Pediatric Scientist Development Program

Building the next generation of pediatrician scientists

2020 Annual Meeting

February 27 – March 1, 2020

The Hilton Long Beach

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WELCOME

On behalf of the Pediatric Scientist Development Program (PSDP), it is a great pleasure to welcome you to the 2020 Annual meeting, hosted by the Association of Medical School Pediatric Department Chairs (AMSPDC) in Long Beach, California. The meeting has been organized to facilitate discussion on all aspects of the Pediatric Physician-Scientist mission, primarily through platform sessions and a poster session. We are honored to have all participants at this year's meeting, including PSDP Steering Committee presenters: Drs. Terence Dermody, Adam Ratner, Hilary Haftel and Karen Winer. We would also like to extend appreciation to guest spakers: Drs. Ann Reed, Patrick Brophy, Rasheed Gbadegesin, Jason Nagata and Brian Kalish.

Thank you for your participation in helping build the next generation of pediatric physician-scientists through collaboration and career development.

We hope you enjoy the 2020 conference!

Sallie R Permar

Sallie Permar, MD, PhD
PSDP Program Director
Associate Dean for Physician-Scientist Development
Professor of Pediatrics, Molecular Genetics & Microbiology, and Immunology
Duke University School of Medicine

PSDP Administration

Program Director

Sallie Permar, MD, PhD

Steering Committee - Program Evaluation subcommittee

Lisa Satlin, MD	Mount Sinai School of Medicine (Program Evaluation Chair)
Sherin Devaskar, MD	University of California, Los Angeles
Terence Dermody, MD	Children's Hospital of Pittsburgh
Phyllis Dennery, MD	Brown University
Jordan Orange, MD, PhD	Columbia University

Steering Committee - Selection subcommittee

Adam Ratner, MD, MPH	New York University (Selection Chair)
Wade Clapp, MD	Indiana University
Stephanie Davis, MD	University of North Carolina, Chapel Hill
Mary Leonard, MD	Stanford University
Norman Rosenblum, MD	Hospital for Sick Children, University of Toronto
Alan Schwartz, MD, PhD	Washington University
Joseph St. Geme III, MD	Children's Hospital of Philadelphia

Steering Committee Funding Agency Representatives

Hilary Haftel, MD, MHPE, FAAP	American Academy of Pediatrics
Kelle Moley, MD	March of Dimes
Susan Samuel, MD, MSc	University of Calgary
William Steinbach, MD	American Pediatric Society
Karen Winer, MD	National Institute of Child Health & Human Development

Partner Programs

St. Jude Children's Research Hospital Fellowship Award in Basic and Translational Science

Funded by St. Jude Children's Research Hospital and jointly sponsored by the Pediatric Infectious Diseases Society and St. Jude, the goal of this program is to develop a larger cadre of physician-scientists who are trained to carry out rigorous, high-quality, basic and translational investigation in the field of pediatric infectious diseases. Awardees are pediatric infectious diseases fellows who pursue research opportunities in cutting-edge basic science and translational units, including opportunities outside of departments of pediatrics in laboratories in the U.S. and throughout the world. Fellows supported by the program have fully protected time for research throughout their research years. During the clinical year of training, fellows are offered the option of a one-month rotation on the Infectious Diseases service at St. Jude Children's Research Hospital. This rotation provides an intensive experience in the diagnosis and treatment of infectious complications in patients with compromised immunity. Each year fellows have the opportunity to present their research at the St .Jude-PIDS Pediatric Infectious Diseases Research Conference and the Pediatric Transplant Research Conference, annual events involving leading scientists in microbiology and infectious diseases. They are also welcomed at the AMSPDC annual meeting with PSDP fellows to further explore career development.

CANADIAN CHILD HEALTH CLINICIAN-SCIENTIST PROGRAM

In June 2003, the Canadian Child Health Clinician-Scientist Program (CCHCSP) / Programme Canadien de Cliniciens-Chercheurs en Santé de l'Enfant (PCCCSE) and the PSDP formed a partnership. The goal is to ensure pediatric residents and pe- diatricians-in-training have access to the best North American clinician-scientist training programs. The cooperative interaction between our two programs has led to excellent educational practices and outstanding clinician-scientists working in academic pediatrics. We have devel- oped a number of collaborative venues to expose PSDP and CCHCSP trainees to best practices in clinical and laboratory research, training, and management. Both of our programs are characterized by a talented group of trainees and experi- enced mentors. Our trainees, mentors, and program leaders attend one another's annual symposia, providing a shared forum for training workshops and exchange of research ideas and practices. Participation by PSDP and CCHCSP trainees in these symposia has been highly acclaimed by trainees and mentors alike. At each symposium, trainees participate in career development workshops, present research, and receive critical feedback. These activities enrich the training expe- riences in both the PSDP and the CCHCSP.

AMSPDC and the PSDP welcome CCHCSP Administration and two trainees to the 2020 PSDP meeting activities and the FIS Session.

CCHCSP Administration

Susan Samuel, MD, MSc, Principal Investigator
Associate Professor, Departments of Paediatrics, Department of Community Health Sciences
Linda Pires,
CCHCSP Program Manager
The University of Calgary
Alberta Children's Hospital Research Institute

General Announcements

Oral Presentations

Talks scheduled for Thursday February 27 will be held in International II. Talks scheduled for Friday February 28 will be held in International III-V. It is important that the participants do not exceed the allocated time.

Posters

The poster session is scheduled for Friday February 28 at 6pm in the Promenade. Presenters begin setting up at 3pm. All posters will be displayed for the entirety of the poster session. Posters are numbered according to the abstract portion of this program book, so please be sure to hang your poster on the board assigned to this number (clips will be provided). Posters must be removed from the boards prior to dinner on Friday night.

Catering

Throughout the conference, we will offer breakfast, lunch and snacks. On Thursday evening, there will be a conference reception in the Catalina room. The PSDP Conference attendees will have dinner at 7:30pm at Bueno's Pizza. On Friday evening, we will offer hors d'oeuvres during the poster session, followed by dinner at 7:30pm in the International Room.

Evaluations

Participants will be asked to evaluate the conference. These evaluations provide important feedback and help the organizers improve future meetings.

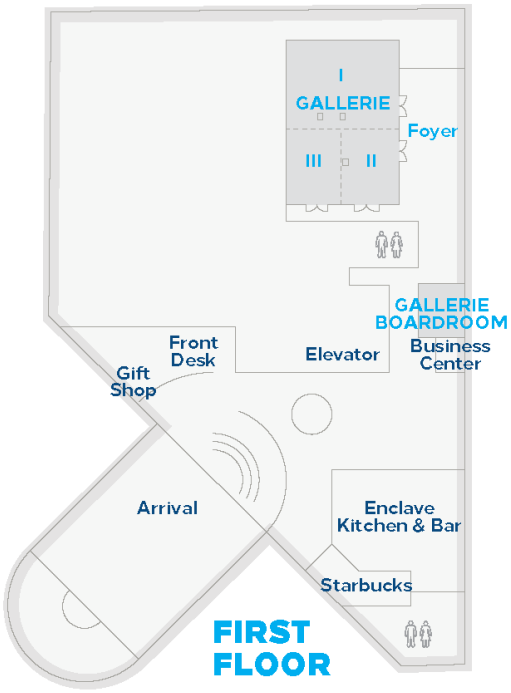
WIFI

Complimentary Wireless Internet:
Network - AMSPDC Annual Meeting
Password - AMSPDC20

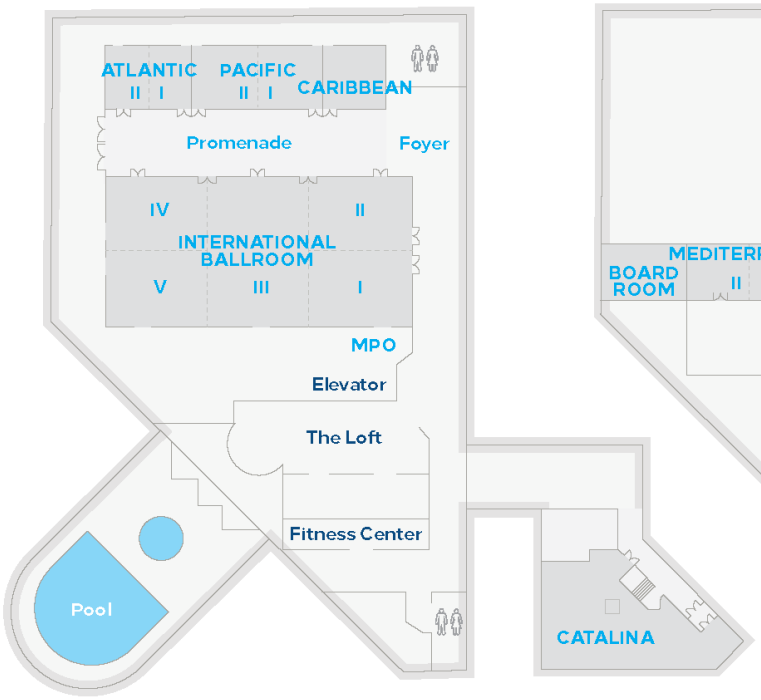
Acknowledgements

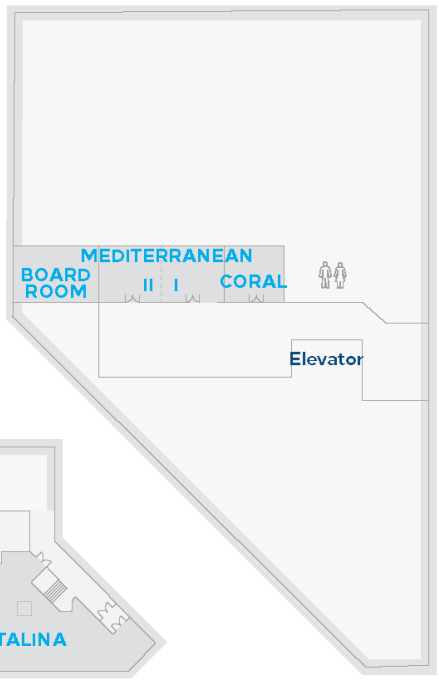
We are very grateful for all of the people who have worked diligently behind the scenes to make this meeting success, as well as the support of the Association of Medical School Pediatric Department Chairs.

VENUE / FLOOR PLAN



Hilton
LONG BEACH

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THIRD
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CONFERENCE AGENDA – Thursday February 27, 2020

6:30 – 8:00am	**Breakfast Buffet**	Atlantic I	PSDP, CCHCSP and St. Jude Fellows
7:00 – 7:30am	**Advising Session Orientation Breakfast**	Atlantic II	PSDP Steering Committee
7:30 – 10:00am	**Advising Sessions** *Locations are noted on your personal agenda		1st, 2nd & 3rd Year PSDP Fellows and Advisors
8:00 – 8:45am	**PSDP Orientation** *Sallie Permar, MD, PhD* *PSDP Program Director*	International II	PSDP Incoming Fellows
9:00 – 10:00am	**Career in Pediatrics and Panel on Negotiating a Faculty Job** *Sallie Permar, MD, PhD (PSDP Program Director);* *Ann Reed, MD (Chair, Dept. of Pediatrics, Duke);* *Patrick Brophy, MD (Chair, Dept. of Pediatrics, University of Rochester);* *Jason Nagata, MD (Assistant Professor of Pediatrics, UCSF)*	International II	PSDP Incoming, CCHCSP, and St. Jude Fellows
10:00 – 10:15am	**BREAK**		
10:15 – 11:45am	**PSDP Advisory Session Debrief**	Boardroom	PSDP Advisory Session Participants
10:15 – 11:15am	**Kick Starting PS Careers: Primer on K Award** *Rasheed Gbadegesin, MBBS, MD, FASN* *(Professor of Pediatrics and Medicine, Duke University School of Medicine)*	International II	PSDP, CCHCSP and St. Jude Fellows
11:30-12:30pm	**Lunch**	Loft Dining Room	PSDP, CCHCSP and St. Jude Fellows; Steering Committee
12:30-1:30pm	**Maintaining Effective Mentoring Relationships** *Terence Dermody, MD* *(Chair, Department of Pediatrics UPMC Children's Hospital of Pittsburgh)*	International II	PSDP, CCHCSP and St. Jude Fellows
1:00-1:30pm	**Selection Sub-Committee Meeting**	Atlantic I	Selection Sub-Committee
1:30-2:15pm	**Keeping all the Balls in the Air: Managing Your Time When You Are Already Overextended** *Hilary Haftel, MD* *(Senior Vice President of Education, AAP)*	International II	PSDP, CCHCSP and St. Jude Fellows
2:15 – 2:30pm	**BREAK**		
2:30-3:30pm	**Communicating Your Science** *Adam Ratner, MD, MPH* *(Associate Professor of Pediatrics & Microbiology, New York University School of Medicine)*	International II	PSDP, CCHCSP, and St. Jude Fellows
3:30-4:15pm	**Identifying NIH Funding Opportunities** *Karen Winer, MD* *(Director, Endocrinology & Bone Programs, Pediatric Growth & Nutrition Branch, NICHD)*	International II	PSDP, CCHCSP and St. Jude Fellows
3:30-4:30pm	**Joint Committee Meeting**	Boardroom	PSDP Steering Committee
6:00-7:00pm	**Joint Networking Reception**	Catalina	New Chairs, PSDP, PLDP, CCHCSP and St. Jude Fellows
7:30pm	**PSDP Conference Dinner**	Bueno's Pizza	PSDP, CCHCSP and St. Jude Fellows, and Steering Committee Members

CONFERENCE AGENDA – Friday February 28, 2020

7:00 – 8:00am	**Breakfast Buffet**	International I-II	FIS, PSDP, CCHCSP and St. Jude Fellows
8:00 – 11:00am	**PSDP Research Presentations** **ALL TALKS WILL BE HELD IN INTERNATIONAL III-V** *ATTENDEES: FIS, PSDP, CCHCSP and St. Jude Fellows*		
8:00am	**Opening Remarks** *Sallie Permar, MD, PhD (PSDP Program Director)*		
8:05am	**Selective CDK inhibitors alter gammaherpesvirus reactivation and lytic infection in vitro** *Joy Gibson, MD, PhD (University of Colorado School of Medicine)*		
8:20am	**Surprising Efficacy of β-lactamase inhibitors against Multidrug-Resistant Gram- Negative Pathogens Occurring Through Innate Immune Sensitization** *E.R. Chulie Ulloa, MD, MSc (University of California, San Diego)*		
8:35am	**MvaT and MvaU act coordinately as global repressors of genic and intragenic transcription in Pseudomonas aeruginosa** *Andrew M. Lippa, MD, PhD (Boston Children's Hospital)*		
8:50am	**Transgenic TCR Adoptive Cellular Therapy for Treatment of Solid Tumors** *Theodore Scott Nowicki, MD, PhD (University of California, Los Angeles)*		
9:05am	**Nutrient availability drives community dynamics in the vaginal microbiota** *M. Indriati Hood Pishchany, MD, PhD (Boston Children's Hospital)*		
9:20am	**Boys, Bulk, and Body Ideals: Epidemiology of Muscle-Enhancing Behaviors in Adolescents and Young Adults** *Jason M. Nagata, MD, MSc (University of California, San Francisco)*		
9:35am	**Maternal Immune Activation Disrupts Proteostasis in the Fetal Brain** *Brian Kalish, MD (Harvard Medical School)*		
9:50am	**BREAK**		
10:00am	**Human Antibodies Neutralize Enterovirus D68 and Protect Against Infection and Paralytic Disease** *Matthew R. Vogt, MD, PhD (Vanderbilt University Medical Center)*		
10:15am	**The effect of an International competitive leaderboard on self-motivated simulation-based CPR practice** *Catharine Walsh, MD, MEd, PhD (Hospital for Sick Children, Toronto, Canada)*		
10:30am	**The Pediatric Scientist Development Program** *Sallie Permar, MD, PhD (PSDP Program Director)*		
11:00 – 12:00pm	**What They Didn't Teach You in Medical School – Key Leadership and Administrative Skills for Success** *Brian Kalish, MD (Harvard Medical School)* *Jason Nagata, MD (University of California, San Francisco)*		
11:00 – 12:00pm	**PSDP Steering Committee Meeting**	Pacific II	PSDP Steering Committee Members
12:00 – 1:30pm	**FIS Meet the Professor Lunch**	Atlantic I-II	FIS, PSDP, CCHCSP and St. Jude Fellows
2:00 – 5:00pm	**Frontiers in Science Symposium**	International III-V	FIS, PSDP, CCHCSP and St. Jude Fellows
6:00 – 7:30pm	**Reception and Poster Presentations** Pediatric Scientist Development Program, Canadian Child Health Clinician Scientist Program and St. Jude Research Fellowship Program	Promenade	FIS, PSDP, CCHCSP and St. Jude Fellows
7:30-9:30pm	**PSDP, CCHCSP, St. Jude & FIS Dinner**	International I-II	FIS, PSDP, CCHCSP and St. Jude Fellows

GUEST SPEAKERS

Sallie Permar, MD, PhD

Associate Dean for Physician-Scientist Development
Professor of Pediatrics
Professor of Molecular Genetics and Microbiology
Professor in Immunology
Professor of Pathology
Director, Children's Health & Discovery Initiative

Dr. Permar is a physician scientist focusing on the prevention and treatment of neonatal viral infections. She leads a research laboratory investigating immune protection against vertical transmission of neonatal viral pathogens, namely HIV and cytomegalovirus (CMV), using human cohorts and nonhuman primate models. Dr. Permar has made important contributions to the development of vaccines for prevention of vertical HIV transmission, defining both innate and adaptive immune responses that are associated with protection against infant HIV acquisition. Dr. Permar has a PhD in Microbiology/Immunology from Johns Hopkins Bloomberg School of Public Health in Baltimore, an M.D. from Harvard Medical School and completed her clinical training in pediatric infectious diseases at Children's Hospital in Boston. She is an institutional and national leader in physician-scientist training, serving as the Associate Dean of Physician-Scientist Development at Duke University Medical School and was selected by the Association of Medical School Pediatric Department Chairs (AMSPDC) as the next Director of the national Pediatric Scientist Development Program in 2019.

Ann M. Reed, M.D.

Samuel L. Katz Distinguished Professor of Pediatrics
Chair, Department of Pediatrics
Physician-in-Chief, Duke Children's Hospital
Duke University Medical Center

Dr. Reed joined Duke in August of 2014 from the Mayo Clinic where she was Chair of the Department of Pediatric and Adolescent Medicine, and Physician-in-Chief for the Mayo Clinic Children's Center in Rochester, Minnesota. She is a Professor of Pediatrics at Duke University and remains a Professor of Pediatrics and Medicine at the Mayo Medical School and a consultant in the Division of Rheumatology in both the Departments of Pediatric and Adolescent Medicine and Internal Medicine. She has spent her career caring for children with autoimmune disease and immune dysfunction. Her work focuses on caring for children with juvenile dermatomyositis and auto inflammatory disorders. Dr. Reed has overseen a research program for 24 years studying the genetics and cause of human autoimmune disease, focused on dermatomyositis in children and adults.

Patrick D. Brophy, MD, MHCDS

Williams H. Eilinger Chair, Department of Pediatrics
Physician-In-Chird, Golisano Children's Hospital
Professor of Pediatrics
University of Rochester Medical Center

Dr. Brophy's clinical interests include; Neonatal and congenital genetic renal anomalies, Acute Kidney Injury/Critical Care Nephrology, Lifecourse healthcare delivery, Healthcare Delivery Sciences as well as Technology use in improving healthcare access and reducing disparity. Dr. Brophy serves as the President-elect of the American Society of Pediatric Nephrology (ASPN); Member of the American Board Pediatrics (ABP). Is a member of the Association of Medicine School Pediatric Department Chairs (AMSPDC) and serves on the finance committee of the American Board of Medical Specialties (ABMS) and on the program planning committee for the Society for Pediatric Research (SPR)

Rasheed Gbadegesin, MD, MBBS

Professor of Pediatrics and Medicine
Faculty Director & PI Physician-Scientist Institutional Award

Dr Gbadegesin is a Tenured Professor of Pediatrics, Division of Nephrology, Duke University, Durham, NC, an Investigator at the Duke Molecular Physiology Institute (DMPI). He is the Co-Director of the Duke CTSA KL2 program and the Program Director of the Duke Pediatric Research Scholar (DPRS/PSTP) program. The overarching objective of his research program is to understand the genetic basis, the pathogenesis, and the determinants of variable therapy response in hereditary and idiopathic nephrotic syndrome. In the last ten years, Dr Gbadegesin and his collaborators have identified at least five novel genetic causes of steroid resistant nephrotic syndrome/Focal Segmental Glomerulosclerosis (FSGS) and other kidney diseases. More recently, using the strategy of extreme phenotyping, his group identified the first exome-wide locus for childhood onset steroid sensitive nephrotic syndrome (SSNS). He has established a large biorepository of phenotype data and biosamples from children with nephrotic syndrome and he is currently collaborating with other Investigators around the World to determine the biologic basis for ethnic disparities in the incidence and severity of nephrotic syndrome with the ultimate goal of developing strategies for personalized treatment of nephrotic syndrome. He has presented his research at both international and national meetings and published many peer reviewed journal articles with multiple first and senior author papers related to the molecular pathogenesis of nephrotic syndrome. Dr Gbadegesin is the Principal Investigator on multiple NIH, foundation, and industry grants. He was elected into the American Society for Clinical Investigation (ASCI) in 2016.

Jason Nagata, M.D., M.Sc.

Assistant Professor of Pediatrics
Division of Adolescent and Young Adult Medicine
University of California, San Francisco

Dr. Nagata is an expert in the medical management of eating disorders and researches body image in understudied populations including males, sexual minorities, and gender minorities. His research leverages nationally representative longitudinal cohort studies to understand downstream health consequences of adolescent and young adult behaviors to prevent disease in later adulthood. He has published over 75 articles in academic journals including *The Lancet* and *JAMA*. He serves as Co-Chair of the International Association for Adolescent Health Young Professionals Network and on the Board of Directors of the Society for Adolescent Health and Medicine. He is the recipient of the 2019 American Academy of Pediatrics Emerging Leader in Adolescent Health Award and the Society for Pediatric Research Fellow's Clinical Research Award.

Brian Kalish, MD

Attending Neonatologist, Division of Newborn Medicine
Instructor of Pediatrics
Harvard Medical School

Brian Kalish is an Attending Neonatologist at Boston Children's Hospital and an Instructor of Pediatrics at Harvard Medical School. Originally from Chicago, Brian studied Public Health at Johns Hopkins University prior to obtaining his medical degree at Harvard Medical School. Brian completed pediatrics training on the Accelerated Research Pathway in the Boston Combined Residency Program, followed by fellowship in the Harvard Neonatal-Perinatal Medicine training program. His research focuses on developmental neurobiology and the impact of adverse early life experiences on long term neurodevelopment.

PSDP RESEARCH SPEAKERS

Terence S. Dermody, M.D., is the Vira I. Heinz Professor and Chair of the Department of Pediatrics at the University of Pittsburgh School of Medicine as well as Professor of Microbiology and Molecular Genetics. He also is physician-in-chief and scientific director of Children's Hospital of Pittsburgh of UPMC. Dr. Dermody is a virologist with interests in viral pathogenesis and vaccine development. He has focused mainly on reovirus, an important experimental model for studies of viral encephalitis in infants. The work in Dr. Dermody's lab has encompassed several inter-related themes including the structural basis of viral attachment and cell entry; mechanisms of genome replication and packaging; patterns of cell signaling and gene expression occurring in response to viral infection; mechanisms of virus-induced apoptosis and its significance in the viral life cycle; and the role of viral receptor distribution and utilization in disease pathology. The lab also is developing viral vectors for oncolytic and vaccine applications.

Hilary M. Haftel, MD, MHPE, FAAP serves as Senior Vice President for Education, overseeing education across the continuum, from medical students through continuing medical education (CME), as well as CME accreditation and the AAP's electronic educational platforms. She is Professor Emerita of Pediatrics and Health Learning Sciences at the University of Michigan Medical School, where she has spent her entire academic career prior to moving to the AAP. She served as Director of the Division of Pediatric Rheumatology and Associate Chair and Director of Education in the Department of Pediatrics and Communicable Diseases at the University of Michigan, as well as being the Program Director for the Categorical Residency program

Adam J. Ratner, MD, MPH, is the Director of Pediatric Infectious Diseases at Hassenfeld Children's Hospital and Associate Professor of Pediatrics and Microbiology at NYU-Langone Health. Adam received his B.A. from Yale and his M.D. and M.P.H. degrees from Columbia University. He completed his pediatric residency at Columbia and his subspecialty training in infectious diseases at Children's Hospital of Philadelphia. Dr. Ratner heads an active, NIH-funded translational research laboratory focused on understanding and preventing infections during pregnancy and early childhood. He is the immediate past chair of the Clinical Research and Field Studies in Infectious Diseases review panel at NIH, serves on the Board of Directors of the Pediatric Infectious Diseases Society, and is President-Elect of the Infectious Diseases Society of New York.

Karen Winer, M.D is a pediatric endocrinologist and senior medical officer at the NICHD. She completed her pediatric residency training at the Mount Sinai Medical Center in New York and subspecialty training in pediatric endocrinology in the NICHD's Division of Intramural research (DIR). As a clinical investigator in the DIR, she was the first to study parathyroid hormone replacement therapy for treatment of hypoparathyroidism. Dr. Winer joined NICHD's Pediatric Growth and Nutrition Branch in 1998 to direct and develop programs in pediatric endocrinology and bone health. She established the Diabetes Research in Children Network (DirecNet) to develop the artificial pancreas and to characterize dysglycemia effects on young children. Additionally, she initiated and directed the Bone Mineral Density in Childhood Study, a multicenter network that established pediatric reference curves for bone density. Dr. Winer has a special interest in training early stage investigators and is the program director for the Pediatric Scientist Development Program. She has received multiple NIH Director's awards for her work and is a member of the editorial board of the Journal of Clinical Endocrinology and Metabolism for the past 6 years.

SPEAKER ABSTRACTS

Name and Degree(s))	Joy Gibson, MD, PhD
Research Mentor(s)	Linda van Dyk, PhD
Presentation Title	**Selective CDK inhibitors alter gammaherpesvirus reactivation and lytic infection *in vitro*.**

Background: Infection with the gammaherpesvirus EBV leads to lifelong viral latency with intermittent reactivation that is typically controlled through cell-mediated immunity. Following transplantation, viral reactivation may be poorly controlled, leading to a spectrum of lymphoproliferative disorders. Cyclins are necessary for viral reactivation and pathogenesis, suggesting that the cyclin binding partners, cyclin-dependent kinases (CDKs), likely also contribute to viral pathogenesis.

Aims: The aims of this research are to determine the impact of clinical CDK inhibitors on viral reactivation and lytic infection, which will yield insight regarding their clinical use and on the mechanistic role of CDKs in viral pathogenesis.

Methods: We make use of the mouse model and a latently infected B cell line, A20-HE2.1, to evaluate viral reactivation *in vitro*. With this system, we chemically induce viral reactivation and analyze the effect of CDK inhibition. Using quantitative PCR and flow cytometry readouts, we can assess viral gene expression and replication in the presence and absence of CDK inhibitors.

Results: We show that treatment with broad spectrum CDK inhibitors (such as Dinaciclib, inhibitor of CDK 1/2/5/9) potently reduce viral reactivation in the A20-HE2.1 cell line. However, the specific CDK4/6 inhibitor, Palbociclib, significantly increases viral reactivation when given 24 hours prior to reactivation induction, but decreases reactivation when given concurrently with reactivation inducers. Membrane permeability analyses indicate that Palbociclib-mediated differences in reactivation induction are not related to a loss of viability. The effect of inhibitors on reactivation is consistent across the mouse model gammaherpesvirus68 and EBV. Interestingly, lytic infection is consistently inhibited by both broad spectrum inhibitors and CDK4/6 inhibitors regardless of treatment timing.

Conclusions: These findings indicate that CDK 4/6 plays a unique role in viral reactivation relative to other CDKs. Palbociclib and other specific CDK 4/6 inhibitors are currently in use clinically as an adjunctive chemotherapy for a number of cancers, including leukemia and lymphoma. Our findings suggest that these inhibitors may function as useful components of treatment for EBV complications following transplantation.

Name and Degree(s)	E.R. Chulie Ulloa, MD, MSc
Research Mentor(s)	Victor Nizet, MD UC San Diego George Sakoulas, MD UC San Diego
Presentation Title	Surprising Efficacy of β-lactamase inhibitors against Multidrug-Resistant Gram-Negative Pathogens Occurring Through Innate Immune Sensitization

Background: Infections caused by New Delhi metallo-β-lactamases (NDM)-producing strains of multidrug-resistant (MDR) *Klebsiella pneumoniae* are a global public health threat lacking reliable therapies. NDM is impervious to all existing β-lactamase inhibitor (BLI) drugs, including the non-β-lactam structure BLI, avibactam (AVI). Though lacking direct activity against NDM enzymes, AVI can interact with penicillin-binding protein (PBP) 2 in a manner that may influence bacterial cell wall dynamics that may render MDR pathogens more susceptible to killing by the innate immune system.
Aim: Given the selective binding of AVI to PBP2 in *K. pneumoniae* and a precedent for certain PBPs in modulating bacterial susceptibility to import innate immune components such as antimicrobial peptides, we explored the influence of this Food and Drug Administration (FDA)–approved non–β-lactam BLI on *K. pneumoniae* interactions with the human defense peptide LL-37 and

other soluble and cellular effectors of innate immunity. As an exemplar of urgent public health concern, we performed our studies by using an NDM-1–expressing *K. pneumoniae* strain for which prevailing logic would predict BLI monotherapy to be fruitless.
Methods: We performed LL-37 kill curves and killing assays with human serum, neutrophils and platelets in the presence or absence of AVI 4 µg/mL against NDM-producing *K. pneumoniae.* Bacterial cytological profiling, a high-resolution fluorescence microscopy technique, was used to assess LL-37 bacterial cell binding and cytotoxicity in the presence or absence of AVI. Lastly, AVI monotherapy was tested in an *in vivo* murine model of infection.
Results: We found that exposure of NDM *K. pneumoniae* to AVI led to striking bactericidal interactions with human antimicrobial peptide LL-37, a frontline component of host innate immunity. Moreover, AVI markedly sensitized NDM *K. pneumoniae* to killing by freshly isolated human neutrophils, platelets, and serum when complement was active. Microscopy demonstrated a *K. pneumoniae* morphologic transition from rod to sphere in the presence of AVI. AVI-treated cells also demonstrated increased LL-37 binding, leading to widespread bacterial cell death. Finally, AVI monotherapy reduced lung NDM *K. pneumoniae* counts in a murine pulmonary challenge model.
Conclusions: AVI has immune sensitizing activities against NDM *K. pneumoniae* not appreciated by standard antibiotic testing and meriting further study. Better *in vitro* assays are needed to improve predictability of the *in vivo* activity of antibiotics.

Name and Degree(s)	Andrew M. Lippa, MD, PhD
Research Mentor(s)	Simon L. Dove
Presentation Title	MvaT and MvaU act coordinately as global repressors of genic and intragenic transcription in *Pseudomonas aeruginosa*

Background: The H-NS-like proteins MvaT and MvaU act coordinately as global repressors in *Pseudomonas aeruginosa* by binding to DNA, polymerizing laterally, and forming bridges between distant genomic regions. These proteins regulate diverse cellular processes required for virulence, including quorum-sensing and biofilm formation. Identifying the combined effect of MvaT/MvaU has been limited because they play partially redundant functions. Further, because either MvaT or MvaU effectively represses transcription/production of a conserved, endogenous prophage Pf4, cells do not tolerate the absence of both proteins. In other bacteria, in addition to acting as repressors of transcription H-NS family members have been shown to repress transcription initiating from promoters housed within genes. MvaT and MvaU are thought to act similarly, though a role in repressing aberrant transcription from cryptic intragenic promoters has not been described.
Aims: To identify the effect of loss of both MvaU and MvaT on transcription profiles and on aberrant transcription initiation in cells.
Methods: High throughput sequencing and molecular biology techniques were used.
Results: Compared to the parental strain, deletion of *mvaU* alone had minimal effect on the transcriptome by RNA-seq; however, loss of both *mvaT* and *mvaU* altered expression of ~10% of known genes. Further, we also observed increased antisense, intragenic, and antisense-intragenic transcription from regions normally occupied by MvaT or MvaU in wild-type cells by ChIP-seq. As expected, occupancy of the RNA polymerase σ70 subunit, a proxy for promoter location, was increased upstream of transcripts that were upregulated in the Δ*mvaT* Δ*mvaU* cells, but σ70 occupancy was also at intragenic loci. Transcription start site mapping for a candidate operon demonstrated sense and antisense initiation sites that correlated with σ70 enrichment peaks, both upstream of and within genes.
Conclusions: MvaT and MvaU act together in *P. aeruginosa* to globally silence gene expression, but also to prevent transcription initiation from cryptic intragenic and antisense promoters.

Name and Degree(s)	Theodore Scott Nowicki, M.D., Ph.D.
Research Mentor(s)	Antoni Ribas, M.D., Ph.D.
Presentation Title	Transgenic TCR Adoptive Cellular Therapy for Treatment of Solid Tumors

Background: Transgenic T-cell receptor (TCR) cell therapies consisting of genetically engineered T cells expressing tumor antigen-specific TCRs displays robust initial antitumor activity, followed by frequent disease relapse. There is an urgent need to characterize the biological changes in transgenic T cells which occur over time in vivo which contribute to this loss of antitumor activity.

Aims/Methods: Clinical circulating T cell samples were obtained from patients treated at UCLA for melanoma and sarcoma utilizing transgenic T cells expressing the MART-1 and NY-ESO-1 TCR, respectively. Baseline and longitudinal changes in transgenic T cell functional phenotype in clinical samples were determined via flow cytometry and mass cytometry. Persistence was determined via fluorescent MHC dextramer binding with the transgenic TCR.

Results: Cells which utilized shorter ex vivo culture protocols displayed increased proportions of naïve and memory T cells (which are phenotypically "younger" and more effective T cells), and cells which were administered fresh displayed superior persistence and expansion of these phenotypes over time in vivo. All transgenic T cells displayed overall increases in terminally differentiated effector/exhausted phenotypes over time, correlating with loss of therapeutic efficacy.

Conclusions: Chronic antigen stimulation leading to hypofunctional phenotypic progression is associated with loss of therapeutic efficacy in transgenic TCR cell therapy for advanced cancers. A new clinical protocol providing a renewable source of these cells via transducing of the TCR within the hematopoietic stem cell niche may provide a long-term, renewable source of these cells in vivo.

Name and Degree(s)	M. Indriati Hood Pishchany, MD, PhD
Research Mentor(s)	Seth Rakoff-Nahoum and Raina Fichorova
Presentation Title	Nutrient availability drives community dynamics in the vaginal microbiota

Background: Nutrient utilization is both critical for niche occupation and is the driver of competitive and cooperative interactions in microbial communities. The female reproductive tract (FRT) is replete with host-associated sugars in the form of glycoproteins, epithelial glycogen stores and the breakdown products of these glycans. We hypothesized that host-associated sugars drive environment, microbe-microbe and host-microbe interactions in the FRT.

Aims: We aimed to define the carbohydrate utilization traits of diverse FRT taxa and to determine to contribution of interspecies competition for sugars in community composition and cdynamics.

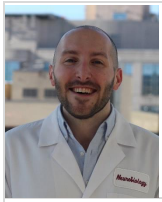
Methods: We have developed scalable, high-throughput culturing methods to empirically define the substrate utilization traits from more than sixty unique bacterial species that colonize the vagina. In addition, we applied batch and continuous cultivation of multispecies communities to study vaginal bacteria within complex community, that closely recapitulate salient dynamics observed *in vivo*.

Results: Demonstrating the power of these *in vitro* models, we have defined the carbohydrate utilization profiles of hundreds of unique FRT isolates, identifying species and strain-level variation in utilization of host-derived carbohydrates. Given the known abundance of glycogen in the vaginal epithelium, I hypothesized that utilization of host-associated glycogen represents an adaptation to the vaginal environment. Indeed, we identify glycogen degradation enzymes in diverse species resident in the reproductive tract, and find enrichment in genes encoding glycogen-degrading enzymes in *L. crispatus* strains derived from vaginal as opposed to intestinal sites. Metatranscriptomic analyses from human samples demonstrate that bacterial glycogen and maltose (a breakdown product of glycogen) utilization genes are highly expressed in the vagina. To empirically investigate the impact of glycogen availability and glycogen utilization in FRT microbiota communities, I assembled type strains or co-resident consortia into model, polymicrobial communities *in vitro.* These studies demonstrate that among health-associated *L. crispatus* strains, those that use glycogen have a competitive advantage during growth in complex community.

Conclusions: Taken together, these data establish that strain-level variability in glycan utilization contributes to competitive fitness during growth in community, and suggest that these traits may influence community stability or persistence *in vivo*. Moreover, the methods we have developed provide a scalable system in which to empirically study ecological dynamics within complex community.

Name and Degree(s)	Jason M. Nagata, MD, MSc
Research Mentor(s)	Kirsten Bibbins-Domingo, PhD MD MAS and Andrea Garber PhD
Presentation/Project Title	Boys, Bulk, and Body Ideals: Epidemiology of Muscle-Enhancing Behaviors in Adolescents and Young Adults

Background: Research on the epidemiology of muscle-enhancing behaviors among adolescents and young adults is limited and has not yet been examined using nationally representative samples in the United States. Many muscle-enhancing substances are not regulated by the Food and Drug Administration and thus long-term health outcomes associated with use of these substances are largely unknown.
Aims: To determine the prevalence, predictors, and outcomes of muscle-enhancing behaviors in adolescents and young adults using a nationally representative sample in the United States.
Methods: We analyzed prospective cohort data from the National Longitudinal Study of Adolescent to Adult Health, Waves I through IV (1994-2008). Engagement in muscle-enhancing behaviors including dietary changes, exercise and weightlifting, supplement use, legal performance-enhancing substances (creatine, andro, monohydrate), and anabolic androgenic steroids were recorded. Multiple logistic regression models using generalized estimating equations were used to determine associations with muscle-enhancing behaviors across three data collection waves.
Results: Of the 18,922 adolescents at baseline, 29.2% of males and 7.0% of females reported weight gain attempts, while 25.2% of males and 3.8% of females reported any muscle-enhancing behavior. Muscle-enhancing behaviors peaked at ages 17-19 years in males at 30.7%. Among young men 18-26 years old, 15.6% reported using legal performance-enhancing substances and 2.7% reported using androgenic anabolic steroids. Factors recorded at adolescence that were prospectively associated with higher odds of muscle-enhancing behaviors in both sexes included Black race, self-perception of being underweight, and lower body mass index z-score. Use of legal performance-enhancing substances at 18-26 years was prospectively associated with androgenic anabolic steroid use (AOR 3.19, 95% CI 1.90-5.32) and binge drinking (AOR 1.45, 95% CI 1.15-1.82) at seven-year follow-up.
Conclusions: Muscle-enhancing behaviors ranging from dietary changes to supplement and androgenic anabolic steroid use are common among adolescent and young adult males. Clinicians should consider screening for muscle-enhancing behaviors in these populations.



Name and Degree(s)	Brian Kalish, MD
Research Mentor(s)	Michael Greenberg, PhD
Presentation/Project Title	Maternal Immune Activation Disrupts Proteostasis in the Fetal Brain

Background: Maternal infection and inflammation during pregnancy is associated with neurodevelopmental disorders in offspring, but little is understood about the molecular mechanisms underlying this epidemiologic phenomenon.
Aims: The purpose of this study was to interrogate the mechanisms by which intrauterine inflammation cause long term neuro-behavioral phenotypes in mice.
Methods: Using a combination of single cell transcriptomics, proteomics, and ribosome profiling, we systematically quantified transcriptional and translational changes in the maternal immune activation (MIA) model of neuropsychiatric disease. We used genetic and pharmacologic approaches to target specific signaling pathways and assessed behavioral changes in offspring.
Results: We found that MIA results in cell type- and gender-specific transcriptional responses in the fetal brain, with a notable disruption in translation-associated gene expression. Moreover, we found that activation of the integrated stress response (ISR) via phosphorylation of eIF2α is responsible for a gender-specific arrest of mRNA translation in the fetal brain. Blockage of the ISR protects against social behavioral phenotypes in MIA offspring.
Conclusions: Our data suggest that therapeutic targeting of the ISR may be beneficial in reducing maternal inflammation-associated neurodevelopmental disorders.

Name and Degree(s)	Matthew R. Vogt, M.D., Ph.D.
Research Mentor(s)	James E. Crowe, Jr., M.D.
Presentation Title	Human Antibodies Neutralize Enterovirus D68 and Protect Against Infection and Paralytic Disease

Background: Enterovirus D68 (EV-D68) causes worldwide outbreaks of human respiratory illness with spatio-temporally related outbreaks of acute flaccid myelitis (AFM). The major circulating clade of EV-D68 has changed since the largest ever recorded outbreak, which occurred in the United States in 2014. Numerous seroepidemiology studies show that essentially all adult humans have EV-D68-neutralizing antibodies in their serum, even in serum collected prior to large outbreaks. But some studies show a lack of neutralizing antibodies in pediatric populations.
Aims: We aimed to isolate human monoclonal antibodies (mAbs) from B cells in peripheral blood mononuclear cells (PBMCs) of immune subjects, induced by natural infection, to further understand human humoral immunity to EV-D68.
Methods: We obtained PBMCs from donors with known infection during 2014. We used EV-D68 virus isolates from this outbreak as antigen in an indirect ELISA to screen immortalized PBMCs for antigen-specificity, then fused B cells with myeloma cells to create hybridomas.
Results: We isolated > 60 naturally occurring anti-EV-D68 human mAbs. These mAbs exhibit diverse binding affinities when compared across clades of recent EV-D68 isolates. Many mAbs neutralize EV-D68 quite potently *in vitro*, with [ng/mL] half maximal effective concentrations. Some mAbs like EV68-228 neutralize diverse clades, whereas others are highly clade-specific. Binding of antibodies to at least three, but likely more, major antigenic sites on the virus leads to neutralization. When given as either prophylaxis before or treatment after EV-D68 inoculation, mAb EV68-228 protects mice from both respiratory and neurologic disease, whereas equivalent doses of polyclonal human immunoglobulin protect only partially.
Conclusions: We observed a qualitative difference among antibodies isolated from patients who had a history of natural infection. These differences could contribute to certain individuals being susceptible to respiratory disease and AFM. These studies of humoral immunity are especially important for a disease with nearly universal apparent seroprotection, in which the virus somehow persistently causes outbreaks across the world. Further, no licensed vaccines or treatments exist for EV-D68. These mAbs have therapeutic benefit *in vivo* and show promise for the prevention and/or treatment of EV-D68 related diseases in humans. We are pursuing the use of mAb EV68-228 in humans with partners in industry and government agencies.

Name and Degree(s)	Catharine Walsh, MD, MEd, PhD
Research Mentor(s)	Brian Feldman
Presentation Title	The effect of an International competitive leaderboard on self-motivated simulation-based CPR practice

Background: Little is known about how best to motivate healthcare professionals to engage in frequent cardiopulmonary resuscitation (CPR) refresher skills practice. A competitive leaderboard for simulated CPR can encourage self-directed practice on a small scale.
Aims: The study aimed to determine if a large-scale, multi-center leaderboard improved simulated CPR practice frequency and CPR performance among healthcare professionals.
Methods: This was a multi-national, randomized cross-over study among 17 sites using a competitive online leaderboard to improve simulated practice frequency and CPR performance. All sites placed a Laerdal1 ResusciAnne or ResusciBaby QCPR manikin in 1 or more clinical units– emergency department, ICU, etc. – in easy reach for 8 months. These simulators provide visual feedback during 2-minute compressions-only CPR and a performance score. Sites were randomly assigned to the intervention for the first 4-months or the second 4-months. Following any CPR practice by a healthcare professional, participants uploaded scores and an optional 'selfie' photo to the leaderboard. During the intervention phase, the leaderboard displayed ranked scores and high scores earned digital badges. The leaderboard did not display control phase participants. Outcomes included CPR practice frequency and mean compression score, using non-parametric statistics for analyses.
Results: Nine-hundred nineteen participants completed 1850 simulated CPR episodes. Exposure to the leaderboard yielded 1.94 episodes per person compared to 2.14 during the control phase ($p = 0.99$). Mean CPR performance participants did not differ between phases: 90.7 vs. 89.3 ($p = 0.19$).
Conclusions: A competitive leaderboard was not associated with an increase in self-directed simulated CPR practice or improved performance.

POSTER ABSTRACTS

POSTER 1

 **Name and Degree(s)**	Ori Scott, MD
Research Mentor(s)	Supervisor: Dr. Ronald Cohn; Mentor: Dr. Chaim Roifman
Poster Title	Harnessing CRISPR/Cas9 for Interrogation of the Immunodeficiency STAT1 Gain-of-Function (GOF)

Authors and Affiliations: Ori Scott, MD[1,2,3]; Chaim Roifman, MD[1,4]; Evgueni Ivakine, PhD[2]; Roald Cohn, MD[1,2,5]

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Aims: STAT1 is a transcription factor pivotal in interferon (IFN)-induced immunity. Gain-of-function (GOF) point mutations in *STAT1* lead to its enhanced phosphorylation and alter its transcriptional activity, resulting in a disorder of profound immune dys-regulation. Unfortunately, adequate disease models of STAT1 GOF are scarce. This study aimed to generate cell models of STAT1 GOF recapitulating various human mutations. Subsequently, we have investigated the functional impact of aberrant STAT1 activation in our novel cell models.

Methods: Databases of STAT1 GOF mutations were reviewed, and transition mutations (A→G or C→T) were selected for modelling by CRISPR/Cas9-base editing. Mutations were generated in HAP1, a cell line derived from human chronic myeloid leukemia, which has been previously used for interrogation of IFN signaling. Mutations were validated by Sanger sequencing. Levels of total and phospho-STAT1, both at rest and following IFN stimulation, were measured using immunoblotting. Quantitative real time PCR was used (qRT-PCR) to determine the impact of IFN stimulation on STAT1-dependent gene expression. Comparison was made with wild-type (WT) HAP1 cells.

Results: We have generated cell models of known human mutations, spanning various gene domains. Mutants show increased levels of phospho-STAT1 compared with WT, both at baseline and following IFN stimulation, verifying a GOF phenotype. Interestingly, following stimulation mutants display excessive and sustained elevation not only of phospho-STAT1, but also of total STAT1, persisting for over 48 hours. Transcriptional output downstream of STAT1 shows differential gene expression between mutants and WT cells, with a mixed pattern of up- and down-regulation observed.

Conclusions: CRISPR/Cas9 base-editing can be successfully employed to generate cell models of STAT1 GOF mutations, recapitulating disease phenotype on the molecular level. These cell models provide a deeper insight into disease pathophysiology, and will allow the study of STAT1 impact on other pathways, as well as interrogation of potential therapeutic targets.

POSTER 2

Name and Degree(s)	Megan Culler Freeman, MD, PhD
Research Mentor(s)	Carolyn Coyne, PhD
Poster Title	**Human intestinal tract serves as an unrecognized route of infection for EV-D68**

Authors and Affiliations: **Megan Culler Freeman**[1, 2], Carolyn B. Coyne[1, 2]

[1]Department of Pediatrics, [2]Division of Pediatric Infectious Disease, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania, US

Aims: To define intestinal replication of enterovirus D68 (EV-D68) and discover contributions to pathogenesis.

EV-D68, responsible for a recent outbreak of severe respiratory disease and acute paralysis in children, is unique as it is thought to spread via the respiratory route with limited fecal shedding. The role of intestinal replication, if any, is unknown. *We hypothesized that despite successful replication in the human airway, EV-D68 is also able to replicate in the human intestine, representing an unrecognized method of viral spread.*

Methods: We utilize primary human-derived enteroid cultures and polarized primary human bronchial epithelial cells to compare infection kinetics and polarity of infection. We also exposed virus to intestinal conditions to monitor the effects on replication.

Results: Evaluation of multiple EV-D68 ATCC and local clinical isolate strains from 2018 have robust replication in both primary airway models and human-derived enteroid cultures. In addition to the capacity of EV-D68 to enter and replicate in intestinal derived cells, EV-D68 was able to tolerate incubation with simulated intestinal fluids with similar results to traditional enteroviruses, such as echovirus 11, to the same conditions.

Conclusions: These results suggest that EV-D68 has the capacity to replicate in the human intestine, a previously unrecognized route of infection in addition to the human airway. Continued work in this model will provide insights into the contributions of airway versus intestinal viral replication and host cellular response to EV-D68 and impart crucial information about EV-D68 tissue tropism, viral spread, and infection control.

POSTER 3

Name and Degree(s)	Drew Schwartz, MD, PhD
Research Mentor(s)	Gautam Dantas, PhD
Poster Title	**Death from antibiotics is microbiota dependent in a humanized mouse model of late onset neonatal sepsis**

Authors and Affiliations:

Schwartz, D.[1,2], D'Souza, A.[1], Crofts, T.[3], Ning, J.[1], Shalon, N.[1], Robinson, J.[4,5], Henderson, J.[4,5,6], Warner, B.[1], Tarr, P.[1,6], Dantas, G.[2,6,9,10]

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Aims: Premature infants receive antibiotics frequently for culture negative sepsis, which diminishes gut microbial diversity and increases susceptibility to antibiotic resistant pathogens. We hypothesize that antibiotics (1) enrich for pathobionts

within the gut, 2) promote a systemic, proinflammatory host response, and 3) cause death in an antibiotic specific manner in a gnotobiotic mouse model.
Methods: We colonized germ free (GF) dams and sires with stools from preterm infants. Mouse pups acquire this neonatal microbiota, and at 10 days of life (DOL), we treat them with clinically-relevant doses of antibiotics subcutaneously for 3 days. We use metagenomic shotgun sequencing to ascertain phylogenetic composition, and use flow cytometry to determine the local and peripheral immune response.
Results: Using two representative microbiota from human neonates (microbiota A or B), we show that 94% of pups given microbiota A survive versus 64% given microbiota B after meropenem/probenecid treatment ($p<0.05$). 40% of pups given microbiota A treated with ampicillin/gentamicin/probenecid survived ($p<0.01$). *Klebsiella* species dominated the gut microbiota of microbiota A-humanized pups who succumbed, and *Enterococci* dominated the gut microbiota of microbiota B-humanized pups who died during treatment. Pups colonized with microbiota B had increased peripheral CD4+ T cells compared to microbiota A-humanized pups (61% versus 44% of circulating T cells, $p<0.0005$).
Conclusions: Our model of preterm microbiota development and perturbation by antibiotics demonstrates potential bacterial translocation, proinflammatory immune response, and death dependent on the microbiota-antibiotic combination. Our transgenerational humanized-microbiota mouse model can be utilized to determine antibiotic by microbiota perturbation and examine risks of late onset sepsis from antimicrobials.

POSTER 4

Name and Degree(s)	Bradford A Becken III, MD
Research Mentor(s)	Raphael Valdivia, PhD
Poster Title	***Akkermansia muciniphila*: Its Genotypic and Phenotypic Diversity in a Pediatric Cohort with Obesity and Investigating its Potential Role in Promoting the Lean State**

Authors and Affiliations:
Bradford A Becken III MD, Duke University Pediatric Infectious Disease, Duke University Department of Molecular Genetics and Microbiology, Per Malkus PhD, Duke University Department of Molecular Genetics and Microbiology, Lauren Davey PhD, Duke University Department of Molecular Genetics and Microbiology, Katherine Mueller, Duke University Department of Molecular Genetics and Microbiology, Raphael Valdivia PhD
Background: The prevalence of childhood obesity has tripled over the last several decades, with currently over 20% of adolescents now obese according to the CDC. There is growing evidence that enteric microbes play a vital role in energy regulation and metabolism, as well as maintenance of a healthy gut mucosal barrier and immunity. One such organism is *Akkermansia muciniphila*, a mucin-degrading anaerobic bacterium that has been implicated in both obesity and type 2 diabetes mellitus. In this study we are examining the bacterial microbiome of pediatric patients with obesity undergoing various anti-obesity interventions, as well as samples from age matched controls, to learn more about colonization rates and strain differences, at the genotypic and phenotypic levels, that may elucidate *A. muciniphila's* role in promoting the lean state.
Aims:
Aim 1. Identify the patterns of *A. muciniphila* colonization among obese children and age-matched lean controls and those associated with weight loss interventions.
Aim 2. To identify *A. muciniphila* genes associated with stimulation of common receptors of the enteric innate immune system from wild-type *A. muciniphila* isolated from pediatric patients with obesity and lean age-matched controls, and using transposon mutagenesis, identify genes responsible for interaction with the innate immune system.
Methods: Stool samples from pediatric patients with obesity and lean age matched controls were used to innoclulate mucin media, in which mucin was the sole source of carbon and nitrogen, allowing for the enrichment of *A. muciniphila*. Once *A. muciniphila* was isolated, it underwent whole 16s and clade specific analysis. These strains were also grown in a plate reader to assess for growth rates, and underwent testing for biofilm formation and capsule. Once the phenotypic characterization of the strains was complete, the isolated were exposed to various TLR expressing HEK cells: TLR2 (2/1/6), TLR2/1, TLR2/6, TLR2 (1/6 double knock-out) TLR3, TLR4, TLR5 and TLR9, and their relative activation was analyzed compared to both the type strain and a negative control. Select isolated, based on growth and TLR activation patterns then underwent whole genome sequencing (WGS). Based on WGS results were are selecting strains for transposon mutagenesis

in an attempt to identify genes and gene products that may be responsible for the varied TLR activation. These strains will then be used in the murine model of obesity (Spring 2020).
Results: We have identified over 80 wild-type strains present in our pediatric cohort that represent multiple clades of *A. muciniphila.* Whole genome sequencing on a subset of these isolates has shown significant genetic diversity. Additionally, our analysis of the strains has shown widely differing growth characteristics involving growth rates, biofilm formation and capsule formation. These strains, when tested against several toll-like receptor lines, have shown differential activation, leading us to believe that the potential beneficial effects from *A. muciniphila* may be unique to specific strains of the bacteria.
Conclusions: There does not appear to be a correlation between the *A. muciniphila* abundance and the lean state. However, from a library of over 80 unique wild-type strains, we have been able to identify unique patterns of interaction with the toll-like receptors commonly expressed in the intestinal epithelium.

POSTER 5

Name and Degree(s)	Elizabeth Crouch, MD, PhD
Research Mentor(s)	Eric J Huang, MD, PhD
Poster Title	Single-cell RNA sequencing of blood vessel cells in the developing human brain reveals a novel neurovascular progenitor

Authors and Affiliations: **Elizabeth E Crouch[1]***, Aparna Bhaduri[2,3], Madeline Andrews[2,3], Jiapei Chen[4], Cristina Mora[5], Arnold Kriegstein[2,3], Mercedes F Paredes[2], Eric J Huang[5]

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Background: Brain blood vessel cells play key roles in both physiological and pathological states. As a striking example, approximately 20% of premature babies born between 24-28 gestation weeks will develop germinal matrix hemorrhage (GMH). The Germinal Matrix is a transient, sub-cortical area with many neural stem cells, and hemorrhage in this location confers high risk for intellectual disability, cerebral palsy, and death. Currently, this devastating condition has no treatments. To study the relationship between blood vessel cells and brain parenchyma, adult mice have served as the predominant model organism. With this tractable model, blood vessel cells are now understood to regulate the formation of the blood brain barrier, secrete factors which influence neural stem cells, and modulate blood flow in response to neuronal activity. However, there are critical gaps in our understanding of the origin of brain blood vessel cells and how the vasculature interacts with the developing neural tissue. Additionally, little is known about human brain vasculature at any age. Given the discrete location and timing of GMH in the premature infant, we hypothesize that human brain blood vessel cells display regional and stage-specific differences during development. Additionally, we propose that a novel neurovascular progenitor in the human brain serves as a previously unrecognized source of both neural and vascular lineage cells. Understanding the developmental trajectory and potential of brain vascular cells will allow us to understand the functional implications of brain angiogenesis and envision therapies for neonatal brain injury.
Aims:

1. Define the morphological maturation of blood vessels in the developing human brain, focusing on the germinal matrix (GM) and the cortex.
2. Use Fluorescence-Activated Cell Sorting (FACS) coupled with single cell RNA sequencing (scRNAseq) to characterize the subtypes and molecular repertoire of endothelial and mural cells from the developing human brain.
3. Evaluate the potential of human brain vascular cells with 2D and 3D culture models and transplants into brain organoids.

Methods: To understand the role of blood vessel cells in the developing human brain, we focused on endothelial and mural cells. Taking advantage of the UCSF Pediatric Neuropathology Consortium (http://ucpnc.pathology.ucsf.edu), we

performed immunostaining for endothelial (CD31+) and mural cells (NG2+ or PDGFR-β+) in tissue sections from postmortem brain tissues between 15-39GW. To define the molecular mechanisms of vascular maturation, I developed a Fluorescence Activated Cell Sorting (FACS) strategy followed by single cell RNA sequencing (scRNA-seq) to characterize the transcriptomes of endothelial and mural cells in the GM and cortex, a control region. To perform FACS experiments, tissue is digested with collagenase/dispase and a Percoll centrifugation step follows to remove debris. The single-cell suspension is then stained with CD45 to eliminate microglia and blood cells, CD31 to label endothelial cells, and CD146 to simultaneously purify mural cells (high expression) and radial glia (low expression). Single-cell sequencing is performed with the 10x Chromium system. To test the vascular and neurogenic potential of human brain vascular cells, endothelial and mural cells are cultured in EGM2 media on collagen (vascular conditions) or radial glia media on Matrigel (neurogenic conditions). To test the ability of endothelial and mural cells to make tube-like structures in vivo, vascular cells are immersed in 3D Matrigel using Ibidi angiogenesis slides. To transplant vascular cells into brain organoids, endothelial or mural cells are infected with a GFP-expressing virus (AAV-CMV-GFP) and co-cultured for 2 weeks. The organoids are then embedded and sectioned to determine the cell fate of transplanted vascular cells.
Results: Immunostaining experiments revealed a predominance of vascular cells with angiogenic features directly adjacent to the ventricle. As the brain matures, this angiogenic zone disappears. FACS of endothelial and mural cells followed by scRNAseq demonstrated distinct vascular subtypes by the end of the second trimester. Strikingly, this strategy also uncovered a novel progenitor population that express both vascular and neural stem cell markers, suggesting that common neurovascular progenitor that contributes to both angiogenesis and neurogenesis. Consistent with these results, immunohistochemistry in the GM showed that putative neurovascular progenitors are perivascular cells located in the ventricular zone amongst canonical neural stem cells. Moreover, FACS-purified mural cells produced smooth muscle cells, neurons, and astrocytes as well as form organoid-like structures in neural media *in vitro*.
Conclusions: Human vascular cells are heterogeneous and dynamic during development. In contrast to the traditional model, a novel perivascular neurovascular progenitor may produce both neural and vascular lineage cells which then co-migrate outward to populate the human brain. Future experiments will characterize the impact of GMH on different vascular subtypes including neurovascular progenitors.

POSTER 6

Name and Degree(s)	Pamela Good, MD
Research Mentor(s)	Fangming Lin, MD, PhD & Franklin Costantini, PhD
Poster Title	Generation of New Mouse Models of Low Nephron Endowment to Study the Development of Chronic Kidney Disease in Humans Born Preterm

Authors and Affiliations: Good, Pamela I.[1]; Hurst, Holly[1]; Bateman, David A.[1]; D'Agati, Vivette[2]; Costantini, Franklin[2]; Lin, Fangming[1]
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Background: Humans born preterm have low nephron endowment and increased risk for chronic kidney disease (CKD). The pathogenesis of CKD in this population is poorly understood due to limited animal models.
Aims: We generated new mouse models of low nephron number that simulate humans born preterm to test the hypothesis that low renal reserve and sustained glomerular hyperfiltration result in maladaptive changes leading to CKD.
Methods: We took chemical and genetic approaches to reduce ureteric bud (UB) branching during kidney development by deleting the Ret gene or inhibiting Ret tyrosine kinase. Because Ret signaling induces UB branching, which in turn determines nephron number, interfering with Ret signaling generates kidneys with low nephron endowment. We deleted the Ret gene in $Ret^{flox/flox}$ mice using UB specific *Hoxb7rtTA* to drive expression of *TetOcre*, treating pregnant dams with DOX at E15.5, E16.5 or E17.5 until delivery. Alternatively, when the Ret gene is not deleted by Cre, the encoded Ret protein has one amino acid substitution that renders it susceptible to a small molecule inhibitor, NA-PP1. This allows manipulation of nephron number by either genetic or chemical approach. We examined renal morphology and function in offspring, and counted glomerular number (N_{glom}) using the acid maceration method.
Results: Pregnant dams treated with at DOX E15.5, E16.5 or E17.5 had offspring with a significant reduction in N_{glom} (mean N_{glom} 3,977; 5,473; 8,888 vs. control 13,468; $p<0.05$ for all comparisons). We chose DOX starting at E16.5 for detailed

analyses. DOX E16.5 offspring had glomerular enlargement (mean glomerular area 59.4% larger than controls; p=0.002) and tubular hypertrophy (mean tubular diameter 7.81 µm larger than controls; p=0.007). Glomeruli in the outer cortex exhibited obsolescence, suggesting developmental arrest. Serum creatinine (sCr) was higher at 6w (mean 0.16 vs 0.08 in controls; p=0.03). By 12w, lesions of focal segmental glomerulosclerosis (FSGS) developed and urinary albumin excretion increased (mean Ualb:cr 15.5 vs 5.98 in controls; p=0.02). Alternatively, treating pregnant dams with NA-PP1 (daily intraperitoneal injection from E 16.5 through delivery) to inhibit Ret activity led to a dose dependent decrease in N_{glom}. To detect neonatal kidney structural changes, we examined kidneys on the day of birth in offspring exposed to 50 mg/kg of NA-PP1. The results showed truncated UB branching with qualitatively decreased number of UB tips.
Conclusions: We generated novel mouse models of congenitally reduced N_{glom} and showed that they develop functional and structural changes of CKD by 6-12w of age. These mice are useful tools to study the renal consequences of preterm birth.

POSTER 7

Name and Degree(s)	Devan Jaganath, MD MPH
Research Mentor(s)	Adithya Cattamanchi
Poster Title	The Detection of Breath-based Biomarkers for Pulmonary Tuberculosis Diagnosis

Authors and Affiliations:
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Background: A non-sputum, non-invasive biomarker-based diagnostic for pediatric TB is urgently needed. Breath-based volatile organic compounds (VOCs) that are metabolic byproducts of *M. tuberculosis* are promising biomarkers for TB diagnosis and/or screening in children.
Aims: In a cohort of adults and children being evaluated for pulmonary TB, we 1) assessed the feasibility and safety of collecting breath in children for VOC testing; 2) determined the minimum volume needed to measure methyl nicotinate (MN) and methyl p-anisate (MPA) via gas chromatography/mass spectrometry (GC/MS), and 3) developed approaches to detect VOCs using a novel nanosensor.
Methods: Symptomatic children (under 15 years old) and adults (18 years old) being evaluated for pulmonary TB were enrolled in Kampala, Uganda at the Mulago National Referral Hospital and surrounding community clinics and hospitals. A clinical survey was completed, and sputum, gastric aspirate or nasopharyngeal aspirate was collected for Xpert MTB/RIF or Ultra, solid and liquid culture. Up to 20L of breath was collected, and presence of the VOCs was detected via gas chromatography/mass spectrometry (GC/MS). Nanosensors were developed using titanium dioxide nanotube arrays.
Results: We collected breath in 117 children, with median age 3.3 years (IQR 1.2-6.2), 61 (52%) male, 14 (12.5%) HIV positive, and 72 (62%) underweight. None had adverse events from breath collection. Among 47 adults (8 (17%) HIV positive, 30 (64%) with TB), MN and MPA were not detected if 5L of breath was collected, whereas MN was detected in 18 (49%) participants and MPA in 22 (59%) if at least 10L was collected. Three approaches to nanosensor testing (Amperometry, Cyclic Voltammetry, and Square Wave Voltammetry) of MN and MPA have been developed and are undergoing comparative evaluation in adults and children in Kampala, Uganda.
Conclusions: Breath collection in children with respiratory symptoms is safe and feasible for VOC measurement. A minimum of 10L is likely needed to detect MN and MPA using GC/MS for TB evaluation. Nanosensors using three different electrochemical methods can detect MN and MPA and are ready for field testing.

POSTER 8

Name and Degree(s)	Steven D. Rhodes, M.D., Ph.D.
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Poster Title	Molecular origins of malignant peripheral nerve sheath tumor in neurofibromatosis type 1

Authors and Affiliations:

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Background: Malignant peripheral nerve sheath tumors (MPNST) are treatment refractory neurofibrosarcomas that represent the leading cause of death in patients with neurofibromatosis type 1 (NF1). Developing therapeutic strategies to not only treat MPNST, but ultimately to prevent their occurrence is paramount to improve survival, reduce suffering, and alleviate the burden of care for individuals living with this devastating cancer predisposition syndrome. The development of atypical neurofibroma (ANNUBP) from an existing plexiform neurofibroma precursor can be an early warning sign that precedes MPNST, and these tumors frequently harbor loss of *CDKN2A* (*INK4A*) and its alternate reading frame (*ARF*). However, the role of *INK4A/ARF* loss in driving the progression of atypical neurofibroma and MPNST remains ambiguous. Furthermore, a comprehensive understanding of the genomic and transcriptomic landscape of these tumors is lacking due to limited availability of patient samples.

Aims: 1) To determine whether combined loss of *Nf1* and *Ink4a/Arf* in neural crest derived Schwann cells is necessary and sufficient to drive the development of ANNUBP and MPNST in genetically engineered mice; 2) To isolate the tumorigenic cell of origin for *Nf1-Ink4a/Arf* driven nerve sheath tumors from the embryonic nerve roots of genetically engineered mice and establish a non-germline model of ANNUBP and MPNST to serve as a platform for *in vivo* evaluation of experimental therapeutics; 3) To investigate the role of the *Let7*-HMGA2 axis in driving the malignant transformation of ANNUBP precursor tumors and to establish whether genetic and/or pharmacologic inhibition of HMGA2 can treat existing MPNST and/or event prevent MPNST progression.

Methods: We developed novel genetically engineered mouse models driving conditional loss of *Nf1* and *Ink4a/Arf* in embryonic neural crest derived Schwann cells driven by Cre-recombinase under the control of a 3.9kb fragment of the Periostin promoter. We performed in depth transcriptomic and chemical proteomic characterization of tumors arising in these models to systematically identify putative molecular drivers of tumor progression. Techniques to isolate, expand, manipulate, and reimplant *in vivo* the cells of origin for these nerve sheath tumors have allowed us to establish a robust non-germline model to mechanistically delineate the role of *Ink4a/Arf* as well as other modifier genes implicated in disease progression.

Results: We found that *Ink4a/Arf* plays a critical role in maintaining plexiform neurofibroma indolence by triggering senescence-mediated growth arrest within the tumorigenic cell of origin. Conditional ablation of *Nf1* and *Ink4a/Arf* in neural crest-derived Scwhann cells allowed escape from senescence, leading to the development of tumors that phenocopy human ANNUBP and progress to MPNST with high penetrance. Transcriptome (RNAseq) analysis of these tumors further illuminated profound alteration in an oncofetal microRNA-protein axis (*Let7*-HMGA2) as plexiform and atypical neurofibroma precursor tumors undergo malignant transformation. Knockdown of HMGA2 resulted in growth arrest and triggered apoptosis in human MPNST cell lines and in primary *Nf1-Ink4a/Arf* mutant cancer stem cells.

Conclusions: These mouse models are the first to recapitulate the spontaneous and stepwise evolution of MPNST from plexiform and atypical neurofibroma precursor tumors, thus allowing for interrogation of interventions that can both treat as well as prevent MPNST. We hypothesize that *Let7*-HMGA2 deregulation reprograms broad transcriptional and kinase networks to drive MPNST progression by reactivating programs of embryonic growth in their tumor initiating cells of origin. By isolating this unique population of cancer stem cells from the embryonic nerve roots, we are exploring how *Let7*-HMGA2 perturbation alters broad networks of protein kinases and transcription factors to drive the aggressive behavior of these tumors. In future studies, through both genetic and pharmacologic methods, we will establish whether HMGA2 inhibition can treat and/or prevent the progression of MPNST from plexiform and atypical neurofibroma precursor tumors *in vivo*.

Pediatric Scientist Development Program

UNDER THE AEGIS OF THE ASSOCIATION OF MEDICAL SCHOOL PEDIATRIC DEPARTMENT CHAIRS (AMSPDC)

TRAINING THE NEXT GENERATION OF PEDIATRIC SCIENTISTS

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https://amspdc-psdp.org

Since 1987, the Pediatric Scientist Development Program (PSDP) has provided research training and career development support for pediatricians committed to careers in academic medicine. Today, PSDP alumni include an Assistant Secretary, HHS, one associate dean, four pediatric department chairs, two Howard Hughes Medical Institute Investigators, the President of the Gladstone Institute for Cardiovascular Disease, and other highly productive pediatric physician scientists at more than 80 institutions across the US and Canada. In fact, the PSDP program graduates are 2 1/2 times more likely to secure NIH funding than non-graduates. We hope you consider joining them on the leading edge of scientific discovery.

The PSDP is an intense, full-time experience in basic, translational, or clinical research training. After the completion of one or two years of clinical fellowship, the PSDP provides two years of research support during which MD or MD/PhD pediatricians address central problems in child health with mentors from eminent laboratories in North America. In the second year of PSDP support, PSDP fellows have the option of applying for a third year of research support from their sponsoring departments, where they are appointed as instructors or equivalent rank. Dedicated to careers with a major focus in basic, translational, and clinical research, these pediatric scientists will translate research advances to improvements in clinical care for children. The active involvement and support of pediatric department chairs in the nominations, application process, and career development of PSDP scholars are essential to the success of this program.

Physicians who wish to train in basic, translational, clinical or health services research with an established investigator/mentor are encouraged to apply, as are candidates who seek training in epidemiology/statistics, informatics, health services, or health policy. A commitment to an investigative academic career is essential. Prior research experience is not required.

Candidates who anticipate 12 months of clinical work in fellowship should apply in March of the PGY-3 year (15 1/2 months prior to the start of the longitudinal protected continuous research time). Candidates in sub-specialties requiring more than 12 months clinical time should apply as first-year fellows. Candidates who are fast-tracking in pediatrics should identify themselves in the autumn of the PGY-2 year. Please see the PSDP website https://amspdc-psdp.org/ for FAQ and additional information.

The PSDP is made possible with the support of AMSPDC, the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development, the American Academy of Pediatrics, the American Pediatric Society and the March of Dimes

2021 Annual Meeting

March 4 - 7, 2021 St. Petersburg, FL

The Hilton St. Petersburg Bayfront

Joint meeting with the Association of Administrators in Academic Pediatrics (AAAP) on Saturday, March 6, 2021

DOWNTOWN LONG BEACH

MEMBER RESTAURANTS

RESTAURANTS

1 555 EAST STEAKHOUSE
2 BEACHWOOD BBQ AND BREWING
3 BEER BELLY
4 BERLIN BISTRO
5 BO BEAU KITCHEN + ROOF TAP
6 BUBBA GUMP SHRIMP CO.
7 BUONO'S AUTHENTIC PIZZERIA
8 CAFE SCUBA
9 CAFE' SEVILLA SPANISH RESTAURANT & TAPAS BAR
10 CALIFORNIA PIZZA KITCHEN - PIKE-RAINBOW
11 CHELSEA CHOWDER HOUSE & BAR @ THE QUEEN MARY
12 CUPPA CUPPA
13 DISTRICT WINE
14 DOG HAUS BIERGARTEN LONG BEACH
15 DOLY'S DELECTABLES
16 FICKLEWOOD CIDERWORKS (Opening Spring 2019)
17 FOURTH & OLIVE
18 FOUNDATION SANDWICH SHOP
19 FUEGO @ The Hotel Maya
20 GEORGES GREEK CAFE (PINE AVE)
21 GLADSTONE'S
22 GREAT SOCIETY CIDER & MEAD
23 HAMBURGER MARY
24 HOOTERS of LONG BEACH
25 ICE CREAM & FROZEN YOGURT ON THE BOARDWALK
26 ISLANDS FINE BURGERS AND DRINKS
27 JAMES REPUBLIC
28 JOHNNY ROCKETS
29 KING'S FISH HOUSE - PINE AVENUE
30 LONG BEACH CAFE
31 L'OPERA RISTORANTE
32 LOUISIANA CHARLIE'S BBQ & CAJUN CUISINE
33 MICHAEL'S DOWNTOWN
34 MODICA'S
35 NAREE THAI RESTAURANT
36 OMELETTE INN
37 PADRE'
38 PARKERS' LIGHTHOUSE
39 PIER 76 FISH GRILL
40 POKE' BAR
41 POKI CAT
42 PROMENADE CAFE
43 Q'S SMOKEHOUSE
44 QUEENSVIEW STEAKHOUSE
45 ROCK BOTTOM RESTAURANT and BREWERY
46 ROMEO CHOCOLATES
47 SHANNON'S ON PINE
48 SHENANIGANS IRISH PUB & GRILLE
49 SIP LOUNGE
50 SIR WINSTON'S @ THE QUEEN MARY
51 SUPER MEX
52 TACO BEACH
53 TEQUILA JACK'S
54 THAI DISTRICT
55 THE AULD DUBLINER IRISH PUB
56 THE BEACHWOOD BLENDERY
57 THE BREAKFAST BAR
58 THE BROKEN DRUM BAR
59 THE FEDERAL
60 THE FUNNEL HOUSE
61 THE PIE BAR
62 THE PROMENADE CAFE @ THE QUEEN MARY
63 THE REEF ON THE WATER
64 THE SPOON BISTRO - AUTHENTIC THAI CUISINE
65 TIDES ON PINE RESTAURANT @ Hyatt
66 TOKYO WAKO
67 UTOPIA RESTAURANT
68 WABA GRILL
69 WOKCANO ASIAN RESTAURANT & LOUNGE
70 YARD HOUSE

Attractions | Excursions | Getaways

City sights

The Aquarium of the Pacific is home to more than 11,000 inhabitants of the Pacific Ocean. Explorer's Cove features Lorikeet Forest with colorful and friendly miniature parrots that can be hand-fed by visitors and the Shark Lagoon, where nine shark species can be viewed from above and below water. Go behind the scenes with your group to see how the animals are cared for and bred up close.

Catch LB Transit Passport | www.aquariumofpacific.org
Time Allotted: General Aquarium experience 2-3 hours
Aquarium Add-ons: 3-D Movie: 1.5 hours, Behind-the-Scenes Tour: 1.5 hours, Animal Encounter: 2 hours
Location: Rainbow Harbor, Downtown

The Queen Mary with Soviet Scorpion Submarine.

Catch LB Transit Passport | www.queenmary.com
Time Allotted:
Behind the Scenes: 45 min. to 1 hour
World War ii: 1 hour
Scorpion Submarine: Self-guided
Ghosts & Legends: 35-40 minutes
Location: Across the Bay, 1.6 miles from Downtown

Daily Behind-the-Scenes Tours, annual festivals and a host of hands-on educational programs are offered.

Enjoy:

June Keyes Penguin Habitat

Touching Sea Creatures

Whale Watching and Harbor Cruises

Animal Encounters

Scuba Diving

The Ocean Science Center

One of the most recognized attractions in Long Beach is The Queen Mary, one of the world's most luxurious ships that once sailed the Atlantic Ocean.The ocean liner features a 314-stateroom hotel, historical tours and several fine restaurants.

Princess Diana
Princess Diana will forever be remembered for her beauty, classic style, compassion and charitable works.

Diana: Legacy of a Princess captures Diana's true essence, showcasing a priceless collection of her gowns and other cherished memorabilia.

Located on Queen Mary's Sun Deck the exhibilt chronicles Diana's private and public life from her days as a student at university, to her engagement and historic marriage to Prince Charles, to her reign as Princess of Wales and her extraordinary charity work. This very personal and rare exhibit includes many never before seen items and offers a more in-depth and up-close look into the life of a Princess everyone thought they knew.

The Princess Diana Collection includes an elegant dressing room featuring nine of Princess Diana's dresses and gowns. Stunningly displayed and featuring a story behind each item, these iconic dresses have transcended pop culture becoming historical artifacts of the beloved princess.

Also included are handwritten letters and notes from Princess Diana and Prince Charles, personal photographs of Prince Charles and Princess Diana, the hand-carved wooden bench that Prince Charles gave Diana as a wedding gift, wedding items including recreation of Diana's wedding bouquet, the seating chart, invitations, "thank you" notes from the royal family and more.

In the Royal Family exhibit, guests will get an inside look at Queen Mary (for whom the ship was named), as well as King George, Queen Mother Elizabeth and Queen Elizabeth II, through personal documents, letters, newspapers, royal memorabilia and more.

Cruises | Culture |History

Points of interest

Suggested Itineraries -

Aquarium/Harbor Cruise

Experience life above and below the ocean on a day tour featuring the Aquarium of the Pacific and a scenic harbor cruise. Get up close with the animals during a Behind-the-Scenes tour of the Aquarium. Grab lunch at one of the many surrounding restaurants in Shoreline Village or Rainbow Harbor before your narrated cruise of the Long Beach harbor. Be sure to bring along your camera to capture the breath-taking views.

The Queen Mary

One of the most recognized attractions in Long Beach is The Queen Mary, one of the world's most luxurious ships that once sailed the Atlantic Ocean. Be sure to visit the Russian Scorpion Submarine docked just outside. Have lunch at the Promenade Café and enjoy picturesque views of the harbor and city skyline. Browse specialty shops before experiencing the *Diana: Legacy of a Princess* exhibit, and the Ghosts & Legends Tour, taking you through areas of the ship known to be haunted.

Tour de Arts

Art lovers will appreciate the culture and creativity of the Long Beach art scene. After a short drive down scenic Ocean Boulevard, arrive at the Long Beach Museum of Art. Explore the two floors of galleries on a scheduled docent led tour then make your way to the Museum of Latin American Art (MoLAA) downtown. Those who prefer to go it alone can rent an mp3 player or use their mobile phone to access an automated audio tour as well. Just a short walk from MoLAA is the always popular Pacific Island Ethnic Art Museum (PIEAM), where both group and individual tours are available.

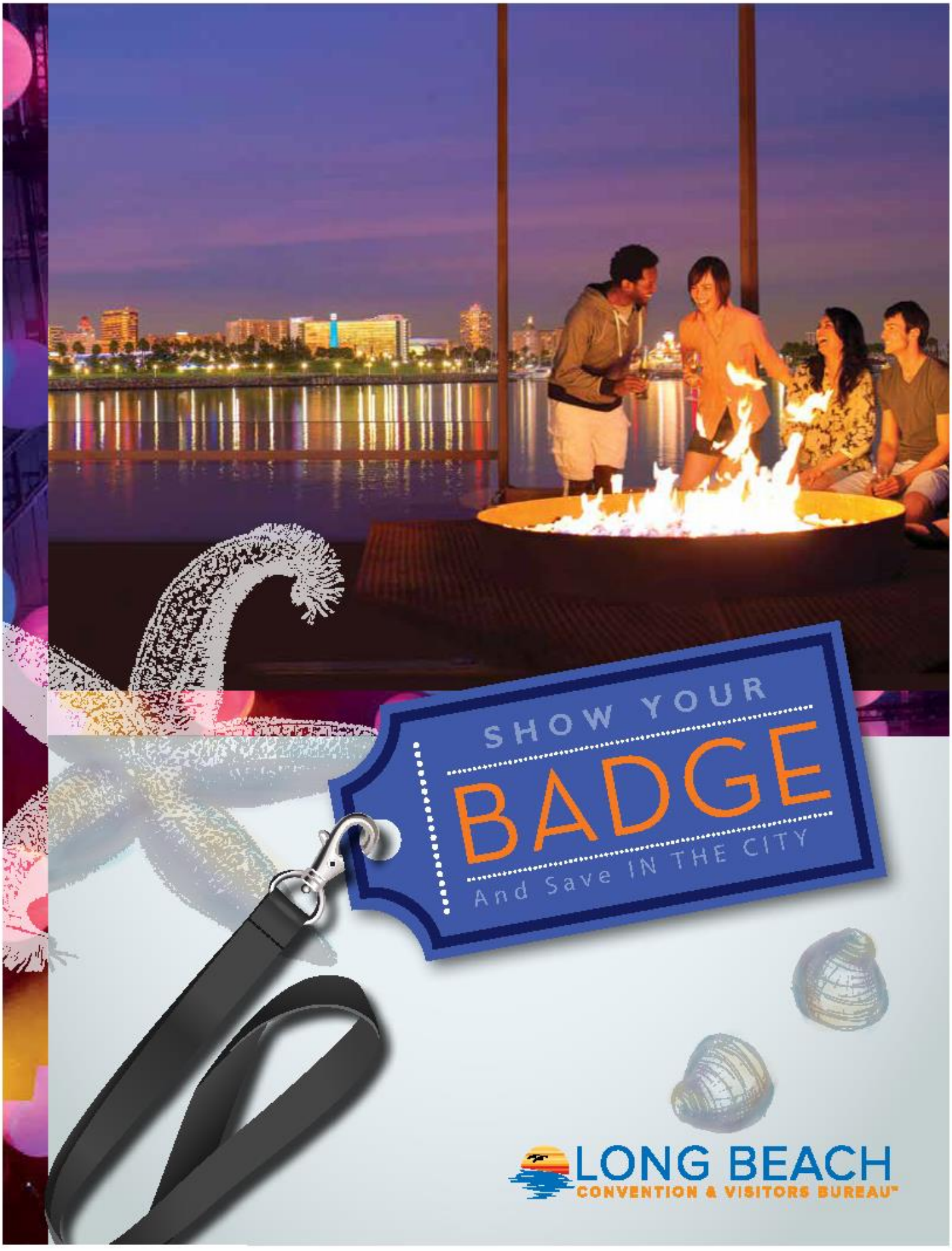
Garden Tours

Begin your day at the Earl Burns Miller Japanese Gardens with a tour of the garden's waterfalls, tea house, Zen Garden and Koi pond. Next stop, the gardens of Rancho Los Alamitos located just up the hill from campus. Take a tour of the four-acre garden designed by preeminent landscape architects of the 1920s-1940s. A short drive takes you to Rancho Los Cerritos, a National, State and Long Beach Historic site.

Day Trip to Catalina Island

Board a speedy catamaran to the island from the Catalina Express Landing. One short hour and 22 miles later, arrive at the City of Avalon on beautiful Catalina Island. Hike above the hills of Catalina or glimpse the world below the waves on a classic glass bottom boat tour. Deep sea fish, kayak, scuba dive, snorkel or simply lie out on the beach and watch the waves roll in. With several daily sailings between Long Beach and Avalon, it's easy to customize the length of your trip to fit your needs.

www.catalinaexpress.com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PLAY

Battleship IOWA Museum
250 S. Harbor Blvd., San Pedro
(877) 446-9261 pacificbattleship.com
$11 general admission tour ticket (approx. 50% off)

Gondola Getaway
5437 E Ocean Blvd
(562) 433-9595 gondolagetawayinc.com
25% Discount

Harbor Breeze Cruises
100 Aquarium Way
(562) 432-4900 2seewhales.com
25% Off full fare adult whale watching or 45 minute sightseeing cruise

International City Theatre
67 Long Beach Blvd
(562) 436-4610 ictlongbeach.org
$10 off any performance. One ticket per badge.

Long Beach Boat Rentals
401 Shoreline Village Dr
(562) 491-7400 boatsrent.com
10% Off Sat-Sun; 20% off weekdays; Not valid Holiday or Holiday weekends

Long Beach Waterbikes
110 N Marina Dr, Long Beach
(562) 545-2493 lbwaterbikes.com
BOGO - Buy one ride, get second free. Walk-in only.

Sunseeker Tours
(562) 331-1230 sunseekertours.us
10% Off

The Laugh Factory
151 S Pine Ave
(562) 495-2844 Ext 1 laughfactory.com
Upgrade from General Admission to Priority Admission

Wheel Fun Rentals
429-G Shoreline Village Dr
(805) 650-7770 wheelfunrentals.com
2nd Hour Free (rent one product for one hour, and get the 2nd hour free)

DINE

Alegria Cocina Latina
115 Pine Ave
(562) 436-3388 alegriacocinalatina.com
15% Discount

Beer Belly
255 Long Beach Blvd
(562) 436-2337 beerbellyla.com
50% Off one draft beer & any appetizer

Berlin Bistro
420 E 4th St
(562) 439-0600 berlincoffeehouse.com
15% Off on all items

Bo Beau Kitchen + Rooftop
144 Pine Ave
(562) 983-0055 eatmorestaurants.com
15% Off food

Bubba Gump Shrimp Co.
87 Aquarium Way
(562) 437-2434 bubbagump.com
Complimentary Chef's choice Appetizer with purchse of two entrees. One per table

Buono's Pizzeria
250 W Ocean Blvd, 250-A
(562) 432-2211 buonospizza.com
10% Discount including alcoholic beverages

Cafe Sevilla
140 Pine Ave
(562) 495-1111 cafesevilla.com
15% off entire FOOD bill. Cannot be combined with any other offer or promotion

District Wine
144 Linden Ave
(562) 612-0411 districtwine.com
10% Discount

Foundation Sandwich Shop
160 The Promenade N
(562) 349-0282 foundationsandwichshop.com
15% Discount

Fuego at the Maya
700 Queensway Drive
(562) 435-7676 hotelmayalongbeach.com
10% Discount

All offers subject to availability/change. Discounts do not include sales tax, gratuity or alcoholic beverages unless otherwise specified.

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George's Greek Café
135 Pine Ave
(562) 437-1184 georgesgreekcafe.com
Complimentary Baklava dessert

Great Society Cider & Mead
601 E Broadway
(562) 270-5625 greatsocietycider.com
10% Off food, beverages, and bottles to go. Excludes happy hour menu

Ice Cream & Yogurt...
429 Shoreline Village Dr
(562) 495-0121 icecreamontheboardwalk.com
$2 Off smoothies, frozen yogurt & ice cream with $5 min purchase

King's Fish House
100 W Broadway
(562) 432-7463 kingsfishhouse.com
15% Off Food and Non Alcoholic Beverages

Long Beach Cafe
615 E Ocean Blvd
(562) 436-6037 thelongbeachcafe.com
10% Off any meal

Michael's Downtown Italian Kitchen
210 E 3rd St
(562) 491-2100 michaelspizzeria.com
$1.3 Pizza Margherita & Soft Drink, 10% off any party of 5 or more - one check

Modica's
455 E Ocean Blvd
(562) 436-7011 modicas.com
10% Off all food excluding beverages and desserts

Parkers' Lighthouse
435 Shoreline Village Dr
(562) 432-6500 parkerslighthouse.com
10% Off with purchase of an entrée. Not valid with other offers or discounts.

Rock Bottom Brewery
One Pine Ave
(562) 308-2255 rockbottom.com
Purchase 2 entrees and receive up to $10 off any appetizer

Romeo Chocolates
460 Pine Ave
(562) 500-5097 romeochocolates.com
10% Discount on total purchase

Shannon's on Pine
209 Pine Ave
(562) 436-4363 shannonspine.com
15% Discount

The Attic
3441 E Broadway
(562) 433-0153 theatticonbroadway.com
10% Off Mon to Fri Only. Excludes Weekends and Holidays

The Funnel House
425 Shoreline Village Dr
(562) 901-1835 thefunnelhouse.com
Buy one get one (menu item of equal or lesser value) FREE. $4 minimum purchase

Utopia Restaurant
445 E 1st St
(562) 432-6888 utopiarestaurant.net
20% Discount

Fourth & Olive Restaurant
743 E 4th St
(562) 269-0731 4thandolive.com
Fresh Baked Pretzel and Pickle Plate with purchase of any entrée

L'Opera Ristorante
101 Pine Ave
(562) 491-0066 lopera.com
Complimentary Bruschetta for each guest with a badge

Restauration
2708 E. 4th Street
(562) 439-8822 restaurationlb.com
Free share plate per two guests or 50% off a bottle of wine

Shenanigans Irish Pub & Grille
423 Shoreline Village Dr
(562) 437-3734 shenaniganslb.com
15% off not to be combined with any other discounts or specials

SIP Bar + Lounge
111 E Ocean Blvd
(562) 437-5900 renaissance-hotels.marriott.com/renaissance-long-beach-hotel
10% off

Taco Beach
211 Pine Ave
(562) 983-1337 tbeantina.com
15% Off

The Crooked Duck
5095 E Pacific Coast Hwy
(562) 494-5118 thecrookedduck.com
15% off entire check (happy hour and other promotions excluded)

All offers subject to availability/change. Discounts do not include sales tax, gratuity or alcoholic beverages unless otherwise specified.

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The Pie Bar
450 Pine Ave
(562) 444-8PIE thepiebarlongbeach.com
10% Off

Wokcano
199 Promenade North
(562)951-9652 wokcanorestaurant.com
10% off (excluding alcohol)

SHOP

The Pike Outlets
95 South Pine Ave
(562) 432-8325 facebook.com/thepikeoutlets
Special shopping & dining coupons in our directory brochures located around the mall

The Pirates Cove & LB Souvenirs
419 C Shoreline Village Drive
(562) 435-2210 facebook.com/piratescovelongbeach
15% off all non sale merchandise (excludes cigarettes)

Raindance
419-K Shoreline Village Dr
(562) 432-0199 raindance.us.com
15% Off on all merchandise

World Trade Office Supplies
100 Oceangate, Ste 360
(562) 435-9153 worldtradeofficesupplies.com
20% Discount on selected items

SAVE

Blow It Out with Vicki and Friends
5530 South Street, Lakewood
(562) 866-7761 blowitoutwithvickiandfriends.com
Complimentary eyelashes with hair and makeup. Blowouts starting at $35. Men's Grooming $22

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Save $4 off one way or $8 off round trip Airport transfer with PROMO CODE: LBSYBS

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(800) 897-1952 securetransportation.com
10% Discount off airport and hourly transportation

Shore Business Center
5318 E 2nd St
(562) 987-4777 shorebizcenter.com
Free Packaging when you ship with us (Fedex US Mail, DHL or UPS)

Pedestrian and bicycle friendly, Long Beach is ranked as one of the "Most 'Walkable' Neighborhoods in America," by WalkScore.com

Superior Transportation: Passport shuttles free throughout downtown, 1st Street Transit Gallery is the hub for Long Beach, LA and Orange County bus lines.

Major attractions: Queen Mary, Aquarium of the Pacific, Whale Watching, Museums, Ranchos, dozens of performing arts groups and cultural events.

Perfect weather: 345 days of sunshine (moderate temps/low humidity)

The Long Beach Airport: Lowest U.S. airfares, best dining (USA Today), most beautiful (BBC)—if you haven't flown LGB, you just have to experience it!

Center of Southern California whale watching cruises and the gateway to Catalina Island

Discover Belmont Shore, Naples Island, Alamitos Bay, the East Village, 4th Street's Funky Retro Row.

Long Beach boasts four distinctive museums, two Spanish-era ranchos, many performing arts companies, including a Symphony Orchestra, Ballet, Municipal Band and Opera.

All offers subject to availability/change. Discounts do not include sales tax, gratuity or alcoholic beverages unless otherwise specified.

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