
2023 International TSC
Research Conference
Fueling the Future
Washington, DC
September 7-9, 2023
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2023 International TSC Research Conference

Fueling the Future

Washington, DC
September 7–9, 2023

Welcome

The TSC Alliance® welcomes you to Washington, DC, for the **2023 International TSC Research Conference: Fueling the Future**, presented by Nobelpharma and Marinus Pharmaceuticals. We are thrilled to be able to be able to once again exchange ideas in person and learn from one another face to face.

The Conference Organizing Committee has worked hard to present a comprehensive three-day agenda, including plenary sessions, breakout group discussions, a poster session and reception, and a number of breaks to help you forge professional relationships and further collaborations.

A highlight of this year's conference is the Early Career Research Symposium. During this session, early-stage investigators will have the opportunity to report on their cutting-edge research, network, and learn about the diverse paths forward in the field of TSC research, including a panel discussion of career and funding opportunities.

Thursday night's dinner will feature a keynote presentation by Martina Bebin, MD, MPA, who will discuss the outcomes and impact of the PREVeNT Study (Preventing Epilepsy using Vigabatrin in Infants with TSC), the first preventative trial in the United States for any form of epilepsy. On Saturday, the conference will conclude with a panel discussion between researchers and individuals and families affected by TSC to highlight the impact of TSC research and how it directly relates to their needs and concerns.

The TSC Alliance is grateful to our planning committee, Early Career Research Symposium Co-Chairs, speakers and poster presenters, our partners from TSC International as well as the multiple sponsors who made this year's research conference possible. They include Nobelpharma America, LLC; Marinus Pharmaceuticals; Jazz Pharmaceuticals; Neurelis; UCB; Upsher-Smith Laboratories, LLC; Aeovian Pharmaceuticals; GeneDx; Grin Therapeutics; Longboard Pharmaceuticals; Noema Pharma; Ovid Therapeutics; PsychoGenics; Seizure Tracker; and Total CareRX.

We would also like to recognize the National Institutes of Health, the National Institute of Neurological Disorders and Stroke, National Center for Advancing Translational Sciences, the National Cancer Institute, and National Heart, Lung, and Blood Institute, all of which provided grant support for this year's conference.

Sincerely,

Kari Luther Rosbeck
President & CEO

Steven L. Roberds, PhD
Chief Scientific Officer

Shafali Spurling Jeste, MD
Conference Co-Chair

Rebecca Ihrie, PhD
Conference Co-Chair

Conference Organizing Committee

Conference Co-Chairs

Shafali Spurling Jeste, MD, Chief, Division of Neurology, Co-Director, Neurological Institute, Children's Hospital, Los Angeles

Rebecca Ihrie, PhD, Associate Professor, Cell & Developmental Biology and Neurological Surgery, Vanderbilt University School of Medicine

Organizing Committee Members

Jamie K. Capal, MD, Associate Professor of Pediatrics and Neurology, University of North Carolina at Chapel Hill

Laura Farach, MD, Associate Professor, Division of Medical Genetics, Department of Pediatrics, University of Texas Health Science Center at Houston

Zoë Fuchs, Manager, Translational Research, TSC Alliance

Gina Lee, PhD, Assistant Professor, Microbiology & Molecular Genetics, UC Irvine School of Medicine

Carmen Priolo, MD, PhD, Assistant Professor of Medicine, Brigham and Women's Hospital

Tracee Ridley-Pryor, DNP, APRN, PMHNP-BC, Psychiatric-Mental Health Nurse Practitioner, Le Bonheur Children's Hospital, and Assistant Professor, University of Tennessee Health Science Center

Steven L. Roberds, PhD, Chief Scientific Officer, TSC Alliance

Kari Luther Rosbeck, President & CEO, TSC Alliance

Katie Smith, Director, Government & Global Affairs, TSC Alliance

Daniel Vogt, PhD, Assistant Professor, Department of Pediatrics and Human Development, Michigan State University

Oded Volovelsky, MD, PhD, Pediatric Nephrology Unit Head, Hadassah Hebrew University Medical Center

Anne Wolfe, Senior Manager, Strategic Projects, TSC Alliance

Jane Yu, PhD, Professor, Pulmonary, Critical Care and Sleep Medicine, University of Cincinnati

Early Career Researcher Symposium Co-Chairs

Nicole McDonald, PhD, Assistant Clinical Professor, UCLA Semel Institute

Uchenna John Unachukwu, PhD, Associate Research Scientist, Columbia University Medical Center

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2023 International TSC Research Conference Agenda

September 7	Day One, Thursday — Blue Ballroom
1:30 pm - 1:45 pm	**Welcome and Opening Remarks:** Kari Luther Rosbeck, President & CEO, TSC Alliance **TSC Community Speaker:** Kevin Waller
Plenary Session 1: Chemical and Cell Biology	
1:45 pm - 2:15 pm	**Welcome and Introduction**: Jane Yu, PhD, University of Cincinnati Ziyang Zhang, PhD, University of California, Berkeley – **Design of novel mTORC1 inhibitors**
2:15 pm - 2:30 pm	Katharina Maisel, PhD, University of Maryland – **Intranasal adjuvant immunotherapy to treat TSC-associated LAM tumors**
2:30 pm - 3:00 pm	Ben Philpot, PhD, Department of Cell Biology & Physiology, University of North Carolina, Chapel Hill – **Identification of novel therapeutics for neurodevelopmental disorders**
3:00 pm - 3:15 pm	Charilaos (Harry) Filippakis, PhD, University of New England, College of Osteopathic Medicine – **Tryptophan-mediated macropinocytosis is a metabolic vulnerability in tuberous sclerosis complex**
3:15 pm - 3:45 pm	***Break***
3:45 pm - 4:00 pm	Joohwan Kim, PhD, University of California, Irvine – **mTORC1-dependent RNA methylation confers rapamycin resistance in TSC tumors**
4:00 pm - 4:15 pm	Nicola Alesi, MD, PhD, Brigham and Women's Hospital and Harvard Medical School – **TFEB drives mTORC1 hyperactivation and kidney disease in tuberous sclerosis complex**
4:15 pm - 4:30 pm	David Ritter, MD, PhD, Cincinnati Children's Hospital Medical – **A precision dosing strategy for overcoming challenges to the dosing of mTOR Inhibitors in TSC infants in clinical practice and clinical trials**
4:30 pm - 4:45 pm	Ulrike Rehbein, PhD, Msc, BSc, University of Innsbruck – **A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression**
4:45 pm - 5:00 pm	Anil Kumar Kavala, PhD, Texas Tech University Health Sciences Center – **TSC-null extracellular vesicles facilitate metastable phenotypes of LAM cells and formation of lung metastatic niche**
	Close of Plenary Sessions for Day 1
5:30 pm - 6:30 pm	**Reception — Blue Room Prefunction**
6:30 pm - 9:00 pm	**Dinner and Keynote Presentation — Hampton Ballroom**
	Emcee: Steven L. Roberds, PhD, TSC Alliance **Presentation of the Vicky H. Whittemore Travel Awards:** Shafali Spurling Jeste, MD, Children's Hospital, Los Angeles, and Rebecca Ihrie, PhD, Vanderbilt University School of Medicine **Presentation of the Manuel R. Gomez Award:** Kari Luther Rosbeck, TSC Alliance **Keynote Presentation:** Martina Bebin, MD, MPA, University of Alabama at Birmingham – **Outcomes and impact of the PREVeNT trial**

September 8 Day Two, Friday — Blue Ballroom

Plenary Session 2: Biomarkers and Ethics in Early Intervention

8:30 am - 8:45 am	**Welcome and Introduction:** Shafali Spurling Jeste, MD, Children's Hospital Los Angeles **TSC Community Speakers:** Ryan and Andrea Beebe
8:45 am - 9:15 am	Connie Kasari, PhD, University of California, Los Angeles – **Early intervention in autism**
9:15 am - 9:45 am	Kate MacDuffie, PhD, MA, Treuman Katz Center for Pediatric Bioethics, Seattle Children's Research Institute and University of Washington School of Medicine – **Ethical and social impacts of advances in neuroscience on children and families impacted by neurodevelopmental disorders**
9:45 am - 10:15 am	Sergiusz Jóźwiak, MD, PhD, Medical University of Warsaw – **Impact of early intervention in TSC**
10:15 am - 10:45 am	***Break***
10:45 am - 11:30 am	Drs. Bebin, Jóźwiak, Kasari, and MacDuffie – **Panel discussion on ethics of early intervention**
11:30 am - 11:45 am	Tanjala Gipson, MD, University of Tennessee Health Science Center – **Early vocal development and autism in TSC**
11:45 am - 12:00 pm	Jenny Do, MS, CGC, University of Texas Medical Branch – **Parental stress in tuberous sclerosis complex**
12:00 pm - 1:00 pm	***Lunch — Hampton Ballroom***

Breakout Session Discussions

1:00 pm - 2:30 pm	**Transition from pediatric to adults** – Calvert Room **Session Co-Chairs:** Jamie K. Capal, MD, University of North Carolina at Chapel Hill, and Elizabeth Thiele, MD, PhD, Massachusetts General Hospital
1:00 pm - 2:30 pm	**Neurodevelopment and early interventions** – Governors Boardroom **Session Co-Chairs:** Shafali Spurling Jeste, MD, Children's Hospital Los Angeles, and Daniel Vogt, PhD, Michigan State University
1:00 pm - 2:30 pm	**Big data and single cell approaches/analysis** – Blue Ballroom **Session Co-Chairs:** Rebecca Ihrie, PhD, Vanderbilt University, and Laura Farach, MD, University of Texas Health Science Center at Houston
1:00 pm - 2:30 pm	**Cellular energetics and metabolic (core cell bio)** – Capitol Room **Session Co-Chairs:** Oded Volovelsky, MD, PhD, Hadassah Hebrew University Medical Center, and Gina Lee, PhD, University of California Irvine
2:30 pm - 3:00 pm	***Break***

Plenary Session 3: TAND (TSC-Associated Neuropsychiatric Disorders) and Epilepsy Research

3:00 pm - 3:15 pm	**Welcome and Introduction:** Tracee Ridley-Pryor, DNP, APRN, PMHNP-BC, Le Bonheur Children's Hospital and University of Tennessee Health Science Center **TSC Community Speaker:** Rahul Vipparthi
3:15 pm - 3:45 pm	Nola Chambers, PhD, Centre for Autism Research in Africa (CARA), Division of Child and Adolescent Psychiatry, University of Cape Town – **TANDem project outcomes and next steps**
3:45 pm - 4:00 pm	Agnies M. van Eeghen, MD, PhD, Emma Children's Hospital, Amsterdam University Medical Center – **Behavioral outcomes of treatment with cannabidiol oral solution in individuals with seizures associated with tuberous sclerosis complex: design of an ongoing phase 4 trial (EpiCom)**
4:00 pm - 4:15 pm	Michael Wong, MD, PhD, Washington University School of Medicine – **Cerebral vascular and blood brain-barrier abnormalities in a mouse model of epilepsy and tuberous sclerosis complex**
4:15 pm - 4:45 pm	Howard Weiner, MD, Baylor College of Medicine – **Impact of advances in neurosurgery in TSC**
4:45 pm - 5:00 pm	**Poster Previews**
5:00 pm - 8:00 pm	**Poster Session and Reception — Hampton Ballroom**

September 9 Day Three, Saturday — Blue Ballroom

Plenary Session 4: Organoids and Cell Development

8:30 am - 8:45 am	**Welcome and Introduction:** Rebecca Ihrie, PhD, Vanderbilt University **TSC Community Speakers:** Chip and Kristin Burkhalter
8:45 am - 9:15 am	Manoocher Soleimani, MD, The University of New Mexico Health Sciences Center – **Mechanisms of renal cystogenesis**
9:15 am - 9:30 am	Tasnim Olatoke, University of Cincinnati – **Stat1 promotes survival and lung metastasis of TSC2-null cells in lymphangioleiomyomatosis (LAM)**
9:30 am - 10:00 am	Jennifer Sucre, MD, Vanderbilt University – **Early lung development**
10:00 am - 10:30 am	***Break***
10:30 am - 11:00 am	Nina Corsini, PhD, Institute for Molecular Biotechnology – **Contributions of interneuron progenitor cells to neurological disease**
11:00 am - 11:15 am	Jeffrey Calhoun, PhD, Northwestern University – **Multimodal framework to resolve variants of uncertain significance in TSC2**
11:15 am - 12:00 pm	**Report on Breakout Sessions**
12:00 pm - 1:00 pm	***Lunch — Hampton Ballroom***

Plenary Session 5: Hot Topics Advancing TSC Research and Clinical Care

1:00 pm - 1:30 pm	**Welcome and Introduction:** Carmen Priolo, MD, PhD, Brigham and Women's Hospital **Short presentations of ongoing clinical studies**
1:30 pm - 1:45 pm	Joseph Bateman, PhD, King's College London – **TSC and the UK Rare Disease Research Platform**
1:45 pm - 2:15 pm	Isaac Rodriguez-Chavez, PhD MHS – **Clinical research approaches to increase diversity of research participants**
2:15 pm - 2:30 pm	Meredith Rose, MS, Cincinnati Children's Hospital Medical Center – **Healthcare provider recognition of pregnancy related risks and management considerations in patients with tuberous sclerosis complex**
2:30 pm - 3:00 pm	***Break***
3:00 pm - 4:00 pm	**Joint session with individuals and families attending the Regional TSC & LAM Conference** Conference Co-Chairs Shafali Spurling Jeste, MD, Children's Hospital Los Angeles, and Rebecca Ihrie, PhD, Vanderbilt University, will moderate a panel discussion and Q&A session to convey the relevance and impact of the research conference to scientific and non-scientific attendees alike. **Community Speakers:** Iris Mustich and Mary Vasseghi

Opening Night Keynote Speaker

Martina Bebin, MD, MPA
Professor of Neurology and Pediatrics
University of Alabama at Birmingham
Birmingham, AL

Martina Bebin MD, MPA, is Professor of Neurology and Pediatrics at the University of Alabama at Birmingham. Her primary research interest is in tuberous sclerosis complex (TSC); she serves as co-director of the UAB TSC Clinic, which is a Center of Excellence and offers comprehensive multidisciplinary TSC care for all ages. She has been involved in clinical epilepsy and TSC research for more than 20 years. Currently, she is the Principal Investigator (PI) for the NIH-funded PREVeNT Trial, the first epilepsy prevention trial for infants with TSC. Also, she is an original member of the TSC Clinical Research Consortium and a site PI for several NIH-funded TSC clinical research efforts over the past 10 years.

Invited Speaker Biographies

Dean Aguiar, PhD
Vice President, Translational 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 (IND) and investigational device exemption (IDE), 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 co-founded 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 and completed post-doctoral training at the University of Minnesota.

Nicola Alesi, MD, PhD
Instructor in Medicine
Brigham and Women's Hospital
Harvard Medical School
Boston, MA

Nicola Alesi, MD, PhD, is a physician/scientist born and raised in Italy. He is a member of the fantastic Henske Lab Team, where he has developed a line of research regarding the role of lysosomes in the pathogenesis of TSC/LAM.

Joseph Bateman, PhD
Professor of Molecular Neuroscience
King's College London
London, United Kingdom

Joseph Bateman is Professor of Molecular Neuroscience in the department of Basic and Clinical Neuroscience at King's College London. Joe did his BSc and PhD at University College London. He then moved to the University of Texas Southwestern Medical Center as a postdoc to work on mitochondrial genetics. After three years in the USA, he was awarded a Cancer Research UK Postdoctoral fellowship to work at the CRUK London Research Institute in Helen McNeil's lab studying Drosophila neurodevelopment. Joe started his own lab as a Lecturer at King's in 2005 and was promoted to Professor in 2021. Joe's lab studies how cellular signaling pathways regulate nervous system development and function. He has a longstanding interest in mTOR signaling and how mTOR regulates nervous system development. Joe leads the mTOR Pathway Diseases node, part of the UK Rare Disease Research Platform.

Jeffrey Calhoun, PhD
Research Assistant Professor
Northwestern University
Chicago, IL

Jeff received a PhD in Cellular and Molecular Biology at the University of Michigan in 2013 under the mentorship of Dr. Lori Isom. For his postdoctoral training, he studied the genetics of epilepsy in mice and humans in the laboratories of Dr. Jennifer Kearney and Dr. Gemma Carvill. His research interests focus on improving the genetic diagnosis of epilepsy through genome sequencing, machine learning, and functional characterization of variants of uncertain significance.

Jamie K. Capal, MD
Associate Professor of Pediatrics and Neurology
University of North Carolina at Chapel Hill
Chapel Hill, NC

Dr. Capal is an Associate Professor of Pediatrics and Neurology at the University of North Carolina at Chapel Hill and the Carolina Institute for Developmental Disabilities (CIDD). She is director of the Tuberous Sclerosis Complex Clinic at UNC and the TAND Clinic at the CIDD. She is also Co-Founder and Co-Director of the CIDD Clinical Trials Program. Her clinical and research focus is in neurogenetic conditions resulting in neurodevelopmental disabilities across the lifespan and developing clinical trials to identify, characterize, and develop preventative, disease modifying treatments.

Nola Chambers, PhD
Senior Research Officer
Centre for Autism Research in Africa (CARA), Division of Child and Adolescent Psychiatry, University of Cape Town
Cape Town, South Africa

Dr. Chambers is a speech-language pathologist based in the Centre for Autism Research in Africa (CARA) at the University of Cape Town, South Africa. Her research interests include early childhood development and assessment, early signs of autism, and caregiver-mediated interventions for young children with autism. She has been a member of the TAND Consortium since the start of the TANDem Project in 2019, is the autism-like cluster group leader, and has been part of the Action Group since September 2022. She will be presenting an update on the TANDem Project on behalf of the TAND Consortium.

Nina Corsini, PhD
Research Associate
Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA)
Vienna, Austria

Nina Corsini is a neurobiologist who explores human brain development using stem cell models. Nina received her PhD from the University of Heidelberg working with Ana Martin-Villalba on mouse adult neurogenesis. She performed her post-doctoral work in the lab of Juergen Knoblich at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences in Vienna, Austria working on stem cell differentiation and brain development using human cerebral organoids. Currently, Nina uses cerebral organoid technology to gain insight into the development of childhood epilepsies and pediatric hemispheric tumors with a focus on how human late born interneurons contribute to these pathologies.

Jenny Do, MS, CGC
Genetic Counselor
University of Texas Medical Branch
Houston, TX

Jenny Do is a genetic counselor for the division of Medical Genetics at the University of Texas Medical Branch. She received her Bachelor of Arts from Middlebury College in Middlebury, Vermont. After two years working as a post-baccalaureate research fellow at the National Human Genome Research Institute, she then received her Master of Science in Genetic Counseling from The University of Texas MD Anderson Cancer Center Graduate School of Biomedical Science. Her research interests include TSC, neurogenetics, metabolic disorders, and the psychosocial underpinnings that accompany these topics.

Agnies M. van Eeghen, MD, PhD
Intellectual Disability Physician
Emma Children's Hospital, Amsterdam University Medical Center and Advisium, 's Heeren Loo
Amsterdam, The Netherlands

Agnies van Eeghen is an intellectual disability physician, a novel medical specialty in the Netherlands. She provides clinical care for children and adults with rare genetic neurodevelopmental disorders. Also, she is PI of a research group focussing on neuropsychiatric trajectories, trial design, outcome measures and guideline development in rare disorders including TSC. She takes part in the TANDem consortium and is chair of the Guideline Working group of European Reference Network – ITHACA.

Laura S. Farach, MD
Associate Professor
Division of Medical Genetics, Department of Pediatrics, McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth Houston) and Children's Memorial Hermann Hospital
Houston, TX

Dr. Farach is a medical geneticist and associate professor at UTHealth in Houston with both a clinical and research focus in TSC. A wonderful mentor piqued her interest in TSC during her combined medical genetics and pediatrics residency and she has had a love for the patients and study of TSC ever since. Through her experience seeing patients in the UT- Memorial Hermann TSC Center of Excellence, she witnessed how the knowledge gap regarding the cause of the underlying variable expressivity and resultant difficulty with prognosis prediction negatively impacted her patients/families. Thus, her primary focus is using genetics to help explain the variability of neurological features in TSC. Projects exploring genotype-phenotype correlations have led to discoveries of specific variants that confer milder phenotypes, as well as genotypes that increase risk for developmental delay. She is also evaluating the role of modifier genes as an explanation for the underlying variable expressivity and is using risk prediction models to help uncover some of the milder/moderate effects of these genes. Her work has been funded by the Rare Disease Clinical Research Network Scholars Program, Department of Defense, and TSC Alliance.

Charilaos (Harry) Filippakis, PhD
Assistant Professor
University of New England, College of Osteopathic Medicine
Biddeford, ME

Dr. Filippakis is an Assistant Professor in the Department of Biomedical Sciences at the University of New England, College of Osteopathic Medicine. The Filippakis laboratory aims to understand how mTORC1-hyperactive cells acquire and process nutrients, and how these processes go awry in TSC and LAM. We focus on elucidating the molecular mechanisms that drive nutrient uptake via macropinocytosis, lysosomal metabolism, and autophagy in TSC, integrating metabolomic, proteomic, and transcriptomic methodologies to discover novel therapeutic modalities. Dr. Filippakis has been funded by The LAM Foundation, and the Department of Defense Kidney Cancer Research Program.

Tanjala Gipson, MD
Director, TSC-Associated Neuropsychiatric Disorders Clinic, Le Bonheur Children's Hospital
Associate Professor, Division of Developmental Pediatrics, University of Tennessee Health Science Center
Memphis, TN

Dr. Gipson is an internationally recognized expert in Tuberous Sclerosis Associated Neurodevelopmental Disorders (TAND). In her current position, she is working collaboratively with Le Bonheur Children's Hospital, the Boling Center for Developmental Disabilities, and the University of Tennessee Health Sciences Center to further her clinical and research efforts for this unique population. Her mission is to provide the latest in innovative clinical care for this population; conduct research that will lead to improved treatments and work toward the goal of finding a cure. Although Dr. Gipson is in Memphis, TN, she remains dedicated to the mission of serving families affected by TSC worldwide.

Marina K. Holz, PhD
Dean, Graduate School of Biomedical Sciences
New York Medical College
Valhalla, NY

Dr. Marina K. Holz, Professor of Cell Biology and Anatomy and Dean of the Graduate School of Biomedical Sciences came to NYMC from Stern College of Yeshiva University where she served as the Doris and Dr. Ira Kukin Chair in Biology, chair of the Division of Natural Sciences and Mathematics, and holder of a joint appointment in the Department of Molecular Pharmacology of the Albert Einstein College of Medicine. She received her B.Sc. in microbiology and immunology with Great Distinction from McGill University and completed her Ph.D. in cell and developmental biology at Harvard Medical School. Bringing to NYMC her passion and leadership in biomedical research and education, Dr. Holz leads an NIH-funded laboratory studying the mechanisms of signaling by hormones and growth factors in breast cancer and lymphangioleiomyomatosis (LAM), a rare lung disease. Her work spans basic science and clinical applications and has been published in leading journals. The Holz lab has been previously funded by grants from the American Cancer Society (ACS), LAM Foundation, Wendy Will Case Cancer Fund, American Association for Cancer Research (AACR), Mindlin Foundation, National Cancer Center, and Atol Foundation. Dr. Holz also serves as a peer reviewer for multiple journals and participates in grant review study sections for the NIH, Department of Defense, ACS, The LAM Foundation, and many international funding agencies.

Rebecca 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 with Dr. Laura Attardi, and a postdoctoral Fellowship at the University of California San Francisco with Dr. Arturo Álvarez-Buylla. 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 signaling and fate decisions in 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. Current projects in the group focus on applying these approaches to mouse and iPSC-based models of Tuberous Sclerosis. Previously, the Ihrie 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 lab and collaborators developed a new imaging analysis workflow for highly multiplexed imaging data to classify cellular neighborhoods across patient brain tissue specimens. Dr. Ihrie is a member of the Vanderbilt Brain Institute, Vanderbilt Center for Stem Cell Biology, and the Vanderbilt-Ingram Cancer Center, and holds an adjunct faculty appointment at Meharry Medical College.

Shafali Spurling Jeste, MD
Chief, Division of Neurology
Co-Director, Neurological Institute
Las Madrinas Chair
Professor of Neurology and Pediatrics
Keck School of Medicine of USC
Children's Hospital
Los Angeles, CA

Dr. Jeste is a behavioral child neurologist specializing in autism and related neurodevelopmental disorders. She is Professor of Pediatrics and Neurology at the USC Keck School of Medicine, and the Las Madrinas Chair, Chief of Neurology and Co-Director of the Neurological Institute at CHLA. After earning a BA in philosophy from Yale University in 1997 and her MD from Harvard Medical School in 2002, Dr. Jeste completed a residency in child neurology and a fellowship in behavioral child neurology at Boston Children's Hospital. She joined the faculty at UCLA in 2010, and then moved to CHLA in 2021. Dr. Jeste's research is focused on developing methods to improve precision in the diagnosis and treatment of neurodevelopmental disorders. Her lab studies neurodevelopmental disorders from early infancy through late childhood. Dr. Jeste has designed innovative studies in early predictors of autism in tuberous sclerosis tomplex (TSC) that integrate biomarkers with behavior to define atypical development prior to the onset of autism. This work in TSC has led to the first randomized controlled clinical trial of behavioral intervention for these infants and has paved the way for other early intervention trials in rare genetic syndromes. Dr. Jeste's research is directly inspired by her clinical work. To address the many gaps in medical care for rare genetic forms of neurodevelopmental disorders, she developed the KiNDD (Kids with Neurogenetic and Developmental Disabilities) Clinic at CHLA. Dr. Jeste's work is funded by the National Institutes of Health, the Department of Defense and the Simons Foundation. She holds several national and international leadership positions including the Board of Directors of the National Organization for Rare Disorders and Board of Directors of the TSC Alliance, and she recently served as the Chair of the International Baby Siblings Research Consortium. In 2019 she was awarded the Presidential Early Career Award for Scientists and Engineers for her innovations in research in early predictors and intervention for genetic neurodevelopmental disorders.

Prof. Sergiusz Jóźwiak, MD, PhD
The Children's Memorial Health Institute
Warsaw, Poland

Sergiusz Jóźwiak is a professor at The Children's Memorial Health Institute in Warsaw. He received his Medical Degree from the Medical University Warsaw in 1983 and doctoral degree from the Children's Memorial Health Institute in 1990. Prof. Jóźwiak completed his habilitation at the same institute in 1995 and was appointed Head of the Paediatric Neurology and Epileptology Department in 1997 in the same institution. He held this position until May 2015, when he moved to Warsaw Medical University. In years 2009-2014 he served as a National Consultant in Paediatric Neurology. Prof. Jóźwiak's research focuses mainly on neurocutaneous disorders and epilepsy, especially infantile spasms. For more than 25 years, he has led a special programme for tuberous sclerosis patients and worked out practical guidelines for TSC management. In 2009 Prof. Jóźwiak received the prestigious Manuel Gomez Award established by the TSC Alliance for "creative or pioneering efforts that have appreciably improved either the understanding of the disease or the clinical care available for individuals with tuberous sclerosis." In years 2013-2019 he was a coordinator of the large-scale European Commission Project EPISTOP evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy–tuberous sclerosis complex (www.EPISTOP.eu). Prof. Jóźwiak is an active member of numerous international organisations and has published more than 300 papers in national and international peer reviewed journals. Prof. Jóźwiak is on the editorial boards of several professional journals as Pediatric Neurology (USA), European J.Paediatric Neurology (Amsterdam), Journal of Child Neurology (USA).

Anil Kumar Kalvala, PhD
Postdoctoral Fellow
Texas Tech University Health Sciences Center
Abilene, TX

Currently, Anil Kumar Kalvala, PhD, is working as a postdoctoral research associate at Texas Tech University under the supervision of Dr. Magdalena Karbowniczek. Using small extra cellular vesicles (EVs) obtained from TSC2 null and TSC2 add back patient-derived angiomyolipoma cells, we are currently investigating the molecular pathways involved in the evolution of LAM disease. We standardized EV isolation by employing multiple techniques, such as the Sucrose cushion method combined with ultracentrifugation, and ultrafiltration followed by size exclusion chromatography. To characterize isolated EVs, we have used robust methods consistent with the recommendations made by the International Society for Extracellular Vesicles (ISEV) for doing both functional and fundamental molecular research. EVs derived from TSC-2 null patient derived angiomyolipoma cells have a strong cancerogenic phenotype in promoting low grade cancer; LAM disease, and we are currently validating our research with advanced methodology and making significant efforts to identify specific molecular pathways involving in LAM progression using EV proteomics and RNA sequencing analysis. We anticipate that this study will provide a new treatment target and advance our understanding of the molecular basis for the development of LAM, the etiology of which remains a mystery for decades.

Connie Kasari, PhD
Distinguished Professor of Human Development and Psychology
University of California, Los Angeles
Los Angeles, CA

She received her PhD from the University of North Carolina at Chapel Hill and has been on the faculty at UCLA where she teaches both graduate and undergraduate courses and has been the primary advisor to more than 70 PhD students. She is a founding member of the Center for Autism Research and Treatment at UCLA. Her research aims to development novel, evidence-tested interventions implemented in community settings. Recent projects include targeted treatments for early social communication development in at risk infants, toddlers and preschoolers with autism, and peer relationships for school aged children with autism. She leads several large multi-site studies including a network on interventions for minimally verbal school aged children with ASD, and a network that aims to increase equity in access to interventions for children with ASD who are under-represented in research trials. She is on the board of directors of the International Society of Autism Research.

Joohwan Kim, PhD
Postdoctoral Fellow
University of California, Irvine
Irvine, CA

Joohwan Kim, PhD, is interested in understanding the molecular mechanisms of human diseases. During his PhD at Kangwon National University in Korea, hestudied tumor angiogenesis and microRNA biogenesis. As a post-doc in Gina Lee lab at University of California Irvine (UCI), he is currently studying the pathogenesis and potential treatments for kidney diseases in TSC. His primary focus is exploring RNA modification, an innovative and exciting field that he believes will enhance our understanding of TSC and improve TSC patient care.

Tracy King, MD, MPH
Medical Officer
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
Bethesda, MD

Tracy King is a medical officer in the Intellectual and Developmental Disabilities Branch (IDDB) at the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). She currently oversees the NICHD portfolio on Fragile X syndrome. She is also closely involved with the NIH Rare Disease Clinical Research Network, and her portfolio includes a number of other rare genetic conditions associated with IDDs, including TSC. Dr. King earned a bachelor's degree in biological sciences from Stanford University and a medical degree from Baylor College of Medicine. Following pediatric residency training at the Boston Medical Center and Boston Children's Hospital, she completed a research fellowship in general academic pediatrics and a master's in public health at Johns Hopkins. Prior to joining NICHD in 2015, she was a faculty member in General Pediatrics at Johns Hopkins and conducted research on improving early identification of children with developmental delays in primary care settings.

Renata Lazarova, MD
VP Clinical Development, Pediatric Programs
Noema Pharma AG
Basel, Switzerland

Dr. Lazarova is a pharmaceutical leader with clinical experience as a primary care pediatrician and comprehensive understanding of pharmaceuticals. In her current role at Noema, she is responsible for establishing and implementing strategy for development programs to address medical needs of pediatric population in rare neurological diseases. She is passionate about working with rare disease patient communities to ensure patient insights are shaping the clinical program design, development and its execution. Dr Lazarova leads clinical program to develop mGlu5 receptor antagonist for control of seizures associated with tuberous sclerosis complex.

Gina Lee, PhD
Assistant Professor
University of California Irvine
Irvine, CA

Throughout her scientific career, Gina Lee, PhD, has dedicated herself to investigating the intricate interplay between oncogenic signaling, RNA biogenesis, and metabolic pathways. Her primary focus has centered on studying mTORC1 signaling, the major oncogenic signaling in TSC and LAM tumors. Using proteomic, transcriptomic, and metabolomic analyses of cancer cells, patient samples, and mouse models, we have unveiled the downstream biological processes of oncogenic mTORC1 signaling. Our findings have brought to light the critical role of mTORC1 in the metabolic adaptation of TSC and LAM cells, specifically by enhancing RNA splicing, stability, and translation of vital metabolic enzymes. By uncovering the new connections between signal transduction, cellular metabolism, and RNA processing, she hopes her work will inspire groundbreaking therapeutic approaches for the treatment of human cancers including TSC and LAM.

Fueling the Future

Andy Liu, MD, MS
Assistant Professor in Neurology and Pathology
Director of Biomarker Discover, Department of Neurology
Duke University School of Medicine
Durham, NC

Andy Liu, MD, MS is a clinician-scientist who specializes in behavioral neurology at Duke University School of Medicine, Department of Neurology. Clinically, he evaluates patients who experience cognitive changes which includes adult TSC patients. Further exploring these cognitive changes is a significant research interest of mine. Investigating cognitive changes in adult TSC patients will allow him to link the pathophysiology of a neurodevelopmental disorder and apply it to a neurodegenerative process.

Kate MacDuffie, PhD, MA
Assistant Professor
Treuman Katz Center for Pediatric Bioethics, Seattle Children's Research Institute and University of Washington School of Medicine.
Seattle, WA

Kate MacDuffie, PhD, MA, is an Assistant Professor at the Treuman Katz Center for Pediatric Bioethics at Seattle Children's Research Institute, and in the Division of Bioethics & Palliative Care, Department of Pediatrics, University of Washington School of Medicine. Trained in clinical psychology and bioethics, Dr. MacDuffie's research is focused on understanding the ethical and social impacts of advances in neuroscience on children and adults affected by psychiatric, neurological, and neurodevelopmental disorders.

Katharina Maisel, PhD
Assistant Professor
University of Maryland
College Park, MD

Dr. Maisel obtained her BSE in Materials Science and Engineering from the University of Michigan and PhD in Biomedical Engineering from Johns Hopkins University. She completed her postdoctoral training at the University of Chicago in lymphatic and respiratory immunobiology prior to joining the Fischell Department of Bioengineering at the University of Maryland as faculty in 2019. The Mucosal Associated Immune System Engineering and Lymphatics (MAISEL) Lab's research integrates materials science, immunology, mucosal barrier physiology, and drug delivery to design nanoparticles to take advantage of and study the interface between biological barriers, particularly the lymphatics, interstitial tissue, and mucosal surfaces, and nanoparticles. Dr. Maisel has won a number of awards, including NSF GRFP and NIH F32 fellowships as a trainee, the American Lung Association Dalsemer Award, The LAM Foundation Career Development Award, NSF CAREER Award, and NIH NIGMS Maximizing Investigator Research Award. Her work has led to numerous high-impact publications, particularly in the field of drug delivery and mucosal and lymphatic immunoengineering, and several patents.

Nicole McDonald, PhD
Assistant Professor
UCLA Division of Child and Adolescent Psychiatry
David Geffen School of Medicine at UCLA
University of California, Los Angeles
Los Angeles, CA

Dr. Nicole McDonald is an Assistant Professor in the Division of Child and Adolescent Psychiatry at the David Geffen School of Medicine at UCLA. She is a licensed clinical psychologist who specializes in the early identification of ASD. As an Attending Psychologist at the UCLA Child and Adult Neurodevelopmental (CAN) Clinic, Dr. McDonald conducts ASD-focused evaluations, primarily in young children, and family-based treatment of behavior problems in preschool-aged children (e.g., PCIT), and she oversees the clinic practicum program. Dr. McDonald's research integrates brain-based (fNIRS, EEG) and naturalistic behavioral methods to study early social and emotional development in infants with elevated risk for ASD. She is a member of the Baby Siblings Research Consortium, with much of her past research focusing on infants with familial risk for ASD and, more recently, children with genetic conditions (e.g., TSC). Currently, she has a K23 award from the National Institute of Child Health and Human Development to longitudinally study early brain and social development in high-risk infants who experienced extended NICU hospitalizations. It is Dr. McDonald's eventual goal to apply the information gained from her longitudinal studies and clinical experiences to develop family-based interventions to improve early social development in at-risk infants.

Annelike Müller
Graduate Student
Amsterdam UMC location University of Amsterdam, Department of Pediatrics
Amsterdam, The Netherlands

Annelieke Müller is a clinical researcher in the field of interventions and outcome measures for rare genetic neurodevelopmental disorders. She earned her B.S. in Neuroscience and obtained a Master in Neurobiology at the University of Amsterdam, the Netherlands. She has worked at the Netherlands Brain Bank and performed research at the University of British Columbia, Vancouver, Canada. She is currently finishing her Ph.D. (supervised by Dr. Agnies van Eeghen) at the University of Amsterdam. Her research focuses on innovative and alternative trial designs, such as the N-of-1 design, and (personalized) outcome measures.

Shui-Lin (Stan) Niu, PhD
Program Manager, Congressional Directed Medical Research Programs
U.S. Army Medical Research and Development Command

Dr. Niu currently serves as the Program Manager for the Tuberous Sclerosis Complex Research Program (TSCRP), a federal program that specifically funds TSC research within the Congressionally Directed Medical Research Programs (CDMRP), U.S. Army Medical Research and Development Command. He is responsible for the strategic planning and execution of these programs under the guidance of congressional intent and the vision and mission of the programs. TSCRP received a total appropriation of $113M and funded 205 awards. Prior to managing TSCRP, Dr. Niu has managed a variety of disease programs including autism, bone marrow diseases, neurotrauma, psychological health, and cancer as a Science Officer at CDMRP. Dr. Niu holds multidisciplinary experiences in biomedical research, technology transfer and commercialization, and biomedical research management. Before joining CDMRP, Dr. Niu served as a Technology Transfer Specialist at the National Institutes of Health (NIH), where he managed a portfolio of intellectual properties including diagnostics, devices, therapeutics, and vaccine products. He has spent more than a decade at NIH conducting basic research on GPCR signaling and n-3 fatty acids on brain function. He received several research awards from NIH. Dr. Niu holds a Technology Transfer and Biomedical Development certificate from NIH. He obtained his PhD in Biophysical Chemistry from University of Hawaii under Dr. Randy W. Larsen, where he studied the structure and function of heme proteins. His undergraduate degree was in Organic Chemistry from Tongji University in Shanghai.

Fueling the Future

Tasnim Olatoke
PhD Student
Graduate Research Assistant
University of Cincinnati
Cincinnati, OH

Tasnim Olatoke is a PhD Student in the Systems Biology and Physiology program at the University of Cincinnati where she works on delineating molecular mechanisms underlying Lymphangioleiomyomatosis (LAM) and Tuberous Sclerosis Disease. Her dissertation research focuses on elucidating uterine HOX/PBX/STAT regulatory circuits and their functional impact on LAM pathogenesis.

Ben Philpot, PhD
Kenan Distinguished Professor
Associate Director, UNC Neuroscience Center
Department of Cell Biology & Physiology
University of North Carolina, Chapel Hill
Chapel Hill, NC

Dr. Ben Philpot is a Kenan Distinguished Professor in the Neuroscience Center and Department of Cell Biology & Physiology at the University of North Carolina. He earned his Ph.D. in psychobiology from Dr. Peter Brunjes at the University of Virginia and performed a postdoctoral fellowship in the laboratory of Dr. Mark Bear at Brown University and M.I.T., where he made important contributions to our understanding of experience-dependent brain development. He is currently the Associate Director of the UNC Neuroscience Center and a member of the Carolina Institute for Developmental Disabilities, for which he directs a cross-disciplinary postdoctoral training grant for neurodevelopmental disorders. Dr. Philpot's research seeks to understand the pathophysiology underlying monogenic neurodevelopmental disorders, and he uses this information to develop small molecule and gene therapies to treat these disorders. His research focuses on early-stage development of treatments for Pitt-Hopkins, Dup15q, and Angelman syndromes. Dr. Philpot has made key therapeutic discoveries, including developing an approach to unsilence the epigenetically-repressed paternal UBE3A allele as a novel treatment strategy for Angelman syndrome. Dr. Philpot has >100 peer-reviewed scientific publications. He has advised prominent biotech and pharmaceutical companies, and he serves on the scientific advisory committee for the Angelman Syndrome Foundation. He has won multiple awards, including the NARSAD Young Investigator Award, a Whitehall Foundation fellowship, the Dr. Claudia Benton Award for Scientific Research, an IMPACT award from the Carolina Institute for Developmental Disabilities, and he is currently a SFARI Investigator of the Simons Foundation.

Carmen Priolo, MD, PhD
Assistant Professor of Medicine
Brigham and Women's Hospital
Boston, MA

Carmen Priolo is an Assistant Professor of Medicine in the Division of Pulmonary and Critical Care Medicine at the Brigham and Women's Hospital and Harvard Medical School, and an Associate Member of the Broad Institute of MIT and Harvard. She received her MD from the University of Messina and her PhD from the Catholic University of Rome and completed residency in Medical Oncology at the University of Messina and the Regina Elena Cancer Institute, Rome (Italy). Dr. Priolo has studied cancer cell metabolism for more than 15 years. Her laboratory studies novel molecular mechanisms in the pathogenesis of tuberous sclerosis complex (TSC) and lymphangioleiomyomatosis (LAM). Her goal is to target these mechanisms therapeutically and to test their diagnostic potential through various technologies, including positron emission tomography (PET)-based metabolic imaging. Dr. Priolo is currently leading a [11C]acetate – PET imaging clinical trial in patients with TSC and LAM.

Tracee Ridley-Pryor, DNP, APRN, PMHNP-BC
Psychiatric-Mental Health Nurse Practitioner
Le Bonheur Children's Hospital
Assistant Professor
University of Tennessee Health Science Center
Memphis, TN

Tracee Ridley-Pryor is a doctoral-prepared board-certified Psychiatric-Mental Health Nurse Practitioner at Le Bonheur Children's Hospital and Assistant Professor in the College of Nursing at the University of Tennessee Health Science Center (UTHSC) in Memphis. She also holds a joint appointment as an Instructor in the Department of Psychiatry, College of Medicine also at UTHSC. Her clinical and research interests include attention-deficit and hyperactivity disorders, anxiety, depression, autism spectrum disorder, trauma and stress-related disorders, functional neurological disorders, with an emphasis on psychogenic non-epileptic events (PNEE) and suicide.

Ulrike Rehbein, PhD, MSc, BSc
Post-Doctoral Fellow
University of Innsbruck
Innsbruck, Austria

Dr. Ulrike Rehbein, currently postdoctoral scientist in the lab of Prof. Dr. Kathrin Thedieck in Innsbruck, obtained her dual PhD degree in Biology at the University Medical Center of Groningen (UMCG), the Netherlands and the Carl-von-Ossietzky University Oldenburg, Germany. During her PhD she focused on identifying and characterizing novel molecular mechanisms and interactors regulating the metabolic master regulator MTOR kinase. Now in Innsbruck, she investigates the crosstalk of metabolic signaling and mRNA stability and its implication in diseases such as neurological disorders and cancer with a focus on TSC.

David Ritter, MD, PhD
Assistant Professor of Pediatrics and Neurology
Cincinnati Children's Hospital Medical Center
Cincinnati, OH

David Ritter, MD, PhD, is an Assistant Professor of Pediatrics and Neurology at Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine. He attended Thomas Jefferson University in Philadelphia, PA for his MD and PhD degrees and completed residency in pediatrics and child neurology at Cincinnati Children's. During residency, he was awarded the M. Richard Koenigsberger Award from the Child Neurology Society and a Research Innovation in Support of Excellence Award from Cincinnati Children's. After training, Dr. Ritter joined the Tuberous Sclerosis Clinic at Cincinnati Children's, where he strives to give each patient and family an individualized approach and is researching early phenotypes and treatment in tuberous sclerosis complex. Outside of research and clinic, he likes to spend time with his family, play basketball, run, and garden.

Steven L. Roberds, PhD
Chief Scientific Officer
TSC Alliance
Silver Spring, MD

Steve joined the TSC Alliance staff in 2011 as Chief Scientific Officer. In this role, Steve leads the development and execution of the TSC Alliance's research strategy through partnerships and conversations with all stakeholders, including individuals and families affected by TSC, basic and clinical researchers in academia and industry, healthcare providers, government agencies involved in medical research, and other non-profit organizations. Steve led the implementation of the 2012 TSC Clinical Consensus Conference and an update to the consensus guidelines in 2021. Together with the CEO, CFO, and Board, he developed a Research Business Plan beginning in 2019 to grow the TSC Alliance's research programs, including a Preclinical Consortium to accelerate testing of potential new treatments, a Biosample Repository to collect and share biosamples from individuals enrolled in the TSC Natural History Database, a Clinical Research Consortium, a TSC Learning Healthcare System, Innovation Workshops, conferences, and grants. Steve was awarded his PhD in Pharmacology from Vanderbilt University in 1992, after which he was introduced to rare disease research as a postdoctoral fellow at the University of Iowa studying the biology and genetics of muscular dystrophies. He then began a 16-year research career in the pharmaceutical industry. Immediately prior to joining the TSC Alliance, Steve was an Associate Research Fellow and project leader responsible for driving global project teams toward new human proof-of-concept studies to repurpose Pfizer compounds for new indications.

Isaac Rodriguez-Chavez, PhD, MHS, MS
Independent Consultant
Rockville, MD

Dr. Isaac R. Rodriguez-Chavez is a scientific and regulatory leader with expertise in Rare Diseases, Infectious Diseases, Viral Immunology, Viral Oncology, and Vaccinology. His experience covers the entire life cycle of medical products from basic, preclinical, interventional clinical research (phase I – IV), non-interventional clinical research, and post-marketing studies. He also has expertise in digital health technologies (DHTs), modern clinical research strategy and operations, regulatory affairs, approval or licensure of medical products, and quality control and quality assurance. Currently, he is an independent consultant for clinical research (scientific-, clinical-, regulatory-affairs), and DHTs. Past positions over the last 25 years include: Senior Vice President for Scientific, Clinical Affairs, leading the Strategy of the Global Center of Excellence for decentralized clinical trials (DCTs) and Digital Medicine at PRA Health Sciences and ICON plc; FDA, CDER Senior Officer for Clinical Research Methodology, Regulatory Compliance and Policy Development modernizing clinical research through DCTs and Real World Evidence (RWE) enabled by DHTs and electronic Clinical Outcome Assessments (eCOAs); CEO and Founder, 4Biosolutions Consulting; Vice President, Research, Texas Biomedical Research Institute; Director of HIV Clinical Research Programs at National Institute of Allergy and Infectious Diseases (NIAID) and National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH); Senior Clinical Scientist, Schering Plough Corp. – Merck & Corp.; Scientist, Columbia University; Scientist, Polar Biotechnology Company and Venezuelan Institute for Scientific Research (IVIC). He has a PhD in Virology and Immunology; a MS in Microbiology; a MHS in Clinical Research and Health Sciences; and a B.S. in Biology. He also has 5 years of postdoctoral specialty experience in Clinical Research (phases 1 & 2 trials) linked to AIDS Malignancies, Immuno-Oncology and Viral Immunology (Infectious Diseases) done at the U.S. NIA and NCI, NIH.

Kari Luther Rosbeck
President & Chief Executive Officer
TSC Alliance
Silver Spring, MD

Kari joined the TSC Alliance in June 2001 and became President and CEO in November 2007. She is responsible for the overall management and administration of the organization. This includes strategic planning, implementation of organizational strategies and evaluation of results, in conjunction with the board of directors, to ensure the organization meets its mission to find a cure for tuberous sclerosis complex (TSC) while improving the lives of those affected. Kari has been involved in nonprofit fund-raising and volunteer management for more than 35 years. During Kari's tenure as President and CEO, the TSC Alliance established a comprehensive research program fostering collaboration with industry and academia to move treatments for TSC forward in a more expedited way. The "Unlock the Cure" research strategy focuses on key points along the drug discovery path and has also served as a capital fundraising campaign with more than $24 million raised for TSC research since August 2011. Because of her leadership, the organization has taken an active role in educating the TSC community about clinical trials to diminish the time for recruitment, including pivotal trials that have led to three FDA-approved drugs specifically for TSC. Kari previously served as Executive Vice President, overseeing the national volunteer outreach program and was responsible for fund development. She developed and implemented a vast national network of more than 30 volunteer branches called Community Alliances; increased volunteer participation from 90 individuals to more than 2,000; and through her involvement in special events and major gift fundraising, helped increase the annual revenue by more than 50 percent. Kari graduated with a BA degree in Theatre from the State University of New York at Albany and upon graduation founded a theatre company with fellow graduates in New York, NY. After the loss of her first child, Noell, to sudden infant death, she dedicated her career to helping other families.

Meredith M. Rose, MS, LGC
Genetic Counselor
Mary Bridge Children's Hospital
Tacoma, WA

Meredith completed her master's degree in medical genetics at the University of Cincinnati & Cincinnati Children's Hospital Medical Center (CCHMC) in 2023. During that time, she worked closely with CCHMC's TSC Center of Excellence both by seeing patients clinically and developing her thesis research. She has always had a passion for pediatric genetics, and currently works as a pediatric genetic counselor at Mary Bridge Children's Hospital in Tacoma, WA.

Manoocher Soleimani, MD
Professor, Department of Medicine
University of New Mexico School of Medicine
Albuquerque, NM

Dr. Manoocher Soleimani is a Professor at the Department of Medicine, University of New Mexico School of Medicine. In addition, he is a Senior Clinician Scientist Investigator with the Department of Veterans Health Administration (2022-2030). He is a nephrologist by training and his research focuses on cloning and characterization of acid-base transporting molecules in the kidney and gastrointestinal tract, identification of genes mediating tissue damage in ischemic or toxic injury in kidney and liver, and identification of fructose-absorbing molecules in the small intestine and kidney. His research on TSC focuses on the identification of genes and pathways unique to the kidney and essential to cystogenesis in mouse models of TSC.

Jennifer Srygley Sucre, MD
Assistant Professor of Pediatrics and Cell and Developmental Biology
Vanderbilt University School of Medicine
Nashville, TN

Jennifer Sucre, MD, is an Assistant Professor of Pediatrics and Cell and Developmental Biology at Vanderbilt University School of Medicine. She graduated with degrees in creative writing and genetics from the University of Georgia, graduated from Harvard Medical School, trained in pediatrics at Washington University in St. Louis, and completed fellowship in Neonatal-Perinatal Medicine at UCLA. Since joining the Vanderbilt faculty in 2016, she has established a research program focused on understanding the molecular mechanisms of normal lung development and lung disease with a particular focus on bronchopulmonary dysplasia, the leading complication in survivors of preterm birth. Her clinical experience treating premature infants provides a unique perspective for studying lung development, and she has cultivated new ex vivo, in vitro, and in vivo models of lung injury. Dr. Sucre has combined these models with single-cell biology, spatial transcriptomics, and 4-dimensional live imaging to gain paradigm-shifting insights into cellular specialization and dynamics in the developing lung, elucidate age-regulated host susceptibility factors to infection, and define previously unrecognized cell states in chronic respiratory diseases. Her research group integrates cell biology, informatics, and large human datasets with mathematical modeling to study cellular behavior during organogenesis and how early life lung injury disrupts development, with a goal of harnessing the mechanisms of normal lung development to promote lung regeneration after injury across the lifespan.

Elizabeth Thiele, MD, PhD
Physician Investigator, Interdisciplinary Brain Center, Mass General Research Institute
Professor of Neurology, Harvard Medical School
Neurologist, Neurology, Massachusetts General Hospital
Pediatrician, Pediatric Neurology, Massachusetts General Hospital
Cambridge, MA

Dr. Elizabeth A. Thiele is a neurologist and epileptologist at Massachusetts General Hospital. She received her medical training at Johns Hopkins University School of Medicine in Baltimore, Maryland, and completed an internship and residency in pediatrics at the Johns Hopkins Hospital. She completed a second residency in child neurology and a postdoctoral research fellowship in neurology at Children's Hospital in Boston. Dr. Thiele organized and established the Herscot Center for Tuberous Sclerosis Complex, a multidisciplinary comprehensive clinical program for TSC, as well as a ketogenic diet clinic to treat and manage patients with epilepsy. She is also the Director of the Pediatric Epilepsy Service at Mass General and a Professor in Neurology at Harvard Medical School. Dr. Thiele's research and clinical interests include the role of diet in epilepsy treatment, genotype-phenotype correlation in TSC, the role of epilepsy surgery in management of intractable epilepsy, outcomes following infantile spasms, and neuropsychological profiles in relationship to tuber number and location in TSC.

Daniel Vogt, PhD
Assistant Professor
Michigan State University
Grand Rapids, MI

Dr. Vogt attained his PhD in Neuroscience at Case Western Reserve University and then pursued a postdoc at the University of California San Francisco. Dr. Vogt joined Michigan State University as an Assistant Professor in 2017 and started an independent lab to investigate the molecular and cellular mechanisms underlying rare neurological syndromes, with a particular interest in understanding how mutations in these syndromic genes lead to neuropsychiatric symptoms. Some of these syndromes regulate cellular signaling events, including the TSC1&2 genes that underlie TSC. Dr. Vogt's lab is currently investigating how cellular signaling events are involved in normal brain development and the impacts of syndromic gene mutants on these processes.

Uchenna John Unachukwu, PhD
Associate Research Scientist
Columbia University Medical Center
New York, NY

Uchenna John Unachukwu, PhD is an early-stage investigator studying molecular mechanisms underlying disease development and stem cell mediated-tissue repair in the mammalian lung and other pathology-specific organs. His research specialty is in understanding biochemical signaling driving neoplastic growth in lymphangioleiomyomatosis (LAM), and how they can be modulated to accelerate the development of effective therapies.

Oded Volovelsky, MD, PhD
Director of Pediatric Nephrology Unit
Chairman, Israeli Society of Pediatric Nephrology
Hadassah Hebrew University Medical Center
Jerusalem, Israel

Oded Volovelsky, MD, PhD, is the Israeli Pediatric Nephrology Society chairman and the TSC multidisciplinary clinic co-director at Hadassah Hebrew University Medical Center. He leads a research lab looking for ways to prevent kidney disease in TSC at its early stages.

Howard Weiner, MD
Chief of Neurosurgery
George A. Peterkin Jr. Endowed Chair in Neurosurgery
Texas Children's Hospital
Professor and Vice Chair of Neurosurgery
Baylor College of Medicine
Houston, TX

Howard L. Weiner, MD, is Chief of Neurosurgery and the George A. Peterkin Jr. Endowed Chair in Neurosurgery at Texas Children's Hospital (TCH), where he leads a team of 10 pediatric neurosurgeons, the largest group of its kind in the nation, and is Professor (with Tenure) and Vice Chair of Neurosurgery at Baylor College of Medicine (BCM) in Houston, Texas. TCH neurosurgery/neurology is ranked 2nd nationally among all Children's Hospitals by the US News and World Report. Prior to this, he was Professor of Neurosurgery at NYU Langone Medical Center, where he worked for 27 years. A graduate of the Ramaz School in Manhattan, he received his BA from the University of Pennsylvania and MD from Cornell University Medical College, graduating from both with highest academic standing in his class. He completed neurosurgery residency and pediatric neurosurgery fellowship at NYU and was a research fellow in the labs of Ed Ziff, PhD, at NYU and Nicole Le Douarin, PhD, in Paris. He conducted basic brain tumor research and was awarded an NIH grant. Howard has a national and international surgical practice in pediatric epilepsy and brain tumor surgery, has served on the editorial board of several leading medical journals, and is a widely sought-after mentor and academic leader. He is considered an expert in tuberous sclerosis complex and has worked consistently over the last 25 years to advance the care and scientific knowledge for these children. Recognized consistently in "Best Doctors" lists, Dr. Weiner has been invited to speak at numerous national and international meetings and academic medical centers and has been a Visiting Professor both in the US and abroad. Patients are drawn from around the world not only to his novel neurosurgical treatment approaches, but also to his warm, caring, down-to-earth, and highly attentive bedside manner. He has worked intensely over the years to impart this holistic philosophy of "hospitality" in pediatric neurological surgery to the many residents, fellows, and students whom he has mentored.

Michael Wong, MD, PhD
Allen P. and Josephine B. Green Professor of Pediatric Neurology; Professor of Neurology, Pediatrics, and Neuroscience
Washington University School of Medicine
St. Louis, MO

Michael Wong, MD, PhD, is the Allen P. and Josephine B. Green Professor of Pediatric Neurology at Washington University School of Medicine. He received a B.A. in Biology from Princeton University and MD and PhD (Neuroscience) from University of Texas Southwestern Medical School. He is a board-certified pediatric epileptologist and clinical neurophysiologist. He is also director of the Washington University Tuberous Sclerosis Center of Excellence, which provides multidisciplinary care for children and adults with tuberous sclerosis complex and conducts both basic science and clinical research in TSC. His laboratory investigates mechanisms of epileptogenesis and seizure-induced brain injury in epilepsy. His lab helped establish the role of the mechanistic target of rapamycin (mTOR) pathway in epileptogenesis in TSC, as well as in animal models of acquired epilepsy due to brain injury. As a translational application of his basic research, he has served as a site principal investigator for a number of clinical trials, such as the EXIST-3 trial of the mTOR inhibitor everolimus for epilepsy in TSC and the PREVeNT trial for preventing neurological manifestations of TSC. He is past recipient of the American Academy of Neurology Dreifuss-Penry Epilepsy Research Award and is Chief Editor of Epilepsy Currents. His lab has been funded continuously by NIH and other sources for 20 years and has published more than 100 research papers and reviews on epilepsy and TSC.

Jane Yu, PhD
Professor
University of Cincinnati College of Medicine
Cincinnati, OH

Dr. Yu leads a research laboratory focusing on investigation of the role of tumor suppressor proteins tuberin (TSC2) and hamartin (TSC1) in steroid action, cell survival, cellular metabolism, tumorigenesis and metastasis, and signaling transduction pathways in the progression of TSC and LAM. Her laboratory also develops animal models to test the efficacy of FDA-approved drugs on the progression and metastasis of mTORC1 hyperactive cells, lung inflammation and lung remodeling.

Ziyang Zhang, PhD
Assistant Professor of Chemistry and Chemical Biology
University of California, Berkeley
Berkeley, CA

Ziyang graduated from Peking University with a BSc in Chemistry in 2011 where he studied natural product synthesis with Profs. Zhen Yang, Jiahua Chen and Yefeng Tang. As an HHMI Predoctoral Fellow, he did his PhD training with Prof. Andrew Myers at Harvard University and developed a platform for the synthesis of new macrolide antibiotics. He then joined Prof. Kevan Shokat's lab at UCSF as a Damon Runyon postdoc fellow, where he devised chemical strategies for the brain-specific inhibition of kinases and the mutant-specific targeting of KRAS-driven cancer. He started his independent research at UC Berkeley in 2022. His lab creates chemical tools to tweak our immune system and enable new therapeutic mechanisms for cancer and autoimmune diseases. .

Plenary Session 1 Abstracts: Chemical and Cell Biology

Intranasal adjuvant immunotherapy to treat TSC-associated LAM tumors

Katharina Maisel

University of Maryland, College Park, MD

Lymphangioleiomyomatosis (LAM) is cystic lung disease affecting primarily women of reproductive age, and approximately 50% of women with TSC will develop LAM. Currently there is no cure for this disease and only modest progress has been made with the introduction of rapamycin as LAM treatment, but close to 40% of patients only have partial or no response to treatment. Recently, we and others have identified that LAM causes immunosuppression, and that checkpoint inhibitor treatment can increase survival in mouse models of LAM. Here we investigated the use of toll-like receptor (TLR) agonists, a class of immune adjuvants, as a treatment for LAM. We found that the TLR 9 agonist CpG increased median survival from 33 to 60 days in a mouse model of LAM. This was accompanied with a >50% reduction in the number of LAM nodules and a significant increase in activated T cell infiltration in the lungs. We have found that these effects are at least in part mediate by plasmacytoid dendritic cells (pDCs), as pDC depletion results in reduced survival of mice with LAM as well as mice with LAM treated with CpG. We found that CpG treatment reduced regulatory T cell infiltration in LAM lungs, suggesting that this treatment successfully increases anti-LAM immunity. We also found that CpG treatment increases survival at early and late stage disease in mice, with late stage disease survival increasing a more moderate 25% from 32 to 40 days. CpG has been successfully combined with checkpoint inhibitor immunotherapies, and here we found that CpG is synergistic with checkpoint inhibitor anti-PD1 therapy in LAM, leading to an additional ~20% increase in survival compared to each treatment alone. Finally, we have exciting new data demonstrating that rapamycin and CpG are also synergistic and increase survival in a mouse model of LAM. In summary, our data suggest that CpG could be a new treatment strategy for LAM, as it increases the anti-LAM immune response. This treatment could be combined with standard of care rapamycin treatment to synergistically increase patient responses and improve therapeutic outcomes in LAM.

Tryptophan-mediated macropinocytosis is a metabolic vulnerability in tuberous sclerosis complex

Sarah Lafleur, Windrie Cox, Elizabeth P. Henske, Charilaos Filippakis

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Tuberous sclerosis complex (TSC) is an autosomal dominant disease that affects multiple organ systems, including the kidney, brain, heart, skin, and lung. In the lung, TSC can manifest as Lymphangioleiomyomatosis (LAM), a progressive lung disease resulting in destruction of the lung parenchyma. In TSC and LAM, biallelic loss of *TSC1/2* leads to constitutive activation of the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is active on the lysosome and has a profound impact on cellular metabolism via several inter-connected mechanisms, including enhanced glucose and glutamine utilization, nucleic acid, protein and lipid synthesis. *TSC2*-deficient cells have increased macropinocytosis, an actin-dependent endocytic process that facilitates uptake of extracellular material and processing at the lysosome. L-Tryptophan (Trp) is an essential amino acid that plays a critical role in maintaining cellular metabolism and growth. We found that Trp stimulates macropinocytosis ~2.5-fold ($p<0.0001$) in *TSC2*-deficient cells, but not *TSC2*-expressing cells. Interestingly, Trp increased the proliferation of *TSC2*-deficient cells by 7-fold ($p<0.0001$), compared to *TSC2*-expressing cells (2.5-fold). Over 95% of Trp is metabolized via the kynurenine (Kyn) pathway, which is regulated by the rate-limiting enzymes indoleamine-2, 3-dioxygenase (IDO1), IDO2 and tryptophan-2, 3-dioxygenase 2 (TDO2). Targeting IDO1 (Linrodostat; 10uM) or TDO2 (LM10; 10uM) selectively inhibited the proliferation of TSC2-deficient cells by ~60% ($p<0.0001$). Trp-derived Kyn binds to the transcription factor aryl hydrocarbon receptor (AhR), which then translocates to the nucleus to regulate cellular metabolism and growth. In *TSC2*-deficient cells, *AhR*, *Ido1*, and *Tdo2* gene expression is increased 50-fold ($p<0.01$), 7-fold ($p<0.01$), and 45-fold ($p<0.0001$) respectively, compared to *TSC2*-expressing cells, and was sensitive to rapamycin treatment (20nM; 24h). Furthermore, *AhR* was localized in the nucleus of *TSC2*-deficient cells, while inhibition using SR-1 (2uM) or CH223191 (5uM) blocked macropinocytosis and selectively inhibited the proliferation of *TSC2*-deficient cells (70%; $p<0.0001$). Collectively, our data indicate that Trp and Kyn are key regulators of macropinocytosis, metabolic reprogramming and cell growth in TSC. Targeting macropinocytosis by blocking the kynurenine pathway may represent a novel therapeutic approach for the treatment of TSC and LAM.

mTORC1-dependent RNA methylation confers rapamycin resistance in TSC tumors

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Tuberous sclerosis complex (TSC) is a genetic tumor syndrome that causes the formation of benign tumors in multiple organs. While rapamycin is the most potent therapy for TSC, rapamycin faces several clinical challenges such as drug resistance and tumor regrowth. One of the suggested mechanisms for the limited efficacy of rapamycin is the feedback activation of pro-growth signaling such as PI3K, RAS, and ERK, through protein phosphorylation-dependent mechanisms. In this study, we identified RNA methylation of MAPK13 as a new mechanism limiting rapamycin's tumor suppressive effects. N6-adenosine methylation (m6A) is the most abundant mRNA modification that controls gene expression through diverse mechanisms. Accordingly, m6A-dependent regulation of oncogenes and tumor suppressors contribute to tumor development. However, the role of m6A-dependent gene regulation upon drug treatment or resistance is poorly understood. Here, we investigated m6A RNA methylation-mediated rapamycin resistance mechanisms in TSC patient-derived cell lines, LAM 621-101 and UMB1949. We found that m6A modification of mitogen-activated protein kinase 13 (MAPK13) mRNA determines the sensitivity of TSC cells to rapamycin. Mechanistically, the mammalian target of rapamycin complex 1 (mTORC1) induces m6A modification of MAPK13 mRNA at its 3' untranslated region (3'UTR) through the methyltransferase-like 3 (METTL3)-METTL14-Wilms' tumor 1-associating protein (WTAP) methyltransferase complex, facilitating its mRNA degradation via m6A reader protein YTH domain family protein 2 (YTHDF2). Rapamycin treatment blunts this process, which stabilizes and increases MAPK13 expression. On the other hand, inhibition of MAPK13 by genetic knockdown or chemical inhibitor on top of rapamycin treatment synergistically suppressed cell growth and proliferation of LAM 621-101 and UMB1949 cells. Together, our data indicate that rapamycin-mediated MAPK13 mRNA stabilization underlies rapamycin resistance and limits its efficacy, and MAPK13 should be considered as a promising co-therapeutic target to sensitize TSC tumors to rapamycin therapy.

TFEB drives mTORC1 hyperactivation and kidney disease in tuberous sclerosis complex

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Hyperactivation of mechanistic target of rapamycin complex 1 (mTORC1) is believed to play a crucial role in tumor formation in TSC. mTORC1 phosphorylates the Transcription Factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy, at Ser211. In Alesi et al. Nature Communications, 2021 we found that lysosomal biogenesis is increased in pulmonary LAM, renal tumors, kidneys from *Tsc2+/-* mice, and *TSC1/2*-deficient cells via a TFEB-dependent mechanism. In *TSC1/2*-deficient cells, TFEB is paradoxically hypo-phosphorylated at the mTORC1-dependent site S211, resulting in nuclear localization and hyperactivation. In unpublished work, we found that TFEB is the primary driver of kidney tumorigenesis in two novel mouse models of TSC. Specifically, we developed a mouse model in which TFEB is knocked out in the kidney (KspCre; $Tsc2^{fl/fl}$), resulting in cystic kidney disease and a 10-fold increase in kidneys/body weight ratio. Double knockout of *TSC2* and TFEB (KspCre; $Tsc2^{fl/fl}$ $Tfeb^{fl/fl}$) completely abolished kidney cysts, normalized the kidney/body weight ratio, renal function, and extended lifespan. We also studied an inducible total body model of TSC (Cagg-CreERT2; $Tsc2^{fl/fl}$) in which Tamoxifen is administered at day P1-3. These mice have decreased body size (~50%) and cystic kidneys. TFEB knockdown (CreERT2; $Tsc2^{fl/fl}$ $Tfeb^{fl/fl}$) normalized body weight and eliminated the cystic renal disease. RNA sequencing of the *Tsc2*-deficient mouse kidneys revealed that the TFEB-dependent upregulated genes (35%) are also Rapamycin-dependent, indicating, for the first time, that the benefit of Rapamycin in TSC may be directly mediated by the relocalization of TFEB from nucleus to cytoplasm. In *TSC2*-null HeLa and HEK293T cells inhibition of mTORC1 Rapamycin or Rheb knockdown resulted in a paradoxical increase (3-fold) phosphorylation of TFEB at the mTORC1 site and relocalization from the nucleus to the cytoplasm. This non-canonical effect of Rapamycin on TFEB was confirmed in the mouse models. Moreover, we found that mTORC1 hyperactivation (measured as the ratio of p4EBP1 over total 4EBP1, and pS6k over total S6K) in *TSC2*-deficient kidneys was decreased by TFEB knockdown and in vitro, in HeLa cells with *TSC2* knockout and angiomyolipoma derived *TSC2*-deficient 621-102 cells. In addition to revealing a novel and unexpected mechanism through which TFEB upregulates mTORC1 activity in TSC, these data identify TFEB inhibition as a therapeutic strategy for TSC.

A precision dosing strategy for overcoming challenges to the dosing of mTOR Inhibitors in TSC infants in clinical practice and clinical trials

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Rationale: Conventional dosing of sirolimus and everolimus in TSC infants is primarily by "trial and error", extrapolated from studies conducted in older children and adults. The exclusion of infants from most clinical trials in TSC has perpetuated this uncertainty. Here we compare sirolimus and/or everolimus dosed conventionally vs. a novel PK-based precision dosing protocol in TSC infants less than 2 years of age.

Methods: TSC infants less than 2 years with clinical indication for treatment with mTOR inhibitor (everolimus n=9; sirolimus n=4) dosed conventionally between 2015-2022 were followed prospectively to collect additional dosing metrics, safety labs, side effects, and clinical outcomes (Early mTOR). TSC infants treated with sirolimus up to 24 months of age enrolled into the Stopping TSC Onset and Progression 2 (STOP2A) clinical trial between 2020-2022 were dosed following a PK-guided model with Bayesian adaptive control methodology (n=5). Primary outcomes were time to achievement of target blood trough level (5-15 ng/ml) and the ability to maintain blood trough levels within the same range throughout the remainder of the treatment period.

Results: Starting age of TSC infants dosed conventionally ranged from age 2-18 months of age (mean 8.6 +/- SD 5.3) with dosing that ranged from 0.47-1.92 mg/m2/day for sirolimus and 1.16-4.88 mg/m2/day for everolimus. Dosing frequency also was highly variable, ranging from 2x/day to as little as 3x/week. By comparison, TSC infants with precision dosing began sirolimus treatment between 1-4 months of age (mean 2.0 +/- 1.2) with a starting dose ranging from 0.50-0.90 mg/m2/day, all dosed at a frequency of 2x/day. 7 of 13 infants conventionally dosed reached blood trough levels within the target range, taking an average time of 63.6 +/- 52.0 days to reach target and maintaining levels within the target range 53% of the time. 5 of 5 with precision dosing reached target trough levels, all within 7-14 days (average 9.8 +/- 3.8 days) and maintained levels within the target range 94% of time.

Conclusions: Precision dosing of mTOR inhibitors, especially sirolimus, is a feasible means for improving the time to achieving and subsequently maintaining target blood trough levels in TSC infants. The STOP2B: TSC-STEPS clinical trial continues to evaluate and refine this precision dosing methodology and develop tools for facilitating its use in clinical practice and future clinical trials.

A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression

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Tuberous sclerosis complex (TSC) is caused by mechanistic target of rapamycin complex 1 (mTORC1) hyperactivity associated with disease phenotypes related to cellular overgrowth, migration, and neuronal excitability. TSC results from heterozygous loss of function variants in the *TSC1* and *TSC2* genes that encode components of the TSC protein complex. In healthy cells, the TSC complex acts as a relay for nutritional signals to suppress mTORC1, at mTORC1's central signaling platform, the lysosomes. We report that Ras GTPase-activating protein-binding proteins (G3BPs) act as a lysosomal tether of the TSC complex. G3BPs are primarily recognized as RNA-binding proteins that constitute core components of stress granules (SGs), and only a few SG-independent functions have been reported. They act in a non-redundant manner to anchor the TSC complex to lysosomes and hence, suppress mTORC1 activation. Using biochemical approaches and in silico protein interaction predictions we find that the G3BP-TSC axis mediates metabolic signals to mTORC1, suggesting a novel mode of TSC-mediated nutrient sensing. Like in TSC, G3BP1 deficiency elicits phenotypes related to mTORC1 hyperactivity. In the context of neuronal excitability G3bp1 inhibition in zebrafish disturbs neuronal development and function, leading to white matter heterotopia and neuronal hyperactivity. Furthermore, low G3BP1 levels enhance mTORC1-driven breast cancer cell motility and correlate with adverse outcomes in patients. The G3BP1-TSC tumor suppressor axis is highly preserved in breast cancer patients. Therefore, targeting this axis could increase the efficacy of current therapies. Thus, G3BPs are not only core components of SGs but also a key element of lysosomal TSC-mTORC1 signaling (PMID: 33497611). In the presentation we will give an update on metabolic signals and mechanisms that are being transduced through the G3BP-TSC axis. We will also present data on the clinical impact of our findings in TSC and breast cancer. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 754688 (MESI-STRAT) and is co-funded by the European Union (ERC, BEYOND STRESS, 101054429). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

TSC-null extracellular vesicles facilitate metastable phenotypes of LAM cells and formation of lung metastatic niche

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Lymphangioleiomyomatosis (LAM) is a low-grade cancer of smooth muscle cells that primarily affects reproductive-aged women. The mTOR inhibitors, Rapamycin, and its analogs (Everolimus), have been approved by FDA for LAM therapy. Although research shows that this treatment improves a patient's lung function, discontinuing treatment has led to a progressive loss in some patient's lung function, indicating the need for discovery of new therapeutic targets. Exosomes (EVs) are small extracellular vesicles (50-150 nm) with a lipid bilayer membrane that are secreted by all cell types and thought to have a multi vesicular endosomal origin. It is well established that EVs mediate immune responses, reprogramme stromal cells, remodel the extracellular matrix, and normalize cell phenotypes by transferring bioactive molecules between cancer and various cells in local and distant microenvironments. We previously reported that EVs derived from *TSC1*–null neuronal progenitors block differentiation of recipient wild-type progenitors via activation of the Notch1/mTOR pathways, phenocopying *TSC1*-null cells. Despite the growing interest in the role of EVs in modifying cancer growth, their impact on LAM progression remains largely unknown. Therefore, in this study, we aimed to compare the impact of EVs from *TSC2* null LAM patient-derived cells (621-101) with EVs isolated from *TSC2* add-back cells (621-103) on LAM progression. EVs derived from 621-101 cells demonstrated increased expression of Integrin-beta1, Integrin-alpha6, proto-oncogene c-Src, focal adhesion kinase, SRY-related HMG-box-10, Rheb, CD-44, Rab27b, ALIX, flotillin-1 and flotillin-2 compared to 621-103 EVs. The treatment of 621-101 cells with *TSC*-null EVs increases (1) F-actin polymerization, (2) migration, and (3) cancer stem cell-like phenotypes of these cells, including sphere forming ability, anoikis resistance and ALDH activity when compared to treatment with *TSC2*-add-back EVs. The blockade of EV uptake or biogenesis using Dyngo-4a or Tipifarnib, respectively reduced sphere size. In vivo, intravenous injection of *TSC*-null EVs to NCG mice led to higher seeding and retention of luciferase expressing 621-101 cells in the lungs by live imaging, as well as an increase in collagen deposition in this organ based on Masson trichrome staining. This data suggests that EVs from *TSC*-null cells may play a pivotal role in the lung extracellular matrix remodeling and, therefore, lung seeding by these cells.

Plenary Session 2 Abstracts: Biomarkers and Ethics in Early Intervention

Early vocal development and autism in TSC

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Rationale: Autism in TSC has been reported in up to 50-60%. Language deficits impact as many as 70% of those with TSC. However, much of the research has focused on autism, but very little is known about vocal development in this population. One way to assess early vocal development is through measuring volubility (number of syllables used by an infant per minute), canonical babbling (the number of consonant-vowel combinations, eg 'ba') and the canonical babbling ratio (CBR, i.e., canonical syllables/total syllables) within the first year. The threshold for entry into the canonical stage has been reported to be greater than or equal to 0.15. Speech and language delays have been reported in children with a CBR lower than this and those who were delayed in achieving this milestone. The purpose of the current study is to compare early vocal development in infants with TSC with and without autism at 12 months of age.

Methods: Audio-video recordings of infants with TSC engaged in developmental testing were shared by the TACERN (TSC Autism Centers of Excellence Research Network). Seventy-four of these recordings from 40 infants were randomly selected and analyzed by human observers using a software-based coding environment to determine volubility, canonical babbling, and the canonical babbling ratio (CBR). We then compared these measures of early vocal development in infants with and without autism at age 12 months.

Results: There were 6 infants with autism and 30 without autism. Autism data was not reported for the remaining 4 infants. For infants without autism, mean volubility (syllables per minute) was 4.10 (SD 2.14), and the mean canonical babbling ratio was 0.12 (SD 0.15). For infants with autism, mean volubility (syllables per minute) was 3.94 (SD 2.49), and the mean canonical babbling ratio was 0.12 (SD 0.14). The differences between the groups were not significant.

Conclusions: The findings suggest delays in early vocal development in children with TSC with and without autism and warrant further follow up. Our ongoing prospective study Baby Talk is focused on characterizing early vocal development in infants with TSC starting at 9 months of age and comparing it to other early measures of language in terms of predicting language and developmental outcomes at later stages.

Parental stress in tuberous sclerosis complex

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Background: Tuberous sclerosis complex (TSC) is a multi-systemic genetic disorder with great clinical variability. As the needs of one child with TSC may vastly differ from another, parenting demands may similarly differ. Characterizing parental stress, or emotional maladaptation arising from parenting duties, can enable healthcare providers to efficiently assist parents of children with TSC. This may ultimately improve both parent and child health outcomes.

Methods: This study surveyed 269 parents of children (aged 0-12 years) with TSC and received the following information: children's TSC clinical features, parent demographics, and a Parent Stress Index (PSI) score.

Results: Parents reported higher stress levels for children with certain skin and ocular TSC features, intractable epilepsy with or without status epilepticus, low adaptive functioning, TSC-Associated Neuropsychiatric Disorders (TAND), and parent race and income. These variables accounted for 69% of variability in parent's PSI scores. Overall, 50% of parents achieved a clinically relevant PSI. Thematic analyses identified stressors consistent with survey findings and noted that parents face uncertainty and a lack of personal or healthcare support as additional stressors.

Conclusion: Utilizing this data to improve parent's healthcare experience can be achieved in multiple methods: improving coordination between counseling and school services with a focus on parent-child interactions, assessing barriers to healthcare or accessing early childhood intervention, and providing psychosocial assessment to all parents with a low threshold for referral to a mental health specialist. These additional considerations may efficiently ameliorate parental stress and ultimately improve quality of life for families and patients with TSC.

Plenary Session 3 Abstracts: TAND (TSC-Associated Neuropsychiatric Disorders) and Epilepsy Research

Behavioral outcomes of treatment with cannabidiol oral solution in individuals with seizures associated with tuberous sclerosis complex: design of an ongoing phase 4 trial (EpiCom)

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Background: There are limited treatments for tuberous sclerosis complex (TSC)–associated neuropsychiatric disorders (TAND), which affect ~90% of individuals with TSC. Cannabidiol (CBD; Epidiolex/Epidyolex) treatment reduced TSC-associated seizure frequency and improved patients' overall clinical condition in trials. Anecdotal reports in individuals with TSC, and studies in other rare neurogenetic disorders suggest positive effect on behavioral manifestations such as irritability. EpiCom is a multicenter, open-label, phase 4 study designed in collaboration with patient advisory groups and healthcare professionals to evaluate behavioral outcomes associated with add-on CBD treatment in individuals with TSC-associated seizures.

Methods: Participants (aged 1–65 years) starting CBD treatment for seizures and with moderate/severe behavioral challenges on Caregiver Global Impression of Severity scale are eligible. After screening, participants will receive CBD (up to 25 mg/kg/d based on individual response and tolerability) for 26 weeks. Following this 26-week treatment period, participants will have the option to continue CBD with standard of care for up to 52 weeks. Key behavioral efficacy endpoints include a change from baseline on the Aberrant Behavior Checklist (eg, irritability subscale) and the most problematic behavior on the TAND-Self-Report, Quantified Checklist (TAND-SQ). Changes in executive function, sleep, quality of life, family functioning, seizure outcomes (symptom severity, retention rate, responder rates, seizure-free days), and safety will also be evaluated.

Results: The trial will enroll ~75 participants at ~20 sites across the US, the UK, The Netherlands, Canada, Israel, and Poland.

Conclusions: The EpiCom study will describe changes in neuropsychiatric outcomes in individuals with TSC who experience seizures and are using CBD. This may inform future studies evaluating potential pharmacotherapy for behavioral outcomes in TSC and similar populations. FUNDING Jazz Pharmaceuticals, Inc.

Cerebral vascular and blood brain-barrier abnormalities in a mouse model of epilepsy and tuberous sclerosis complex

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Background: Tuberous sclerosis complex (TSC) is a genetic disorder, characterized by tumor formation in the brain and other organs, and severe neurological symptoms, such as epilepsy. Abnormal vascular endothelial growth factor (VEGF) expression may promote angiogenesis in kidney and lung tumors in TSC and has been identified in brain specimens from TSC patients, but the role of VEGF and vascular abnormalities in neurological manifestations of TSC is poorly defined. We investigated abnormalities in brain VEGF expression, cerebral blood vessel anatomy, and blood-brain barrier (BBB) structure and function and the role of these abnormalities in epileptogenesis in a mouse model of TSC.

Methods: *Tsc1GFAPCKO* mice were used to investigate VEGF expression and vascular abnormalities in the brain by western blotting and immunohistochemical analysis of vascular and BBB markers. In vivo two-photon imaging was used to assess BBB permeability to normally impenetrable fluorescently-labeled compounds. The effect of mechanistic target of rapamycin (mTOR) pathway inhibitors, VEGF receptor antagonists, or BBB modulators were assessed in some of these assays, as well as on seizures by video-EEG.

Results: VEGF expression was elevated in cortex of *Tsc1GFAPCKO* mice, which was reversed by the mTOR inhibitor, rapamycin. Tsc1GFAPCKO mice exhibited increased cerebral angiogenesis and vascular complexity in cortex and hippocampus, which were reversed by the VEGF receptor antagonist, apatinib. BBB permeability was abnormally increased and BBB-related tight junction proteins, occludin and claudin-5, were decreased in *Tsc1GFAPCKO* mice, also in an apatinib-dependent manner. A BBB modulator (RepSox), but not the VEGF antagonist, decreased seizures in *Tsc1GFAPCKO* mice.

Conclusions: Increased brain VEGF expression is dependent on mTOR pathway activation and promotes cerebral vascular abnormalities and increased BBB permeability in a mouse model of TSC. BBB modulation may affect epileptogenesis in TSC, but functional consequences of these vascular abnormalities require further investigation.

Plenary Session 4 Abstracts: Organoids and Cell Development

STAT1 promotes survival and lung metastasis of TSC2-null cells in lymphangioleiomyomatosis (LAM)

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Lymphangioleiomyomatosis (LAM) is a rare lung disease that predominantly affects premenopausal women, with an estimated global prevalence of 3-8 per million women. LAM is caused by infiltration of metastasizing atypical smooth muscle-like cells into the lungs, leading to progressive respiratory failure. Though the origin of these cells remains elusive, LAM cells carry loss-of-function mutations in the Tuberous sclerosis complex (*TSC1/TSC2*) genes, leading to hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1) and aberrant cell growth. There is no cure for LAM. The greatest challenge to finding a cure for LAM is its undetermined mechanism. A deeper understanding of network pathways dysregulated in LAM is crucial to finding a cure. Our integrative single cell omics analyses of LAM lung identified the activation of *HOX/PBX/STAT* transcriptional network in LAMCORE cells compared to other lung cells. We confirmed *HOX/PBX/STAT* activation in LAM tissues. Our network prediction analysis showed that *STAT1* and *STAT3* are PBX1 targets, and work with PBX1 to promote LAM cell survival. STAT proteins are involved in several pathways involved in cell proliferation and apoptosis; thus, their dysregulation is implicated in tumorigenesis. The objective of this study is to delineate cellular and molecular mechanisms by which STAT signaling may regulate LAM pathogenesis, with hopes to find potential therapeutic options for LAM and TSC-associated disorders. We hypothesize that *STAT1* suppression attenuates LAM patient-derived (*TSC2*-null) cell viability and lung metastasis in LAM. We first confirmed our network prediction analysis by showing that aberrant PBX1 activation upregulate *STAT1* in LAM cells and tissues. Using cell viability assays including crystal violet assay and MTT, we found that Fludarabine—an FDA approved *STAT1* inhibitor decreased *TSC2*-null cell growth and viability in vitro (*$p<0.05$, n=6/treatment group), and induced apoptosis compared to controls. Finally, using a xenograft mouse model of LAM, we tracked lung metastasis of *TSC2*-null LAM cells in vivo (Xenogen IVIS Spectrum System) and quantified total photon flux in the chest region. We found that *STAT1* suppression attenuates LAM cell lung metastasis and tumor burden (*$p<0.05$, n=4/group). Our results indicate that *STAT* signaling is essential for LAM progression and should be investigated as a possible treatment target for LAM and TSC-associated disorders.

Multimodal framework to resolve variants of uncertain significance in TSC2

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Rationale: Tuberous sclerosis complex (TSC) affects 1 in 6,000 individuals born in the United States and is a multisystem mTORopathy characterized by benign tumors, drug-resistant epilepsy, and other cognitive manifestations. This condition is caused by loss-of-function (LOF) genetic variants in the *TSC1/2* complex which results in constitutive mTOR activity. Obtaining a precise genetic diagnosis in TS is a challenge as many missense *TSC2* variants are considered variants of uncertain significance (VUS). More than 2,500 *TSC2* VUS have been documented in the ClinVar database. To address the growing challenge presented by VUSs, there is a critical need to develop tools to resolve their functional impact. We have developed a multimodal approach incorporating gene-specific machine learning (ML) methods and a *TSC2* multiplex assay of variant effect (MAVE) to improve *TSC2* VUS resolution.

Methods: We have developed a gene-specific model using a random forest algorithm trained on known benign and pathogenic missense *TSC2* variants. For our *TSC2* MAVE, haploid HAP1 cells are generated each expressing a single *TSC2* variant using prime editing and a plasmid library. Cells are serum starved overnight, fixed, and labeled with an antibody for phosphorylated S6 (P-S6), a well-characterized biomarker of mTORC1 complex activity. Fluorescence activated cell sorting (FACS) is used to sort cells with constitutive mTORC1 activity (P-S6HIGH), followed by next generation sequencing (NGS) of *TSC2* amplicons to examine which *TSC2* variants are enriched in the sorted cell pool relative to unsorted cells.

Results: Our gene-specific ML model accurately predicts variant class in 93% of a test dataset which was not used in model training. Our proof-of-concept prime editing of *TSC2* exon 17 generated cells with 22 different *TSC2* variants. Importantly, pathogenic variants, but not benign variants, were enriched in sorted P-S6HIGH cell pools.

Conclusions: We have developed a MAVE for *TSC2* based on mTORC1 activity which distinguishes loss-of-function variants from benign variants. Our goal is to generate enrichment scores for hundreds of *TSC2* missense variants using this approach. This data will be used to test the validity of our ML pathogenicity predictions and to refine the performance of this classifier. This gene-specific workflow for improving the rate of VUS resolution can be adapted to other mTORopathy genes, such as NPRL2, MTOR, and DEPDC5.

Plenary Session 5 Abstracts: Hot Topics Advancing TSC Research and Clinical Care

TSC and the UK Rare Disease Research Platform

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TSC is one of a group of rare diseases with a shared underlying molecular mechanism: hyperactivation of the mechanistic target of rapamycin (mTOR) pathway. mTOR pathway diseases are a group of 14 rare, early-onset genetic diseases, including TSC, with symptoms ranging from benign tumours in multiple organs to brain malformations causing epilepsy. Although rare diseases may be individually rare, they are collectively common, with 1 in 17 people being affected by a rare disease at some point in their lives. To coordinate and address tractable challenges in rare diseases and achieve a step change in the mechanistic understanding, diagnosis and therapy of rare diseases, the UK Medical Research Council (MRC) and National Institute for Health and Care Research (NIHR) recently launched the 'UK Rare Disease Research Platform', consisting of 11 nodes and a coordinating hub. The 'mTOR Pathway Diseases' node is part of the UK Rare Disease Research Platform and over 5 years aims to transform the mechanistic understanding, diagnosis and treatment of mTOR pathway diseases, including TSC. We will present how the node is tackling the lack of connectivity between clinicians and researchers working on mTOR pathway diseases by bringing together all the UK clinical specialists and scientists in the field, covering 13 universities, research institutions and NHS Trusts. We are also working with the Tuberous Sclerosis Association and Epilepsy Research UK, and industrial partners with research interests in TSC and mTOR pathway diseases. The node also aims to benefit the international community and work with new collaborators to benefit TSC patients worldwide. We will describe the node enabling science projects focused on understanding underlying mechanisms, improving diagnosis and treatment. Utilising the unique ability of the NHS England National Disease Registration Service to access patient records, we will build a registry of patients with TSC and other mTOR pathway diseases across the UK. Working with clinical partners, we will assemble a tissue and peripheral blood mononuclear cell resource to facilitate research and study TSC patient cerebral organoids to understand differing responses to anti-seizure medications. We will also develop new non-invasive genetic testing techniques for mosaic mTOR pathway diseases such as focal cortical dysplasia and TSC mosaicism. We will describe how these enabling science projects will benefit the international TSC research community.

Healthcare provider recognition of pregnancy related risks and management considerations in patients with tuberous sclerosis complex

Meredith Rose, David Ritter, Nishant Gupta, Leandra Tolusso, Paul Horn, Emily Wakefield, Jennifer Glass

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Patients with tuberous sclerosis complex (TSC) face an increased risk of maternal health complications and worsening disease manifestations during pregnancy. However, many of these risks are poorly understood and there are no established consensus guidelines that address pregnancy management in patients with TSC. In the absence of established guidelines, TSC healthcare providers rely on their individual experience and preference to derive treatment decisions when caring for this population. We sought to ascertain provider recognition of pregnancy related maternal complications in patients with TSC and the common evaluations and management strategies employed to address these complications. We conducted an anonymous cross-sectional survey of healthcare providers. Descriptive analysis was used to analyze provider recognition of maternal risks/complications and recommendations before and during pregnancy. We received responses from 87 providers from 11 countries, with 40.7% (n=35) seeing >30 TSC patients yearly. Most providers (n=70, 88.6%) deemed that a patient with TSC needed expert care beyond the standard of care for typical pregnancy, with over 25% of providers reporting that they have seen lymphangioleiomyomatosis (LAM) exacerbation, seizures, and preterm labor in pregnant patients with TSC at an increased rate. Providers who managed mTOR inhibitors (mTORi) also agreed that mTORi use should be stopped prior to pregnancy (n=45, 68.2%) but there was uncertainty about when to stop the mTORi (one month 28.9%, two months 11.1%, three months 42.2%, and 6-12 months 2.2%). Additionally, there was no consensus on restarting mTORi in response to disease progression. Our study found that healthcare providers recognize that patients with TSC are at an increased risk for maternal health complications during pregnancy. However, with 71.6% (n=53) of providers stating that they do not have a clear protocol for management decisions for patients with TSC before/during pregnancy, there are wide inter-individual variances in practice especially pertaining to decisions regarding mTORi use. There is a critical need to better understand the implications of pregnancy for patients with TSC and to draft consensus recommendations to guide management decisions.

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   J. Chris Kingswood (presenting author), Nicholas M.P. Annear, Lydia Israel, Rita Bhandari, Frances Elmslie

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

   Jerome S. Arceneaux, Rohit Khurana, Asa A. Brockman, Mary-Bronwen L. Chalkley, Laura C. Geben, Robert P. Carson, Bret C. Mobley, Kevin C. Ess, Rebecca A. Ihrie

3. **AMPK mediates oncogenic transformation in TSC**

   Kaushal Asrani (presenting author), Juhyung Woo, Adrianna A. Mendes, Thiago Vidotto, Leon Deng, Ethan Schaffer, Kewen Feng, Tamara L. Lotan

4. **S6 Kinase is involved in TSC-related epilepsy through the regulation of TREK-1 potassium channel**

   Francesco Avanzi, Stefano Fumagalli, Mario Pende

5. **Ultraviolet signature mutations in TSC skin tumors are increased in sun-exposed body sites, reinforcing recommendations for good sun protection**

   Devin Barzallo, Xijun Zhang, Ji-an Wang, Alexander Cartron, Amanda Jones, Patricia Julien-Williams, Hai-Ping Wu, Matthew Wilkerson, Clifton Dalgard, Joel Moss, Thomas Darling

6. **Tryptophan-mediated macropinocytosis is a metabolic vulnerability in tuberous sclerosis complex**

   Sarah Lafleur, Windrie Cox, Elizabeth P. Henske, Charilaos Filippakis

7. **A novel rapamycin cream formulation improves facial angiofibromas associated with tuberous sclerosis complex: a double-blinded, randomized, placebo-controlled trial**

   Ioana Stanescu, Phillip Aitken, Laura Boddington, Caroline Mahon, Andras Fogarasi, Yi-Hua Liao, Marta Ivars , Ester Moreno-Artero, Doris Trauner, Steven T. DeRoos, Jasna Jancic, Milos Nikolic, Patrícia Balážová, Harper N. Price, Kinga Hadzsiev, Kate Riney, Stacie Stapleton, Megha M. Tollefson, Derek Bauer, Blanka Pinková, Hartley Atkinson

8. **Burden of illness in patients with facial angiofibroma associated with tuberous sclerosis complex: a systematic review**

   Eric Beresford, Denny John, Lawrence A. Schachner, Adelaide A. Hebert, Deepti Rai, Abhirup Dutta Majumdar, Sreedevi Boggarapu

9. **Topical sirolimus 0.2% gel for the management of tuberous sclerosis complex-related cutaneous manifestations: an interim analysis of postmarketing surveillance in Japan**

   Eric Beresford, Akemi Egami, Shinji Takahashi, Takeshi Kokubo, Sreedevi Boggarapu

10. **Healthcare resource utilization and cost burden of facial angiofibroma associated with tuberous sclerosis complex: a real-world claims database analysis in the United States**

    Sreedevi Boggarapu, Adelaide A. Hebert, Caleb Paydar C, Ashis K Das, Eric Beresford

11. **Management of facial angiofibroma associated with tuberous sclerosis complex: a systematic review of evidence of clinical effectiveness and cost-effectiveness**

    Sreedevi Boggarapu, Denny John, Lawrence A. Schachner, Joyace Teng, Md Sohail Aman, Saswata Paul Choudhury, Eric Beresford

12. **The relationship between tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) and seizure characteristics: a US-based cross-sectional study**

    Anna Jansen, Sally Bowditch, Lisa Moore-Ramdin, Katie Smith, Elizabeth Cassidy, Annabel Nixon, Petrus J. de Vries

13. **Gyrification index in TSC: a new marker of cortical disarrangement**

    Francesco Brunello, Nicolò Trevisan, Margherita Nosadini, Stefano Sartori, Maria Federica Pelizza, Fabio Sambataro, Renzo Manara, Irene Toldo

14. **TSC-associated neuropsychiatric disorders (TAND) assessment and the neuropsychological cluster in a group of patients from Brazil**

    Laís F. M. Cardozo, Ana Paula A. de Pereira, Mariana R. Schwind, Luiz G. Dufner-Almeida, Luciana A. Haddad, Ana Chrystina de S. Crippa, Sérgio A. Antoniuk

15. **Lysine metabolism as an emerging therapeutic target for epilepsy in tuberous sclerosis complex (TSC)**

    Felix Chan, Li-Jin Chew, Richard Heath, Hugh Delaney, Luca Bartolini, Shane Evans, Qing Wu, Caroline Lewis, Scott Shaffer, Chima Oluigbo, William Gaillard, Mark Cunningham, Wyatt Yue, Darcy Krueger, Judy Liu

16. **The role of CTHRC1 in the pathogenesis of TSC**

    Pieter Cory, Dean M. Rosenthal, Damir Khabibullin, Michel Alchoueiry, Eli Akl, Heng-Jia Liu, Yan Tang, Julia F. Charles, Volkhard Lindner, Nicola Alesi, Elizabeth P. Henske

17. **Parental stress in tuberous sclerosis complex**

    Jenny Do, Syed Hashmi, Deborah Pearson, Hope Northrup, Laura S. Farach, Kate Richardson

18. **Lipomorphosis from everolimus treatment in renal angiomyolipoma associated with tuberous sclerosis complex**

    Richard A. Dixon, Jacob Whitworth, Ne-Ron Loh

19. **Evaluating the etiology of cortical tubers in tuberous sclerosis complex**

    Nada A. Elsayed, Jerome S. Arceneaux, Mary-Bronwen L. Chalkley, Laura C. Geben, Asa A. Brockman, Madeline J. Hayes, Todd Bartkowiak, Justine Sinnaeve, Grant Westlake, Sierra M. Lima, Mustafa Sahin, Jonathan M. Irish, Rebecca A. Ihrie, and Kevin C. Ess

20. **Van Andel Institute's biospecimen core resource for the TSC Alliance®**

    Tori Evans, Dan Rohrer, Galen Hostetter, Melissa DeHollander, Alex Blanski, Meghan Kirchhoff, Brianne Livermore, Christina Missler, Lisa Turner, Sydney Whitford, Bree Berghuis, Elizabeth Cassidy, Zoë Fuchs, Dean Aguiar, Steve Roberds

21. ***TSC2* coordinates differentiation**

    David M. Feliciano, Victoria A. Riley, Vijay Shankar, Jennie C. Holmberg, Aidan M. Sokolov, Victoria N. Neckles, Kaitlyn Williams, Rachel Lyman, Trudy F.C. Mackay

22. **Characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment in TSC**

    Marie Girodengo, Simeon R. Mihaylov, Pranetha Baskaran, Laura Mantoan Ritter, Sila K. Ultanir, Joseph M. Bateman.

23. **Tuber functional connectivity with somatomotor vs visual cortices as well as the cerebellum is associated with differential ADOS subscale severity in TSC**

    Wendy Xiao Herman, William Drew, Gillian Miller, Mustafa Sahin, Jurriaan M Peters, Simon K Warfield, Darcy A Kreuger, E Martina Bebin, Hope Northrup, Joyce Wu, and Alexander L Cohen on behalf of the Tuberous Sclerosis Complex Autism Center of Excellence Network Study Group

24. **Everolimus enhances pro-metastatic potentials of LAM-derived exosomes**

    Apoorva Kasetti, Anil Kumar Kalvala, Ashok Silwal, Bhaumik Patel, Jane J. Yu, Maciej Markiewski, Magdalena Karbowniczek

25. **Design of a double-blind, randomized, cross-over, placebo-controlled Phase 2 trial of basimglurant as adjunctive therapy in patients with seizures associated with tuberous sclerosis complex (GALENE)**

    Renata Lazarova, Rachel Gurrell, Amine Tahiri, George Garibaldi, Ali Mostajelean

26. **Altered differentiation and astrocyte reactivity in a human brain organoid model of tuberous sclerosis complex**

    Thomas L. Li, John D. Blair, Gerald A. Grant, Brenda E. Porter, Dirk Hockemeyer, Helen S. Bateup

27. **Sleep and its association with temperament and sensory processing in early TSC**

    Natasha Lindsay, Abigail Runicles, Tony Charman, Mark H. Johnson, Emily J. H. Jones, Patrick F. Bolton, EDiTS Study team, Charlotte Tye

28. **Discovering the neuropathological etiology of tuberous sclerosis complex-associated neuropsychiatric disorders**

    Andy Liu, Jamie Capal, Darcy Kreuger, David Ritter, Klaus Werner

29. ***TSC1* mutant bladder cancer is characterized by a TSC-associated gene expression signature due to TFE3 transcriptional activity**

    Magdalena Losko, Clemens K. Probst, Elio Adib, Heng Du, Michelle S. Hirsch, Krinio Giannikou, David J. Kwiatkowski

30. **Single-cell transcriptomic analysis of tuberous sclerosis complex (TSC)**

    Qianqian Ma, Xiaolong Jiang

31. **Translatome analysis of tuberous sclerosis complex patient-derived isogenic neural progenitor cells**

    Roberta L. Beauchamp (presenting author), Pauline Martin, Krzysztof J. Szkop, Francis Robert, Inci S. Aksoylu, Nicholas E. Redmond, Serkan Erdin, Jerry Pelletier, Ola Larsson, Vijaya Ramesh

32. **Co-creation of EpiCom Phase 4 study design in collaboration with the tuberous sclerosis complex (TSC) community of patients, caregivers, healthcare professionals, and a pharmaceutical company**

    Lisa Moore-Ramdin, Shelly Meitzler, Pooja Takhar, Petrus de Vries, Joanne Stevens, Agnies van Eeghen

33. **OV329, a next-generation GABA-AT inhibitor, is a potent anticonvulsant in mouse models of epilepsy**

    Jay Mukherjee, Christopher Bope, Shing Hong Lin, Ray Colburn, Paul A. Davies, Patrick Sarmiere

34. **Cannabidiol (Epidyolex) for severe behavioral manifestations in individuals with TSC: Protocol for a series of N-of-1 trials**

    Annelieke R. Müller, Floor E. Jansen, Marie-Claire Y. de Wit, Leontine W. ten Hoopen, André B. Rietman, Bram Dierckx, Frits A. Wijburg, Marion M.G. Brands, Agnies M. van Eeghen

35. **Understanding the impact of TSC: development and validation of the TSC-PROM**

    Annelieke R. Müller, Michiel A.J. Luijten, Lotte Haverman, Wendela L. de Ranitz-Greven, Peter Janssens, André B. Rietman, Leontine W. ten Hoopen, Laura C.G. de Graaff, Marie-Claire de Wit, Anna C. Jansen, Tanjala Gipson, Jamie K. Capal, Petrus J. de Vries, Agnies M. van Eeghen

36. **Modeling tuberous sclerosis complex with 3-D human cortical organoids**

    Weibo Niu, Shaojun Yu, Xiangru li, Michael J Gambello, Zhexing Wen

37. **Development of a novel mouse model to study *TSC2* mutation in hematopoietic cells and its impact on TSC lesions**

    Reena Rani, Hui Chen, Hong-Jun Wang, Elizabeth Henske, Wei Shi

38. **A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression**

    Ulrike Rehbein, Kathrin Thedieck

39. **Continued demonstration of safety of mTOR Inhibitors in infants and young children with TSC**

    David M. Ritter, David N. Franz, Molly Griffith, Adrienne Victory, Madeleine Robben, Karen Agricola, Paul S. Horn, Darcy A. Krueger, Jamie K. Capal

40. **Earlier surgery is associated with improved post-operative language development in children with tuberous sclerosis complex (TSC)**

    Sina Sadeghzadeh, Jurriaan Peters, Brenda Porter, S. Katie Ihnen

41. **Long-term safety and efficacy of add-on cannabidiol (CBD) for seizures associated with tuberous sclerosis complex (TSC): 3-year results from GWPCARE6 open-label extension (OLE)**

    Elizabeth A. Thiele, E. Martina Bebin, Francis Filloux, Floor E. Jansen, Patrick Kwan, Rachael Loftus, Farhad Sahebkar, Steven Sparagana, John Lawson, James Wheless

42. **Long-term efficacy and safety of cannabidiol (CBD) in patients with tuberous sclerosis complex (TSC): 4-year results from the Expanded Access Program (EAP)**

    Elizabeth A. Thiele, Arie Weinstock, E. Martina Bebin, Daniel Checketts, Gary D. Clark, Jerzy P. Szaflarski, Laurie Seltzer, Farhad Sahebkar

43. **Machine learning drive TSC disease modeling and reversion screening in an iPSC derived disease model**

    Jordi Serrats, Santiago Akle, Rahul Atmaramani, Ci Chu, Tommaso Dreosi, Lexie Ewer, Ariel Kauss, Ajamete Kaykas, Daphne Koller, Yvonne Kwan, Bobby Leitmann, Sahil Malpotra, Shahin Mohammadi, Angela Pisco, Ninad Ranadive, Nav Ranu, Max Salick, Santosh Satapati, Eilon Sharon, Srinivasan Sivanandan, Shengjiang Tu, Joyce Yang

44. **Exosomal ITGB1 regulates LAM cancer stem cell properties via ITG-c-Src-FAK-cdc42 axis**
    Kirti Shetty, Anil Kumar Kalvala, Ashok Silwal, Jane J. Yu, Maciej Markiewski, Magdalena Karbowniczek

45. **Managing visual disturbances in TSC patients with astrocytic hamartomas**
    Amy Shteyman, Julie Shabto, Brian Marr, Vlad Diaconita

46. **mTORC1-dependent RNA methylation confers rapamycin resistance in TSC tumors**
    Joohwan Kim, Yujin Chun, Cuauhtemoc Ramirez, Cholsoon Jang, Gina Lee

47. **The adverse events of everolimus in tuberous sclerosis complex patients treated for renal angiomyolipoma/subependymal giant cell astrocytoma**
    Jeng-Dau Tsai, Shuo-Yan Gau

48. **Vigabatrin acts on cilia pathways in TSC2-deficient neurons**
    Kagistia Hana Utami, Velda Han, Nevin Tham, Sarah R. Langley, Mahmoud A. Pouladi

49. **Voices from Ireland**
    Mary Vasseghi, Miriam Galvin, Eilish Burke, Catherine Darker, Claire Behan, Colin P. Doherty

50. **The Latin American TSC Consortium: an ongoing collaborative strategic initiative**
    Zacil Vilchis-Zapata, Joao Garcia-Martínez, Darcy Kruger Ary Agami, Janeth Correa, María Silva, Wesley Gómez, Jonny Cuevas, Aynara Wulsin, Alejandro Claros-Bustamante, Rosalba Sevilla-Montoya.

51. **Arginine metabolism in tuberous sclerosis complex-associated kidney disease**
    Amleh A, Chen HP, Watad L, Abramovich I, Agranovich B, Gottlieb E, Ben-Dov IZ, Nechama M, Volovelsky O.

52. **Epilepsy and TAND in the tuberous sclerosis complex are caused by an alteration in microglial polarity to M1 and sirolimus cures them by returning microglial polarity to M2**
    Emi Kaneda, Makiko Koike-Kumagai, Mari Wataya-Kaneda

53. **Implementation of a clinical and research consortium to achieve early diagnosis, adequate disease surveillance and management across the lifespan of patients with TSC in a middle-income country**
    Aynara C. Wulsin, Zacil Vilchis-Zapata, Darcy Krueger, Rosalba Sevilla-Montoya

54. **Understanding phenotype heterogeneity of tuberous sclerosis for potential precision medicine**
    Seungyeul Yoo, Yu Liu, Eunjee Lee, Quan Chen, Stephen McGee, Li Wang, and Jun Zhu

55. **Understanding dynamic molecular regulations in brains with regard to tuberous sclerosis diseases**
    Seungyeul Yoo, Yu Liu, Eunjee Lee, Quan Chen, Stephen McGee, Li Wang, and Jun Zhu

56. **Pulmonary explant cultures for ex vivo testing of LAM therapeutics**
    Mohamed A. Youssef, Rohan S. Shivde, Octavio K. Bartos, Gina M. Scurti, Daniel F. Dilling, Michael I. Nishimura, I. Ca

57. **A precision dosing strategy for overcoming challenges to the dosing of mTOR Inhibitors in TSC infants in clinical practice and clinical trials**
    David M. Ritter, Jamie K. Capal, David Neal Franz, Molly Griffith, Karen Agricola, Bridget Kent, Kristn Currans, E Martina Bebin, Hope Northrup, Mary Kay Koenig, Tomoyuki Mizuno, Alexander A. Vinks, Stephanie Galandi, Wujuan Zhang, Kenneth D. R. Setchell, Carlos E. Prada, Kelly Kremer, Katherine Holland-Bouley, Hans Greiner, Paul S. Horn, Darcy A. Krueger

58. **Healthcare provider recognition of pregnancy related risks and management considerations in patients with tuberous sclerosis complex**
    Meredith Rose, David Ritter, Nishant Gupta, Leandra Tolusso, Paul Horn, Emily Wakefield, Jennifer Glass

59. **STAT1 promotes survival and lung metastasis of TSC2-null cells in lymphangioleiomyomatosis (LAM)**
    Tasnim Olatoke, Erik Y. Zhang, Aristotelis Astrinidis, Minzhe Guo, Yan Xu, Jane J. Yu

Poster Session Abstracts

1. Real-world experience of long-term treatment of TSC-Related kidney angiomyolipomas (AMLs) with an mTOR inhibitor

J. Chris Kingswood (presenting author), Nicholas M.P. Annear, Lydia Israel, Rita Bhandari, Frances Elmslie

TSC Service, St. George's University Hospitals NHS Foundation Trust (NMPA, LI, JCK); St. George's, University of London (NMPA, RB, FE, JCK); Royal Sussex County Hospital (FE, JCK)

Introduction: Angiomyolipomas (AMLs) are the most common TSC-associated kidney lesions, developing in around 80% of patients by adulthood. Untreated, AMLs may cause severe bleeding in half of these patients. Two mTOR inhibitor medications (mTORi) - Everolimus and Sirolimus - have been shown to shrink and de-vascularise AMLs, preventing bleeding. With careful management, initial side-effects from mTORi treatment can be successfully ameliorated to allow continuous treatment. Outside of specialist prescribing centres, there remains concern about side-effects, resulting in a reluctance to start, or to continue what is an effective, targeted treatment in this condition.

Methodology: TSC patients commenced on mTORi - Everolimus 5 mg (85 %) or Sirolimus 2 mg (15 %) daily - were seen after one month, then 3-monthly thereafter. The dose was increased if blood levels were below measurable levels, or more commonly reduced if there were persistent or severe side-effects. Blood test monitoring was performed at every follow-up; and repeat MRI scans at 3-6 months, then annually. Median time on mTORi was 59 months (range 4-190 months).

Results: Of 137 patients (86(64%) Female) on mTORi, treatment of kidney AMLs was the primary indication for mTORi in 131(96%). Side-effects included mouth ulcers (40%), GI disturbances (26%), infections (34%), respiratory infections (19%), acne (15%) and skin rash (16%). All were low grade, CTCAE 1-2. No significant differences in mouth ulcers were found between the EXIST-2 clinical trial follow-up study and the researched cohort, but there were significantly fewer headaches, and a lower incidence of reported acne in patients on treatment for longer than 12 months, and less than 25 months. Adverse events (AEs) were commonest in the first 12 months, and progressively less common thereafter. Sixty-three percent of patients continued on their initial mTORi dose, 11% had it increased, 23% reduced, and 3% discontinued permanently. No patient had a kidney bleed on treatment, and in all, AMLs either shrank or stopped enlarging. Two patients died of unrelated causes (One bowel cancer; the other a community acquired pneumonia). Patient kidney function was stable in all but 4 patients, with one commencing haemodialysis over the study period.

Conclusion: Long term mTORi treatment is effective and safe if managed with frequent supervision. It is far superior to the alternatives of embolisation or surgery for the majority of TSC patients.

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

Jerome S. Arceneaux, Rohit Khurana, Asa A. Brockman, Mary-Bronwen L. Chalkley, Laura C. Geben, Robert P. Carson, Bret C. Mobley, Kevin C. Ess, and Rebecca A. Ihrie

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Tuberous sclerosis complex (TSC) arises due to heterozygous mutations in *TSC1* or *TSC2* and affects approximately 1 in 6000 births. Neuropsychiatric symptoms of this disorder include autism spectrum disorder (ASD), developmental delay, intellectual disability, and epilepsy. Mutations in *TSC2* are often associated with worse symptoms and severity. Epilepsy in TSC patients is often refractory to drug treatment, sometimes 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 exhibit loss of heterozygosity (LOH) of *TSC1/2* in otherwise heterozygous tissue. Though mutations in *TSC1/2* lead to 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 broad TSC cohorts at the

protein level. In addition, how "balloon cells" may influence their microenvironment to produce epileptogenic foci is poorly understood. High-dimensional approaches such as imaging mass cytometry (IMC) offer the opportunity to directly assess thirty or more proteins and signaling events in single cells while documenting spatial relationships within the tissue. Using a custom antibody panel, where each of thirty-six (36) antibodies was successfully tested on known positive and negative controls, we have identified through immunofluorescence probable colocalization of lineage markers as well as signaling readouts of mTORC1 hyperactivation. In addition, we developed customized machine-learning workflows that 1) identify prospective "balloon cells" with 93% precision and 69% efficiency within archived cortical tubers and 2) can map the cytoarchitecture and signaling perturbations within tissue samples, with a specific focus on "balloon cells" and their immediate neighbors. These data will comprise a rich dataset for understanding the abundance, structure, and signaling activity of progenitor-like, neuronal, and glial cells within archived tubers and perituberal tissues, enabling quantitative comparisons of TSC with other mTORopathies and assaying possible therapeutic targets.

3. AMPK mediates oncogenic transformation in TSC

Kaushal Asrani (presenting author), Juhyung Woo, Adrianna A. Mendes, Thiago Vidotto, Leon Deng, Ethan Schaffer, Kewen Feng, Tamara L. Lotan

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The serine-threonine kinase and cellular energy biosensor, 5'AMP-activated protein kinase (AMPK) is central regular of energy homeostasis and is known to promote lysosomal biogenesis and tumorigenesis via activation of the MiT/TFE transcription factors (TFEB/ TFE3). We and other groups have previously shown that TFEB and TFE3 are constitutively activated in the setting of *TSC1/2* loss (www.nature.com/articles/s41467-022-34617-7). Here, we examined the expression and function of AMPK in renal cells and tumors driven by loss of the TSC tumor suppressors. Phosphorylation of AMPK and its substrates (p-ACC[S79] and p-ULK1[S555]) was constitutively increased in HEK293T cells with genomic deletion of *TSC1*, *TSC2* and *TSC1/2* and was further enhanced in response to glucose deprivation or the lysosomal AMPK activator Aldometanib, in an mTORC1-sensitive manner. Expression of p-AMPK (T172) and p-ACC (S79) was increased in murine renal cysts and tumors in $TSC2^{+/-}$ A/J mice. Multiple AMPK-dependent CLEAR genes sets were positively enriched by GSEA in *TSC2* KO compared to WT xenografts, and negatively enriched in the TFEB/ TFE3 double KO xenografts compared to *TSC2* KO xenografts. We used CRISPR-Cas9 genome editing to knockout the two genes encoding the alpha catalytic subunits of AMPK (AMPKa1 and AMPKa2) in *TSC2* KO cells, and isolated and characterized 3 AMPK double-knockout (DKO) CRISPR clones. AMPK depletion in the *TSC2* KO background significantly reduced cellular proliferation (IncuCyte ZOOM live cell imaging and clonogenic assays) and cellular ATP levels (CellTiter-Glo). These findings suggest that AMPK may be an important mediator of tumorigenesis in TSC.

4. S6 kinase is involved in TSC-related epilepsy through the regulation of TREK-1 potassium channel

Francesco Avanzi, Stefano Fumagalli, Mario Pende

Necker Enfants Malades Institute (INEM), Paris, France (FA, SF, MP); Université de Paris Cité, Paris, France (FA, SF, MP)

Epilepsy is the most common neurological manifestation of tuberous sclerosis complex (TSC), a genetic disease caused by loss-of-function mutations on the *TSC1* or *TSC2* genes that results in the mTOR pathway hyperactivation. The molecular mechanisms downstream of aberrant mTOR signaling that are responsible of the alterations of neuronal excitability are largely unknown. To study the role of the downstream mTOR-effector S6 Kinase (S6K), we generated a model of TSC based on AAV9-Cre-mediated deletion of the *TSC1* gene in the brain of $TSC1^{f/f}$ and $TSC1^{f/f}S6K^{-/-}$ mice. Interestingly, epilepsy development and premature death of TSC mice is completely reverted upon the deletion of the *S6K1/2* genes. To identify new potential downstream targets of S6Ks we performed, in collaboration with Cell Signaling Technology, a phosphoproteomic analysis and we observed an enrichment of proteins known to be involved in autism and seizure with a specific phosphorylation consensus sequence for S6K (RXXS*/T*). We focused on TREK-1, a potassium channel, whose deletion increases seizure susceptibility. We demonstrated that TREK-1 is phosphorylated on Ser333 in a rapamycin-sensitive way by S6K in HEK293 cells and in primary cortical neurons upon glutamatergic stimulation. Moreover, by electrophysiology recording, we observed that TREK-1 is phosphorylated and kept in a closed conformation after amino acids stimulation, while is opened in presence of rapamycin. Finally, overexpression of TREK-1-S333A is able to revert the induction of cFos – a common marker of neuronal activity – in in vitro model of TSC. Our work will provide a mechanism for epilepsy development and new potential therapeutic approaches for TSC patients.

5. Ultraviolet signature mutations in TSC skin tumors are increased in sun-exposed body sites, reinforcing recommendations for good sun protection

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Ultraviolet (UV) light is a mutagen implicated in the development of skin cancers, generating C>T and CC>TT mutations and mutational signatures known as SBS7a and SBS7b. UV light also contributes to the formation of facial angiofibromas (FAFs) in individuals with tuberous sclerosis complex (TSC), as evidenced by CC>TT mutations as second hits in the TSC genes in fibroblast-like cells derived from FAFs. The role of UV light in TSC tumorigenesis may be expected to be the most pronounced in sun-exposed skin and could be reduced by an individual's constitutive pigmentation, since greater melanin content is known to reduce the penetration of UV light. It has not been elucidated whether UV-signature mutations across the genome in TSC skin tumors differ by body site or race. We performed whole-genome sequencing of DNA from fibroblast-like cells grown from 39 tumor samples from 27 patients, including 2 Black individuals, 1 Hispanic individual, and 24 White individuals. TSC lesions were grouped as sun exposed (FAF and fibrous cephalic plaque of the face and neck) or sun protected (e.g. shagreen patches, ungual fibromas, fibrous cephalic plaque of the scalp). The median number [and interquartile ranges] of UV mutations across the genome for the TSC skin lesions in sun-exposed skin (SBS7a: 2,485 [447-6,465], SBS7b: 3,736 [738-9,042], n=15) were greater than those in sun-protected skin (SBS7a: 55 [27-163], SBS7b: 6 [0-55], n=24). Among FAFs, the median mutation rates for the samples from White individuals (SBS7a: 4,198 [1,621-9,394], SBS7b: 6,855 [2,348-10,957], n=8) were similar to that observed in the Black individual (SBS7a: 14,949, SBS7b: 13,533]). In addition, the FAF from the Black individual showed UV signature mutations in *TSC2* as second-hit pathogenic variants. UV-signature mutations in TSC skin tumors vary with body location, with a greater number of mutations in sun-exposed areas such as the face, suggesting that sun protection measures will reduce mutational burden and severity of skin disease. However, our preliminary results suggest that constitutive pigmentation does not provide adequate protection against the effects of UV light. These observations emphasize the importance of counseling on sun protection among all individuals with TSC to reduce the formation of FAFs.

6. Tryptophan-mediated macropinocytosis is a metabolic vulnerability in tuberous sclerosis complex

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See abstract on page 21.

7. A novel rapamycin cream formulation improves facial angiofibromas associated with tuberous sclerosis complex: a double-blinded, randomized, placebo-controlled trial

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Background: Tuberous sclerosis complex (TSC) is a rare genetic disorder which causes non-cancerous tumours in the brain, kidneys, and skin. Topical rapamycin can successfully treat Facial Angiofibroma (FA) in patients with TSC. Few topical formulations are available and compounded formulations are inadequate since rapamycin rapidly degrades. A novel stabilized topical cream which protects rapamycin from oxidation has been developed in 0.5% and 1% concentrations.

Methods: This multicenter, double-blind, randomized, placebo-controlled, dose-response phase II/III study with a parallel design aimed to assess the efficacy and safety of the topical cream. 107 participants aged 6-65 years with mild-to-moderate FA associated with TSC were randomized to receive topical rapamycin 0.5% (n=36), 1% (n=33), or placebo (n=38) once daily for 26 weeks. Efficacy was assessed using an Investigator Global Assessment (IGA) scale, the facial angiofibroma severity index, subject and clinician-reported percentage-based scales, and categorical improvement.

Results: Both strengths of cream led to statistically significant and clinically meaningful improvements across multiple efficacy assessments at 26 weeks. 60.6% and 55.6% of patients treated with 1% and 0.5% rapamycin cream, respectively, achieved a measurable improvement on the IGA scale. 71.4% and 90% of patients reported they were “Moderately” or “Significantly” better following treatment with 1% or 0.5% rapamycin cream, respectively. Both strengths were well tolerated, with mild application site irritation the most common adverse event.

Conclusions: This novel, stable, rapamycin cream is effective at reducing angiofibromas and is well-tolerated.

8. Burden of illness in patients with facial angiofibroma associated with tuberous sclerosis complex: a systematic review

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Limited clinical evidence exists regarding the burden of the disease of facial angiofibroma, a prominent manifestation of tuberous sclerosis complex (TSC), an autosomal genetic disorder. Our systematic literature review assessed the global epidemiology and the humanistic and economic burden of facial angiofibroma associated with TSC to identify current evidence gaps. A systematic review was performed per PRISMA guidelines, and we queried for studies (March-May 2022) via PubMed, OVID, EMBASE, Cochrane CENTRAL, EconLit, and HTA databases and conferences. Several checklists assessed methodological quality and bias risk for humanistic and economic outcomes. Meta-analyses for pooled epidemiological outcomes were quantitatively synthesized per the MOOSE guidelines. Of 8657 studies, 58 were included, presenting epidemiological (n=42), humanistic (n=14), and economic (n=6) outcomes. Ten different measures of TSC diagnosis were identified across all studies. The prevalence of facial angiofibroma among patients with TSC was 34%–90% across community and hospital-based studies. In a meta-analysis, the pooled proportion of facial angiofibroma associated with TSC ranged from 50%–59% across all diagnostic criteria. Considerable heterogeneity was observed across all diagnostic criteria for both community-based ($p<0.001$) and hospital-based ($p<0.01$) studies. The Health-Related Quality-of-Life (HRQoL) impact of the disease was examined based on disease-specific instruments such as Dermatology Life Quality Index (DLQI; n=3), Children’s DLQI (n=4), Family DLQI (n=1), Childhood Atopic Dermatitis Impact Scale (n=1), and SKindex-teen (n=1). Across studies examining the humanistic impact of facial angiofibroma, in 8 studies, variability in using HRQoL instruments prevented comparability. Studies examining the economic burden of patient management presented direct costs related to 0.1% sirolimus (n=6). The per unit cost of 0.1% sirolimus was $113.8 to $620.7, and the annual cost of treatment ranged between $2458.58 and $13,112. No studies reported incremental cost analyses. The prevalence of facial angiofibroma associated with TSC requires further characterization of the patient population. Lack of evidence was observed across the epidemiological, humanistic, and economic outcomes due to the notable absence of global studies, providing the opportunity for future research.

9. Topical sirolimus 0.2% gel for the management of tuberous sclerosis complex-related cutaneous manifestations: an interim analysis of postmarketing surveillance in Japan

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Background: We assessed the real-world effectiveness and safety of a topical sirolimus 0.2% gel treatment for TSC-related cutaneous manifestations.

Methods: An interim analysis of postmarketing surveillance over 52 weeks was conducted in Japan. A total of 635 and 630 patients were included in the safety and efficacy analysis sets, respectively. Improvement rate of all cutaneous manifestations, responder rate of improvement in individual lesions, adverse events (AEs), adverse drug reactions (ADRs), patient satisfaction level of topical sirolimus 0.2% gel use and patient characteristics associated with the improvement rate of cutaneous manifestations or safety were evaluated.

Results: The mean age of the patients was 22.9 years and 46.1% were men. The overall improvement rate was 74.8% and the responder rate was the highest for facial angiofibroma (86.2%) over 52 weeks treatment. The incidence rates of overall AEs and ADRs were 24.6% and 18.4%, respectively. Efficacy was associated with age (<15, >=15 to <65, and >=65 years, p=0.010), duration of use (p<0.001), and total dosage (p=0.005). Safety was associated with age (< 15, >=15 to <65, and >=65 years, p = 0.011) and duration of use (p<0.001). However, the incidence of ADRs was similar across the age groups without significant differences when the broad age group (>=15 to <65) was subcategorized by 10-year interval. Hepatic impairment, renal impairment or concomitant use of systemic mTOR inhibitors had no effect on the effectiveness or safety. Overall, 53% of patients were “very satisfied” or “satisfied” with the treatment.

Conclusions: Topical sirolimus 0.2% gel is effective in the management of TSC-related cutaneous manifestations and generally well tolerated. Age and duration of usage had a significant association with the effectiveness or safety of topical sirolimus 0.2% gel, whereas total dosage had a significant association with the effectiveness.

10. Healthcare resource utilization and cost burden of facial angiofibroma associated with tuberous sclerosis complex: a real-world claims database analysis in the United States

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Objectives: The disease burden of facial angiofibroma (FA), a predominant cutaneous manifestation of TSC, is substantial. We aimed to document healthcare resource utilization (HCRU) and costs related with management of TSC and in a subset of TSC patients with FA.

Methods: This cross-sectional, retrospective study included prevalent and incident TSC with =1 inpatient or =2 outpatient claims for TSC (International Classification of Diseases, 10th Revision, Clinical Modification diagnosis code: Q85.1) using the Symphony Health Solution Integrated Dataverse (IDV®) database (November 2018–October 2021). TSC-related HCRU (inpatient hospitalizations, office and emergency department [ED] visits, outpatient services, and prescription fills) and costs were estimated based on medical claims with TSC as the primary diagnosis, stratified by TSC patients with/without FA, therapy for FA, age group, and payer types, and assessed over 1-year follow-up period.

Results: In total, 4,446 patients (1,390 [31.3%]/3,056 [68.7%] with/without FA; female 52.4%) were included. Medicaid was the most common insurance type (43.3%), and most common comorbidities involved kidney (16.4%) and heart (8.4%). The TSC cohort with FA vs. overall TSC patients had 982/3,352 inpatient hospitalizations with a per visit hospitalization charge of $20,695/$29,254; 1042/3,538 ED visits with a per visit charge of $2,280/$3529; 9,113/26,232 office visits with a per visit charge of $475/$410; 17,393/50,235 non-ED/office outpatient services with a per service charge of $3,552/$3,746; and 56,664/152,013 pharmacy claims with a per claim charge of $982/$3,352. TSC-related HCRU and cost outcomes across different stratifications were aligned with overall findings for both patient cohorts.

Conclusions: TSC patients and those with FA face substantial disease, HCRU and cost burden, with total costs being driven by outpatient pharmacy claims and other outpatient services, highlighting their treatment needs in the US healthcare setting.

11. Management of facial angiofibroma associated with tuberous sclerosis complex: a systematic review of evidence of clinical effectiveness and cost-effectiveness

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Background: Treatment of facial angiofibroma associated with tuberous sclerosis complex (TSC), a rare autosomal dominant disorder, is challenging due to the lack of established and easy-to-administer therapeutic opportunities. OBJECTIVES: We conducted a systematic review to determine the clinical and cost-effectiveness of medical and physical treatments for managing facial angiofibroma associated with TSC.

Methods: Multiple academic databases, health technology assessment databases, trial registries, and conferences were searched between March-April 2022 for randomized controlled trials (RCTs), non-RCTs, observational and cost-effectiveness studies. The risk of bias and study quality was assessed using standard checklists. Random effects meta-analysis was conducted using Residual Maximum Likelihood to compute study variances. Findings were reported using Synthesis Without Meta-analysis guidelines.

Results: Of 12,459 studies, 8,155 and 401 were screened at title-abstract and full-text levels, respectively, and 91 were included (6 RCTs, 7 non-RCTs, 78 observational). Topical rapamycin 0.1% was most frequently examined (2 RCTs, 1 non-RCTs, 8 observational), followed by sirolimus 0.2% (3 RCTs, 3 observational). Quantitative improvements, such as a change from baseline using facial angiofibroma severity index (FASI) scores, showed improvement in 2 RCTs, 6 cohort studies, 1 case series, and case report, each with topical sirolimus (0.05%, 0.1%, 0.2%, 0.4%, 1%). Patients with any treatment-emergent adverse event (TEAE) ranged from 1.6%-62.5% and serious adverse effects from 3.33%-5.55%. Four trials reported recurrence following treatment cessation. Of the 7 intention-to-treat trials identified, 2 showed a high risk of bias, 2 were moderate risk, and 3 were low risk. Among cohort studies, 3 were low, 4 moderate, and 8 of high quality. Most case series and case reports scored >5 using the Joanna Briggs Institute (JBI) checklists for critical appraisal. Topical sirolimus 0.2% was associated with lower risk versus placebo from a pooled analysis of 2 RCTs [risk difference: -0.63 (0.78-0.47)]. None of the studies screened for cost-effectiveness met the inclusion criteria.

Conclusions: In this systematic review, we comprehensively analyzed all published evidence examining the clinical effectiveness and safety of the therapeutic options available for managing facial angiofibroma associated with TSC, which can help contribute to establishing treatment.

12. The relationship between tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) and seizure characteristics: a US-based cross-sectional study

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In tuberous sclerosis complex (TSC), there is an acknowledged correlation between seizures and TSC-associated neuropsychiatric disorders (TAND); however, the association between current seizures and TAND clusters is unknown. This ongoing cross-sectional study aims to explore these potential associations in patients with TSC enrolled in the US-based, multicenter TSC Alliance Natural History Database. Adult (>=18 years) caregivers of patients with TSC are invited to complete an online survey consisting of a bespoke questionnaire and TAND-SQ, comprising six levels quantifying TAND difficulties. Interim analyses (including data to May 22, 2023) include descriptions and correlations of current seizure characteristics with current TAND cluster severity scores (seven natural and overall psychosocial: 0–10 scale, with higher scores denoting greater dysfunction). Of 44 surveys, the mean (standard error [SE]) age of participants with TSC was 17.3 (2.20) years; 52% were female. Genetic tests were available for 91% (n=40); gene mutations included *TSC1* (14%; n=6), *TSC2* (59%; n=26), PKD1, and *TSC2* (2%; n=1); other participants had no (9%; n=4) or unknown mutations (7%; n=3). Seizure history was reported in 98% of participants, with 53% (n=23) in the past month (several times a day [n=11]; once a day [n=4]; 1–6 times per week [n=2]; <1 per week [n=6]). Seizure severity in the past month (0–10 scale; 'not at all' to 'extremely') was mostly 0 (47%; n=20) or 1–5 (44%; n=19); no scores were >7. Mean (SE) TAND cluster severity scores in the past month were 5.0 (0.8; scholastic [n=27]), 3.9 (0.52; neuropsychological [n=38]), 3.2 (0.41; autism like [n=41]), 3.3 (0.44; overactive/impulsive [n=41]), 2.8 (0.41; dysregulated behavior [n=41]), 2.6 (0.33; mood/anxiety [n=41]), and 2.6 (0.33; eat/sleep [n=40]). The combined mean (SE) psychosocial cluster score was 5.3 (0.48 [n=38]). Numerically higher current neuropsychological (P=0.08), autism-like (P=0.08), and combined psychosocial (P=0.05) scores were associated with current seizure severity. The low number of patients with high seizure severity is a key limitation of these interim analyses. However, findings suggest that there may be an association between current seizure severity and some TAND-SQ clusters. The full multivariate analyses will provide additional insight to enhance our understanding of the relationship between seizures and TAND manifestations. Funding: Jazz Pharmaceuticals, Inc.

13. Gyrification index in TSC: a new marker of cortical disarrangement

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Background: Gyrification brain abnormalities are considered a prognostic marker of early neurodevelopmental alterations and a predictor of poor outcome in psychiatric disorders. Tuberous sclerosis complex (TSC) is an autosomal dominant multisystemic disorder in which the neurological and neuropsychiatric involvement is present in about 90% of cases. TSC-associated neuropsychiatric disorders (TANDs) represent, together with drug resistant epilepsy (DRE), the major disease burdens for patients and caregivers.

Objectives: To evaluate brain gyrification in a group of patients with TSC, identifying possible differences when compared with a control group; analyze the possible correlations between brain gyrification and the clinical and neuroradiological variables in patients with TSC; evaluate the possible prognostic role of gyrification for neurological and neuropsychiatric outcomes in patients with TSC.

Methods: Retrospective analysis of a population of 45 patients (20 males, 25 females) affected by TSC, with average age, at last magnetic resonance imaging (MRI), of 14 ± 6.3 years (range 4-31 years). For each patient we evaluated the clinical characteristics, including in particular the neurological and neuropsychiatric phenotype, and the brain MR images (in particular volumetric T1 sequences). Gyrification index (GI) was measured using the CAT12 software.

Results: Patients showed significantly higher gyrification when compared with healthy controls both on the left and the right hemisphere. Significant correlations were found between gyrification and clinical and neuroradiological variables in TSC patients such as: abnormal neurological examination, epilepsy, intellectual disability, TSC-associated neuropsychiatric disorders (TAND) and number of cortical tubers.

Conclusions: Gyrification differs greatly between TSC patients and healthy controls. Gyrification and number of cortical tubers showed a clear correlation; this was expected because tubers determine a profound alteration of the cortical structure. Interestingly, we found that some TAND categories had a more accurate correlation with the gyrification index: for this reason, gyrification index and number of tubers might have an independent prognostic role in TSC patients. Further studies are needed to identify specific pathological patterns of gyrification in TSC; local gyrification index could be used to better understand the role of specific cortical area in the pathogenesis of TAND.

14. TSC-associated neuropsychiatric disorders (TAND) assessment and the neuropsychological cluster in a group of patients from Brazil

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There is a gap in worldwide evaluation and treatment of TAND manifestations in tuberous sclerosis complex (TSC) patients. A cross-sectional study was conducted with prospective data collected from TSC patients from the South of Brazil. The aims were (i) to identify the prevalence of TAND signs, and (ii) to relate clinical history with neuropsychiatric characteristics. The research was conducted at the Pediatric Neurology Center from a tertiary hospital, in Curitiba, Brazil. Forty-four patients aged 6 to 44 years with a definite clinical diagnosis of TSC were enrolled in the study after providing signed informed consent. In 37 cases genetic testing was performed. The participants were remotely assessed by the TAND checklist and clinical data was obtained. Statistical analysis has so far been performed with descriptive and bivariate analyses - Fisher's exact test and Mann Whitney U test for comparing the clinical variables with the TAND clusters. Significance threshold at 0.05. Among 44 participants, 40 (90.9%) had a history of seizures and 26 (65%) reported controlled seizures at the time of assessment. Thirty (81.1%) had a pathogenic alteration identified in the TSC2 gene, three (8.1%) in TSC1, and four (10.8%) had no mutation identified (NMI). The most frequent variant types were frameshift (21.3%), nonsense (21.2%), and missense (18.2%). All participants had at least one impairment related to TAND. Regarding specifically the neuropsychological cluster, forty-two (95.5%) participants had at least one positive response, and 13 (29.5%) affirmatively answered all questions. Thirty-eight (86.4%) had difficulties in executive functions, 33 (75%) in dual-tasking, 33 (75%) in paying attention/concentrating, 31(70.5%) in neuropsychological attention, 28 (63.6%) in memory, 26 (59.1%) in getting oriented, and 24 (54.5%) in visuo-spatial tasks. Significant associations were observed between visuospatial skills and executive functions with (1) the number of medications previously used (p<0,001), (2) the age of the first symptoms (p=0.01 and p=0.01), and (3) the type of genetic variant (p=0.03 and p=0.03). Our results are in accordance with the literature regarding the high frequency of TAND manifestations and neuropsychological difficulties, and significant association between TAND manifestations and a greater number of employed antiepileptic drugs. Further analyses will be conducted to obtain more detailed information about the TAND profile in this Brazilian sample.

15. Lysine metabolism as an emerging therapeutic target for epilepsy in tuberous sclerosis complex (TSC)

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Tuberous sclerosis complex (TSC) is a rare neurodevelopmental disease characterised by the presence of tubers in the brain which are highly epileptogenic. This causes TSC patients to suffer from severe intractable focal epilepsy. The causative genes of TSC are known as inhibitors of the mammalian target of rapamycin complex I (mTORC1) pathway. Thus, in TSC brain, there is hyperactivation of mTORC1. Inhibitors of mTORC1 such as everolimus have been the focus of mTOR therapy and while it is effective for other symptoms of TSC, seizure control on everolimus remains relatively low (30-60%). Thus, there is a need to look beyond the mTORC1 pathway to identify epilepsy-driving pathway for novel drug development in TSC. Our study uses fresh-frozen surgical tuber samples from patients with epilepsy. Integrated transcriptomics-metabolomics revealed increased lysine breakdown in the TSC brain to generate a byproduct called 2-aminoadipate. This is driven by an upregulation of the lysine catabolic enzyme, alpha-aminoadipic acid semialdehyde synthase (AASS). We observe this same increase in lysine breakdown to 2-aminoadipate in cell and animal models of TSC. Interestingly, 2-aminoadipate appears to be pro-convulsant and neurotoxic. Thus, the accumulation of 2-aminoadipate in the TSC brain could be correlated with the epilepsy observed in TSC patients. Finally, genetic knockdown of AASS appears to reduce mTORC1 activity in cell models, suggesting potential target for a new TSC treatment. We have identified small-molecule inhibitor of AASS and preliminary results appear to show promising effect in suppression of mTORC1 activity from resultant AASS inhibition. To conclude, our data suggests that lysine metabolism is increased in TSC brain to generate accumulation of pro-epileptogenic byproduct, 2-aminoadipate. Reducing accumulation of 2-aminoadipate through inhibition of the catabolic enzyme, AASS, may be a promising new approach to treat epilepsy in TSC. Future works will further characterise the mechanism by which 2-aminoadipate is pro-epileptogenic in TSC.

16. The role of CTHRC1 in the pathogenesis of TSC

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease caused by mutational inactivation of either the *TSC1* or *TSC2* tumor suppressor genes, resulting in hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1). Collagen triple helix repeat containing 1 (CTHRC1) is a secreted protein that is highly expressed in many cancers and associated with poor prognosis. CTHRC1 is implicated in tumor cell proliferation, migration, and invasion in several cancers. We have observed a 10-fold increase of CTHRC1 expression in patient-derived angiomyolipoma tissue compared to healthy kidney tissue ($p<0.0001$). Cthrc1 mRNA expression is also elevated in cellular and murine models of TSC, ranging from 6-60-fold, in *TSC1*$^{-/-}$ MEFs, *TSC2*$^{-/-}$ MEFs, and *TSC2*-deficient LAM patient-derived 621-102 cells, relative to controls. At the protein level, CTHRC1 protein is highly expressed in *TSC1/2*-deficient cells. Consistent with this, CTHRC1 extra-cellular secretion is upregulated 4-fold in TSC-deficient cells. Furthermore, Cthrc1 mRNA is elevated ~250 fold ($P < 0.0001$) in cystic kidney tissue from a ROSACreERT2 *TSC2* mouse model. CTHRC1 knockdown in *TSC2*$^{-/-}$ MEFs decreased proliferation (~2.5-fold, $P < 0.0001$) and colony formation (~7-fold, $P < 0.0001$) with no effect on *TSC2*$^{+/+}$ MEFs, suggesting that CTHRC1 may be an important diver of cell growth in TSC. Pharmacological inhibition of mTORC1 with rapamycin treatment and genetic inhibition of mTORC1 with RAPTOR knockdown did not decrease CTHRC1 mRNA or protein, indicating that the upregulation of CHTRC1 in TSC is mTORC1-independent. Transcription factors EB and E3 (TFEB/TFE3) are master coordinators of lysosomal biogenesis and autophagy; the transcriptional activity of both is hyperactivated in TSC. TFEB/TFE3 recognize a promoter sequence termed the "coordinated lysosomal expression and regulation" (CLEAR) motif. CTHRC1 contains six CLEAR motifs upstream of its transcriptional start site. Consistent with this, CTHRC1 mRNA and protein expression are decreased by TFEB/TFE3 knockdown in *TSC1*$^{-/-}$ MEFs (~2-fold, $p < 0.0001$) and 621-102 cells (~2.5-fold, $p < 0.01$). In aggregate, our results indicate that CTHRC1 expression in TSC is driven by TFEB/TFE3, and that CTHRC1 promotes cell proliferation and invasion. CTHRC1 mRNA and protein expression is insensitive to mTORC1 inhibition, suggesting a novel and potentially important role for CTHRC1 in the pathogenesis and therapy of TSC.

17. Parental stress in tuberous sclerosis complex

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See abstract on page 27.

18. Lipomorphosis from everolimus treatment in renal angiomyolipoma associated with tuberous sclerosis complex

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Background: Tuberous sclerosis complex is a genetic condition characterised by overactivation of mammalian target of rapamycin (mTOR) pathway. This aberration underlies the growth of renal angiomyolipoma (AML). In the United Kingdom National Health Service (NHS) funding for the mTOR inhibitor, Everolimus, is provided for patients with AMLs at significant risk of haemorrhage. Current guidelines state Lesions of at least 3cm and that have exhibited growth on interval imaging are eligible for treatment. Ongoing funding requires evidence of the reduction or stabilisation of AML size. After conducting a service evaluation we propose that size analysis of AML alone may be too simplistic. We present a case series in which AML size increased on Everolimus, however, the ratio of the components changed in a favourable way for the patient.

Method: Patients with renal AMLs treated with everolimus between 2016 and 2020 were identified to be included in the study. Imaging at baseline (CT or MRI) was compared with subsequent scans carried out following 6 months or longer of treatment as part of the patient's standard clinical evaluation. Patients displaying an increase in AML size were selected for further analysis of the underlying components.

Results: We identified 47 patients during the time period, of which 31 were female. Radiological analysis demonstrated an increase in AML size in 7 patients whilst on Everolimus. Further analysis showed growth was due to an increase in the lipid component of the AMLs. Reassuringly, there was a reduction of the vascular component of the AMLs.

Limitations of study: This was a retrospective review in which data was collected from patient's clinical notes during routine treatment and surveillance. There was no standardisation of imaging protocol as patients were usually imaged in their local health facilities.

Conclusions: Clinicians need to be aware that contrary to previous large studies, e.g. EXIST 2, some patients appear to experience growth of their AMLs on Everolimus treatment. However, life-saving mTORi therapy should not be withdrawn due to the increase in the total volume of AML alone. Treatment success should be measured by reduction in bleeding risk. Given the lack of vascularity in fat-rich AMLs within this patient cohort, despite the observed growth, the risk of bleeding remains low. These findings are in keeping with those previously described by Watanabe with sirolimus, another mTOR inhibitor.

19. Evaluating the etiology of cortical tubers in tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is a genetic neurodevelopmental disorder caused by mutations in the *TSC1/TSC2* genes and characterized by benign growths in many organ systems. Within the brain, dysplastic cortical lesions called tubers emerge during fetal development. Although tuber growth appears to stop postnatally, repercussions do not. TSC patients suffer debilitating neurological sequelae from these tubers, including epilepsy, autism, and neuropsychiatric disorders. The exact molecular mechanisms underlying tuber development remain unclear. In this study, we sought to understand TSC-specific changes in cellular identity during cortical development and their association with tuber formation. Using CRISPR-Cas9 technology, we generated isogenic lines of TSC patient-derived induced pluripotent stem cells (iPSCs) with pathogenic mutation in the *TSC2* gene. iPSCs were differentiated into neural lineages and collected at several timepoints for mass cytometry analysis. Compared to $TSC2^{+/+}$ and $TSC2^{+/-}$ neural lineage cells, a subset of *TSC2* mutant cells with a biallelic hypomorphic six amino acid deletion possessed a distinct cellular identity with unique populations exhibiting upregulated mTOR-specific and mTOR-independent markers, including p-S6 240/244, p-STAT3 S727, and YAP1. Mass cytometry analysis of resected tubers from TSC patients with epilepsy also showed distinct cellular identities with unique populations of cells exhibiting elevated expression of mTOR downstream targets and combinations of stem cell / astrocytic markers. Taken together, these results indicate that cell-specific changes during cortical development may play a role in tuber formation and can be adequately modeled using human iPSCs.

20. Van Andel Institute's biospecimen core resource for the TSC Alliance®

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TSC Alliance, Silver Spring, MD, USA (EC, ZF, DA, SR); Van Andel Insititute, Grand Rapids, MI, USA (TE, DR, GH, MD, AB, MK, BL, CM, LT, SW, BB)

Background: The TSC Alliance is an internationally recognized nonprofit that does everything it takes to improve the lives of people with TSC. They drive research, improve quality care and access and advocate for all affected by the disease. The TSC community is their strongest ally. The collaboration of individuals and families, along with the partnership of other organizations, fuels their work and ensures people navigating TSC have support—and hope—every step of the way. The Van Andel Institute's (VAI) Pathology and Biorepository Core (PBC) serves as the Biospecimen Core Resource (BCR) for the TSC Alliance. The TSC BCR houses biosamples linked to detailed clinical data in the TSC Natural History Database, which will enable researchers to discover biomarkers, establish human cell lines or tissue arrays for drug testing, and search for clues to understand why TSC differs from person to person.

Methods: The VAI PBC laboratories for histology, molecular processing, kit making, and biorepository, and a robust quality management program serve large research and clinical trial projects, such as NIH and NCI projects. The PBC provides and manages, standard operating procedures (SOP), biospecimen collection kits tailored specific to each project, tracks shipping, specimen receipt and adherence to protocol verification, specimen processing, such as plasma, serum, cell separation, and tissue pathology verification followed by downstream extraction of nucleic acids, when requested. The VAI PBC BCR uses the Biological Specimen Inventory (BSI) system developed by Information Management Systems (IMS) and uses the Leica Biosystems Aperio digital pathology platform for slide imaging and review. The VAI PBC is accredited by College of American Pathologists (CAP) for both the Biorepository Accredited Program (BAP) and CLIA.

Results: As of April 2023, The BCR has stored over 34,000 samples from over 1,500 subjects. These samples have come from several TSC studies including RDCRN, NHD, PREVeNT, and STEPS.

Conclusions: The Van Andel Institute's PBC provides a proficient and valuable resource to the TSC Alliance, and other foundation funded, federally funded, and clinical trial biorepository programs.

21. *TSC2* coordinates differentiation

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Multipotent neural stem cells (NSCs) that reside within the ventricular-subventricular zone (V-SVZ) of the brain generate a variety of cell types. The number and diversity of cell types produced is controlled by genetically encoded transcription factors that regulate transcriptional programs and orchestrate core mRNA availability. The uncoupling of transcript availability and mRNA translation occurs during the progression from stem to differentiated states. The mTORC1 kinase pathway acutely controls proteins that regulate mRNA translation. Inhibiting mTORC1 during differentiation is hypothesized to be critical for brain development since somatic mutations of negative regulators of mTORC1 result in abnormal brain architecture. Here it is demonstrated that in vivo loss of *TSC2* which occurs in the developmental disorder tuberous sclerosis complex (TSC) causes striatal hamartomas. Hamartomas contained a variety of cell types including cytomegalic neurons with hypertrophic dendrite arbors. Whole transcriptome profiling of NSCs was performed to uncover mechanistic insight into how loss of *TSC2* leads to hamartoma formation. We found that *TSC2* removal altered the abundance of NSC translation factor encoding mRNA transcripts. However, the amount of translation factor transcripts did not accurately predict the respective abundance of encoded proteins. This discordance was associated with altered mutant NSC translatomes and aberrations in translational efficiency. Single nuclei RNA sequencing following in vivo loss of *TSC2* confirmed changes in translation factor mRNAs and NSC activation states. Ultimately, the inability to decouple mRNA transcript availability and translation delayed differentiation leading to the retention of immature phenotypes in hamartomas. Importantly, new studies utilizing single nuclei RNA sequencing of human patient SEGAs reveal convergent mechanisms involved in TSC hamartoma formation. Taken together, *TSC2* is required for translational repression and differentiation, which may have physiological and pathophysiological significance.

22. Characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment in TSC

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Tuberous sclerosis complex (TSC) is caused by mutations in the genes *TSC1* or *TSC2* and characterised by the formation of benign tumours in multiple organs, including the brain. In patients, the greatest morbidity comes from neurological lesions including cortical tubers, subependymal nodules and subependymal cell astrocytomas (SEGAs), as well as manifestations like epilepsy, developmental delays and neuropsychiatric disorders. *TSC1* and *TSC2* are negative regulators the mTOR complex 1 (mTORC1) signaling pathway. Manipulation of mTORC1 signaling in animal models has shown that this pathway has key roles in neurogenesis. However, the molecular mechanisms linking mTORC1 hyperactivity to the neurological features of TSC are poorly understood, in part because the targets of mTORC1 during neurodevelopment have not been systematically characterised. To systematically characterise the mTORC1-dependent phosphoproteome during neurodevelopment, we used an unbiased Tandem Mass Tag and quantitative mass spectrometry approach on embryonic day 16.5 brain tissue from mice with a conditional knockout of Tsc1 in the brain using Nestin-Cre (*Tsc*1cKO). In two independent experiments, we performed total proteomics and phosphoproteomics of *Tsc1*cKO mice compared to controls, and *Tsc1*cKO mice treated with rapamycin compared to vehicle, to activate and inhibit the mTORC1 pathway respectively. We identified over 30,000 phosphopeptides and over 7000 proteins in both experiments and observed expected changes in phosphorylation of established mTORC1 targets including rpS6 and 4E-BP2, validating this approach. We then developed an analysis pipeline to identify high confidence, novel mTORC1 targets. Through this pipeline we identified 25 novel targets of mTORC1 in the developing brain. The functions of these proteins include transcriptional regulation, chromatin remodelling, regulation of ubiquitination and regulation of small GTPases. Moreover, mutations in several of these novel mTORC1 targets in patients cause neurodevelopmental disorders associated with epilepsy and intellectual disability. To validate the novel mTORC1 targets in TSC we also performed quantitative phosphoproteomic analysis of surgical tuber and SEGA tissue from TSC patients. Our characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment reveals novel molecular mechanisms and identifies potential new therapeutic targets for the neurological manifestations in TSC.

23. Tuber functional connectivity with somatomotor vs visual cortices as well as the cerebellum is associated with differential ADOS subscale severity in TSC

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Boston Children's Hospital: WXH, GM, MS, JP, SKW Center for Brain Circuit Therapeutics, Brigham and Women's Hospital: WD Cincinnati Children's Hospital: DK University of Alabama at Birmingham: MB McGovern Medical School at University of Texas Health Science Center: HN Ann & Robert H. Lurie Children's Hospital of Chicago: JW

Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting 1:54 people in the United States that results in altered social communication, restrictive/repetitive patterns of behavior, interests, and activities. Individuals with tuberous sclerosis complex (TSC), a rare genetic disorder resulting from *TSC1/TSC2* mutations, are at high risk of developing ASD (40%) and have focal brain lesions present from birth that affect surrounding brain tissue function. Because ASD in individuals with TSC is behaviorally indistinguishable from idiopathic/non-syndromic ASD, these individuals may provide unique lesion-based insight into the neuroanatomical substrate of ASD symptoms. Here, we leverage consortium-level data, high-quality consensus autism scoring, and lesion network mapping to identify tuber locations in 115 children with TSC with available Autism Diagnostic Observation Scale (ADOS) scores. We found that social affect (SA) severity scores are related to positive functional connectivity between tuber distributions in somatomotor areas, the medial prefrontal cortex, and a specific location in the cerebellum, and negative functional connectivity with visual association areas. Conversely, more severe repetitive and restrictive behaviors (RRB) severity scores were associated with an opposing network of cortical functional connectivity. Total ADOS score is comprised of the sum of the SA and RRB scores, and with the same analysis did not identify strong functional connectivity networks. The identification here of opposing SA- and RRB-related networks, leveraging the unique focal lesion-associated nature of TSC, may help to explain why neuroimaging research of ASD using overall severity has produced heterogenous results. The findings here have significant implications for the development of biomarkers for early diagnosis and focal treatment targets for autism.

24. Everolimus enhances pro-metastatic potentials of LAM-derived exosomes

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Lymphangioleiomyomatosis (LAM) is metastatic low-grade sarcoma that primarily affects reproductive-aged women. LAM can be sporadic or in association with tuberous sclerosis complex (TSC). TSC is caused by germline mutations in the *TSC1* or *TSC2* tumor suppressor genes and manifests as multiple central nervous system tumors, renal angiomyolipomas, and pulmonary LAM. Sporadic LAM and angiomyolipoma are caused by somatic *TSC1* and *TSC2* mutations resulting in dysregulation of the mechanistic target of rapamycin (mTOR), therefore, rapamycin analogs (rapalogs), which inhibit mTORC1, are approved for TSC and LAM. While studies have shown that this therapy stabilizes the lung function, its discontinuation resulted in progressive decline in the lung function among some patients with LAM, indicating the need for discovery of new therapeutic targets. Exosomes (EVs) are natural membranous vesicles with unique biological and pharmacological properties. It is well established that EVs contribute to cancer progression and metastasis by reprogramming stromal cells, remodeling the architecture of extra cellular matrix, and normal cell phenotypes through the transfer of bioactive molecules between cancer and cells in local and distant microenvironments. We previously reported that EV derived from *TSC1*–null neuronal progenitors block differentiation of recipient wild-type progenitors via activation of the Notch1/mTOR pathways, phenocopying *TSC1*-null cells. Therefore, in this study we investigated the effect of Everolimus on *TSC*-null EV release and content to determine its impact on progression of LAM. Here, we show that treatment with Everolimus of *TSC2* null surrogate LAM cells (621-101) increased extracellular vesicle (EV) release and enriched these EVs with the Integrin-beta1, Integrin-alpha6, proto-oncogene cellular tyrosine kinase Src (c-Src), SRY-box transcription factor 10 (SOX10), MMP-2, focal adhesion kinase and CD44. In addition, EVs derived from Everolimus treated 621-101 cells increased stem cell-like characteristics of LAM surrogate cells, such as increased sphere-forming ability, aldehyde dehydrogenase (ALDH) activity, migration and invasion. Taken together, these results imply that EVs released by LAM surrogate cells treated with Everolimus promote EV biogenesis and LAM cancer stem-like phenotypes by modulating integrins and integrin-mediated signaling pathways.

25. Design of a double-blind, randomized, cross-over, placebo-controlled Phase 2 trial of basimglurant as adjunctive therapy in patients with seizures associated with tuberous sclerosis complex (GALENE)

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Purpose: Emerging evidence indicate that overactivation of metabotropic glutamate receptor type 5 (mGluR5) is involved in a number of central nervous diseases, in particular tuberous sclerosis complex (TSC). Here we describe the design of an ongoing phase 2 trial assessing basimglurant, a selective negative allosteric modulator (NAM) of mGluR5, in patients with TSC.

Method: Basimglurant is a potent mGluR5 NAM with selectivity > 1000-fold compared to other mGluR NAMs. An optimized modified release oral formulation demonstrates robust brain occupancy, good bioavailability, and half-life, and allows once daily dosing. In previous clinical trials, basimglurant has been well tolerated in children and adolescents. GALENE is a Phase 2B, multicenter, 30-week, prospective, cross-over, double-blind, randomised placebo-controlled trial (NCT05059327) to evaluate the efficacy and safety of basimglurant. Children, adolescents, and young adults with a documented history of TSC with seizures currently taking at least 1 antiseizure medication (ASM) are being enrolled. After a 4-week stabilization (Period 1), participants are randomized to basimglurant or placebo for 12 weeks (Period 2). After a 2-week wash-out (Period 3), they are randomized to the other treatment arm for another 12 weeks (Period 4). Eligible participants are offered access to a one-year open label extension period.

Results: Enrolment is ongoing aiming to enrol 54 participants. The primary endpoint is monthly seizure counts during the 12-week maintenance dosing in Period 2 and Period 4. Secondary efficacy endpoints include impact on functioning, number of treatment responders, and longest seizure free interval. In addition to safety and tolerability assessments, impact on seizure type is assessed. Conclusion: The GALENE trial will characterise the efficacy and safety profile of the novel mGluR5 NAM basimglurant in patients with uncontrolled seizures associated with TSC. This trial is funded by Noema Pharma AG.

26. Altered differentiation and astrocyte reactivity in a human brain organoid model of tuberous sclerosis complex

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In this work, we used genetically-engineered human brain organoid models of TSC to determine how loss of *TSC2* affects transcriptional programs and cell fate during neural development. Loss of heterozygosity of *TSC2* is one hypothesis explaining the emergence of cortical tubers in patients with TSC, which arise during brain development and contain aberrant cell types such as dysmorphic neurons, dysplastic astrocytes and giant cells. While these tubers are a significant source of morbidity, the developmental origins and molecular mechanisms underpinning tuber cell formation are not well understood. We generated a conditional knock-out model consisting of stem cells with one non-functional *TSC2* allele and one functional allele that can be disrupted through the addition of Cre recombinase. Exposure to a low dose of Cre during neural differentiation generates a subpopulation of $TSC2^{-/-}$ cells within a background of $TSC2^{+/-}$ cells in the same organoid. Using single-cell RNA sequencing, we find that $TSC2^{-/-}$ cells preferentially generate astrocyte progenitors and astrocytes compared to $TSC2^{+/-}$ cells in the same organoid. Additionally, we find that $TSC2^{-/-}$ cells have numerous general and cell type-specific gene expression changes, including in genes associated with proteostasis, autophagy, and reactive astrogliosis, which were validated in primary tuber samples resected from TSC patients. Together this work reveals that loss of *TSC2* during development strongly biases developmental trajectories toward the generation of astroglial cells that show transcriptional hallmarks of reactivity.

27. Sleep and its association with temperament and sensory processing in early TSC

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Background and Aim: Tuberous sclerosis complex (TSC) is multisystem genetic disorder which is associated with a range of cognitive, behavioural and neurological manifestations. In children with TSC, sleep problems are well documented however its association with other behavioural manifestations, such as temperament traits and sensory dysregulation are not well characterised. The established relationship between sleep disruption and daytime behaviours indicates that prospective measurement of temperament and sensory processing may be key to understanding the mechanisms which underlie sleep problems in TSC. Here, we aimed to establish the association between sleep parameters, temperament and sensory processing in TSC.

Methods: The Early Development in Tuberous Sclerosis Complex study involved completion of home visits at ages 3, 5, 8, 10, 14, 18 and 24 months to collect data on infants' behavioural and neurocognitive development. A total of 33 infants with TSC and 34 typically developing infants were recruited up to 14 months of age to take part in a minimum of three consecutive visits. Here, we will be focusing on data at 10, 14 and 24 months old. Parent-report questionnaires and interviews were used to collect data on: (1) Sleep duration, onset latency and settling (Sleep and Settle Questionnaire and a Sleep Diary at 10, 14 and 24 months), (2) temperament (Early Childhood Behaviour Questionnaire at 24 months) and (3) sensory processing (Infant Toddler Sensory Profile at 10, 14 and 24 months).

Results: At 14 months old, morning sleep was significantly longer in TSC infants ($p=.006$) compared to TD infants. Longer duration of daytime settling in infants with TSC at 14 months old was associated with reduced attentional focus ($p=.02$), but no other dimensions of temperament. Both longer morning sleep and daytime settling were associated with reduced visual ($p<.003$) and auditory processing ($p<.006$) at 14 months old. Longer duration of night awakenings at 24 months old in TSC infants ($p=.04$) was associated with negative affect ($p=.03$) and effortful control ($p=.01$). No significant group differences were observed in sleep patterns at 10 months old.

Discussion: Early sleep parameters in TSC are associated with specific dimensions of temperament and visual/auditory processing difficulties. Early comprehensive assessments of behavioural manifestations may be key for improving sleep outcomes in TSC and may aid in the design and targeting of effective early intervention.

28. Discovering the neuropathological etiology of tuberous sclerosis complex associated neuropsychiatric disorders

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Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder that affects 50,000 people in the United States and nearly 1,000,000 people worldwide. These patients experience nonmalignant masses involving various organ systems due to mutations in TSC1 or TSC2 with an autosomal dominant inheritance pattern that leads to the hyperactivation of the mammalian Target of Rapamycin (mTOR) pathway. The mTOR pathway is a centralized intracellular pathway that regulates cell growth. Additionally, TSC patients experience neuropsychiatric symptoms collectively named Tuberous sclerosis complex Associated Neuropsychiatric Disorders (TAND). In 2019 as the lead author, we published an article in JAMA Neurology which established that TAND symptoms and cerebrospinal fluid biomarkers in TSC patients significantly overlap with a variant of Alzheimer's Disease (AD). Neuropsychological testing in TSC subjects from this study revealed significant impairment in the executive function and memory domains more commonly seen in AD. Furthermore, cerebrospinal fluid biomarkers in adult TSC subjects showed significantly elevated phosphorylated tau-181 (pTau-181) levels with normal amyloid-beta (Aß42) levels. We believe that TSC represents a novel human model to study AD-related pTau independent of amyloid accumulation. In 2021, it is estimated that 6.2 million Americans aged 65 or older live with Alzheimer's Disease dementia. The well-established neuropathological substrates include extracellular amyloid plaques composed of Aß42 and intracellular neurofibrillary tangles comprised of pTau. There is currently an unmet need in AD research to establish a human model that displays the accumulation of pTau in brain tissue independent of amyloid accumulation. This is because accumulation of pTau, but not Aß42, closely correlates with progression of clinical symptoms in AD. Investigating TSC can fill this need since a recently published neuropathological study by our group further investigated TAND symptoms in TSC patients. Results from this study showed that TSC brain tissue accumulates an AD-related pTau in brain tissue. Unlike AD, Aß42 staining in TSC brain tissue was absent unlinking Aß42 from AD-related pTau pathology. We currently believe that TSC accumulates a tauopathy commonly seen in AD that develops independent of amyloid accumulation.

29. *TSC1* mutant bladder cancer is characterized by a TSC-associated gene expression signature due to TFE3 transcriptional activity

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Mutations and inactivation of the tumor suppressor gene *TSC1* are recurrent (6-10%) events in bladder cancer, but the mechanism by which *TSC1* loss promotes bladder cancer development beyond mTORC1 activation is uncertain. Here, we characterized *TSC1*-mutated bladder cancers (*TSC1*mtBLCA, n=26) with *TSC1* wildtype tumors (*TSC1*wtBLCA, n=382) from The Cancer Genome Atlas (TCGA) data set and compared them to an internal cohort of *TSC1/TSC2*-mutated tumors (n=48). We identified 26 genes upregulated in *TSC1*mt-BLCA out of 60 genes commonly upregulated in tuberous sclerosis complex (TSC) tumors. Among them, GPNMB showed the greatest increase in expression. Gene Set Enrichment Analysis (GSEA) and differential gene expression (DESeq2) analyses implicated activation of both mTORC1 and lysosomal pathways in *TSC1*mt-BLCA. Expression differences in several genes, including GPNMB, and in pS6 were confirmed by immunohistochemistry (IHC) of 5 independent *TSC1*mt-BLCA and *TSC1*wt-BLCA specimens each. TFE3, a key regulator of lysosomal gene expression, was elevated in expression and localized to the nucleus in *TSC1*mt-BLCA by IHC. Molecular studies of *TSC1*-mt/wt BLCA isogenic cell lines recapitulated the phenotype found in human tumors. TFE3 was both post-translationally modified (assessed by immunoblot) and predominantly nuclear in *TSC1*-null cell lines compared to *TSC1*wt cells, and the localization was partially reversed by rapamycin treatment. *TSC1*mt-BLCA HCV29 cells showed higher expression of multiple genes increased in *TSC1*mt-BLCA tumors (GPNMB, SQSTM1, GNPDA1). TFE3 knock-out HCV29 clones showed markedly reduced growth, which was not seen with TFE3 KO in *TSC1*-addback HCV29 cells. GSEA of RNA-seq data indicated that TFE3 KO led to significant reduction in expression of lysosome genes, as well as those involved in DNA/RNA-related processes. TFE3 CUT&RUN confirmed that TFE3 binds to promoter regions of lysosome genes, thereby directly regulating these pathways. In contrast, TFE3 KO led to an increase in inflammatory and cellular stress genes that could impede *TSC1*-null cell growth. Our findings revealed that *TSC1*mt-BLCA tumors and cell lines share a transcriptional signature with other TSC-associated tumors and show nuclear localization and transcriptional activation of TFE3. Aberrant TFE3 activation likely contributes to *TSC1*mt-BLCA development and may therefore be amenable to targeted therapy.

30. Single-cell transcriptomic analysis of tuberous sclerosis complex (TSC)

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Tuberous sclerosis complex (TSC) is a multi-organ genetic disorder, with up to 90% developing intractable epilepsy at a very early age that often requires special medical attention. Seizures generally arise from cortical tubes (CTs), a hallmark neuropathological manifestation in TSC. Despite the clear involvement of TSC gene mutations (*TSC1/TSC2*) in the pathogenesis, it is unclear how these molecular lesions drive the genesis of CTs and maintain the prominent cellular abnormalities in CTs that may underlie epileptogenicity. To this end, we conducted single-nucleus RNA sequencing analysis of surgically resected CT specimens to establish a comprehensive taxonomy of transcriptomic cell types within this epileptogenic brain lesion. In parallel, the same approach was applied to age/sex-matched autopsy cases as controls to generate a transcriptomic atlas against CT data to identify specific cell subpopulations and cell type-specific transcriptomic signatures in CTs. To link each molecularly distinct cell population to CT-specific morpho-electric cell types including balloon cells and cytomegalic cells, Patch-seq combined with a spatial transcriptomic approach was applied to the same CT specimen. While CT is conceived as abnormal tumor-like tissue, our analysis indicated that cortical cellular composition is generally preserved in CT, and all major cortical cell types defined in the normal cortex could find their molecular correspondences in CT. However, we revealed that 40% of Patch-seq cells identified as L2/3 excitatory neurons exhibited a transcriptomic profile characteristic of L5/6 excitatory neurons, suggesting a potential source of dyslamination. Additionally, the majority of Patch-seq balloon cells were mapped to a molecular subtype of reactive astrocytes. Furthermore, by comparing with age- and sex-matched autopsy controls, we uncovered the profound cell-type specific transcriptomic signatures due to *TSC1/2* deficiency. One of the most significant signatures was upregulation or aberrant expression of drug transporters (ABC genes) at specific cell types. Specifically, Pgp (ABCB1) was aberrantly expressed in excitatory and inhibitory neurons in CTs, while MRP (*ABCC1*) gene was upregulated in astrocytes. In summary, by applying an integrative scRNA-seq approach, our study provided a multi-level, single-cell view of CT, one of the most readily available epileptogenic brain lesions for research, providing an unprecedented insight into how single-gene mutation.

31. Translatome analysis of tuberous sclerosis complex patient-derived isogenic neural progenitor cells

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Tuberous sclerosis complex (TSC) is caused by mutations in *TSC1* or *TSC2* genes and patients are often diagnosed with neuro-developmental (ND) manifestations termed TSC-associated neuropsychiatric disorders (TAND) that includes autism spectrum disorder (ASD). Hamartin (*TSC1*) and tuberin (*TSC2*) proteins form a complex inhibiting mechanistic target of rapamycin complex 1 (mTORC1) signaling. Loss of *TSC1* or *TSC2* activates mTORC1 that, among several targets, controls protein synthesis by inhibiting translational repressor eIF4E-binding proteins (4E-BPs). Using *TSC1* patient-derived neural progenitor cells (NPCs), we recently reported early ND phenotypic changes, including increased cell proliferation, and altered neurite outgrowth in CRISPR-modified *TSC1*-null vs isogenic WT NPCs, which were unaffected by the mTORC1 inhibitor rapamycin. Here, employing polysome-profiling to quantify changes in translational efficiencies at a transcriptome-wide level, we observed numerous *TSC1*-dependent alterations in mRNA translation in NPCs, which were largely recapitulated in post-mortem ASD donor brain samples. While rapamycin treatment partially reversed the altered *TSC1*-associated translation, most genes related to neural activity/synaptic regulation or ASD remained rapamycin-insensitive. Treatment with the third-generation, bi-steric mTORC1 inhibitor RMC-6272 revealed inhibition of rapamycin-insensitive translation and rescue of *TSC1*-associated ND phenotypes of increased proliferation and neurite outgrowth. More recently, as with our *TSC1* NPCs, we have generated CRISPR-modified isogenic *TSC2* patient-derived NPCs and observed similar mTORC1 signaling signatures and ND phenotypic changes in *TSC2*-null vs isogenic WT cells. Interestingly, polysome-profiling of *TSC2*-null NPCs has revealed significant overlap in translationally regulated genes between *TSC1* and *TSC2* NPCs, as well as regulation distinct to *TSC1* or *TSC2*. Gene enrichment analysis of translatome data has uncovered a subset of regulated genes associated with ASD and neurodevelopmental disorders, and further analysis of these data is underway. In summary, our work demonstrates *TSC1*-dependent translation overlapping with *TSC2*-null NPC, as well as distinct regulation. In addition, we also show rescue of rapamycin-insensitive ND phenotypes by RMC-6272, thus unveiling potential implications for more efficient targeting of mTORC1 as a superior treatment strategy for TAND.

32. Co-creation of EpiCom Phase 4 study design in collaboration with the tuberous sclerosis complex (TSC) community of patients, caregivers, healthcare professionals, and a pharmaceutical company

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Background: Treatment options for TSC-associated neuropsychiatric disorders (TAND) are limited. Psychiatric and behavioral manifestations associated with TAND are difficult to measure with generic instruments because of limited validation, acceptance, and relevance. Plant-derived highly purified cannabidiol (CBD; Epidiolex, 100 mg/mL oral solution) is approved for treatment of seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, and TSC. The TSC community of patients, caregivers, and healthcare professionals (HCPs) have also reported anecdotal evidence of other neuropsychiatric benefits, including improvements in behavior, cognition, and psychiatric symptoms. To evaluate behavioral outcomes associated with add-on CBD in people with TSC-associated seizures, this phase 4 study was designed in collaboration with the TSC community.

Methods: The study design approach began by identifying HCPs through conference activities, publications, and clinical trials and finding patient and caregiver participants through patient advisory groups (PAG) at national and international levels. An initial advisory (Ad) board with PAGs provided feedback on the need to investigate the impact of antiseizure medications on TAND and ways to collaborate with patients/caregivers. Through pre-meeting surveys, participants helped co-create Ad board topics, including prioritization of TAND elements for evaluation, study design and feasibility, study population, and measurement tool suitability and feasibility. Feedback from a patient/caregiver Ad board was incorporated into an HCP Ad board. Integrated input from both Ad boards was used to design the study. A joint patient–HCP steering group was established to advise throughout the study period.

Results: Input from 9 US and European patient organizations and 8 global HCPs was used to design EpiCom (Epilepsy Comorbidities), a multicenter, open-label, single-arm, decentralized phase 4 study (with flexibility of virtual/in-person visits) to assess behavioral outcomes associated with add-on CBD in people with TSC-associated seizures.

Conclusions: The co-creation study design approach can strengthen the collaboration between patients, caregivers, and HCPs, which in turn may increase the potential for efficient study execution, data dissemination, and impact on patient care. It may also provide a template for design of other trials incorporating input from all stakeholders to directly impact the future of patient care.

33. OV329, a next-generation GABA-AT inhibitor, is a potent anticonvulsant in mouse models of epilepsy

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GABA, the primary inhibitory neurotransmitter in the adult CNS, is catabolized by GABA-amino transferase (GABA-AT). Therefore, inactivation of GABA-AT can elevate GABA levels within the CNS and potentially reduce neuronal hyperexcitability associated with seizures. For example, vigabatrin (VGB), a GABA-AT inhibitor, was approved to treat infantile spasms (IS) and seizures, two common clinical manifestations associated with tuberous sclerosis complex disorder. Due to potential adverse ocular effects of VGB, broader clinical application has been limited. Compared to VGB, OV329 is highly potent and holds the potential to be the best in-class drug with efficacy without adverse ocular effects at potential therapeutic doses. The efficacy of OV329 in controlling seizures was tested in two mouse models; 1) NMDA-induced IS and 2) kainic acid (KA)-induced mesial temporal lobe epilepsy (MTLE). In the IS model, vehicle treated mice (postnatal day 13), injected with NMDA, exhibited seizure activity ranging from Grade 1 (twitching, tail flicking) through to Grade 5 (severe convulsions with uncontrollable bouncing) in the chamber. OV329-treated mice at all doses (0.01, 0.1 and 1.0 mg/kg) had significant decreases in overall seizure severity following NMDA injections. The decrease in seizure severity also corresponded to increased animal survival. OV329 was highly effective at delaying the onset of NMDA-induced flexion seizures and reduced the total number of flexion seizures during the observation period. To test the efficacy of OV329 in a focal seizure animal model, MTLE was induced by injecting KA into the dorsal hippocampus and hippocampal paroxysmal discharges (HPDs) were monitored by depth electrode recordings. Four weeks after injury, OV329 (0.3, 1.0, or 3.0 mg/kg/day) or vehicle was administered for 8 days followed by a ~2-week washout period. HPD numbers and duration were evaluated up to 23 days. Once daily dosing of 3 mg/kg OV329 for 8 days significantly reduced the number of HPDs (70 ± 9% at day 8) compared to the baseline. A similar treatment also resulted in significant reduction (~35%) of GABA-AT activity in brain. In conclusion, OV329 was highly effective in reducing both flexion seizures and HPDs, characteristics of IS and MTLE respectively. Finally, low and multiple doses of OV329 may be a viable strategy for treating patients, which may also help avoid potential adverse effects without compromising efficacy.

34. Cannabidiol (Epidyolex) for severe behavioral manifestations in individuals with TSC: protocol for a series of N-of-1 trials

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Introduction: TSC-associated neuropsychiatric disorders (TAND) can be severe, and are often refractory to therapy. Cannabidiol (CBD) has been shown to be effective as an add-on therapy for refractory seizures associated with TSC. There are also indications from previous (case) studies and patient reports that CBD may be an effective treatment for severe behavioral manifestations, although clear evidence is lacking. Our objective is to examine the effectiveness of CBD on irritability and other behavioral manifestations in children and adults with TSC, proposing an alternative trial design since traditional randomized controlled trials are complex in rare and heterogeneous patient populations.

Methods: We aim to conduct a series of N-of-1 studies, which are placebo-controlled, double-blind, randomized, multiple cross-over trials. The treatment is oral CBD (Epidyolex) twice daily with dosages based on an individual titration phase at the start of the trial. Based on a power analysis, we aim to include 12 children and adults aged =6 years with clinically or genetically confirmed TSC and suffering from severe behavioral manifestations as assessed with a minimum score of 4 on the Clinical Global Impression severity scale. The primary outcome measure is the subscale irritability of the Aberrant Behavior Checklist. Secondary outcomes include behavioral and psychiatric outcomes, such as aberrant behavior, anxiety, depression, mood, communication and sensory processing, and quality of life. The impact of TSC on all life domains will be measured using the recently developed TSC-PROM. Also, personalized treatment goals, parental stress, seizure frequency, and adverse effects of CBD will be measured. Statistical analysis includes a mixed model analysis. All participants receive an assessment of their individual treatment effect and data will be aggregated to investigate the effectiveness of CBD for behavioral manifestations in TSC.

Conclusions: This N-of-1 study addresses an unmet patient need and will provide information on the effectiveness of CBD for severe behavioral manifestations in TSC. This protocol can be used as an example to empower other researchers to conduct N-of-1 studies in TSC and other rare genetic disorders, providing a much-needed bridge between science and practice to optimize evidence-based and personalized care.

35. Understanding the impact of TSC: development and validation of the TSC-PROM

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Introduction: More insight into the impact of TSC on functioning will help improve care and surveillance. The impact of TSC can be measured by patient-reported outcome measures (PROMs). The aim of this study was to develop a TSC-specific PROM for adults that captures the impact of TSC on all relevant life domains, including physical functioning, mental functioning, activities and participation, social support, and quality of life.

Methods: COSMIN methodology was used to develop a self-report and proxy-report version. In the developmental phase, relevant themes were identified using patient-driven data, the TSC-Associated Neuropsychiatric Disorders (TAND) checklist, Lifetime Version, literature and expert groups. The International Classification of Functioning and Disability was used as a framework. Content validity was examined by a multidisciplinary expert group and cognitive interview study. Structural and construct validity, and internal consistency were examined in a large international cohort, using confirmatory factor analysis, hypotheses testing, and Cronbach's alpha.

Results: The study resulted in an 82-item self-report version and 75-item proxy-report version of the TSC-PROM with four subscales. Sufficient results were found for structural validity. With regard to construct validity, 82% of the hypotheses were met for the self-report version and 59% for the proxy-report version. The TSC-PROM showed good internal consistency (Cronbach's alpha 0.78-0.97).

Conclusion: We developed a PROM for adults with TSC, named TSC-PROM, showing sufficient evidence for internal consistency reliability and validity that can be used both in clinical and research settings to systematically gain insight into the experiences of adults with TSC. It is the first PROM in TSC that addresses the impact of specific TSC manifestations on all life domains, providing a valuable, patient-centered addition to the current clinical outcomes.

36. Modeling tuberous sclerosis complex with 3-D human cortical organoids

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutations in either *TSC1* or *TSC2*, presenting with systemic growth of benign tumors. In addition to the brain lesions, neurologic impairment causes the greatest morbidity in TSC patients. Approximately 50% of TSC patients are affected with autism spectrum disorders (ASDs), including wide range of autistic symptoms overlapping with that in idiopathic ASD patients. Studies from animal or human stem cell models with *TSC1/2* knockout suggest that hyper-activation of mTORC1 signaling due to TSC deficiency may lead to aberrant neurodevelopment. However, how the causal variants of *TSC1/2* genes identified in TSC patients affect human cortical development and how they contribute to the neurological manifestations in TSC remain largely unexplored. To address these questions, here we generated 3-D cortical organoids from induced pluripotent stem cells (iPSCs) derived from TSC patients carrying *TSC2* mutations as well as healthy controls. To investigate how *TSC2* mutations affect the molecular trajectories during cortical development, we generated transcriptome profiles across several developmental stages of TSC and control organoids. Gene regulatory network analyses at four stages (day 35, 56, 98, and 126, respectively) revealed significantly up-regulated gene pathways involving neurogenesis, and chemical synaptic transmission during long-term organoid development compared to controls. We observed that multiple genes involved in neurodevelopment, synapse, and glial cells, including *SYN, SYT1, BCL11B, GRIN1, NNAT*, and *S100B*, were upregulated during organoid development. Moreover, the differentially expressed genes in TSC organoids are significantly enriched among genes associated with ASD, schizophrenia, intellectual disability, and epilepsy. To further understand the specificity and complexity of the impact of TSC2 mutations on human brain development at the single-cell level, we performed single-cell RNA-seq (scRNA-seq) on TSC and control organoids. The scRNA-seq and time trajectory analyses suggest that the *TSC2* mutations lead to alternative and differential developmental progression. Additionally, multielectrode array recording analyses demonstrate increased neuronal network activity in TSC organoids. Collectively, our study illustrated that disease-associated *TSC2* mutations may impair neurodevelopment by the perturbation of gene regulatory networks during early cortical development.

37. Development of a novel mouse model to study *TSC2* mutation in hematopoietic cells and its impact on TSC lesions

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Tuberous sclerosis complex (TSC) is caused by germline loss of function mutation of either the *TSC1* or *TSC2* tumor suppressor genes, affecting multiple organs including the brain, heart, kidney, lung and skin. The hallmark of *TSC1* and *TSC2* deficient cells is therefore hyperactivation of mammalian target of rapamycin complex 1(mTORC1), which is believed to be primary driver of tumorigenesis in TSC. Finding a suitable preclinical model is crucial for studying TSC pathologies. To determine the role of *TSC2* mutation in hematopoiesis and its impact on other organs, we deleted *TSC2* in hematopoietic cells using a Vav-iCre driver line.The cell specificity of Vav-iCre-driven *TSC2* deletion was assessed by including a Cre-mediated reporter mT-mG and analyzing mG expression by FACS. Consistently with the previous publications, Vav-iCre specifically targets hematopoietic cells and some endothelial cells in certain organs such as bone marrow. However, pulmonary vascular endothelial cells were not affected. In Vav-iCre/*TSC2* knockout mice, a new pulmonary phenotype with perivascular cell infiltration and proliferation was detected, which appeared as epitheloid cell clusters. These cells were negative for epithelial cell marker, (e.g., Cdh1), endothelial cell marker, (e.g., Pecam1/CD31), and lymphatic endothelial cell marker, (e.g., Lyve1) by immunofluorescence staining. Interestingly, they had strong expression of lysosomal marker Npc1, Further characterization of pulmonary and extrapulmonary lesions are undergoing. Our data suggest that *TSC2* mutation in hematopoietic cells leads to pulmonary cell infiltration and abnormal growth, potentially contributing to tumorigenesis, this new genetic model could potentially be valuable in understanding molecular and cellular mechanisms and designing better therapeutic strategies for TSC and other related diseases.

38. A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression

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See abstract on page 24.

39. Continued demonstration of safety of mTOR inhibitors in infants and young children with TSC

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Background: mTOR inhibitors are used to treat many aspects of TSC, but data on the safety of mTOR inhibitors is limited in infants and young children. A previous retrospective study suggested that mTOR inhibitors are safe in young children (STOP1, Krueger et al., 2018). Here we sought to show the continued safety of mTOR inhibitors in two prospective cohorts of very young TSC patients treated with sirolimus or everolimus.

Methods: TSC patients <2 years of age and treated clinically with an mTOR inhibitor were enrolled between 2015-2022 in a prospective, observational study (Early mTOR) at Cincinnati Children's. Parents tracked adverse events (AE), reported at subsequent clinical follow-up and research study visits, and classified by category, severity, and treatment association (CTCAE 5.0). Data were compared to results from the prospective, phase 1 clinical trial (STOP2A) completed at Cincinnati Children's between 2020-2022.

Results: 13 subjects with treatment initiation at 8.6 +/- 5.3 months of age were enrolled in Early mTOR and continued treatment for an average 1.7 +/- 0.6 years. During this time, there were 96 reported AEs (31% grade 1 and 58% grade 2). Respiratory (URIs and/or cough, N=26) was the most common category, followed by general (fevers, teething, and/or irritability; N=25) and infections (conjunctivitis, otitis media, and/or pneumonia N=20). Two serious adverse events (SAE) occurred, hospital admissions for respiratory infections that resolved. Less than 1/3 of AE were related to mTOR inhibitor treatment. Previously reported side effects in older children and adults with TSC, mouth sores (N=2) and GI upset (N=5) were rare. Two individuals (15%) stopped treatment due to side effects. In comparison, there were 91 AE reported from 5 TSC infants treated with sirolimus between 1-12 months of age in STOP2A, with similarly low severity (98% grade 1 or 2), relatedness to treatment (29%), and cases of mouth sores (N=9) or GI upset (N=15).

Conclusion: mTOR inhibitors in infants and young children with TSC are well-tolerated and most AEs are mild and unlikely to be related to treatment. These results are consistent with STOP1 results, with the increased number of AE overall reflective of the prospective study design of Early mTOR and STOP2A. The ongoing phase 2, double-blind, placebo-controlled STOP2B: TSC-STEPS study should provide additional insight into mTOR inhibitor treatment-related side effects in very young patients with TSC.

40. Earlier surgery is associated with improved post-operative language development in children with tuberous sclerosis complex (TSC)

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Introduction: Early life epilepsy in TSC has been strongly associated with lifelong intellectual disability. While improved seizure control correlates with improvements in quality of life and cognition, the importance of surgery timing in young children has not been well-studied. Objective: We evaluated the impact of surgery timing and duration of epilepsy prior to surgery on neurocognitive outcome in a cohort of children followed in the TSC Autism Center of Excellence Network (TACERN) study who underwent epilepsy surgery.

Method: Changes in patients' preoperative (0-3 months) to postoperative (36 months) scores in neuropsychologic tests—including the Mullen Scales of Early Learning (MSEL), Vineland Adaptive Behavior Scales, Second Edition (VABS-II), and the Preschool Language Scales, 5th addition (PLS-5)—were calculated. Pearson correlation and multivariate linear regression models were used to correlate changes in test scores with age at surgery and separately with duration of epilepsy prior to surgery. Analysis was limited to those patients who demonstrated improved seizure control post-operatively (n=18).

Result: Age at surgery had a statistically significant negative correlation with changes in the combined verbal sub-tests of the MSEL (R=-0.41, p=0.04) and predicted the change in that measure outcome in a multivariate linear regression model (ß: -0.09, p = 0.03). Similar trends were seen for the change in the total language score of the PLS-5 (R=-0.38, p=0.06; ß: -0.21, p = 0.07). Results were similar when the duration of epilepsy prior to surgery was assessed as the independent variable. However there were no clear trends with the VABS-II questionnaires, even in language domains.

Conclusion: Our data shows that successful earlier surgery and shorter epilepsy duration prior to surgery are both associated with improved post-operative language development in a small cohort of patients with TSC. Prospective clinical trials are necessary to further elucidate this relationship.

41. Long-term safety and efficacy of add-on cannabidiol (CBD) for seizures associated with tuberous sclerosis complex (TSC): 3-year results from GWPCARE6 Open-Label Extension (OLE)

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Background: Add-on CBD demonstrated efficacy with an acceptable safety profile in patients with TSC in the phase 3 randomized controlled trial (RCT) GWPCARE6 (NCT02544763). To evaluate long-term safety and efficacy of CBD, patients who completed the RCT were enrolled in an OLE, for which we report safety for the full follow-up and efficacy through 156 wks.

Methods: Patients in OLE received CBD (Epidiolex; 100 mg/mL oral solution) at a starting dose 25 mg/kg/d, with titration up to a maximum 50 mg/kg/d. The primary endpoint was safety. Secondary endpoints included percentage change in TSC-associated (countable focal and generalized) seizures, responder rates, and Subject/Caregiver Global Impression of Change (S/CGIC).

Results: Of 201 RCT completers, 199 (99%) entered OLE. At baseline, median (range) age was 10.7 y (1.1–56.8) and number of antiseizure medications (ASMs) was 3 (0–5). Most common concomitant ASMs were valproate (43%), vigabatrin (37%), and clobazam (35%). Baseline median (Q1, Q3) monthly TSC-associated seizure frequency was 57 (28, 109). Thirty-four patients (17%) completed treatment in OLE and 165 (83%) withdrew. The most common reason for withdrawal was transition to commercial product (93 [56%]). Median (range) treatment time was 631 d (18–1462). Mean (SD) modal dose was 28 mg/kg/d (9); 140 (70%) were treated with modal dose =25 mg/kg/d. Adverse events (AEs) were reported in 96% of patients, serious AEs in 28%, and 9% discontinued treatment due to an AE. Most common AEs were diarrhea (47%), seizures (30%), pyrexia (24%), and decreased appetite (24%). Overall, 7% of patients had elevation in ALT levels and 5% in AST levels. There was 1 death due to cardiopulmonary failure, deemed not treatment related. Median reduction from baseline in TSC-associated seizures ranged from 53%–90% across 12-wk windows through 156 wks. Results for last observation carried forward analysis ranged from 52%–62%. Seizure reductions ranged from 53%–93% for patients with a modal dose =25 mg/kg/d. Seizure responder rates (=50%, =75%, and 100%) ranged from 52%–78%, 29%–69%, and 6%–31% across 12-wk windows through 156 wks. Improvements on S/CGIC were reported by 89% and 93% of subjects/caregivers at 52 and 104 wks.

Conclusions: Add-on CBD treatment was well tolerated, and the frequency of TSC-associated seizures remained lower than the RCT baseline throughout the OLE treatment period, supporting long-term use of CBD for treatment of seizures associated with TSC.

42. Long-term efficacy and safety of cannabidiol (CBD) in patients with tuberous sclerosis complex (TSC): 4-year results from the Expanded Access Program (EAP)

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Backgound: The EAP, initiated in 2014, provided open-label CBD to patients with treatment-resistant epilepsies at 25 US epilepsy centers. Here we report results from the analysis of 34 patients with TSC through January 2019; the objective was to evaluate the long-term efficacy (up to 192 weeks) and safety (up to 233 weeks) of add-on CBD in the cohort of patients with TSC in the EAP final analysis.

Methods: Patients received plant-derived highly purified CBD (Epidiolex; 100 mg/mL oral solution) increasing from 2–10 mg/kg/d to tolerance or maximum 25–50 mg/kg/d dose, depending on the study site. Efficacy endpoints were the percentage change from baseline in convulsive, focal, and total seizures, and responder rates across visit windows (12-week windows). Safety endpoints were measured by adverse events (AEs).

Results: Of 892 patients in the safety data set, 34 had TSC. Of those with TSC, 8 (24%) patients withdrew because of lack of efficacy (n=4), AE of diarrhea (1), and other reasons (3). Patients (mean age, 12.4 [range, 1.8–31.2] years) were taking a median of 3 (range, 1–7) concomitant antiepileptic drugs (AEDs) at baseline. Most common AEDs were clobazam (20 [59%]), lamotrigine (14 [41%]), levetiracetam (11 [32%]), and valproate (6 [18%]). The median (Q1, Q3) top CBD dose was 40 (25, 50) mg/kg/d. The median (Q1, Q3) duration of exposure to CBD was 1102 (414, 1341) days. Baseline median (Q1, Q3) monthly seizure frequency for the efficacy analysis set was 46 (18, 76) for convulsive, 37 (24, 84) for focal, and 64 (31, 148) for total seizures. Median percent reduction in seizure frequency during the first 48 weeks was 48%–55% for convulsive, 61%–75% for focal, and 44%–56% for total seizures; the overall pattern of response was maintained through 192 weeks. Responder rates (=50%) for convulsive (47%–100%), total (26%–80%), and focal (19%–80%) seizures were maintained through 192 weeks. AEs were reported in 94% of patients and serious AEs in 47%; there were no deaths. Most common AEs were somnolence (32%), diarrhea (29%), convulsion (18%), and vomiting (18%). One patient (3%) had a liver-related AE of abnormal liver function test.

Conclusions: CBD treatment in patients with TSC was generally well tolerated, and the AE profile was consistent with the overall EAP population and the randomized controlled trial. Add-on CBD was associated with sustained seizure reduction in patients with TSC for up to 192 weeks with an acceptable safety profile.

43. Machine learning driven TSC disease modeling and reversion screening in an iPSC derived disease model

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Tuberous sclerosis complex (TSC) is a multisystem genetic disorder, with high disease penetrance in the neurological system, highlighted by epilepsy in more than 80% of patients. TSC is caused by mutations in *TSC1* or *TSC2* which lead to mTORC1 pathway hyperactivity. Current treatment options are limited. Here in an attempt to build a predictive in vitro disease model, we introduced *TSC2* genetic knockout (KO) into human iPSC derived cortical neurons, collected multi-modal and longitudinal molecular and imaging data (biomarker, bulk and scRNAseq, fluorescent and live cell imaging), and built machine learning (ML) phenotypic models that can describe the biological states of the sick neurons and the healthy isogenic controls. We showed that the ML phenotypic disease models can be used for target / drug discovery by demonstrating time and dose dependent phenotypic reversion using a mTOR inhibitor, and by identifying known disease-reverting genes via pooled transcriptomic and optical genetic screens. Lastly, we verified the relevance of in vitro ML disease models to in vivo pathology by correctly classifying transcriptomic data generated from primary samples extracted from TSC patients. Thus, we demonstrated a rapid and scalable approach to engineering predictive iPSC based disease models that can be leveraged for multi-modal, ML-driven phenotypic drug discovery.

44. Exosomal ITGB1 regulates LAM cancer stem cell properties via ITG-c-Src-FAK-cdc42 axis

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Lymphangioleiomyomatosis (LAM) is a rare, lung-metastatic, low-grade malignancy affecting reproductive-aged women. LAM can be sporadic or in association with tuberous sclerosis complex (TSC). TSC is caused by germline mutations in the *TSC1* or *TSC2* tumor suppressor genes and sporadic LAM are caused by somatic *TSC1* and *TSC2* mutations resulting in dysregulation of the mechanistic target of rapamycin (mTOR), therefore, rapamycin analogs (rapalogs), which inhibit mTORC1, are approved for TSC and LAM. Although research shows that this treatment improves a patient's lung function, discontinuing treatment has led to a progressive loss in some patients' lung function, necessitating the discovery of new therapeutic targets. Tumor derived extracellular vesicles (EVs), including exosomes are known to metabolically reprogram cells in premetastatic niche, and mediate organ-specific metastasis, with *TSC*-null EVs conferring disease phenotype in cells with normal genome. Integrins are implicated in multifaceted properties of tumor cells from signaling molecule to metastasis. We found that LAM surrogate cell (621-101) derived EVs are enriched with integrin alpha6 (ITGA6) and integrin beta1 (ITGB1), thus we examine the effect of ITGB1 depletion in 621-101 cells on EV cargo and its effect on cancer stem cell (CSC) characteristic of LAM cells. ITGB1 depletion in 621-101 cells using short hairpin RNAs (shRNA) resulted in depletion of ITGB1 expression in EVs derived from these cells. The EV ITGB1 depletion also associated with decreased EV expression of (1) ITGA6, (2) SRY-box transcription factor 10 (SOX-10), (3) matrix metalloproteinase-2 and proteins involved in cell migration such as (4) proto-oncogene cellular tyrosine kinase Src (c-Src), and (5) focal adhesion kinase (FAK). Furthermore, the treatment of *TSC*-null cells with *TSC*-null EVs depleted of ITGB1 decreased migration, which associated with reduced actin polymerization and activity of ITGB1- c-Src-FAK-cdc-42 axis. Additionally, the treatment of *TSC*-null cells with *TSC*-null EVs depleted of ITGB1 reduced cancer stem cell (CSC) characteristics of these cells indicated by decreased sphere forming ability, migration, and invasion of sphere-derived LAM cells. Taken together, our data suggest that depletion of ITGB1 in LAM surrogate cells reduces EV expression of ITGB1 and mitigates CSC characteristics of LAM cells via ITGß1-c-Src-FAK-cdc42 axis.

45. Managing visual disturbances in TSC patients with astrocytic hamartomas

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Columbia University Medical Center

Retinal astrocytic hamartomas are tumors of astrocyte origin that often present. This case series reports the clinical presentations, treatment modalities and outcomes for three patients with symptomatic astrocytic hamartomas. All three patients had a previous diagnosis of TSC and presented with vision deficits and persistent cystoid macular edema (CME). Patient A (27-year-old female) reported a floater in her right eye and blurry vision and was found on exam to have a yellow/white retinal lesion with neovascularization and pre-retinal hemorrhage. The patient was treated with monthly bevacizumab injections and later a 3-dose course of faricimab-svoa. Patient B (56-year-old male) had a history of retinal hemorrhage and astrocytic hamartoma treated with 1-year of bevacizumab injections and persistent CME. Treatment continued with Sirolimus as well as intravitreal aflibercept injections every four weeks. Patient C (27 year-old male) presented with an enlarging blind spot and reported circular ring flash when closing his eyes. He had worsening visual acuity and was found to have a vitreous hemorrhage. He received aflibercept and bevacizumab injections every one to two months for one year. All three patients have been treatment-responsive to repeated anti-VEGF therapy. This case series documents three patients with symptomatic astrocytic hamartomas and a variety of clinical presentations who were responsive to treatment with anti-VEGF therapies. secondary to phacomatoses such as tuberous sclerosis complex (TSC). Astrocytic hamartomas are typically asymptomatic but can lead to visual deficits when they result in subretinal hemorrhage, retinal detachment, neovascularization, fluid build-up, or lipid exudation affecting either the retina or optic nerve. Previously reported therapies for patients with symptomatic retinal astrocytic hamartomas include argon laser photocoagulation, vitrectomy to remove blood from a hemorrhage, and photodynamic therapy. Intravitreal anti-VEGF injection has also been shown to improve retinal astrocytic hamartoma visual deficits due to its anti-neovascular properties.

46. mTORC1-dependent RNA methylation confers rapamycin resistance in TSC tumors

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See abstract on page 22.

47. The adverse events of everolimus in tuberous sclerosis complex patients treated for renal angiomyolipoma/subependymal giant cell astrocytoma

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Background: Though regarded as potential efficient approach to address tuberous sclerosis complex (TSC)-associated complications, adverse event profile of everolimus has not been fully constructed. The current study aims to clarify the adverse events spectrum in TSC patients using everolimus for common indications comparing with everolimus non-users with TSC.

Materials and Methods: TSC patients annually followed up at TSC integrated clinics or referred for medical assistance at the three branches of Chung Shan Medical University Hospital, Taiwan were recruited. Medical reviews and laboratory investigations were performed at baseline and annually by clinical physicians. The adverse events were assessed as per the National Cancer Institute Common Terminology Criteria for Adverse Events.

Results: For Everolimus users, common adverse events including hypercholesterolemia (55%), gingivostomatitis (50%), proteinuria (50%) and hyperglycemia (40%) were observed. When compared with everolimus non-users with TSC, the occurrence of gingivostomatitis and proteinuria were significantly higher in everolimus users with TSC (gingivostomatitis, p=0.02; proteinuria, p=0.02). Within Everolimus users, 12 patients were reported to present level I in CTCAE level and 5 patients were reported to be in level II. No everolimus users presented CTCAE level III above.

Conclusion: Everolimus users with TSC had higher tendency developing gingivostomatitis and proteinuria than non-users, whereas difference in the occurrence of other adverse events were not noted between everolimus users and non-users.

48. Vigabatrin acts on cilia pathways in *TSC2*-deficient neurons

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Tuberous sclerosis complex (TSC) is a rare autosomal dominant multisystemic disorder with predominant neurological impairment, including seizures, autism and intellectual disability. Mutations in *TSC1* or *TSC2* genes lead to the hyperactivation of the mechanistic mammalian target of rapamycin (mTOR) pathway, affecting multiple signalling networks, resulting in altered neuronal migration. Epilepsy develops in 70-90% of children with TSC and is often refractory to medication. Vigabatrin has been found to be extremely effective in treating TSC-related infantile spasms, however, the exact mechanism on its specificity to TSC is largely unknown. Unfortunately, the use of vigabatrin is associated with irreversible peripheral vision loss. There is currently a lack of studies that analyse the mechanisms of vigabatrin in TSC, despite its recent success in early intervention studies. In this study, we employed a human pluripotent stem cell model harboring mutation in exon 5 of *TSC2* gene, resulting in a premature stop codon and silencing its protein expression. Molecular profiling was performed on neurons derived from controls and *TSC2*-deficient hPSCs treated with Vigabatrin and Rapamycin, as a positive control. Among the differentially expressed genes between the groups, the top 10 GO pathways were enriched in axon development, neuron differentiation and cilia organization, in which cilium movements were predominantly upregulated. Interestingly, Vigabatrin-treatment reversed the gene expression within the cilium pathway in the *TSC2*-deficient neurons. In the Rapamycin-treated neurons, we observed a similar reversal but at a smaller degree in these cilia and axonal pathways. Cilia deficits have been previously characterized in TSC neurons. Neuronal cilia act as antennas surveying the extracellular milieu and have central roles in multiple signalling pathways. Neuronal cilia dysfunction is now linked to certain genetic epilepsies and autism spectrum disorder. However, the link between cilia deficits and seizures in TSC is currently not known. Here, we report a novel mechanism of action of Vigabatrin on TSC neurons through cilia pathways. Further characterization of cilia deficits in *TSC2* and impact of Vigabatrin on cilia represent a window of opportunity to understand key mechanisms and to generate disease-modifying treatments in TSC.

49. Voices from Ireland

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Purpose: To explore the lived experience of individuals with a diagnosis of Tuberous Sclerosis Complex (TSC) and their carers/families in the Republic of Ireland.

Method: 30 interviews were conducted. Adults with TSC (n=11) Family/carers of adults with TSC (n= 7) , Parents of children with TSC (n=12). Semi structured interviews were organised to suit participants, with the majority conducted in individuals' homes. Interviews were audio recorded, transcribed and thematically analysed.

Results: Experiences differ widely and are associated with symptom variability and where/how healthcare is delivered. Epilepsy and tuberous sclerosis complex-associated n neuropsychiatric disorders (TAND) are identified as key challenges, as are poor access to respite and services for individuals with special needs. From the interviews, it is clear that managing TSC and the unknown trajectory of the disease can be very demanding for patients and families. Lack of coordinated care causes numerous communication complexities, difficulties in accessing care, and is perceived as stressful. Diagnosis is frequently experienced as traumatic. Although the care and commitment of healthcare professionals is appreciated, low awareness and lack of knowledge about TSC amongst healthcare professionals are identified as concerns.

Conclusion: There is broad variation in the lived experience of individuals/families affected by TSC. The management of epilepsy and TAND are identified as significant issues. Coordinated care is desired and may alleviate many reported concerns. Education of healthcare professionals about TSC is seen as very important. Increased support for patients and families might assist them in coping with the impacts of this disease.

50. The Latin American TSC Consortium: an ongoing collaborative strategic initiative

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TSC Alliance of Mexico (ZVZ, JGM, AA) Asociacion Colombiana de Esclerosis Tuberosa (JC) Asociacion Argentina de Esclerosis Tuberosa (MS) Asociacion Brasileña de Esclerosis Tuberosa (WG) Esclerosis Tuberosa Panamá (JC) Cincinnati Children´s Hospital, Cincinnati OH, USA (AW, DK) Holos Genetica (ZVZ, ACB) Instituto Nacional de Perinatologia, Mexico City, Mexico (RSM)

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with multisystem manifestations that include benign tumors of the heart, kidneys, lungs, skin, and brain. About 90% of the TSC patients experience neurological, renal and neuropsychiatric abnormalities, which represent a major cause of morbidity and mortality. Latin America (LA) is a region with a large variety of ethnic groups, comprising 23 countries, and showing significant demographic growth in the last few years. Over the past several years, North America and European countries have developed robust registries and multicenter collaborative networks to improve the care of patients with TSC. There is a need for LA countries to develop effective programs, policies and national laws to improve the quality of life of patients with TSC. To this aim, TSC Alliance Mexico, and representatives from 6 different LA countries (Colombia, Argentina, Brazil, Costa Rica, Ecuador y Panama) have begun working together under the Latin America TSC Consortium (LATSC) to facilitate global collaborations that will improve healthcare decision making for TSC patients in Latin America. Four opportunity areas were identified by this group through interviews with LA patient advocacy groups of rare diseases including TSC: 1) Strengthen the Latin American Tuberous Sclerosis Network, which is currently made up of non-profit organizations that care for patients with this condition 2) To promote TSC Patient Registry Programs for low and middle-income countries. The program may begin locally following Rare Disease Patient Registries, and then integrate with a multi-national registry. This strategy will allow LATSC to make more patients visible, support evidence-based public health policies, as well as acknowledging the specific challenges associated with this condition 3) Empower regional research partnerships for scientific and clinical collaboration that can help us better understand the evolution, follow-up, and treatment of patients with TSC, especially on TAND research, 4) Provide workshops and educational courses about the International TSC Consensus Group in Surveillance and Management Recommendations to medical professionals 5) Facilitate and improve access to drugs. The development of our Consortium combines efforts made all around LA to benefit the entire community, giving them access to better clinical practices and to contribute to the global effort towards clinical research on TSC worldwide.

51. Arginine metabolism in tuberous sclerosis complex-associated kidney disease

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Introduction: Kidney disease affects most patients with tuberous sclerosis complex disease (TSC) and is a leading cause of death in adulthood. Mutations in either *TSC1* or *TSC2* induce mTOR activation in TSC, resulting in cell growth and tumorigenesis as renal angiomyolipoma and cystic disease. However, the exact mechanisms leading to cyst formation remain poorly understood. Metabolic reprogramming is an important mechanism by which cells promote proliferation and cell growth. Here we aimed to analyze the metabolic reprogramming in TSC-associated kidney disease.

Methods: *Six2Cre*$^{+/tg}$*Tsc1*$^{-/-}$ and control offspring, treated with either vehicle or rapamycin, were dissected, and kidneys were excised for metabolite extraction and analyzed by liquid chromatography/inline tandem mass spectrometry. Kidneys were also used for histology, RNA/protein extraction, and immunofluorescence.

Results: Whole kidney metabolome and PTCs extracted metabolome analysis from TSC mice showed significant perturbation in the arginine biosynthesis pathway. These changes were associated with increased urea cycle metabolites and the rate-limiting enzyme Argininosuccinate Synthase 1 (ASS1) expression levels. High ASS1 expression was demonstrated in kidney lysates of TSC mice compared to control mice, and ASS1 was specifically localized in cyst lining cells in the TSC kidney. Knockdown of *Tsc1* in the HK2 cell line emphasized the contribution of the *Tsc1*-mTORC1 pathway to ASS1 expression. Arginine depletion in vivo and in vitro reduced the mTOR signaling pathway, cell proliferation, and cystic kidney load.

Discussion: We show that TSC kidneys exhibit a major shift in their metabolic state, associated with different metabolic pathways, mainly the arginine biosynthesis pathway. We show that dysregulated mTOR pathway in TSC PTCs induces the arginine biosynthesis pathway by over-expression of ASS1 to support the high arginine demand in PTCs. Arginine depletion ameliorates the PTCs cell signaling and cell proliferation which are major contributors to cyst development in TSC, with the potential for immediate translational and clinical impact.

52. Epilepsy and TAND in the tuberous sclerosis complex are caused by an alteration in microglial polarity to M1 and sirolimus cures them by returning microglial polarity to M2

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Osaka University

Tuberous sclerosis complex (TSC) is a multi-system genetic disorder characterized by systemic hamartomas, epilepsy and TSC-associated neuropsychiatric disorders (TAND). Epilepsy and TAND are common symptoms and suffer patients and their families. However, unlike hamartomas, the pathogenesis of epilepsy and TAND is not well known. Furthermore, mTORC1 inhibitors (mTORi) such as sirolimus and everolimus are very effective for hamartomatous lesions, such as SEGA (subependymal giant cell astrocytoma), renal angiomyolipoma and facial angiofibroma, but have limited efficacy in epilepsy and TAND. The mechanism by which mTORi suppress epilepsy and TAND is also unknown. Recently, different types of glial cells have been increasingly recognized to play important roles in the phenotypes of TSC. Among them, the involvement of microglias and astrocytes has attracted attention, but the detailed mechanisms have not yet been elucidated. To investigate the role of glia cells in epilepsy and TAND in TSC, Mitf-M specific *TSC2* conditional knockout (*Tsc2cKO*) mice showing epilepsy and TAND were constructed, and the mice were cured by sirolimus treatment. Morphological, immunohistochemical staining of brain tissue specimens of control and *Tsc2cKO* mice with or without sirolimus treatment and quantitative RT-PCR of microglia in *Tsc2KO* mouse revealed that microglial abnormality was involved in the TSC neurological symptoms. Microglia is known to have two distinct phenotypes, M1 and M2. The M1-phenotype microglia induces inflammation and, exhibits CD16, CD32, CD86 and iNOS, whereas the M2 phenotype involved in tissue repair exhibits arginase-1, Ym1, Fizz, IL-10, MHC II, CD163, CD206 and TGF-ß. Then, we investigated the microglia phenotypes. Morphological and immunohistochemical analysis of microglia of *Tsc2cKO* mouse showed that changes in microglial polarity were involved in the epilepsy and TAND in TSC. Quantitative RT-PCR analysis of microglia confirmed the conclusion. That is, microglias in *Tsc2cKO* mice were tilted to M1 phase, but sirolimus treatment returned them to M2 phase, curing epilepsy and TAND. Current treatments for epilepsy mainly control neuronal mechanisms, such as direct control of neuronal excitation. Shifting microglial polarization from the M1 to the M2 phenotype may be an effective strategy in the treatment of epilepsy and TAND in TSC.

53. Implementation of a clinical and research consortium to achieve early diagnosis, adequate disease surveillance and management across the lifespan of patients with TSC in a middle-income country

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Tuberous sclerosis complex (TSC) affects ~2 million people worldwide. Epilepsy is the most common neurological manifestation (up to 90% of patients) and a high source of morbidity in TSC. There is clinical evidence for the use of vigabatrin and current clinical trials using mTOR inhibitors as potential anti-epileptogenic therapies capable to change the natural course of the disease. In Mexico, the median age of diagnosis is 6, with patients presenting with refractory epilepsy. In order to implement early therapeutic interventions, there is a stark need for improved strategies to ensure early detection and long-term surveillance across the country. Working together with TSC Alliance Mexico, we have designed a multidisciplinary clinic and research consortium among three Institutes within the larger Mexican National Health Institutes Network (NHIN). We hope to become a National TSC Center of Excellence for both research and multidisciplinary care. The consortium located in Mexico City, is comprised of the National Institute of Perinatology (INPeR) focused on pre-natal diagnosis and early post-natal care, the National Institute of Pediatrics (INP) open to pediatric patients, and the National Institute of Neurology and Neurosurgery (INNN) for adults. These institutes provide high-quality, low-cost care in multiple specialties and have the infrastructure to lead innovative research projects and clinical trials. Along with the three existing TSC multidisciplinary clinics (two in the north in Monterrey and one in the south in Merida), the new consortium in Mexico City (central region) will ensure that all regions of Mexico have access to multidisciplinary care. For day-to-day care, patients will be assigned to local neurologists and geneticists, members of the TSC National Network of Physicians. This network of physicians communicates with the head of the assigned region multidisciplinary clinic, and has access to a panel of experts through the TSC Alliance Mexico Clinical Board to consult on difficult cases. Mexico will join USA, Canada and India by participating in the TSC Natural History Database and Biorepository with the purpose of generating large cohort data to inform future investigations and identify patients eligible for clinical trials. We believe that our proposed consortium will help lower the age of diagnosis and improve the care and clinical outcomes of Mexican children with TSC; a model that could be implemented in other middle-income countries.

54. Understanding phenotype heterogeneity of tuberous sclerosis for potential precision medicine

Seungyeul Yoo, Yu Liu, Eunjee Lee, Quan Chen, Stephen McGee, Li Wang, and Jun Zhu

Data Science Department, GeneDx, Stamford, CT (SY, YL, EL, QC, LW, JZ); Production Bioinformatics, GeneDx, Gaithersburg, MD (SM)

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic condition affecting 1 in 6000 newborns in the US and is driven by variants in *TSC1* or *TSC2*. The brain is the primarily affected tissue with related neurologic disorders including epilepsy, developmental delay, and autism. These brain disorders can occur early in life and have a high impact on the quality of life of patients and families. Clinical manifestations of TSC are very heterogeneous. The severity and age of onset of epilepsy are correlated with intellectual disability and autism. To understand the molecular mechanisms of TSC heterogeneity, we integrated diverse omics data at bulk tissue and single cell level to identify key molecular regulations underlying disease heterogeneity. The mTOR pathway is essential to cell growth regulation in all types of cells, but transcription regulations of *TSC1*/*TSC2*/mTOR and mTOR complex 1 (mTORC1) activity in the brain are unique. The age of epilepsy onset is a surrogate biomarker for the disease severity of TSC. To access whether *TSC1*/*TSC2* variants are associated with early- (< 2 yrs) vs. late- (>= 2 yrs) onset of epilepsy, we compared *TSC1*/*TSC2* variants of TSC patients in the GeneDx database and found no clear difference in variants associated with early vs. late onset of epilepsy. Next, we compared transcription profiles of brain tissues from TSC patients and identified a signature differentiating patients with early vs. late onset of epilepsy. The genes upregulated in the brains of TSC patients with early-onset epilepsy were enriched for genes in the immune response. To test whether baseline immune pathway activity is different between TSC patients with early vs. late onset of epilepsy, we imputed the brain gene expression profile of each patient based on genotypes derived from whole exome sequencing data and then refined the brain gene expression profiles based on constructed brain molecular causal networks. The results suggest that the immune system plays an important role in epilepsy age of onset in TSC. Both genetic and environmental factors affect the molecular state of the immune system in the brain. Routine brain biopsy of TSC patients is impractical, thus whole genome sequencing or whole exome sequencing beyond sequencing *TSC1* and *TSC2* is necessary to infer immune system activity in the brain of TSC patients and critical to developing personalized medicine for TSC patients.

55. Understanding dynamic molecular regulations in brains with regard to tuberous sclerosis diseases

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder affecting 1 in 6000 newborns in the US driven by variants in *TSC1* or *TSC2*. These variants can affect diverse organ systems including the brain, eye, heart, kidney, skin, and lung. The primary affected tissue/organ is the brain which results in several neurologic disorders such as epilepsy, developmental delay, and autism. Proteins encoded by *TSC1* and *TSC2* form a complex that inhibits the mTOR pathway, in turn regulating many pathways essential for cell growth in all cells. To understand the relationship between TSC and mTOR pathway in the brain and other organ systems, we first investigated cell type-specific expression of *TSC1*, *TSC2*, and mTOR and inferred mTOR complex 1 pathway (mTORC1) activity. Across 79 human cell types, cells in the brain and eye had the lowest mTORC1 activity while these cells had the highest gene expression levels of *TSC1*, *TSC2*, and mTOR. The results suggest that brain- and eye-specific cells are more likely to be affected by TSC-mTOR pathway dysregulation. In the GTEx database, the three genes were tightly correlated in brain tissues but less correlated in other tissues (t-test p=1.9×10-19). To understand the co-regulation in the brain, we identified ARNT2, ETV5, and BHLHE41 as key transcription factors contributing to the brain-specific co-regulation. ARNT2 plays a role in the development of brain, visual, and renal function, consistent with the top tissues affected in tuberous sclerosis diseases. The tightly co-transcriptional regulation of mTOR, *TSC1*, and *TSC2* results in stable mTORC1 activity in the brain. Variants in *TSC1* or *TSC2* will disturb the stable state among mTORC1 activity and result in lower or higher mTORC1 activity. To test the hypothesis, we checked microcephaly (lower mTORC1 activity) and macrocephaly (higher mTORC1 activity) phenotypes in individuals with *TSC2* variants. In the GeneDx database, among patients with *TSC2* variants, 291 patients were with microcephaly, and 185 were with macrocephaly. The corresponding variants in *TSC2* were not enriched in any *TSC2* structure domain, suggesting that the lower or higher mTORC1 activity was determined by other genetic or environmental factors. For example, rs1042602 in tyrosinase was significantly associated with macrocephaly among patients with *TSC2* variants, suggesting whole genome sequencing or whole exome sequencing is necessary to understand the disease heterogeneity.

56. Pulmonary explant cultures for ex vivo testing of LAM therapeutics

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Finding suitable models for lymphangioleiomyomatosis research can be challenging. We maintained tissue samples of ~6mm3 at the air-liquid interphase atop smooth muscle cell media in a humidified incubator at 5% CO2 for one week. Explants settled overnight before adding 50 mL of vehicle alone, 10 mM rapamycin, or 106 untransduced T cells in RPMI with 100 IU/mL of rhIL-2 or T cells transduced to express a chimeric antigen receptor targeting TYRP1. TYRP1 is a melanosomal protein overexpressed on the surface of melanoma and LAM cells. Upon antigen stimulation, TYRP1 CAR T cells secrete IFN?, and the ~80% CD8 T cells exhibit polyfunctional cytokine responses including MIP1a and-ß, RANTES, granzyme B and TNFa, suggestive of their cytotoxic potential. Rapamycin was added every other day before harvesting explants on day 8 (N=4). T cells were applied twice, 4 days apart (N=3 per group). Tissues were snap frozen and 8 mm cryosections were acetone fixed before staining with antibodies HMB45 to PMEL/gp100, UCTH1 to human CD3 or polyclonal rabbit Ab to phospho-S6 ribosomal protein (Ser240/244). Tissue morphology was well maintained. Added T cells were detected at end point by CD3 staining. Rapamycin treatment induced a trend towards 2/3 reduced abundance of gp100-expressing cells over vehicle alone (p=0.2) while transduced T cells significantly reduced the abundance of PS6 expressing cells by 75% (p=0.009). We propose that LAM explants offer a viable option to evaluate LAM etiology and treatment ex vivo. NDRI and LAM patients are acknowledged for enabling this research.

57. A precision dosing strategy for overcoming challenges to the dosing of mTOR Inhibitors in TSC infants in clinical practice and clinical trials

David M. Ritter, Jamie K. Capal, David Neal Franz, Molly Griffith, Karen Agricola, Bridget Kent, Kristn Currans, E Martina Bebin, Hope Northrup, Mary Kay Koenig, Tomoyuki Mizuno, Alexander A. Vinks, Stephanie Galandi, Wujuan Zhang, Kenneth D. R. Setchell, Carlos E. Prada, Kelly Kremer, Katherine Holland-Bouley, Hans Greiner, Paul S. Horn, Darcy A. Krueger

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See abstract on page 23.

58. Healthcare provider recognition of pregnancy related risks and management considerations in patients with tuberous sclerosis complex

Meredith Rose, David Ritter, Nishant Gupta, Leandra Tolusso, Paul Horn, Emily Wakefield, Jennifer Glass

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See abstract on page 33.

59. STAT1 promotes survival and lung metastasis of TSC2-null cells in lymphangioleiomyomatosis (LAM)

Tasnim Olatoke, Erik Y. Zhang, Aristotelis Astrinidis, Minzhe Guo, Yan Xu, Jane J. Yu

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Early Career Researcher Symposium Agenda

September 7	Thursday — Hampton Ballroom
8:30 am - 8:45 am	**Welcome and Introductions:** Nicole McDonald, PhD, UCLA Semel Institute, and Uchenna John Unachukwu, PhD, Columbia University Medical Center **TSC Community Speaker:** Matt Olear
8:45 am - 9:45 am	**Presentations by Early Career Researchers – Group 1** Jerome Arceneaux, BS, Department of Biochemistry, Cancer Biology, Neuroscience, and Pharmacology, Meharry Medical College – **Mapping the cellular composition of resected cortical tubers and perituberal tissues** Francesco Avanzi, Necker Enfants Malades Institute (INEM – **S6 Kinase is involved in TSC-related epilepsy through the regulation of TREK-1 potassium channel** Marie Girodengo, MSCi, Francis Crick Institute – **Characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment in TSC** Lais Cardozo, MSc, Federal University of Paraná – **TSC-associated neuropsychiatric disorders (TAND) assessment and the neuropsychological cluster in a group of patients from Brazil** **Moderated Discussion and Q&A with Group 1 Selected Talk Authors** **Moderator:** Nicole McDonald, PhD, UCLA Semel Institute
9:45 am - 10:00 am	***Break***
10:00 am - 11:00 am	**Presentations by Early Career Researchers – Group 2** Apoorva Kasetti, MS, BPharm, Texas Tech University Health Sciences Center – **Everolimus enhances pro-metastatic potentials of LAM-derived exosomes** Kaushal Asrani, PhD, MBBS, Johns Hopkins University School of Medicine – **AMPK mediates oncogenic transformation in TSC** Devin Barzallo, MSc, Uniformed Services University of the Health Sciences – **Ultraviolet signature mutations in TSC skin tumors are increased in sun-exposed body sites, reinforcing recommendations for good sun protection** Magdalena Losko, PhD, Brigham and Women's Hospital – **TSC1 mutant bladder cancer is characterized by a TSC-associated gene expression signature due to TFE3 transcriptional activity** **Moderated Discussion and Q&A with Group 2 Selected Talk Authors** **Moderator:** Uchenna John Unachukwu, PhD, Associate Research Scientist, Columbia University Medical Center
11:00 am - 12:00 pm	**Career Development Panel** **Moderators:** Nicole McDonald, PhD, UCLA Semel Institute, and Uchenna John Unachukwu, PhD, Columbia University Medical Center **Panelists:** Dean J. Aguiar, PhD, TSC Alliance; Tracy King, MD, MPH, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NICHD; Shui-Lin (Stan) Niu, PhD, Congressionally Directed Medical Research Programs, U.S. Department of Defense; Jane Yu, PhD, University of Cincinnati
12:00 pm - 1:00 pm	**Networking Lunch and Informal Roundtable Discussions**

Early Career Researcher Symposium Abstracts

Mapping the cellular composition of resected cortical tubers and perituberal tissues

Jerome S. Arceneaux, Rohit Khurana, Asa A. Brockman, Mary-Bronwen L. Chalkley, Laura C. Geben, Robert P. Carson, Bret C. Mobley, Kevin C. Ess, and Rebecca A. Ihrie

Department of Biochemistry, Cancer Biology, Neuroscience, and Pharmacology, Meharry Medical College (JSA); Departments of Cell and Developmental Biology (RK, AAB, MBLC, KCE, RAI) and Pharmacology (LCG, RPC), Vanderbilt University; Departments of Neurology (RPC, KCE), Pediatrics (RPC, KCE), Neuropathology (BCM), and Neurological Surgery (RAI), Vanderbilt University Medical Center

Tuberous sclerosis complex (TSC) arises due to heterozygous mutations in *TSC1* or *TSC2* and affects approximately 1 in 6000 births. Neuropsychiatric symptoms of this disorder include autism spectrum disorder (ASD), developmental delay, intellectual disability, and epilepsy. Mutations in *TSC2* are often associated with worse symptoms and severity. Epilepsy in TSC patients is often refractory to drug treatment, sometimes 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 exhibit loss of heterozygosity (LOH) of *TSC1/2* in otherwise heterozygous tissue. Though mutations in *TSC1/2* lead to 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 broad TSC cohorts at the protein level. In addition, how "balloon cells" may influence their microenvironment to produce epileptogenic foci is poorly understood. High-dimensional approaches such as imaging mass cytometry (IMC) offer the opportunity to directly assess thirty or more proteins and signaling events in single cells while documenting spatial relationships within the tissue. Using a custom antibody panel, where each of thirty-six (36) antibodies was successfully tested on known positive and negative controls, we have identified through immunofluorescence probable colocalization of lineage markers as well as signaling readouts of mTORC1 hyperactivation. In addition, we developed customized machine-learning workflows that 1) identify prospective "balloon cells" with 93% precision and 69% efficiency within archived cortical tubers and 2) can map the cytoarchitecture and signaling perturbations within tissue samples, with a specific focus on "balloon cells" and their immediate neighbors. These data will comprise a rich dataset for understanding the abundance, structure, and signaling activity of progenitor-like, neuronal, and glial cells within archived tubers and perituberal tissues, enabling quantitative comparisons of TSC with other mTORopathies and assaying possible therapeutic targets.

S6 Kinase is involved in TSC-related epilepsy through the regulation of TREK-1 potassium channel

Francesco Avanzi, Stefano Fumagalli, Mario Pende

Necker Enfants Malades Institute (INEM), Paris, France (FA, SF, MP); Université de Paris Cité, Paris, France (FA, SF, MP)

Epilepsy is the most common neurological manifestation of tuberous sclerosis complex (TSC), a genetic disease caused by loss-of-function mutations on the *TSC1* or *TSC2* genes that results in the mTOR pathway hyperactivation. The molecular mechanisms downstream of aberrant mTOR signaling that are responsible of the alterations of neuronal excitability are largely unknown. To study the role of the downstream mTOR-effector S6 Kinase (S6K), we generated a model of TSC based on AAV9-Cre-mediated deletion of the *Tsc1* gene in the brain of $Tsc1^{f/f}$ and $Tsc1^{f/f}S6K^{-/-}$ mice. Interestingly, epilepsy development and premature death of TSC mice is completely reverted upon the deletion of the *S6K1/2* genes. To identify new potential downstream targets of S6Ks we performed, in collaboration with Cell Signaling Technology, a phosphoproteomic analysis and we observed an enrichment of proteins known to be involved in autism and seizure with a specific phosphorylation consensus sequence for S6K (RXXS*/T*). We focused on TREK-1, a potassium channel, whose deletion increases seizure susceptibility. We demonstrated that TREK-1 is phosphorylated on Ser333 in a rapamycin-sensitive way by S6K in HEK293 cells and in primary cortical neurons upon glutamatergic stimulation. Moreover, by electrophysiology recording, we observed that TREK-1 is phosphorylated and kept in a closed conformation after amino acids stimulation, while is opened in presence of rapamycin. Finally, overexpression of TREK-1-S333A is able to revert the induction of cFos – a common marker of neuronal activity – in in vitro model of TSC. Our work will provide a mechanism for epilepsy development and new potential therapeutic approaches for TSC patients.

Characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment in TSC

Marie Girodengo, Simeon R. Mihaylov, Pranetha Baskaran, Laura Mantoan Ritter, Sila K. Ultanir, Joseph M. Bateman.

Francis Crick Institute, London, United Kingdom (MG, SRM, SKU); King's College London, London, United Kingdom (MG, PB, LMR, JMB).

Tuberous sclerosis complex (TSC) is caused by mutations in the genes *TSC1* or *TSC2* and characterised by the formation of benign tumours in multiple organs, including the brain. In patients, the greatest morbidity comes from neurological lesions including cortical tubers, subependymal nodules and subependymal cell astrocytomas (SEGAs), as well as manifestations like epilepsy, developmental delays and neuropsychiatric disorders. *TSC1* and *TSC2* are negative regulators the mTOR complex 1 (mTORC1) signaling pathway. Manipulation of mTORC1 signaling in animal models has shown that this pathway has key roles in neurogenesis. However, the molecular mechanisms linking mTORC1 hyperactivity to the neurological features of TSC are poorly understood, in part because the targets of mTORC1 during neurodevelopment have not been systematically characterised. To systematically characterise the mTORC1-dependent phosphoproteome during neurodevelopment, we used an unbiased Tandem Mass Tag and quantitative mass spectrometry approach on embryonic day 16.5 brain tissue from mice with a conditional knockout of *Tsc1* in the brain using Nestin-Cre (*Tsc1c*KO). In two independent experiments, we performed total proteomics and phosphoproteomics of *Tsc1*cKO mice compared to controls, and *Tsc1*cKO mice treated with rapamycin compared to vehicle, to activate and inhibit the mTORC1 pathway respectively. We identified over 30,000 phosphopeptides and over 7000 proteins in both experiments and observed expected changes in phosphorylation of established mTORC1 targets including rpS6 and 4E-BP2, validating this approach. We then developed an analysis pipeline to identify high confidence, novel mTORC1 targets. Through this pipeline we identified 25 novel targets of mTORC1 in the developing brain. The functions of these proteins include transcriptional regulation, chromatin remodelling, regulation of ubiquitination and regulation of small GTPases. Moreover, mutations in several of these novel mTORC1 targets in patients cause neurodevelopmental disorders associated with epilepsy and intellectual disability. To validate the novel mTORC1 targets in TSC we also performed quantitative phosphoproteomic analysis of surgical tuber and SEGA tissue from TSC patients. Our characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment reveals novel molecular mechanisms and identifies potential new therapeutic targets for the neurological manifestations in TSC.

TSC-associated neuropsychiatric disorders (TAND) assessment and the neuropsychological cluster in a group of patients from Brazil

Laís F M Cardozo, Ana Paula A de Pereira, Mariana R Schwind, Luiz G Dufner-Almeida, Luciana A Haddad, Ana Chrystina de S Crippa, and Sérgio A Antoniuk

Department of Psychology, Federal University of Paraná, Curitiba, PR, Brazil (LFMC, AAAP); Pediatric Neurology Center, Hospital de Clínicas, Federal University of Paraná, Curitiba, PR, Brazil (MRS, ACSC, SAA); Human Genome and Stem Cell Research Center, Department of Genetics and Evolutionary Biology, Instituto de Biociências, Universidade de São Paulo, SP, Brazil (LGDA, LAH)

There is a gap in worldwide evaluation and treatment of TAND manifestations in tuberous sclerosis complex (TSC) patients. A cross-sectional study was conducted with prospective data collected from TSC patients from the South of Brazil. The aims were (i) to identify the prevalence of TAND signs, and (ii) to relate clinical history with neuropsychiatric characteristics. The research was conducted at the Pediatric Neurology Center from a tertiary hospital, in Curitiba, Brazil. Forty-four patients aged 6 to 44 years with a definite clinical diagnosis of TSC were enrolled in the study after providing signed informed consent. In 37 cases genetic testing was performed. The participants were remotely assessed by the TAND checklist and clinical data was obtained. Statistical analysis has so far been performed with descriptive and bivariate analyses - Fisher's exact test and Mann Whitney U test for comparing the clinical variables with the TAND clusters. Significance threshold at 0.05. Among 44 participants, 40 (90.9%) had a history of seizures and 26 (65%) reported controlled seizures at the time of assessment. Thirty (81.1%) had a pathogenic alteration identified in the *TSC2* gene, three (8.1%) in *TSC1*, and four (10.8%) had no mutation identified (NMI). The most frequent variant types were frameshift (21.3%), nonsense (21.2%), and missense (18.2%). All participants had at least one impairment related to TAND. Regarding specifically the neuropsychological cluster, forty-two (95.5%) participants had at least one positive response, and 13 (29.5%) affirmatively answered all questions. Thirty-eight (86.4%) had difficulties in executive functions, 33 (75%) in dual-tasking, 33 (75%) in paying attention/concentrating, 31(70.5%) in neuropsychological attention, 28 (63.6%) in memory, 26 (59.1%) in getting oriented, and 24 (54.5%) in visuo-spatial tasks. Significant associations were observed between visuospatial skills and executive functions with (1) the number of medications previously used (p<0,001), (2) the age of the first symptoms (p=0.01 and p=0.01), and (3) the type of genetic variant (p=0.03 and p=0.03). Our results are in accordance with the literature regarding the high frequency of TAND manifestations and neuropsychological difficulties, and significant association between TAND manifestations and a greater number of employed antiepileptic drugs. Further analyses will be conducted to obtain more detailed information about the TAND profile in this Brazilian sample.

Everolimus enhances pro-metastatic potentials of LAM-derived exosomes

Apoorva Kasetti, Anil Kumar Kalvala, Ashok Silwal, Bhaumik Patel, Jane J. Yu, Maciej Markiewski, Magdalena Karbowniczek

Department of Biotechnology and Immunotherapeutics, School of Pharmacy, Texas Tech University Health Sciences Center, 1718 Pine Street, Abilene, TX 79601 (AK, AKK, AS, BP, MM, MK); Department of Internal Medicine, University of Division of Pulmonary, Critical Care and Sleep Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 45267 (JJY)

Lymphangioleiomyomatosis (LAM) is metastatic low-grade sarcoma that primarily affects reproductive-aged women. LAM can be sporadic or in association with tuberous sclerosis complex (TSC). TSC is caused by germline mutations in the *TSC1* or *TSC2* tumor suppressor genes and manifests as multiple central nervous system tumors, renal angiomyolipomas, and pulmonary LAM. Sporadic LAM and angiomyolipoma are caused by somatic *TSC1* and *TSC2* mutations resulting in dysregulation of the mechanistic target of rapamycin (mTOR), therefore, rapamycin analogs (rapalogs), which inhibit mTORC1, are approved for TSC and LAM. While studies have shown that this therapy stabilizes the lung function, its discontinuation resulted in progressive decline in the lung function among some patients with LAM, indicating the need for discovery of new therapeutic targets. Exosomes (EVs) are natural membranous vesicles with unique biological and pharmacological properties. It is well established that EVs contribute to cancer progression and metastasis by reprogramming stromal cells, remodeling the architecture of extra cellular matrix, and normal cell phenotypes through the transfer of bioactive molecules between cancer and cells in local and distant microenvironments. We previously reported that EV derived from *TSC1*–null neuronal progenitors block differentiation of recipient wild-type progenitors via activation of the Notch1/mTOR pathways, phenocopying *TSC1*-null cells. Therefore, in this study we investigated the effect of Everolimus on *TSC*-null EV release and content to determine its impact on progression of LAM. Here, we show that treatment with Everolimus of *TSC2* null surrogate LAM cells (621-101) increased extracellular vesicle (EV) release and enriched these EVs with the Integrin-beta1, Integrin-alpha6, proto-oncogene cellular tyrosine kinase Src (c-Src), SRY-box transcription factor 10 (SOX10), MMP-2, focal adhesion kinase and CD44. In addition, EVs derived from Everolimus treated 621-101 cells increased stem cell-like characteristics of LAM surrogate cells, such as increased sphere-forming ability, aldehyde dehydrogenase (ALDH) activity, migration and invasion. Taken together, these results imply that EVs released by LAM surrogate cells treated with Everolimus promote EV biogenesis and LAM cancer stem-like phenotypes by modulating integrins and integrin-mediated signaling pathways.

AMPK mediates oncogenic transformation in TSC

Kaushal Asrani (presenting author), Juhyung Woo, Adrianna A. Mendes, Thiago Vidotto, Leon Deng, Ethan Schaffer, Kewen Feng, Tamara L. Lotan

Johns Hopkins University School of Medicine, Baltimore, MD, USA

The serine-threonine kinase and cellular energy biosensor, 5'AMP-activated protein kinase (AMPK) is central regular of energy homeostasis and is known to promote lysosomal biogenesis and tumorigenesis via activation of the MiT/TFE transcription factors (TFEB/TFE3). We and other groups have previously shown that TFEB and TFE3 are constitutively activated in the setting of *TSC1/2* loss (www.nature.com/articles/s41467-022-34617-7). Here, we examined the expression and function of AMPK in renal cells and tumors driven by loss of the TSC tumor suppressors. Phosphorylation of AMPK and its substrates (p-ACC[S79] and p-ULK1[S555]) was constitutively increased in HEK293T cells with genomic deletion of *TSC1*, *TSC2* and *TSC1/2* and was further enhanced in response to glucose deprivation or the lysosomal AMPK activator Aldometanib, in an mTORC1-sensitive manner. Expression of p-AMPK (T172) and p-ACC (S79) was increased in murine renal cysts and tumors in $TSC2^{+/-}$ A/J mice. Multiple AMPK-dependent CLEAR genes sets were positively enriched by GSEA in*TSC2* KO compared to WT xenografts, and negatively enriched in the TFEB/TFE3 double KO xenografts compared to *TSC2* KO xenografts. We used CRISPR-Cas9 genome editing to knockout the two genes encoding the alpha catalytic subunits of AMPK (AMPKa1 and AMPKa2) in*TSC2* KO cells, and isolated and characterized 3 AMPK double-knockout (DKO) CRISPR clones. AMPK depletion in the *TSC2* KO background significantly reduced cellular proliferation (IncuCyte ZOOM live cell imaging and clonogenic assays) and cellular ATP levels (CellTiter-Glo). These findings suggest that AMPK may be an important mediator of tumorigenesis in TSC.

Ultraviolet signature mutations in TSC skin tumors are increased in sun-exposed body sites, reinforcing recommendations for good sun protection

Devin Barzallo, Xijun Zhang, Ji-an Wang, Alexander Cartron, Amanda Jones, Patricia Julien-Williams, Hai-Ping Wu, Matthew Wilkerson, Clifton Dalgard, Joel Moss, Thomas Darling

Dermatology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States. (DB, JW, AC, TD); Anatomy, Physiology, and Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States. (XJ, MW, CD); Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, United States. (DB, AC, AJ, PJW, HPW, JM)

Ultraviolet (UV) light is a mutagen implicated in the development of skin cancers, generating C>T and CC>TT mutations and mutational signatures known as SBS7a and SBS7b. UV light also contributes to the formation of facial angiofibromas (FAFs) in individuals with tuberous sclerosis complex (TSC), as evidenced by CC>TT mutations as second hits in the TSC genes in fibroblast-like cells derived from FAFs. The role of UV light in TSC tumorigenesis may be expected to be the most pronounced in sun-exposed skin and could be reduced by an individual's constitutive pigmentation, since greater melanin content is known to reduce the penetration of UV light. It has not been elucidated whether UV-signature mutations across the genome in TSC skin tumors differ by body site or race. We performed whole-genome sequencing of DNA from fibroblast-like cells grown from 39 tumor samples from 27 patients, including 2 Black individuals, 1 Hispanic individual, and 24 White individuals. TSC lesions were grouped as sun exposed (FAF and fibrous cephalic plaque of the face and neck) or sun protected (e.g. shagreen patches, ungual fibromas, fibrous cephalic plaque of the scalp). The median number [and interquartile ranges] of UV mutations across the genome for the TSC skin lesions in sun-exposed skin (SBS7a: 2,485 [447-6,465], SBS7b: 3,736 [738-9,042], n=15) were greater than those in sun-protected skin (SBS7a: 55 [27-163], SBS7b: 6 [0-55], n=24). Among FAFs, the median mutation rates for the samples from White individuals (SBS7a: 4,198 [1,621-9,394], SBS7b: 6,855 [2,348-10,957], n=8) were similar to that observed in the Black individual (SBS7a: 14,949, SBS7b: 13,533]). In addition, the FAF from the Black individual showed UV signature mutations in *TSC2* as second-hit pathogenic variants. UV-signature mutations in TSC skin tumors vary with body location, with a greater number of mutations in sun-exposed areas such as the face, suggesting that sun protection measures will reduce mutational burden and severity of skin disease. However, our preliminary results suggest that constitutive pigmentation does not provide adequate protection against the effects of UV light. These observations emphasize the importance of counseling on sun protection among all individuals with TSC to reduce the formation of FAFs.

TSC1 mutant bladder cancer is characterized by a TSC-associated gene expression signature due to TFE3 transcriptional activity

Magdalena Losko, Clemens K. Probst, Elio Adib, Heng Du, Michelle S. Hirsch, Krinio Giannikou, David J. Kwiatkowski

Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA (ML, CKP, EA, HD, KG, DJK); Department of Neurological Sciences, Larner College of Medicine, University of Vermont, Burlington, VT, USA (CKP); Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA (MSH); Division of Hematology and Oncology, Cancer and Blood Disease Institute, Children's Hospital Los Angeles, Los Angeles, CA, USA (KG)

Mutations and inactivation of the tumor suppressor gene *TSC1* are recurrent (6-10%) events in bladder cancer, but the mechanism by which *TSC1* loss promotes bladder cancer development beyond mTORC1 activation is uncertain. Here, we characterized *TSC1*-mutated bladder cancers (*TSC1*mtBLCA, n=26) with *TSC1* wildtype tumors (*TSC1*wtBLCA, n=382) from The Cancer Genome Atlas (TCGA) data set and compared them to an internal cohort of *TSC1/TSC2*-mutated tumors (n=48). We identified 26 genes upregulated in *TSC1*mt-BLCA out of 60 genes commonly upregulated in tuberous sclerosis complex (TSC) tumors. Among them, GPNMB showed the greatest increase in expression. Gene Set Enrichment Analysis (GSEA) and differential gene expression (DESeq2) analyses implicated activation of both mTORC1 and lysosomal pathways in *TSC1*mt-BLCA. Expression differences in several genes, including GPNMB, and in pS6 were confirmed by immunohistochemistry (IHC) of 5 independent *TSC1*mt-BLCA and *TSC1*wt-BLCA specimens each. TFE3, a key regulator of lysosomal gene expression, was elevated in expression and localized to the nucleus in *TSC1*mt-BLCA by IHC. Molecular studies of *TSC1*-mt/wt BLCA isogenic cell lines recapitulated the phenotype found in human tumors. TFE3 was both post-translationally modified (assessed by immunoblot) and predominantly nuclear in *TSC1*-null cell lines compared to *TSC1*wt cells, and the localization was partially reversed by rapamycin treatment. *TSC1*mt-BLCA HCV29 cells showed higher expression of multiple genes increased in *TSC1*mt-BLCA tumors (GPNMB, SQSTM1, GNPDA1). TFE3 knock-out HCV29 clones showed markedly reduced growth, which was not seen with TFE3 KO in *TSC1*-addback HCV29 cells. GSEA of RNA-seq data indicated that TFE3 KO led to significant reduction in expression of lysosome genes, as well as those involved in DNA/RNA-related processes. TFE3 CUT&RUN confirmed that TFE3 binds to promoter regions of lysosome genes, thereby directly regulating these pathways. In contrast, TFE3 KO led to an increase in inflammatory and cellular stress genes that could impede *TSC1*-null cell growth. Our findings revealed that *TSC1*mt-BLCA tumors and cell lines share a transcriptional signature with other TSC-associated tumors and show nuclear localization and transcriptional activation of TFE3. Aberrant TFE3 activation likely contributes to *TSC1*mt-BLCA development and may therefore be amenable to targeted therapy.

Vicky H. Whittemore Travel Awards

Platform Presentations

Nicola Alesi, MD, PhD
Brigham and Women's Hospital/ Harvard Medical School
Boston, MA
TFEB drives mTORC1 hyperactivation and kidney disease in tuberous sclerosis complex

Jeffrey Calhoun, PhD
Northwestern University
Chicago, IL
Multimodal framework to resolve variants of uncertain significance in TSC2

Jenny Do, MS, CGC
University of Texas Medical Branch
Houston, TX
Parental stress in tuberous sclerosis complex

Anil Kumar Kalvala, PhD
Texas Tech University Health Sciences Center
Abilene, TX
TSC-null extracellular vesicles facilitate metastable phenotypes of LAM cells and formation of lung metastatic niche

Joohwan Kim, PhD
University of California, Irvine
Irvine, CA
mTORC1-dependent RNA methylation confers rapamycin resistance in TSC tumors

Andy Liu, MD, MS
Duke University School of Medicine
Durham, NC
Discovering the neuropathological etiology of tuberous sclerosis complex-associated neuropsychiatric disorders

Katharina Maisel, PhD
University of Maryland
College Park, MD
Intranasal adjuvant immunotherapy to treat TSC-associated LAM tumors

Tasnim Olatoke
University of Cincinnati
Cincinnati, OH
STAT1 promotes survival and lung metastasis of TSC2-null cells in lymphangioleiomyomatosis (LAM)

Ulrike Rehbein, PhD
University of Innsbruck
Innsbruck, Austria
A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression

David Ritter, MD, PhD
Cincinnati Children's Hospital Medical Center
Cincinnati, OH
A precision dosing strategy for overcoming challenges to the dosing of mTOR inhibitors in TSC infants in clinical practice and clinical trials

Poster Presentations

Jerome Arceneaux, MD, PhD
Meharry Medical College/Vanderbilt University
Nashville, TN
Mapping the cellular composition of resected cortical tubers and perituberal tissues

Devin Barzallo
National Institutes of Health
Bethesda, MD
Ultraviolet signature mutations in TSC skin tumors are increased in sun-exposed body sites, reinforcing recommendations for good sun protection

Lais Cardozo
Federal University of Paraná
Curitiba, Paraná, Brazil
TSC-associated neuropsychiatric disorders (TAND) assessment and the neuropsychological cluster in a group of patients from Brazil

Marie Girodengo, PhD
The Francis Crick Institute Ltd
London, England
Characterisation of the mTORC1-dependent phosphoproteome during neurodevelopment in TSC

Natasha Lindsay
King's College London
London, England
Sleep and its association with temperament and sensory processing in early TSC

Qianqian Ma
Baylor College of Medicine
Houston, TX
Single-cell transcriptomic analysis of tuberous sclerosis complex (TSC)

Kagistia Hana Utami, PhD
Keio University
Tokya, Japan
Vigabatrin acts on cilia pathways in TSC2-deficient neurons

Aynara Wulsin, MD, PhD
Cincinnati Children's Hospital Medical Center
Cincinnati, OH
Implementation of a clinical and research consortium to achieve early diagnosis, adequate disease surveillance and management across the lifespan of patients with TSC in a middle-income country

Manuel R. Gomez Award Recipients

In 2001, the TSC Alliance® began presenting the Manuel R. Gomez Award to individuals who have made a significant impact on our understanding of TSC in research and/or have impacted the delivery of clinical care for individuals with TSC. The award is granted in memory of Manuel R. Gomez, MD (1928-2006), the "Father of TSC" in the United States. A dedicated physician-scientist, Dr. Gomez provided care and compassion for hundreds of individuals with TSC while conducting some of the pioneering research in the field.

Kari Luther Rosbeck, TSC Alliance

Darcy Krueger, MD, PhD, Cincinnati Children's Hospital

Mark Nellist, PhD, Erasmus MC

Chris Kingswood, MB, BS, FRCP, Brighton and Sussex University Hospitals NHS Trust

Julian Sampson, DM, FRCP, FMedSci, Cardiff University

Paolo Curatolo, MD, Tor Vergata University Hospital of Rome

John J. Bissler, MD, University of Tennessee Health Science Center

E. Martina Bebin, MD, MPA, University of Alabama at Birmingham

Petrus J. de Vries, MBChB, MRCPsych, PhD, University of Cape Town

Howard Weiner, MD, Texas Children's Hospital (formerly at New York University Medical Center)

Mark Mausner, MD

Sergiusz Jóźwiak, MD, PhD, Medical University of Warsaw

Elizabeth A. Thiele, MD, PhD, Carol and Jim Herscot Center for Children and Adults with TSC at Massachusetts General Hospital

Ann Hunt, Tuberous Sclerosis Association

David Neal Franz, MD, Cincinnati Children's Hospital Medical Center

Elizabeth Petri Henske, MD, Brigham and Women's Hospital (formerly at Fox Chase Cancer Center)

Hope Northrup, MD, University of Texas Health Science Center at Houston

E. Steve Roach, MD, The Ohio State University College of Medicine (formerly at Wake Forest University)

David Kwiatkowski, MD, PhD, Brigham and Women's Hospital

Vicky Whittemore, PhD, National Institute of Neurological Disorders and Stroke (formerly at the TSC Alliance)

About the TSC Alliance®

The TSC Alliance is an internationally recognized nonprofit that does everything it takes to improve the lives of people with tuberous sclerosis complex. We drive research, improve quality care, increase access and advocate for everyone affected by the disease. The collaboration of individuals and families, along with the partnership of other organizations, fuels our work to ensure people navigating TSC have support—and hope—every step of the way.

Our work wouldn't be possible without the commitment of our community. Advancing research requires dedicated investigators, individuals with TSC, government and industry. Improving quality of and access to care demands healthcare professionals and dedicated volunteers. Raising awareness takes the work of families, individuals, and volunteers.

We use a comprehensive approach to improve quality of life for people with TSC—fueling promising research while making sure that, day-to-day, individuals are diagnosed early and receive the highest quality care available. We also have a voice in policy around healthcare access and federal funding for TSC research.

The TSC community is our strongest ally. With the power of families and the support of donors, volunteers, healthcare providers, 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.

Learn more at tscalliance.org.

Research Overview

The TSC Alliance stimulates, coordinates, and drives research toward a cure for TSC while improving the lives of those affected. Since 1984, the TSC Alliance has invested more than $34 million into TSC research projects through grants and contracts:

- $20.1 million in research grants
- $4.8 million into the Natural History Database and Biosample Repository
- $7.9 million into the Preclinical Research Consortium
- $1.3 million into the Clinical Research Consortium

With TSC community input and support, the TSC Alliance launched a Research Business Plan in 2019 with the following aims: focus research to better understand the course of TSC and identify new treatments; alter the course of the disease through prenatal diagnosis and preventative treatments to improve the quality of life of those with TSC; and educate medical professionals to achieve more rapid diagnosis and better treatment aligned with evidence-based standards of care and consensus guidelines.

Research Resources

TSC Biosample Repository and Natural History Database: The TSC Alliance built the TSC Biosample Repository to accelerate research into why TSC is so variable among individuals and how we might determine which individuals respond better or poorly to certain treatments. Samples in the repository – such as blood, DNA, and tissue – are linked to detailed clinical data in our TSC Natural History Database and are available to qualified researchers worldwide. As of December 1, 2022, the Natural History Database contained 2,546 participants enrolled across 21 TSC clinic sites or by the TSC Alliance. Samples are housed at and distributed from the Van Andel Institute in Grand Rapids, Michigan, under control of the TSC Alliance.

TSC Clinical Research Consortium: In 2012, the TSC Alliance helped launch a TSC Clinical Research Consortium in partnership with investigators running clinical studies to ensure clinical research in TSC is as efficient and effective as possible. Consortium investigators applied for and were awarded more than $39 million by the NIH and Food and Drug Administration (FDA) through competitive grant processes. The TSC Alliance raises community awareness about clinical studies to help identify potential participants and has provided supplemental financial support to accelerate or expand NIH-funded studies. In the coming months, the TSC Alliance will take ownership of coordinating the Clinical Research Consortium to broaden the scope in terms of types of clinical studies and participating sites.

TSC Preclinical Consortium: Translational research takes early discoveries and facilitates its entry into clinical care through coordinated and directed research to evaluate the effectiveness and safety of candidate therapeutics. A key component of this research is conducted in the TSC Preclinical Consortium, which encourages collaboration between a multidisciplinary team of researchers, including clinical researchers. The consortium facilitates drug testing in cell and animal models of TSC. Those compounds that prove efficacious and safe are referred to the TSC Clinical Research Consortium to consider for clinical testing. A goal of the Preclinical Consortium is to advance at least two candidate compounds into clinical trials by 2025.

Research Grants Program: The TSC Alliance invests in early-career researchers to drive innovative TSC research and to foster a diverse group of researchers dedicated to our shared mission to catalyze new treatments and ultimately find a cure for TSC. Most TSC Alliance research grants support focused projects enabling investigators to develop innovative ideas into preliminary data capable of garnering funding from larger organizations such as the National Institutes for Health and Tuberous Sclerosis Complex Research Program as the U.S. Department of Defense.

For more information about the TSC Alliance's research efforts and accessing research resources, contact Steven L. Roberds, PhD, Chief Scientific Officer, at sroberds@tscalliance.org.

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TSC International (TSCi) Member Organizations

Tuberous Sclerosis Complex International (TSCi) is a world-wide consortium of existing tuberous sclerosis complex associations and organizations, serving as an avenue to empower those affected by tuberous sclerosis complex (TSC), including individuals, families, caregivers, educators, and health care providers. In addition, TSCi provides a forum to share information, exchange ideas and methods, co-fund research projects and promote increased international awareness of TSC. Additional information can be found at www.tscinternational.org.

Europe

European Tuberous Sclerosis Complex Association (ETSC)
Established in 2012, ETSC is a federation of Tuberous Sclerosis Associations. ETSC believes in total "patient experience" and that interaction with all healthcare providers and relative agencies is paramount.

Website: www.e-tsc.eu
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Argentina

Asociación Argentina de Esclerosis Tuberosa (ARGET)
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Australia

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Email: info@tscchina.org

Denmark

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Website: http://tsdanmark.dk
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Finland

TS-YHDISTYS
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France

French TSC association – Association française Sclérose tubéreuse de Bourneville (ASTB)
Website: www.astb.asso.fr
Email: contact@astb.asso.fr

Germany

German Tuberous Sclerosis Association (TSDe.V) – Tuberöse Sklerose Deutschland e.V.
Website: www.tsdev.org
Email: info@tsdev.org

Greece

Tuberous Sclerosis Association of Greece – Ελληνική Εταιρεία Οζώδους Σκληρύνσεως (Ε.Ε.Ο.Σ.)
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Hungary

Hungarian Foundation for Tuberous Sclerosis
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The Portuguese Tuberous Sclerosis Association – Associação de Esclerose Tuberosa em Portugal (AETN)
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Russia

Association of patients with tuberous sclerosis – Ассоциация больных туберозным склерозом
Website: epileptologhelp.ru/about/association-of-patients-with-tuberous-sclerosis
Email: tsc.rus@mail.ru

Serbia

Tuberous Sclerosis Association of Serbia – Udruženje za tuberoznu sklerozu Srbije
Email: 2013utss@gmail.com

Slovakia

ASTUS – Asociácia tuberóznej sklerózy (ASTUS, n.o.)
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Spanish Tuberous Sclerosis Association – Asociación Nacional de Esclerosis Tuberosa
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Thank You to the National Institutes of Health

Funding for this conference was made possible in part by 1R13NS134255-01 from National Institutes of Health, the National Institute of Neurological Disorders and Stroke, National Center for Advancing Translational Sciences, the National Cancer Institute, and National Heart, Lung, and Blood Institute.

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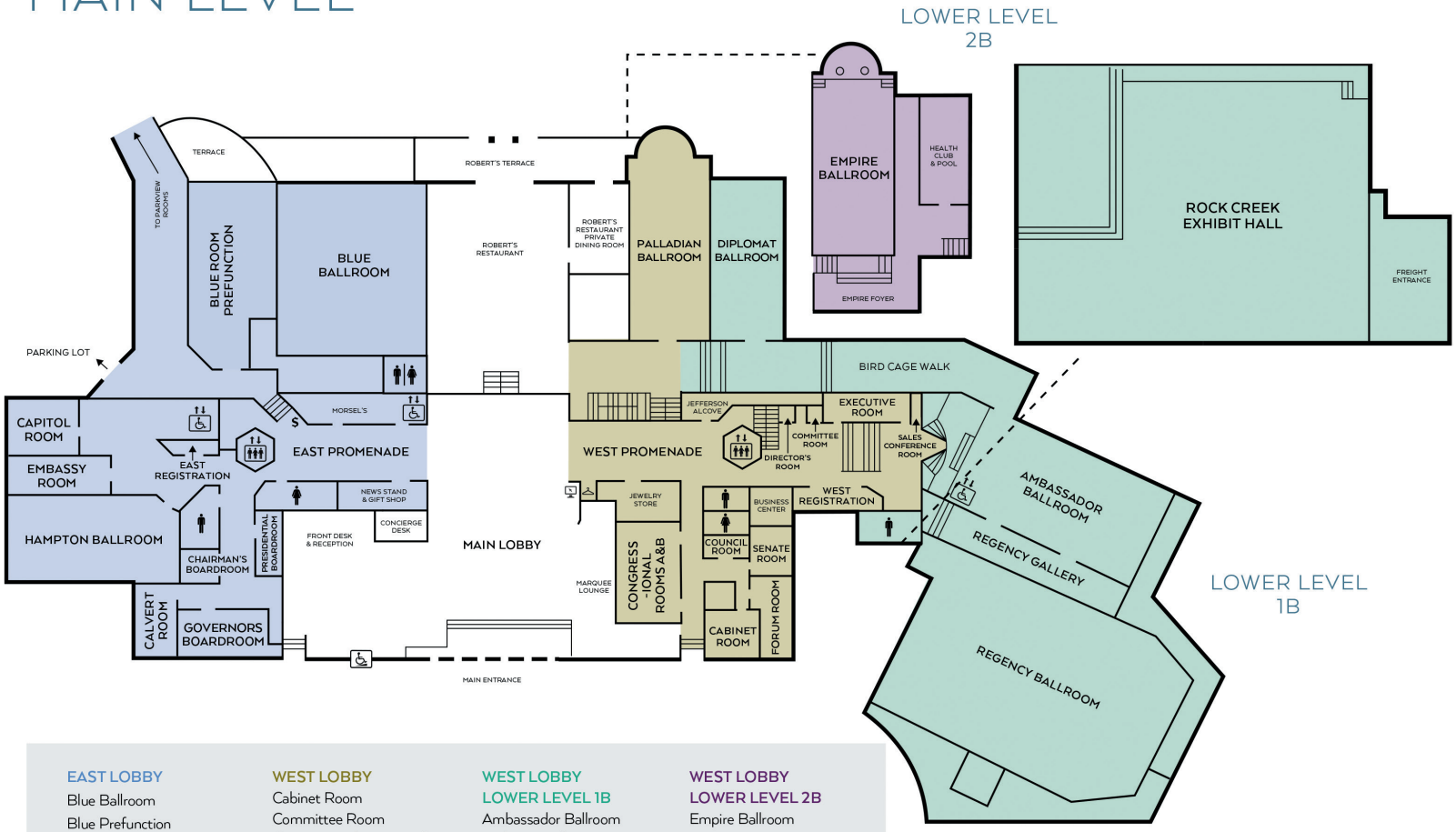

EAST LOBBY
Blue Ballroom
Blue Prefunction
Calvert Room
Capitol Room
Chairman's Boardroom
Embassy Room
Governor's Boardroom
Hampton Ballroom
Presidential Boardroom
East Registration

WEST LOBBY
Cabinet Room
Committee Room
Congressional Rooms A&B
Council Room
Director's Room
Executive Room
Forum Room
Palladian Ballroom
Sales Conference Room
Senate Room
West Registration

WEST LOBBY
LOWER LEVEL 1B
Ambassador Ballroom
Diplomat Ballroom
Regency Room
Regency Gallery
Rock Creek Exhibit Hall

For access to Diplomat Room, please use the elevators on the west side and go to level 1B.

WEST LOBBY
LOWER LEVEL 2B
Empire Ballroom

For access to the Empire Room and Health Club/ Outdoor Pool please use the elevators on the west side and go to level 2B.

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