihrie, with participation from the Tuberous Sclerosis Association's June conference Co-Chairs, Profs. Anna Jansen and Chris Kingswood. While the conference's live sessions end October 30, all sessions and materials will be accessible on our virtual conference platform for 90 additional days.

We sincerely thank our sponsors who generously supported this conference, including Greenwich Biosciences; The Rothberg Institute for Childhood Diseases; Nobelpharma; UCB; PsychoGenics, Inc.; Upsher-Smith Laboratories, LLC; Noema Pharma; Seizure Tracker; BridgeBio; and Novartis.

Finally, we salute our speakers and poster presenters for their contributions to making the conference a success. And of course, we sincerely thank all attendees for choosing to spend time away from their institutions, laboratories, and practices to help advance LAM and TSC research.

Sincerely,

Kari Luther Rosbeck
President & CEO
TSC Alliance



Susan Sherman, MHA
Executive Director & CEO
The LAM Foundation

Conference Organizing Committee

Co-Chairs

Nishant Gupta, MD
University of Cincinnati

Rebecca A. Ihrie, PhD
Vanderbilt University

Committee Members

Helen Bateup, PhD
University of California, Berkeley

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New York Medical College

Simon Johnson, DM
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Early-Career Research Symposium Co-Chairs

Charilaos "Harry" Filippakis, PhD
Brigham and Women's Hospital

Gerta Hoxhaj, PhD
UT Southwestern

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2021 International TSC & LAM Research Conference Agenda

(All times: Eastern Daylight Time, United States)

Thursday, October 28, 2021

10:00 AM – 12:10 PM **Cell biology, metabolism, and cellular communication in TSC and LAM**
Session Chairs: Marina Holz, PhD and Mark Nellist, PhD

10:00 – 10:10 AM — **Opening remarks and TSC/LAM community speaker** – Kari Luther Rosbeck, President & CEO, TSC Alliance, introducing Nicole Seefeldt

10:10 – 10:40 AM — Kathrin Thedieck, PhD (University of Innsbruck) – **Stress granule proteins meet TSC-mTOR signaling: an unexpected mechanism of lysosomal TSC tethering**

10:40 – 10:55 AM — Adam Pietrobon (Ottawa Hospital Research Institute) – **Three-dimensional drug screen identifies HDAC inhibitors as therapeutic agents in a tissue-engineered model of lymphangioleiomyomatosis**

10:55 – 11:10 AM — Uchenna Unachukwu, PhD (Columbia University) – **Novel neural crest markers characterize tumors in *Tsc2*$^{+/-}$ mouse model of TSC**

11:10 – 11:25 AM — Krinio Giannikou, PhD (Brigham and Women's Hospital) – **Kidney angiomyolipomas are defined by a unique transcriptional profile and H3K27ac chromatin state**

11:25 – 11:40 AM — Mayowa Amosu (University of Maryland, College Park) – **Intranasal administration of CpG increases survival and counters immunosuppression in a murine model of metastatic lymphangioleiomyomatosis (LAM)**

11:40 AM – 12:10 PM — Vivi M. Heine, PhD (Amsterdam UMC) – **Neuron-glia iPSC models for TSC**

12:10 – 12:40 PM — ***Break*** – opportunity for Sponsored Exhibit Time

12:40 – 3:00 PM **TOPIC-BASED DISCUSSION: Extracting meaning from multiomics datasets: How do we bring together multiomics data? What is the question we are trying to answer?**
Session Moderator: Rebecca A. Ihrie, PhD (Vanderbilt University)

12:40 – 1:20 PM — **Panelist presentations**
Rebecca A. Ihrie, PhD (Vanderbilt University)
Vera P. Krymskaya, PhD, MBA (University of Pennsylvania)
Peter E. Davis, MD (Boston Children's Hospital)
Yan Xu, PhD (University of Cincinnati)

1:20 – 1:50 PM — **Moderated Q&A**

1:50 – 2:30 PM — **Breakout Discussions**

2:30 – 3:00 PM — **Breakout Report Back**

3:00 – 4:00 PM **POSTER SESSION 1 AND EXHIBITS OPEN**

Friday, October 29, 2021

10:00 AM – 12:10 PM **Predicting and preventing manifestations of TSC and LAM**
Session Chairs: Carmen Priolo, MD, PhD & Peter Tsai, MD, PhD

10:00 – 10:10 AM — **Opening remarks and TSC/LAM community speaker** – Sue Sherman, Executive Director & CEO, The LAM Foundation, introducing Ray Marco, Secretary, Tuberous Sclerosis Canada Sclérose Tubérose (TS Canada ST)

10:10 – 10:40 AM — Simon Johnson, DM (University of Nottingham) – **Predicting and preventing manifestations of TSC and LAM**

10:40 – 10:55 AM — I. Caroline LePoole, PhD (Northwestern) – **A PDX model of adoptive T cell therapy to treat lymphangioleiomyomatosis**

10:55 – 11:10 AM — Anushka Palipana (University of Cincinnati) – **Predicting individualized lung disease progression in lymphangioleiomyomatosis**

Friday, October 29, 2021 (cont.)

11:10 – 11:25 AM	Katarzyna Klonowska, PhD (Brigham and Women's Hospital) – **UV mutation drives a high prevalence of subclinical facial tumors in TSC – focus on mutation signatures**
11:25 – 11:40 AM	Mary Beth Brown, PT, PhD (University of Washington) – **Blood oxygen saturation during a mobile health exercise program in women with lymphangioleiomyomatosis**
11:40 AM – 12:40 PM	**KEYNOTE ADDRESS – Daphne Koller, PhD (Insitro, Inc.) – Transforming drug discovery using digital biology**
12:40 – 12:50 PM	***Break***
12:50 – 3:00 PM	**TOPIC-BASED DISCUSSION: Informing and enabling clinical trials, from bench to randomized clinical trials** **Session Moderator: Nishant Gupta, MD (University of Cincinnati)**
12:50 – 1:30 PM	**Panelist Presentations** Kevin C. Ess, MD, PhD (Vanderbilt University) Dean Aguiar, PhD (TSC Alliance) Elizabeth P. (Lisa) Henske, MD (Brigham and Women's Hospital) Ajamete "Aj" Kaykas, PhD (Insitro, Inc.)
1:30 – 2:00 PM	**Moderated Q&A**
2:00 – 2:40 PM	**Breakout Discussions**
2:40 – 3:00 PM	**Breakout Report Back**
3:00 – 4:00 PM	**POSTER SESSION 1 AND EXHIBITS OPEN**

Saturday, October 30, 2021

10:00 AM – 11:55 AM	**Clinical, translational, and basic research for epilepsy** **Session Chairs: Tanjala Gipson, MD & Brenda Porter, MD, PhD**
10:00 – 10:10 AM	**Opening remarks and TSC/LAM community speaker** – Peter Crino, MD, PhD, Chair, TSC Alliance Board of Directors and Director, TSC Center of Excellence at the University of Marylan, introducing Lesley Holmes
10:10 – 10:40 AM	Helen Bateup, PhD (University of California, Berkeley) – **The multi-faceted roles of TSC-mTOR signaling in neural development, function, and disease**
10:40 – 10:55 AM	Lena Nguyen, PhD (Yale University) – **Juvenile 4E-BP1 expression attenuates epilepsy and neurophysiological a bnormalities associated with mTOR hyperactivation in mice**
10:55 – 11:10 AM	Mary Bronwen Chalkley (Vanderbilt University) – **Early neurodevelopment and cytoarchitecture is altered in TSC**
11:10 – 11:25 AM	Vasiliki Karalis (University of California, Berkeley) – **Raptor downregulation rescues neuronal phenotypes in mouse models of TSC**
11:25 – 11:55 AM	Jamie K. Capal, MD (University of North Carolina) – **Clinical, translational, and basic research for epilepsy**
11:55 AM – 12:30 PM	***Break*** – opportunity for Sponsored Exhibit Time
12:30 – 2:00 PM	**CLOSING PANEL SESSION** **Panel Moderators: Rebecca A. Ihrie, PhD and Nishant Gupta, MD** **Panelists: Session Chairs and Co-Chairs from the UK Tuberous Sclerosis Association June 2021 Conference**

Early-Career Researcher Symposium Agenda

(All times: Eastern Daylight Time, United States)

Saturday, October 30, 2021

2:05 – 4:30 PM **Selling Science**

Session Chairs: Gerta Hoxhaj, PhD (UT Southwestern) and Charilaos "Harry" Filippakis, PhD (Brigham and Women's Hospital)

2:05 – 2:10 PM **Opening remarks and TSC/LAM community speaker** – Charilaos (Harry) Filippakis, PhD (Brigham and Women's Hospital) and Gerta Hoxhaj, PhD (UT Southwestern) introducing Kendall Shields

2:10 – 3:00 PM **Talks selected from abstracts**

- 2:10 – 2:20 PM Bailey Fessler (University of Cincinnati College of Medicine) – **Prevalence of thoracoabdominal imaging findings in tuberous sclerosis complex**
- 2:20 – 2:30 PM Rohit Khurana (Vanderbilt University) – **Computational identification of balloon cells in whole slide images of cortical tubers**
- 2:30 – 2:40 PM Shikshya Shrestha, PhD (Brigham and Women's Hospital) – **ETV2 is a critical regulator of cell death in Tsc2–deficient cells**
- 2:40 – 2:50 PM **Group discussion of the talks**

2:50 – 3:00 PM **Introduction of Career Development Panel**
Rebecca Ihrie, PhD (Vanderbilt)
Mark Keezer, MD, PhD (Université de Montréal Hospital Centre)
Gina Lee, PhD (University of California, Irvine)
Dario Lemos, PhD (Brigham and Women's Hospital)
Alexander Valvezan, PhD (Rutgers)

3:00 – 3:30 PM **Workshop-style activities with Career Development Panel**

3:30 – 3:55 PM **Open Q&A with Career Development Panel**

3:55 – 4:00 PM **Wrap up**

4:00 – 4:30 PM **Open Zoom for networking (optional)**

Invited Speaker Biographies

Dean Aguiar, PhD

Director, Preclinical Research
TSC Alliance
Silver Spring, MD

Dean joined the TSC Alliance in November 2018 with more than 17 years of research and development (R&D) leadership in biopharmaceutical and medical device industries, leading teams and technologies from discovery to investigational new drug and investigational device exemption, a pre-requisite for clinical trial evaluation. He brings an entrepreneurial and collaborative approach to R&D, identifying opportunity, defining strategy and developing a scientific data package to warrant clinical translation of technologies to benefit the patient, the family and care provider. In Dean's prior role as Program Director at The Hartwell Foundation, he gained significant experience in pediatric disease from oncology and inflammation to neurodevelopmental disorders including autism, ADHD and epilepsy. He provided guidance to academic investigators regarding the scientific evidence and regulatory path required to successfully translate technologies toward commercial viability. He also established partnerships with industry to gain access to proprietary drugs and a possible path for licensing. Dean cofounded Pendant Biosciences, a Johnson & Johnson Innovation JLABS company, with a mission to develop a novel polymer biomaterial for targeted drug delivery improving efficacy and minimizing toxicity. Dean contributed to the leadership team that identified opportunities for the company's core technology to address unmet patient needs and was responsible for developing an R&D strategy and data package to attract strategic partners. In addition, Dean has a breadth of pharmaceutical R&D experiences from early discovery to clinical translation that provides a unique perspective on project management and mitigating risk. As the former Director of Product Development at Biomimetic Therapeutics, a medical device company, he led a team focused on the development of drug-device combination products and drug-only products bridging the regulatory framework for a drug and a device. Dean's early career landed him at Pfizer, where he led cross-functional project teams in the areas of inflammation spanning discovery to preclinical development of both small molecules and protein biologic drugs. He played a key role while ensuring a robust decision funnel for translatable cell, animal models and biomarkers of human disease. Dean earned his PhD in Biochemistry from Rush University at Rush Presbyterian St. Luke's Medical Center in 1996 and completed post-doctoral training at the University of Minnesota. Dean and his wife, Missie, have three boys – Ryan, Cole and Chase.

Helen Bateup, PhD

Associate Professor of Neurobiology
University of California, Berkeley
Berkeley, CA

Dr. Bateup graduated from the honors program at Penn State University in 2000 with a BS degree in Biobehavioral Health and minor in Neuroscience. She completed a one-year post-baccalaureate research fellowship at NIMH prior to joining the graduate program at Rockefeller University 2001. In 2007, Dr. Bateup received her PhD, completing her dissertation work in Dr. Paul Greengard's lab where she established novel genetic mouse models to determine the cell type-specific consequences of dopamine signaling on striatal function. From there she joined Dr. Bernardo Sabatini's lab as a post-doctoral fellow at Harvard Medical School to study how TSC-mTOR signaling affects synaptic function and excitatory/inhibitory balance in the hippocampus. In 2013, Dr. Bateup started her lab at the University of California, Berkeley in the Department of Molecular and Cell Biology and became a member of the Helen Wills Neuroscience Institute. In 2019 she was named a Chan Zuckerberg Biohub Investigator. The goal of Dr. Bateup's research is to elucidate the cellular and molecular basis of neurodevelopmental disorders with a focus on TSC. To accomplish this, her lab takes a multi-faceted approach incorporating molecular, biochemical, electrophysiological, and behavioral analyses in genetic mouse models. In addition, her lab is investigating the early developmental alterations that may contribute to TSC and related disorders using genetically engineered human brain organoids.

Jamie K. Capal, MD

Associate Professor of Pediatrics and Neurology – Child Neurology, Neurodevelopmental Disabilities
University of North Carolina and Carolina Institute of Developmental Disabilities
Chapel Hill, NC

Dr. Capal is an Associate Professor of Pediatrics and Neurology. She is board-certified in Pediatrics, Neurology with special qualification in Child Neurology, and Neurodevelopmental Disabilities. She received her training at Cincinnati Hospital Medical Center and stayed on as faculty there until the spring of 2020. While at Cincinnati Children's Hospital, she was a member of the multidisciplinary Tuberous Sclerosis Complex Clinic and was involved in several research studies funded by the National Institutes of Health and TSC Clinical Research Consortium (TACERN), including determining biomarkers for ASD in young children with TSC, ages birth to 36 months; determining the timing and relationship between seizures, cognition, and development of ASD; evaluating the broader autism phenotype in ASD; and examining common features of ASD among different single-gene disorders with high prevalence of ASD (TSC, Phelan-McDermid syndrome, PTEN Hamartoma syndrome).

She has since joined the University of North Carolina at Chapel Hill where she has a joint appointment in neurology and the Carolina Institute for Developmental Disabilities. Her clinical and research interests focus on children with neurodevelopmental and neurogenetic conditions, including autism spectrum disorders, tuberous sclerosis complex, and Angelman syndrome. She has transitioned to UNC Chapel Hill with a vision to improve care for individuals with TSC. She has also started a TAND clinic.

Peter E. Davis, MD

Instructor in Neurology
Boston Children's Hospital
Boston, MA

I am a pediatric neurologist. My research uses clinical data and computational EEG methods to identify risk factors for epilepsy and neurodevelopmental disorders in children with tuberous sclerosis complex. I am a member of the international TANDem consortium. Clinically, I see children with autism, intellectual disabilities, epilepsy, and other neurodevelopmental disorders in the Multi-disciplinary TSC Clinic and Autism Spectrum Center at Boston Children's Hospital.

Kevin C. Ess, MD, PhD

Principal Investigator, Gerald M. Fenichel Chair in Neurology Pediatrics
Division Chief, Division of Pediatric Neurology
Associate Professor, Department of Neurology; Department of Pediatrics
Associate Professor, Department of Cell and Developmental Biology
Vanderbilt University
Nashville, TN

Dr. Ess has dedicated his career to understanding the genetic control of brain development and how aberrations in developmental processes lead to epilepsy, and autism. For the past 12 years at Vanderbilt, his research has focused on tuberous sclerosis complex (TSC) as patients with this disease have prominent brain malformations, white matter disease and a very high prevalence of epilepsy and autism. Resulting from mutations in either the *TSC1* or *TSC2* genes, this disorder involves dysregulation of the mTOR kinase with striking signaling abnormalities of both the mTORC1 and mTORC2 signaling pathways. He has also been interested in a recently defined disorder, alternating hemiplegia of childhood (AHC), a devastating neurodevelopmental disorder due to mutations in the ATP1A3 gene. To study abnormal developmental processes in TSC and AHC, he has utilized diverse model systems including transgenic mouse, zebrafish, and human induced pluripotent stem cells (iPSCs). Principally employing iPSCs, his basic and translational research approaches to TSC and AHC should culminate in advanced knowledge about pediatric neurological disorders and hopefully lead to the development of novel and more effective therapies.

Nishant Gupta, MD

Director of the LAM Clinic Network
Assistant Professor of Medicine
University of Cincinnati
Cincinnati, OH

Nishant Gupta completed his medical school training in India, followed by residency in internal medicine at Tennessee and fellowship in the field of Pulmonary and Critical Care Medicine at the University of Cincinnati. In addition, he has completed fellowship training in the field of rare lung diseases, as well a Masters degree in Clinical and Translational Research at the University of Cincinnati. Dr. Gupta is currently an Associate Professor in the Division of Pulmonary, Critical Care and Sleep Medicine at the University of Cincinnati, where he serves as the director of the interstitial and rare lung diseases program. Dr. Gupta is also the medical director of the international LAM clinic network and has recently assumed the role of the scientific director of The LAM Foundation. Dr. Gupta's clinical and research focus is in the field of rare lung diseases such as LAM, and his work is aimed at better defining the natural history, improving detection, and developing novel treatment modalities and monitoring strategies for patients with LAM.

Vivi M. Heine, PhD

Associate Professor, Clinical genetics
Associate Professor, Amsterdam Neuroscience - Cellular & Molecular Mechanisms
Associate Professor, Amsterdam Neuroscience - Compulsivity, Impulsivity & Attention
Amsterdam UMC
Amsterdam, Netherlands

Dr. Heine studies developmental brain disorders with glial dysfunction involvement using human induced pluripotent stem cell (iPSC)-based models. So far research is mainly focused on neurons, while glia dysfunction is increasingly shown to contribute to various neurological disorders. Therefore we need better understanding of how neuron-glia cross talk contribute to brain network dysfunctions in genetic brain disorders, such as axon-myelin and neurotrophic interactions. The Heine lab uses optimized procedures to generate 2D or 3D iPSC-derived neuron-glia co-cultures, which functionality is confirmed with advanced microscopy, electrophysiology, proteomics and/or high content cellular screening. She aims for a comprehensive strategy to improve iPSC research to drive future basic and translational research in the field of developmental brain disorders. iPSCs form an attractive translational tool to compensate for shortcomings of, or replace, current in vivo models, and to increase the translational value of target identification and validation. But, to live up to the high promise of iPSCs, it will be important to align criteria and efforts to consolidate the technology to create reliable and therapeutically valid disease models. One of her research lines focuses on developing iPSC-based models for TSC.

Elizabeth P. (Lisa) Henske, MD

Director, Center for LAM Research and Clinical Care
Co-Director, Pulmonary Genetics Center
Professor, Harvard Medical School
Brigham and Women's Hospital
Boston, MA

Elizabeth (Lisa) Petri Henske is the Director of the Center for LAM Research and Clinical Care at Brigham and Women's Hospital. She is Professor of Medicine at Harvard Medical School, an Associate Member of the Broad Institute of MIT and Harvard, and a practicing medical oncologist at the Dana-Farber Cancer Institute. She earned her undergraduate degree summa cum laude from Yale University, where she majored in Molecular Biophysics and Biochemistry and her MD from Harvard Medical School. She completed a residency in Internal Medicine and a fellowship in Hematology/Oncology at the Massachusetts General Hospital. Dr. Henske's laboratory discovered that lymphangioleiomyomatosis (LAM) is caused by mutations in the tuberous sclerosis complex (TSC) genes. She also was the first to discover that the TSC1 and TSC2 proteins physically interact. Her research laboratory is focused on the cellular, metabolic, and immunologic mechanisms underlying the pathogenesis of angiomyolipomas and LAM. She is a member of the American Society for Clinical Investigation and the Association of American Physicians, and serves on the Board of Directors of The LAM Foundation and the Professional Advisory Board of the TSC Alliance. Dr. Henske has received awards for her research from the TSC Alliance, The LAM Foundation, the American Thoracic Society, and the Society for Women's Health Research (the Medtronic Prize).

Rebecca A. Ihrie, PhD

Associate Professor of Cell and Developmental Biology
Associate Professor of Neurological Surgery
Vanderbilt University
Nashville, TN

Dr. Ihrie completed undergraduate studies in Biochemistry with Honors at the University of Michigan, a Ph.D. in Cancer Biology at Stanford University, and a postdoctoral Fellowship at the University of California San Francisco. Her work has been recognized by Stanford's Lieberman Award, the Damon Runyon Cancer Research Foundation, the American Association for Cancer Research/National Brain Tumor Society, the Southeastern Brain Tumor Foundation, and the Ben & Catherine Ivy Foundation for Brain Tumor Research. Since 2012, the Ihrie Lab at Vanderbilt University has studied the stem cells of the brain and stem-like cells in brain tumors using approaches that measure tens to hundreds of features on millions of cells at a time. Her group revealed intrinsic differences in per-cell signaling capacity between groups of neural stem cells, and showed that this difference is linked to the ability of these cells to form brain tumors in TSC. The laboratory also demonstrated that tumor contact with the brain's stem cell niche is a key independent predictor of patient outcome. Recently the Ihrie lab and collaborators used new machine learning algorithms to identify and deeply characterize novel, risk stratifying populations of brain tumor cells. Dr. Ihrie is a member of the Vanderbilt Brain Institute, Vanderbilt Center for Stem Cell Biology, and the Vanderbilt-Ingram Cancer Center.

Simon Johnson, DM

Professor of Respiratory Medicine
Head of Division of Respiratory Medicine, Faculty of Medicine & Health Sciences
University of Nottingham
Nottingham, England

Simon Johnson's laboratory focus is upon proteolytic mechanisms of lung destruction. The group has published extensively on the role of matrix metalloproteinases, cell-cell and cell-matrix interactions in LAM and other chronic lung diseases. Prof. Johnson is director of the National Centre for LAM, which provides comprehensive clinical care for more than 250 patients. Funded by NHS England, the LAM Centre runs a prospective research cohort allowing access to clinical data, physiology, imaging, tissue and DNA. Clinical research has examined biomarkers and methods to predict outcomes and complications in LAM. Prof. Johnson's contribution to LAM research was recognised with The LAM Foundation's Scientific Advancement Award in 2014. He is Medical advisor to LAM Action, a Professional advisor to the Tuberous Sclerosis Association and a member of The LAM Foundation Scientific board.

Ajamete "Aj" Kaykas, PhD

Chief Technology Officer
Insitro, Inc.
San Francisco, CA

As Chief Technology Officer, Aj is responsible for producing high-quality data sets to use in for machine learning-based target and drug discovery. He leads insitro's wet lab activities which consists of functional genomics, disease modeling, phenotyping, automation, and process engineering. Ajamete has spent more than 28 years in both industry and academia, working in the areas of proteomics, genomics, and stem cell biology. Before joining insitro, Aj led the early target discovery team at Novartis Institutes for Biomedical Research in the Neuroscience unit. His team efforts have led to the discovery of multiple new disease targets and the development of better predictive preclinical models. He conducted his postdoc with Dr. Randy Moon at the University of Washington/Howard Hughes Medical Institute on Wnt-signaling. While in Randy's lab, he conducted one of the first ever genome-wide RNAi screens and studied the role of Wnt-signaling in human disease and stem cell biology. He completed his graduate work at the University of Wisconsin-Madison in Dr. Bill Sugden's lab where he studied virology, immunology, and oncology. In his free time, Aj enjoys traveling, kayaking, sailing, biking, making whiskey and most of all his family.

Daphne Koller, PhD
Founder and CEO
Insitro, Inc.
San Francisco, CA

Daphne Koller is CEO and Founder of insitro, a machine-learning enabled drug discovery company. Daphne is also co-founder of Engageli, was the Rajeev Motwani Professor of Computer Science at Stanford University, where she served on the faculty for 18 years, the co-CEO and President of Coursera, and the Chief Computing Officer of Calico, an Alphabet company in the healthcare space. She is the author of more than 200 referenced publications appearing in venues such as *Science*, *Cell*, and *Nature Genetics*. Daphne was recognized as one of *TIME Magazine*'s 100 most influential people in 2012. She received the MacArthur Foundation Fellowship in 2004 and the ACM Prize in Computing in 2008. She was inducted into the National Academy of Engineering in 2011 and elected a fellow of the American Association for Artificial Intelligence in 2004, the American Academy of Arts and Sciences in 2014, and the International Society of Computational Biology in 2017.

Vera P. Krymskaya, PhD, MBA
Professor of Medicine
University of Pennsylvania
Philadelphia, PA

Dr. Krymskaya is a Professor of Medicine with tenure at the University of Pennsylvania Perelman School of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine in Philadelphia, PA USA. Krymskaya's lab discovered the TSC2 function as a negative regulator of the mTOR and was the first to establish primary human LAM cell cultures to perform preclinical testing of rapamycin for inhibition of mTOR and abrogating LAM cell growth. Investigation into immunity in LAM, led to identification of PD-L1 upregulation in LAM lungs, and performance of preclinical study of anti-PD1 antibody to improve survival of immunocompetent mouse model of LAM. Dr. Krymskaya's study of LAM lung cell composition and novel TSC-LAM-relevant genetic animal model lays the groundwork for developing a novel mechanistic understanding of TSC-LAM pathology. Dr. Krymskaya has received the Science Advancement Award and Established Investigator Awards from The LAM Foundation, continued funding from NHLBI, DOD, and The American Lung Association. Dr. Krymskaya serves on the Board of Directors and Scientific Advisory Board of The LAM Foundation, the National Disease Research Interchange, and the Castleman's Disease Collaborative Network. She served as a member of the Department of Defense TSC Research Program Scientific Peer Review Council. Dr. Krymskaya received the Science Advancement Award from The LAM Foundation and is an elected fellow of the College of Physicians of Philadelphia.

Kathrin Thedieck, PhD
Full Professor and Institute Head for Biochemistry, University of Innsbruck
University of Innsbruck
Innsbruck, Austria

Kathrin Thedieck is Professor and Institute Head of Biochemistry at the University of Innsbruck, Austria. Her research spans the field of metabolic signaling with a focus on networks converging on the metabolic master regulator mechanistic target of rapamycin (mTOR) and its suppressor, the TSC complex. Her team pioneers crosstalk of TSC-mTOR signaling with RNA-protein networks, and conducts systems studies to unravel TSC-mTOR network topology as well as its responses to metabolism and therapy. She coordinates and partners several European consortia that develop systems approaches for precision medicine in cancer and in congenital diseases.

Yan Xu, PhD
Director of Bioinformatics Core, Neonatology & Pulmonary Biology; Perinatal Institute
Professor, UC Department of Pediatrics
University of Cincinnati
Cincinnati, OH

I am a Professor of Pediatrics and Bioinformatics, and my research interest is to develop and apply bioinformatics and systems biology approaches to understand pulmonary development and disease. I have a broad background training in Medicine, Molecular Biology, and Bioinformatics. My current lines of research center on the revealing of cell-cell communication, regulatory circuits, and networks controlling normal lung maturation and the pathogenesis of Lymphangioleiomyomatosis (LAM) using integrative single-cell omics approaches. I am also devoting my efforts to develop a web-based lung cell atlas to facilitate sharing and usage of the ever-growing omics data resources for the lung research community. My research activities are supported by NHLBI, LungMAP consortium, The LAM Foundation, CFF, and Chan Zuckerberg Initiative. I have considerable experience in gene expression profiling and transcriptional network studies and have published more than 100 peer-reviewed articles.

Plenary Session 1 Abstracts

Stress granule proteins meet TSC-mTOR signaling: an unexpected mechanism of lysosomal TSC tethering

Kathrin Thedieck, PhD
University of Innsbruck
Innsbruck, Tyrol, Austria

The tuberous sclerosis protein (TSC) complex acts as a tumor suppressor by restricting signaling through the metabolic master regulator mTORC1 (mechanistic target of rapamycin complex 1). Mutations in the *TSC1* or *TSC2* genes frequently occur in cancer and hereditary TSC disorder, characterized by tumor formation in multiple organs. The TSC complex senses anabolic signals, and suppresses pro-tumorigenic processes by inhibiting mTORC1 at its central signaling platform – the lysosomes. We report that G3BP1 and G3BP2 (Ras GTPase-activating protein-binding proteins, G3BPs) act in a non-redundant manner to anchor the TSC complex to lysosomes and suppress activation of mTORC1 by nutritional signals. Like the TSC complex, deficiency of G3BP1 elicits phenotypes related to mTORC1 hyperactivity in the context of breast cancer and neuronal dysfunction. G3BP1 prevents mTORC1-driven cancer cell motility, and in agreement, low G3BP1 expression correlates with poor outcome in metastatic breast cancer. G3bp1 inhibition in zebrafish disturbs neuronal development and function, leading to white matter heterotopia and neuronal hyperactivity. Thus, G3BPs are not only core components of SGs but also a key element of lysosomal TSC-mTORC1 signaling.

Reference including full list of coauthors: Prentzell MT, Rehbein U, Cadena Sandoval M et al. Cell. 2021 Feb 4;184(3):655-674.e27. doi: 10.1016/j.cell.2020.12.024.

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Three-dimensional drug screen identifies HDAC inhibitors as therapeutic agents in a tissue-engineered model of lymphangioleiomyomatosis

Adam Pietrobon, Julien Yockell-Lelièvre, Nicole Melong, Laura J. Smith, Sean P. Delaney, Nadine Azzam, Chang Xue, Nishanth Merwin, Eric Lian, Alberto Camacho-Magallanes, Carole Doré, Gabriel Musso, Lisa M. Julian, Arnold S. Kristof, Roger Y. Tam, Jason N. Berman, Molly S. Shoichet, William L. Stanford

Ottawa Hospital Research Institute, Ottawa, Canada (AP, JY, SPD, EL, AC, CD, WLS); CHEO Research Institute, Ottawa, Canada (NM, NA, JNB); Donnelly Centre for Cellular and Biomolecular Research, Toronto, Canada (LJS, CX, MSS); Biosymetrics Inc, Toronto, Canada (NM, GM); Simon Fraser University, Burnaby, Canada (LMJ); Research Institute of the McGill University Health Centre, Montreal, Canada (ASK); Health Canada, Ottawa, Canada (RYT)

Lymphangioleiomyomatosis (LAM) is a rare disease involving cystic lung destruction by invasive LAM cells. These cells harbor loss-of-function mutations in *TSC2*, conferring constitutive mTORC1 signalling. Rapamycin is the only clinically approved disease-modifying treatment, but its action is cytostatic and disease progresses upon its withdrawal. There is a critical need to identify novel agents that prevent the invasive phenotype and/or eradicate the neoplastic LAM cells. Here, we employed novel cellular and extracellular models to screen for candidate therapeutics in a physiologically relevant setting. We found that lung-mimetic hydrogel culture of pluripotent stem cell-derived diseased cells more faithfully recapitulates human LAM biology compared to conventional culture on two-dimensional tissue culture plastic. Leveraging our culture system, we conducted a three-dimensional drug screen using a custom 800-compound library, tracking cytotoxicity and invasion modulation phenotypes at the single cell level. We identified histone deacetylase (HDAC) inhibitors as a group of anti-invasive agents that are also selectively cytotoxic towards *TSC2*$^{-/-}$ cells. Unexpectedly, we observed that next generation ATP-competitive mTORC1/2 inhibitors potentiate invasion. We determined anti-invasive effects of HDAC inhibitors to be independent of genotype, while selective cell death is mTORC1-dependent and mediated by apoptosis. Drug performance was subsequently evaluated at the single cell level in zebrafish xenografts. We observed consistent therapeutic efficacy *in vivo* at equivalent concentrations to those used *in vitro*, substantiating HDAC inhibitors as potential therapeutic candidates for pursuit in patients with LAM.

Novel neural crest markers characterize tumors in the *Tsc2*$^{+/-}$ mouse model of tuberous sclerosis complex

Uchenna Unachukwu, Jeanine D'Armiento
Columbia University Medical Center, New York, NY, USA (UU, JDA)

Precise identification of the origin of cells causing tuberous sclerosis complex (TSC) has since eluded investigators amidst conflicting results of the ontogenesis of these disorders from multiple studies. As such, the development of effective prophylactic and therapeutic interventions has been limited compounding the disease burden. Prevailing postulates of a neural crest origin of TSC instigated our development of a *Tsc2*$^{+/-}$ reporter mice model in which tdTomato epifluorescence is driven by a myelin protein zero (Mpz) promoter marking neural crest cells in spontaneously developing renal tumors. In these mice, distinct neural crest cell (NCC) subpopulations were identified by the expression of neural crest markers Cd57 and Tfap2-alpha in renal tumors. We postulated that these NCCs initiate and spur tumorigenesis in the mouse model. To test our hypothesis, single cell RNA-seq analysis of tumors excised from our *Tsc2*$^{+/-}$ reporter mice was performed on 6708 cells with 52657 mean reads/cell. Cells were clustered based on differential gene expression and results were validated using RT-qPCR and immunohistochemistry on *Tsc2*$^{+/-}$ mouse renal tumor and human Lymphangioleiomyomatosis (LAM) uterine and pulmonary tissue samples. We next initiated bi-allelic Tsc2 and NCC marker mutations in the O9-1 neural crest cell line using CRISPR/Cas9 and determined the tumorigenic potential of the mutated versus wildtype NCCs and control mouse embryonic fibroblasts (MEFs) using EdU proliferation, transwell invasion, scratch and teratoma assays. We observed a small cluster of NCCs within the renal tumors of the *Tsc2*$^{+/-}$ mice based on characteristic NCC marker expression supporting our hypothesis. These cells differentially expressed tumorigenic and NCC-specific genes including SRY-Box Transcription Factor 9 (Sox9), lipocalin (Lcn2), N-Myc Downstream Regulated 1 (Ndrg1), and WAP Four Disulfide Core Domain (Wfdc2). During validation experiments, the expression of these genes significantly increased with the age of *Tsc2*$^{+/-}$ mice tumors and their protein forms were detected in both the *Tsc2*$^{+/-}$ mice renal tumors and human LAM tissue samples. Comparative assessment of the tumorigenic potential of mutated NCCs and MEFs is ongoing. We conclude that *Tsc2* mutations potentially drive neural crest cells to form tumors in the *Tsc2*$^{+/-}$ mouse model thus asserting the neural crest origin of TSC tumors. Sox9 and Ndrg1 could serve as potential therapeutic targets in these tumors to stymy TSC disease progression.

Kidney angiomyolipomas are defined by a unique transcriptional profile and H3K27AC chromatin state

Krinio Giannikou, Clemens Probst, Xintao Qiu, Melissa Duarte, Nikolas Kesten, Mahsa Zarei, Magdalena J, Losko, Heng Du, Zach Hebert, Raga Vadhi, Alba Font-Tello, Paloma Cejas, Charles H. Yoon, Chin-Lee Wu, Myles Brown, Elizabeth Henske, Henry Long, David J. Kwiatkowski

Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA (KG, CP, MJL, HD, EH, DJK); Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, MA, USA (KG, XQ, NK, ZH, RV, AFT, PC, MB, HL); Texas A&M University, College Station, TX, USA (MZ); Cutaneous Oncology & Melanoma, Division of Surgical Oncology, Brigham and Women's Hospital and Harvard Medical School, USA (CHY); Urology Research Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA (CLW)

Background:
Kidney angiomyolipomas (AML) are benign mesenchymal tumors that are commonly seen in tuberous sclerosis complex (TSC), but also occur sporadically. Kidney AMLs are due to *TSC1/TSC2* biallelic loss, whereas other somatic genetic events are rare and do not contribute to tumor development. We hypothesized that the chromatin state and master transcription factors (TFs) are also drivers of kidney angiomyolipoma growth, alongside mTORC1 activation.

Material and Methods:
We performed RNA-seq on 28 kidney AMLs, and ChIP-seq for H3K27ac (a histone modification that marks open chromatin and and regulates high transcription of nearby genes) on 25 AMLs, a human kidney AML derived *TSC2* null cell line (621-101), and the most pigmented melanoma cell line (SK-MEL30). MITF ChIP-Seq was also carried out on three kidney AMLs and SK-MEL30 cells.

Results:
Differential expression analyses of kidney AML compared to The Cancer Genome Atlas (TCGA) tumors and GTEx normal tissues revealed 347 differentially expressed genes (DEGs), including 18 TFs (FDR<0.05). MITF-A and PPRAG, known oncogenes, were found highly expressed in kidney AML (4th and 1st out of 27 TCGA tumor types, respectively). In addition, 6 of 10 top DEGs in kidney AMLs are known MITF targets including CTSK, PMEL, and GPNMB. ROSE (Ranking of Super Enhancers) and regulatory potential (RP) analysis of H3K27ac ChIP-seq data compared to human normal tissues (Epigenome Roadmap), identified MITF-A, PPARG, CTSK and GPNMB as genes with extended open regulatory chromatin regions, known as 'super-enhancers', suggesting they are critical for AML development. Gene set enrichment analysis (GSEA) of all 347 DEGs showed enrichment in pathways reflecting kidney AML nature/cell composition including epithelial-mesenchymal transition, myogenesis, adipogenesis, estrogen response (q-value< 6.54 e-9). Immunohistochemistry demonstrated positive staining for nuclear MITF, ARID5B, MEIS2, NR2F2, NFIC TFs, and cytoplasmic GPNMB in kidney AML sections and other TSC tumors, compared to adjacent normal tissue. Highly level of expression were confirmed by western plot in protein lysates by AML tumors. SiRNA for selected top TFs MEIS2 and ARID5B showed reduction in cell growth in vitro.

Conclusions:
Our studies have identified unique chromatin signatures, and several highly-expressed TFs, including MITF-A and PPARG, which likely are essential for AML development enabling novel strategies with more effective therapeutic potential.

Intranasal administration of CpG increases survival and counters immunosuppression in a murine model of metastatic lymphangioleiomyomatosis (LAM)

Mayowa Amosu, Bennett Yang, Jacob McCright, Katharina Maisel
University of Maryland College Park, College Park, MD, USA (MA, BY, JM, KM)

Introduction:
Pulmonary Lymphangioleiomyomatosis (LAM) is a slow progressing, metastasizing neoplasm primarily affecting women of reproductive age. LAM is characterized by the abnormal growth of smooth muscle-like cells leading to cystic destruction of the lungs. Rapamycin, the only FDA approved treatment for LAM, slows disease progression and ~40% of patients have partial or no response to treatment. Thus, there is a critical need for new LAM treatments. Research has shown that LAM has cancer hallmarks like expression of melanoma markers and immune checkpoint receptors. We and others have shown that immune checkpoint inhibition (ICI), such as anti-PD1 therapy, enhances survival in murine LAM, but is not curative. This suggests that other immune stimulating anti-cancer strategies, like toll like receptor (TLR) activation, could be used to treat LAM. Here, we investigate intranasal administration of the adjuvant CpG, a TLR9 agonist, as anti-LAM therapy.

Methods:
We used a murine model of metastatic LAM to determine survival after biweekly intranasal CpG (10μg) treatment or combination treatment with systemic anti-PD1. We used flow cytometry and immunofluorescence to determine how CpG alters the immune response in LAM.

Results:
We found that CpG treatment enhances median survival from 32 to 45 days in murine LAM. Histological analysis shows that CpG treatment increases tissue-wide inflammation, and decreases overall LAM nodule burden. Lower dose CpG (5μg) further enhances survival to 64 days, likely by reducing lung inflammation. Combination therapy also significantly increases survival to 64 days over treatment with CpG (10μg) alone.

In more progressive LAM disease, we observed a decrease in macrophage, eosinophil, and dendritic cell infiltration in lungs of CpG (10μg) treated vs untreated LAM mice. CpG treatment decreases FoxP3 expression in regulatory T cells in LAM lungs and increases expression of T cell activation marker, ICOS.

Conclusions:
In LAM, CpG immunotherapy reduces immunosuppression and diminishes innate immune cell infiltration that is often associated with tumor growth/progression. Our findings suggest that activating adjuvant immunotherapy, like CpG, may offer new treatment avenues for LAM, and that benefits of local CpG treatment are dose dependent.

Neuron-glia iPSC models for TSC

Vivi M. Heine, PhD
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Tuberous sclerosis complex (TSC) is caused by mutations in the *TSC1* or *TSC2* genes, leading to gray and white matter defects in the brain. Previous studies have shown that hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway can lead to excitation/inhibition (E/I) balance changes. To study the involvement of neuron and glia cells in TSC phenotypes, we generated induced pluripotent stem cell (iPSC)-based cultures, i.e. neuron-oligodendrocyte and neuron-astrocyte co-cultures. TSC neuron cultures showed hyperactivity, as measured by calcium transients and multi-electrode array analysis. However, in co-cultures with oligodendrocytes, neuronal defects became more apparent, showing cellular hypertrophy and increased axonal density. Pharmacological intervention with the mTOR regulator rapamycin suppressed these defects. As astrocytic dysfunction can cause changes in the E/I balance, we also generated control and TSC iPSC-derived astrocytes. TSC astrocytes show increased proliferative activity and differential gene expression revealed by RNA sequencing analysis. Control neurons presented an altered synaptic balance, when these were cultured in astrocyte-conditioned medium (ACM) of TSC astrocytes. These neuron cultures with TSC ACM showed an increased percentage of GABAergic synapses. Together, our patient iPSC-based models show that cellular TSC phenotypes arise from the interaction between neuronal and glial cells and provide a platform for disease modeling and therapeutic target investigations.

Topic-Based Discussion 1 Summary

Extracting meaning from multiomics datasets: How do we bring together multiomics data? What is the question we are trying to answer?

Moderator:
Rebecca A. Ihrie, PhD (Vanderbilt University)

Panelists:
Vera P. Krymskaya, PhD, MBA (University of Pennsylvania)
Peter E. Davis, MD (Boston Children's Hospital)
Yan Xu, PhD (University of Cincinnati)

The intent of this session is to discuss the many available single-cell omics and imaging modalities in use across TSC research, highlighting tools and approaches that can be used to bring multiple "big data" platforms together for analyses and to consider how these rich datasets can be shared and used going forward.

This topic-based discussion will feature presentations from panelists, followed by a question-and-answer period where attendees may ask specific questions to each panelist. After the whole-group Q&A, conference attendees will be randomly sorted into breakout rooms led by conference panelists. Breakout groups will be given a series of prompts to stimulate conversation leading to potential action items for the TSC and LAM research community and their advocacy partners.

Plenary Session 2 Abstracts

Predicting and preventing manifestations of TSC and LAM

Simon Johnson, DM
University of Nottingham
Nottingham, Nottinghamshire, England

Treatments are available for lung function decline, pneumothorax, angiomyolipoma and lymphatic complications in LAM however as LAM is a highly variable disease and not all patients will suffer all these complications or need treatment. This uncertainty is difficult for patients and may result in excess treatment costs and side effects. To address this, we have used machine learning to identify subgroups or endotypes within LAM patients and identified three clusters of patients with differing clinical features. These clusters are clinically recognisable and are associated with different risks of pneumothorax, need for angiomyolipoma intervention and survival. The results could be used to give better clinical advice, improve screening programs and treatment. We go on to show that making treatment decisions using serial lung function may be associated with loss of lung function and rapamycin resistance. Finally, we describe our current work to use machine learning to predict lung function decline and need for mTOR inhibitor treatment. Current models are examining the relevance of baseline features and biomarkers to future lung function decline and need for mTOR inhibitor therapy. These methods have the potential to stratify care for women with LAM reducing uncertainty and improving outcomes.

A PDX model of adoptive T cell therapy to treat lymphangioleiomyomatosis

Caroline Le Poole, Ancy Thomas, Rohan Shivde, Emilia Dellacecca, Gina Scurti, Prathyaya Ramesh, Mike Nishimura, Jose Guevara, Masha Kocherginsky, Daniel Dilling, Lisa Henske, Vera P. Krymskaya, Wenan Qiang.

Dept. of Dermatology/ Lurie Cancer Center, Northwestern University (CLP, AT, RS, ED, PR), Department of Surgery, Loyola University Chicago (GS, MN, JG), Department of Preventive Medicine, Northwestern University (MK), Department of Medicine, Loyola University Chicago (DD), Department of Medicine, Harvard Medical School (LH), Department of Medicine, University of Pennsylvania (VK), 7. Department of Obstetrics and Gynecology and Pathology, Northwestern University (WQ).

Lymphangioleiomyomatosis (LAM) patients present with pulmonary cysts and benign tumors with hyperactive mTORC1. Treatment with rapalogs can halt disease progression, but a true cure has yet to be developed. LAM was historically diagnosed by HMB45 staining of a lung biopsy, pointing to occult expression of gp100 by lesional cells. As gp100 is a recognized target for immunotherapy in melanoma, we proposed to develop adoptive T cell therapy for LAM using TCR transgenic (Tg) T cells reactive with gp100. We prepared a retroviral vector encoding our SILv44 T cell receptor to gp100, which imparts a Tc17 profile on host CD8 T cells. As the target molecule gp100 is also expressed by normal melanocytes, we proposed to avoid autoimmunity by including a CCR2 transgene to favor trafficking to the lung. CCR2 alsomediates macrophage infiltration of LAM lung. To enable preclinical testing of patient-derived Tg T cells, we developed patient derived xenograft models to reflect the complexity of LAM lesions in mice, and to test the anti-LAM activity of our Tg human T cells. We established 3 LAM PDX models, and treated them with SILv44-Tg T cells. Maintenance of LAM features in PDX mice was tested by immunohistology. We also evaluated the migration and cytotoxicity of Tg T cells in vitro. The first model that was studied in detail revealed that PDX tissues retained phosphoS6 and gp100 expression over 2 passages for at least 12 weeks, in stark contrast to LAM cells losing expression of gp100 within days of in vitro culture. We found that 5 ml of patient blood will suffice to obtain >10(8) purified SILv44-Tg T cells. T cells expressing both SILv44 and CCR2 exhibited increased migration towards CCL2 in transwell migration assays. Mice bearing PDX tissues in second passage were treated with 10(6) Tg T cells twice, one week apart, supported by 5000 IU IL-2 IP per 2 days, resulting in significantly reduced pS6+, gp100 expressing LAM lung tissue, and infiltration by T cells. Our data suggest that (A) PDX mice can offer a remarkably effective model of LAM for preclinical testing of promising therapies; (B) human T cells can be functionally driven to migrate towards CCL2 and importantly, (C) adoptive T cell therapy targeting gp100 can treat advancing LAM lesions. These studies provide support for the concept, that benign tumors are amenable to treatment by T cell based immunotherapy, offering an opportunity to truly eradicate LAM lesions.

Predicting individualized lung disease progression in lymphangioleiomyomatosis

Anushka K. Palipana, Rhonda D. Szczesniak, Simon R. Johnson, Nishant Gupta

Cincinnati Children's Hospital Medical Center, Ohio, USA (AKP, RDS), University of Cincinnati, Ohio, USA (AKP, RDS, NG), University of Nottingham, Nottingham, UK (SRJ), National Centre for Lymphangioleiomyomatosis, Nottingham, UK (SRJ)

Rationale:
Clinical decision making in LAM is challenging due to the marked inter-individual variability in disease progression in patients with Lymphangioleiomyomatosis (LAM).
Objectives:
To develop a dynamic prediction model and an easy-to-use online calculator estimating the probability of clinically meaningful lung-function declines (rate of change in FEV1 thresholds of 60, 75, and 100ml/year), and to simultaneously characterize lung-function trajectories and predict mortality in patients with LAM.

Methods:
Patients observed in the US National Heart, Lung and Blood Institute (NHLBI) LAM Registry were included. We employed novel stochastic modeling and performed covariate selection, evaluating predictive probabilities for clinically relevant FEV1 drops. We formed predictive probabilities of transplant-free survival by jointly modeling longitudinal FEV1 and lung transplant or death. The results to predict FEV1 decline were externally validated using the UK LAM natural history cohort. Lastly, we estimated life expectancy of patients on the basis of LAM subtype (TSC vs. S-LAM) using Kaplan-Meier curves.

Results:
In the NHLBI cohort (n=216), higher baseline lung function (FEV1 and diffusion capacity for carbon monoxide) corresponded to better lung function over time, whereas increasing age at diagnosis was associated with lower lung function. Premenopausal status was associated with a faster rate of decline in FEV1. Areas under the receiver-operating characteristic curve (AUCs) were 0.81, 0.80, 0.82 for predicting drops of 100, 75 and 60 mL/yr, respectively, and mean absolute percentage error (MAPE) for FEV1 values was 3.3%. In the UK validation cohort (n=194), AUCs were roughly 0.97 for the selected FEV1 decline thresholds, and MAPE was 4.9%. The overall survival time following LAM diagnosis [95% CI] was similar in women with TSC-LAM compared with S-LAM: 24.22 [20.38 - 28.06] vs. 22.62 [20.91 – 24.32] years following LAM diagnosis. The average life expectancy for S-LAM and TSC-LAM patients was 63.36 and 64.96 years, respectively, compared to the average life expectancy of 81 years for women in the US.

Conclusions:
Predictive probabilities calculated from longitudinal modeling of routinely collected demographic and clinical data can aid in individualized LAM prognostication and therapeutic decision-making.

UV mutation drives a high prevalence of subclinical facial tumors in TSC – focus on mutation signatures

Katarzyna Klonowska, Joannes M. Grevelink, Krinio Giannikou, Barbara Ogorek, Zachary T. Herbert, Aaron R. Thorner, Thomas Darling, Joel Moss, David J. Kwiatkowski

Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA (KK, KG, BO, DJK); Massachusetts General Hospital, Boston, MA, USA (JMG); Molecular Biology Core Facilities, Dana-Farber Cancer Institute, Boston, MA, USA (ZTH); Center for Cancer Genomics, Dana-Farber Cancer Institute, Boston, MA, USA (ART); Uniformed Services University, Bethesda, MD, USA (TD); National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA (JM)

Introduction:
Previously we had shown by Massively Parallel Sequencing (MPS) that UV-induced mutation is a consistent second hit in TSC facial angiofibromas (FAFs). Here, we explored the extent and range of effects of UV-induced mutation in the TSC facial skin.

Methods:
For ultra-sensitive detection, we developed Multiplex High-sensitivity PCR Assay (MHPA), enabling mutation detection at variant allele frequencies (VAF) <0.1%.

Results:
We performed MHPA analysis for several sample sets, including TSC FAF (n=24) and nonTSC normal sun-exposed skin (nonTSC NS) (n=8). MHPA analysis of 24 TSC FAFs, led to the identification of 112 low VAF (0.01–8%, median:0.08%,) somatic indels/point mutations. Remarkably, two or more somatic *TSC2* mutations were identified in 19 of 24 (79%) FAFs, suggesting that these small (2mm diameter) FAF biopsies contained at least two FAF clones. 34 of 112 (30%) mutations were CC:GG>TT:AA indicative of UV radiation causation. We also developed an MHPA assay for TP53; an average of 10.1 TP53 mutations were identified per whole skin FAF (likely in keratinocytes), and correlated with the number of *TSC2* mutations in the same sample (r=0.63, p=0.005), likely reflecting the role of UV in the generation of both genes' mutations. Examination of the mutation signatures revealed that the SNV signatures for TSC FAF and nonTSC NS are similar to the canonical UV signature SBS7b (COSMIC) with predominance of C>T substitutions. A modest enrichment for G:C>T:A substitutions in variable sequence contexts was seen in the TSC FAF set, less so for nonTSC NS, likely resulting from reactive oxygen species (ROS) generated by sunlight. Dinucleotide variant (DNV) signatures matched very well with the DBS-1 canonical UV signature, with CC:GG>TT:AA substitutions accounting for >90% of all DNVs. The indel signature was different than the reference COSMIC UV-related ID13 signature, dominated by single nucleotide deletions [23 of 38 (61%)]. Finally, we identified a recurrent novel complex mutation signature pattern in TP53, consisting of both an SNV or DNV, and a deletion.

Conclusions:
MHPA analysis showed that unique signatures of *TSC2* UV-induced point mutations are highly prevalent in facial skin of TSC subjects, generating hundreds of thousands of incipient facial tumors (subclinical 'micro-FAFs'), a small proportion of which develop into observable tumors. The study was funded by FY2020 TSC Alliance Postdoctoral Fellowship Award (KK) and Engles Family Fund (DJK).

Blood oxygen saturation during a mobile health exercise program in women with lymphangioleiomyomatosis

Mary Beth Brown, Haley Sizelove, Morgan Kelly, SiWei Luo, Amanda Wise, Lawrence Ho, Ylinne Lynch

University of Washington, Seattle, WA, USA (MBB, HS, MK, SWL, AW, LH, YL)

Purpose:
Dyspnea and reduced blood oxygen saturation (SpO2) during exercise are common in patients with lymphangioleiomyomatosis (LAM). Here we characterize SpO2 responses measured during home exercise sessions performed over 12 wks and relationship to training variables and training responses.

Methods:
15 women with LAM were enrolled and 14 have been completed to date. A 12-wk mobile health (mHealth) intervention was conducted which consisted primarily of smartphone-guided progressive aerobic exercise (30-45 min, 65-75% of heart rate reserve, 4 days/wk) and interfacing wearable and home monitoring devices. Initial and final assessments included maximal cardiopulmonary exercise testing (CPET) and 6 Min Walk Test (6MWT) with measurement of SpO2, pulmonary function testing (PFT), and surveys. Fingertip pulse oximetry with Bluetooth communication to the mHealth platform provided SpO2 during all home exercise sessions. Frequency analysis characterized proportion of each session spent in defined desaturation levels: none (SpO2>95%), mild (SpO2 90-95%), moderate (SpO2 84-89%), severe (SpO2 78-83%), and very severe (SpO2 <78%). Data are mean±SD.

Results:
Patients spent the majority (55±22%) of their home exercise sessions in 'mild' desaturation, with only 16±19%, 1.3±0.9%, and 0.3±0.5% in 'moderate', 'severe', and 'very severe' desaturation, respectively. This distribution did not change ($p>0.05$) across the 12 wks, nor did it when expressed as average SpO2 in workouts over 12 wks. SpO2 at end of 6MWT did not change significantly from initial (90.6±4%) to final (88.6±7%) ($p=0.23$) study visit. Proportion of home exercise sessions spent in 'very severe' desaturation related to poorer baseline FEV1/FVC ($r= -0.72$), end SpO2 values in 6MWT ($r= -0.75$) and CPET ($r= -0.68$), and worse exit survey ratings for aerobic program satisfaction ($r= -0.54$) and feasibility ($r= -0.55$).

Conclusion:
Patients with LAM exhibited oxygen desaturation in CPET and 6MWT not improved by training, consistent with previous literature. Even with exercise intensity prescribed relative to aerobic fitness and heart-rate-guided, patients were in at least mild desaturation for the majority of their home exercise sessions. Desaturation during sessions did not resolve with training, but may have contributed to improved exercise tolerance over time. SpO2 response to 6MWT and CPET may be useful to identify patients likely to desaturate more during a home exercise program and find it less tolerable.

Transforming drug discovery using digital biology

Daphne Koller, PhD
Insitro, Inc.
San Francisco, CA, USA

Modern medicine has given us effective tools to treat some of the most significant and burdensome diseases. At the same time, it is becoming consistently more challenging and more expensive to develop new therapeutics. A key factor in this trend is that the drug development process involves multiple steps, each of which involves a complex and protracted experiment that often fails. We believe that, for many of these phases, it is possible to develop machine learning models to help predict the outcome of these experiments, and that those models, while inevitably imperfect, can outperform predictions based on traditional heuristics. To achieve this goal, we are bringing together high-quality data from human cohorts, while also developing cutting edge methods in high throughput biology and chemistry that can produce massive amounts of in vitro data relevant to human disease and therapeutic interventions. Those are then used to train machine learning models that make predictions about novel targets, coherent patient segments, and the clinical effect of molecules. Our ultimate goal is to develop a new approach to drug development that uses high-quality data and ML models to design novel, safe, and effective therapies that help more people, faster, and at a lower cost.

Topic-Based Discussion 2 Summary

Informing and enabling clinical trials, from bench to randomized clinical trials

Moderator:
Nishant Gupta, MD (University of Cincinnati)

Panelists:
Kevin C. Ess, MD, PhD (Vanderbilt University)
Dean Aguiar, PhD (TSC Alliance)
Elizabeth P. "Lisa" Henske, MD (Brigham and Women's Hospital)
Ajamete "Aj" Kaykas, PhD (Insitro, Inc.)

The intent of this session is to discuss a variety of preclinical approaches and resources being utilized in the field of TSC and LAM to identify and prioritize therapeutic targets, and to have an open discussion on the key aspects of preclinical assessments necessary to ensure the most promising new therapies in the field are rapidly advanced to clinical trials.

This topic-based discussion will feature presentations from panelists, followed by a question-and-answer period where attendees may ask specific questions to each panelist. After the whole-group Q&A, conference attendees will be randomly sorted into breakout rooms led by conference panelists. Breakout groups will be given a series of prompts to stimulate conversation leading to potential action items for the TSC and LAM research community and their advocacy partners.

Plenary Session 3 Abstracts

The multi-faceted roles of TSC-mTOR signaling in neural development, function, and disease

Helen Bateup, PhD
University of California, Berkeley
Berkeley, CA, USA

Research in my lab broadly aims to understand the cellular and molecular basis of neuropsychiatric disorders. We have a particular interest in tuberous sclerosis complex (TSC) and related "mTORpathies", which are caused by mutations in the mTOR signaling pathway. To understand how mutations in *TSC1* and *TSC2* lead to brain malformations, epilepsy, cognitive impairments, and autism spectrum disorder we use genetically-engineered mouse and human cellular models in combination with a variety of techniques spanning molecular profiling to behavioral analysis. Our goal is to generate a mechanistic understanding of how TSC-associated mutations affect the cell biology and physiology of specific types of neurons and glia, and how altered neuronal activity impacts circuit function and behavior. In this talk, I will present our work using human brain organoid models of TSC to understand the early developmental changes that lead to the formation of cortical tubers. I will discuss how deregulation of mTORC1 signaling during early cortical development leads to altered cellular differentiation and the formation of enlarged and dysmorphic tuber cells.

Juvenile 4E-BP1 expression attenuates epilepsy and neurophysiological abnormalities associated with mTOR hyperactivation in mice

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Tuberous sclerosis complex (TSC) is caused by mutations in *TSC1* or *TSC2* genes, leading to hyperactivation of the mechanistic target of rapamycin (mTOR) signaling pathway. The mTOR pathway is a master regulator of cell growth and development, and increased mTOR signaling during fetal neurodevelopment results in focal cortical malformations and intractable epilepsy. Recent evidence suggests a role for dysregulated 4E-BP/eIF4E-mediated translation, downstream of mTOR complex 1 (mTORC1), in the formation of cortical malformations and seizures. However, it is unknown whether modifying translation once the developmental pathologies are established can reverse neuronal abnormalities and seizures. Here, we examined whether genetic modulation of a downstream mTORC1 effector, eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), can reduce seizures in a mouse model of mTORopathy. To model mTOR-induced focal malformations in mice, we expressed a constitutively active Rheb (RhebCA) in developing neurons via in utero electroporation. Rheb is the direct activator of mTORC1, and expression of RhebCA results in persistent mTORC1 hyperactivation and increased phosphorylation (and thus, repression) of 4E-BP1. To compensate for the repressed 4E-BP1, we co-expressed a constitutively active form of 4E-BP1 (4E-BP1CA) that resists phosphorylation by mTOR. We used a tamoxifen-inducible Cre/lox system which allows for time-regulated, conditional expression of 4E-BP1CA in juvenile mice after the onset of cellular and behavioral phenotypes. We found that 4E-BP1CA expression in juvenile RhebCA mice reduced neuronal cytomegaly and corrected several neuronal electrophysiological alterations, including depolarized resting membrane potential, irregular firing pattern, and aberrant expression of HCN4 ion channels. 44% of the RhebCA mice showed no seizures following 4E-BP1CA expression (vs. 9% in RhebCA only mice) and the mean seizure frequency was significantly decreased by >50%. Overall, our findings support 4E-BP1 dysregulation as a crucial contributor to mTOR-related epilepsy, and targeting 4E-BPs may represent a novel treatment strategy for epilepsy in TSC.

Early neurodevelopment and cytoarchitecture is altered in tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the heart, eyes, lungs, kidney, skin, and brain are all hallmarks of the disease, often the most severe symptoms of TSC are neurological, including seizures, autism, psychiatric disorders, and intellectual disabilities. TSC is caused by a loss of function mutation in the *TSC1* or *TSC2* genes, which encode the hamartin/tuberin proteins respectively. Patients are heterozygous for mutations, but the most widely accepted model is that second hit mutations in *TSC1/2* occur in tumorigenesis. Hamartin/tuberin function as a heterodimer that negatively regulates mechanistic Target of Rapamycin Complex 1 (mTORC1). While TSC neurological phenotypes are well-documented, it is not yet known how early in neural development *TSC1/2*-mutant cells diverge from the typical developmental trajectory, and whether such phenotypes are seen in the heterozygous-mutant populations that comprise the majority of cells in patients. To examine early neurodevelopmental phenotypes in a cell-based model of TSC, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) that harbor a heterozygous microdeletion mutation in TSC2. To model this state, CRISPR was used to create a similar deletion mutation in the other *TSC2* allele, producing a homozygous mutant line. A TALEN system was also used to correct the heterozygous mutant to wild type, creating a set of isogenic lines. This isogenic series was then compared to a second allelic series in which *TSC2* was deleted using CRISPR editing. Using immunofluorescent microscopy, immunoblotting, and flow cytometry, we observed aberrant early neurodevelopment in both sets of *TSC2* mutant iPSCs. Homozygous mutant neural progenitors exhibit altered behavior as in vitro differentiation proceeds, including changes in multicellular structures within the first 10 days with misexpression of key transcription factors associated with lineage commitment. As expected, mutant cells have more active mTORC1, with increased phosphorylation of ribosomal S6 protein, than heterozygous and wild-type cell lines. Collectively, these data suggest that mutation or loss of *TSC2* has early effects on proper neural development. Understanding precisely when development is disrupted in *TSC1/2*- mutant brain will be essential to tailoring treatment and determining whether prenatal diagnosis or treatment should be pursued.

Raptor downregulation rescues neuronal phenotypes in mouse models of tuberous sclerosis complex

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TSC is associated with significant neurological and psychiatric problems, which can include epilepsy, variable intellectual disability, and psychiatric conditions including autism. The *TSC1* and *TSC2* genes are known to form a protein complex that negatively regulates mTOR complex 1 (mTORC1) signaling. Current pharmacological approaches to treat TSC are based on rapamycin and its analogs, which suppress mTORC1 activity. However, chronic rapamycin treatment inhibits both mTORC1 and mTOR complex 2 (mTORC2), is associated with systemic side-effects, and may not be effective for treating all neurologic aspects of TSC. In this study we examined which mTOR complex is most relevant for TSC-related brain phenotypes by selectively reducing neuronal mTORC1 or mTORC2 in mouse models of TSC via genetic deletion of Raptor or Rictor, respectively. We found that reduction of the mTORC1 component Raptor, but not the mTORC2 component Rictor, rebalanced mTOR signaling in cultured *Tsc1* knock-out (KO) hippocampal neurons. We tested whether Raptor reduction could improve TSC-related neurodevelopmental phenotypes in vivo and found that it was sufficient to ameliorate neuronal hypertrophy, macrocephaly, impaired myelination, network hyperactivity, and premature mortality in forebrain-specific *Tsc1* KO mice. By contrast, deletion of Rictor did not rescue brain phenotypes in *Tsc1* KO mice or improve survival. This work provides novel information regarding the relationships between *Tsc1/2*, mTORC1, and mTORC2 signaling in neurons and suggests an alternative therapeutic strategy for treating TSC and potentially other mTOR-related disorders.

Clinical, translational, and basic research for epilepsy

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Epilepsy is one of the most common neurologic manifestations seen in tuberous sclerosis complex (TSC). Epilepsy has been associated with developmental delay and intellectual disability, particularly when seizures occur early in life. The presentation discusses findings from the TSC Autism Center of Excellence Research Network (TACERN), a multicenter, prospective, observational study, specifically seizure variability and severity to determine early epilepsy profiles. The role of early preventative treatment is discussed including clinical trials using vigabatrin to assess prevention of seizures and impact on development, as well as an upcoming clinical trial evaluating the efficacy and safety of sirolimus in delaying or preventing the onset of seizures in infants with TSC with secondary aims looking at the impact on development, autism risk, EEG and MRI biomarkers, and precision dosing.

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    Anushka K. Palipana, Rhonda D. Szczesniak, Simon R. Johnson, Nishant Gupta

Poster Session Abstracts

1. Evolution of the TSC Alliance TSC Biosample Repository

Gabrielle V. Rushing, Jo Anne Nakagawa, Zoë Fuchs, Dana R. Valley, Daniel C. Rohrer, Scott D. Jewell, Steven L. Roberds

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The TSC Alliance Biosample Repository (BSR) began collecting biosamples in early 2016 and as of March 2021 houses a collection of more than 1,600 biospecimens including plasma, white blood cell pellets, DNA isolated from white blood cells and buccal cells, and remnant surgical tissue including brain, kidney, and liver, and a subset of postmortem tissues for use by researchers studying tuberous sclerosis complex (TSC). Many DNA and blood samples were collected in collaboration with the TSC Clinical Research Consortium's clinical trials. Our biosamples are linked to detailed clinical data in the Natural History Database (NHD), allowing applicants to request subsets of biosamples based on clinical phenotypes, age, biological sex, and other criteria. Additional data from the NHD relevant to the project may be requested for each sample. A condition of receiving biosamples is that data must be shared back with the TSC Alliance, creating a continually growing dataset on these samples to be shared with researchers who request samples in the future. The BSR also provides researchers access to the *TSC1*- and *TSC2*-knockout HEK293T cell lines from the Nellist laboratory at Erasmus MC. All biosamples are stored at the Van Andel Institute in Grand Rapids, MI. More than 1000 samples have been shared with researchers, and a clinical trial was initiated in 2020 based on results from BSR plasma samples. The TSC Alliance introduced mobile phlebotomy in December 2019 as part of the Waxlax Biosample Collection Initiative to permit anyone with a confirmed diagnosis of TSC to participate in our research projects regardless of where they live or where they go for their TSC care. As of March 2021, 80 constituents have provided samples this way, increasing the geographic diversity of the BSR. In this poster, we will present the latest BSR data as of June 1, 2021.

2. Cellular and molecular features of TSC2 null neural crest cells demonstrate characteristics of pulmonary lymphangioleiomyomatosis

Alberto Camacho Magallanes, Sean P. Delaney, Adam Pietrobon, Julien Yockell-Lelièvre, William L. Stanford

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Pulmonary lymphangioleiomyomatosis (P-LAM) is a rare mesenchymal tuberous sclerosis related neoplasm characterized by slow invasion of large smooth muscle-like and melanocytic-like cells into the lung parenchyma, causing cystic destruction and progressive respiratory decline. The genetic basis of LAM is a loss-of-function mutation in either the *TSC1* or *TSC2* gene, leading to hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1). Currently, the only treatment available for patients is sirolimus (i.e. rapamycin), yet this treatment is cytostatic. The discovery of new therapeutics to eradicate LAM cells has been hampered by the difficulty in culturing primary patient cells or effectively modelling LAM in cell or animal models. It is well-established that LAM cells express markers of the neural crest lineage, suggesting that *TSC2* null neural crest cells (NCC) could be used to model LAM. Here, we present a comprehensive analysis of *TSC2* knockout ($TSC2^{-/-}$) human pluripotent stem cell lines differentiated to NCCs. Using a combination of temporal RNA-seq and immuno-fluorescence, we find that the $TSC2^{-/-}$ NCCs recapitulate numerous LAM phenotypes. First, immunofluorescence staining revealed that NCCs express key markers associated with LAM including PMEL, SMA, HMGA2, MLNA (MART-1), and GD3. Second, $TSC2^{-/-}$ NCCs were much larger, more motile, and have a higher rate of protein synthesis. Finally, comparing our RNA-seq analysis to recently characterized P-LAM gene signature identified by single cell-RNA-seq of primary samples isolated from multiple LAM patient lungs. We found that both WT and $TSC2^{-/-}$ NCCs show a high degree of overlap with the P-LAM dataset. Together, these data suggest the NCCs can be used to gain deeper insight into the pathogenesis and therapeutic discovery for the mesenchymal manifestations of TSC and LAM.

3. Tuberous sclerosis complex renal lesion pleiotropy arises from multiple aberrant developmental processes

Adam Pietrobon, Julien Yockell-Lelièvre, Trevor Flood, William L. Stanford

Ottawa Hospital Research Institute, Ottawa, Canada (AP, JYL, TF, WLS)

Tuberous sclerosis complex (TSC) is a multisystem tumor-forming disorder caused by loss of *TSC1* or *TSC2*. Renal manifestations predominately include cysts and angiomyolipomas. Despite a well-described monogenic etiology, the cellular pathogenesis has remained elusive. Here, we report a novel genetically-engineered human renal organoid model which recapitulates pleiotropic features of TSC kidney disease in vitro and upon orthotopic xenotransplantation. Using a combination of temporal scRNA-seq, flow cytometry, and whole mount immunofluorescence, we find that loss of *TSC1/2* affects multiple developmental processes in the renal epithelial, stromal, and glial compartments. First, loss of *TSC1/2* leads to an expanded stroma by favouring stromal cell fate acquisition and alters terminal stromal cell identity. Second, epithelial cells in the *TSC1/2*$^{-/-}$ organoids exhibit a rapamycin-insensitive epithelial-to-mesenchymal transition. Third, a melanocytic population forms exclusively in *TSC1/2*$^{-/-}$ organoids, branching from MITF+ Schwann cell precursors of a *bona fide* neural crest-to-Schwann cell differentiation trajectory. Together, these results illustrate the pleiotropic developmental consequences of biallelic inactivation of *TSC1/2* and offer insight into the pathogenesis of TSC kidney lesions.

4. Elevated expression of serum Cathepsin K is associated with active disease and inhibition shows potential as a novel therapy for LAM

Suzanne Miller, Roya Babaei-Jadidi, Debbie Clements, Vera P. Krymskaya, Simon R. Johnson

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Introduction:
LAM is a rare multisystem disease of women characterised by lung cysts, lymphatic abnormalities and angiomyolipomas. Sirolimus is the mainstay treatment, however this only slows the decline in lung function and is not a cure. Therefore, new therapeutic targets are greatly needed. Cathepsin K is expressed in LAM and we previously identified Cathepsin K gene expression was 40-fold higher in LAM compared to control lung tissue ($p < 0.0001$), increased with worsening disease and could be activated in LAM lungs. To determine if Cathepsin K is involved in the pathogenesis of LAM, we assess Cathepsin K levels in serum from a national cohort of LAM and evaluate inhibition of Cathepsin K in TSC2-null cells and a murine model of LAM.

Materials and Methods:
53 women with LAM were recruited from the National Centre for LAM in the UK. Serum samples with linked phenotype and lung function were collected. Active LAM was defined as a decline in FEV1 =100 ml/year and stable LAM an FEV1 = 100ml/year. Protein levels were quantitated using Cusabio Human Cathepsin K ELISA (CSB-E09438h). Inhibition of Cathepsin K by Odanacatib was performed in vitro using TSC2-null cells (621-101 and TTJ cells). In vivo, an immunocompetent TSC2-null murine model of LAM was treated with Odanacatib (2mg/kg) and tumor burden assessed.

Results:
Serum Cathepsin K levels for 53 women with LAM were 7.8 – 84.68 pg/ml (35.28 ± 17.47). Cathepsin K was higher in 27 patients with active LAM (12.44 – 84.68 pg/ml, 40.83 ± 19.01) compared to 26 patients with stable disease (7.8 – 62.06, 29.53 ± 13.50), $p = 0.034$. A trend was observed for higher levels of Cathepsin K in patients with lower FEV1 ($p = 0.067$). Cathepsin K levels were not associated with the level of serum VEGFD ($p = 0.133$). In vitro, treatment with 10nM Odanacatib inhibited the viability of TTJ cells ($p = 0.001$). In vivo, Odanacatib treatment over 4 weeks significantly decreased the size of lung nodules in a murine model of LAM ($p < 0.0001$).

Conclusion:
Our findings suggest that Cathepsin K may be involved in lung damage in LAM and inhibition of Cathepsin K should be investigated further as a treatment for LAM.

5. Engineered 3D hydrogel for studying LAM disease in-vitro

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Lymphangioleiomyomatosis (LAM) is a rare lung disease that primarily affects women of childbearing age. LAM is characterized by the abnormal metastasis of immature, proliferative smooth muscle-like cells into the lungs. Diagnosis of LAM is typically delayed 5-6 years due to the similarity of its symptoms with asthma or chronic obstructive pulmonary disease (COPD). Currently, there is no effective treatment for this fatal disease. However, there is only one FDA-approved drug available on the market for the treatment of LAM disease: the mTOR inhibitor, rapamycin, which could only slow down the disease progression instead of a cure. Advance research and drug screening are urgently needed. We designed a novel biomimetic 3D HA-based hydrogel platform to replicate human LAM progression *in-vitro* to concur with the limitation of the lack of a good animal model. These 3D hydrogels mimic the native niche *in-vivo*, enable multiple modes of invasion, and delineate phenotypic provide a biomimetic microenvironment difference between healthy and diseased cells. This 3D hydrogel system has the ability to expand the viscoelastic and mechanically recoverable properties that will allow potential multicellular co-culture for advanced mechanism study and drug screening.

6. ETV2 is a critical regulator of cell death in Tsc2–deficient cells

Shikshya Shrestha, Anthony Lamattina, Gustavo Pacheco-Rodriguez, Julie Ng, Xiaoli Liu, Abhijeet Sonawane, Jewel Imani, Kosmas, Kosmas, Kimberly Glass, Joel Moss, Kelan Tantisira, Souheil El-Chemaly

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Lymphangioleiomyomatosis (LAM) cells are smooth muscle-like cells with mutations in the tuberous sclerosis complex genes, *Tsc1* or *Tsc2* that inactivates the genes' regulatory function and leads to hyperactivation of mammalian target of rapamycin complex 1 (mTORC1) leading to uncontrolled cell proliferation and survival. Currently, the mTORC1 inhibitors, rapalogs, remain the only therapy in LAM. Since rapalogs are cytostatic not cytocidal there is a need for continuous and long-term treatment to maintain a stable lung function. Here, we investigate mTORC1–independent molecular pathways regulating LAM pathogenesis and identified E26 transformation specific (ETS) Variant Transcription Factor 2 (ETV2) as a potential novel therapeutic target in LAM. Our laboratory had previously demonstrated the therapeutic potential of targeting spleen tyrosine kinase (Syk) in tuberin-deficient cells, the effects of which were similar to that of rapamycin. We utilized gene expression and regulatory network analyses to compare Syk- and mTORC1-inhibitions and discovered that ETV2 is regulated only by Syk–inhibition, and not mTORC1–inhibition in *TSC2*-null cells. We report Syk inhibition drives ETV2 to the nucleus, where it induces transcriptional upregulation of poly(ADP-ribose) polymerase 1 (PARP1) binding protein (PARPBP) mRNA and protein expressions. We showed that ETV2 is a critical regulator of *Tsc2*-deficient cell survival in vitro and in vivo and that silencing of ETV2 or PARPBP in *Tsc2*–deficient cells induced ER stress and increased cell death. To assess the translational relevance of our findings, we analyzed published single cell RNA-sequencing data from LAM samples and examined gene expression in human cells isolated from LAM lungs with loss of heterozygosity for *TSC2 in vitro* and confirmed the expression of ETV2 in these cells. To conclude, nuclear translocation of ETV2 and its regulation of PARPBP is a novel mTORC1–independent signaling that notably promotes a cytocidal response in Tsc2–deficient cells. Further elucidation of this mechanism and identification of agent(s) targeting ETV2 localization or expression may provide novel and more effective alternative therapy of LAM.

7. Mapping the cellular composition of resected cortical tubers and perituberal tissues

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Clinical symptoms of tuberous sclerosis complex (TSC) include developmental delay, intellectual disability, and epilepsy, and mutations in *TSC2* are often associated with increased symptom severity. Unfortunately, epilepsy associated with mutations in *TSC1/2* is often refractory to drug treatment, requiring surgical resection. Within resected brain tissues from patients with TSC, detection of enlarged "balloon" cells is diagnostic for this disorder. Analysis of tubers and perituberal tissues indicates seizures in TSC originate in the perituberal tissues, and "balloon" cells may contain loss of heterozygosity (LOH) of *TSC1/2* compared to surrounding tissue. Though mutations in *TSC1/2* produce epilepsy and cause mTORC1 hyperactivation, unified criteria to identify "balloon" cells and infer their lineage are lacking, and these diagnostic cells have not been studied across large TSC cohorts at the protein level. In addition, how "balloon" cells influence their microenvironment to produce epileptogenic foci is poorly understood. High-dimensional approaches like imaging mass cytometry (IMC) and cyclic immunostaining offer the opportunity to directly assess 30+ proteins and signaling events in single cells while documenting spatial relationships within the tissue. We have developed a custom imaging panel and computational workflow to identify "balloon" cells within archived cortical tubers. We are currently mapping cytoarchitecture and signaling perturbations within these samples, with a specific focus on "balloon" cells and their immediate neighbors. These data will represent a rich dataset for understanding the abundance, structure, and signaling activity of neuronal, glial, and immune cells within archived tubers and perituberal tissues, enabling quantitative comparison of TSC with other mTORopathies.

8. Computational identification of balloon cells in whole slide images of cortical tubers

Rohit Khurana, Asa A. Brockman, Bret C. Mobley, Kevin C. Ess, Rebecca A. Ihrie.

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Balloon cells (BCs) are a prominent subpopulation of abnormal cells found in cortical tubers of patients with Tuberous Sclerosis Complex (TSC). They are phenotypically characterized by a large cytoplasmic body and peripheral nucleus and are suspected to frequently have loss of heterozygosity of the *TSC1/2* allele. Given their unresolved molecular profile, further study is needed to explain their contribution to TSC pathogenesis. Identification and study of BCs using highly multiplexed approaches such as digital spatial profiling, imaging mass cytometry (IMC), or imaging mass spectrometry are potential starting points for such studies. Hematoxylin and eosin (H&E) staining is considered the gold standard for BC identification by a trained pathologist. However, H&E staining is not suitable for combination with highly multiplexed imaging techniques such as IMC. Additionally, manual annotation of a large number of images can be highly time-consuming. Thus, we developed a new computational workflow to automatically identify BCs across whole slide images to isolate regions of interest for further analysis. A pixel classification approach was adopted using toluidine blue (TB) stain given its full compatibility with IMC and ability to stain nuclei and some cytoarchitectural features within tissue. Whole slide images of H&E and TB were first registered to allow cell-to-cell matching across the staining modalities. A training set of H&E images was annotated by an expert pathologist to establish ground truth BCs and a pixel classifier was trained solely on the registered TB image. Probability maps were generated for each image tile, indicating the relative confidence subsets of pixels were representative of a true BC. Putative BCs were subsequently segmented on the probability maps and outlines were overlaid onto its respective TB image. The resulting images were reassembled to form a new compressed multichannel whole slide image. The final product highlights the spatial organization of these cells across the tuber sample, allowing future researchers to confidently and accurately excise BC hotspot regions.

9. Spectrum of germline and somatic mitochondrial DNA variants in tuberous sclerosis complex

Krinio Giannikou, Katie Martin, Ahmad Abdel-Azim, Thomas R. Hougard, Yan Tang, Jeffrey MacKeigan, David J. Kwiatkowski, Hilaire C. Lam

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Background:
Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease causing multisystem growth of hamartomatous tumors in brain, heart, skin, lung and kidney and it is known to be due to genetic alterations in either *TSC1* or *TSC2*. While the nuclear genome has extensively been studied in TSC, there is limited knowledge regarding the role of mitochondrial DNA (mtDNA) in TSC pathogenesis.

Aim:
To examine the prevalence and spectrum of mtDNA variants in TSC patients and correlate them with clinical features and disease severity since mtDNA variants may act as a disease modifier contributing to tumor development and the remarkable phenotypic heterogeneity seen in TSC.

Methods:
We analyzed mtDNA from buccal swabs from 102 TSC patients (44 male, 54 female, 4 unknown, median age: 31 years; 11 familial cases) using deep coverage amplicon massively parallel sequencing (median read coverage: 7,349). mtDNA analysis was also performed in 100 TSC related tumors (58 kidney angiomyolipoma, 24 SEGA, 11 cortical tubers, 2 LAM, 5 TSC-RCC) with matching normal sample (n=9) from 70 patients; 80 tumors had exome data available. Alterations in mitochondrial copy number were determined by qPCR in tumor and matching normal.

Results:
A median of 21 non-synonymous mtDNA variants were identified in 102 buccal swabs, with high homoplasmy (median: 99.62% allele frequency) mainly missense of unknown significance. A pathogenic variant (UUR;MT-TL1; m.3243A>G, heteroplasmy 12%) was identified in one male TSC patient. Five VUS small indels with >97% heteroplasmy were identified in five individuals. Large mtDNA deletions were not detected. Analysis of TSC tumors demonstrated similar spectrum of mtDNA variants as seen in buccal swabs. mtDNA variants did not correlate with any pathological TSC features. qPCR analysis did not reveal changes in mitochondrial content between tumors and corresponding normal tissue.

Conclusions:
Our study provides insight into the mtDNA landscape of TSC for first time, demonstrating that mtDNA genome is stable within the tumors analyzed and across different tissues.

10. UV mutation drives a high prevalence of subclinical facial tumors in TSC – focus on mutation signatures

Katarzyna Klonowska, Joannes M. Grevelink, Krinio Giannikou, Barbara Ogorek, Zachary T. Herbert, Aaron R. Thorner, Thomas Darling, Joel Moss, David J. Kwiatkowski

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Introduction:
Previously we had shown by Massively Parallel Sequencing (MPS) that UV-induced mutation is a consistent second hit in TSC facial angiofibromas (FAFs). Here, we explored the extent and range of effects of UV-induced mutation in the TSC facial skin.

Methods:
For ultra-sensitive detection, we developed Multiplex High-sensitivity PCR Assay (MHPA), enabling mutation detection at variant allele frequencies (VAF) <0.1%.

Results:
We performed MHPA analysis for several sample sets, including TSC FAF (n=24) and nonTSC normal sun-exposed skin (nonTSC NS) (n=8). MHPA analysis of 24 TSC FAFs, led to the identification of 112 low VAF (0.01–8%, median:0.08%,) somatic indels/point mutations. Remarkably, two or more somatic *TSC2* mutations were identified in 19 of 24 (79%) FAFs, suggesting that these small (2mm diameter) FAF biopsies contained at least two FAF clones. 34 of 112 (30%) mutations were CC:GG>TT:AA indicative of UV radiation causation. We also developed an MHPA assay for TP53; an average of 10.1 TP53 mutations were identified per whole skin FAF (likely in keratinocytes), and correlated with the number of TSC2 mutations in the same sample (r=0.63, p=0.005), likely reflecting the role of UV in the generation of both genes' mutations. Examination of the mutation signatures revealed that the SNV signatures for TSC FAF and nonTSC NS are similar to the canonical UV signature SBS7b (COSMIC) with predominance of C>T substitutions. A modest enrichment for G:C>T:A substitutions in variable sequence contexts was seen in the TSC FAF set, less so for nonTSC NS, likely resulting from reactive oxygen species (ROS) generated by sunlight. Dinucleotide variant (DNV) signatures matched very well with the DBS-1 canonical UV signature, with CC:GG>TT:AA substitutions accounting for >90% of all DNVs. The indel signature was different than the reference COSMIC UV-related ID13 signature, dominated by single nucleotide deletions [23 of 38 (61%)]. Finally, we identified a recurrent novel complex mutation signature pattern in TP53, consisting of both an SNV or DNV, and a deletion.

Conclusions:
MHPA analysis showed that unique signatures of *TSC2* UV-induced point mutations are highly prevalent in facial skin of TSC subjects, generating hundreds of thousands of incipient facial tumors (subclinical 'micro-FAFs'), a small proportion of which develop into observable tumors. The study was funded by FY2020 TSC Alliance Postdoctoral Fellowship Award (KK) and Engles Family Fund (DJK).

11. Brain magnetic resonance spectroscopy (MRS) evaluation of children with tuberous sclerosis complex

Tena Rosser, Robert Brown, Vijay Vishwanath, Stefan Bluml

Children's Hospital Los Angeles (TR, VV, SB); Nurix, San Francisco, CA (RB)

Objective: The aim of this study was to establish baseline metabolic profiles of normal appearing brain tissue in a pediatric TSC population using magnetic resonance spectroscopy (MRS). MRS is a non-invasive technique which has the potential to serve as a biomarker for TSC-associated neurologic complications and could potentially help guide treatment options.

Methods:
Eighteen subjects with TSC (9 males, 9 females) ages 3-18 years (mean 11.1 +/- 4.1 years) were enrolled in the study. Results were compared to those of a control population of 54 children (29 males, 26 females) with a mean age of 9.2 +/- 4.3 years. In vivo MR spectra of parieto/occipital grey matter, parietal white matter, frontal white matter, and hippocampus were obtained with a standard single-voxel PRESS sequence (echo time TE=35ms). Fully automated LCModel software was used for processing and absolute metabolite concentration were obtained using the water signal as an internal concentration reference. A total of 119 spectra (grey matter: 49, parietal white matter: 60, frontal WM: 10) from control subjects were acquired.

Results:
In TSC subjects, the axonal/neuronal marker N-acetylaspartate (NAA) appeared to be slightly decreased whereas creatine was mildly elevated. Of the metabolites with weaker signal, the ketone body acetone was highly consistently reduced in TSC ($p = 0.00002$) which is a novel finding.

Conclusion:
MRS of unremarkable appearing brain tissue in TSC shows small abnormalities when compared with controls. Reduced NAA is consistent with a reduced axonal/neuronal density but the mechanism causing low acetone is unclear.

12. Prevalence of thoracoabdominal imaging findings in tuberous sclerosis complex

Bailey K. Fessler, David M. Ritter, Daniel Ebrahimi-Fakhari, Jun Wei, David N. Franz, Darcy A. Krueger, Andrew T. Trout, Alexander J. Towbin

Cincinnati Children's Hospital Medical Center (BKF, DMR, DEF, JW, DNF, DAK, ATT, AJT) University of Cincinnati College of Medicine (BKF, DMR, DNF, DAK, ATT, AJT)

Background:
Tuberous sclerosis complex (TSC) results in neurodevelopmental phenotypes, benign tumors, and cysts throughout the body. Recent studies show numerous rare findings in TSC. Guidelines suggest routine abdominal and chest imaging to monitor for these thoracoabdominal findings. However, imaging is not uniformly done across centers, and thus the prevalence of many findings is not known. To answer this, we categorized the clinical reads of 1398 thoracoabdominal scans from 649 patients of all ages in the Cincinnati Children's Hospital TSC Database.

Results:
Typical TSC findings were present in most patients: kidney cysts (72%), kidney fat-containing AMLs (51%), kidney lipid-poor AMLs (27%), liver AMLs (19%), and lung nodules thought to represent multifocal micronodular pneumocyte hyperplasia (MMPH) (18%). While many features were more common in TSC2 patients, TSC1 patients had a higher prevalence of MMPH than TSC2 patients (24% versus 13%, $p=0.05$). Many rare findings (e.g., lymphatic malformations and liver masses) are more common in TSC than in the general population. Additionally, most thoracoabdominal imaging findings increased with age except kidney cysts decreased, with the 0-10 years age group having the highest percentage (69% 0-10 years, 49% 10-21 years, 48% 21+ years, $p<0.001$). Finally, in our population, no patient had renal cell carcinoma found on abdominal imaging.

Conclusions:
These results show that regular thoracoabdominal scans in TSC may show several findings that should not be ignored or, conversely, over-reacted to when found in patients with TSC. Female sex, *TSC2* mutation, and age are risk factors for many thoracoabdominal findings. The data suggest novel interactions of genetic mutation with pulmonary nodules and age with renal cysts. Finally, in agreement with other works, these findings indicate that several rare thoracoabdominal imaging findings occur at higher rates in the TSC population than in the general population. Overall, this work supports obtaining detailed thoracoabdominal imaging in patients with TSC.

13. Factors associated with facial angiofibroma related to tuberous sclerosis complex and use of topical mTOR inhibitor in the United States: A retrospective analysis of the TSC Natural History Database

Eric Beresford, Steven L. Roberds, Jo Anne Nakagawa, Sreedevi Boggarapu

Nobelpharma America, LLC Bethesda Maryland (EB, SB); TSC Alliance, Silver Spring, Maryland (SLR, JAN)

This retrospective analysis of the TSC Alliance's Natural History Database (NHD) aimed to evaluate the factors associated with facial angiofibroma and the use of topical mechanistic target of rapamycin (mTOR) inhibitor. The TSC Alliance's NHD, the longest-running repository of longitudinally-studied individuals with TSC, is an IRB-approved research database implemented in 2006. Of the 2240 individuals (data extracted in May 2020), 2088 individuals were enrolled from the United States (18 clinical sites) and data from 2057 individuals were included in this retrospective analysis. Univariate and multivariate logistic regression was performed to evaluate the association of demographics and baseline characteristics with facial angiofibroma or topical mTOR inhibitor used for the treatment of facial angiofibroma. Out of 2057 individuals, facial angiofibroma was noted in 1329 (64.4%) individuals. In the univariate analyses, a higher risk of facial angiofibroma was associated with the age groups 18–45 years and =46 years, the 18–45 years age group at diagnosis, being white, and the presence of TSC-related manifestations such as focal seizures, angiomyolipoma, renal cysts, SEGA, autism, retinal hamartoma, ADHD, anxiety, or liver hamartoma. The 6–17 years age group, *TSC 1* mutation, and rhabdomyoma were associated with a significantly decreased risk of facial angiofibroma. In the multivariate regression, the 6–17 years age group, the 18–45 years age group, *TSC 2* mutation, angiomyolipoma, or renal cysts were significantly associated with higher risk, while the presence of arrhythmias was significantly associated with a lower risk of facial angiofibroma. Of the individuals with facial angiofibroma, topical rapamycin use was noted for 329 (24.8%) individuals. Topical rapamycin use was most commonly associated with the 6–17-year-old age group, the 0–2 years age group at diagnosis, TSC 2 gene mutation, being white, and the presence of manifestations such as renal cysts, angiomyolipoma, renal cysts, rhabdomyoma, retinal hamartoma, and ADHD. In the multivariate analyses, Asians, whites, and individuals with angiolipoma were significantly associated with higher use of topical rapamycin. In conclusion, the association of TSC-related manifestations in individuals with facial angiofibroma illustrates the importance of accurate diagnosis by dermatologists and referral to a comprehensive multi-disciplinary TSC Clinic for surveillance and management of other TSC manifestations.

14. Predicting individualized lung disease progression in lymphangioleiomyomatosis

Anushka K. Palipana, Rhonda D. Szczesniak, Simon R. Johnson, Nishant Gupta

Cincinnati Children's Hospital Medical Center, Ohio, USA (AKP, RDS), University of Cincinnati, Ohio, USA (AKP, RDS, NG), University of Nottingham, Nottingham, UK (SRJ), National Centre for Lymphangioleiomyomatosis, Nottingham, UK (SRJ)

Rationale:
Clinical decision making in LAM is challenging due to the marked inter-individual variability in disease progression in patients with Lymphangioleiomyomatosis (LAM).

Objectives:
To develop a dynamic prediction model and an easy-to-use online calculator estimating the probability of clinically meaningful lung-function declines (rate of change in FEV1 thresholds of 60, 75, and 100ml/year), and to simultaneously characterize lung-function trajectories and predict mortality in patients with LAM.

Methods:
Patients observed in the US National Heart, Lung and Blood Institute (NHLBI) LAM Registry were included. We employed novel stochastic modeling and performed covariate selection, evaluating predictive probabilities for clinically relevant FEV1 drops. We formed predictive probabilities of transplant-free survival by jointly modeling longitudinal FEV1 and lung transplant or death. The results to predict FEV1 decline were externally validated using the UK LAM natural history cohort. Lastly, we estimated life expectancy of patients on the basis of LAM subtype (TSC vs. S-LAM) using Kaplan-Meier curves.

Results:
In the NHLBI cohort (n=216), higher baseline lung function (FEV1 and diffusion capacity for carbon monoxide) corresponded to better lung function over time, whereas increasing age at diagnosis was associated with lower lung function. Premenopausal status was associated with a faster rate of decline in FEV1. Areas under the receiver-operating characteristic curve (AUCs) were 0.81, 0.80, 0.82 for predicting drops of 100, 75 and 60 mL/yr, respectively, and mean absolute percentage error (MAPE) for FEV1 values was 3.3%. In the UK validation cohort (n=194), AUCs were roughly 0.97 for the selected FEV1 decline thresholds, and MAPE was 4.9%. The overall survival time following LAM diagnosis [95% CI] was similar in women with TSC-LAM compared with S-LAM: 24.22 [20.38 - 28.06] vs. 22.62 [20.91 – 24.32] years following LAM diagnosis. The average life expectancy for S-LAM and TSC-LAM patients was 63.36 and 64.96 years, respectively, compared to the average life expectancy of 81 years for women in the US.

Conclusions:
Predictive probabilities calculated from longitudinal modeling of routinely collected demographic and clinical data can aid in individualized LAM prognostication and therapeutic decision-making.

Awardees Selected from Submitted Abstracts

Platform Presentations

Mayowa Amosu
University of Maryland, College Park
College Park, MD, USA

Intranasal administration of CpG increases survival and counters immunosuppression in a murine model of metastatic lymphangioleiomyomatosis (LAM)

Mary Bronwen Chalkley
Vanderbilt University
Nashville, TN, USA

Early neurodevelopment and cytoarchitecture is altered in tuberous sclerosis complex

Vasiliki Karalis
University of California, Berkeley
Berkeley, CA, USA

Raptor downregulation rescues neuronal phenotypes in mouse models of tuberous sclerosis complex

Lena Nguyen, PhD
Yale University
New Haven, CT, USA

Juvenile 4E-BP1 expression attenuates epilepsy and neurophysiological abnormalities associated with mTOR hyperactivation in mice

Adam Pietrobon
Ottawa Hospital Research Institute
Ottawa, Ontario, Canada

Three-dimensional drug screen identifies HDAC inhibitors as therapeutic agents in a tissue-engineered model of lymphangioleiomyomatosis

Poster Presentations

Alberto Camacho Magallanes
Ottawa Hospital Research Institute
Ottawa, Ontario, Canada

Cellular and molecular features of TSC2 null neural crest cells demonstrate characteristics of pulmonary lymphangioleiomyomatosis

Suzanne Miller, PhD
University of Nottingham
Nottingham, Nottinghamshire, England

Elevated expression of serum Cathepsin K is associated with active disease and inhibition shows potential as a novel therapy for LAM

About the Conference Hosts

The TSC Alliance is an internationally recognized nonprofit that does everything it takes to improve the lives of people with tuberous sclerosis complex (TSC).

TSC is a rare genetic disease that causes tumors to grow in different organs, from the brain and heart to the lungs and kidneys to the skin and eyes. Nearly one million people worldwide have TSC. Some live independently with few symptoms while others require complex care.

We are a source of hope and connection for all affected by TSC. We drive research, increase care quality and access and advocate with and for people affected by the disease. Through our collaboration and partnerships, we've advanced FDA-approved treatments and created support systems around the world so no one has to navigate TSC alone.

The TSC community is our strongest ally. With the power of families and the support of donors, volunteers, researchers, educators, industry partners, and more, we can create a future where everyone with TSC can realize their full potential—no matter how complex their journeys are to get there. **Join us at tscalliance.org or contact us at info@tscalliance.org.**

TSC Alert

TSC Alert is a monthly e-newsletter for researchers and healthcare professionals. Subscribe to be the first to hear about our funding opportunities, research resources and conferences.

tscalliance.org/researchers/tsc-alert/

About the Conference Hosts

THE LAM FOUNDATION
A BREATH OF HOPE

The LAM Foundation is the global leader in the fight against LAM (lymphangioleiomyomatosis), a rare lung disease that strikes young women, often in the prime of their lives. A 501(c)(3) non-profit, the Foundation's mission is: To urgently seek safe and effective treatments, and ultimately a cure, for lymphangioleiomyomatosis (LAM) through advocacy and the funding of promising research. We are dedicated to serving the scientific, medical, and patient communities by offering information, resources, and a worldwide network of hope and support.

Founded in 1995 in Cincinnati, Ohio, as a grassroots effort. The LAM Foundation has evolved into an organization that is described by the National Heart, Lung and Blood Institute (NHLBI) as "a model for voluntary health agencies." The Foundation has provided a mechanism to organize and focus the LAM community to facilitate patient support, informed clinical care, scientific interchange, and research.

Highlights of The LAM Foundation's accomplishments include:

- The LAM Foundation has registered nearly 3,415 patients over the past 26 years, including roughly 2,400 patients currently living in the United States. Additionally, an international network, the Worldwide LAM Patient Coalition, was created to support LAM patients globally. There are now 16 patient organizations around the world.
- The LAM patient and family community is supported by The LAM Foundation through a robust website, monthly e-newsletters, a 30-member LAM Liaison Network that organizes 20+ virtual regional educational and support meetings each year.
- The LAM Foundation has developed a network of 63 LAM clinics (34 in the US and 29 international), each led by a dedicated LAM Clinic Director, to facilitate high quality, multidisciplinary care of LAM patients and provide a platform for research.
- Research supported by The LAM Foundation formed the basis for clinical trials which have resulted in the discovery of an effective, FDA-approved treatment, sirolimus, now available in 40 countries. This research advanced knowledge of the mTOR pathway (important in regulating the cell division cycle) and has proven central to understanding other, much more common diseases such as cancer, diabetes, and obesity.
- More than $17 million of the $27 million raised to date has been committed directly to LAM research including grants awarded for 149 projects to unique investigators to study the basic mechanisms of disease in LAM, as well as clinical trial support.
- A NHLBI-funded tissue bank has been established at the National Disease Research Interchange (NDRI), including a system for tissue acquisition and distribution. The NHLBI has supported both an extensive intramural basic re search program for LAM and a highly successful intramural clinical protocol, which has collected natural history data on more than 700 LAM patients.
- The LAM Foundation provides resources to LAM patients considering lung transplant through the Circle of Hope Transplant Support Program. This initiative helps patients and families navigate the complex and stressful journey of transplant while also assuring that rare and valuable tissues are made available to LAM researchers.
- The LAM Foundation and the NHLBI have co-sponsored LAM scientific conferences since 1999, these meetings have immeasurably strengthened and focused the efforts of the scientific community to understand the disease.

Looking forward, The LAM Foundation's work is far from complete, and much remains to be done. Future research priorities include:

- Maximizing the effectiveness of the current treatment, sirolimus (related to optimal dosage and long-term effectiveness), while continuing the search for a cure.
- More clinical trials are needed to determine if there are combination or alternate therapies for the treatment and cure of LAM.
- An untold number of women with LAM remain undiagnosed; we need to find them and shorten time to diagnosis in the United States and globally.
- Great opportunities exist for The LAM Foundation to serve as a model for other rare disease organizations, and to share knowledge to propel treatments and cures in rare and common diseases.

For more information about The LAM Foundation, contact: Sue Sherman, Executive Director and CEO, at ssherman@thelamfoundation.org at 513-777-6889.

LAM Clinic and Research Network

The LAM Foundation seeks to help improve the diagnosis and treatment of all LAM patients. Toward this end, it has a program to develop regional LAM Clinics throughout the world. The objective of The LAM Foundation Clinic and Research Network is to focus on LAM care to medical institutions or hospitals that have the interest and expertise to deliver state of the art, coordinated, multidisciplinary LAM care, and to perform cooperative research with other LAM clinics.

United States

Birmingham, Alabama: University of Alabama at Birmingham (UAB)
Clinic Director(s): Joseph Barney, MD
Tel.: 205-996-5864

Phoenix, Arizona: St. Joseph's Hospital and Medical Center
Clinic Director(s): Sofya Tokman, MD; Rajat Walia, MD
Tel.: 602-406-8187

Los Angeles, California: University of California
Clinic Director(s): Ariss DerHovanessian, MD; Joseph Lynch, MD; Elinor Lee, MD
Tel.: 310-206-9149

Los Angeles, California: Keck Medicine of USC
Clinic Director(s): Richard Lubman, MD
Tel.: 323-442-9590

San Diego, California: University of California
Clinic Director(s): Bernie Sunwoo, MD
Tel.: 855-355-5864

San Francisco, California: University of California
Clinic Director(s): Rupal Shah, MD; Paul Wolters, MD
Tel.: 415-353-2577

Stanford, California: Stanford University Medical Center
Clinic Director(s): Stephen Ruoss, MD
Tel.: 650-723-6983

Denver, Colorado: National Jewish Health
Clinic Director(s): Gregory Downey, MD; Kevin Brown, MD; Matthew Koslow, MD; Jeff Swigris, DO
Tel.: 303-398-1912

Gainesville, Florida: University of Florida
Clinic Director(s): Ali Ataya, MD; Mark Brantly, MD
Tel.: 352-273-8740

Jacksonville, Florida: Mayo Clinic
Clinic Director(s): Charles Burger, MD; Augustine Lee, MD
Tel.: 904-953-0860

Atlanta, Georgia: Emory University School of Medicine
Clinic Director(s): Srihari Veeraraghavan, MD; Ria Gripaldo, MD
Tel.: 404-778-5736

Chicago, Illinois: Loyola University Medical Center
Clinic Director(s): Daniel Dilling, MD; James Gagemeier, MD; Emily Gilbert, MD
Tel.: 708-216-4946

Indianapolis, Indiana: Indiana University School of Medicine
Clinic Director(s): Tim Lahm, MD; Ryan Boente, MD
Tel.: 317-278-0064

Iowa City, Iowa: University of Iowa Health Care
Clinic Director(s): Kam Ussavarungsi, MD; Nabeel Hamzeh, MD
Tel.: 319-356-8133

Kansas City, Kansas: University of Kansas Medical Center
Clinic Director(s): Mark J. Hamblin, MD; Chase Hall, MD
Tel.: 913-945-8536

Bethesda, Maryland: National Institutes of Health
Clinic Director(s): Joel Moss, MD, PhD
Tel.: 301-496-3632

Boston, Massachusetts: Brigham and Women's Hospital
Clinic Director(s): Elizabeth Henske, MD; Souheil El-Chemaly, MD
Tel.: 617-732-6770

Ann Arbor, Michigan: University of Michigan
Clinic Director(s): MeiLan Han, MD, MS; Kevin Flaherty, MD, MS; Bonnie Wang, MD
Tel.: 734-647-7840

Rochester, Minnesota: Mayo Clinic
Clinic Director(s): Jay Ryu, MD; Teng Moua, MD; Misbah Baqir, MBBS
Tel.: 507-284-2079

St. Louis, Missouri: Washington University School of Medicine
Clinic Director(s): Adrian Shifren, MD
Tel.: 314-747-9523
Alt. Tel.: 314-454-8917

New York, Brooklyn: NYU Langone Health
Clinic Director(s): Luis Angel, MD; Melissa Lesko, MD
Tel.: 866-838-5864

New York, New York: Presbyterian/Columbia
Clinic Director(s): Jeanine D'Armiento, MD, PhD; Monica Goldklang, MD
Tel.: 212-305-3745

Rochester, New York: University of Rochester Medical Center
Clinic Director(s): Mary Anne Morgan, MD
Tel.: 585-273-5460

Cincinnati, Ohio: University of Cincinnati Medical Center/UC Health
Clinic Director(s): Francis McCormack, MD; Nishant Gupta, MD
Tel.: 513-558-4831

Cleveland, Ohio: Cleveland Clinic
Clinic Director(s): Joseph Parambil, MD
Tel.: 216-445-8615

Portland, Oregon: Oregon Health and Science University
Clinic Director(s): Matthew Drake, MD; Alan Barker, MD
Tel.: 503-494-1620

Philadelphia, Pennsylvania: University of Pennsylvania
Clinic Director(s): Maryl Kreider, MD; Robert Kotloff, MD
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Charleston, South Carolina: Medical University of South Carolina
Clinic Director(s): Charlie Strange, MD; Rachana Krishna, MD
Tel.: 843-792-2543

Nashville, Tennessee: Vanderbilt University Medical Center
Clinic Director(s): Justin Hewlett, MD
Tel.: 615-322-0774

Dallas, Texas: University of Texas Southwestern Medical Center
Clinic Director(s): Carlos E. Girod, MD
Tel.: 214-645-7003
Alt. Tel.: 214-645-2100

Houston, Texas: University of Texas Health Center
Clinic Director(s): Rosa M. Estrada-Y-Martin, MD
Tel.: 832-325-7396

Salt Lake City, Utah: University of Utah School of Medicine
Clinic Director(s): Robert Paine III, MD; Barbara Cahill, MD
Tel.: 801-587-6014

Seattle, Washington: Swedish Medical Center
Clinic Director(s): George Pappas, MD
Tel.: 206-320-6500

Morgantown, West Virginia: West Virginia University School of Medicine
Clinic Director(s): Rahul Sangani, MD
Tel.: 304-598-4853

International Sites

Sydney, Australia: St. Vincent's Hospital
Clinic Director(s): Deborah Yates, MD; Allan Glanville, MD
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Sao Paulo, Brazil: Heart Institute (InCor), Hospital das Clinicas, University of Sao Paulo
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Beijing, China: Peking Union Medical College Hospital
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Mexico City, México: Instituto Nacional de Enfermedades Respiratorias
Clinic Director(s): Ivette Buendia Roldan, MD; Mayra Mejia, MD
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Nieuwegein, Netherlands: Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital
Clinic Director(s): Jan C Grutters, MD; Marcel Veltkamp, PhD
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Warsaw, Poland: National Tuberculosis and Lung Diseases Research Institute
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Moscow, Russia: First Moscow State Medical University
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St. Petersburg, Russia: University of St. Petersburg
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Lausanne, Switzerland: Lausanne University Hospital (CHUV)
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Nottingham, United Kingdom: University of Nottingham
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TSC Alliance Research Resources

innovative RESEARCH

The TSC Alliance endeavors to stimulate, support and coordinate research that will lead to a cure for TSC while improving the lives of those affected. Most TSC Alliance research grants support small, focused projects that allow investigators to develop preliminary data to compete for funding from larger organizations such as the National Institutes of Health. Other TSC Alliance research grants support post-doctoral fellowships to encourage young investigators to engage in TSC research.

For more information, contact Zoë Fuchs – zfuchs@tscalliance.org.

NATURAL HISTORY database

Implemented in 2006, the TSC Natural History Database captures clinical data to document the impact of the disease on a person's health over their lifetime. More than 2,000 people with TSC are enrolled in the project among 18 U.S.-based clinical sites. The TSC Alliance provides funding to participating clinics to perform data entry, monitors the integrity of the database, and makes data available to investigators to answer specific research questions and identify potential participants for clinical trials and studies.

For more information, contact Gabrielle Rushing, PhD – grushing@tscalliance.org.

biosample REPOSITORY

The TSC Biosample Repository houses human biological materials such as blood, DNA, and tissues linked to detailed clinical data in the TSC Natural History Database. High-quality biosamples and their associated clinical data will enable researchers to discover biomarkers, establish human cell lines or tissue arrays for drug testing, and search for clues to understand why TSC is so different from person to person.

For more information, contact Gabrielle Rushing, PhD – grushing@tscalliance.org.

preclinical CONSORTIUM

To spur development of new ideas leading to novel drugs, the TSC Alliance funds research grants to help learn more about the mechanisms of pathology in TSC. To facilitate the translation of basic and mechanistic research into clinical trials and new treatments for TSC, the TSC Alliance created a TSC Preclinical Consortium in collaboration with industry and academia to test the efficacy of candidate therapeutic drugs and advance the best to the clinical stage.

For more information, contact Dean Aguiar, PhD – daguiar@tscalliance.org.

clinical RESEARCH CONSORTIUM

The execution of clinical studies requires extensive planning, cooperation, and collaboration to protect the safety of participants and the integrity of valuable data. To initiate and implement clinical studies more quickly and effectively, researchers from five TSC Clinics together with the TSC Alliance formed the TSC Clinical Research Consortium in 2012. The consortium has expanded to include additional sites for the PREVeNT trial.

For more information, please contact Steve Roberds, PhD – sroberds@tscalliance.org.

TSC International (TSCi) Member Organizations

Argentina
Asociación Argentina de Esclerosis Tuberosa (ARGET)
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