AAP / ASCI / APSA

JOINT MEETING 2019

Meeting Program & Abstracts

APRIL 5 - 7, 2019
FAIRMONT CHICAGO MILLENNIUM PARK
CHICAGO, ILLINOIS

JointMeeting.org
#JointMeeting2019
Network: **Fairmont_Meeting** Code: **jointmtg**

APSA
American Physician Scientists Association

THE PREMIER MEETING FOR PHYSICIAN-SCIENTISTS

SPECIAL EVENTS
AT THE 2019 AAP/ASCI/APSA JOINT MEETING

FRIDAY, APRIL 5

ASCI President's Reception Honoring Donald Seldin and Holly Smith
6:00 p.m. – 7:00 p.m. Gold Room

ASCI Dinner and New Member Induction Ceremony *(Ticketed event)*
7:30 p.m. – 9:45 p.m. Rouge Room, Lobby Level
After-Dinner Speaker: **Victor J. Dzau, MD**, *National Academy of Medicine*

APSA Welcome Reception *(All attendees welcome; Ticketed event; ID required)*
9:00 p.m. – Midnight Mid-America Club, Aon Center *(Off-site)*

SATURDAY, APRIL 6

ASCI Food and Science Evening *(Ticketed event; ID required)*
6:30 p.m. - 9:30 p.m Mid-America Club, Aon Center *(Off-site)*
Featuring Poster Presentations by the ASCI's 2019 Young Physician-Scientist Award Recipients

AAP Banquet and New Member Induction Ceremony *(Ticketed event, formal attire required)*
7:00 p.m. – 9:30 p.m. Imperial Ballroom, Level B2
Health Equity: Accelerating the Bend toward Justice
After Dinner Speaker: **Claire Pomeroy, MD, MBA,** *Albert and Mary Lasker Foundation*

APSA Dinner *(Ticketed event)*
7:30 p.m. - 9:00 p.m. Rouge Room, Lobby Level
On Virulence
Dinner Speaker: **Arturo Casadevall, MD PhD,** *Johns Hopkins University*

Dessert Reception and Best Poster Awards *(Open to all)*
9:45 p.m. – 11:30 p.m. Imperial Lobby, Level B2

SUNDAY, APRIL 7

APSA Residency Luncheon
12:30 p.m. - 2:30 p.m. Rouge Room, Lobby Level

PROGRAM **CONTENTS**

GENERAL **PROGRAM INFORMATION**

Registration Desk Hours

Friday, April 5	7:00 a.m. – 6:30 p.m.
Saturday, April 6	7:00 a.m. – 5:00 p.m.
Sunday, April 7	7:30 a.m. – 10:00 a.m.

Americans with Disabilities Act

Event staff will be glad to assist you with any special needs (i.e., physical, dietary, etc.). Please contact the Registration Desk at the meeting if you require any special assistance.

Joint Meeting Evaluations

The AAP/ASCI/APSA Joint Meeting Planning Committee relies on your input to enhance its meetings. Following the Joint Meeting an online meeting evaluation will be emailed to all attendees. APSA attendees will receive a separate survey to help it planning committee enhance APSA-sponsored events at future AAP/ASCI/APSA Joint Meetings. Your participation in this survey is greatly appreciated.

AAP/ASCI/APSA Joint Meeting Code of Conduct

We value your attendance. Our conference is dedicated to providing a harassment-free experience for everyone, regardless of gender, gender identity and expression, age, sexual orientation, disability, physical appearance, body size, race, ethnicity, or religious preference. AAP/ASCI/APSA do not tolerate harassment of conference participants in any form. A participant engaging in harassing behavior will be warned and may be asked to leave the conference with no refund. If you are being harassed, notice that someone else is being harassed, or have any other concerns, please contact a member of conference staff at the registration desk immediately. Conference staff and organizers are dedicated to making all participants feel safe for the duration of the conference.

Poster Session Schedule

(Location: B2, Imperial Ballroom)

Friday, April 5

1:00pm – 3:00pm	Poster Setup
6:15pm – 9:30pm	Informal Viewing: Presenters do not need to be at posters

Saturday, April 6
TWO Poster Presentation Sessions

8:00 am – 9:00 am	Poster Session & Continental Breakfast ***Odd Numbered posters presented***
11:45 am – 1:30 pm	Poster Session & Lunch ***Even Numbered posters presented***
1:30 pm – 2:00 pm	Poster Dismantle

Poster presenters should plan to be available on Saturday for their appointed poster presentation session and the resulting awards program later in the evening.

Best Poster Awards

Best Poster Awards will be given in the amount of $1000 each. Members of the AAP, ASCI and APSA will judge posters on scientific novelty, quality and clarity of presentation. Awards will be presented on Saturday, April 6, from 9:45pm – 11:30pm in the Imperial Ballroom Foyer on Level B2. Poster presenters should plan on attending for award presentation.

Wi-Fi Log-In & Code

Network: **Fairmont_Meeting**
Code: **jointmtg**

CONTINUING **MEDICAL EDUCATION INFORMATION**

Target Audience

By its nature, translational medicine -- the main focus of the Joint Meeting -- draws on many different disciplines in order to better expose the basis of normal physiology and disease-state pathology. Participants in the Joint Meeting learn about advances in areas of biomedical research in which they are actively engaged, but they also learn about advances in areas outside their specific focus. Presentations are focused on the scientific method and implementation of research strategies, which have broad application. Physician-scientists (MD, MD/PhD, or other comparable training), ranging from early faculty appointment through tenured investigators, are the target audience.

Learning Objectives

At the conclusion of this activity, participants should be able to:

- Describe important recent advances in the scientific basis of disease and therapy
- Describe novel strategies to address challenges to the physician-scientist
- Be prepared to address gender discrimination in the medical workplace
- Explain recent breakthrough advances in the application of immunotherapy in human disease
- Describe the roles that improved understanding of immunology advances and strategies can play in the potential treatment of human disease

Activity Goal

This activity is designed to address the following core and team competencies:
Medical Knowledge and Professionalism

Disclosure

Cine-Med adheres to accreditation requirements regarding industry support of continuing medical education. Disclosure of the planning committee and faculty's commercial relationships will be made known at the activity. Speakers are required to openly disclose any limitations of data and/or any discussion of any off-label, experimental, or investigational uses of drugs or devices in their presentations. - All Cine-Med employees and activity planners in control of content have indicated that they have no relevant financial relationships to disclose.

Non Endorsement Statement

Cine-Med verifies that sound education principles have been demonstrated in the development of this educational offering as evidenced by the review of its objectives, teaching plan, faculty, and activity evaluation process. Cine-Med does not endorse or support the actual opinions or material content as presented by the speaker(s) and/or sponsoring organization.

Accreditation

In support of improving patient care, this activity has been planned and implemented by Cine-Med and the Association of American Physicians, the American Society for Clinical Investigation, and the American Physician-Scientists Association. Cine-Med is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC), to provide continuing education for the healthcare team.

Ciné-Med designates this live activity for a maximum of 9.5 *AMA PRA Category 1Credit(s)™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

All other healthcare professionals will receive a Certificate of Participation. For information on the applicability and acceptance of Certificates of Participation for activities designated for *AMA PRA Category 1 Credits™*, consult your professional licensing board.

Support

Supported by a grant from the National Institute of General Medical Sciences of the National Institutes of Health.

Faculty Disclosure Summary

The following speakers have all indicated that they have No Relevant Financial Relationships to Disclose:

E. Abel, MD, PhD; N. Brown; V. Dzau; L. Fan; B. Haynes, MD; R. Jagsi, MD; T. Johnson, MD; E. Miller, MD, PhD; D. Pan; J. Rodrigues; D. Salvo; D. Segev, MD, PhD

Continued On Page 4...

CONTINUING **MEDICAL EDUCATION INFORMATION**

Faculty Disclosure Summary Continued

Last	First	Suffix	Commercial Interest	For what role?
Butte	Atul		*See Opening Disclosure Slide*	*See Opening Disclosure Slide*
Califf	Robert	MD	Verily Life Sciences/Alphabet	Senior Advisor
			Cytokinetics	Board Member
			Merck / Astra Zeneca / Sanofi / Lilly	Consultant
Diamond	Michael	MD, PhD	Moderna	SAB member, equity options
			Inbos / Atreca	Consultant
Domchek	Susan	MD	AstraZeneca / Clovis	Honoraria, research funding for a clinical trial to my institution
			BMS	Honoraria
Ebert	Benjamin	MD, PhD	Celgene	Research Grant
Erzurum	Serpil	MD	ABIM	Chair of the ABIM Pulmonary Disease Board
Greka	Anna	MD, PhD	Goldfinch Bio	Financial interest
June	Carl	MD	Novartis	Royalty payments for intellectual property license
			Tmunity Therapeutics	Scientific Founder with founder's equity
Lazar	Mitchell	MD, PhD	Eli Lilly / Pfizer	Board Member
			Novartis	Consultant
Lowy	Doug	MD	GlaxcoSmithKline / Merck / Indian Immunologicals Ltd	Patent Royalties
Nijhawan	Deepak	MD, PhD	Peloton Therapeutics	Research Grant, Consultant, Stock holder
			Barricade Therapeutics	Stock Holder
Payne	Aimee	MD, PhD	Cabaletta Bio	Equity Founder, inventor on licensed patents, chair of Scientific Advisory Board
			Novartis	Inventor on licensed patents in the field of autoimmunity
Riddell	Stanley	MD	Celgene	Consultant, Stockholder
			Adaptive Biotechnology	Consultant
			Lyell Immunopharma	Consultant, Equity holder
Ridker	Paul		Novartis, KOWA, NHLBI	Research Grant
			Novartis / Amgen / Merk / CiviBio / Corvidia / Sanofi	Consultant
Rowe	Steven	MD	Vertex Pharmaceuticals / Novartis	Research grant, Consultant
			Galapagos/Abbvie	Consultant
Wu	Gary	MD	Danone / BioCodex	Scientific Advisory Board
			Seres Therapeutics / Intercept Pharmaceuticals / Takeda	Research Grant
			Hitachi	Consultant

JOINT PROGRAM PLANNING COMMITTEE & APSA EVENTS COMMITTEE

Joint Program Planning Committee

Members

From the AAP:

John Carethers, MD
AAP President
University of Michigan

Mary Klotman, MD
AAP Vice President
Duke University

Serpil Erzurum, MD
AAP Immediate Past President
Cleveland Clinic

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John Hopkins School of Medicine

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Dana-Farber Cancer Institute

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APSA President
Virginia Commonwealth University

Jillian Liu
APSA Immediate Past President
The Ohio State University

Abhik Banerjee
APSA President-Elect
University of Southern-California
California Institute of Technology

Jeremie Lever
APSA Events Co-Chair
University of Alabama

Lillian Zhang
APSA Events Co-Chair
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Events Committee Member
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Michigan State University College of Osteopathic Medicine (MSUCOM)

Events Committee Member
Francesca LoBianco (2nd year MD/PhD)
University of Arkansas for Medical Sciences

Events Committee Member
Jeff Chen (5th year MD/PhD)
University of Kentucky

Member-At-Large Social Sciences and Humanities
Joshua Franklin (5th year MD/PhD)
University of Pennsylvania

Resident Liaison Lead
Julia (Erin) Wiedmeier (PGY2)
Mayo Clinic

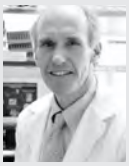
Undergraduate Liaison Lead
Mona Chatrizeh (Undergraduate)
University of California, Los Angeles

SCIENTIFIC **PROGRAM SCHEDULE**

Friday, April 5, 2019

Time	Event	Location
7:00 a.m. - 6:30 p.m.	**Registration Desk Hours**	International Foyer - Level 2
8:30 a.m. - 11:00 a.m.	**APSA Business Meeting** *(open to all APSA Members)*	Lobby Level: Rouge
11:00 a.m. - 1:00 p.m.	**APSA Session I** Moderators: **Abhik Banerjee & Jeremie Lever**	International Ballroom - Level 2
11:00 a.m. - 11:45 a.m.	 Invited Speaker: **The Transformation of Cystic Fibrosis Therapy (and Opportunities for Other Diseases of Mucus)** **Steven M. Rowe, MD, MSPH** *University of Alabama at Birmingham*	International Ballroom
12:00 p.m.- 12:45 p.m.	 Invited Speaker: **Of Math and Medicine: Big data in action** **Dorry Segev, MD, PhD** *Johns Hopkins University*	International Ballroom
1:00 p.m. - 3:00 p.m.	**Poster Setup**	Imperial Ballroom - Level B2
1:00 p.m. - 6:00 p.m.	**Plenary Session I: Big Data** Moderators: **Mary Klotman, Kieren Marr & Lillian Zhang**	International Ballroom
1:00 p.m. – 1:30 p.m.	 Invited Speaker: **Medicine in the 4th Industrial Revolution** **Robert M. Califf, MD, MACC** *Duke University*	International Ballroom
1:30 p.m. – 2:00 p.m.	 Invited Speaker: **Mitochondria and Cardiovascular Complications of Diabetes** **E. Dale Abel, MD, PhD** *University of Iowa*	International Ballroom
2:00 p.m. - 2:30 p.m.	**AAP New Member Presentations**	International Ballroom
2:00 p.m. - 2:15 p.m.	 **Inherited Susceptibility to Breast Cancer: Risk Prediction to Therapy** **Susan M. Domchek, MD** *University of Pennsylvania*	International Ballroom
2:15 p.m. - 2:30 p.m.	 **Sexual Violence Prevention in the #MeToo Era** **Elizabeth Miller, MD** *University of Pittsburgh School of Medicine*	International Ballroom

Friday, April 5, 2019 *(continued)*

Time	Event	Location
2:30 p.m. - 3:00 p.m.	**Presentations from the 2018 Donald Seldin-Holly Smith Award for Pioneering Research Recipients**  **Using Chemistry To Identify New Cancer Targets** **Deepak Nijhawan, MD, PhD** *UT Southwestern Medical Center*  **Never Say Never Again: Toward Targeted Therapies for Kidney Diseases** **Anna Greka, MD, PhD** *Brigham and Women's Hospital, Harvard Medical School*	International Ballroom
3:00 p.m. - 3:30 p.m.	**Break**	International Foyer
3:30 p.m. - 4:00 p.m.	Moderators: **Daniel Barnett, Mitch Lazar & W. Kimryn Rathmell**  **ASCI/Harrington Prize Lecture** **CAR T Cells: New Kid On The Block For Cancer Therapy And Beyond?** **Carl H. June, MD** *Perelman School of Medicine, University of Pennsylvania*	International Ballroom
4:00 p.m. - 4:30 p.m.	 **APSA Lasker Award Winner Lecture** **Preventing HPV-Associated Cancers by Vaccination** **Douglas R. Lowy, MD** *National Cancer Institute*	International Ballroom
4:30 p.m. - 5:00 p.m.	 **ASCI Presidential Address** **The Ownership Paradox: Nurturing Continuity and Change For the Future ASCI** **Kieren A. Marr, MD, MBA** *John Hopkins School of Medicine*	International Ballroom
5:00 p.m. - 5:30 p.m.	 **ASCI/Stanley J. Korsmeyer Award Lecture** **Michael S. Diamond, MD, PhD** *Washington University School of Medicine in St. Louis*	International Ballroom
5:30 p.m. - 5:45 p.m.	**APSA Founder's Award Presentation**	International Ballroom
5:45 p.m. - 7:00 p.m.	**Inaugural Resident, Fellow, and Junior Faculty Committee Working Session**	State Room - Level 2

SCIENTIFIC **PROGRAM SCHEDULE**

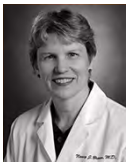
Friday, April 5, 2019 *(continued)*

Time	Event	Location
6:00 p.m. – 7:00 p.m.	**ASCI President's Reception Honoring Donald Seldin and Holly Smith**  **John H. Dirks, CM, MD, FRCPC** *University of Toronto Faculty of Medicine* **Remarks on Donald W. Seldin, MD**  **Arthur Weiss, MD, PhD** *Howard Hughes Medical Institute, University of California, San Francisco, School of Medicine* **Remarks on Lloyd H. Smith Jr., MD**	Gold Room - Level 2
6:15 p.m. – 9:30 p.m.	**Poster Viewing**	Imperial Ballroom
7:00 p.m. – 9:00 p.m.	**AAP President's Dinner** *(off-site; by invitation only.)*	Mid-America Club *Enter via Aon Center on B1*
7:30 p.m. – 9:45 p.m.	**ASCI Dinner and New Member Induction Ceremony**  **Frontiers in Biomedical Science & Technology: A Brave New World** After-Dinner Speaker: **Victor J. Dzau, MD** *National Academy of Medicine*	Rouge
9:00 p.m. – 12:00 a.m.	**APSA Welcome Reception** *(all attendees welcome)*	Mid-America Club

Saturday, April 6, 2019

Time	Event	Location
7:00 a.m. - 5:00 p.m.	**Registration Desk Hours**	International Foyer
7:00 a.m. - 8:00 a.m.	**AAP Council Meeting**	State Room - 2nd Level
7:00 a.m. - 8:00 a.m.	**Research Mentoring Breakfast**	Rouge
8:00 a.m. - 9:00 a.m.	**Poster Session and Continental Breakfast** ODD number posters will be presented/judged. Special thanks to our photo booth sponsor BURROUGHS WELLCOME FUND	Imperial Ballroom
9:00 a.m. - 11:45 a.m.	Plenary Session II: **Big Data II and The Scientific Workplace** Moderators: **Abhik Banerjee, John Carethers & Kieren Marr**	International Ballroom
9:00 a.m. – 9:30 a.m.	 Invited Speaker: **Translating a Trillion Points of Data into Therapies, Diagnostics, and New Insights into Disease** **Atul Butte, MD, PhD** *University of California San Francisco*	International Ballroom

Saturday, April 6, 2019 *(continued)*

Time	Event	Location
9:30 a.m. - 10:30 a.m	**National Academies Report on Sexual Harassment and Gender Diversification**	International Ballroom
9:30 a.m.- 9:40 a.m.	 **Why the National Academies Sponsored the Report** **Victor J. Dzau, MD** *National Academy of Medicine*	International Ballroom
9:40 a.m.- 9:46 a.m.	 **Sexual Harassment: Definitions and Methods of Measurement** **Reshma Jagsi, MD, DPhil** *University of Michigan*	International Ballroom
9:47 a.m.- 9:53 a.m.	 **Addressing Sexual Harassment and Unconscious Bias** **Nancy J. Brown, MD** *Vanderbilt University*	International Ballroom
9:54 a.m.- 10:00 a.m.	 **Changing the Culture and Climate** **Timothy R. B. Johnson, MD** *University of Michigan*	International Ballroom
10:00 a.m.- 10:30 a.m.	**Panel Discussion and Audience Questions**	International Ballroom
10:30 a.m.- 10:45 a.m.	**Break**	
	Moderators: **Hossein Ardehali, Robert Brown & Tyler McCaw**	
10:45 a.m.- 11:00 a.m.	 APSA Trainee Oral Abstract **T Cells Promote Peripheral Nerve Regeneration via Regulation of IL-4** **Deng Pan** *Washington University in St. Louis*	International Ballroom
11:00 a.m. - 11:30 a.m.	 Invited Speaker: **On Target: Cellular Immunotherapy for Pemphigus** **Aimee S. Payne, MD, PhD** *University of Pennsylvania*	International Ballroom
11:30 a.m. - 11:45 a.m.	**Recognition of the 2019 Donald Seldin~Holly Smith Award for Pioneering Research**  **Vijay G. Sankaran, MD, PhD** *Harvard Medical School, Boston Children's Hospital*	International Ballroom

SCIENTIFIC **PROGRAM SCHEDULE**

Saturday, April 6, 2019

Time	Event	Location
11:45 a.m.– 1:30 p.m.	**Poster Session with Lunch** *EVEN number posters will be presented/judged.*	Imperial Ballroom
12:45 p.m. - 1:30 p.m.	**Poster Reviewer Meeting**	Royal Room - Level B2
1:30 p.m. - 2:45 p.m.	Plenary Session III: **Immunology, Immune Therapy, and Inflammation** Moderators: **Audra Iness Christovich, Mary Klotman & Lorraine Ware**	International Ballroom
1:30 p.m. – 2:00 p.m.	 Invited Speaker: **Diet and the Gut Microbiome in Health and Disease** **Gary D. Wu, MD** *University of Pennsylvania*	International Ballroom
2:00 p.m. - 2:15 p.m.	 APSA Trainee Oral Abstract **Skeletal Muscle Krüppel-like Factor 15 and PPARδ Cooperate to Regulate Skeletal Muscle Lipid Metabolism** **Liyan Fan** *Case Western Reserve University*	International Ballroom
2:15 p.m. - 2:45 p.m.	 Invited Speaker: **Big Science, Big Data and HIV Vaccine Development** **Barton F. Haynes, MD** *Duke University*	International Ballroom
2:45 p.m. - 3:15 p.m.	**Break**	
3:15 p.m. - 5:30 p.m.	**Plenary Session IV: Immunology, Immune Therapy and Inflammation** Moderators: **David Ginsburg, Sarah Groover & W. Kimryn Rathmell**	International Ballroom
3:15 p.m. - 3:45 p.m.	 Invited Speaker: **Inflammation and Atherosclerosis: From Theory to Proven Intervention** **Paul M. Ridker, MD, MPH** *Brigham and Women's Hospital*	International Ballroom
3:45 p.m. - 4:15 p.m.	 Invited Speaker: **Engineering T Cells for Cancer Therapy** **Stanley R. Riddell, MD** *Fred Hutchinson Cancer Research Center*	International Ballroom

Saturday, April 6, 2019 *(continued)*

Time	Event	Location
4:15 p.m. - 4:45 p.m.	 AAP Presidential Address **Diversification in the Medical Sciences Fuel Growth of Physician-Scientists** **John M. Carethers, MD,** *University of Michigan*	International Ballroom
4:45 p.m - 5:15 p.m.	**AAP/Kober Medal Presentation**  Recipient: **C. Ronald Kahn, MD** Harvard Medical School  Presenter: **Jeffrey S. Flier, MD** Harvard Medical School	International Ballroom
5:15 p.m. - 5:30 p.m.	**AAP Business Meeting**	International Ballroom
5:45 p.m. - 7:00 p.m.	APSA Panel: **The Dos and Don'ts of MSTP Admissions** Moderator: **Jose A. Rodrigues**  Panelist: **Kenneth Ramos, MD, PhD** *University of Arizona Health Sciences*  Panelist: **Richard Steinman, MD, PhD** *University of Pittsburgh*  Panelist: **Lawrence (Skip) Brass, MD, PhD** *University of Pennsylvania*  Panelist: **Sandra Lemmon, PhD** *University of Miami*	Crystal Room

SCIENTIFIC **PROGRAM SCHEDULE**

Saturday, April 6, 2019 *(continued)*

Time	Event	Location
5:45 p.m. - 7:00 p.m.	APSA Panel: **Physician Scientist Training Program/ Research in Residency (PSTP/RiR)** Moderators: **Abhik K. Banerjee & Audra Iness Christovich**  Panelist: **Robert Baiocchi, MD, PhD** *The Ohio State University*  Panelist: **Patrick Hu, MD, PhD** *Vanderbilt University*  Panelist: **Audrea Burns, PhD** *Baylor College of Medicine*  Panelist: **Rebecca Baron, MD** *Brigham and Women's Hospital*	Ambassador Room
6:30 p.m. - 9:30 p.m.	**ASCI Food and Science Evening:** *Featuring Poster Presentations by the ASCI's 2019 Young Physician-Scientist Award Recipients*	Mid America Club
7:00 p.m. – 9:30 p.m.	 **AAP Banquet and New Member Induction Ceremony** After Dinner Speaker: **Claire Pomeroy, MD, MBA** *Albert and Mary Lasker Foundation* **Health Equity: Accelerating the Bend toward Justice**	Imperial Ballroom
7:30 p.m. - 9:00 p.m.	 **APSA Dinner** **On Virulence** **Arturo Casadevall, MD, PhD** *Johns Hopkins University*	Rouge
9:00 p.m. - 9:30 p.m.	**APSA Trainees Join AAP Banquet for After Dinner Speaker**	Imperial Ballroom
9:45 p.m. – 11:30 p.m.	**Dessert Reception and Best Poster Awards** *(open to all attendees)*	Imperial Lobby

Sunday, April 7, 2019

Time	Event	Location
7:30 a.m. - 10:00 a.m.	**Registration Desk Hours**	International Foyer
7:30 a.m. - 8:15 a.m.	**ASCI Town Hall** *(all ASCI members welcome)*	Cuvee Room - Level B2
8:00 a.m. - 9:00 a.m.	**APSA Board Meeting**	Regal Room
8:00 a.m. - 12:00 p.m.	**APSA Session II** Moderator: **Jose Rodrigues**	International Ballroom Gold Room Ambassador Room
8:00 a.m. - 9:30 a.m.	**Specialty Interest Mentoring Breakfast**	International Ballroom
8:30 a.m. - 9:30 a.m.	**Society Leadership Wrap-Up Meeting**	State Room
9:30 a.m. - 10:00 a.m.	 Invited Speaker: **Reflections on Physician-Scientist Training and Careers** **Donna M. Martin, MD, PhD** *University of Michigan*	Gold Room
10:00 a.m. - 12:00 p.m.	**APSA Research Residency Directors' Meeting**	State Room
10:00 a.m. - 11:00 a.m.	APSA Panel: **Women in Science** Moderator: **Francesca V. LoBianco**  Panelist: **Vineet Arora, MD** *University of Chicago*  Panelist: **Sara Shalin, MD, PhD** *University of Arkansas for Medical Sciences*  Panelist: **Susan Smyth, MD, PhD** *University of Kentucky*	Gold Room

SCIENTIFIC **PROGRAM SCHEDULE**

Sunday, April 7, 2019 *(continued)*

Time	Event	Location
10:00 a.m. - 11:00 a.m.	APSA Panel: **Fellowship and Specialties** Moderators: **Abhik K. Banerjee & Audra Iness Christovich**  **Panelist: Todd Florin, MD** *Ann and Robert H. Lurie Children's Hospital of Chicago*  Panelist: **Bruce Bochner, MD** *Northwestern Universty*  Panelist: **Anisha Dua, MD, MPH** *Northwestern University*  Panelist: **Kenneth Cohen, MD** *University of Chicago*	Ambassador Room
11:00 a.m. - 12:00 p.m.	APSA Panel: **Policy (Addressing Sex and Gender Health Disparities in Medical Research)** Moderator: **Jeff Chen**  Panelist: **Lauren Walter, MD** *University of Alabama at Birmingham*  Panelist: **Robert Garofolo, MD, MPH** *Ann and Robert H. Lurie Children's Hospital of Chicago*  Panelist: **Kim Templeton, MD** *University of Kansas*	Gold Room

Sunday, April 7, 2019 *(continued)*

Time	Event	Location
11:00 a.m. - 12:00 p.m.	APSA Panel: **Social Sciences and Humanities (Big Data: Social and Cultural Perspectives)** Moderator: **Trevor C. Hunt**  Panelist: **Susan L. Erikson, PhD** *Simon Fraser University*  Panelist: **Lauren Carruth, PhD** *American University*	Ambassador Room
12:30 p.m. - 2:30 p.m.	**Residency Luncheon**	International Ballroom

	Program Name	Program Representative
1	Vanderbilt University School of Medicine Internal Medicine Physician Scientist Training Program (PSTP)	Patrick Hu, MD, PhD
2	The Ohio State University College of Medicine Internal Medicine Physician Scientist Training Program (PSTP)	Robert Baiocchi, MD, PhD
3	University of Alabama at Birmingham School of Medicine ABIM Research Pathway Program	Sonya Heath, MD
4	University of Minnesota School of Medicine Physician Scientist Training Program (PSTP)	Clifford Steer, MD
5	University of Pennsylvania Perelman School of Medicine Physician Scientist Residency Program (PSTP)	Peter Klein, MD, PhD
6	University of Iowa Carver College of Medicine Physician Scientist Training Pathway (Multidisciplinary)(PSTP)	David Stoltz, MD, PhD
7	University of Cincinnati Internal Medicine Physician Scientist Training Program (PSTP)	Jack Rubinstein, MD
8	Nationwide Children's Hospital Integrated Research Pathway	Brian Becknell, MD, PhD
9	Harvard Medical School Massachusetts General Hospital Internal Medicine Program	Jay Vyas, MD, PhD; Jay Rajagopal, MD; Caroline Sokol, MD, PhD
10	University of California Los Angeles David Geffen School of Medicine Specialty Training and Advanced Research (STAR) Program (Multidisciplinary)	Olujimi Ajijola, MD, PhD
11	Beth Israel Deaconess Medical Center Physician Scientist Track	Steven Freedman, MD, PhD
12	Brigham and Women's Hospital Internal Medicine Residency Program	Brittany Weber, MD, PhD
13	Weill Cornell Medicine Medical Research Track	Kyu Rhee, MD, PhD
14	Baylor College of Medicine Pediatrician Scientist Training and Development Program (Pediatrics)(PSTDP)	Will Parsons, MD, PhD, Audrea Burns, PhD
15	Vanderbilt University School of Medicine Pediatrics Physician Scientist Training Program (Pediatrics)(PSTP)	Mark Denison, MD
16	National Institutes of Health Clinical Center Office of Clinical Research Training and Medical Education (OCRTME) Residency and Fellowship Training Programs (Multidisciplinary)	Thomas Burklow, MD

SPEAKER **BIOGRAPHIES**

E. Dale Abel, MD, PhD

Dr. Abel is the Chair and Department Executive Officer of the Department of Internal Medicine, Director of the Division of Endocrinology & Metabolism in the Department of Internal Medicine, and Director of the Fraternal Order of Eagles Diabetes Research Center (FOEDRC) at the University of Iowa. He is a Professor of Medicine, of Biochemistry, and of Biomedical Engineering, and holds the John B. Stokes III Chair in Diabetes Research and the François M. Abboud Chair in Internal Medicine. Dr. Abel has had a distinguished career in endocrine related research. His pioneering work on glucose transport in the heart guides his current research interests: molecular mechanisms responsible for cardiac dysfunction in diabetes. He directs a focused research group examining the molecular mechanisms leading to cardiac dysfunction in diabetes and the regulation of myocardial growth and metabolism by insulin signaling. His studies have elucidated mechanisms responsible for mitochondrial dysfunction displayed by the heart as a result of insulin resistance and insulin action in the heart. These findings provide insight into the pathogenesis of cardiac dysfunction in the diabetic heart. Dr. Abel is an established investigator of the American Heart Association (AHA) and his research program has been continually funded by the National Institutes of Health since 1995, by the AHA, the American Diabetes Association, and the Juvenile Diabetes Research Foundation. He is an elected member of the American Association of Physicians (AAP), the American Society for Clinical Investigation (ASCI), National Academy of Medicine (NAM), and the American Clinical and Climatological Association (ACCA). He is a member of the Board of Directors of Keystone Symposia, the immediate past-chair of the Scientific Advisory Committee, and immediate past-Chair of the Board of Directors of the Sarnoff Cardiovascular Research Foundation. He serves as a council member for the North American Section of the International Society for Heart Research (ISHR) and is currently a member of the Advisory Council of the National Heart Lung and Blood Institute. Dr. Abel was recently elected as President of the Endocrine Society. Additionally, Dr. Abel has received many scholastic honors which are too numerous to mention by name.

Vineet Arora, MD

Dr. Vineet Arora a board certified internist, is an academic hospitalist, Assistant Dean of Scholarship & Discovery, and Director of GME Clinical Learning Environment and Innovation at University of Chicago. Through her leadership roles, she bridges educational and hospital leadership to integrate trainees and frontline staff into the quality, safety, and value missions of the institution. An accomplished researcher, she is current PI of FDA and NIH grants to developed and evaluated novel interventions that combine systems change with adult learning theory to improve care and learning in healthcare with a focus on interprofessional quality improvement projects. She is PI of an FDA U01 to improve generic prescribing among primary care physicians and nurse practitioners. In addition, with NIH funding, she has developed an implemented an interprofessional intervention to improve patient sleep in hospitals through engaging residents, hospitalists and nurses. Lastly, with funding from AMA and ACGME, she is leading innovations to engage trainees in interprofessional QI and learning through projects like IGNITE (Improving GME Nursing Interprofessional Team Experiences). She has authored over 100 peer-reviewed publications, with widespread coverage in the New York Times, NPR, and the Associated Press. She currently serves on the Board of Directors for the American Board of Internal Medicine. In 2011, she was named to "20 People Who Make American Healthcare Better" by HealthLeaders Magazine. Dr. Arora earned her medical degree at the Washington University in St. Louis and completed her residency, chief residency, and Masters in Public Policy at the University of Chicago.

Robert Baiocchi, MD, PhD

Dr. Baiocchi joined the faculty at The Ohio State University (OSU) in 2005 and is currently a fully tenured Professor of Internal Medicine and Associate Director for Translational and Clinical Science in the Division of Hematology. Dr. Baiocchi leads the Physician Scientist Training Program (PSTP) and serves as Assistant Residency Program Director for Research in the Department of Internal Medicine. He has led the PSTP at OSU for 8 years and where he has developed novel inroads for diversifying physician scientist trainees. From an educational standpoint, Dr. Baiocchi is a member of the Integrated Biomedical Graduate Program at OSU and mentors several graduate students in the PhD program. He actively participates in education of undergraduate, graduate and medical students, residents and fellows and has been the recipient of several educational and mentoring awards recognizing his contribution and commitment to educational efforts. Dr. Baiocchi currently mentors post-doctoral fellows supported by the NIH T32 program, the American Association of Cancer Research, and American Society of Hematology. He also provides research mentorship to 4 undergraduate students, 3 graduate PhD candidates, 3 internal medicine residents, and 3 post-doctoral fellows at The Ohio State University, most of who have received independent funding supporting their research. Dr. Baiocchi's laboratory focuses on three major areas: (1) epigenetics of B-cell lymphomas; (2) experimental therapeutics of cancer and (3) immune surveillance of EBV-driven diseases. He has over 20 years of experience investigating the pathogenesis of EBV-driven cancers and experimental therapeutics of lymphoma in immune compromised patients. Current translational projects involve: (1) vaccine strategies to prevent EBV-driven lymphomas in high risk patients; (2) experimental

therapeutic strategies using selective PRMT5 inhibitors to target cancer; and (3) characterizing immune reconstitution in HIV+ patients following autologous and allogeneic stem cell transplantation. He has developed first-in-class EBV-vaccines and small molecule inhibitors of the PRMT5 enzyme and is collaborating with industry partners to translate these novel strategies to prevent and treat patients with cancer on phase I clinical trials. His laboratory is fully funded by the NIH/NCI, ACS, QNRF, and the Leukemia Lymphoma Society.

Rebecca Baron, MD

Dr. Baron is a physician-scientist with clinical and research interests in sepsis and lung injury. Her clinical work is in the Medical Intensive Care Unit, and her laboratory focuses on pathways underlying initiation and resolution of inflammation in ARDS. She studies pre-clinical models of lung injury, as well as clinical studies with the goal of identifying novel therapeutic targets and functional biomarkers in critical illness. She attended college at Stanford University, obtained her M.D. from Harvard Medical School, and completed internship, residency, and fellowship at Brigham and Women's Hospital, where she is currently an Associate Program Director for the Internal Medicine Residency Program focusing on residency related research activities.

Bruce Bochner, MD

Dr. Bochner, attended medical school at the University of Illinois College of Medicine in Chicago, and graduated with honors. After completing Internal Medicine residency training at the same institution, he began his postdoctoral allergy and immunology training at Johns Hopkins in the Division of Allergy and Clinical Immunology of the Department of Medicine, where he joined the faculty in 1988. In 1999, he became Professor of Medicine at Johns Hopkins, and from 2003-2013 was the Director of the Division of Allergy and Clinical Immunology. As of August 2013, Dr. Bochner moved to Chicago to become the Samuel M. Feinberg Professor of Medicine in the Division of Allergy and Immunology at the Northwestern University Feinberg School of Medicine. Dr. Bochner is a member of the American Society for Clinical Investigation and the Association of American Physicians; and President of the Collegium Internationale Allergologicum and the International Eosinophil Society. He is co-Editor-in-Chief for the Allergy and Immunology Section of the online resource UpToDate. He has been steadily funded by NIH and other sources, and is a former standing member of multiple NIH study sections. His primary research interests focus on the biology of human eosinophils and mast cells, and how they can be targeted for therapeutic benefit. He is the author of more than 280 peer-reviewed publications, reviews, and book chapters. He also sees patients, with a particular interest in the diagnosis and treatment of eosinophil and mast cell-related disorders and is board-certified in both internal medicine and allergy-immunology.

Lawrence (Skip) Brass, MD, PhD

Dr. Lawrence (Skip) Brass is a graduate of Harvard College and Case Western Reserve University, where he received his MD and a PhD in biochemistry. After residency training in internal medicine he became a fellow in Hematology-Oncology at the University of Pennsylvania where he served as Vice Chair for Research in the Department of Medicine from 2004 to 2007, and is currently Professor of Medicine and Professor of Systems Pharmacology and Translational Therapeutics. He has led the NHLBI-funded Hematology Research Training Program since 1994 and became Associate Dean for Combined Degree and Physician Scholars Programs and Director of Penn's MSTP in 1998. Hehas been active at the national level in the development of training programs for physician-scientists, has served asPresidentof the National Association of MD-PhD Programs, Chair of the AAMC GREAT section on MD-PhD training and was a member of the NIH Physician-Scientist Workforce advisory group in 2013-2014. He is also a practicing hematologist whose research and clinical interests are in the fields of hemostasis and vascular biology. He has been continuously funded by the NIH HLBI since the mid-1980's, has been elected to the American Society for Clinical Investigation and the Association of American Physicians, was an Established Investigator of the American Heart Association and has received the Christian R. and Mary F. Lindback Award for Distinguished Teaching from the University of Pennsylvania (2001), the Distinguished Career Award from the International Society of Hemostasis and Thrombosis (2013), the inaugural Bert Shapiro Award for Leadership, Dedication and Service to the Physician-Scientist Community from the National Association of MD/PhD Programs (2015), the Distinguished Educator Award from the Association of Clinical and Translational Science (2018), and numerous teaching awards from students at the Perelman School of Medicine.

Nancy J. Brown, MD

Dr. Brown serves as chair of the Vanderbilt Department of Medicine and physician-in-chief of Vanderbilt University Hospital. A graduate of Yale College and Harvard Medical School, Dr. Brown also leads a translational research program that focuses on developing new pharmacological strategies to prevent vascular disease in patients with high blood pressure and diabetes. Dr. Brown has worked to promote the career development of physician-scientists. She established the Vanderbilt Master of Science in Clinical Investigation in 2000. From 2006-2010, Dr. Brown served as the Associate Dean for Clinical and Translational Scientist Development and established infrastructure to promote the development of physician-scientists. Dr. Brown has served as a member of the NIH National Advisory Research Resources Council and currently serves National Heart, Lung, and Blood Advisory Council. Her research has been recognized by the American Heart Association (Harriet Dustan Award), the E.K. Frey-E. Werle Foundation, the American Society of

Hypertension and the American Federation for Clinical Research. In 2018, she was named the Robert H. Williams, MD, Distinguished Chair of Medicine by the Association of Professors of Medicine. Dr. Brown is a fellow in the American Association for the Advancement of Science and a member of the American Society for Clinical Investigation, the American Association of Physicians, and the National Academy of Medicine.

Audrea Burns, PhD

Dr. Burns received her Bachelor's degree in Biology from Xavier University of Louisiana. After completing her graduate studies in Immunology in the Department of Biological Sciences at The University of Chicago, she completed her postdoctoral fellowship at Baylor College of Medicine (BCM) in the Research Education and Career Horizon (REACH) - Institutional Research Academic Career and Development Award (IRACDA) program, which included developing courses in the natural sciences and teaching at The University of Houston Downtown, national and local undergraduate conference planning, and also conducting research in cancer immunology. She also is currently completing the BCM Master Teachers Fellows Program for Medical Educator researchers. Dr. Burns co-developed the curriculum and serves as the Associate Program Director for the Pediatrician-Scientist Training & Development Program at BCM where she has been faculty since September 2013. Her research focuses on Professionalism with a focus in Professional Identity Formation and also serves as the Co-Chair of the National Physician-Scientist Collaborative Workgroup and the Co-Chair of the Council on Pediatric Subspecialties Work Action Task Force for Recruiting and Sustaining Junior Faculty in their Research Paths. Her interests in graduate medical education include threshold concepts, retention and support strategies for underrepresented minorities, and physician-scientist pipeline development.

Atul Butte, MD, PhD

Dr. Butte is the Priscilla Chan and Mark Zuckerberg Distinguished Professor and inaugural Director of the Bakar Computational Health Sciences Institute (bchsi.ucsf.edu) at the University of California, San Francisco (UCSF). Dr. Butte is also the Chief Data Scientist for the entire University of California Health System, with 17 health professional schools, 6 medical centers, and 10 hospitals. Dr. Butte has been continually funded by NIH for 20 years, has authored over 200 publications, with research repeatedly featured in the New York Times, Wall Street Journal, and Wired Magazine. Dr. Butte was elected into the National Academy of Medicine in 2015, and in 2013, he was recognized by the Obama Administration as a White House Champion of Change in Open Science for promoting science through publicly available data. Dr. Butte is also a founder of three investor-backed data-driven companies: Personalis, providing medical genome sequencing services, Carmenta (acquired by Progenity), discovering diagnostics for pregnancy complications, and NuMedii, finding new uses for drugs through open molecular data. Dr. Butte is a principal investigator of two major programs: (1) the California Initiative to Advance Precision Medicine, implementing Governor Brown's vision to promote precision medicine in California; and (2) ImmPort, the clinical and molecular data repository for the National Institute of Allergy and Infectious Diseases. Dr. Butte trained in Computer Science at Brown University, worked as a software engineer at Apple and Microsoft, received his MD at Brown University, trained in Pediatrics and Pediatric Endocrinology at Children's Hospital Boston, then received his PhD from Harvard Medical School and MIT.

Robert M. Califf, MD, MACC

Dr. Califf is the Donald F. Fortin, MD, Professor of Cardiology. He is also Professor of Medicine in the Division of Cardiology and remains a practicing cardiologist. Dr. Califf was the Commissioner of Food and Drugs in 2016-2017 and Deputy Commissioner for Medical Products and Tobacco from February 2015 until his appointment as Commissioner in February 2016. Prior to joining the FDA, Dr. Califf was a professor of medicine and vice chancellor for clinical and translational research at Duke University. He also served as director of the Duke Translational Medicine Institute and founding director of the Duke Clinical Research Institute. A nationally and internationally recognized expert in cardiovascular medicine, health outcomes research, healthcare quality, and clinical research, Dr. Califf has led many landmark clinical trials and is one of the most frequently cited authors in biomedical science, with more than 1,200 publications in the peer-reviewed literature. Dr. Califf is a Member of the National Academy of Medicine (formerly known as the Institute of Medicine (IOM)) in 2016, one of the highest honors in the fields of health and medicine. Dr. Califf has served on numerous IOM committees, and he has served as a member of the FDA Cardiorenal Advisory Panel and FDA Science Board's Subcommittee on Science and Technology. Dr. Califf has also served on the Board of Scientific Counselors for the National Library of Medicine, as well as on advisory committees for the National Cancer Institute, the National Heart, Lung, and Blood Institute, the National Institute of Environmental Health Sciences and the Council of the National Institute on Aging. He has led major initiatives aimed at improving methods and infrastructure for clinical research, including the Clinical Trials Transformation Initiative (CTTI), a public-private partnership co-founded by the FDA and Duke. He also served as the principal investigator for Duke's Clinical and Translational Science Award and the NIH Health Care Systems Research Collaboratory coordinating center and co-PI of the Patient Centered Outcomes Research Institute Network.

SPEAKER **BIOGRAPHIES**

John M. Carethers, MD

Dr. Carethers is the C. Richard Boland Distinguished University Professor and Chair of the Department of Internal Medicine at the University of Michigan. He received his M.D. from Wayne State University, completed residency in internal medicine at Massachusetts General Hospital and gastroenterology fellowship at the University of Michigan. Prior to becoming Chair at Michigan, he was Chief of Gastroenterology at UC San Diego. John's laboratory focuses on genetics of colorectal cancer, and in particular, DNA mismatch repair where he has gleamed important insights into how this DNA repair system recognizes chemotherapy for the killing cancer cells, and how inflammation influences DNA repair. He is a member of the National Academy of Medicine and the American Academy of Arts & Sciences, and is President of AAP for 2018-2019. He was awarded the 2019 Robert H. Williams Distinguished Chair of Medicine Award from the Association of Professors of Medicine.

Lauren Carruth, PhD

Dr. Lauren Carruth is a medical anthropologist specializing in humanitarian assistance, global health, nutrition, food security, and displacement. Her ongoing research focuses on emerging infectious diseases as well as the lasting social, political, and health effects of episodic humanitarian interventions. She is currently writing a book tentatively titled, ""Saving lives and making a living: local aid workers and humanitarian labor in the Horn of Africa"" highlighting, for one, the important but invisible contributions local aid workers make to relief operations and the collection of scientific and programmatic data in humanitarian emergencies.

Arturo Casadevall, MD, PhD

Dr. Casadevall is Bloomberg Distinguished Professor and Chair of the W. Harry Feinstone Department of Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health. Dr. Casadevall's major research interests are in fungal pathogenesis and the mechanisms of antibody action. In the area of biodefense, he has an active research program to understand the mechanisms of antibody-mediated neutralization of Bacillus anthracis toxins. Dr. Casadevall is the editor-in-chief of mBio and Deputy Editor for the Journal of Clinical Investigation and is on the editorial board of several journals including the Journal of Infectious Diseases and the Journal of Experimental Medicine. He has also served in numerous NIH committees including those that drafted the NIAID Strategic Plan and the Blue Ribbon Panel on Biodefense Research. He served on the National Academy of Sciences panel that reviewed the science on the FBI investigation of the anthrax terror attacks of 2001, the National Science Advisory Board for Biosecurity from 2005-2014 and from 2015-2017 and as a Commissioner to the National Commission on Forensic Science, the United States Department of Justice. Dr. Casadevall has received numerous honors including election to the American Society for Clinical investigation, American Academy of Physicians, Fellow of the American Association for Advancement of Science, National Academy of Medicine and the American Academy of Arts and Sciences.

Kenneth Cohen, MD

Dr. Cohen received a B.S. in Molecular Biochemistry and Biophysics from Yale College and his medical degree from the University of Pennsylvania School of Medicine. He pursued internal medicine residency training at the Massachusetts General Hospital (MGH) in Boston followed by adult Hematology/Oncology fellowship training at the Dana-Farber Cancer Institute/Partners CancerCare program. He received research training in the stem cell lab of Dr. David Scadden at MGH where his research focused on the role of bone marrow-derived angiogenic cells in tumor growth and vascularization. After fellowship training he was promoted to Instructor at the Massachusetts General Hospital and continued additional mentored research training. He was recruited to the University of Chicago Section of Hematology-Oncology where he pursued research focused on understanding the role of the tumor vascular microenvironment in regulating lymphocyte populations. He currently is an associate professor of medicine and serves as the program director for the Adult Hematology-Oncology Fellowship training program.

Michael S. Diamond, MD, PhD

Dr. Diamond's research focuses on the interface between viral pathogenesis and the host immune response. Two globally important mosquito-borne human pathogens are studied, the West Nile encephalitis and Dengue hemorrhagic fever viruses. They are single-stranded positive-polarity RNA viruses, and closely related to the viruses that cause yellow fever, St. Louis and Japanese encephalitis, and hepatitis C. Studies with West Nile and Dengue viruses have focused on investigating their pathogenesis and the immune system response that controls infection. Using in vitro models of infection in primary neurons, we are studying the mechanisms by which West Nile virus causes direct injury to different populations of neurons. Using a mouse model we have defined critical roles for interferon, antibody, complement, CD4+, and CD8+ cell in the control and eradication of West Nile virus infection. More recently, we have begun to study the structural basis of antibody-mediated neutralization of West Nile and Dengue virus. By combining our structural and pathogenesis data, we have developed and humanized a monoclonal antibody that has strong therapeutic activity against WNV even after the virus has disseminated into the central nervous system. This data is also being applied to the development of novel strategies for vaccine development.

SPEAKER **BIOGRAPHIES**

John H. Dirks, CM, MD, FRCPC

Dr. Dirks received his MD from the University of Manitoba (1957) and a Fellowship in Medicine from the Royal College of Physicians (1963). He trained in nephrology research at the NIH (1963-1965) with Dr. Robert Berliner and held a Medical Research Council of Canada grant from 1965 to 1987 for his work in renal pathophysiology. Now Emeritus Professor of Medicine at the University of Toronto, Dr. Dirks held a number of major academic positions, including Director of Nephrology, McGill University (1965-1976); Head, Department of Medicine, University of British Columbia (1976-1987); Dean of Medicine, University of Toronto (1987-1991); and Dean-Rector, Aga Khan University in Pakistan (1994-1996). From 1994 to 2005, Dr. Dirks chaired the International Society of Nephrology's Commission for the Global Advancement of Nephrology, a major educational-clinical outreach program in over 100 countries. Dr. Dirks is Emeritus President and Scientific Director of the Gairdner Foundation, having held the role of President from 1993 until his retirement in 2016; in 2011, the Foundation created the John Dirks Canada Gairdner Global Health Award in his honor. Among other recognition, Dr. Dirks is a recipient of the International Distinguished Medal from the US National Kidney Foundation (2005), the International Society of Nephrology's Roscoe R. Robinson Award (2004), the Order of Canada (2006), and honorary doctorates in science from the University of Manitoba and the University of Toronto. He is an elected member of the American Society for Clinical Investigation (1971), the Association of American Physicians (1977), and the American Academy of Arts and Sciences (2008). In 2012 he was inducted into the Canadian Medical Hall of Fame and received the Queen Elizabeth II Diamond Jubilee Medal.

Susan M. Domchek, MD

Dr. Domchek is the Basser Professor in Oncology at the University of Pennsylvania. She serves as Executive Director of the Basser Center for BRCA at the Abramson Cancer Center. Her work focuses on the translation of genetic information related to cancer susceptibility into clinical practice, ranging from risk assessment and prevention to cancer therapeutics. A significant contributor to the oncology literature, she has authored/co-authored more than 250 articles appearing in scholarly journals including the New England Journal of Medicine, Lancet, the Journal of the American Medical Association and the Journal of Clinical Oncology. Dr. Domchek is an elected member of the American Society of Clinical Investigation as well as the National Academy of Medicine.

Anisha Dua, MD, MPH

Dr. Dua is an Associate Professor of Rheumatology at Northwestern University where she is the Director of the Rheumatology fellowship Program and Associate Director of the Vasculitis Center. She has previously served as the Rheumatology Fellowship Program Director at The University of Chicago and the Director of Medical Education at Allegheny Health Network in Pittsburgh. Dr. Dua has been focused on education throughout her career, serving as the secretary/treasurer for the Chicago Rheumatism society and volunteering for the American College of Rheumatology Committee on Training and Workforce as well as the In-Training Exam committee. She completed her Rheumatology training at Rush Medical Center as well as a fellowship in medical education (Medical Education Research, Innovation, Teaching and Scholarship) at The University of Chicago. Her clinical interests are in vasculitis, where she is the PI of ongoing clinical trials and is a member of the Vasculitis guidelines committee for the American College of Rheumatology.

Victor J. Dzau, MD

Dr. Dzau is President of the National Academy of Medicine (formerly Institute of Medicine). He is Chancellor Emeritus of Duke University and former CEO of the Duke University Health System. Previously, Dr. Dzau was the Professor and Chairman of Medicine at Harvard Medical School as well as at Stanford University. His important work on cardiovascular medicine led to the development of widely used, lifesaving drugs. In his role as a leader in health care, Dr. Dzau has led efforts in innovation to improve health, including the development of the Duke Translational Medicine Institute, Duke Global Health Institute, the Duke-National University of Singapore (NUS) Graduate Medical School, and the Duke Institute for Health Innovation. He has served on the Advisory Committee to the Director of National Institutes of Health (NIH) and chaired the NIH Cardiovascular Disease Advisory Committee. He served on the Governing Board of the Duke-NUS and the Board of Health Governors of the World Economic Forum and chaired its Global Agenda Council on Personalized and Precision Medicine. Currently, he is a member of the Board of Directors of Singapore Health System, Expert Board of Imperial College Health Partners, UK, and the Biomedical Science Council of Singapore. Among his many honors are Gustav Nylin Medal from the Swedish Royal College of Medicine, Distinguished Scientist Award of American Heart Association, Max Delbruck Medal, Ellis Island Medal of Honor, and the Henry Freisen International Prize for Health Research. In 2014, he received the Public Service Medal from the President of Singapore. He has received nine honorary doctorates. He is a member of the National Academy of Medicine, American Academy of Arts and Sciences and the European Academy of Sciences and Arts.

SPEAKER **BIOGRAPHIES**

Susan L. Erikson, PhD

Dr. Erikson is an anthropologist and former international affairs expert who has worked in Africa, Europe, Central Asia, and North America. She is currently an Associate Professor at Simon Fraser University in British Columbia. Global Health Data and Global Health Financialization are Dr. Erikson's two current research foci. Dr. Erikson is the founding director of the Global Health Affairs Program at the Korbel School of International Studies at the University of Denver, and was voted Best Professor there in 2004. She joined the Faculty of Health Sciences at Simon Fraser University in 2007, where she was awarded the Graduate Teaching and Mentorship Excellence Award in 2012. In 2013, she was awarded the Society for Medical Anthropology's (SMA) Virchow Prize for her publication, ""Global Health Business: The Production and Performativity of Statistics in Germany and Sierra Leone."" As an academic, Dr. Erikson combines her practical work experience with a critical study of the relations of power informing global health scenarios. Early in her research career, Dr. Erikson's findings showed that health outcomes are inextricable from economic, political and techno-scientific interests. Since 2013, Dr. Erikson has conducted fieldwork research in Sierra Leone on the production, use, and global circulation of health data. She was in Freetown in February 2014 studying local and global data use when news of Ebola infections in neighboring Guinea first reached the capital city. Findings from that research led to a project on the financialization of humanitarian response, specifically the 'Ebola bond.'

Liyan Fan

Liyan Fan is currently a fourth year MSTP student at Case Western Reserve University School of Medicine in Cleveland, OH. She is completing her graduate studies in the laboratory of Dr. Mukesh Jain and is a NIH T32 Pre-doctoral Trainee Award Recipient through the CWRU Cardiovascular Research Training Program. Liyan's research focuses on the mechanisms underlying skeletal muscle regulation of lipid metabolism and their consequent impact on systemic metabolic health and disease. Specifically, she is investigating the skeletal muscle intrinsic role of the transcription factor Krüppel-like factor 15 and its interaction with the nuclear receptor PPAR in affecting lipid metabolism.

Jeffrey S. Flier, MD

Dr. Flier became the 21st Dean of the Faculty of Medicine at Harvard University on September 1, 2007. His term as Dean ended in 2016 after nine years.

Flier, an endocrinologist and an authority on the molecular causes of obesity and diabetes, is the Caroline Shields Walker Professor of Medicine at Harvard Medical School. Previously he had served as Harvard Medical School Faculty Dean for Academic Programs and Chief Academic Officer for Beth Israel Deaconess Medical Center, a Harvard teaching affiliate. Flier is one of the country's leading investigators in the areas of obesity and diabetes. His research has produced major insights into the molecular mechanism of insulin action, the molecular mechanisms of insulin resistance in human disease, and the molecular pathophysiology of obesity.

Todd Florin, MD

Dr. Todd Florin is Associate Professor of Pediatrics and Director of Research in the Division of Pediatric Emergency Medicine at Ann and Robert H. Lurie Children's Hospital of Chicago and Northwestern University Feinberg School of Medicine. He is also Head of the Grainger Initiative in Pediatric Emergency Medicine Research at Lurie Children's Hospital. Dr. Florin completed medical school at the University of Rochester School of Medicine and Dentistry in 2005. He completed pediatrics residency, chief residency and a pediatric emergency medicine fellowship at The Children's Hospital of Philadelphia from 2005-2012, in addition to a Master's degree in Clinical Epidemiology from the Center for Clinical Epidemiology and Biostatistics at the University of Pennsylvania. He was an Associate Professor of Pediatrics and Director of Research Operations for Emergency Medicine at Cincinnati Children's Hospital Medical Center from 2005-2012 before moving to Lurie Children's. Dr. Florin's work focuses on improving the diagnosis, management and outcomes of children with common, serious infections in the emergency department. His current efforts are concentrated on pediatric lower respiratory tract infections, namely bronchiolitis and pneumonia.

Dr. Florin is the principal investigator of Catalyzing Ambulatory Research in Pneumonia Etiology and Diagnostic Innovations in Emergency Medicine (CARPE DIEM), a prospective cohort study of children who present to the ED with community-acquired pneumonia with the overall objectives of using clinical and translational methodology to understand CAP pathophysiology, improve prediction of CAP severity, and enhance differentiation of CAP etiology. This work has been funded by a KL2 mentored career development award through the University of Cincinnati Center for Clinical and Translational Science and Training (CCTST), a grant from the Gerber Foundation and a K23

from the National Institute of Allergy and Infectious Diseases.His work has also centered on resource utilization, variation in care, and use of clinical trials to improve treatments for respiratory tract infections. Dr. Florin is a past recipient of the Academic Pediatric Association Young Investigator Award, in addition to research awards from the Sections of Emergency Medicine and Hospital Medicine of the American Academy of Pediatrics. He serves on the Council for the Society for Pediatric Research and is the Protocol Review Chair for the American Academy of Pediatrics Pediatric Emergency Medicine Collaborative Research Committee.

Robert Garofolo, MD, MPH

Dr. Robert Garofalo is a Professor of Pediatrics and Preventive Medicine at Northwestern University's Feinberg School of Medicine in Chicago, Illinois. He is also an attending physician at the Ann & Robert H. Lurie Children's Hospital, where he serves as the Director of the Research Center of Excellence for Gender, Sexuality, and HIV Prevention and as the Division Chief of Adolescent Medicine. He co-directs the gender and sexual development clinical program at Lurie Children's Hospital — the first comprehensive program providing multidisciplinary care to transgender/gender-nonconforming children and adolescents in the Midwest. His research focuses on HIV prevention, mostly targeting either young men who have sex with men (MSM) or transgender individuals. He has more than 25 years of research experience in this field and is a national authority on LGBT health issues, adolescent sexuality, and HIV clinical care and prevention. In 2010, he was appointed to the National Academy of Science/ Institute of Medicine on LGBT Health Issues and Research Gaps and Opportunities. Dr. Garofalo's research has been generously funded by the National Institutes of Health. He is or has been the Principal Investigator on 13 NIH-funded Investigator initiated research grants and a Co-Investigator on an additional 14 other NIH-funded research projects. He is currently a member of a number of professional organizations and scientific associations including the American Academy of Pediatrics, the Society for Adolescent Medicine, the American Medical Association, and the World Professional Association for Transgender Health. Dr. Garofalo is the Editor-in-Chief of the journal Transgender Health. He has over 150 publications in scholarly journals. In addition to his academic work, Dr. Garofalo is founder of Fred Says (named after his dog), a 501©3 non-profit charity that since 2013 has raised and donated back to the community over $300.000 to support care and services for HIV+ youth.

Anna Greka, MD, PhD

Dr. Greka is a physician-scientist leading the translation of scientific discoveries from the laboratory to clinical trials. She is an Associate Professor at Harvard Medical School (HMS); an Associate Physician in the Renal Division in the Department of Medicine at Brigham and Women's Hospital (BWH); and the founding director of Kidney-NExT, a Center for Kidney Disease and Novel Experimental Therapeutics at BWH. Dr. Greka is also an Institute Member of the Broad Institute of MIT and Harvard, where she directs the institute's Kidney Disease Initiative (KDI) and the ion channel therapeutics interest group (CHAnnel Therapeutics, CHArT). The Greka laboratory specializes in the development of precision therapies for difficult-to-treat diseases with a special interest in genetically defined disorders. Specifically, her lab studies mechanisms of cell survival and metabolic regulation, including calcium signaling and transient receptor potential (TRP) ion channel biology. The Greka laboratory is also interested in using the modern tools of genomics and other multi-omic approaches to understand disease mechanisms, including mechanisms of disrupted cellular metabolism, with important connections to obesity and diabetes. Finally, the study of ion channel biology remains an active area of investigation, with a special focus on harnessing the considerable therapeutic potential of ion channels for a wide range of diseases, from kidney to neurologic disorders. Dr. Greka has been the recipient of several honors, including the ASCI's 2018 Seldin-Smith Award for Pioneering Research, a 2017 Presidential Early Career Award for Scientists and Engineers (PECASE), a 2014 Top 10 Exceptional Research Award from the Clinical Research Council, and a 2014 Young Physician-Scientist Award from the ASCI. She also serves on the Harvard-MIT MD-PhD Program Leadership Council. Dr. Greka holds an AB in biology from Harvard College and an MD and PhD in neurobiology from HMS. She received her medical and scientific training in the Harvard-MIT program in Health Sciences and Technology (HST) in the laboratory of David Clapham, MD, PhD, where, as a Howard Hughes Medical Institute (HHMI) predoctoral fellow, she explored the role of TRP channels in neuronal growth cone motility.

Barton F. Haynes, MD

Dr. Haynes is the Frederic M. Hanes Professor of Medicine and Immunology, and Director of the Human Vaccine Institute in the Duke University School of Medicine. Prior to leading the Vaccine Institute at Duke, Dr. Haynes served as Chief of the Division of Rheumatology, Allergy and Clinical Immunology and later as Chair of the Department of Medicine. As Director of the Duke Human Vaccine Institute, Bart Haynes is leading a team of investigators working on vaccines for emerging infections, including tuberculosis, pandemic influenza, and HIV/AIDS. To work on the AIDS vaccine problem, his group has been awarded two large consortium grants from the NIH, NIAID knownas the Center for HIV/AIDS Vaccine Immunology (CHAVI) (2005-2012), and the Center for HIV/AIDS Vaccine Immunology-Immunogen Discovery (CHAVI-ID) (2012-2019) to conduct discovery science to speed HIV vaccine development.

Patrick Hu, MD, PhD

Dr. Hu is an Associate Professor of Medicine and Cell and Developmental Biology and Director of the Physician-Scientist Training Program/Harrison Society in the Department of Medicine at Vanderbilt University Medical Center. He earned an A.B. from Harvard College and M.D. and Ph.D. degrees from New York University, where he studied PI 3-kinase signaling in Joseph Schlessinger's lab. After completing clinical training in Internal Medicine at The Johns Hopkins Hospital and Adult Oncology at the Dana-Farber Cancer Institute and Massachusetts General Hospital, he did postdoctoral research on insulin-like growth factor signaling in the nematode Caenorhabditis elegans in Gary Ruvkun's lab. He spent eleven years on the faculty of the University of Michigan Medical School prior to his recruitment to Vanderbilt in 2016. Dr. Hu is a member of the American Society for Clinical Investigation. Patrick Hu, M.D., Ph.D., is an Associate Professor of Medicine and Cell and Developmental Biology and Director of the Physician-Scientist Training Program/Harrison Society in the Department of Medicine at Vanderbilt University Medical Center. He earned an A.B. from Harvard College and M.D. and Ph.D. degrees from New York University, where he studied PI 3-kinase signaling in Joseph Schlessinger's lab. After completing clinical training in Internal Medicine at The Johns Hopkins Hospital and Adult Oncology at the Dana-Farber Cancer Institute and Massachusetts General Hospital, he did postdoctoral research on insulin-like growth factor signaling in the nematode Caenorhabditis elegans in Gary Ruvkun's lab. He spent eleven years on the faculty of the University of Michigan Medical School prior to his recruitment to Vanderbilt in 2016. Dr. Hu is a member of the American Society for Clinical Investigation. Patrick Hu, M.D., Ph.D., is an Associate Professor of Medicine and Cell and Developmental Biology and Director of the Physician-Scientist Training Program/Harrison Society in the Department of Medicine at Vanderbilt University Medical Center. He earned an A.B. from Harvard College and M.D. and Ph.D. degrees from New York University, where he studied PI 3-kinase signaling in Joseph Schlessinger's lab. After completing clinical training in Internal Medicine at The Johns Hopkins Hospital and Adult Oncology at the Dana-Farber Cancer Institute and Massachusetts General Hospital, he did postdoctoral research on insulin-like growth factor signaling in the nematode Caenorhabditis elegans in Gary Ruvkun's lab. He spent eleven years on the faculty of the University of Michigan Medical School prior to his recruitment to Vanderbilt in 2016. Dr. Hu is a member of the American Society for Clinical Investigation. Patrick Hu, M.D., Ph.D., is an Associate Professor of Medicine and Cell and Developmental Biology and Director of the Physician-Scientist Training Program/Harrison Society in the Department of Medicine at Vanderbilt University Medical Center. He earned an A.B. from Harvard College and M.D. and Ph.D. degrees from New York University, where he studied PI 3-kinase signaling in Joseph Schlessinger's lab. After completing clinical training in Internal Medicine at The Johns Hopkins Hospital and Adult Oncology at the Dana-Farber Cancer Institute and Massachusetts General Hospital, he did postdoctoral research on insulin-like growth factor signaling in the nematode Caenorhabditis elegans in Gary Ruvkun's lab. He spent eleven years on the faculty of the University of Michigan Medical School prior to his recruitment to Vanderbilt in 2016. Dr. Hu is a member of the American Society for Clinical Investigation.

Reshma Jagsi, MD, DPhil

Dr. Jagsi is Professor and Deputy Chair in the Department of Radiation Oncology and Director of the Center for Bioethics and Social Sciences in Medicine at the University of Michigan. After graduating first in her class from Harvard College, she pursued her medical training and radiation oncology residency at Harvard Medical School. She also served as a fellow in the Center for Ethics at Harvard University and completed her doctorate in Social Policy at Oxford University as a Marshall Scholar. Dr. Jagsi has devoted a substantial portion of her scholarly effort to investigations regarding gender equity in academic medicine. Of the over 200 articles she has authored in peer-reviewed journals, over 50 focus on gender issues in the medical profession. Her research in this area has been funded by independent grants from the NIH, the Doris Duke Foundation, the Robert Wood Johnson Foundation, the AMA, and others. She has published on the specific subject of sexual harassment in JAMA and the New England Journal of Medicine, and she is a frequently invited speaker who has delivered talks on this subject at dozens of institutions, professional specialty societies, and leadership groups within medicine. A former member of the Steering Committee of the AAMC's Group on Women in Medicine in Science, she is a member of ASCI.

Timothy R.B. Johnson, MD

Dr. Johnson served as Chair of Obstetrics and Gynecology and Bates Professor of Diseases of Women and Children at the University of Michigan from 1993-2017. He remains Arthur F. Thurnau Professor; Professor, Women's Studies, and Research Professor, Center for Human Growth and Development. His education and training have been at the University of Michigan, University of Virginia and Johns Hopkins. He is author of over three hundred articles, chapters and books and has served on numerous editorial boards, study sections, professional committees, societies and boards. He is active in international teaching and training, especially in Ghana, and is honorary fellow of the West African College of Surgeons and the Ghana College of Surgeons. Doctor Johnson received the Distinguished Service Award, the highest honor of the American College of Obstetricians and Gynecologists

(ACOG), Distinguished Merit Award, highest honor of the International Federation of Gynecology and Obstetrics (FIGO), Fellowship ad eundem of the Royal College of Obstetricians and Gynaecologists (London) and membership in the Society of Scholars of the Johns Hopkins University. He is Past President of the Association of Professors of Gynecology and Obstetrics, receiving their Lifetime Achievement Award in 2012, and Editor-Emeritus of the International Journal of Gynecology and Obstetrics (official journal of FIGO) serving as Editor from 2007-2014. Doctor Johnson is an elected member of the National Academy of Medicine of the National Academy of Science. His research interests are fetal behavior and assessment, principles of prenatal care, global health ethics, and international academic faculty development and capacity building.

Carl H. June, MD

Dr. June is the Richard W. Vague Professor in Immunotherapy in the Department of Pathology and Laboratory Medicine. He is currently Director of the Center for Cellular Immunotherapies at the Perelman School of Medicine, and Director of the Parker Institute for Cancer Immunotherapy at the University of Pennsylvania. He is a graduate of the Naval Academy in Annapolis, and Baylor College of Medicine in Houston, 1979. He had graduate training in Immunology and malaria with Dr. Paul-Henri Lambert at the World Health Organization, Geneva, Switzerland from 1978-79, and post-doctoral training in transplantation biology with E. Donnell Thomas and John Hansen at the Fred Hutchinson Cancer Research Center in Seattle from 1983 - 1986. He is board certified in Internal Medicine and Medical Oncology. He maintains a research laboratory that studies various mechanisms of lymphocyte activation that relate to immune tolerance and adoptive immunotherapy for cancer and chronic infection. In 2011, his research team published findings detailing a new therapy in which patients with refractory and relapsed chronic lymphocytic leukemia were treated with genetically engineered versions of their own T cells. The treatment has also now also been used with promising results to treat children with refractory acute lymphoblastic leukemia. He has published more than 350 manuscripts and is the recipient of numerous prizes and honors. He was elected to the ASCI in 1992, the AAP in 2006, the Institute of Medicine in 2012, and the American Academy of Arts and Sciences in 2014. He is recipient of the William B Coley award, the Richard V. Smalley Memorial Award from the Society for Immunotherapy of Cancer, the AACR-CRI Lloyd J. Old Award in Cancer Immunology, the Philadelphia Award in 2012, the Taubman Prize for Excellence in Translational Medical Science in 2014 (shared with S. Grupp, B. Levine, D. Porter), the Paul Ehrlich and Ludwig Darmstaedter Prize (shared with J. Allison), the Novartis Prize in Immunology (shared with Z. Eshaar and S. Rosenberg), the Karl Landsteiner Memorial award, the Debrecen Award and a lifetime achievement award from the Leukemia and Lymphoma Society.

C. Ronald Kahn, MD

Dr. Kahn is a world recognized expert in diabetes and obesity research, as well as a preeminent investigator in the area of insulin signal transduction and mechanisms of altered signaling in diabetes and metabolic disease. Dr. Kahn is Senior Investigator, Head of the Section on Integrative Physiology and Metabolism at Joslin Diabetes Center and the Mary K. Iacocca Professor of Medicine at Harvard Medical School. Dr. Kahn served as Research Director of the Joslin Diabetes Center from 1981 to 2000, and served as President of Joslin from 2001 to 2007. He is currently the Center's Chief Academic Officer.

Sandra Lemmon, PhD

Dr. Sandra K. Lemmon received her BA from the Univ. of Rochester and her PhD from Washington Univ., St. Louis. She did postdoctoral training at Carnegie Mellon Univ. (1983-1985) and continued at CMU as Research Assistant Professor (1985-1988). From 1988-2003 Dr. Lemmon was a faculty member in the Dept. of Molecular Biology & Microbiology at Case Western Reserve University (CWRU). In 2003 she was recruited to the Univ. of Miami Miller School of Medicine (UMMSM), where she is currently Professor of Molecular & Cellular Pharmacology. She served on several NSF and NIH grant review panels and has been on the editorial board of Molecular Biology of the Cell since 2006. She has been federally funded for over 30 years. Dr. Lemmon is a cell biologist whose research focuses on the molecular mechanisms of membrane traffic. Dr. Lemmon has been involved in MD/PhD training since 1994. While at CWRU Dr Lemmon was on the Steering Committee of the MSTP, and served as Associate Director from 1999-2003. When she moved to the University of Miami in 2003, she joined the MD/PhD Program Committee and was appointed MD/PhD Program Director in 2006. Under her leadership, in 2017 the UMMSM MD/PhD program was awarded a MSTP T32 training grant from the NIH. She has been on the program organizing committee for several meetings of the AAMC Group on Graduate Education and Training (GREAT) and MD/PhD Section of GREAT. She has been a member of the MD/PhD GREAT Section Communications Committee since 2014. Dr. Lemmon has also been a member of the Steering Committee of the MD/PhD Section of the GREAT Group since 2014, and is currently serving as Chair (2018-2019).

SPEAKER **BIOGRAPHIES**

Douglas R. Lowy, MD

Dr. Lowy has been the Deputy Director of the NIH National Cancer Institute since 2010. Dr. Lowy served as NCI's acting director from 2015-2017 and has been helping lead NCI's key scientific initiatives as deputy director since 2010. His more than 40 year-track record of cancer research excellence has earned him the National Medal of Technology and Innovation from President Barack Obama in 2014 for his work that led to the development of the human papillomavirus vaccine and, in turn, left a tremendous positive impact on population health.

Dr. Lowy received his medical degree from New York University School of Medicine. He then trained in internal medicine at Stanford University and dermatology at Yale University. As chief of the Laboratory of Cellular Oncology in the Center for Cancer Research at the NCI, Lowy's research includes the biology of papillomaviruses and the regulation of normal and neoplastic growth. His laboratory, in close collaboration with Dr. John T. Schiller, was involved in the development, characterization, and clinical testing of the preventive virus-like particle-based HPV vaccines that are now used in the three FDA-approved HPV vaccines. Dr. Lowy is a member of the National Academy of Sciences and the National Academy of Medicine. For their pioneering work, Lowy and Schiller received numerous honors in addition to the National Medal, including the 2017 Lasker~DeBakey Clinical Medical Research Award. Today, Dr. Lowy will give his talk titled, "Preventing HPV-Associated Cancers by Vaccination.

Kieren A. Marr, MD, MBA

Dr. Marr is a Professor of Medicine and Oncology and serves as the Director of the Transplant and Oncology Infectious Diseases Program at Johns Hopkins. She serves as Vice Chair of Medicine for Innovation in Healthcare Implementation. The overarching theme of Dr. Marr's research program is to reduce infectious morbidity in medically immunosuppressed hosts, by using a bi-directional translational approach, integrating laboratory and clinical trials to develop and optimize diagnostic tools and prevention strategies. Studies have focused specifically on pulmonary fungal infections that have significant morbidity. Her laboratory made pivotal observations describing innate risks for pulmonary fungal infections in transplant patients and mechanisms of host-pathogen interaction, advancing personalized strategies to prevent transplant–associated infection. Her group discovered a new fungal pathogen now recognized as a widely distributed drug-resistant organism (Aspergillus lentulus). Current laboratory efforts are focused on optimizing diagnostics, including immunologic methods to detect both active and latent infections. Her work was instrumental in optimizing current commercially available tests, and new technology has led to establishment of a start-up company focused on developing tests and drugs to prevent fungal infections. Altogether, Dr. Marr's efforts have advanced our understandings of pulmonary fungal infections and have resulted in important diagnostic tools and drugs used in practice today. In 2015, she was named by Thomson Reuters as one of the "World's Most Influential Scientific Minds", based on 11-year citation data.

Donna M. Martin, MD, PhD

Dr. Martin is Interim Chair, Department of Pediatrics and the Donita B. Sullivan, MD Research Professor of Pediatrics. She also holds a secondary appointment as Professor of Human Genetics. She is a graduate of Michigan Technological University (BS in Mathematics and Foreign Languages) and received her PhD in Neuroscience (1992) and MD (1996) from the University of Michigan. She completed internship and residency training in Pediatrics (1999) and Medical Genetics (2001) at Mott Children's Hospital in Ann Arbor, and served as Associate Director of the Medical Scientist Training Program from 2012 through 2018. Her research focus is on developmental disorders of the nervous system. Her laboratory has contributed to the understanding of roles for the ATP dependent chromatin remodeler, CHD7, in the pathogenesis of CHARGE Syndrome, the most common monogenic cause of deaf-blindness. Dr. Martin is a member of the Cellular & Molecular Biology Graduate Program and the Neuroscience Graduate Program. She is an Attending Physician in the Division of Medical Genetics, Metabolism, and Genomic Medicine in the Department of Pediatrics, where she cares for children and adults with a wide variety of developmental, metabolic, and genetic disorders on the inpatient and outpatient services, as well as providing outreach clinical care twice yearly in Marquette and Traverse City, Michigan. She also teaches in the third year medical school pediatrics curriculum, and mentors residents and genetics fellows in outpatient clinics and on the inpatient consultation service. Dr. Martin has trained 5 PhD students (including one MSTP student) and 8 postdoctoral fellows. Dr. Martin is a former member of the NIH Developmental Brain Disorders Study Section (2009-2015). She is a Taubman Scholar and serves on the Taubman Medical Institute Scientific Advisory Board. Nationally, she serves on the council of the American Society for Clinical Investigation (2018-2011) and the board of the American Society of Human Genetics (2018-2011).

SPEAKER **BIOGRAPHIES**

Elizabeth Miller , MD

Dr. Elizabeth Miller is Professor of Pediatrics, Public Health, and Clinical and Translational Science at the University of Pittsburgh School of Medicine, Director of Adolescent and Young Adult Medicine, and director of community and population health at UPMC Children's Hospital Pittsburgh. She holds the Edmund R. McCluskey Endowed Chair in Pediatric Medical Education. Trained in Internal Medicine and Pediatrics and medical anthropology, she has over 15 years of practice and research experience in addressing gender-based violence among adolescents and young adults in clinical and community settings. She is also involved in developing and testing primary violence prevention programs, including one titled "Coaching Boys into Men" which involves training coaches to talk to their male athletes about stopping violence against women.

Deepak Nijhawan, MD, PhD

Dr. Nijhawan is currently Assistant Professor in the Departments of Biochemistry and Hematology and Oncology at UT Southwestern Medical Center, where he joined the faculty in 2012. He received his MD/PhD in 2005 from UT Southwestern, followed by an internship and residency in internal medicine at MGH (2007) and a fellowship in medical oncology at the Dana-Farber Cancer Institute (2011). His work has been supported in part by the Sass Foundation for Medical Research, Damon Runyon Cancer Research Foundation, Harrington Discovery Institute, and the National Cancer Institute. He was elected to the ASCI in 2019 and is co-recipient of the the ASCI's 2018 Seldin-Smith Award for Pioneering Research. Dr. Nijhawan focuses on identifying targets for cancer treatment. In particular, his laboratory has investigated indisulam and CD437, identified as anticancer agents in the 1990s but not subsequently developed because their targets were poorly understood. In 2016, Dr. Nijhawan's laboratory identified the target of CD437, followed in 2017 by identification of the target of indisulam and of cancer-cell variants most susceptible to its effects. This has created new interest in these agents, which are now in active development in collaboration with Dr. Nijhawan's team.

Deng Pan

Deng Pan is an MD/PhD student from Washington University in St. Louis. He currently works in the lab of Dr. Susan Mackinnon, studying peripheral nerve regeneration. He has previous worked in R&D at AstraZeneca and Perosphere Inc. He earned his B.S. in Biomedical Engineering from Johns Hopkins University. While he was a student there, he worked under Prof. Hai-Quan Mao to investigate strategies to improve drug delivery. He is born in Zhejiang, China.

Aimee S. Payne, MD, PhD

Dr. Payne is the Albert M. Kligman Associate Professor of Dermatology, Associate Director of the Medical Scientist Training Program, and Core Director of the Skin Biology and Diseases Resource-based Center at the University of Pennsylvania. Dr. Payne received her BS in Biology from Stanford University, her MD and PhD in Molecular and Cellular Biology from Washington University School of Medicine, and her dermatology training at the University of Pennsylvania. Her laboratory has focused on B cell repertoire cloning from patients with the autoimmune disease pemphigus vulgaris to better understand how autoreactivity develops and to develop better targeted therapies for disease. Recently, her laboratory developed a novel genetically engineered cellular immunotherapy for autoimmune disease treatment known as chimeric autoantibody receptor T cell (CAART) therapy, which she is currently advancing toward clinical trials in pemphigus to assess its safety and curative potential. Dr. Payne's work has been recognized with the Charles and Daneen Stiefel Scholar Award in Autoimmune Diseases, the Top 10 Clinical Research Forum Award, and election to the American Society for Clinical Investigation.

Claire Pomeroy, MD, MBA

Dr. Pomeroy is president and CEO of the Albert and Mary Lasker Foundation which is dedicated to accelerating support for medical research. She currently serves on the Board of Trustees for the Morehouse School of Medicine and the Board of Directors for Sierra Health Foundation; Science Philanthropy Alliance; Foundation for Biomedical Research; iBiology, Inc.; New York Academy of Medicine; New York Blood Center, Inc.; and Becton Dickinson.

Kenneth Ramos, MD, PhD

Dr. Kenneth S. Ramos is an accomplished physician-scientist and transformational leader, designated as an associate of the National Academy of Sciences and elected to the National Academy of Medicine. Dr. Ramos obtained his medical degree from the University of Louisville Health Sciences Center and his Ph.D. from the University of Texas at Austin in Biochemical Pharmacology. He has vast depth of experience across the tripartite mission areas of education, research and clinical service, and he is recognized throughout the world for his scientific contributions in the areas of genomics, precision medicine and toxicology. Dr. Ramos served as the MD-PhD director for the University of Arizona College of Medicine from 2014 – 2019 where he mentored students interested in careers as research-intensive physicians. Recently, Dr. Ramos joined Texas A&M University Health Science Center in Houston where he serves as the assistant vice chancellor for health services and executive director of the Institute of Biosciences and Technology. He is the Margaret M. Alkek Chair in Medical Genetics and a distinguished Governors University Research Initiative investigator.

SPEAKER **BIOGRAPHIES**

Stanley R. Riddell, MD

Dr. Riddell is a Member in the Program in Immunology and Director of the Immunotherapy Integrated Research Center at the Fred Hutchinson Cancer Research Center, and Professor in the Department of Medicine at the University of Washington. His research focuses on understanding T cell immunity to pathogens and tumors, and on the development and clinical application of adoptive T cell therapy for cancer by genetically modifying T cells to instruct them to recognize tumor cells.

Paul M. Ridker, MD, MPH

Dr. Ridker has been collaboratively responsible for elucidating the critical role of inflammation in the detection, prevention, and treatment of cardiovascular diseases for the past 25 years. Best known for his pioneering population biology work on inflammatory biomarkers such as high-sensitivity CRP and interleukin-6, the first demonstrations of the anti-inflammatory effects of statins, the guideline changing JUPITER trial in 2008, and ultimately through the CANTOS interleukin-1 inhibition trial in 2017, Dr. Ridker's work has led to a fundamental shift in our understanding of atherosclerosis and to the first proof that targeted anti-cytokine therapies can lower cardiovascular event rates in the absence of lipid lowering. Insights from his group that the magnitude of inflammation inhibition directly relates to the magnitude of clinical benefit has spawned a novel class of cardiovascular therapeutics, led to the clinical recognition that ""residual inflammatory risk"" is a separate and distinct entity from ""residual cholesterol risk"", and opened an entirely novel approach to the treatment of inflammatory lung cancers. Spanning the fields of epidemiology, vascular biology, population genetics, public health, preventive medicine, and clinical trials, Dr. Ridker's career-long focus on inflammatory mechanisms of disease has advanced a controversial concept into a proven clinical intervention. Few clinical investigators have had as much translational influence at the bench, the bedside, and on guidelines for the prevention and treatment of cardiovascular disease. While Dr. Ridker's research efforts are primarily supported by RO1 grants from the National Institutes of Health, he has received additional career support from the Doris Duke Charitable Foundation, the Leducq Foundation, the Donald W Reynolds Foundation, and the American Heart Association from whom he has been the recipient of a Clinician Scientist Award (1992-197), an Established Investigator Award (1997-2002), and a Distinguished Scientist Award (2013). His work on inflammation, CRP, and atherothrombosis garnered recognition by Time magazine as one of America's Ten Best Researchers in Science and Medicine (2001) and selection to the "Time 100" (2004). Dr. Ridker has additionally been the Trial Chairman of several multinational trials funding by the NHLBI or industry including PREVENT, PRINCE, Val-MARC, LANCET, JUPITER, SPIRE-1, SPRE-2, CANTOS, CIRT, and PROMINENT. Dr. Ridker has served on multiple federal scientific review panels for United States Food and Drug Administration and the National Heart Lung and Blood Institute, including a 10-year term on the Board of External Experts. The recipient of several honorary degrees, Dr. Ridker was selected in 2018 to deliver the Distinguished Scientist Lecture at the international American Heart Association meetings. Dr. Ridker is the author of over 800 original manuscripts (h-index > 200) related to cardiovascular medicine and is listed as a co-inventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in the diagnosis and treatment of cardiovascular disease.

Steven M. Rowe, MD, MSPH

Dr. Rowe is a pioneer in the field of personalized therapeutics for cystic fibrosis (CF), cutting-edge discovery in airway disease biology, and translational research in COPD. He is an international authority in the design and conduct of clinical trials targeting the basic CF defect, and has made key advances in the measurement and interpretation of CFTR function in humans and animals. Dr. Rowe has characterized that COPD patients with chronic bronchitis exhibit 'acquired CFTR dysfunction' through a pathway that causes delayed mucociliary clearance and confers chronic bronchitis. To complement this, Dr. Rowe developed the first animal model that exhibits chronic bronchitis using cigarette smoke exposed ferrets. Dr. Rowe co-invented one-micron resolution optical coherence tomography (Micro-OCT) that captures 3D imaging in real-time at the cellular level, and with his collaborators is the first to bring this technique in vivo in humans. Micro-OCT imaging is highly sensitive to the epithelial function of airway tissues and can provide simultaneous and non-invasive measurements of the functional microanatomy of the airway surface. Dr. Rowe is a Professor with tenure in the Departments of Medicine, Pediatrics, and Cell Developmental and Integrative Biology at the University of Alabama at Birmingham (UAB). He is the Director of the Gregory Fleming Cystic Fibrosis Research Center at UAB, which involves over 100 faculty members and has been continuously funded for over 25 years. Dr. Rowe is board certified in Internal Medicine, Pediatrics, Pulmonary Medicine and Critical Care Medicine and serves as a Special Consultant for Translational Science for the Cystic Fibrosis Foundation. He received his M.D. degree from Vanderbilt University, and Residency and Fellowship training at UAB, followed by his Master's Science degree in Public Health (Clinical Research), also at UAB. He presently has a laboratory of over 25 individuals, embracing lung research from basic discovery, to translational science, to clinical application.

SPEAKER **BIOGRAPHIES**

Vijay G. Sankaran, MD, PhD

Dr. Sankaran earned his MD and PhD degrees from Harvard Medical School. He subsequently did a residency in pediatrics at Boston Children's Hospital and Boston Medical Center, followed by a fellowship in pediatric hematology/oncology at Boston Children's Hospital and the Dana-Farber Cancer Institute. He has been an Assistant Professor of Pediatrics at Harvard Medical School since 2014 and an Attending Physician in Hematology/Oncology since 2015 at Boston Children's Hospital and the Dana-Farber Cancer Institute. His research has been supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Heart, Lung, and Blood Institute. He was elected to the ASCI in 2018 and is the recipient of the ASCI's 2019 Seldin-Smith Award for Pioneering Research. Dr. Sankaran's research aims to understand blood cell production in health and disease. His work is focused on genetic variation that impacts this process of blood cell production. Of particular interest is how stem cells produce blood cells, how the hemoglobin genes are regulated during red blood cell production, and how disease alters these processes. From these insights, Dr. Sankaran hopes to develop improved therapies for blood disorders such as sickle cell disease, thalassemia, Diamond-Blackfan anemia, aplastic anemia, myelodysplastic syndromes, myeloproliferative disorders, and childhood leukemia.

Dorry Segev, MD, PhD

Dr. Segev is the Marjory K. and Thomas Pozefsky Professor of Surgery and Epidemiology and Associate Vice Chair of Surgery at Johns Hopkins University. With a graduate degree in biostatistics, he focuses on novel statistical and mathematical methods for simulation of medical data, analysis of large healthcare datasets, and outcomes research. Dr. Segev was the first to demonstrate the survival benefit of incompatible kidney transplantation, the first to estimate attributable risk of ESRD in live kidney donors, and is responsible for the first HIV-to-HIV transplants in the United States. His NIH-funded research includes kidney exchange, desensitization, long-term donor risk, access to transplantation, expanding transplantation including HIV+ donors, geographic disparities, and the intersection between transplantation and gerontology. Dr. Segev received the American Society of Transplantation's Clinical Science Investigator Award. He is a councilor of the American Society of Transplant Surgeons and former chair of the American Transplant Congress. His work has directly influenced policy, including two Congressional bills (the Norwood Act for kidney exchange and the HOPE Act for HIV-to-HIV transplants). Dr. Segev is most inspired by his role as a mentor, having mentored over 100 students, residents, and faculty, and is the only general surgeon in the US funded by an NIH/NIDDK Mentoring Grant.

Sara C. Shalin, MD, PhD

Dr. Sara C. Shalin is an Associate Professor in the Departments of Pathology and Dermatology at the University of Arkansas for Medical Sciences. She serves as the director of the UAMS MD/PhD program and as associate program director of the Dermatopathology fellowship. Dr. Shalin graduated from a combined M.D./Ph.D. program at Baylor College of Medicine in Houston, TX in 2007, with a PhD in neuroscience. She remained in Houston at Baylor College of Medicine where she completed a residency in anatomic and clinical pathology and served as chief resident in 2010-2011. She then completed her dermatopathology fellowship training in Boston at the Harvard Hospitals Combined Dermatopathology program. Dr. Shalin was recruited to UAMS in 2012. Her involvement in the MD/PhD program at UAMS led to her appointment as the program director in 2017, which has turned into one of her most meaningful positions to date. Her academic position is predominantly clinical, but she maintains an active involvement in translational research projects and research collaborations in melanoma pathogenesis and biology and other cutaneous malignancies. Dr. Shalin's daily life is a mix of diagnosing skin disease, teaching and mentoring residents, medical students, and graduate students, and managing operations in the hospital anatomic pathology lab.

Susan Smyth, MD, PhD

Dr. Susan S. Smyth is the Jeff Gill Professor of Cardiology, Chief of the Division of Cardiovascular Medicine, Director of the Linda and Jack Gill Heart and Vascular Institute, and Director of the MD/PhD Program at the University of Kentucky. She also has a part-time appointment as a cardiologist and funded investigator at the Lexington VA Medical Center.

Smyth is a physician scientist who combines clinical practice in cardiology with NIH- , VA-, and industry-funded research focused on the interplay between inflammation and thrombosis in vascular biology. She has authored more than 200 publications and contributed to over a dozen textbooks. Smyth received her A.B. in Biology, summa cum laude, from Mount Holyoke College (South Hadley, Massachusetts), and graduated from the MD/PhD Program at the University of North Carolina (Chapel Hill). After completing training in Internal Medicine, she performed cardiology subspecialty fellowship training at the Mount Sinai School of Medicine (New York, New York) and at the University of North Carolina. She is a member of the American Society of Clinical Investigation, on the council for the Association of University Cardiologists, on the steering committee of the Board of Governors for the American College of Cardiology, and the steering committee for the National Center for Advancing Translational Science CTSA program.

SPEAKER **BIOGRAPHIES**

Richard Steinman, MD, PhD

Dr. Richard Steinman is Associate Professor of Medicine and Pharmacology at the University of Pittsburgh School of Medicine. He completed his undergraduate degree at Haverford College then went on to complete his MD-PhD training at University of Pennsylvania. Dr. Steinman has a longstanding interest in the education of physician scientists, directing the University of Pittsburgh's Physician Scientist Incubator and serving as Director of Pitt's Medical Scientist Training Program since 2012. He has also directed the University of Pittsburgh Physician Scientist Training Program since 2008. Diversity in the physician scientist workforce is another interest; he has initiated and directed a multi-year NIH supported collaborative education and training program between the University of Pittsburgh Cancer Institute and Hampton University, a minority-serving institution. Dr. Steinman's dedication to mentorship and education have earned him several awards including the University of Pittsburgh Chancellor's Distinguished Teaching Award, AAMC Award for Innovations in Research Training and Education, Fraley Award for Mentoring, and the Philip Troen MD Excellence Mentoring Award. Dr. Steinman's laboratory studies the cancer microenvironment with a focus on the molecular and functional interactions between cancer cells, fibroblasts and platelets. He also studies tumor dormancy, modeling factors in host stromal cells that could contribute to breast cancer recurrence and conversion to estrogen receptor negativity in bone.

Kim Templeton, MD

Dr. Kim Templeton is Professor of orthopaedic surgery at the University of Kansas Medical Center in Kansas City, specializing in orthopaedic oncology. Dr. Templeton's research interests include women's health, medical education, and long-term impact of treatment of pediatric sarcomas on bone health. In 2017, Dr. Templeton was elected to a second term on the National Board of Medical Examiners, after spending several years on various committees and task forces, and is now leading part of the research arm of the RENEW task force, to address stress among medical students related to the USMLE exams. Dr. Templeton is a past-president of the American Medical Women's Association. She has also served on the executive committee and chaired the Sex and Gender Women's Health Collaborative, whose mission is to improve the translation of research into sex- and gender-based differences into clinical practice through education and evaluation. Dr. Templeton is an invited founding board member of the Academy of Women's Health. In 2013, Dr. Templeton was named by the National Academy of Sciences to the musculoskeletal work group, reviewing and recommending new venues for sex and gender research for the National Aeronautic and Space Administration (NASA). Dr. Templeton has spoken at venues around the country in the area of sex and gender medicine. She has and continues to serve on expert committees that are working to incorporate this information into health professionals' education.

Lauren Walter, MD

Dr. Lauren Walter graduated from the University of Michigan Medical School in 2005. She completed her residency training in Emergency Medicine in 2009, subsequently staying on as faculty at the University of Alabama at Birmingham (UAB) where she is currently an Associate Professor and Assistant Residency Program Director. Dr. Walter's research interests include the development and integration of sex and gender-based medical education curriculum for both UME and GME. She has created and implemented novel SGBM didactics for medical students, residents, and faculty and in addition, she has lectured regionally and nationally on this topic. Dr. Walter is on the board of the Sex and Gender Health Collaborative, a national, interdisciplinary group aimed at increasing SGBM awareness and integration into medication education. In addition, Dr. Walter is a national research collaborator with the Society for Academic Emergency Medicine's (SAEM) Sex and Gender in Emergency Medicine Interest Group and is on the executive planning team for the upcoming 2020 Sex and Gender in Health Education Summit.

Arthur Weiss, MD, PhD

Dr. Weiss is the Ephraim P. Engleman Distinguished Professor of Rheumatology in the Department of Medicine at the University of California, San Francisco, and has been on the faculty there since 1985. He served as Division Chief of Rheumatology at UCSF from 1988-2011. He has been an Investigator of the Howard Hughes Medical Institute since 1985.

A graduate of Johns Hopkins University (1973), Dr. Weiss received his MD (1979) and PhD (1978) degrees at the University of Chicago. He did his graduate training in the lab of Dr. Frank Fitch where he studied transplantation immunology. Dr. Weiss did his internship and residency in internal medicine at UCSF. During his subspecialty training in rheumatology he worked in the laboratory of Dr. John Stobo where he began his work on characterizing the T cell receptor and its mechanism of signaling transduction.

Dr. Weiss is a leading researcher in the field of signal transduction in the immune system, focusing on the roles of tyrosine kinases and phosphatases in regulating lymphocyte activation. He has studied how abnormalities in tyrosine phosphorylation pathways lead to immunologically-mediated diseases.

SPEAKER **BIOGRAPHIES**

Dr. Weiss is a member of the National Academy of Sciences, the National Academy of Medicine, the American Academy of Arts and Sciences and an associate member of EMBO. He was elected to the ASCI in 1988 and the AAP in 1994. He is a recipient of the Distinguished Investigator Award of the ACR, Arthritis Foundation's Lee C. Howley Prize, and the American Association of Immunologists Meritorious Career Award.

Dr. Weiss a co-founder of Nurix, Inc. and is on the Scientific Advisory/ Review Boards of Five Prime Therapeutics, Genentech, and Portola Pharmaceuticals.

Gary D. Wu, MD

Dr. Wu is the Ferdinand G. Weisbrod Professor in Gastroenterology at the University of Pennsylvania's Perelman School of Medicine where he is the GI Associate Chief for Research, the Associate Director of the Center for Molecular Studies in Digestive and Liver Disease, the Co-Director of the PennCHOP Microbiome Program, and the Director of the Penn Center for Nutritional Sciences and Medicine. With respect to the latter, he is an advisor to NIH, the National Academy of Sciences, and the USDA on topics related to human health, diet, and nutrition. An elected member of both the American Society for Clinical Investigation and the Association of American Physicians, Dr. Wu was the inaugural Director and Chair of the Scientific Advisory Board for the AGA's Center for Gut Microbiome Research and Education and currently serves as member of the AGA's Governing Board as the Basic Research Councilor. Research programs in the Wu laboratory focus on the mutualistic interactions between the gut microbiota and its host with a particular emphasis on metabolism including nitrogen balance, intestinal oxygen regulation, and epithelial intermediary metabolism. As a physician-scientist, he has gained international recognition for his highly innovative multidisciplinary team research approach to translational avenues of investigation that help to guide the development of therapeutic strategies relevant to IBD and metabolic diseases.

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Call for Nomination for the George M. Kober Medal and Lecture

This is a Call for Nomination for the George M. Kober Medal Recipient and George M. Kober Lecture for 2021.

He was active in the early days as a leader of several national organizations including the Association of American Physicians – an early organization founded in the 1885 by seven Physicians (including William Osler) an organization which promotes:

> *"the pursuit of medical knowledge, and the advancement through experimentation and discovery of basic and clinical science and their application to clinical medicine..."*

Please provide a brief cover letter highlighting the major accomplishments of the nominee along with an updated CV and submit by December 1, 2019 to Lori Ennis: admin@aap-online.org

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George M. Kober Lecture

The Association of American Physicians honors Kober and continues to honor him by giving their highest award to an honoree(s) every three years to present the Kober Lecture. This award is given to an AAP member for outstanding research contributions which have extraordinary impact on patients. Among the list of previous speakers, include at least 13 Nobel laureates to date.

To view a list of past recipients go to: http://aap-online.org/kober/

ORAL PRESENTATIONS **& POSTER ABSTRACTS**

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Joint Meeting Oral Presentation
T cells promote peripheral nerve regeneration via regulation of IL-4
Deng Pan

T cells promote peripheral nerve regeneration via regulation of IL-4

Deng Pan, Dan Hunter, Lauren Schellhardt, Sally Jo, Alex Halevi, Katherine Santosa, Anja Fuch, Alison Snyder-Warwick, Susan Mackinnon, Matthew Wood

Department of Surgery, Washington University in St. Louis

Peripheral nerve injury remains a significant public health issue. Traumatic nerve injuries often necessitate surgical repair with nerve grafts. While autologous nerve grafts are the clinical standard, acellular nerve allograft (ANAs) have been increasingly used. ANAs are prepared from nerve obtained from deceased donors then treated with detergents to remove cellular debris and antigenic components. While it has the advantage of being available off-the-shelf, its ability to promote axon regeneration, especially across a long nerve gap, is limited. In this study, we evaluate why nerve regeneration across long nerve gap is limited.

For both rats and mice, we utilized a sciatic nerve transection with ANA graft as model of nerve repair. Two cm (short) and 4 cm (long) ANAs were used. Grafts were analyzed after 4 and 8 weeks in vivo with histology, gene expression, histomorphometry. For mice, grafts were analyzed after 2 or 4 weeks. One cm grafts were used in the mice for repair.

We found that at 8 weeks, rats that were repaired using 2 cm (short) ANAs regenerated significantly more axons than those that received 4 cm (long) ANAs. Interestingly, T cells within long ANAs were significantly fewer than those in short ANAs, angiogenesis was also reduced. To test if T cells impacts regeneration, we utilized RNU rats, which are T cell deficient. Eight weeks after nerve repair using short ANAs, we found that the $RNU^{+/-}$ rats (T cell sufficient) had significantly more regenerated axons than the RNU-/- rats. Similar results in mice deficient in T cells (Rag1-/-) were observed compared to wildtype (WT) control. We also observed reduced Schwann cell accumulation and blood vessels in the Rag1-/- mice compared to WT, with no significant alterations to macrophages. T cell related cytokines, including IL-4 were significantly down-regulated in ANAs from Rag1-/- mice compared to WT. Using IL-4GFP mice, in which cells secreting IL-4 also express GFP, we found that appearance of GFP+ cells on day 14 post surgery correlates with increase in T cells. However, majority of IL-4+ cells were eosinophils (SiglecF+) rather than T cells, suggesting T cell's role in regulating rather than secreting IL-4. Finally, utilizing an IL-4 knockout mice, we found that loss of IL-4 resulted in reduced myelinated axons, as well as reduced angiogenesis. As therapeutic strategy, we demonstrate that IL-4 incorporated within ANAs can be sustained for release for more than 7 days. Incorporation of IL-4 within ANAs resulted in increased angiogenesis and nerve regeneration.

In conclusion, our study uncovered the critical importance of T cells in promoting nerve regeneration. We showed that T cells regulate IL-4 via recruitment of eosinophils, and that loss of IL-4 impact regeneration due to loss of angiogenesis.

Joint Meeting Oral Presentation
Skeletal muscle Krüppel-like factor 15 and PPARδ cooperate to regulate skeletal muscle lipid metabolism
Liyan Fan

Skeletal muscle Krüppel-like factor 15 and PPARδ cooperate to regulate skeletal muscle lipid metabolism

Liyan Fan[1,2], Domenick A. Prosdocimo[1], Mukesh K. Jain[2]

[1]Department of Pathology, Case Western Reserve University, Cleveland, OH, USA; [2]Cardiovascular Research Institute, Case Western Reserve University, and Harrington Heart and Vascular Institute, University Hospitals Cleveland Medical Center, Cleveland, OH, USA

Skeletal muscle metabolism significantly modulates systemic metabolic status through its role in lipid metabolism; unsurprisingly, aberrant skeletal muscle nutrient handling is closely associated with metabolic diseases (e.g. obesity and type II diabetes). However, the mechanisms underlying skeletal muscle regulation of lipid metabolism remain to be fully elucidated. Previous studies have shown that mice deficient in peroxisome proliferator-activated receptor δ (PPARδ), a nuclear receptor, suffer from deranged skeletal muscle lipid handling; a similar phenotype is observed in animals that are deficient in the transcription factor Krüppel-like factor 15 (KLF15). Preliminary data and published studies support the existence of KLF15-PPARδ cooperativity in regulating skeletal muscle lipid metabolism, and thus we sought to 1) define the role of skeletal muscle specific KLF15 in lipid metabolism; and 2) elucidate the molecular basis of KLF15-PPARδ interaction and impact on skeletal muscle lipid metabolism.

We generated a skeletal muscle specific KLF15 knockout (K15-SKO) mouse and characterized the metabolic phenotype of this animal. K15-SKO mice had increased body weight and fat mass, elevated circulating free-fatty acids and triglyceride levels, insulin insensitivity, and glucose intolerance compared to controls. Importantly, K15-SKO mice demonstrated decreased skeletal muscle expression of a number of lipid flux genes, many of which are targets of PPARd, indicating impaired lipid flux.

To determine the necessity of KLF15 for PPARδ-mediated gene expression, we depleted KLF15 in C2C12 cells, a myoblast cell line, and looked at a number of PPARδ targets in the presence and absence of GW501516, a PPARδ agonist. The expression levels of *Fatp1, Cpt1b, and Scl25a20* were attenuated – this response was unchanged in the presence or absence of GW501516. We used Seahorse cell metabolism analyzer to assess fatty acid oxidation in the same cell culture model and observed that knockdown of KLF15 reduces GW501516 induction of palmitate oxygen consumption rates. To further assess the functional cooperativity of KLF15 and PPARδ, we conducted co-transfection studies and determined that KLF15 and PPARδ act synergistically on the *Fatp1* promoter. Co-immunoprecipitation studies confirmed physical interaction between KLF15 and PPARδ. Finally, control and K15-SKO mice were gavaged with GW501516 for 10 days, and concordant with in vitro results, K15-SKO animals demonstrated attenuated induction of a number of PPARδ targets in skeletal muscle. Taken together, these data suggest that skeletal muscle specific KLF15 is critical in the regulation of skeletal muscle lipid handling, and KLF15 is necessary for optimal PPARδ-mediated regulation of skeletal muscle lipid metabolism.

1 Exploring post-zygotic genetic variants in obsessive-compulsive disorder
Sarah Abdallah

Exploring post-zygotic genetic variants in obsessive-compulsive disorder

Sarah Abdallah[1], Carolina Cappi[2], Emily Olfson[3,4], James Noonan[5], Thomas Fernandez[3,4]

[1]School of Medicine, [3]Child Study Center, [4]Department of Psychiatry, and [5]Department of Genetics, Yale University, New Haven, CT, USA, [2]Department of Psychiatry, School of Medicine, University of São Paulo, São Paulo, São Paulo, Brazil

Obsessive-compulsive disorder (OCD) is a debilitating neuropsychiatric disorder with an estimated prevalence of 1-3% worldwide. Current pharmacologic treatments are not completely effective in eliminating symptoms, providing great incentive to study the molecular basis of the disorder. Although OCD is known to be moderately heritable, its genetic etiology and resulting pathogenesis remain poorly understood, limiting development of novel treatments. We previously have demonstrated a significant contribution to OCD risk from likely damaging *de novo* germline DNA sequence variants, which arise spontaneously in the parental germ cells or zygote instead of being inherited from a parent, and we successfully have used these identified variants to implicate new OCD risk genes. Recent studies of autism spectrum disorder and intellectual disability suggest a risk contribution from post-zygotic variants (PZVs) arising *de novo* in multicellular stages of embryogenesis, suggesting these mosaic variants can be used to examine the genetic underpinnings of other neuropsychiatric disorders such as OCD.

We examined whole-exome sequencing (WES) data from peripheral blood of 184 OCD parent-proband trios and 777 control parent-child trios that passed quality control measures. We used the bioinformatics tool MosaicHunter to identify low–allele frequency, potentially mosaic single-nucleotide variants (SNVs) in probands and in control children, only considering variants with the alternate allele not present in parents and with frequency less than 0.05 in the Single Nucleotide Polymorphism Database. We discarded one OCD family with an excess of PZVs and additional variants deemed highly likely to be technical artifacts to reduce the number of false positive PZVs in our final dataset.

We found that the rate of all single-nucleotide PZVs per base pair is not significantly different between OCD probands (5.88×10^{-9}) and controls (5.80×10^{-9}), rate ratio = 1.01 (95% confidence interval = 0.654-1.53), one-sided p = 0.5. Of the putative PZVs identified in OCD probands, none are likely gene disrupting (LGD; alteration of a splice site or stop codon) and 27% are missense mutations predicted by the PolyPhen-2 tool to be probably damaging (Mis-D). The rate of putative damaging PZVs (LGD and Mis-D) also is not significantly different in OCD probands (1.57×10^{-9}) than in controls (1.39×10^{-9}), rate ratio = 1.13 (95% confidence interval = 0.444-2.56), one-sided p = 0.446. We observe no recurrence of PZVs in the same gene in unrelated probands. Finally, using the DAVID functional annotation tool, we found that the set of genes harboring putative damaging PZVs is enriched for genes encoding proteins that interact with magnesium, Benjamini-corrected p = 0.027. Although we did not detect a higher burden of PZVs in blood in individuals with OCD, further studies may benefit from examining a larger sample of families or from looking for PZVs in other tissues.

2 Pluripotent stem cell-based modeling of cigarette smoke injury to the human alveolar epithelium
Kristine M. Abo

Pluripotent stem cell-based modeling of cigarette smoke injury to the human alveolar epithelium

Kristine Abo, Anjali Jacob, Darrell Kotton, Andrew Wilson

Boston University School of Medicine, Boston, MA, USA

Smoking is the most important cause of chronic obstructive pulmonary disease (COPD), which encompasses chronic bronchitis and emphysema. Emphysema is characterized by distal airspace enlargement. Destruction of the alveolar epithelium results in increased lung compliance and decreased elastic recoil, thus generating an obstructive respiratory pattern. Cigarette smoke causes oxidative stress and provokes an inflammatory response, which results in increased protease activity and breakdown of the alveolar architecture. In spite of the clear implication of the alveolar epithelial response to smoke in the etiology of emphysema, there exists no model system of the human alveolar epithelium capable of recapitulating its response to cigarette smoke in a physiologically relevant manner.

Induced pluripotent stem cells represent a patient-specific, genetically tractable, and indefinitely renewable source of cells. Using a 35-day directed differentiation protocol developed by our group, we differentiated human induced pluripotent stem cells to type 2 alveolar epithelial-like cells (iAEC2s) in three-dimensional organoids known as alveolospheres. We then dissociated alveolospheres and re-plated single-cell iAEC2s in a cell culture insert system allowing for physiologically relevant air-liquid interface (ALI) culture. We characterized the cellular makeup of the ALI cultures compared to their parental alveolospheres by immunohistochemistry and RT-qPCR. Using a VitroCell VC1 smoke exposure robot, we exposed iAEC2 ALI cultures to whole, gas-phase cigarette smoke at multiple physiologically relevant, sub-cytotoxic doses. We assessed temporal induction and maintenance of epithelial integrity by trans-epithelial electrical resistance (TEER). We assessed transcriptional perturbations in known and novel smoke-perturbed genes by RT-qPCR, and compared iAEC2 ALI to primary human bronchial epithelial cells cultured at ALI in their transcriptional response to smoke.

iAEC2 ALI cultures generated from day 35 iPSC-derived alveolospheres could be maintained for at least 14 days. iAEC2s at ALI maintain transcript and protein-level expression of surfactant protein C (*SFTPC*), a specific type 2 alveolar epithelial cell marker, compared to their parental alveolospheres. iAEC2s at ALI also maintain mRNA expression of *NKX2-1*, a key lung lineage transcription factor. iAEC2s at ALI gain TEER, a quantitative measure of barrier function, over time, reaching a steady state of approximately $400\Omega.cm^2$ after five days of ALI culture. TEER was significantly reduced in smoke-exposed iAEC2 ALIs in a dose-responsive fashion. Interestingly, iAEC2s at ALI exhibit a unique transcriptional response to smoke compared to primary airway epithe-

lial cells exposed in the same system.

Overall, we were able to successfully develop an air-liquid interface culture protocol for human iPSC-derived type 2 alveolar epithelial cells, expose them to cigarette smoke in a physiologically relevant manner, and identify novel smoke-responsive transcriptional perturbations that are unique from airway epithelial smoke exposure responses.

3 Wolfram Syndrome 1 protein is a key regulator of β-cell function and viability

Damien Abreu

Wolfram Syndrome 1 protein is a key regulator of β-cell function and viability

Damien Abreu[1,3], Matthew Revilla[1], Zeno Lavagnino[1], Cris M. Brown[1], David W. Piston[1], Fumihiko Urano[1,2]

[1]Department of Medicine, Division of Endocrinology, Metabolism, and Lipid Research, Washington University School of Medicine, St. Louis, [2]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, [3]MD-PhD Program, Washington University in St Louis

Endoplasmic reticulum (ER) homeostasis is crucial for proper β-cell function and viability as evidenced by rare monogenic diabetic disorders caused by mutations in key ER molecules. Wolfram syndrome is one such disorder arising from mutation of the ER transmembrane protein, *Wolfram Syndrome 1 (WFS1)*. While many *WFS1* variants are associated with diabetes mellitus, the role of WFS1 in maintaining beta-cell viability and function remains unclear. Our central hypothesis is that WFS1 regulates beta-cell viability through downregulation of ER stress-responsive pro-apoptotic factors and promotes beta-cell function by maintaining beta-cell maturity. To test this hypothesis, we generated beta-cell models of inducible WFS1 knockdown and overexpression using rat insulinoma cell lines to monitor beta-cell function and beta-cell death. We also employed WFS1 knockout (KO) mouse models to assess islet morphometry in relation to the physiological progression of diabetic phenotypes. Interestingly, *WFS1*-KO islets have a reduced beta-cell mass and disrupted islet architecture at glucose intolerance onset, with an alpha-cell/beta-cell ratio of 0.53±0.02 compared to 0.27±0.01 in WT islets. Our *in vivo* and *in vitro* data confirm that beta-cells depleted of *WFS1* exhibit impaired insulin secretion and reduced insulin content. These phenotypes occur together with aberrant Ca^{2+} dynamics in response to glucose stimuli and increased beta-cell death. Conversely, increasing *WFS1* expression *in vitro* increases insulin production and expression of beta-cell maturity factors, while also conferring protection against ER stress-mediated beta-cell death. Our data suggest that WFS1 may preserve beta-cell viability by reducing the expression of the pro-apoptotic factors *CHOP* and *TRIB3*, thereby activating Akt. Future studies seek to clarify the mechanisms by which WFS1 protects beta-cells against metabolic stressors and promotes insulin production. These studies will expand our understanding of the broader mechanisms by which ER dysfunction triggers beta-cell pathology in more common forms of diabetes, and provide novel targets for intervention that center on preserving ER homeostasis.

4 Characterizing inflammatory stimulus encoding by NFκB signaling dynamics

Adewunmi Adelaja

Characterizing inflammatory stimulus encoding by NFκB signaling dynamics

A. Adelaja[1], B. Taylor[2], A. Hoffmann[1]

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Macrophages are the primary coordinators of the innate immune response. They recognize several classes of pathogens: viruses, bacteria, and parasites. Macrophages coordinate a pathogen-appropriate inflammatory response by inducing the activity of the key stimulus-responsive transcription factor, NFκB. Host-derived molecules, such as TNF, also induce NFκB activity. How macrophages distinguish between different NFκB stimuli is unknown.

Single cell studies have revealed that genetically-identical cells produce heterogenous signaling responses to identical stimuli. This challenges the notion that signaling dynamics constitute a signaling code that mediates stimulus-specific cellular responses. In other words, the mechanisms of NFκB signaling that underlie stimulus discrimination in macrophages are unknown.

To examine NFκB signaling with single-cell resolution in primary macrophages, we generated a RelA-Venus knockin mouse, in which NFκB is fused to fluorescent fusion protein. Using live-cell microscopy, we measured NFκB signaling in bone marrow-derived macrophages in response to pathogen-derived molecules (CpG, Pam3CSK4, PolyI:C, LPS) and host-derived molecules (TNF) in real-time. For each stimulus, we quantified NFκB signaling across the full dose-response range. To dissect which features of NFκB signaling confer stimulus-specificity, we trained an ensemble of classification models to learn the relationships between each stimulus and the NFκB signaling response.

Despite response variability, our classification models predict stimulus information from NFκB signaling dynamics with high accuracy. We show that predicting the source of NFκB stimuli (Virus, Bacteria, and Host) is more accurate than predicting the identity (CpG, Pam3CSK4, PolyI:C, LPS, and TNF). Our model identified specific features of NFκB signaling that encode stimulus information, such as NFκB oscillations. We validated our model predictions by examining NFκB signaling dynamics in BMDMs from mutant mice that have deficient NFκB oscillations. Our results show that loss of NFκB oscillations abolishes the accuracy of stimulus encoding: NFκB signaling dynamics fail to predict the stimulus in the absence of NFκB oscillations. These findings show that NFκB oscillations are critical for encoding stimulus information in macrophages. Furthermore, our results show that machine learning is a valuable tool for inferring signaling network properties that underlie robust cellular decision-making in innate immunity.

5 Physiological noise removal in fast functional magnetic resonance imaging without separate physiological signal acquisition

Uday Agrawal

Physiological noise removal in fast functional magnetic resonance imaging without separate physiological signal acquisition

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Technological advances in acquisition protocols have enabled an order of magnitude increase in the speed of functional magnetic resonance imaging (fMRI) measurements. While this new, "fast" fMRI has enormous potential for neuroscientists, the scaling of physiological noise with the improved resolution of fast fMRI limits its applicability. Commonly used pre-whitening and physiologic noise regression techniques in conventional fMRI are insufficient to account for serial correlations in fast fMRI, which may lead to errors in interpretation of the fMRI signal.

Here, we create and test a model of physiological noise based on Harmonic Regression with Autoregressive Noise (HRAN) that utilizes the enhanced sampling of fast fMRI to estimate physiological noise directly from the fMRI data; therefore, it does not require physiological reference signals such as respiration, which are technically challenging to collect. We evaluated HRAN performance in de-noising 1) simulated fast fMRI data with physiological noise, 2) fast resting-state fMRI data collected with physiological reference signals (TR = .367s, 2.5 x 2.5 x 2.5 mm^3, 5mm FWHM Gaussian smoothing), and 3) fast fMRI experiment data in response with a .1 Hz oscillating visual stimulus for 24s where no reference data was collected (TR = .227s, 2 x 2 x 2 mm^3, 5mm FWHM Gaussian smoothing).

In the simulated fast fMRI signal driven by a .1 Hz stimulus, HRAN was able to estimate and remove the added physiological noise and reduce the root mean squared error. In resting-state fast fMRI data, we found that the estimated physiological frequencies derived from the 4th ventricle accurately tracked the average heart rate and respiration rate obtained from the EKG and respiratory belt. HRAN also satisfied goodness of fit criteria with model parameters determined using the Bayesian Information Criterion. At the single-voxel level, HRAN reduced autocorrelations in the residuals as effectively as RETROICOR, with greatest impact in gray matter. Finally, in the fast fMRI experiment with a 0.1 Hz visual stimulus, HRAN was able to estimate physiological frequencies from the lateral ventricle and improve detection of visually-driven voxels, as compared to standard FSL analysis. In one exemplar voxel, physiological noise modelling with HRAN reduced the residual variance by 56%, enabling detection with a voxel-wise corrected threshold of p

We found that HRAN is able to accurately estimate physiological frequencies using the fast fMRI data directly and is as effective as removing autocorrelation as commonly employed techniques, while estimating these noise patterns directly from the data itself. These findings suggest that HRAN is able to successfully remove physiological noise from fast fMRI. Capturing serial correlations using the HRAN framework will not only help to improve interpretations of future fast fMRI experiments, but also help to guide researchers in prospective experimental design.

6 Zoniporide and α-methylnorepinephrine administered in a rat model of cardiac resuscitation influences the amplitude spectral area of the ventricular fibrillation waveform

Salvatore Aiello

Zoniporide and α-methylnorepinephrine administered in a rat model of cardiac resuscitation influences the amplitude spectral area of the ventricular fibrillation waveform

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We present a post-hoc analysis from a study examining the effects of administration of the peripheral selective α_2 adrenoreceptor agonist α-methylnorepinephrine (α-MNE) and the Na^+-H^+ exchanger isoform-1 (NHE-1) inhibitor Zoniporide (ZNP) in a rat model of ventricular fibrillation (VF). We use a VF waveform analysis technique known as amplitude spectral area (AMSA), which reflects the energy state of the myocardium and the likelihood of successful defibrillation. α-MNE was expected to increase the coronary perfusion pressure during chest compression and ZNP to ameliorate reperfusion injury and help preserve left ventricular distensibility enabling hemodynamically more effective chest compression.

VF was induced and left untreated for 8 minutes and followed by 8 minutes of chest compressions and ventilation delivering electrical shocks at the end of chest compression. Rats (n=48) were randomized 1:1:1:1 to receive a 3mg/kg bolus of ZNP or 0.9% NaCl before chest compression and a 100 µg/kg bolus of α-MNE or 0.9% NaCl at minute 2 of chest compression. AMSA is the summed product of individual frequencies (F_i) and their corresponding amplitudes (A_i), reported in mV*Hz. AMSA was measured in the final 2.1 s of each minute during untreated VF, during chest compressions, and immediately preceding the first electrical shock. To calculate AMSA during continuous chest compressions, an ECG parsing technique was used to filter the compression artifact. ECG segments during the off phase of the compression duty cycle (i.e., when the depth of the compression returned to zero) were extracted and compiled into a single continuous ECG segment.

The four groups were analyzed by ANOVA. If data failed the Shapiro-Wilk normality test, the Kruskal-Wallis ANOVA on ranks was used. Dunn's method was used to conduct multiple comparisons versus the control group.

Survival and post-resuscitation hemodynamics were reported in the main analysis. Our post-hoc analysis revealed that AMSA (median [Q1-Q3]) during chest compressions was higher at 7 minutes for ZNP/α-MNE (37.54 [28.14-40.09]) versus 0.9% NaCl/0.9% NaCl (11.39 [9.80-21.58]) (p=0.002) and at 8 minutes for both ZNP/0.9% NaCl (24.60 [22.12-44.88]) and ZNP/α-MNE(25.71 [20.35-51.84]) when compared to 0.9% NaCl/0.9% NaCl (13.92 [9.20-20.45]) (p=0.008). Additionally, there was a statistically higher pre-shock AMSA for ZNP/0.9% NaCl (21.51 [15.64-29.49]) versus 0.9% NaCl/0.9% NaCl (13.09 [9.68-16.63]) (p=0.034).

ZNP alone and α-MNE/ZNP were associated with higher AMSA levels both at the end of chest compressions and in the period immediately preceding the first electric shock. To our knowledge, there are no previous reports demonstrating a potential relationship between drug intervention and AMSA levels. Previous groups have proposed methods to overcome artifact caused by chest compressions by applying frequency filters or waiting until there are pauses in compressions to measure AMSA. Our proposed method allows for continuous compressions with the benefit of measuring the raw ECG recording.

7 Mass cytometry reveals increases in a novel circulating memory T cell population and decreases in CCR10+ regulatory T cells in human hypertension
Matthew R. Alexander

Mass cytometry reveals increases in a novel circulating memory T cell population and decreases in CCR10+ regulatory T cells in human hypertension

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Hypertension is the leading risk factor for morbidity and mortality worldwide. Emerging evidence in animal models demonstrates the importance of a variety of innate and adaptive immune cells in hypertension. We hypothesized that the abundance and phenotype of specific immune cell subsets is altered in human hypertension reflecting disease pathophysiology. We performed unbiased high dimensional, single cell profiling of peripheral blood mononuclear cells in humans with a panel of 31 cell surface markers using mass cytometry. Unsupervised computational analysis from 11 control and 10 hypertensive individuals matched for age, gender, race, and body mass index revealed consistent increases in a novel memory helper T cell subset in hypertension. Manual two dimensional gating revealed that this $CD4^+CD45RO^+CD62L^+CCR7^-CD161^{lo}$ memory cell population is nearly 2-fold increased in hypertensive subjects. As an alternative approach to starting with unsupervised analysis, we also first manually gated for immune cell populations implicated in hypertension such as regulatory T cells ($CD4^+CD25^{hi}CD127^{lo}$). Interestingly, circulating regulatory T cells (Tregs) were decreased by 35% in hypertension by this manual gating approach. An unsupervised analysis using Phenograph to subgroup the Tregs revealed a population of CCR10+ Tregs that are selectively decreased in hypertension. Further manual gating confirmed that these circulating CCR10+ Tregs are decreased by nearly 50% in hypertensives compared to controls. Taken together, results of these studies provide novel evidence for the differential abundance of specific memory and Treg lymphocyte populations in human hypertension and provide new insights into hypertension pathogenesis and potential therapeutic targets.

8 Targeting metabolism to treat breast cancer brain metastases
Ahmed Ali

Targeting metabolism to treat breast cancer brain metastases

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Brain metastasis from human epidermal growth factor receptor 2 (HER2) positive breast cancer respond poorly to anti-HER2 therapy, even when the same therapy can be used to treat extracranial breast cancer. Treatment resistance in the brain is often attributed to inadequate drug delivery across the blood-brain barrier as is the case for anti-HER2 antibodies; however, even brain penetrant small molecule drugs like lapatinib fail to control brain metastasis despite adequate target inhibition. Instead, there is increasing evidence that the brain microenvironment contributes to therapy resistance, and that the metabolic phenotypes of cancer cells is also heavily influenced by nutrients in the environment.

To study how the brain microenvironment might contribute to therapy resistance, we utilized a murine model of HER2 amplified breast cancer, where human cancer cells are implanted to form tumors in the mammary fat pad (MFP) or brain. [18F] FDG-PET uptake experiments show increased glucose uptake in the brain metastasis compared to the same cells forming tumor in the primary breast site. In addition, assessment of 13C-labeled glucose fate in tumor tissue show that the increased glucose uptake is used differently in the brain metastasis, serving as a substrate for increased fatty acid biosynthesis. The observed differences in metabolism correlate with response to phosphoinositide-3-kinase inhibition and we are studying whether genetic or pharmaceutical disruption of lipid biosynthesis could be a vulnerability of breast cancer brain metastasis. These experiments will also provide insight into metabolic dependencies of breast cancer cells growing in different sites. Together, these data illustrate the influence of tumor microenvironment on cell metabolism and present a unique opportunity to leverage site-specific metabolic differences to expand treatment options for patients with breast cancer brain metastasis.

9 The role of microglia in the metabolic outcomes of environmental enrichment in aging mice
Seemaab Ali

The role of microglia in the metabolic outcomes of environmental enrichment in aging mice

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Aging is associated with increased risk for chronic metabolic disease, due to a combination of biologic and environmental factors. Aging results in central nervous system (CNS) inflammation and dysfunction of microglia, the resident immune cell of the CNS. Neuroinflammation and microglial activation in the hypothalamus are associated with the development of obesity and diabetes. The mechanisms underlying aging and age-related disease processes, and their interaction with environmental factors, are not fully understood. Environmental enrichment (EE) provides a model for studying the interaction of lifestyle factors with the progression of age-related metabolic dysfunction. EE results in an anti-obesity phenotype in a variety of mouse models, which is dependent on the hypothalamic sympathoneural adipocyte axis (HSA axis). In this axis, brain-derived neurotrophic factor (BDNF) upregulation in the hypothalamus leads to sympathetic activation, which results in adipose tissue browning. Importantly, microglia have previously been shown to express BDNF in the course of synapse formation, and are thought to be necessary for the processes of synaptic remodeling and neurogenesis which contribute to the neurobiological outcomes of EE. We therefore investigated whether microglia play a role in the HSA-mediated metabolic outcomes of EE. First, we assessed whether microglia were affected by EE housing. Short-term (6 week) and long-term housing (8-12 months) of 10 month old middle-age mice in either standard or EE conditions resulted in a significant reduction of adiposity and overall improvements in glucose tolerance. Long-term EE also reduced expression of hypothalamic cytokines, NFκB pathway genes, as well as major histocompatibility complex class II, as measured by RT-qPCR. Iba1 immunohistochemistry revealed a distinct microglial morphology phenotype in EE housing, characterized by increased ramification and hypertrophy without increases in microglial cell count. Depleting microglia using a colony stimulating factor 1 receptor antagonist, PLX5622, also resulted in improvements in adiposity and glycemic control, indicating that dysfunctional microglia contribute to age-related metabolic decline. EE housing additively improved metabolic outcomes with PLX5622, suggesting that microglia are not essential for the EE phenotype. Taken together, this data shows that EE acts on microglia to change and even improve their neuroinflammatory state, but that these cells are not necessary for the hypothalamic changes responsible for the metabolic outcomes of EE. Both lifestyle modification and removing dysfunctional microglia in old age are potential therapeutic avenues for reducing age-related adipose accumulation and glucose intolerance.

10 Pharmacokinetics of a humanized anti-RNLS monoclonal antibody
Oriyomi Alimi

Pharmacokinetics of a humanized anti-RNLS monoclonal antibody

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Introduction: Cancer cells can overcome signaling that restrains their growth and promotes senescence and cell death. Renalase (RNLS) is a secreted flavoprotein that functions as a survival factor after ischemic and toxic injury, signaling through the plasma calcium channel PMCA4b to activate the PI3K/AKT and MAPK pathways. In addition, recent studies, indicate that dysregulated RNLS signaling promotes survival of melanoma cells due to its capacity to augment expression of growth-related genes and to promote macrophage polarization toward a tumor promoting phenotype. Preliminary data show single agent efficacy of the inhibitory rabbit monoclonal m28-RNLS in PD1 resistant tumors. We humanized m28-RNLS and selected a variant (K5), formatted to human IgG1 containing an Fc portion that promotes complement binding. The purpose of these experiments is to determine the pharmacokinetic profile of a K5, a therapeutic candidate for cancer therapy.

Methods: K5 was produced by transfecting human embryonic 293 kidney cells using standard methods. To determine the pharmacokinetics of K5, mice (n=30) were dosed with 6.4 mg/kg K5 either as a single intravenous injection (i.v) via tail vein or subcutaneous (s.c) injection. Blood was collected at 15 time points and stored at -20°C until assayed for K5 concentrations. K5 concentration was determined using a direct-sandwich ELISA and uses His-tag purified recombinant human renalase as capture antigen and goat anti-human Fc IgG as detection antibodies. 384-well plates were coated with 10 mcg/ml recombinant human RNLS in phosphate buffered saline (PBS pH 7.4) at 4°C, incubated overnight, and then blocked with 0.5% BSA in PBS at room temperature for 1 h. Standards were made in triplicate using known concentrations of antibody injected into mouse serum. Sixteen 2-fold serial dilutions of serum samples were prepared in buffer containing, 1x PBS pH 7.37, 0.05% Tween, 0.5% BSA, and were incubated for 1h and then washed 4x with buffer containing 1x PBS and 0.05% Tween. Bound K5 was detected using horseradish peroxidase (HRP)-labeled goat anti-human IgG (Fc). Optical Density was 450nm using a microplate reader. Standard curves were interpolated using GraphPad Prism. Concentrations were determined from the standard curves. Concentration versus time data were analyzed using a non-compartmental analysis.

Results: IV and s.c pharmacokinetic studies were conducted in mice. Preliminary data shows at a dose of approximately 6.4mg/kg in mice, Tmax was 4 hours and the observed maximum concentration after a single s.c injection was 10µg/mL. Drug was undetectable after 8 days.

Conclusions: Preliminary PK data indicate that K5 may behave in vivo like other commercially available IgG1 molecules. It exhibits high bioavailability, with a rapid distribution phase and a slow elimination phase. Pharmacokinetic data in mice can be used to predict the pharmacokinetics of K5 in humans and can guide dosing in future clinical trials.

11 Enterobacteriaceae blooms in the premature intestine
Sahitya Allam

Enterobacteriaceae blooms in the premature intestine

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Neonatal infections are associated with severe morbidity and mortality in preterm infants. In case control studies, dysbiosis of intestinal microbiota has been recognized as a risk factor for these events. Often, an increase or "bloom" of bacteria specied within the family Enterobacteriaceae (ENT) is observed preceding the event. The frequency with which these blooms occur in individual patients and the clinical factors associated with these ecological changes have not been well characterized.

In a prospective cohort of premature infants narJ) specific primers. A standard curve was generated using known quantities of the *Enteroaggregative Escherichia coli* strain 042. Clinical metadata were collected from the electronic medical record.

Samples from 17 infants with a mean gestational age of 25.8 weeks and birth weight 909 grams were analyzed. Eight patients (47%) had an observed bloom event during the first 60 days of life. Blooms were associated with the following clinical events: bacteremia (one of eight cases), urinary tract infection (one of four), and necrotizing enterocolitis (two of three). To explore factors associated with changes in ENT, 108 week-long intervals from all infants were evaluated. Additionally, higher resolution sampling at smaller, 3-5 day intervals was conducted for eight out of the 17 patients. The majority of %ENT values sampled at smaller intervals were consistent with the prior values collected at weekly intervals. Withholding of enteral feeds (NPO status) was the only clinical factor found to be associated with an increase in relative ENT (mean pre 2.8% vs. post 14.2%, p = .03). Other factors including antibiotic exposure and fortification of breast milk were not associated with changes in ENT abundance.

This work has advanced our understanding of the clinical factors associated with Enterobacteriaceae population changes in premature infants. Future directions include expanding the analysis of clinical factors with higher resolution Enterobacteriaceae abundance data and investigating whether modifying the gut microbiome could protect against disease in premature infants.

12 Myelin regulatory factor is required for proper nodal signaling during left-right patterning
Sarah K. Amalraj

Myelin regulatory factor is required for proper nodal signaling during left-right patterning

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Congenital heart disease (CHD) is the most common major birth defect, affecting nearly 3% of children, and is the leading cause of infant mortality. Heterotaxy (Htx) is a disorder of left-right (LR) patterning, in which organs, including the heart, are mispatterned relative to the LR axis. Htx is associated with severe forms of CHD, but its genetic causes remain largely undefined. A recent genetic analysis of Htx/CHD patients identified numerous candidate genes, including membrane-associated transcription factor Myelin Regulatory Factor (MYRF). This is intriguing, as *MYRF* has an identified role in the generation and maintenance of myelin in the central nervous system, but no known function in cardiac development or LR patterning. Here, we show that depletion of *myrf* using CRISPR based gene modification in *Xenopus tropicalis* embryos results in midline heart looping defects, phenocopying our patients. We then analyzed global LR patterning markers and found abnormal bilateral expression of *pitx2*, but normal *coco* expression in *myrf* depleted embryos. We also depleted *myrf* in one cell of a two-cell embryo and found that left-sided depleted embryos resulted in heart looping defects and abnormal *pitx2* expression, whereas right-sided depleted embryos had no LR patterning defects, suggesting that *myrf* contributes to the midline barrier to maintain LR asymmetry. Looking upstream from *pitx2* in the LR signaling cascade, we observed that although *nodal* was appropriately expressed in the left lateral plate mesoderm (LPM), *nodal* expression intensity was increased in *myrf* depleted embryos. Additionally, nodal signaling persisted in *myrf* depleted embryos at later stages when *nodal* expression normally ceases within the left LPM, indicating that *myrf* is necessary for proper nodal signaling. Lastly, to determine if *myrf* acts as a transcription factor in the context of LR patterning, we created a wild type human *MYRF* RNA construct as well as three patient mutation *MYRF* RNA constructs that contained point mutations within the DNA Binding Domain that had been identified in patients with CHD. When co-injected with CRISPR sgRNA, the wild type *MYRF* RNA construct was able to rescue the abnormal *pitx2* expression, whereas the patient mutation constructs where unable to rescue the LR phenotype. Together, our data suggests that *MYRF* acts in the midline as a transcription factor to repress nodal signaling. Loss of *myrf* allows nodal protein to diffuse into the right side of the embryo, leading to abnormal LR patterning. We conclude that patient driven gene discovery can provide new insights into the molecular mechanisms that drive cardiac patterning and LR axis formation.

13 Mitf and the MIT family restrain B cell autoreactivity
Abhimanyu Amarnani

Mitf and the MIT family restrain B cell autoreactivity

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B cells are central in the development of many autoimmune diseases, such as systemic lupus erythematosus (SLE), through differentiation of autoreactive B cells into antibody-secreting plasma cells. Our lab previously developed a mouse model, called TDN-B, whereby inhibition

of the *microphthalmia* transcription factor (Mitf) and its family members, Tfe3, Tfeb, and Tfec occurs specifically in B cells. Through crossing this model with the SLE-susceptible genetic background B6.lpr mouse model, prior work had shown that inhibition of the MiT family in B cells worsened SLE-like disease as evidenced by accelerated mortality, production of pathologic autoantibodies, and hastened renal disease. To define the mechanisms of gene expression regulated by Mitf and the MiT family, the presented work evaluated both the TDN-B model, and a model in which Mitf is not expressed in any cell type (the VGA.9 mouse model). Studies assessed B cell and T cell subsets (flow cytometry), immunoglobulin and autoantibody serum titers (ELISA), cytokine secretion (luminex), organization of splenic follicles (wide-field and confocal microscopy), and comprehensively investigated changes of mRNA expression in ex-vivo B cells (RNA sequencing). Uniquely, VGA.9 mice, with Mitf absent in all cells, showed increased serum levels of IgG anti-dsDNA, increased splenic germinal center B cells, and increased splenic plasma cells, compared to wildtype. While increased splenic germinal center B cells and plasma cells were not observed in TDN-B mice, increased numbers of pre-B/immature B cells and plasma cells were observed in the bone marrow. While some differences between the models were noted, both TDN-B and VGA.9 mouse models showed increased serum rheumatoid factor, splenomegaly, increased numbers of splenocytes, and disorganization of splenic follicles, compared to wildtype. Investigation of mRNA expression changes in ex-vivo B cells showed that in both models, upregulated mRNA pools were significantly enriched for genes with roles in germinal center growth and/or regulation. Further, pathways related to regulation of cell cycle, MHCII antigen presentation, and cytokine signaling were all significantly enriched for in mRNA from both VGA.9 and TDN-B B cells. Additional experiments in VGA.9 mice demonstrated increased numbers of B cells with surface expression of activation markers (CD69, CD25) and antigen presentation molecules (MHCII, CD86), and that B cells in culture had increased secretion of TNF-alpha after LPS stimulation. Overall, these results demonstrate that functional impairment of Mitf and the MiT transcription factor family can permit B cell autoreactivity through dysregulation of B cell activation, antigen presentation, cytokine secretion, germinal center organization, plasma cell differentiation, and autoantibody production.

14 Progesterone affects the NALCN and Slo2.1 complex, which regulates myometrial excitability
Chinwendu L. Amazu

Progesterone affects the NALCN and Slo2.1 complex, which regulates myometrial excitability

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For the majority of pregnancy, the myometrial smooth muscle cells (MSMCs) of the human uterus are non-contractile. This is largely because the MSMC resting membrane potential is maintained in a negative state by an outward potassium leak current majorly conducted by the sodium-activated potassium channel Slo2.1. The activity of Slo2.1 is counteracted by a slow inward leak of sodium mostly through the channel Sodium Leak Channel Non-Selective (NALCN). We previously found that the hormone progesterone, which promotes uterine quiescence during pregnancy, regulates NALCN and Slo2.1 mRNA levels in a microarray study. Here, we tested the hypothesis that Slo2.1 and NALCN function together in a complex to regulate MSMC membrane potential and are regulated by one form of the progesterone receptor (PR), PRB, the pro-quiescence isoform.

We performed whole-cell patch clamping on the immortalized MSMC line hTERT-HM. Co-localization of Slo2.1 and NALCN was assessed by the *in situ* proximity ligation assay. Expression of Slo2.1 and NALCN mRNA was measured by quantitative Polymerase Chain Reaction (qPCR) and NALCN protein was measured by Western blot in hTERT-HM cells in which the relative ratio of PRA and PRB can be manipulated.

The sodium-activated potassium current carried by Slo2.1 was significantly decreased in the presence of gadolinium, an inhibitor of NALCN. The *in situ* proximity ligation assay revealed that NALCN and Slo2.1 were in proximity in hTERT-HM cells. Progesterone is a major hormone that regulates myometrial quiescence during pregnancy. NALCN and Slo2.1 mRNA expression increased in the presence of PRB and progesterone. Conversely, treatment with the PR antagonist RU486 significantly decreased NALCN mRNA expression. Additionally, NALCN protein expression was increased in the presence of PRB and progesterone, compared to PRB alone.

Our data suggest that NALCN provides the sodium to activate Slo2.1 and that the two channels function in a complex and are regulated by progesterone acting through PRB. Further characterization of this complex will provide an insight into possible targets to modulate uterine quiescence and contractility. Long term, such knowledge will lead to strategies to regulate uterine contractile dysfunction leading to pregnancy complications such as preterm birth and dystocia.

15 Understanding the Role of a Prototypical Splicing Factor, SRSF1, in Hepatic Lipid Metabolism
Waqar Arif

Understanding the Role of a Prototypical Splicing Factor, SRSF1, in Hepatic Lipid Metabolism

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It is becoming increasingly evident that post-transcriptional gene regulation is central to many metabolic functions. This form of regulation is highly diverse and includes regulation mediated by microRNAs, mRNA stability and mRNA processing. A recent study examining SNPs in pa-

tients with NASH revealed a significant association for pathways involved in mRNA splicing. However, the role of alternative splicing in liver function and disease has yet to be explored. To begin understanding how alternative splicing contributes to liver physiology, we decided to study the role of a prototypical splicing factor known as SRSF1. To this end, we created hepatocyte-specific knockout (SRSF1 HKO) mice using a Cre/lox system. Histological and serum analyses of these mice revealed spontaneous and progressive NASH-like liver injury including steatosis, inflammation, and fibrosis. To identify the transcriptome defects causing this pathology, we performed a high-resolution RNA-Seq on livers of ten-day and five-week old livers from both wildtype and SRSF1 HKO mice. Furthermore, to determine the direct mRNA targets of SRSF1 in hepatocytes, we performed CLIP-Seq analysis in hepatocytes isolated from wildtype mice liver. Computational analysis of the data revealed hundreds of genes with altered splicing and expression many of which are related to fatty acid metabolism and lipid trafficking.

16 Pediatric glioma invasion mediated through the Nogo pathway
Razina Aziz-Bose

Pediatric glioma invasion mediated through the Nogo pathway

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Diffuse intrinsic pontine glioma (DIPG) is a devastating childhood brain cancer, in part due to its characteristic aggressive infiltration into surrounding tissue that prevents the option of surgical resection. The lack of surgical options for DIPG contributes to the poor prognosis of the disease – the median survival of patients with DIPG is 10 months after diagnosis. Despite the fact that diffuse infiltration is a defining aspect of this disease, the molecular and cellular determinants of DIPG migration and invasion are not well understood. In adult glioma, inhibition of the Nogo receptor (NgR) has been shown to increase tumor migration in vitro. RNA sequencing studies confirmed that NgR is also expressed in pediatric DIPG tumors. To evaluate the role of NgR in DIPG migration, CRISPR technology was used to delete NgR from a patient-derived metastatic DIPG culture, SU-DIPGXIII frontal lobe. Loss of NgR, confirmed by qPCR and by Western blot, significantly enhanced tumor cell migration at 72 hours in the 3D spheroid migration assay $p < 0.05$). NSG mice xenografted with SU-DIPGXIII NgR null cells exhibited a 33% increased extent of tumor invasion through the brain at 8 weeks compared to mice xenografted with wild-type SU-DIPGXIII control cells ($p < 0.05$). This work elucidates the importance of Nogo pathway signaling in diffuse intrinsic pontine glioma, which may be targeted therapeutically to limit tumor spread.

17 Antibodies elicited by an NS1-based vaccine protect mice against Zika virus
Mark J. Bailey

Antibodies elicited by an NS1-based vaccine protect mice against Zika virus

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Zika virus is a mosquito-borne flavivirus that can cause severe disease in humans including microcephaly in newborns and Guillain-Barré syndrome in adults. Therapeutics and vaccines for treating Zika virus disease are not currently available. Our goal is to better understand the immunological responses required for optimal protection against disease. We have previously shown antibodies targeting the Zika virus NS1 protein to be protective without inducing antibody-dependent enhancement of disease. Now, we plan to determine whether the NS1 protein itself is a viable vaccine target. We designed a prime-boost immunization scheme by vaccinating mice with a plasmid expressing the NS1 protein followed by two adjuvanted protein boosts. This regimen elicited high antibody titers to the Zika virus NS1 protein and protected mice from lethal viral challenge. Moreover, our data suggest that protection is mediated by Fc-effector functions. To study the NS1-specific response in humans, we tested sera from patients in either the acute or convalescent phase of Zika virus infection. We find that the NS1-specific antibody response in humans is robust and remains elevated up to a year post infection. This study highlights the importance of the NS1 protein as a potential vaccine component against Zika virus.

18 The Spen Family Transcription Repressor positively buffers Notch Signaling in early T-cell development
Abhik K. Banerjee

The Spen Family Transcription Repressor positively buffers Notch Signaling in early T-cell development

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Early T-cell development is a highly stereotyped biological process, through which multipotent hematopoietic progenitor cells systematically lose alternative cell fate potential and acquire components of the T-cell developmental program. Notch signaling is critical for early T-cell development and lineage commitment, and dysregulation of the pathway has been linked to a variety of hematologic and solid malignancies, including T-Acute Lymphoblastic Leukemia and Pancreatic cancer etc... Despite the essential role for Notch-mediated gene regulation in early T-cell development, it remains a mystery how the pathway can differentially regulate gene expression given near-uniform expression of its core

signaling components.

One important regulator of Notch signaling is the Spen Family Transcription Repressor (Split Ends Family Transcription Repressor, also known as Spen, SHARP, or MINT). Spen and its homologs have been shown to antagonize Notch-mediated developmental processes in several different contexts, including Marginal versus Follicular B-cell differentiation in mouse, primary neurogenesis in *Xenopus laevis,* among others. Given its ability to physically interact with co-repressive complexes such as Nuclear Receptor Co-Repressor 2, Spen has been largely thought to be a transcriptional repressor of Notch signaling. Despite the evidence supporting its role as a negative regulator, Spen deficiency does not strictly phenocopy Notch gain of function in early T-cell development. In stark contrast to Notch pathway gain of function which results in ectopic T-cell differentiation and tumorigenesis, Spen deficiency results in stage-specific developmental delay.

In order to interrogate the role of Spen and its relationship to Notch signaling during thymopoiesis, we have developed a CRISPR/Cas9-based model of Spen deficiency using the SCID.adh.2c2 T-cell leukemia line. Using a combination of flow cytometry, titrated pharmacologic inhibition of Notch signaling, and RNA-sequencing analysis, we present data demonstrating Spen acts to positively buffer Notch signaling in early T-cells, specifically through an interaction with a downstream target gene called Notch-Regulated Ankryn Protein. In addition to presenting this transcriptional circuit, we also present analyses examining additional downstream targets of Spen and Notch signaling during gain and loss of function perturbations in the SCID.adh.2c2 model system.

19 The influence of endogenous anti-drug antibody to the hu14.18-IL2 immunocytokine on its binding to the GD2+ B78 melanoma
Claire C. Baniel

The influence of endogenous anti-drug antibody to the hu14.18-IL2 immunocytokine on its binding to the GD2+ B78 melanoma

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Some cancer patients treated with monoclonal-antibody (mAb) anti-tumor therapies develop endogenous anti-drug-antibodies (ADA) that recognize, and in some cases neutralize, the therapeutic mAb, interfering with its function. We have developed a therapeutic approach combining local radiotherapy with intratumoral (IT) administration of the hu14.18-IL2 immunocytokine (IC), a fusion protein of an anti-GD2 mAb with IL2. GD2 is over-expressed in melanoma and neuroblastoma. This regimen induces potent destruction of GD2+ tumors in mice and results in the generation of tumor specific memory, thereby converting the GD2+ B78 melanoma into an in situ vaccine. A clinical trial of this regimen in patients with melanoma has now opened.

There are no published data on the potential influence of ADA to modify the tumor-binding capability of a tumor-reactive mAb when given IT. This preclinical study aims to characterize the impact of ADA on treatment efficacy utilizing IT-IC.

We have developed a mouse immunization model that allows us to generate, detect, and characterize ADA against hu14.18-IL2 in C57BL/6 mice. Intravenous (IV) or subcutaneous injection of IC at 15mcg/dose for 5 consecutive daily doses induces an endogenous mouse anti-human antibody (MAHA), an ADA to hu14.18-IL2. This MAHA response is detectable as early as day 7 and is maintained in circulating mouse serum as late as 8 months after initial treatment. MAHA is capable of inhibiting the binding of IC to a 1A7 antibody coated ELISA plate. As 1A7 is a mouse IgG1 anti-idiotype antibody specific for the 14.18 mAb, this result indicates that the MAHA interferes with the antigen binding portion of the hu14.18-IL2. In preliminary in vivo studies we have evaluated by flow cytometry IC binding to B78 melanoma in naïve (MAHA-) vs. IC-immunized (MAHA+) mice. When IC is injected IV, there is a dramatic inhibition of IC binding to the B78 tumor in MAHA+ compared to MAHA- mice. In contrast, when IC is injected IT, there is no significant inhibition of IC binding to the B78 tumor in MAHA+ compared to MAHA- mice. Further studies of the role of MAHA in IC binding when given IT and in the antitumor efficacy of IT-IC therapy are underway. These preliminary results suggest that MAHA (or ADA) may not interfere with the therapeutic activity of tumor-reactive mAbs (like this IC) when given IT. Such a result would have translational significance.

20 Alteration of hematopoietic stem cells underlies germline genetic risk for myeloproliferative neoplasms
Erik L. Bao

Alteration of hematopoietic stem cells underlies germline genetic risk for myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPNs) comprise a group of blood cancers that are characterized by clonal expansion of myeloid cells. While extensive studies over the past decade have uncovered the somatic driver mutations causing MPNs, these diseases curiously have an inherited genetic basis that is poorly understood. For example, MPNs have a sevenfold increased risk of acquisition in first-degree relatives of individuals with the disease, which is among the highest across all cancers, yet the precise genetic variants conferring inherited MPN risk and their underlying mechanisms remain largely unknown. Here, we hypothesized

that germline MPN risk variants alter hematopoietic stem cells (HSCs) – where MPNs arise – to confer increased risk of disease acquisition.

First, we sought to elucidate the genetic landscape of MPN risk by performing the largest genome-wide association study of MPNs to date, involving 2,627 cases and 755,476 controls. We identified twelve independent loci surpassing genome-wide significance, of which six are novel, as well as 16 additional loci reaching suggestive significance (p -6). Heritability analyses revealed that common genetic variants collectively explain ~9.45% of variance in MPN risk. In addition, we observed significant genetic correlations between MPNs and a number of commonly measured blood cell traits.

Second, in order to pinpoint likely causal variants and the cell types in which they act, we performed Bayesian fine-mapping on variants within each region of association and overlapped them with chromatin accessibility profiles of 18 human hematopoietic progenitor populations. Strikingly, MPN risk variants showed the strongest enrichment in accessible chromatin of HSCs, as compared to other more differentiated cell populations.

Third, we prioritized target genes of MPN variants by mapping risk loci to: (1) gene bodies, (2) genes implicated by enhancer-promoter interactions, and (3) chromatin accessibility regions correlated with nearby gene expression. These analyses implicated 34 candidate genes in MPN risk, whose top enriched biological functions included cellular aging and HSC proliferation.

Given the notable involvement of HSCs in both the chromatin accessibility and target gene analyses, we performed functional perturbation on one risk variant, rs621940, which colocalized within an HSC enhancer region. Allele-specific reporter assays of the variant and CRISPR/Cas9 perturbation of the associated regulatory element in primary HSCs demonstrated that it specifically regulates *GFI1B*, a transcription factor necessary for HSC quiescence. Colony-forming assays revealed that disruption of this enhancer significantly increased hematopoietic progenitors, suggesting that rs621940 may alter *GFI1B* expression, leading to an increased number of HSCs and potentially driving MPN risk through this mechanism.

Collectively, our findings both elucidate the genetic architecture of MPN risk and demonstrate that these risk variants predominantly act by modulating HSCs. More broadly, our study illustrates how population-based genetic studies can be applied to better understand inherited predispositions to cancer.

21 Development of a precision oncology single molecule molecular inversion probe (smMIP) panel using a community-consensus approach

Erica K. Barnell

Development of a precision oncology single molecule molecular inversion probe (smMIP) panel using a community-consensus approach

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Background: Clinical targeted sequencing panels are important for identifying actionable variants for cancer patients, however, there are currently no strategies to create impartial and rationally-designed panels to accommodate rapidly growing knowledge within the field. Here we use the Clinical Interpretations of Variants in Cancer database (CIViC) in conjunction with single-molecule molecular inversion probe (smMIP) capture to provide a dynamic and accurate capture panel targeting clinically relevant variants in cancer.

Methods: A CIViC actionability score was created for each variant within the CIViC database to establish whether the existing level of curated evidence warrants inclusion into the smMIPs capture panel. Variants with a CIViC actionability score > 20 points were eligible for panel design. All eligible variants were further categorized by variant length to determine the number of smMIPs probes required to adequately assess each variant. For variants that required hotspot targeting, smMIPs probes were designed for the genomic region indicated in the CIViC database. For all variants that required sparse exon tiling or full exon tiling, overlapping probes were designed to cover all protein coding exons. The CIViC smMIPs capture panel was employed on samples with previously conducted orthogonal sequencing data to validate panel design.

Results: In total, 2,027 smMIPs were designed to target 111 eligible CIViC variants. The total genomic region covered by the panel was 61.5 kb. When compared to existing genome or exome sequencing results (n = 27 cancer samples from 5 tumor types), the CIViC smMIP capture panel demonstrated a 95% sensitivity for variant detection (n = 61/64 variants). Variant allele frequency for variants identified on both sequencing platforms were highly concordant (Pearson correlation = 0.885; n = 61 variants). Moreover, for individuals with paired tumor/normal samples (n = 12), 182 clinically relevant variants missed by original sequencing were discovered by the CIViC smMIPs panel.

Discussion: This panel demonstrates the utility of an open-sourced database built on attendant community contributions for each variant with peer-reviewed interpretations. Use of a public repository for variant identification, probe development, and variant annotation could provide a dynamic and transparent approach to alleviate the analysis bottleneck hindering precision oncology efforts.

22 3D organotypic rafts as an authentic in vitro model for pediatric recurrent respiratory papillomatosis

Mary C. Bedard

3D organotypic rafts as an authentic *in vitro* model for pediatric recurrent respiratory papillomatosis

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Recurrent respiratory papillomatosis (RRP) refers to a condition where benign epithelial neoplasms of various sizes occur along the respiratory tract. It is the most common benign neoplasm of the larynx in children and is associated with fatal complications. Children undergo an average of 4 procedures in the first year alone following diagnosis, and 20% need adjuvant medical therapy. Unfortunately, pharmaceutical options are limited and produce highly variable patient response. Together, the need for multiple non-curative surgical interventions and the lack of an effective single-agent therapeutic options places a burden on both the patients' quality of life and the healthcare system. Pathophysiology has been reported to involve a dysfunctional immune response to Human Papilloma Virus (HPV) low risk strains 6 and 11, but infection with HPV does not necessarily result in RRP and it is unclear what aspects of the virus underlie the clinical phenotype. While 2D monolayer systems are normally used to study diseases *in vitro*, 2D models lack the differentiated mucosa that HPV requires. To address this difficulty, we have developed 3D organotypic epithelial rafts that encapsulate the complex differentiated epithelium and can thus incorporate the contribution of the HPV viral lifecycle.

Our long-term goal is to address the variability of clinical phenotypes and therapeutic responses by defining key components of RRP pathogenesis and by implementing a pipeline of in vitro model systems – consisting of patient samples, derivative primary cells, and 3D models – that bridge the gap from bench to bedside. In conjunction with the expertise at Cincinnati Children's Hospital Medical Center (CCHMC), which cares for one of the largest cohorts of RRP patients in the US, fresh RRP tumor-Normal matched tissue from eight patients was harvested and cultured to establish a pipeline of internally controlled patient-specific models of RRP. Despite similarities of RRP primary cells across donors, 3D rafts generated from RRP primary cells rafts harbored distinct morphologic and biologic features. Rafts were examined qualitatively with histology via HPV-related koilocytic changes and quantitatively by degree of hyperplasia via proliferation markers Ki67, K14, K10. RRP phenotype in 3D culture was found to correlate to patient's clinical severity. In order to inform mechanistic studies, a common disease signature was generated by RNA-sequencing four RRP-Normal matched patient samples. Pathway analysis of differentially expressed genes revealed importance for Wnt and JAK/STAT signaling molecules. Ongoing experiments focus on *in vitro* screens of FDA-approved drugs for alternative treatment avenues directed at these pathways. Altogether, the current work provides a 3D model that incorporates the HPV contribution to RRP pathogenesis to enable, for the first time, the future undertaking of efficient therapeutic screening.

23 BCR-induced Ca2+ signals dictate mature B cell fates through control of NF-kappaB and mTORC1 signaling
Corbett T. Berry

BCR-induced Ca^{2+} signals dictate mature B cell fates through control of NF-kappaB and mTORC1 signaling

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Functional humoral immunity relies on the capacity of populations of B cells to compete for antigen leading to the selection of a subset whose antigen receptors (BCR) have high affinity for antigen. How this specificity and affinity is subsequently encoded as specific intracellular signaling cascades to drive cell proliferation and differentiation or alternatively death, remains unresolved. While the interplay between BCR and costimulatory or co-activating signals orchestrate these B cell fate decisions, antigen receptor induced calcium (Ca^{2+}) signals play a key role integrating these inputs and coordinating a multitude of critical subcellular processes that impact transcription and translation. Despite the established importance of Ca^{2+} signals in B cell fate determination, the underlying molecular mechanisms by which Ca^{2+} fine-tunes such fates are not resolved. Here, we sought to dissect and delineate the mechanisms by which STIM/Orai dependent Ca^{2+} entry regulates mature B cell survival and proliferation following BCR engagement. We establish a mechanism by which BCR-induced Ca^{2+} signals rescue B cells from apoptosis through control of canonical NF-kB activation and drive their subsequent entry into the cell cycle through control of c-Rel and mTORC1 dependent signaling. These findings provide a mechanistic framework for understanding how distinct patterns of Ca^{2+} signaling, which are generated by differences in the affinity of antigen binding to the BCR, can regulate key functional responses of B lymphocytes. We expect these studies will reveal therapeutic targets and novel strategies that can be used to prevent pathophysiological immune dysfunction or enhance insufficient immune responses.

24 Using CLCA1 vWA domain to activate alternate anion currents in cystic fibrosis airway
Kayla N. Berry

Using CLCA1 vWA domain to activate alternate anion currents in cystic fibrosis airway

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In the airway, proper activity of the anion channel CFTR contributes to innate immune defense by maintaining a hydrated and alkaline mucus layer. This allows potentially pathogenic microorganisms to be trapped, quickly killed, and cleared via mucociliary clearance, thus preventing microbial colonization of the lungs. In cystic fibrosis (CF), this activity is impaired, resulting in repeated pulmonary infections that damage the lung and, if severe and prolonged enough, lead to early death without lung transplantation. Available therapies remain focused on targeted rescue of the CFTR mutation. However, given the thousands of mutations found in this patient population, individualized rescue of each would be difficult. An alternative and potentially universal strategy may involve activation of a different chloride channel in lung epithelium to bypass CFTR dysfunction. Toward that end, we recently demonstrated that the vWA domain of CLCA1 (calcium activated chloride channel regulator 1) directly engages the calcium activated chloride channel (CaCC) TMEM16A and stabilizes its surface expression on the order of minutes, thereby increasing anion currents through the channel. We have also discovered that the most closely related CLCA family member, CLCA4, potentiates anion currents through the CaCC TMEM16B and *not* through TMEM16A, indicating that CLCA proteins potentiate specific TMEM16 channels. We are currently pursuing a structural model of the CLCA1-TMEM16A interaction by single-particle cryo-electron microscopy, which would be used to inform future design of therapies based on the CLCA1/TMEM16A interaction and gain insight into the specificity of this interaction. We have made significant progress towards this pursuit by determining the X-ray crystal structure of the CLCA1 vWA domain to 2.05 Å, the first structure of any part of CLCA1. Since CLCA1 directly engages TMEM16A, we hypothesized that this molecular recognition could be utilized to specifically activate anion currents in airway epithelia through TMEM16A to compensate for dysfunctional CFTR channels. In whole cell patch clamp experiments, we demonstrate that CLCA1, and in particular its vWA domain, is able to potentiate TMEM16A currents in primary CF airway epithelial cells from three distinct CF genotypes (ΔF508/2789+5G>A, ΔF508/ΔF508 and ΔF508/2184insA). This effect on TMEM16A, however, is not observed in donor CFTR-sufficient airway epithelial cells, and preliminary evidence indicates that this discrepancy may be due to different TMEM16A isoforms expressed in donor versus CF airway epithelial cells. We furthermore show that purified vWA domain is able to sustain TMEM16A currents in polarized CF airway epithelia of other genotypes (ΔF508/621+1G>T and ΔF508/ΔF508) in Ussing chamber experiments. Together, these studies highlight the exciting potential for universal CF treatment modeled after the CLCA1 vWA domain/TMEM16A interaction, and future work will examine the ability of the interaction to restore healthy mucus properties.

25 Novel cell therapy for brain cancer using patient's own fat-derived stem cells
Adip G. Bhargav

Novel cell therapy for brain cancer using patient's own fat-derived stem cells

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Glioblastoma (GBM) is the most devastating and common brain cancer. There is an urgent need for novel therapies. Highly infiltrative brain-tumor initiating cells (BTICs, also known as brain tumor stem cells) contribute to GBM treatment resistance and recurrence. We have shown that commercial human fat-derived mesenchymal stem cells (MSCs) target BTICs that conventional therapy cannot reach. Moreover, autologous MSCs are safe, non-immunogenic delivery vehicles of therapeutic cargo. We have demonstrated promising preclinical efficacy of the anti-GBM protein Bone Morphogenetic Protein 4 (BMP4) and have strong preliminary data with secretable TNF-Related Apoptosis Inducing Ligand (TRAIL). Here, we sought to engineer MSCs with nanoparticles to secrete these anti-GBM proteins and to test the functionality of patient-derived MSCs on primary, intraoperatively-obtained BTICs as a novel treatment modality.

MSCs were isolated from patient adipose tissue and composition and viability was validated. Optimized Poly-(β-amino)-ester nanoparticle formulations achieved transfection efficiencies superior to commercial reagents and included formulations capable of engineering MSCs to express BMP4 or TRAIL. Nanoengineered MSCs remained viable and demonstrated the ability to migrate and target BTICs. BMP4-secreting MSCs decreased sphere-forming capacity of BTICs ($p=0.028$) and decreased BTIC stem markers indicative of aggressiveness. Using conditioned media (CM) experiments from engineered MSCs, combinatorial effects of TRAIL and BMP4 on proliferation and migration of BTICs were observed that suggest potential synergism. We observed a consistent trend of TRAIL/BMP4 CM providing a larger reduction in proliferation than TRAIL alone compared to control at varying concentrations; however, significance between treatment groups was not observed requiring further investigation of optimal TRAIL:BMP4 dosing ratio to maximize potential synergism. TRAIL CM decreased BTIC migration ($p=0.0419$) whereas BMP4 CM did not ($p=0.6916$), suggesting complementary action on migration. Finally, murine ex vivo and in vivo models were used to explore the utility of engineered MSCs as intraoperative therapy. In an ex vivo organotypic GBM model, MSCs engineered with mCherry retained viability in a gel scaffold and extruded out of the gel into normal and GBM brain tissue, mimicking the desired result in patients. Likewise, in a resection model of human GBM, engineered MSCs encapsulated in an FDA-approved fibrin surgical gel demonstrated safety and feasibility in a treatment flow that could be used in patients at point-of-care in the operating room. A longer median survival is observed for the treatment group (108 days vs 76 days; $p=0.0049$) in this ongoing study.

These findings suggest that autologous MSCs derived from patient fat can be engineered with nanoparticles as vehicles for combinatorial, 'smart' therapy that seeks residual BTICs and overcomes treatment resistance via a multimodal mechanism of action. Using MSCs as a robust, tunable platform for nanoengineering, cellular therapies could be created to combat not only GBM but also other diseases by delivering pathology-specific therapeutic cargo.

26 Towards a Systems Biology Understanding of the Role of Brain Connectivity in Schizophrenia
Daniel Biro

Towards a Systems Biology Understanding of the Role of Brain Connectivity in Schizophrenia

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Schizophrenia is among the most significant causes of disability in the world. The etiology is poorly understood, and can be traced across multiple levels of biological organization. Recent progress has been made in understanding the disease through the use of mapping brain connectivity, both on the macro and micro scales. Broadly speaking, brain connectivity approaches utilize tools such as functional magnetic resonance imaging and electroencephalography to measure interactions between brain regions. These approaches have been combined with computational models and graph theoretic concepts such as network structure in order to examine how changes in both physical and functional connections can be correlated to the clinical changes seen in schizophrenic patients. Advances in the field have allowed for substantial correlation between detailed brain measurements and clinical outcomes and symptoms. However the field is still far from being able to provide detailed mechanistic understanding of how changes in brain structure at the level of network structure determines the clinical picture of schizophrenia. We focus on measures of modularity and introduce a simple model which reproduces certain features of schizophrenia, in particular the properties of schizophrenia which cause it to appear to be a "disease of salience". A network model based on models used in the study of gene regulatory networks was adapted for neural systems. Repeated instances of the networks were generated and selected for their ability to complete tasks and switch between tasks. The successful networks were then either subjected to a constraint on total network modularity, or allowed to continue to evolve without a modularity constraint. The networks that were subjected to a high modularity constraint were shown to better solve and switch between tasks. However, these networks were also shown to have lower robustness, and a larger total possible output space. We believe this results are relevant to the study of schizophrenia as a disease of brain connectivity.

27 Evolving biomechanical properties of tissue engineered vascular grafts
Kevin M. Blum

Evolving biomechanical properties of tissue engineered vascular grafts

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Tissue Engineered Vascular Grafts (TEVGs) represent a powerful development in the treatment of congenital heart malformations. TEVGs are capable of being replaced by neotissue over time as the graft is degraded. The result is the formation of a functional blood vessel capable of growth and remodeling as the patient ages. The purpose of this study is to evaluate the structural and mechanical properties of TEVGs and how these properties change throughout their degradation, as well as how these changes affect the in vivo remodeling of the neotissue within patients treated with a TEVG. TEVGs were fabricated using knitted fibers made of polyglycolic acid (PGA) embedded between two layers of porous poly-(&caprolactone-co-L-lactide) (PCLA). TEVGs were exposed to in vitro accelerated degradation followed by evaluations of (1) mass loss, (2) polymer composition, and (3) mechanical properties. Four pediatric patients were implanted with TEVGs as extracardiac, non-fenestrated Fontan grafts to treat single ventricle physiology. During follow-up care, TEVGs were evaluated by serial ultrasound over 36 months. Ultrasound data was analyzed for (1) internal diameter, (2) wall thickness, and (3) strain changes over the cardiac cycle within the wall of the TEVG. Mechanical compliance of the TEVGs was found to increase rapidly over the accelerated degradation, correlating to the loss of the PGA knit fiber layer. Pediatric implants also showed an increasing compliance during implantation time, as well as internal diameter and wall thickness changes due to neotissue formation and changes in biomechanical properties. Changes in TEVG compliance following implantation were shown to play an important role in the mechanical homeostasis of the neotissue environment. Further investigation and understanding of the interplay between the mechanics and biology of TEVG neotissue formation will be vital towards improving care of current TEVG patients, as well as the development of the next generation of TEVGs.

28 Co-abuse of gabapentin in addition to opioids uniquely alters synaptic development in mouse models of neonatal abstinence syndrome
Taylor C. Boggess

Co-abuse of gabapentin in addition to opioids uniquely alters synaptic development in mouse models of neonatal abstinence syndrome

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Neonatal abstinence syndrome (NAS) has become a major health concern in the United States as a result of the rise in the incidence of opioid abuse in recent years. Studies have shown that a large percentage of infants with NAS are born to mothers who abused one or more drugs in addition to opioids during their pregnancy. Gabapentin, a drug commonly given for the treatment of pain and seizure, has emerged as a

common drug of co-abuse with many substance abuse patients reporting that gabapentin potentiates the high produced by opiates. Recently, Marshall University physicians working with labor and delivery at Cabell Huntington Hospital have noted a specific clinical presentation of NAS (involving tongue thrusting, wandering eye movements, and exaggerated Moro reflex) in infants prenatally exposed to opioids and gabapentin. While the external signs and symptoms associated with NAS have been well documented, the cellular and molecular changes occurring within the central nervous system of affected infants, particularly changes in the formation and maturation of synaptic networks, remain an area of research that requires additional investigation. In addition, the effects of drugs other than opioids, such as gabapentin, on the development and progression of NAS remain unclear. In this study, mouse models of NAS were developed using pregnant mothers transgenic for the gabapentin receptor, α2δ-1, so that the effects of co-abuse of the opiate buprenorphine and gabapentin on synaptic development could be examined.

29 Characteristics of patients diagnosed with sebaceous carcinoma in eastern North Carolina
Nicole Bolick

Characteristics of patients diagnosed with sebaceous carcinoma in eastern North Carolina

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Sebaceous Carcinoma is a rare dermatologic condition in which a patient is diagnosed with an aggressive type of cancer originating from sebaceous glands. We identified patients from a large skin cancer data base who had been diagnosed with at least one sebaceous carcinoma. We looked at the characteristics of these patients to determine similarities between individuals with sebaceous carcinomas.

We performed a retrospective chart review at the Brody School of Medicine of dermatology patients diagnosed with at least one case of sebaceous carcinoma to identify characteristics of these patients.

Brody School of Medicine's EHR was utilized to review patients diagnosed with at least one sebaceous carcinoma. The characteristics of the 16 patients eligible for the study were analyzed to determine correlations.

Of the 16 patients eligible for the study 15 were Caucasian and one patient was Asian. Over half (62.5%) of our patients were males. Five of our patients were diagnosed with sebaceous carcinomas on their cheeks (31.25%), followed by the scalp (25%), and the forehead or nose (each 12.5%). Fewer patients had lesions on their ears, neck, or back (each 6.25%). A history of smoking was common among our patients with 57% being previous smokers and 14% current smokers. Thirteen patients' Fitzpatrick skin types were identified with each patient having a skin type of two. Treatment type varied among our patients with most patients having Mohs micrographic surgery (50%) or an excision (31.25%). Immunosuppressant status was analyzed with 13 of our patients being immunocompetent and three (18.75%) immunocompromised. The average age of our patients was 68 with age ranging from 35 to 89 years.

The data shows that most patients diagnosed with sebaceous carcinoma will be Caucasian, male, and have a Fitzpatrick skin type of two. The most common locations in our population for sebaceous carcinoma was the cheeks and scalp. None of our patients had sebaceous carcinomas of the eyelid, possibly due to referral bias with eyelid carcinomas initially referred to ophthalmology. Treatment type will most commonly be Mohs micrographic surgery or an excision. Individuals with sebaceous carcinoma will be older with the average age of patients being around 70. Many sebaceous carcinoma patients will have a history of smoking. Patients with sebaceous carcinoma require a coordinated multispecialty approach.

30 Striving for greater gender equity in one MSTP: A framework for stemming the leaky pipeline
Katarina M. Braun

Striving for greater gender equity in one MSTP: A framework for stemming the leaky pipeline

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Women are underrepresented in leadership positions throughout science and medicine, especially among physician-scientists at all career stages, despite long-standing gender parity in medical school classes and most graduate fields of biomedical sciences. Given these inequities and the potential for women to contribute to these fields and impact trainees' future careers, we investigated gender differences in several aspects of our own Medical Scientist Training Program (MSTP). We sought to identify potential contributors to the underrepresentation of women among physician-scientists at this early career stage. We analyzed gender differences in student withdrawal rates, speakers at an annual MSTP symposium, student participation in a weekly seminar, and survey responses to queries about question-asking in seminar and perceptions of gender-based discrimination. Results across multiple metrics showed measurable differences in female and male trainees' experiences. Female students withdrew from the program at significantly higher rates than did male students (p

31 The relationship between the slow oscillation and underlying resting state cortical activity during anesthesia and NREM sleep
Lindsey M. Brier

The relationship between the slow oscillation and underlying resting

state cortical activity during anesthesia and NREM sleep

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Similar to hemoglobin based brain mapping, cortical correlation structures in spontaneous calcium dynamics have been shown to represent functional connectivity (FC) in the awake state. Genetically encoded calcium indicators (GECIs) such as GCaMP6, offer cell specific expression and temporal resolution up to 4Hz. While these features allow for neural specific FC mapping up into delta range frequencies (0.4-4.0Hz), they also introduce increased sensitivity to modulated brain state. In particular, during non-rapid eye movement (NREM) sleep as well as under certain types of anesthesia, there is an approximately 1Hz slow oscillation that represents a globally continuous hyper/de-polarization of neurons across cortex. This slow oscillation has previously been found to significantly alter FC architecture during induction by ketamine/xylazine anesthesia, however, it remains unclear if this alteration is consistent across types of anesthesia and natural sleep. Further, it has yet to be reconciled whether there are discrepancies between neural and hemoglobin whole brain mapping and whether these potential discrepancies are brain state or frequency band dependent. Lastly, whether the 1Hz slow oscillation replaces FC typical of the awake state or is rather superimposed remains unclear. To these ends, we use *Thy1*-GCaMP6 mice head fixed under a wide field illumination system (Andor iXon EMCCD camera with an 85mm f/1.4 Rokinon camera lens) to simultaneously collect hemoglobin and neural calcium dynamics (illumination sequence: 454 nm (GCaMP excitation), 523nm, 595nm, and 640nm) across the entire cortex. Mice were imaged under ketamine/xylazine and dexmedetomidine anesthesia, a sleep deprivation protocol was used to collect natural sleep data, and separate awake data as well as data under dexmedetomidine reversed by atipamezole was collected for baseline comparison. We found significant changes across GCaMP6 FC architecture in all spectral bands, while only modest changes in hemoglobin FC. Further, linear decomposition analysis revealed a separable 1Hz slow oscillation that upon removal, the remaining data revealed correlation structures strikingly similar to the awake condition. These results indicate that the slow oscillation superimposes onto canonical awake FC architecture. This delineates two types of spontaneous activity for future study, in particular for understanding how sleep and Alzheimer's disease interact, or how anesthesia influences post-operative delirium.

32 Oncometabolite L-2 Hydroxyglutarate Creates a Metabolic Liability in RCC by Decreasing Activating Transcription Factor 4
Garrett Brinkley

Oncometabolite L-2 Hydroxyglutarate Creates a Metabolic Liability in RCC by Decreasing Activating Transcription Factor 4

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INTRODUCTION: Renal cell carcinoma (RCC) is among the ten most common neoplasias in the United States and is well known to undergo extensive metabolic reprogramming. Previous work by our lab has identified high levels of the oncometabolite L-2 Hydroxyglutarate (L2HG) in RCC. It is currently unknown if we can utilize metabolic liabilities created by oncometabolites for personalized RCC therapy.

OBJECTIVES: The primary objective of this study is to understand the impact of reduced Activating Transcription Factor 4 (ATF4) and reduced non-essential amino acid biosynthesis.

METHODS: This project analyzed normal renal cell line HK2 and renal cancer cell lines (RXF-393, OS-RC-2, A498, 786O, 769P, Caki1, Sn-12Pm6, and A704) using lentiviral transgene or knockdown expression. Proliferation assays were counted over 4 day periods and inhibitor experiments were done at 10mM. Data was analyzed via real-time PCR and western blot. Patient samples were obtained through proper procedures at UAB.

RESULTS: ATF4 is a transcription factor shown to play a role in the amino acid starvation response and apoptosis. Here we identify that L-2HG decreases ATF4, as well as the ATF4 target Phosphoglycerate dehydrogenase (PHGDH). PHGDH, the first and rate-limiting step in the serine synthesis pathway, is commonly reduced in both RCC patient samples and several RCC cell lines. L2HGDH knockdown or re-expression decreases or increases ATF4 and PHGDH levels respectively. Serine and glycine starvation significantly decreased proliferation in RCC cell lines with reduced PHGDH but not in RCC cell lines with higher basal PHGDH.

CONCLUSION: L2HG controls de novo serine synthesis in RCC cell lines via ATF4. Targeting serine and glycine transports looks to be a promising direction for novel, personalized RCC treatments.

33 Exploring neural dynamics of arousal modulation under anesthesia
Jessica B. Briscoe

Exploring neural dynamics of arousal modulation under anesthesia

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Modulation of the arousal state to perform surgical procedures began with the administration of an ether vapor in the mid-nineteenth century, paving the way for pain-free surgical procedures. The drive to optimize anesthetic use during surgery lead to the discovery of various com-

pounds found to be responsible for modulating consciousness, arousal and pain. Modulation of arousal states, however, is also associated with sleep and neurological disorders, like narcolepsy, psychiatric disorders, epilepsy, etc. The underlying mechanisms of arousal state modulation by either anesthetics, sleep or disease are not widely understood. As our aim is to identify key neural pathways and mechanisms responsible for modulation of arousal states, we explore a wide array of anesthetic and sedative drugs with differing modes of action to probe their effects on neural dynamics. Recently, studies have shown that each anesthetic will produce a specific neural dynamic during the unconscious state. This may indicate that while all anesthetics may cause unconsciousness, the means of its production, through which brain regions are affected, may be wide-ranging. Research comparing neural mechanisms across different anesthetics may be able to assess similarities and provide a common pathway for producing different arousal states. In the present study, rodents were administered one of four anesthetics: Isoflurane, dexmedetomidine, propofol and midazolam), while local field potentials were recorded in the awake state in prefrontal cortex and anterior thalamus, during loss-of-consciousness and throughout recovery of consciousness. Utilizing analytical methods, such as inter- and intra-regional phase-amplitude coupling, phase-locking, coherence and granger causality, we attempt to elucidate how brain regions coordinate to produce the different types of loss and recovery of consciousness.

34 Mechanisms of transcriptional regulation by myeloid translocation gene 16 (MTG16) in the intestine
Rachel E. Brown

Mechanisms of transcriptional regulation by myeloid translocation gene 16 (MTG16) in the intestine

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Inflammatory bowel disease (IBD), which affects nearly 1.5 million people in the United States, is a known risk factor for colorectal cancer (CRC) due to chronic intestinal injury leading to mutations and epigenetic alterations in the intestinal epithelium. Myeloid translocation gene on chromosome 16 (MTG16) is a transcriptional co-repressor first identified in translocations driving acute myeloid leukemia. *Mtg16*$^{-/-}$ mice exhibit aberrant secretory lineage differentiation and increased proliferation at baseline, are sensitized to dextran sodium sulfate (DSS)-induced colitis, and develop higher tumor burden in the azoxymethane/DSS model of inflammatory carcinogenesis. MTG16 contains highly conserved Nervy homology regions (NHR1-4) that orchestrate the formation of repression complexes via protein-protein interactions. We hypothesized that the NHRs of MTG16 coordinate repression of transcription programs required for intestinal epithelial maintenance and response to injury. We identified MTG16 occupancy of an enhancer in intron 1 of *LGR5*, a gene important in the maintenance of the intestinal stem cell compartment. *In vivo* studies using the *Lgr5*-EGFP-IRES-creERT2 reporter mouse indicated expansion of the LGR5^{+} intestinal stem cell population in the absence of *Mtg16*, implicating MTG16 as a previously unknown regulator of *Lgr5*. Additionally, we performed a yeast two-hybrid screen for MTG16 binding partners and identified novel interactions with the transcription elongation-associated proteins DOT1L, AFF4, and MLL1. Deletion of NHR4 disabled the MTG16:DOT1L and MTG16:AFF4 interactions, while deletion of NHR3 and NHR4 disabled MTG16:MLL1 complex formation. However, *Mtg16*$^{\Delta NHR4}$ mice did not exhibit significantly increased susceptibility to DSS-induced colitis. These data suggest that MTG16 NHR4 may bind to elongation factors (in addition to putative interactors such as NCoR and SMRT), but this is not crucial to its regulation of intestinal epithelial regeneration in response to injury. Further work will investigate the roles of MTG16 NHR1, NHR3, and NHR4 in intestinal *Lgr5* expression, homeostasis, injury, and tumorigenesis. This work may ultimately help us understand the mechanisms behind IBD and CRC.

35 How the gut senses calories
Kelly L. Buchanan

How the gut senses calories

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One of the most satisfying, yet dangerous, things we do everyday is eat. Overconsumption is linked to diseases including obesity. However, how the gut transduces caloric content of nutrients to the brain remains unknown. Recent studies have shown that post-ingestive signaling from sucrose can elicit a robust preference. But sucralose, a non-nutritive sweetener, does not have the same effect. We believe this discrepancy occurs at the level of sensory transduction at the gut epithelium. The sensory epithelial cell of the gut is the enteroendocrine cell. Though classically studied from an endocrine perspective, we recently discovered that a subset of enteroendocrine cells synapse with vagal neurons. We call them neuropod cells. These cells transduce glucose stimuli using glutamate as a neurotransmitter. Here, we sought to define how the neuropod cell–brain circuit senses the calories from sugar to drive sugar preference. This sensory transduction mechanism forms the basis of a gut sensor for calories.

First, we determined how small intestinal neuropod cells sense and transduce nutritive versus non-nutritive sugars. In whole nerve recordings of the cervical vagus, optogenetic silencing of small intestinal neuropod cells abolished the vagal response to both intraluminal sucrose

and sucralose infusions. Because these cells are necessary to sense and synaptically communicate both caloric and non-caloric sugars, we next defined how neuropod cells sort the signals. Using pharmacological inhibition of nutrient receptors, we found that the sodium glucose co-transporter SGLT1 is necessary for sucrose sensation while the sweet taste receptor T1R2/3 is responsible for sucralose sensation. In addition, by inhibiting glutamate receptors, we found that glutamate release depends on SGLT1 activation. Taken together, these results show that sugar calories activate SGLT1 to trigger glutamate release from neuropod cells. Finally, we sought to determine the role of neuropod cells in preference of caloric over non-caloric sugars. We first used an automated phenotyping system to determine the minute-by-minute development of a sugar preference. When given a choice between a sucrose and sucralose solution, mice develop a strong preference for sucrose over sucralose in the first ten minutes of exposure. These data suggest a post-ingestive signal capable of rapid communication contributes to the development of preference. We next adapted optogenetic tools widely used to probe behavior in the brain, to the gut. This allowed us to specifically target neuropod cells in awake, behaving mice. When neuropod cells are optogenetically silenced, the mice's preference for sucrose over sucralose was significantly reduced. These data show that neuropod cells sense and communicate sugar calories and that inhibition of these cells greatly attenuates calorie preference. This neuroepithelial circuit represents a therapeutic target to alter the sensory transduction of calories from gut to brain and to modulate ingestive choices.

36 The oncogene-induced translational landscape alters stem cell fate choice to restrain oncogenic growth
Elise Yi Cai

The oncogene-induced translational landscape alters stem cell fate choice to restrain oncogenic growth

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Oncogenic lesions are surprisingly common in morphologically and physiologically normal human skin. The epidermis must possess adaptive mechanisms to restrain oncogenes' cancer-driving potential. Recently, our group has revealed that oncogene activation induces stem cell differentiation in response to elevated stem cell proliferation, suppressing tumorigenesis and maintaining long-term epidermal homeostasis. However, the molecular mechanisms behind oncogene-induced differentiation are largely unknown. Intriguingly, the fundamental process of protein synthesis has recently been implicated in stem cell regulation during development and cancer. How oncogene activation alters a stem cell's translation program to disrupt growth and normal fate decisions remains unclear. We examined translational regulation during oncogenic activation of *Hras1*, which is frequently mutated across human cancers. Using a $Hras^{G12V}$ mouse model of squamous cell carcinoma, we established that epidermal $Hras^{G12V}$ activation simultaneously induces progenitor cell differentiation and proliferation. *In vivo* functional screens of translation machinery genes identified initiation factor *Eif2b5* as an oncogene-specific driver of differentiation and proliferation, suggesting that EIF2B5 mediates the translational landscape necessary for oncogene-induced differentiation. Furthermore, $Hras^{G12V}$ activation increases global translation rate and EIF2B5 activity, resulting in epidermal overgrowth. To dissect how oncogene-induced translational changes alter cell fate choice, we performed genome-wide profiling of the $Hras^{G12V}$ progenitor cell translatome and identified a distinct subset of oncogenic genes that are translationally regulated by EIF2B5. Functional screening of this gene set allowed us to segregate genes that specifically promote either proliferation or differentiation. While proliferation promoters were enriched for expected transcription factors, our screens uncovered surprising enrichment of ubiquitination genes amongst differentiation promoters. In particular, E3 ubiquitin ligase *Fbxo32* specifically drives progenitor cell differentiation without affecting proliferation, reducing $Hras^{G12V}$ growth and delaying papilloma formation. Thus, oncogene-induced differentiation operates through EIF2B5-mediated translation of differentiation promoters, allowing the epidermis to rapidly adapt to elevated stem cell proliferation. Here, we have uncovered the oncogene-induced translational landscape that regulates stem cell fate choice to suppress tumor formation and prolong tissue homeostasis.

37 Insulin acutely potentiates M3 muscarinic receptor function in rat tracheal smooth muscle
Gina Calco

Insulin acutely potentiates M3 muscarinic receptor function in rat tracheal smooth muscle

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Background: Obesity increases incidence and severity of asthma, but the underlying mechanisms are not known, making obesity-related asthma difficult to appropriately prevent and treat. We have shown that hyperinsulinemia, which is common in obese individuals, potentiates parasympathetic nerve mediated bronchoconstriction. Parasympathetic nerves release acetylcholine, which binds to M3 muscarinic receptors on airway smooth muscle, causing airway smooth muscle contraction and bronchoconstriction. Here we tested whether insulin, or insulin-like growth factor 1 (IGF-1), potentiate M3 receptor agonist-induced airway smooth muscle contraction. Methods: Tracheal rings isolated from wild-type Sprague Dawley rats were placed in an organ bath. Methacholine-induced (0.1-100 μM) smooth muscle contractions were measured before and after incubation with either 100 nM insulin or 13.1 nM (100 ng/mL) IGF-1 for 30 minutes to 2 hours. Separately, rat tracheal smooth muscle cells (passage 4 - 6) were grown to confluence and loaded with the calcium indicator Fluo4. Methacholine-induced changes in intracellular calcium were measured in the absence or presence of insulin (10 μM for 3 hours). Changes in intracellular calcium in response to insulin without methacholine were also measured. M2 and

M3 muscarinic receptor mRNA expression was quantified from tracheal smooth muscle cells treated with or without 1 μM insulin for 3 hours. Results: Insulin significantly potentiated methacholine-induced contractions in tracheal rings. In contrast, IGF-1 had no effect on tracheal contractions induced by methacholine. In isolated smooth muscle cells, 1 μM insulin potentiated methacholine-induced increase in intracellular calcium but did not change M2 or M3 mRNA expression in cultured smooth muscle cells. Insulin without methacholine did not acutely increased intracellular calcium. Conclusions: Insulin, but not IGF-1, potentiated methacholine-induced tracheal smooth muscle contraction and increased the intracellular calcium response to methacholine without changing muscarinic receptor expression. These data may explain why obese individuals with hyperinsulinemia are more prone to airway hyperreactivity and give insights into future targets for asthma treatment. Funding: R01HL131525, R01HL113023, R01AR061567, R01HL124165, R01ES017592.

38 Computational Analysis of Antidepressant Drug Effects in a Model of the Monoaminergic Neurotransmitter and Stress-steroid Systems

Mariam B. Camacho

Computational Analysis of Antidepressant Drug Effects in a Model of the Monoaminergic Neurotransmitter and Stress-steroid Systems

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Augmentation of selective serotonin reuptake inhibitor (SSRI) action relies heavily on clinical judgment and involves months to years of trial and error. Unfortunately, 10-30% of depressed patients are resistant to all attempted combinations. Here we developed a computational model of the monoaminergic neurotransmitter and sex-steroid hormone systems in order to identify potentially more effective combinations of antidepressants. Our neuroadaptation model was used to simulate the result of chronic administration of antidepressant drug and drug/hormone combinations on monoamine and stress hormone (cortisol) levels by adjusting the strengths of its transmitter-system components (TSCs). We also evaluated the contributions of individual and pairs of TSCs to therapeutic neuroadapted configurations with chronic SSRI administration, and found that therapeutic neuroadaptation is an overdetermined process that depends on the contributions of multiple TSCs, providing a potential explanation for the clinical observation that no antidepressant drug or drug/hormone combination can be used to alleviate depressive symptomology in all patients.

39 Characterization of somatostatin receptors (SSTRs) expression and anti-proliferative effect of somatostatin analogues in aggressive thyroid cancers

Danilea M. Carmona Matos

Characterization of somatostatin receptors (SSTRs) expression and anti-proliferative effect of somatostatin analogues in aggressive thyroid cancers

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Background: Somatostatin (SST) is an inhibitory peptide of natural and ubiquitous presentation in our body that exerts is action by binding somatostatin receptors 1-5 (SSTR1-5). Several human carcinomas have demonstrated distinct expression somatostatin receptors (SSTRs) and have since provided the possibility of diagnostic imaging and therapy with radiolabeled SST analogs (e.g. octreotide, pasireotide and KE-108). Although SSTR expression has been heavily studied in medullary thyroid cancer (MTC) information regarding non-medullary thyroid cancers has been limited and conflicting up until now. The purpose of this study is to characterize SSTR expression in non-medullary thyroid cancers and assess the anti-proliferative effects of somatostatin analogues in them. Methods: Proteins from aggressive anaplastic (Hth7 and 8505c) and follicular (FTC236) thyroid cancer cells were isolated and analyzed for basal expression of SSTR1-5 using capillary immunoblotting system followed by densitometry analysis. The basal mRNA expression levels of SSTR1-5 were measured by quantitative real-time PCR (qRT- PCR). All cell lines were treated for two days with one of three SST analogues: octreotide (OCT), pasireotide (SOM230), and KE108. The anti-proliferative effect and IC50 values were determined using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Expression of SSTR2 was examined in human thyroid tissue microarrays. Results: Capillary immunoblotting analysis demonstrated that all thyroid cancer cell lines expressed SSTR1, SSTR2, SSTR3, and SSTR5 in varying degrees. SSTR3 demonstrated the highest expression among all cell lines while none of them expressed SSTR4. qRT-PCR analysis confirmed the correlation between mRNA expression for SSTR2 and SSTR3 with these proteins. In human primary thyroid samples, SSTR2 was absent in 10 normal thyroid tissues but present in 3 aggressive human thyroid cancers. MTT assay showed that KE108, a pan- somatostatin receptor agonist, demonstrated an $IC_{50}$of 24 uM for 8505c and 100uM for Hth7 and FTC236 cells. SOM230, an SSTR5, SSTR3 and SSTR2 agonist, demonstrated an $IC_{50}$of 50uM for FTC236 and 75 uM for 8505c and Hth7 cells. However, OCT, a SSTR2 agonist, did not inhibit the proliferation of any cell line below the concentration of 250uM. Conclusions: Aggressive anaplastic and follicular thyroid cancer cell lines and human tumors express somatostatin receptors. SST analogs KE108 and SOM230 exhibited the best anti-proliferative activity among these dedifferentiated thyroid cancer cell lines. Our results suggest that somatostatin receptor subtypes (SSTR1-SSTR3 and SSSTR5) are relevant and promising therapeutic targets for aggressive thyroid cancers.

40 Delocalization of GABAergic Synapses After Strain Specific Toxoplasma Gondii Infection
Naomi Carter

Delocalization of GABAergic Synapses After Strain Specific *Toxoplasma Gondii* Infection

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Toxoplasma gondii is an obligate intracellular parasite that infects ~25% of the US population and can cause Toxoplasmosis in immunocompromised individuals. Healthy individuals infected by *Toxoplasma gondii* also exhibit a higher risk of developing neuropsychiatric diseases such as schizophrenia. *Toxoplasma gondii* specifically infects neurons in the brain and infected animals develop seizures suggesting alterations in brain circuitry. Previous studies from our laboratory have shown that certain strains of *Toxoplasma gondii* impair the distribution of the inhibitory neurotransmitter GABA. Here, we performed a blinded analysis of how various strains of *Toxoplasma gondii* contribute to the mislocalization of glutamic acid decarboxylase (GAD67), the essential enzyme that catalyzes GABA synthesis within inhibitory neurons. After cryosectioning brains infected with various hybrid strains of *Toxoplasma gondii*, immunohistochemistry was used to explore the distribution of GAD67 and vesicular glutamate transporter 2 (VGluT2), a marker of excitatory nerve terminals that served as a control in these experiments. We focused our attention on the CA1 region of the hippocampus, where excitatory and inhibitory synapses are restricted to different sublamina. Murine brains were imaged using epifluorescence and confocal microscopy and acquired images were analyzed according to their signal intensity using ImageJ software. Image analysis revealed similar ratios of GAD67 signal intensity between the stratum pyramidale and the surrounding tissues suggesting that the brains analyzed were infected with a more virulent strain of *Toxoplasma gondii*. The next step is to analyze whether these same strains of *Toxoplasma gondii* lead to increased seizure susceptibility or altered behaviors in mice.

43 Neuroleptanalgesia for Acute Abdominal Pain: A Systematic Review
Alberto A. Castro Bigalli

Neuroleptanalgesia for Acute Abdominal Pain: A Systematic Review

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BACKGROUND: Acute abdominal pain (AAP) is the most common reason for U.S. emergency department (ED) visits (6-10%), and the incidence is rising. Admission rates approach 25%, and the majority (44% - 59%) of patients are treated with an opiate/opioid analgesic. Administration of opioids (with later prescription) in the ED has been linked to an increased risk of becoming a recurrent opioid user. Current practice is moving towards recent U.S. Food and Drug Administration goals that emphasize adequate pain control with increased use of multimodal pain regimens and decreased opioid use. Butyrophenones are a subclass of neuroleptic antipsychotic drugs, and their use for analgesia dates back to the 1970s. Haloperidol is believed to exert its analgesic effects, and synergistic analgesic effects, through modulation of NMDA-receptors as well as sigma-1 receptors. Neuroleptanalgesia involves combining an opiate with a neuroleptic drug (eg. haloperidol, droperidol) for analgesia.

OBJECTIVE: The objective of this project is to address the following research question: In patients with acute abdominal pain (Population) does administration of butyrophenone antipsychotics (Intervention) compared to placebo, usual care, or opiates alone (Comparisons) improve analgesia and decrease opiate consumption (Outcomes)?

SEARCH METHODS: A structured search was performed of Cochrane CENTRAL, CINAHL, DARE, DOAJ, Embase, IEEE-Xplorer, LILACS, Magiran, PubMed, SID, Scopus, TÜBİTAK ULAKBİM, and Web of Science. Relevant bibliographies and conference proceedings were also searched. Searches were not limited by date, language, or publication status. To limit publication bias, clinical trial registries were searched (ClinicalTrials.gov, WHO ICTRP, ANZCTR).

SELECTION CRITERIA: Three authors (ACM, AMK, AACB) reviewed the titles and abstracts to determine eligibility for inclusion based on relevance. Eligible studies were prospective randomized clinical trials enrolling patients (age ≥18 years) with AAP treated in acute care environments (ED, ICU, post-operative). The butyrophenone must have been administered either intravenously or intramuscularly. Comparison groups included placebo, opiate only, corticosteroids, non-steroidal anti-inflammatory drugs (NSAID), or acetaminophen.

MAIN RESULTS: We identified 7217 references. No ongoing studies were identified. Six studies were included: one assessing ED patients with AAP associated with gastroparesis, and five assessing post-op patients with AAP including: abdominal hysterectomy (n=4), sleeve gastrectomy (n=1). In ED patients with AAP, neuroleptanalgesia improved analgesia and decreased opiate consumption, while also decreasing ED length-of-stay and admission requirements. Results were particularly pronounced for patients with gastroparesis, cyclical vomiting syndrome, and cannabinoid hyperemesis. In post-operative patients, adding either haloperidol or droperidol to the standard PCA regimen improved patient analgesia and satisfaction with the analgesic regimen, and de-

creased opiate consumption without increasing adverse effects.

CONCLUSION: Based on available evidence, we cannot draw a conclusion on the efficacy or benefit of neuroleptanalgesia in the management of patients with acute abdominal pain, however preliminary data suggests that it may improve analgesia and decrease opiate consumption.

44 Impairment of pigmentation and genomic stability in the development of frequent basal cell carcinomas
Warren H. Chan

Impairment of pigmentation and genomic stability in the development of frequent basal cell carcinomas

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Several cancer-resistance mechanisms, including DNA-repair, tumor-suppressor genes, epigenetic stabilization, pigmentation and apoptosis have evolved to maintain genomic integrity and protect against skin cancer formation. We previously showed that mutations in DNA repair genes are enriched in patients with frequent BCC development by analyzing a panel of 29 DNA-repair genes in 61 patients who develop frequent BCCs. Here, we expand our study to analyze 124 highly-penetrant cancer susceptibility genes in this high frequency BCC (hfBCC) cohort. We found that 69% of patients carried pathogenic mutations in 34 cancer susceptibility genes. 23 of the 34 genes were DNA repair genes, including BRCA1, FANCL, MLH1, MSH2, PMS2, RAD51C, RECQL4, and WRN. Interestingly, 67% of patients carried deleterious mutations in pigmentation genes, MC1R and TYR, compared to 6% in the Exome Aggregation Consortium, highlighting a strong association between pigmentation and frequent BCC development. Mutations in the melanocortin 1 receptor (MC1R) were particularly enriched in our cohort, presenting in 64% of patients. MC1R directs pigment synthesis and nucleotide excision DNA repair in melanocytes; abolishment of its protective functions has been implicated in the red hair color trait and polymorphisms in MC1R have been associated with BCC development. The striking enrichment in pigmentation mutations, including those that disrupt DNA repair mechanisms, reveals a mechanistic interplay between pigmentation and carcinogenesis and sheds light on a possible role of pigmentation in hfBCC pathogenesis.

45 The effect of LRRK2-G2019S expression in an α-synuclein fibril induced model of Parkinson disease
Sidhanth Chandra

The effect of LRRK2-G2019S expression in an α-synuclein fibril induced model of Parkinson disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder worldwide. However, mechanisms of PD are still poorly understood. Mutations in leucine rich-repeat kinase 2 (LRRK2) are among the most common genetic causes of neurodegeneration. Specifically, the G2019S mutation in LRRK2 is the most common genetic cause of Parkinson's disease (PD). Pathogenic mutations in LRRK2, such as G2019S, upregulate LRRK2 protein kinase activity ~3-5 fold. This abnormal increase in kinase activity is thought to be the mechanism whereby LRRK2 is responsible for pathological features associated with PD, such as dopaminergic neurodegeneration, α-synuclein (α-syn) protein aggregation, and neuroinflammation in patients with LRRK2 mutations. Previously, our group and others have reported that the G2019S mutation may accelerate neuropathology formation in various models of PD. However, there has historically been a lack of PD animal models that develop pathology similar to humans with PD.

The discovery that mutations in the *SNCA* gene, which code for α-syn protein, cause PD and the fact that α-syn the primary constituent of protein aggregates found in post-mortem PD brains have led to the development of preclinical models emphasizing pathologic α-syn, such as the α-syn preformed fibril model. Preformed fibrils (PFFs), generated from recombinant α-syn, are able to seed the recruitment of endogenous α-syn into aggregates without the need for over expression. PFFs have been shown to cause development of human-like PD neuropathology in neurons, mice, and rats. However, there has never been a comprehensive time course study of PFF induced neuropathology formation.

Herein, we evaluated the effect of G2019S-LRRK2 expression on PFF induced neuropathology in rats at 1, 3, and 6-month time points. We utilized LRRK2-G2019S rats engineered using bacterial artificial chromosome (BAC) technology. We report injection of PFFs into the substantia nigra pars compacta causes progressive neurodegeneration, protein aggregation and spread, and neuroinflammation in both G2019S and nontransgenic rats. However, we found that PFF injected G2019S rats did not have more severe dopaminergic neuron loss, aggregate spread, or macrophage infiltration than PFF injected nontransgenic rats. This finding contradicts results in models overexpressing α-syn and PFF primary neuron models. However, the PFF rat model is perhaps the most physiologically relevant model of PD to date. Our findings will inform future preclinical studies utilizing PFFs *in vivo* and show that G2019S-LRRK2 expression does not accelerate neuropathology formation.

46 ALR protein, a critical protein in cardiac development, regulates cellular iron homeostasis by altering mitochondrial import of ATP-binding cassette (ABC)-B8
Hsiang-Chun Chang

ALR protein, a critical protein in cardiac development, regulates cellular iron homeostasis by altering mitochondrial import of ATP-binding cassette (ABC)-B8

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Introduction: Iron is an essential molecule for normal cellular physiology,

and altered cellular iron homeostasis is commonly observed in diseases with disruption of iron/sulfur (Fe/S) cluster maturation, such as cardiomyopathy associated with Friedreich's ataxia. Inhibition of Augmenter of Liver Regeneration (ALR), a mitochondrial inter-membrane-space protein involved in mitochondrial protein import, results in cardiac developmental defect in zebrafish, and its mutation is associated with increased oxidative stress and cytosolic Fe/S cluster maturation defects. ABCB8 is one of only two mitochondrial membrane proteins known to regulate cytosolic Fe/S cluster maturation. We hypothesized that ALR is critical for cytosolic Fe/S cluster maturation and iron homeostasis by regulating mitochondrial import of ABCB8.

Results: Downregulation of ALR *in vitro* resulted in reduced cytosolic Fe/S cluster-containing enzyme activities and increased cellular iron uptake. Using a knockdown-rescue approach, we further demonstrated that only the mitochondrial, but not the cytosolic, ALR isoform is involved in the maturation of cytosolic Fe/S clusters. Because Fe/S clusters are synthesized in the mitochondria, we then assessed whether ALR can alter the levels or activity of ABCB7 and ABCB8, the two mitochondrial proteins known to regulate the maturation of cytosolic Fe/S clusters. Downregulation of ALR reduced the mitochondrial levels of ABCB8, while ABCB7 levels were not affected. We further demonstrated that ABCB8 physically interact with the protein import system consisting of ALR and Mia40, and that a reduction in ALR results in defective import of ABCB8 into mitochondria.

Conclusion: Our results indicate that ALR and its interaction partner Mia40 are involved in the transport of ABCB8 into the mitochondria, which in turn regulates cytoplasmic Fe/S cluster maturation. These findings provide insights into cellular iron regulation, with implications in cardiovascular disease and cardiac development.

47 Plasticity of Rhythmic and Modulatory Respiratory Axons Drives Diaphragm Recovery After Focal Brain-Derived Neurotrophic Factor Upregulation
Brittany A. Charsar

Plasticity of Rhythmic and Modulatory Respiratory Axons Drives Diaphragm Recovery After Focal Brain-Derived Neurotrophic Factor Upregulation

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Cervical spinal cord injury (SCI) can lead to severe respiratory compromise by disrupting the supraspinal driving force responsible for diaphragm activation. More than half of all human SCI cases occur in the cervical region, where the respiratory neural circuit is located, including the rostral Ventral Respiratory Group (rVRG)–phrenic motor neuron (PhMN) pathway. This circuit is difficult to restore after damage because of intrinsic and extrinsic inhibitory factors inherent to the adult central nervous system (CNS), which limit return of full diaphragm function. We tested a novel strategy to promote targeted plasticity of descending bulbospinal respiratory axons to promote diaphragm recovery after C2 hemisection in rats by upregulating the chemotactic neurotrophin brain-derived neurotrophic factor (BDNF) specifically at the location of phrenic motor neurons (PhMNs) on the side of injury. We assessed diaphragm recovery via *in vivo* electromyography recordings eight weeks after injury. To assess the effect of BDNF on axonal plasticity, we labeled bulbospinal respiratory axons and revealed accompanying neuroplastic changes in the descending axonal populations providing input to PhMNs, including both excitatory and modulatory pathways. Using *in vivo* anterograde tracing methods, we found sprouting of spared rVRG axons from contralateral supraspinal centers in the brainstem around PhMNs on the side of injury. This sprouting was accompanied by an increase in excitatory synaptic colocalization on PhMNs. This excitatory input provided by rVRG axons is likely enhanced by sprouting of modulatory serotonergic axons around the same PhMNs. These exciting data suggest that using BDNF to enhance PhMN reinnervation from spared respiratory pathways is a promising mode of circuit re-connectivity and diaphragm recovery following SCI.

48 Defining the role of IFN signaling in targeted therapy resistant melanomas
Mona Chatrizeh

Defining the role of IFN signaling in targeted therapy resistant melanomas

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The majority of V600E/K mutant BRAF metastatic melanomas treated with MAPK inhibitor (MAPKi) will acquire resistance within a median of one year of treatment initiation. This is despite the fact that most patients initially show a clinical response to BRAF inhibitors (BRAFi) and their combination with MEK inhibitors (BRAFi+MEKi).

MAPKi resistant tumors can evade therapy through genomic and non genomic pathways. Multiple studies, including ours, have shown MAPK pathway reactivation, PI3K pathway activation, and receptor tyrosine kinase upregulation as examples of such resistance mechanisms. In our recent publication, we show that IFN-inflammatory signatures are enriched in the transcriptomic profiles of tumors from patients that are undergoing MAPKi treatment. Intriguingly, we also saw the same activation in vitro in the earliest melanoma clones showing resistance to MAPKi. This led us to speculate that the IFN pathway activation plays a role in MAPKi resistance. In support of this hypothesis, Benci et al have recently shown that tumor interferon signaling orchestrates multigenic resistance to immune checkpoint blockade therapy. Graeber lab (UCLA) have also shown that melanomas respond to interferon gamma (IFN-g) by dedifferentiating to become less vulnerable to drug-induced stress.

This dedifferentiation contributes to acquired resistance to inhibitors and occurs as a response to inflammatory signaling during immunotherapy.

Despite the presence of interferon signaling in the tumor microenvironment and the increase in data showing interferon signaling regulates resistance, most studies are still only based on MAPKi-only treatment. In addition to lack of incorporation of IFN-g to model systems (in vitro or PDXs), there have been no studies showing the effects of a combinatorial MAPKi + IFN-g treatment in melanoma. To this end, we tested the effects of MAPKi and IFN-g treatment on multiple patient-derived melanoma cell lines. We observed that the combination induced a distinct transcriptomic program from that induced by either MAPKi or IFN-g alone. We now have sent these treated melanoma cells for comprehensive transcriptomic analysis. Our preliminary analysis showed that the transcriptomic signatures of MAPKi and MAPKi + IFN-g treated cells were significantly different. To expand the generality of our result, we are planning to determine the synergistic and antagonistic effects of IFN-g treatment in combination with other conventional drugs. Still, further analysis of interferon signaling and exploitation of findings is needed to improve the efficacy of current therapies in melanoma.

49 Exploration of long non-coding RNAs as synthetic essential targets in Pten-deficient cancers
Jasper Chen

Exploration of long non-coding RNAs as synthetic essential targets in Pten-deficient cancers

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PTEN is one of the most frequently inactivated tumor suppressor genes across all cancer types. The loss of PTEN activates the PI3K/AKT pathway, which inhibits GSK3β, thereby stabilizing Myc, which recruits histone acetyltransferases to increase chromatin accessibility of genes involved in both cell proliferation and apoptosis. Among these histone acetyltransferases, the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex preferentially acetylates (ac) histone H3 lysine 9 (H3K9) and histone H4 lysine 16 (H4K16). A pan-cancer analysis of mutually exclusive gene inactivation patterns identified a previously uncharacterized long non-coding RNA (lncRNA) as synthetic essential in the context of PTEN deficient cancer. PTEN knockout induces overexpression of the lncRNA in SF-763, LN-229, and T47D cell lines. The promoter region of the lncRNA contains binding sites for the Myc transcription factor, suggesting that it is regulated by PTEN through Myc. Key regulatory elements and functional domains were found to be conserved in a putative mouse homolog, further supporting the functional importance of this lncRNA across different species. The lncRNA contains numerous repeat elements interspersed by non-repeat domains. We used CRISPR to excise the most highly conserved domain in the lncRNA, corresponding to a LINE1 transposable element. Concurrent knockout of PTEN and the lncRNA impaired cell viability and proliferation, induced chromosome tetraploidy, and enriched expression of Myc target genes. Excessive Myc signaling induces replication stress and causes profound genomic instability in cancer cells. Genomic instability is a double-edged sword for cancer; it can both generate de novo mutations that provide survival advantages and cause irreparable chromosomal damage that leads to cell death. Cancer cells can only survive if they develop strategies that can help them effectively manage genomic instability; such strategies can involve suppressing the DNA damage response pathways that trigger cell death or even limiting the extent of genomic instability altogether. Chromatin isolation by RNA purification mass spectrometry identified the SAGA subunit TADA2B as a potential binding partner of the lncRNA. This led us to probe H3K9ac and H4K16ac levels to characterize SAGA activity. We found that knockout of the lncRNA led to elevation in both H3K9ac and H4K16ac. These histone acetylation marks are associated with euchromatin, result in greater chromatin accessibility, and are typically suggestive of active gene transcription. Excessively loose chromatin conformation could then lead to genomic instability. I hypothesize that this lncRNA helps cancer cells survive by inhibiting SAGA, resulting in two outcomes: 1. Reduction in coactivation of Myc-induced cell proliferation and apoptosis, thereby limiting the genome destabilizing effects of Myc overexpression and inhibiting apoptotic signaling. 2. Reduction in histone acetylation, thereby promoting chromatin condensation and genomic stability.

50 Genetic and antigenic characterization of human respiratory syncytial virus F and G proteins
Yihui Chen

Genetic and antigenic characterization of human respiratory syncytial virus F and G proteins

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Human respiratory syncytial virus (RSV) is the leading cause of severe lower respiratory tract infection in infants and young children. Though no licensed vaccine is available at present, several candidate vaccines based on the fusion (F) and attachment (G) surface proteins are in phase 1-3 clinical trials. RSV circulates as two antigenically distinct subtypes, RSVA and RSVB; each subtype consists of several identified genotypes that co-circulate globally. Information on the correlation between genetic and antigenic diversity of RSV F and G might educate future vaccine approaches in order to maximize cross-protection against multiple circulating RSV strains. All full genome sequences for RSVA (n=690) and RSVB (n=453) were downloaded from public resources and annotated with date and location of isolation. RAxML v7.2.8 was used to infer maximum likelihood (ML) trees for both datasets, and TreeTime (https://github.com/neherlab/treetime) was used to scale the ML trees by time as well as to infer ancestral nucleotide sequences at internal nodes. Nonsynonymous substitutions in the F and G genes occurring at each node were obtained using custom R and Biopython scripts, and correlated to previously described antigenic regions. Phylogenetic analysis indicates that RSVA and RSVB have distinct clades that co-circulate globally and that correlate with known genotypes. In line

with the relative conservation of RSV F, a small number of mutations in antigenic sites were detected that are located at described epitopes. In comparison to RSV-F, RSV G has more mutations. These are mainly located in the mucin-like hypervariable region, but to a lesser extent in the central conserved domain, which is the main target of anti-G neutralizing antibodies. The clade-defining mutations identified in this study will form the basis of studies on the antigenic variation of RSV as measured by virus neutralization or similar methods.

51 Dissecting the genetic architecture of fetal hemoglobin expression
Aaron Cheng

Dissecting the genetic architecture of fetal hemoglobin expression

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Inducing production of fetal hemoglobin (HbF) is a promising therapeutic approach to ameliorate disease severity in beta-thalassemia and sickle cell disease. While studies have characterized the individual genetic factors affecting fetal hemoglobin levels and begun to elucidate some underlying mechanisms, a full understanding of how these elements interact to influence overall fetal hemoglobin expression levels has yet to be achieved. We hypothesize that fetal hemoglobin expression is the result of complex genetic architecture involving the interaction between multiple common and rare genetic variants. To interrogate the underlying genetic architecture of this complex and clinically-relevant trait, we have performed a large genome-wide association study (GWAS) from the tails of a distribution (995 controls with HbF BCL11A, *HBS1L-MYB*, and *HBB*. Moreover, several novel loci and rare variants, including unique structural variants, appear to be present in our study. We are integrating whole genome sequencing on a subset of samples and in general population controls to better define these loci using imputation approaches, and we will account for the aggregate contribution of rare variants with large effects, including the structural variants we have identified. This work has tremendous promise to improve our understanding of how HbF levels can vary in populations, characterize underlying mechanisms by which this clinically-important factor is regulated, and more generally elucidate how a range of allelic variants can collectively contribute to the genetic architecture of a complex trait.

52 Chromosome bridge resolution requires mechanical forces from actin-based contractility
Anna M. Cheng

Chromosome bridge resolution requires mechanical forces from actin-based contractility

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Chromosome bridges result from errors in cell division and form chromatin threads that connect daughter nuclei after division. These bridges eventually break ("resolve") and the daughter cells inherit broken chromosome fragments. This is thought to initiate a major pathway for oncogene amplification and tumor genome evolution called the "breakage-fusion-bridge" (BFB) cycle. However, we still lack a complete understanding of the mechanism(s) causing chromosome bridges to break in the first place. Here we present new evidence that bridge breakage requires actin-dependent contractile forces. As daughter cells connected by a bridge move away from each other, the bridge is typically stretched over long distances before breakage. Using fibronectin micropatterns to limit daughter cell separation, we were able to block bridge resolution, with over 90% of bridges still intact as the daughter cells entered the next mitosis. In cells not constrained by micropatterns, bridge resolution was similarly blocked by timed addition of inhibitors of actin contractility. We propose that mechanical forces from actin-based contractility play a central role in bridge resolution.

We are also studying the genomic consequences of bridge breakage. BFB cycles have been observed in association with another form of localized mutagenesis called chromothripsis. It has also been shown that individual cells experiencing telomere dysfunction can grow into clonal populations with chromothripsis. These findings suggest a mechanistic link between bridge breakage and chromothripsis, but the details of this relationship are unclear. To address this question, we are using our "Look-seq" approach, which combines long-term imaging with single-cell sequencing. We will discuss whether bridge breakage occurs directly via chromothripsis, or if the relationship is indirect, with chromothripsis occurring as a downstream consequence perhaps through formation of micronuclei in subsequent cell cycles.

53 Development of anti-KIT antibodies and immunotoxins as therapeutics and hematopoietic stem cell transplantation (HSCT) conditioning agents for Pediatric Acute Myeloid Leukemia (AML)
Corey K. Cheung

Development of anti-KIT antibodies and immunotoxins as therapeutics and hematopoietic stem cell transplantation (HSCT) conditioning agents for Pediatric Acute Myeloid Leukemia (AML)

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Acute myeloid leukemia (AML) is a frequent type of hematologic malignancy affecting thousands of children worldwide. Unfortunately, these patients experience poor outcomes with an overall survival of only ~ 50-60%. Moreover, patients experience profound toxicity from the harsh conventional chemotherapy +/- hematopoietic stem cell transplantation (HSCT) used to treat AML today.

To address this clinical need, we developed several new monoclonal antibody (mAb) therapies that target CD117, a highly-expressed receptor on many hematopoietic stem cells (HSCs) and progenitor cells. We have previously shown these antagonistic anti-hCD117 mAbs and hCD117-antibody-drug-conjugates (ADCs) can potently and safely deplete HSCs and certain CD117+ progenitor cells. Given similarities between HSCs and AML leukemic stem cells (LSCs), we hypothesize that these anti-CD117 mAbs may similarly deplete AML LSCs and blasts *in vitro* and *in vivo* leading to potential curative AML treatments.

Here, we show that both AML cell lines (NOMO-1, HL60 and HEL) and primary pediatric AML LSCs have high CD117 expression (> 50% and > 90% respectively). Additionally, we show that anti-hCD117 clone SR1 is an antagonistic mAb that inhibits stem cell factor (SCF) binding, whereas 104D2 clone is non-antagonistic. Interestingly when developed into ADCs through biotin-streptavidin conjugation to saporin (SAP), a ribosome-inhibitor, 104D2-SAP had equal potency as SR1-SAP. Specifically, we found that at 100nM, both hCD117-ADCs led to > 200% increase in *in vitro* cell death of NOMO-1 cells compared to media control (p

Additionally, *in vivo* efficacy of these anti-CD117 mAbs is being explored in xenograft models with and without HSCT. NOMO-1, HL-60 and HEL were all found to retain > 70% CD117 expression post-transplantation into immunodeficient NSG mice expressing hIL-3, hGM-CSF and hSCF. In preliminary experiments, incubation of NOMO-1 cells in 1 uM SR1-SAP was found to completely inhibit engraftment of these cells, whereas incubation with 1 uM naked SR1 had no effect (p = 0.54). Ongoing experiments are exploring *in vitro* efficacy of these mAbs on primary AML patient samples pre- and post-transplantation into NSG mice.

These studies explore whether such agents may be used as standalone drugs or conditioning agents in combination with HSCT in models of AML. If promising, we aim to quickly advance this approach to AML patients, as each of these mAb strategies is in advanced clinical development with naked antagonistic anti-hCD117 mAb AMG191 already being tested in clinical trials for another disease at our home institution. Through such work these therapies may become important future curative AML treatments.

54 HHLA2 is a Tumor-Expressed B7 Family Member the Regulates Tumor Immunity
Jordan M. Chinai

HHLA2 is a Tumor-Expressed B7 Family Member the Regulates Tumor Immunity

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HHLA2 is the newest B7 family member and regulates T cell function. Its expressions has been observed on a variety of cancers but only few normal tissues. The purpose of this study was to assess the function and expression-regulation of HHLA2 in tumors. The function of HHLA2 in the tumor microenvironment is not well understood because it has been reported to both inhibit and stimulate T cells in vitro. The mechanisms controlling its expression on cancer cells are also presently unknown. Challenges to addressing these questions include the lack of a functional HHLA2 gene in mice. Here we assess factors that drive expression of HHLA2 in tumors and present a humanized mouse model designed to study the function of HHLA2 in the tumor microenvironment. Human cancer cell lines of various origin were found to upregulate HHLA2 in response to cytokine stimulation, hypoxia, and 3D growth. To study the in vivo function of tumor-expressed HHLA2, CRISPR-Cas9 was used to genetically delete HHLA2 from tumor cells in a humanized mouse immunotherapy model. Deletion of HHLA2 in tumor cells led to an enhancement of the T lymphocyte infiltrate in the tumor. In conclusion, expression of HHLA2 is driven by tumor-specific environmental factors and this expression appears to have a suppressive influence on the tumor-infiltrating lymphocytes.

55 Cellular specificity of matrix metalloproteinase activation on accumbens medium spiny neurons during heroin relapse
Vivian Chioma

Cellular specificity of matrix metalloproteinase activation on accumbens medium spiny neurons during heroin relapse

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Heroin abuse is a leading cause of drug overdose-related deaths in the United States, highlighting a need for further research elucidating effects of maladaptive neuroadaptations following prolonged heroin use. Activation of the tetrapartite synapse in the nucleus accumbens core (NAcore), which comprises of pre- and postsynapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. Specifically, degradation of the ECM by activated matrix metalloproteinases (MMPs) is involved in extracellular synaptic remodeling both constitutively and transiently. Following chronic cocaine self-administration, cocaine-extinguished

rats exhibit enduring increases in MMP-2 activity in NAcore compared to controls, and MMP-9 activity is transiently increased during cued reinstatement. Interestingly, heroin-extinguished rats do not show constitutive MMP activity, however, transient increases were elicited after 15 mins of cued heroin seeking. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in the accumbens, it is unknown which specific cell types harbor changes in MMP activity under heroin-extinguished and cued reinstatement conditions. We hypothesized that D1-receptor expressing MSNs express increased colocalization with MMPs during transient cued heroin seeking, while D2-receptor expressing MSNs express increased colocalization following extinction. We used an AAV cre-dependent mCherry virus to transfect accumbens MSNs in D1 and D2 cre-dependent rats and measured the colocalization of activated MMP-2,9 after FITC-gelatin microinjection under extinguished and reinstated conditions. For D1 MSNs, we observed increased MMP-2,9 colocalization with dendritic surfaces in both extinguished and reinstated animals compared to yoked saline controls. While D2 MSNs showed increased MMP-2,9 colocalization only in heroin-extinguished animals, but MMP-2,9 colocalization after 15 min reinstatement was reduced to yoked saline levels. These findings reveal how NAcore extracellular matrix signaling underlying constitutive and transient synaptic plasticity relies in part on specific cell-types.

56 Fish oil-mediated hepatoprotection in parenteral nutrition-induced liver injury is not dependent on the presence of Kupffer cells

Bennet S. Cho

Fish oil-mediated hepatoprotection in parenteral nutrition-induced liver injury is not dependent on the presence of Kupffer cells

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Parenteral nutrition (PN) is a life-saving therapy in children with intestinal failure (IF) due to insufficient bowel length or loss of function. However, long-term PN administration can lead to IF-associated liver disease (IFALD), characterized by cholestasis and hepatic inflammation. IFALD can progress to end-stage liver disease requiring liver transplantation. Soybean oil-based lipid emulsions (SOLE) provided with PN is believed to contribute to the development of IFALD. Our laboratory first demonstrated that fish oil-based lipid emulsions (FOLE) are able to reverse IFALD. Omegaven®, a commercially available FOLE, has recently been approved by the Food and Drug Administration for treatment of IFALD. In a murine model of PN-induced liver injury, we have demonstrated that FOLE protects from hepatosteatosis in a G-protein coupled receptor 120 (GPR120)-dependent manner. GPR120 is a long-chain fatty acid receptor that mediates many metabolic and anti-inflammatory pathways. It is highly expressed in adipose tissue, macrophages, and enteroendocrine L cells in the intestine. GPR120 signaling in Kupffer cells has been shown to mediate many anti-inflammatory processes. The goal of this study was to determine whether the loss of Kupffer cells would abrogate the hepatoprotective effects of FOLE treatment in a murine model of PN-induced liver injury.

C57BL/6 adult male mice were fed *ad libitum* chow or PN diet for 19 days. PN-fed mice were administered either saline, FOLE, or SOLE every other day by tail vein injection. To deplete Kupffer cells, mice were treated with clodronate-laden liposomes via intraperitoneal injection every three days. Control were treated with vehicle liposomes.

Livers, spleens, and kidneys were weighed and stained for hematoxylin and eosin (H&E) histologic analysis. Formalin-fixed liver specimens were stained with antibodies against F4/80, a macrophage marker.

Immunohistochemistry demonstrated significant reduction of F4/80-positive cells in livers from clodronate-treated mice. H&E staining revealed marked steatosis in livers from both clodronate- and vehicle-treated mice fed PN from saline and SOLE groups. Both clodronate- and vehicle-treated mice in chow and FOLE groups exhibited preserved hepatic architecture with no evidence of steatosis.

The results of this study indicate that FOLE-mediated hepatoprotection is not dependent on the presence of Kupffer cells. While GPR120 signaling on Kupffer cells has been shown to mediate hepatoprotective effects of FOLE in hepatic ischemia reperfusion injury and to exert potent anti-inflammatory effects, this study demonstrates that FOLE treatment is able to maintain its hepatoprotective effects after clodronate-depletion of macrophages.

57 Integrative analysis of KIF4A, 9, 18A, and 23 and their clinical significance in low-grade glioma and glioblastoma multiforme

SangYeon Cho

Integrative analysis of KIF4A, 9, 18A, and 23 and their clinical significance in low-grade glioma and glioblastoma multiforme

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To determine the prognostic significance of kinesin superfamily gene (KIF) expression in patients with brain cancer, including low-grade glioma (LGG) and glioblastoma multiforme (GBM), we comprehensively analyzed KIFs in 515 LGG and 595 GBM patients. Among KIFs, KIF4A, 9, 18A, and 23 showed significant clinical implications in both LGG and GBM. The mRNA and protein expression levels of KIF4A, 9, 18A, and 23 were significantly increased in LGG and GBM compared with those in the normal control groups. The mRNA expression levels of KIF4A, 9, 18A, and 23 in LGG were significantly increased in the high-histologic-grade group compared with those with a low histologic grade. Genomic analysis showed that the percent of mRNA upregulation of KIF4A, 9, 18A, and 23 was higher than that of other gene alterations, including gene amplification, deep deletion, and missense mutation. In addition, LGG patients with KIF4A, 18A, and 23 gene alterations were significantly associated with a poor prognosis. In survival analysis, the group with high expression of KIF4A, 9, 18A, and 23 mRNA was significantly associated with a poor prognosis in both LGG and GBM pa-

tients. Gene Set Enrichment Analysis (GSEA) revealed that high mRNA expression of KIF4A, 18A, and 23 in LGG and GBM patients showed significant positive correlations with the cell cycle, E2F targets, G2M checkpoint, Myc target, and mitotic spindle. By contrast, high mRNA expression of KIF9 in both LGG and GBM patients was significantly negatively correlated with the cell cycle, G2M checkpoint, and mitotic spindle pathway. However, it was significantly positively correlated with EMT and angiogenesis. This study has extended our knowledge of KIF4A, 9, 18A, and 23 in LGG and GBM and shed light on their clinical relevance, which should help to improve the treatment and prognosis of LGG and GBM.

58 GPx3 is reduced in Eosinophilic Esophagitis patients and regulates esophageal epithelial homeostasis in a 3D organoid model
Yash Choksi

GPx3 is reduced in Eosinophilic Esophagitis patients and regulates esophageal epithelial homeostasis in a 3D organoid model

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Background: Eosinophilic Esophagitis (EoE) is an inflammatory disorder of the esophagus characterized by basal cell hyperplasia (BCH) that is regulated by oxidative stress. Selenoproteins, which protect against oxidative injury, can affect inflammation in the gut. Glutathione peroxidase-3 (GPx3) is the only known extracellular form of glutathione peroxidase, is expressed in a variety of cells, and is present in plasma. The role of GPx3 in EoE is unknown; we hypothesized GPx3 plays a protective role in EoE.

Methods: GPx3 mRNA levels were measured by qPCR in control (n=4), active (n=6), and remission (n=3) EoE samples. Glutathione peroxidase activity was measured in WT and *GPx3*$^{-/-}$ mouse esophagus (n=4 & 5, respectively). A 3D esophageal organoid model was developed to study the effect of GPx3 on the epithelium. Plating efficiency was determined, size of WT and *GPx3*$^{-/-}$ mouse esophagoids was measured using Image J, and reactive oxygen species (ROS) was measured by flow cytometry using cellROX. Expression of basal cell markers was determined by qPCR or IF. Apoptosis and proliferation after IL-13 treatment was determined by IF.

Results: Active EoE has 2-fold lower GPx3 transcript levels in comparison to controls and patients in histologic remission (*p*GPx3$^{-/-}$ as compared with WT mouse esophagus (0.026 vs. 0.052 nmol NADPH/ug.min, *p* GPx3$^{-/-}$ mice demonstrate increased BCH (28.2 µm vs. 18.9, *p* GPx3$^{-/-}$ esophagoids demonstrate increased plating efficiency compared with WT (92.3 vs. 72.6, *p* p p pGPx3$^{-/-}$ esophagoids have increased expression of basal cell markers CD49f (4.1 fold increase, *p* p GPx3$^{-/-}$ esophagoids also show increased proliferation by phosphohistone H3 (pH3) staining (2.7 vs. 1.1 positive cells/esophagoid, *p* GPx3$^{-/-}$ esophagoids have increased basal cell thickness (1.7x thicker, *p* p GPx3$^{-/-}$esophagoids do not. However, *GPx3*$^{-/-}$esophagoids demonstrate increased apoptosis by cleaved caspase 3 IF staining (5.3 vs. 3 CC3 positive cells per esophagoid, *p* p

Conclusion: GPx3 regulates esophageal epithelial homeostasis and protects from the development of BCH with low dose IL-13 treatment and apoptosis with high dose IL-13 treatment in an organoid model.

59 The splicing factor SRSF2 in myelodysplastic syndrome
Stephanie S. Chou

The splicing factor SRSF2 in myelodysplastic syndrome

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Myelodysplastic syndrome (MDS) is the most common type of acquired bone marrow failure. The hallmark feature of MDS is impaired clonal hematopoiesis resulting in cytopenias and risk of transformation to acute leukemia. Currently, the only curative therapy for MDS is hematopoietic stem cell transplantation. Unfortunately, because the median age of diagnosis is 71-76 years old, many patients are ineligible for transplantation due to comorbidities. Transplantation bears the risk of multiple complications, such as graft versus host disease and post-transplant relapse. Consequently, owing to the paucity of curative options, there exists an urgent need to unravel mechanisms of MDS pathogenesis. MDS is preceded by a premalignant state known as clonal hematopoiesis of indeterminate potential (CHIP), a common condition where an individual acquires somatic mutations in oncogenes or tumor suppressors in the hematopoietic system that act as a 'first hit' in MDS pathogenesis, but that do not significantly impair mature cell output. In some patients, further somatic mutations cause CHIP to progress to MDS, with attendant clinical manifestations of anemia, opportunistic infection, and bleeding due to thrombocytopenia. Splicing factor mutations occur in about 70% of cases of MDS and often arise early in MDS evolution, consistent with aberrant splicing serving an essential role in MDS pathogenesis.

To better understand the role of splicing factor mutations in the development of MDS, we generated human induced pluripotent stem cell (hiPSC) lines harboring a heterozygous missense mutation (P95H) in the splicing factor *SRSF2* using gene editing with Cas9. *SRSF2*^{P95H} hiPSCs maintained pluripotency as assessed by expression of the pluripotency markers OCT4, NANOG and TRA-1-60, and formation of teratomas containing derivatives of all three germ layers. Upon hematopoietic differentiation, *SRSF2*^{P95H} hiPSCs more efficiently generated CD34^{+}CD45^{+} primitive hematopoietic stem and progenitor cells and exhibited more robust primary and secondary clonogenicity in methylcellulose culture compared to wild-type control cells. These results demonstrate a new tool for the study of CHIP/MDS that recapitulates hallmarks of the dis-

ease. Future experiments are aimed at assessing the effects of *SRSF2* mutations on cell proliferation and differentiation *in vivo*. Improved understanding of MDS disease mechanisms will guide the rational design of novel therapeutics for MDS.

61 Novel mechanisms of poly-ADP-ribose polymerase (PARP) inhibitor resistance in BRCA2-deficient cancer cells
Kristen E. Clements

Novel mechanisms of poly-ADP-ribose polymerase (PARP) inhibitor resistance in BRCA2-deficient cancer cells

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Cells deficient in the DNA repair pathway homologous recombination (HR) show particular sensitivity to inhibitors of poly-ADP-ribose polymerases (PARPi). Based on this observation, several PARPi, such as olaparib (Lynparza, AstraZeneca), have been FDA-approved for the treatment of BRCA2-mutated breast and ovarian cancer. Indeed, clinical trials have demonstrated that use of these agents significantly improved progression free survival (PFS), for example from 5.5 months to 19 months in one cohort of ovarian cancer patients harboring BRCA2 mutations (SOLO2 trial). However, even in this trial, only 65% of patients, who were predicted to be genetically susceptible to this treatment, attained 12 months PFS. This indicates that sensitivity to PARPi is mediated by more than simply BRCA2 status. Moreover, investigations into mechanisms governing sensitivity and resistance to PARPi continue to further our understanding of basic DNA repair and replication pathways.

We conducted a CRISPR-knockout screen in BRCA2-deficient HeLa cells as an unbiased approach for identifying proteins whose loss confers resistance to PARPi. Briefly, over 19,000 genes were individually knocked out in BRCA2-deficient cells. Then, these cells were treated or not with olaparib. Sequencing and computational analysis were used to identify which genes were lost most often in cells surviving olaparib treatment, yielding hundreds of potential hits. Hits were tested in multiple cell lines using cellular viability (CellTiter Glo) and apoptosis (AnnexinV) assays as well as measurements of DNA breaks (Neutral Comet Assay). Several of these hits were successfully validated, including the transcriptional repressor E2F7. Then, two major mechanisms of PARPi resistance, namely restoration of HR and protection of stalled replication forks, were investigated using double strand break (DSB) repair reporter assays and DNA fiber combing, respectively. Here, we show that depletion of E2F7 increases the amount of RAD51, a protein downstream of BRCA2 in the HR pathway as well as in the protection of stalled replication forks. Functionally, this corresponds to a rescue of the HR defect caused by BRCA2 deficiency. Additionally, the DNA fiber combing assay, which enables visualization of individual tracts of newly synthesized DNA, revealed that depletion of E2F7 also protects stalled replication forks in a manner dependent on RAD51.

We have identified many potential mediators of PARPi resistance in BRCA2-deficient cells using a CRISPR-knockout screen. We also show that depletion of E2F7 leads to increased resistance to PARPi in BRCA2-deficient cells. E2F7 depletion causes an increase in RAD51 levels and subsequent restoration of homologous recombination as well as protection of stalled replication forks. Our work identifies E2F7 as a novel potential biomarker for PARPi response in BRCA2-deficient cells. Additional studies investigating the relationship between E2F7 levels in patient samples and tumor response to PARPi are needed to determine if this holds true in the clinic.

64 Investigating the mechanism of Teneurin-Latrophilin trans-synaptic adhesion and signaling in synapse formation
Shaleeka Cornelius

Investigating the mechanism of Teneurin-Latrophilin trans-synaptic adhesion and signaling in synapse formation

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Increasing evidence suggests that synaptic cell-adhesion molecules shape neural circuit formation and function. Teneurins are a family of large cell-adhesion molecules involved in embryogenesis, axonal guidance and synapse formation. Teneurins are type-II transmembrane proteins that are comprised of five domains at its C-terminal extracellular region (ECR) and a small intracellular domain. Teneurin mutations in humans have been linked to a spectrum of disorders including essential tremor and microphthalmia. Teneurins form high-affinity trans-cellular adhesion complexes with Latrophilins (Lphns), which are postsynaptic adhesion class G-protein coupled receptors (GPCRs) with emerging roles in input-specific synapse formation. Trans-cellular adhesion of Lphns and Teneurins causes downstream signaling that may regulate aspects of synapse formation. Previous studies have identified the N-terminal Lectin domain of Lphns as the key component for binding to Teneurins. Moreover, Teneurin splicing in the ECR regulates trans-cellular adhesion to Lphn and induction of inhibitory synapse formation. However, the molecular mechanism of Teneurin-Lphn trans-cellular adhesion and how signaling via this complex modulates synapse formation remains poorly understood. Our aim is to define the molecular basis of Teneurin-Lphn trans-cellular interaction using a systematic array of Teneurin mutants and truncations in trans-cellular adhesion assays with Lphns. We will subsequently examine the mechanism of inhibitory synapse formation by Teneurin using mutations that modulate binding to Lphns using artificial synapse formation assays. Understanding the basis of the Teneurin-Lphn trans-synaptic complex may reveal insights into the molecular mechanism of synapse formation and neural circuit assembly.

65 Effect of L-citrulline supplementation on CD4+ T cell responses during pulmonary Mycobacterium bovis BCG immunization

Rebecca R. Crowther

Effect of L-citrulline supplementation on CD4$^+$ T cell responses during pulmonary *Mycobacterium bovis* BCG immunization

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In 2017, the WHO reported that tuberculosis (TB) was the leading cause of death due to a single infectious agent and the 9thoverall leading cause of death worldwide. Presently, TB prevention centers on the live attenuated *Mycobacterium bovis* (*Mb*) BCG vaccine, the only licensed TB vaccine available. Upon vaccination or infection, T cell activation is initiated within the draining lymph nodes (LNs), leading to proliferation, cytokine production, and effector cell differentiation. These T cell responses are dependent on the availability of the amino acid L-arginine (L-Arg). Under L-Arg deprivation, T cells become hyporesponsive, ceasing proliferation and cytokine production. T cells possess the cellular machinery necessary to synthesize L-Arg from L-citrulline (L-Cit), a non-canonical amino acid, via the sequential activity of argininosuccinate synthase (Ass1) and argininosuccinate lyase (Asl). Given the increased incidence of TB infection in HIV-positive patients and that mice lacking T cells are more susceptible to *Mb* BCG infection, it is important to gain a better understanding of the anti-mycobacterial CD4$^+$ T cell response to *Mb* BCG immunization and subsequent TB infection.

We have previously shown that CD4$^+$ T cells rely on L-Arg synthesis from L-Cit for activation, and that L-Cit rescues CD4$^+$ T cell function in the absence of L-Arg. To determine if CD4$^+$ T cell L-Arg synthesis is necessary to support anti-mycobacterial immunity, we adoptively transferred anti-mycobacterial specific WT and *Asl* KO (*Asl*$^{flox/flox}$; Tie2-cre) CD4$^+$ T cells into WT mice; we observed that L-Arg synthesis supports anti-mycobacterial CD4$^+$ T cell accumulation post-*Mb* BCG infection. We therefore hypothesize that CD4$^+$ T cells require L-Arg synthesis for optimal priming following *Mb* BCG vaccination.

Studies on the effect of L-Arg supplementation on the immune response have had mixed results, however L-Cit supplementation has not yet been explored in this context. To determine if L-Cit supplementation will enhance the CD4$^+$ T cell response following *Mb* BCG vaccination, WT mice were intranasally inoculated with *Mb* BCG and received L-Cit supplemented drinking water. The effects of L-Cit supplementation on CD4$^+$ T cell viability, proliferation, accumulation, cytokine production, and effector profile were assessed post-*Mb* BCG infection. As early as 2 weeks post-*Mb* BCG infection, we observed an increase in CD4$^+$ T cell accumulation, activation, proliferation, and cytokine production in mice receiving L-Cit supplemented drinking water. Knowledge from this study, as well as further analyses on how L-Cit affects immune responses, can be used to improve cellular immunity following *Mb* BCG immunization as well as novel TB vaccine strategies currently in development.

66 Nonautonomous requirements for JNK signaling in thalamocortical axon pathfinding

Jessica G. Cunningham

Nonautonomous requirements for JNK signaling in thalamocortical axon pathfinding

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Proper function of the cerebral cortex is necessary for a wide variety of behavioral tasks such as the perception of sensory stimuli, orchestration of body movements, and complex decision-making. The cerebral cortex is able to carry out these tasks via synaptic connections to and from different brain regions. The thalamus is the main sensory relay station in the brain, which sends sensory information from the body to the cortex via thalamocortical axons. These axons begin to grow very early in development, and must traverse a large anatomical route to make final synapses in the cortex. Disruptions to thalamocortical input have been implicated in human diseases such as schizophrenia. In our lab, we have previously shown that the c-Jun N-terminal Kinase (JNK) signaling pathway is required for the correct migration of cortical interneurons during embryonic development. To study the requirement for JNK signaling, we developed a conditional triple knockout (cTKO) mouse model where Jnk1 is deleted from interneurons in mice lacking both Jnk2 and Jnk3. In the current study, we have seen disruptions not only to interneurons, but also to thalamocortical axons. The cells that give rise to thalamocortical axons are not targeted by our conditional deletion of Jnk1, however they must traverse the territory which gives rise to cortical interneurons, which is the same region from which we have removed JNK signaling. In embryonic cTKO cortices, thalamocortical axons are unable to traverse through the JNK-deficient territory, and instead misroute ventrally towards the hypothalamus. This suggests a nonautonomous requirement for JNK signaling in thalamocortical axon pathfinding. In our knockout model, we have collected and analyzed in vivo cortices from a range of developmental time points spanning embryonic day 12.5 (E12.5) to postnatal day 0. The misrouting of axons begins at E12.5, which is when the axons are first beginning to extend. In our model, this phenotype persists all the way to P0, and is unable to recover. In addition to axon pathfinding defects, we have also begun to characterize structures within the ventral telencephalon, such as the striatum and globus pallidus, which appear to be hypomorphic and misplaced along the rostral caudal axis. Furthermore, disruptions to the cortex itself are evident at later embryonic time points. Through further characterization of the cTKO brain, we will further define the roles of the three JNK genes in cortical development. Understanding the genetic regulation of brain development will help uncover potential causes of neurodevelopmental disorders, and can ultimately lead to better treatment of these devastating diseases.

68 Defining specificity of antibodies elicited by the 2017-2018 vaccine in children
Amy K.F. Davis

Defining specificity of antibodies elicited by the 2017-2018 vaccine in children

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Annually, seasonal influenza A viruses (IAV) present a major health concern, infecting millions and resulting in sizable morbidity and mortality. Vaccine effectiveness can range widely depending on the year, typically ranging between 40-60%. However, age plays a large role in vaccine effectiveness. The 2017-2018 influenza season exemplifies this; while cumulative vaccine effectiveness was low, vaccines had virtually no effectiveness in individuals born in the early 2000's. Mismatch between the H3N2 vaccine and circulating H3N2 strain account for poor population-wide responses, but it is unclear why there was particularly low effectiveness in 10-18 year-olds.

Previously, our lab has shown that vaccination boosts an individual's antibody response towards epitopes that are conserved in strains that they were first exposed to in early childhood. This can have the effect of focusing the antibody response to particular epitopes on the influenza surface protein, hemagglutinin. This focusing is thought to occur because recall responses preferentially target epitopes conserved between primary and secondary exposures. We hypothesize that during the 2017-2018 season, for some individuals, vaccination recalled an antibody response towards an epitope that was mismatched between the vaccine and circulating strains.

Here, we studied a cohort of children, born 2003-2011, with known primary exposure and influenza vaccination history. We found that most individuals in this cohort mounted protective antibody responses against the 2017-2018 vaccine strain, but not against the 2017-2018 circulating strain. To address if 2017-2018 vaccination recalls antibody responses that bind to strains encountered in early childhood, we measured pre- and post-vaccination titers to an H3N2 strain that circulated in the early 2000's. We found that vaccination boosted antibody responses against this older H3N2 strain; additionally, we found that these antibodies were directed towards an epitope that is conserved between this older H3N2 and the 2017 H3N2 vaccine strain, but differs in 2017 H3N2 circulating strain. Together, these data suggest that prior exposures in 10-18 year olds might have contributed to particularly low H3N2 vaccine effectiveness during the 2017-2018 influenza season.

69 Subcellular localization of Hexokinase I dictates glucose utilization between anabolic and catabolic metabolism
Adam De Jesus

Subcellular localization of Hexokinase I dictates glucose utilization between anabolic and catabolic metabolism

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Aerobic glycolysis is the preferential metabolic adaptation of cancer cells and is vital for proper immune cell activation and effector function. Unlike cancer cells, which utilize aerobic glycolysis to sustain rapid proliferation, immune cells harness this metabolic program to support innate and adaptive effector functions. As the first rate-limiting step in glycolysis, hexokinase-1 (HK1) is poised as a key regulator of glucose fate and is requisitely upregulated in cancer cells and effector immune cells. HK1 contains an N-terminal mitochondrial binding domain (MBD) that restricts its localization to the outer mitochondrial membrane, however the metabolic consequence of its subcellular localization remains largely unexplored. We have discovered that removal of HK1s MBD rewires cellular metabolism by shunting glucose away from catabolic processes (lactate and TCA cycle) and into anabolic pathways (pentose phosphate pathway-PPP). We show that this increase in PPP increases proliferative potential in cancer cells and enhances cytokine production in activated monocytes. Our studies provide novel insight into the convergent metabolic rewiring of cancer and immune cells and its effect on their respective characteristic functions.

We generated stable cell lines using GFP fusion lentivirus constructs of full length HK1 (FLHK1), MBD null HK1 (TrHK1), and a MBD null kinase dead HK1 (TrMuHK1) construct along with a GFP empty vector control (EV-GFP). We show that TrHK1 cells have lower glycolytic capacity via seahorse extracellular acidification rate (ECAR), extracellular lactate, and intracellular pyruvate production compared to FLHK1, TrMuHK1, and EV-GFP. We then performed steady state metabolomics on our HEPG2 stable cell line and show enrichment of nucleotide and PPP metabolites and a decrease in lactate, pyruvate, and TCA cycle metabolites with TrHK1 overexpression as compared to controls. We also observed higher proliferative capacity in TrHK1 under high and low glucose. Additionally, we generated a novel mouse model of MBD deleted FLAG-tag substituted N-terminal HK1 using CRISPR/Cas9. We isolated bone marrow derived macrophages (BMDM) from these mice and recapitulated the lower ECAR, lactate, and pyruvate we observed in our cancer cell model. Surprisingly, we saw increased mRNA expression of IL-1beta, IL-6, and TNF-alpha along with increased IL-1beta secretion in LPS activated BMDMs. Overall, we find that altering the subcellular localization of HK1 shifts global cellular metabolism in favor of anabolic pathways with a concomitant increase in proliferation in cancer cells and effector immune response in macrophages.

70 Fasting in mice enables abdominal radiation dose escalation in the setting of pancreatic cancer by mitigating small intestinal toxicity
Marimar de la Cruz Bonilla

Fasting in mice enables abdominal radiation dose escalation in the setting of pancreatic cancer by mitigating small intestinal toxicity

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Surgical resection is the only potentially curative treatment for pancreatic cancer, but only 15-20% of patients have resectable tumors. In unresectable cases, stereotactic body radiotherapy (SBRT) may be used to give tumor-directed radiotherapy (RT). Unfortunately, this can cause severe gastrointestinal (GI) toxicity due to proximity of the pancreatic head to the duodenum. Protecting the intestine from the toxic side-effects of radiation may enable dose-escalation that could achieve more effective local control of disease. We and others have previously shown that a fast of 24 hours protects mice from lethal doses of the DNA-damaging agent etoposide. In this study, we demonstrate that a 24 hour fast also protects mice from lethal doses of total-abdominal radiation. Histologic analyses using the Withers-Elkind microcolony assay show that fasting protected small intestinal (SI) stem cells from radiation damage and promoted early regeneration. To show a proof-of-principle for the use of this radioprotective maneuver in cancer therapy, we used an orthotopic model of pancreatic cancer using KPC tumor cells syngeneic to C57BL/6. Here, we show that fasting-mediated intestinal protection enabled dose escalated SBRT for treatment of these orthotopic tumors. RT with fasting-mediated radioprotection delayed tumor growth and improved survival compared to controls. Given this robust phenotype, we developed a 3D culture ex vivo assay using intestinal stem cell-enriched epithelial spheroid cultures. We modified these intestinal spheroids with a bioluminescent reporter and used these cells to develop a modified clonogenic assay for 3D culture that can be used to identify novel radioprotectors, such as a fasting mimetic. Taken together, these results suggest that fasting protects small intestinal stem cells, allowing animals to receive potentially curative doses of abdominal radiation that would otherwise be lethal. Future work will aim to identifying the mechanisms by which fasting confers intestinal protection and drug candidates that can be used to mimic this fasting-mediated protection.

71 Analysis of the "centrosome-ome" reveals potential causes of centrosome amplification in human cancer
Ryan A. Denu

Analysis of the "centrosome-ome" reveals potential causes of centrosome amplification in human cancer

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The centrosome is the microtubule organizing center of human cells and facilitates a myriad of cellular functions including organization of the mitotic spindle to ensure faithful chromosome segregation during mitosis, cell polarization and migration, and primary cilia formation. A numerical increase in centrosomes, or centrosome amplification (CA), is common in cancer and correlates with more aggressive clinical features and worse patient outcomes. CA is thought to arise by two major mechanisms: (1) centriole overduplication and (2) cell doubling events. To better assess the relative contributions of these two mechanisms, we analyzed 79 melanomas compared to 17 benign nevi and 60 prostate cancers and 20 benign prostate samples. We probed these samples for pericentrin (to mark all centrosomes) and CEP170 (to mark centrosomes with mature centrioles). If cell doubling is the predominant mechanism leading to CA, then we would expect most centrosomes to express CEP170; conversely, if centriole overduplication is predominant, we would expect one centrosome in a cell to express CEP170 and the rest to lack CEP170. We find a decrease in CEP170-positive centrosomes in tumor samples compared to benign samples, indicating that centriole overduplication is the predominant mechanism leading to CA in human cancer. Given this finding, we next sought to determine the predominant molecular mechanisms leading to centriole overduplication in human cancer. Many previous studies have identified ways to amplify centrioles *in cellulo*, such as overexpression of PLK4, but the clinical relevance of these mechanisms is unclear and has not been demonstrated. To address this question, we analyzed mutations, copy number alterations, and RNA expression data in the 366 proteins reported to localize to the centrosome using TCGA data. We identified a list of candidate centrosome proteins that are most frequently altered in cancer. Furthermore, given that cells with CA arrest unless other compensatory alterations are made, such as loss of p53, we considered the fold enrichment in p53 mutant versus p53 wild type tumors. We identified the following candidates: gain of function of CEP19, CEP72, CTNNB1, PTK2, NDRG1, SPATC1, TBCCD1; and loss of function of CEP76, MCPH1, NEURL4, NPM1. *In cellulo* analysis of these candidates reveals that loss of MCPH1 causes the most robust increase in centriole number. MCPH1 deep gene deletions are seen in 5-15% of human cancers, depending on the anatomic site of the tumor. Mechanistic experiments demonstrate that loss of MCPH1 causes an increase in CDK2 activity, which reduces βTrCP -mediated degradation of STIL, thereby increasing STIL levels at the centrosome and driving CA. We conclude that loss of MCPH1 is a common and penetrant cause of CA in human cancer.

72 Acidosis, zinc, and HMGB1 in sepsis: A common connection involving sialoglycan recognition
Chirag Dhar

Acidosis, zinc, and HMGB1 in sepsis: A common connection involving sialoglycan recognition

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Normal blood pH is tightly regulated in a narrow range of 7.35-7.45. Lactic acidosis, low levels of zinc and release of HMGB1 from activated/necrotic cells are all indicators of poor prognosis in sepsis. Surprisingly, we observed that HMGB1 added to hirudin-anticoagulated whole blood at physiological pH did not bind to leukocytes that are known to carry receptor sites. However, when lactic acid was added to lower whole blood pH mimicking sepsis conditions, binding of HMGB1 to leukocytes occurred. Additionally, neutrophils were activated by HMGB1 only at reduced pH. These findings imply the presence of natural inhibitor(s) of HMGB1 that prevent its interaction with receptors at normal pH. Independent studies have shown that glycoproteins such as CD52/CD24 presenting high levels of sialic acids can engage HMGB1 in a sialic acid-dependent manner. We noted that the buffer used in such studies included millimolar concentrations of manganese, a feature likely carried over from unrelated work on the binding of nuclear HMGB1 to DNA. Testing micromolar concentrations of many divalent cations we found that only zinc supported robust binding with sialylated glycoproteins. Further characterization of HMGB1 as a sialic acid-binding lectin suggested optimal binding takes place at physiological blood pH and is markedly reduced when pH is adjusted with lactic acid to levels found in sepsis. Glycan array studies further confirmed the binding of HMGB1 with multiple sialylated glycans again dependent on zinc and normal blood pH. The hypothesis arising from all these findings is that HMGB1-mediated hyper-activation of innate immunity in the bloodstream during sepsis requires lowering of blood pH and that addition of micromolar amounts of zinc might partially protect against this effect. We suggest that the potent inflammatory effects of HMGB1 are normally kept in check via sequestration by plasma sialylated glycoproteins at physiological pH and zinc levels, and triggered when pH and zinc levels fall in the late stages of sepsis. Notably, acute phase response to inflammation results in high production of hypersialylated molecules such as alpha-1-acid glycoprotein from the liver and endothelium, which may then act as a negative feedback loop. Current clinical trials that are independently studying zinc supplementation or pH normalization may be more successful if these approaches are combined with HMGB1 inhibition, and perhaps supplemented by infusions of heavily sialylated molecules like CD52.

73 Clinically Tracking White Matter in Neuro Imaging: Visualizing the Acoustic Radiation in Subjects with Normal Hearing and Hearing Loss

Bryn Dhir

Clinically Tracking White Matter in Neuro Imaging: Visualizing the Acoustic Radiation in Subjects with Normal Hearing and Hearing Loss

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Background: The acoustic radiation (AR) is the final stage of the auditory pathway. It consists of white matter (WM) fibers coursing from the medial geniculate body (MGB) of the thalamus to the Heschl's gyrus (HG) in the temporal lobe. The AR presents a challenge for tracking WM fibers in diffusion tensor imaging (DTI) as it is partly obscured by the optic radiation and crossing fibers from surrounding tracts. Visualization of the AR is complicated (Maffei *et al.* 2017) and it is thought to be impossible to generate from single-fiber analysis (Behrens *et al.* 2007; Berman *et al.* 2013). One probabilistic method of tracking, known as dynamic programming (DP) can deal with such types of crossing fibers (Ratnanather *et al.*, 2013). Li *et al.* (ARO 2013) previously showed that DP could be used to generate the AR but with reference to a single-subject atlas. This extended study shows that it is possible for clinicians and researchers to reliably visualize the AR and anatomical topography using DP with single-fiber analysis in native space of patients with normal hearing (NL) and hearing loss (HL) at 1.5T and 3T MR scans.

Methods: DWI and T1 scans at 1.5T for 10 subjects with no HL and five subjects with HL were acquired, as well as 1.5T and 3T NH atlas, and 3T for a subject with HL. Scans were rigidly registered and DWI data was processed through DTIStudio and MRICloud (Jiang *et al.* 2006; Mori *et al.*, 2016) to yield whole brain images of fractional anisotropy (FA), color orientation maps, eigenvalues and eigenvectors. The MGB and HG were manually segmented in the color map and T1 image, respectively. The AR was generated via DP (Li *et al.* 2014) and reconstructed in 3D for visualization.

Results: The post-mortem and in-vivo tractography studies reported by Maffei *et al.* (2017) were confirmed across all 17 scans, and visualizations were reliably replicated in lateral, posterior, anterior and superior 3D views. Anatomical topography identified three components of the auditory bundle, the genu, stem and fan, and was replicated at 3T. Analyses showed statistical significance for FA between left and right sides and results were comparable to other studies investigating the auditory pathway by Rueckriegel et al., (2016), Lin et al., (2008)., Kurtcan et al., (2007).

Conclusions: It is possible to reconstruct and visualize the AR in clinical DTI scans across several subjects and at different scanner strengths. The ability to visualize the AR may allow for applications in clinical pathology, such as in vestibular schwannomas, multiple sclerosis and stroke. The software used in this study is readily and easily available for clinicians and researchers.

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74 Identification and characterization of Trichomonas vaginalis cellular targets of metronidazole

Fitz Gerald I. Diala

Identification and characterization of *Trichomonas vaginalis* cellular targets of metronidazole

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Trichomonas vaginalis is an obligate, extracellular, sexually-transmitted parasite that causes trichomoniasis, afflicting ~250 million men and women annually. Symptomatic infections typically present as vaginitis and cervicitis in women, and urethritis in men, and are treated with 5-nitroimidazole drugs such as metronidazole (Mz). Mz is a prodrug and is activated in anaerobic organisms into a radical intermediate, which adducts to protein targets, ultimately killing parasite. The lethal targets of the drug in *T. vaginalis* are unknown. With drug resistance tied to less drug activation, identifying Mz targets would provide information necessary for developing effective, next-generation antimicrobials. To this end, we adapted a terminal alkyne analog of Mz (Mz-alkyne) that retains the capacity to be activated and to kill *T. vaginalis*. Terminal alkynes can be reacted with azides to form a covalent bond in a copper(I)-catalyzed click reaction. We treated Mz-resistant and Mz-sensitive *T. vaginalis* strains with the Mz-alkyne and reacted the lysates with azide-agarose beads under Cu(I) catalysis. Clicked proteins were then stringently enriched and subjected to quantitative mass spectrometric analysis. As control, we used Mz in mock click reaction to establish background. Using this method, we identified 107 and 94 significantly Mz-adducted proteins in Mz-sensitive strain and Mz-resistant strain, respectively. In addition, 38 proteins are shared between the two strains. These proteins fall into several pathways including, but not limited to, redox, glucose and amino acid metabolism. We will investigate these targets to understand how their disruption results in *T. vaginalis* death.

75 Understanding the Protein-Protein Interactions important for the Initiation of HSV-1 DNA Synthesis
Katherine A. DiScipio

Understanding the Protein-Protein Interactions important for the Initiation of HSV-1 DNA Synthesis

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Herpes Simplex Viruses type 1 (HSV-1) is important human pathogen that can cause a wide range of pathologies ranging from the common cold sore to disseminated end-organ disease. HSV-1 poses a significant public health due to the rise of acyclovir resistance, particularly within the ever-increasing immunocompromised patient population. It is therefore of great interest to develop new potential therapies against these viruses. Initiation, or the unwinding of dsDNA at the origin of replication, is the first step in viral DNA synthesis. Initiation is a rate-limiting step and therefore may be a good target for the development of new antiviral agents. Two viral proteins are known to be essential for initiation: the origin binding protein UL9 and the single-stranded DNA binding protein ICP8. Despite the importance of this process, we still lack a detailed understanding of the molecular mechanisms and protein-protein interactions that drive initiation. In particular, we seek to map the interaction between ICP8 and UL9. The C-terminal 27 amino acids of UL9 are essential for ICP8 interaction and for the initiation of origin-dependent DNA synthesis. However, the specific residues within this region necessary for interaction with ICP8 are unknown. We are testing the hypothesis that a conserved stretch of amino acids within this region forms a linear motif (VNF, a.a. 846-848) that is essential for the ICP8-UL9 interaction. We have constructed a VNF to AAA UL9 mutant. This mutant expressed at similar levels to WT UL9 and localized properly to the nucleus in Vero cells upon transfection. Interestingly, the VNF mutant was not able to complement the growth of an UL9-null virus, suggesting that these residues are important in the context of infection and may be essential for the ICP8-UL9 interaction. Although we know that the C-terminus of UL9 interacts with ICP8, the complementary binding surface on ICP8 has not been identified. We hypothesize that the VNF motif interacts with a conserved hydrophobic pocket on ICP8 defined by the residues F843 and W844. Interestingly, an ICP8 mutant with alanine substitutions in residues F843 and W844 was unable to bind to the C-terminus of UL9 by far western analysis. Additionally, this ICP8 mutant was unable to stimulate UL9 ATPase activity *in vitro*. Together these data support the hypothesis that the VNF motif within the C-terminus of UL9 and the conserved hydrophobic pocket on ICP8 may define the ICP8-UL9 interaction interface.

76 The Cognitive Role of Insula Volume and Asymmetry
Phillip Dmitriev

The Cognitive Role of Insula Volume and Asymmetry

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The insula is a brain region critical for attention, salience, and the integration of sensorimotor information. The insula has been shown to be asymmetric across hemispheres in healthy adults. However, a comprehensive understanding of the role of this asymmetry has yet to be demonstrated. In our study, we confirm the innate hemispheric asymmetry of the insula in aged normal controls (NC). Additionally, we show that patients with early-stage Parkinson's Disease (PD) show significantly decreased left and right whole insula volume. Right to left asymmetry is increased in older PD patients and those with poorer motor performance, indicating that the Parkinson's Disease pathology may lead to or exacerbate loss of left insula volume. Parcellation of volume analysis supports a rightward asymmetry in the anterior insula, and a left-ward asymmetry in the posterior insula. Asymmetry measures in the whole and anterior insula correlate with cognitive measures of attention and memory in Parkinson' Disease. Finally, while females show a greater bilateral insula volume, males have an increased insula asymmetry. These results indicate that insula volume is inherently asymmetric across volumes, and that this asymmetry is sensitive to disease processes, potentially leading to cognitive dysfunction.

77 Sociocultural barriers to medical care for pregnant, Latina women with diabetes in Eastern NC
Noopur Doshi

Sociocultural barriers to medical care for pregnant, Latina women with diabetes in Eastern NC

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Introduction: Latina women in NC are at significantly increased risk of developing gestational diabetes in comparison to non-Hispanic whites. Little is known about possible sociocultural factors that may explain this health disparity for this population, especially in rural settings. The purpose of this pilot study was to examine possible behavioral and sociocultural barriers to care among pregnant Latina women with a current diagnosis of gestational diabetes in (rural) Eastern North Carolina. Methods: Participants were patients at a Regional Perinatal Center. They were approached during their non-stress test appointment and asked to complete an anonymous 2-page survey in either English or Spanish. The survey assessed basic information about current and past pregnancies, diabetes-related knowledge and behaviors, current access to medical care, and perceived barriers to medical care. Results: The average participant was in their 3rd pregnancy, with 40% reporting gestational diabetes in prior pregnancies. Knowledge of the seriousness of diabetes was moderate (50%), but knowledge of glucometer use and current medication adherence were both high (90%), and the majority (80%) knew where to get care. Significant barriers to care included problems paying for the cost of medical care (75%), lack of social support to get to appointments (50%), and problems with transportation (45%). Language was not perceived as a significant barrier by the majority of the sample, although 70% of the sample opted to complete the survey in Spanish. Conclusion/Implications: These preliminary findings suggest that cost-reducing or transportation interventions may be the most useful targets for future interventions for this population, but a bigger sample is needed to implement any intervention with certainty.

78 PrimerID based measurement of within patient HIV diversity for estimating timing of HIV infection in infants
Sara Drescher

PrimerID based measurement of within patient HIV diversity for estimating timing of HIV infection in infants

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Often, significant time elapses between HIV infection and diagnosis. Accurate estimates of infection timing can be critical for studying the progression of infection, immune response, and response to treatment. Several previous approaches to estimating the timing of HIV infection have relied on measurement of intrapatient viral diversity, which increases with the length of infection. Here we attempt to extend this approach to a new population - infants infected with HIV - and develop a method of accurate determination of within patient viral diversity using primerID. PrimerID places a unique barcode onto each cDNA molecule to accurately assess the total number of viral genomes sampled and allows the identification and elimination of PCR (polymerase chain reaction) and sequencing errors.

We adapted primers previously developed for subtype B HIV samples to enable amplification of subtypes A, C and D, as these are the most common subtypes in Africa. These primers were tested for efficiency using a plasmid containing a known subtype A viral sequence and PCR conditions were optimized. The reverse primers were subsequently extended to include an 8-nucleotide random primerID sequence and previously tested primer landing pad segment. We were able to amplify a 3.1 kilobase region including full-length *pol* with from 1,200 copies of HIV RNA with and without attached PrimerID, which will allow us to determine the impact of primerID on measures of within patient HIV diversity.

We will use this method to sequence HIV RNA diversity in 25 samples from the Nairobi Breastfeeding Trials (NBT) with known infection dates (five each with infection at birth, six weeks, 14 weeks, six months, and 12 months). In the NBT, blood samples were obtained in the first week of life, at six weeks, 14 weeks, six months, and every three months until the age of two years, allowing good characterization of infection timing. We will then create a model of infection time versus viral diversity (calculated as average pairwise distance) and apply this model to a cohort of Kenyan infant samples with unknown dates of infection.

With the development of a protocol to sequence full length *pol* with primer ID, we hope to have precise estimates of HIV diversity to provide a small enough estimated window of infection time to differentiate between pre-, peri-, and post-natal infection in infants and children with HIV.

79 Brain Circular RNAs are significantly associated with Alzheimer's Disease
Umber Dube

Brain Circular RNAs are significantly associated with Alzheimer's Disease

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Background: Circular RNAs (circRNAs) are a class of RNAs highly expressed in the nervous system and enriched in synapses. CircRNAs result from backsplicing events, in which the 3' end of transcripts are covalently spliced with the 5' ends. The resulting backsplice junctions allow for the detection of circRNAs in ribosomal RNA-depleted RNA sequencing (RNA-seq) data. A recent study demonstrated that deletion of a single circRNA – *circCDR1-as* – impacted synaptic function. Interestingly, *circCDR1-as* has been reported to be downregulated in the frontal cortex of Alzheimer's Disease (AD) patients. To address the open question of whether other circRNAs are associated with AD, we performed a circular transcriptome-wide analysis of circRNA differential expression in AD using two independent, brain-derived RNA-seq datasets.

Methods: We generated paired-end 150nt RNA-seq data from

post-mortem parietal cortex tissue donated by 83 individuals with AD and 13 controls. AD case or control status was confirmed via neuropathological diagnosis. We aligned this data to the human reference genome (GRCh38) using STAR software and called circRNAs using DCC software. We then identified circRNA differential expression using DESeq2 software. We replicated our discovery findings using publicly available, inferior frontal gyrus (Brodmann Area 44) RNA-seq data from the Mount Sinai Brain Bank (89 AD cases and 47 controls) and performed a meta-analysis. Finally, we explored the pathological relevance of our findings by analyzing circRNA co-expression with linear transcripts.

Results: On meta-analysis, we identified 84 circRNAs differentially expressed between AD cases and controls at a false discovery rate (FDR) of 0.05. These included novel associations as well as the previously reported *circCDR1-as* (p-value: 1.81 × 10-12). Among the most significant of the novel associations were *circHOMER1* (p-value: 4.78 × 10-10), *circPICALM* (p-value: 5.29 × 10-10), *circDOCK1* (p-value: 3.37 × 10-06), and *circFMN1* (p-value: 1.68 × 10-05). We also observed circRNAs co-expressing with AD-related genes and pathways. For example, *circFMN1* co-expressed with *APP*, which encodes the precursor protein that forms the characteristic plaques of AD. Similarly, *circHOMER1* co-expressed with linear transcripts of genes significantly associated with AD (KEGG Alzheimer's Disease, 66/156 genes, adjusted p-value: 1.07 × 10-15).

Conclusion: We identified replicable and highly significant circRNA differential expression in AD brain tissues. These AD-associated circRNAs co-express with AD-relevant genes and pathways. Consequently, future analyses of circRNAs may yield novel biomarkers or therapeutic targets for AD or other neurological disorders.

80 Elucidating the role of the circadian clock gene Bmal in myometrium function during pregnancy
Thu V Q Duong

Elucidating the role of the circadian clock gene Bmal in myometrium function during pregnancy

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Pre-term birth (PTB) is a devastating issue affecting both the mother and the baby. PTB accounts for most cases of neonatal morbidity and mortality. Due to the developmental complications associated with PTB, it is critical that we develop an understanding of the mechanisms leading to preterm labor (PTL), allowing us to delay birth and continue development of the fetus in utero. In rodents, primates, and humans, birth preferentially occurs at the end of the rest cycle, and in women, absence of uterine circadian rhythms in the third trimester of pregnancy is associated with PTB. On a molecular level, uterine circadian rhythms are generated through a complex transcription-translation feedback loop of "clock" transcription factors, represented by Bmal, Per2, Cry and Clock. This "molecular clock" generates a close to 24h rhythm within each cell, allowing cell autonomous time-keeping and timed cell specific gene expression. Our study explores the role of circadian rhythms in myometrium function. Interestingly, we found the pregnant mouse uterus increases the expression level of molecular clock-genes during pregnancy, with a peak expression level the day before labor onset. This increase in molecular clock genes was associated with enhanced circadian rhythms in the pregnant myometrium, as evaluated in the Per2::luciferase knock-in mouse, where we continuously monitor circadian rhythm generation in live myometrial tissue. Deletion of the molecular clock gene Bmal in the myometrium, the uterine muscle allowing contractions, increases the frequency of PTL. We hypothesize that Bmal controls uterine circadian rhythm generation through its control of myometrial receptors regulating labor onset. To determine how Bmal knock-out impacts uterine anatomy, we performed H&E on control and Bmal KO uteri (n=3), and found abnormal uterine anatomy in the Bmal KO. To determine if the altered anatomy of the Bmal KO uterus was associated with changed uterine expression of progesterone receptor (PR), a receptor promoting myometrial relaxation, we performed PR immunohistochemistry. We found increased PR staining in the Bmal KO uterus (n=3). Future experiments will confirm this observation with qPCR and functional tests in Bmal depleted myometrial samples. To elucidate the mechanisms by which Bmal regulates PR expression and myometrial contraction patterns, we will use a human myometrial cell line. To study the effects of progesterone on the expression of Per2, we performed transient transfection on the pregnant human myometrial cell line PHM1-41 with Per2::luciferase plasmids, recorded the signal under progesterone treatment and compare the result to that under vehicle treatment.

81 Secretion of interleukin-6 by human Acute Myeloid Leukemia inhibits normal erythropoiesis
Ritika Dutta

Secretion of interleukin-6 by human Acute Myeloid Leukemia inhibits normal erythropoiesis

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Acute Myeloid Leukemia (AML) is an aggressive cancer often characterized by infections, fatigue, and bleeding due to cytopenias, caused by the failure of the bone marrow (BM) to generate mature blood cells. The common assumption for AML-induced BM failure is overcrowding due to clonal expansion of immature myeloid blasts, leading to failure of normal hematopoiesis. However, in a cohort of 293 AML patients, we found that disease burden (% of blasts) does not predict severity of cytopenias, arguing against physical crowding as the main mechanism underlying BM failure. Thus, the goal of our study is to identify novel mechanism(s) associated with AML-induced BM failure, thus enabling new therapies to improve AML management and reverse morbidity.

Conventional xenograft models of human AML do not typically exhibit cytopenias due to splenic extramedullary hematopoiesis, making them unsuitable to study BM failure. We developed a novel mouse model in which we splenectomized NSG mice prior to AML engraftment. Sple-

nectomized NSG mice engrafted with primary human AML ($NSG^{spln\text{-}AML}$) develop severe anemia compared to sham-operated AML-engrafted controls, resulting in early mortality (p

Utilizing our model, $NSG^{spln\text{-}AML}$ demonstrate depletion of erythroid progenitors including proerythroblasts (1.39 fold), normoblasts (4.96 fold), late normoblasts (10.0 fold), and reticulocytes (4.34 fold). The relative preservation of proerythroblasts and depletion of normoblasts indicates that AML blasts impart a specific *in vivo* erythroid differentiation blockade. To explore mechanisms by which AML blasts inhibit erythroid differentiation, we generated conditioned media (CM) from primary AML blasts, and found that AML-CM suppressed erythroid colony formation from normal hematopoietic stem/progenitors (HSPCs) (3.1-5.1 fold). These experiments demonstrate that AML imparts an erythroid differentiation blockade in a cell non-autonomous fashion.

We then took an unbiased approach to identify factors differentially secreted by AML which could account for the differentiation blockade. RNA-seq analysis revealed elevated IL-6 levels in AML patients compared to normal CD34+ HSPCs. Using cytokine array analysis, we also identified elevated IL-6 levels in AML-CM (7.80 fold increase) compared to $CD34^+$-derived CM. Elevated IL-6 was similarly found in BM aspirates from $NSG^{spln\text{-}AML}$ compared to mice engrafted with $CD34^+$ HSPCs. Inhibition of IL-6 restored erythroid colony formation in the presence of AML-CM. Treatment of $NSG^{spln\text{-}AML}$ with an IL-6 blocking antibody (siltuximab) increased hemoglobin levels compared to mice treated with isotype control and conferred a survival advantage (p=0.0037). These experiments demonstrate that IL-6 produced by AML acts as a paracrine factor to suppress erythropoiesis.

Together, our data suggest that AML blasts play a previously unrecognized role in imparting an erythroid differentiation blockade through secretion of IL-6. Our results position IL-6 blockade as a promising therapeutic strategy to improve anemia in AML patients.

82 The role of RMTg mediated aversion in addiction
Maya Eid

The role of RMTg mediated aversion in addiction

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Over 90% of Americans have had some exposure to drugs of abuse, but only 15-32% of individuals exposed to the major classes of abused drugs go on to become addicted.. Much basic research has been directed at understanding individual animals who have already progressed into addiction-like behaviors, with relatively less study of what protective factors may help prevent acquisition of drug use in the first place.

Although cocaine's aversive responses are less widely acknowledged than its rewarding effects, they are experimentally robust. Particularly elegant experiments by Ettenberg have shown that single doses of cocaine produce an initial rewarding phase followed by an aversive crash about 15' later that is sufficient to condition a net aversion to cocaine that is strong enough to overcome cocaine's rewarding effects. In our lab, we investigated behavioral responses to cocaine in rats performing a runway operant task that is particularly suited for assessing the combined rewarding and aversive properties of cocaine. In this task rats traverse a 5-foot long corridor to obtain a single daily dose of cocaine. After 4-7 trials, we found large variations in animals responses to cocaine, where some animals slowed down dramatically (high avoiders) and others remained fast (low avoiders).

In recent years, our lab and others have demonstrated that cocaine avoidance depends critically on the rostromedial tegmental nucleus (RMTg) and its afferents. The RMTg is a major GABAergic midbrain input to midbrain dopamine (DA) neurons that plays major roles in avoidance. We have thus shown that there are individual differences in RMTg neurons firing rate that correlate with cocaine-conditioned avoidance behavior. Indeed, compared to low cocaine avoiders, high avoider animals have similar RMTg inhibition during the rewarding phase of the drug (5' post injection), but have significantly higher RMTg firing rates during its aversive phase (15' post-infusion). To investigate the molecular driver of these differences in the RMTg, we used in vitro electrophysiology and demonstrated that low avoiders have less RMTg firing due to aberrant functioning of the GluR1 subunit of the AMPA receptor. Indeed, when we inhibited this subunit pharmacologically, all animals become low avoiders on the runway task, whereas when we activate this subunit, most animals become high avoiders. Finally, we found that the aversive effects of cocaine were much better predictors of cocaine seeking were high avoiders were less likely to acquire drug self administration, but were more likely to reinstate, suggesting that relapse is not just a reward seeking behavior, but also a means to alleviate negative symptoms through negative reinforcement.

83 Atg14 protects the intestinal epithelium from TNFα-triggered villous atrophy
J. Steven Ekman

Atg14 protects the intestinal epithelium from TNFα-triggered villous atrophy

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Regulation of intestinal epithelial turnover is a key component of villus maintenance in the intestine. The balance of cell turnover can be perturbed by various extrinsic factors including the cytokine TNFα, a cell signaling protein that mediates both proliferative and cytotoxic outcomes. Defects in autophagy are associated with TNF-mediated cell death and tissue in the intestinal epithelium, but primarily under conditions of infection and damage; a direct role for this pathway within the context of enterocyte cell death during homeostasis is lacking. Here, we generated mice lacking ATG14, autophagy related gene 14, within the intestinal epithelium (*Atg14*$^{f/f}$ *Villin*-Cre (*VC*)+). These mice developed

spontaneous villus loss and intestinal epithelial cell death within the small intestine. Based on marker studies, the increased cell death in these mice was due to apoptosis. *Atg14$^{f/f}$ VC+* intestinal epithelial cells demonstrated sensitivity to TNFα-triggered apoptosis in vitro. Both TNFα blocking antibody and genetic deletion of *Tnfr1* rescued villus loss and cell death phenotype in *Atg14$^{f/f}$ VC+* mice. Lastly, we identified a similar pattern of spontaneous villous atrophy and cell death when *Fip200* was conditionally deleted from the intestinal epithelium (*Fip200$^{f/f}$ VC+*). Overall, these findings are consistent with the hypothesis that factors that control entry into the autophagy pathway are also required during homeostasis to prevent TNFα triggered death in the intestine.

85 Not too much, not too little: the just right amount of IQGAP1 protects against hepatic tumorigenesis
Hanna L. Erickson

Not too much, not too little: the just right amount of IQGAP1 protects against hepatic tumorigenesis

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IQ motif-containing GTPase Activating Protein 1 (IQGAP1) is a large, ubiquitously expressed scaffolding protein that is overexpressed in a number of cancers, including liver cancer, and is associated with many pro-tumorigenic processes including cell proliferation, motility, and adhesion. It's ability to scaffold, and thus integrate, multiple signaling pathways via its five protein binding domains suggests that IQGAP1 could be an effective anti-tumor target. However, additional data shows that reduced IQGAP1 expression in stromal cells can increase cancer cell proliferation in the liver. Therefore, the objective of our study was to examine the role of IQGAP1 in liver tumorigenesis and determine whether there is a dose-dependent effect of IQGAP1 on hepatic tumor initiation and promotion.

We utilized the gold standard diethylnitrosamine (DEN)-induced model of liver cancer. Male *Iqgap1$^{+/+}$*, *Iqgap1$^{+/-}$*, and *Iqgap1$^{-/-}$* mice on a 129/SVJ background were administered 10 mg/kg body weight DEN by intraperitoneal injection at 14 days of age to initiate hepatic tumorigenesis and were followed for 50 weeks. This treatment resulted in visible tumors in 9/13 (70%) of *Iqgap1$^{+/+}$* mice, which were not observed 20 weeks after injection indicating that there was no early onset of tumor incidence. As expected, the expression of *Iqgap1* in healthy liver corresponded to the number of alleles present and was induced approximately twofold in hepatic tumors of *Iqgap1$^{+/+}$* mice compared to the surrounding healthy tissue. On the other hand, expression of *Iqgap1* homologs *Iqgap2* and *Iqgap3* decreased and increased, respectively, in tumor tissue compared to healthy liver tissue and were not affected by IQGAP1-deletion.

We hypothesized that reduced IQGAP1 expression in all cells would result in fewer and smaller tumors. Surprisingly, we did not find any significant difference in tumor burden between *Iqgap1$^{+/+}$* and *Iqgap1$^{-/-}$* animals. Interestingly, *Iqgap1$^{+/-}$* mice displayed significantly lower incidence of hepatic tumors (11/24) compared to *Iqgap1$^{-/-}$* mice (14/16) (P Iqgap1$^{+/-}$ mice compared to the *Iqgap1$^{-/-}$* mice (P = 0.02, Tukey's multiple comparisons test). This suggests that intermediate expression of IQGAP1 in the liver is protective against tumor development. We did not find any difference in β-catenin activation, proliferative index, or collagen deposition in these livers indicating that these pathways may not contribute towards the protective effect observed in the *Iqgap1$^{+/-}$* mice.

Overall, our finding highlights the need to understand how cellular and pathological context can affect IQGAP1 function and also uncovers that reducing IQGAP1 levels by half can be beneficial during hepatic tumorigenesis.

86 Utility of image cytometry in determining the therapeutic potential of a cell penetrating peptide for the treatment of glioblastoma
Nicholas J. Eustace

Utility of image cytometry in determining the therapeutic potential of a cell penetrating peptide for the treatment of glioblastoma

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Introduction: Glioblastoma (GBM) is an aggressive and incurable brain neoplasm in part because of its heterogeneous composition of enhanced survival signaling, dysfunctional programmed cell death, and upregulations in efflux transporters, which promotes resistance to conventional and targeted therapeutics. We utilized various image cytometry techniques to determine the ability of a cell penetrating peptide therapy, derived from Myristoylated alanine-rich C-kinase substrate (MARCKS) effector domain (ED), to be an effective treatment against GBM.

Objective: 1) Demonstrate the value of image cytometry techniques when determining the cytotoxic effects of cancer therapy. 2) Determine the therapeutic potential of using a cell-penetrating MARCKS ED peptide therapy (MED2) in the treatment of GBM.

Methods: Using molecularly classified GBM patient-derived xenografts (PDX) lines cultured in stem media, and both fluorescently labeled and non-fluorescent MED2, we compared MED2 effects on GBM to its effects on normal human astrocytes (NHA). The Xcyto10 (Chemometec) image cytometer was used to study cytotoxicity and cellular accumulation of MED2 using a combination of high-resolution imaging, fluorescent multiplexing with quantification, and data analysis tools in both adherent and suspension cells. Cytotoxic characteristics of MED2 investigated include cell morphology, cell cycle, caspase activation, annexin V staining and plasma membrane permeability, intracellular calcium alterations, and ATP luminescence. Blood-brain barrier penetration and intratumoral accumulation of MED2 were assessed *in vivo* using a tumor naive and orthotopic GBM model with intravenous delivery of

the peptide.

Results: MED2 had dose-dependent cytotoxic effects against GBM PDX lines at low micromolar concentrations, which remained non-toxic in normal human astrocytes. MED2 was found to rapidly induce membrane ruffling, cytoplasmic contraction, the formation of Annexin V^{pos} blebs, and concurrent Annexin V^{pos}/$Sytox^{pos}$ staining, with cytotoxic effects resistant to caspase inhibition. We found MED2 to be similarly cytotoxic to adherent or suspension cells, and MED2 triggered a more robust and sustained increase in intracellular calcium in GBM over NHA's. Quantitative fluorescent imaging revealed abundant punctate accumulations forming at the plasma membrane of GBM, and not common in NHA's. Tail-vein delivery of TAT-ED in athymic mice revealed dose-dependent increases in CY7 fluorescent in the brain of tumor naïve mice and accumulation in intracranial tumors. Overall, this study utilizes imaging cytometry to qualitatively and quantitatively characterize MED2 cytotoxic effects.

87 The Effect of Epigenetic Pharmacological Agents on Abnormal Nuclear Morphologies in Cancer
Aliasger Ezzi

The Effect of Epigenetic Pharmacological Agents on Abnormal Nuclear Morphologies in Cancer

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Abnormal nuclear shapes are hallmarks of many diseases. Nuclear size and shape are prognostic and diagnostic indicators of cancer. Irregular nuclear shapes can be characterized by blebs, bulges, and concavities in the contour. To identify families of proteins which may play a role in nuclear shaping, our lab conducted a high-content RNAi screen of 615 epigenetic-related genes and screened for nuclear shape. Building on the results of the siRNA screen which revealed that chromatin regulators, particularly those that regulate histone modifications, play a substantial role in nuclear shaping, we then asked if pharmacological agents with chromatin-regulating targets could similarly dysregulate nuclear shape. To investigate this possibility, we are systematically screening 146 drugs in MCF-10A and MDA-MB-231 cells using confocal and epifluorescence microscopy and assayed for nuclear shape. MCF-10A (non-tumorigenic breast epithelial) cells have regular nuclear shapes compared to MDA-MB-231 (human breast adenocarcinoma) cells. We hope to find that drugs that inhibit similar molecules as those that produced irregular nuclear shapes when knocked down in the siRNA screen, also produce irregular nuclei in MCF-10A cells when treated with the drug. We are also interested in identifying if drugs that have the opposite effect on these molecules can make the nucleus more regular in cancer cells with irregular nuclei. This would allow us to use pathway analysis software to identify potential pathways involved in regulating nuclear shaping in breast epithelial cells. Many epigenetic drugs require several cell divisions cycles for the effects to be apparent. Thus, an incubation period of seven days is being used to allow for approximately seven cell cycles.

88 Inhibition of the Akt1-mTORC1 axis alters venous remodeling to improve arteriovenous fistula patency
Arash Fereydooni

Inhibition of the Akt1-mTORC1 axis alters venous remodeling to improve arteriovenous fistula patency

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Introduction: Arteriovenous fistulae (AVF) are the most common access created for hemodialysis, but only up to 50% of AVFs mature and thereby enable dialysis, suggesting a need to improve AVF maturation. In a mouse model, Akt1 expression increases during AVF maturation and reduced Akt1 expression *in vivo* reduces fistula wall thickness and diameter while improving patency. Mammalian target of rapamycin (mTOR) is a key regulatory protein that integrates signals from the Akt pathway to coordinate cell growth and proliferation. We hypothesized that inhibition of the Akt1-mTORC1 axis alters venous remodeling that is associated with failure of AVF maturation.

Methods: A C57BL6/J mouse aortocaval fistula model was used (male, 9-12 weeks). Mice were injected with 100 µg of vehicle or rapamycin (intraperitoneal) daily. The AVF (venous limb) of control- and rapamycin-injected mice were harvested at days 0, 3, 7 and 21 for analysis. Post-operative vessel remodeling was assessed using serial ultrasound measurements of the fistula diameter and computer morphometry to measure vessel wall thickness. AVF were compared for leukocyte, M1 and M2 macrophage surface markers and expression level of mTOR signaling proteins using Western blot and immunofluorescence (IF) intensity.

Results: Rapamycin reduced AVF wall thickness at days 7 and 21 (p

Rapamycin treatment was associated with diminished phosphorylation of the mTORC1 pathway, with less phosphorylation of mTOR (Ser2481), Akt1, 4EBP1 and p70S6K (p0.4; n=6). Mice treated with rapamycin showed decreased colocalization of p-Akt1/α-actin and p-mTORC1/α-actin immunoreactivity at days 7 and 21 (p

Rapamycin improved AVF patency by day 42 (p=0.0495; n=13-14). These AVF showed persistently less thickening (p0.52; n=5) immunoreactivity compared to control AVF.

Conclusion: Rapamycin improves AVF patency by reducing early inflammation and wall thickening through the Akt1-mTORC1 signaling pathway. Rapamycin may be a translational strategy to improve AVF patency.

89 Structural Analysis of a Novel Inhibitor and a Substrate Bound to Acinetobacter-derived Cephalosporinase (ADC-7)

Erin R. Fish

Structural Analysis of a Novel Inhibitor and a Substrate Bound to *Acinetobacter*-derived Cephalosporinase (ADC-7)

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Present day bacteria have developed many resistance mechanisms to combat β-lactam antibiotics. One of these is the production of β-lactamases which break down the antibiotics, rendering them ineffective. In *Acinetobacter baumannii* infections, the production of *Acinetobacter*-derived cephalosporinase (ADC) β-lactamases provide a bacterial mechanism for deactivating antibiotics. In order to design and characterize molecules that inhibit the ADC enzyme, it's important to investigate the various interactions between the inhibitors and the active site residues. To accomplish this, we have characterized the structure/function relationship with some boronic acid transition state inhibitors (BATSIs), as well as the antibiotic ceftazidime, bound in the active site of ADC-7. These studies will contribute to the characterization of novel inhibitor compounds that can help in the alleviation of antibiotic resistance in *Acinetobacter baumannii*.

90 Pro-efferocytic nanoparticles prevent atherosclerosis

Alyssa M. Flores

Pro-efferocytic nanoparticles prevent atherosclerosis

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Efferocytosis refers to the phagocytic removal of apoptotic cells. Because the body carefully ensures that only apoptotic cells are removed and healthy cells are not, efferocytosis is a highly regulated process, achieved by balancing "eat-me" and "don't eat me" signals.

CD47 is a key anti-efferocytic molecule that is ubiquitously expressed on healthy cells. Upon interaction with phagocytes, CD47 conveys a "don't-eat-me" signal that negatively regulates phagocytosis. Recently, we found that the upregulation of CD47 within the necrotic core is a key driver for the accumulation of apoptotic debris in the atherosclerotic plaque. Blocking CD47 with anti-CD47 antibodies can reactivate efferocytosis and prevent atherosclerosis. However, this antibody-based therapy can also cause the off-target clearance of red blood cells, thus compromising the therapeutic potential of systemic pro-efferocytic therapies.

To overcome this barrier, we developed a "precision" therapy which interrupts CD47 signaling locally in the atherosclerotic plaque. Here, we report a therapeutic nanomedicine that comprises single-walled carbon nanotubes (SWNTs) nanoparticles loaded with inhibitors of the CD47 signaling axis. We demonstrate that SWNTs home to the inflamed lesional macrophage and block CD47 signaling specifically at the diseased vessel. We find that SWNTs loaded with the pro-efferocytic therapy accumulate in the atherosclerotic plaque, enhance macrophage-mediated phagocytosis of vascular cells, and prevent atherosclerosis in atheroprone *apoE*$^{-/-}$ mice. Furthermore, pro-efferocytic SWNTs reduced plaque burden without systemic toxicities, suggesting they may form the basis of a new platform of precision nanotherapies for cardiovascular disease.

91 The Influence of Nato3 on Genes Involved in Dopamine Neurogenesis and Maturation

Melina Frantzeskakis

The Influence of Nato3 on Genes Involved in Dopamine Neurogenesis and Maturation

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Dopamine neurons arise from the floor plate of the midbrain, these midbrain dopamine (mDA) neurons are responsible for the development of Parkinson's disease when they cease to function. Genes that promote the formation of mDA have potential to be used for clinical therapy development; this is due to the influence they have on gene regulation. Transcription factors effect expression of genes that encourage mDA development. Neurogenesis and maturation of mDA are influenced by multiple genes such as: FOXA1/2, LMX1, WNT1, and several others. One gene involved in dopamine neuron maturation is the basic helix-loop-helix transcription factor Nato3 (N3), however, its mechanism of action is unknown. We hypothesized that Nato3 had the ability to upregulate genes involved in mDA neurogenesis and maturation in vivo and in the SN4741 cell line and is therefore able to drive dopamine neurogenesis. Previous data produced by our lab using qPCR and immunostaining showed that overexpression of N3 upregulates LMX1 genes in vivo. The upregulation of the genes involved in mDA neurogenesis and maturation by Nato3 overexpression was mimicked in the SN4741 cell line, shown through qPCR data. This upregulation of these genes (such as Nurr1, En1, and FOXA1/2) indicates that Nato3 influences dopamine neurogenesis.

92 Exploring the role of Arid1a in Kras-mediated transformation

Scott C. Friedland

Exploring the role of *Arid1a* in *Kras*-mediated transformation

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Objective: Pancreatic Adenocarcinoma (PDAC) is an almost universally fatal disease. The SWI/SNF complex (an ATP-dependent nucleosome remodeling complex) is deleteriously mutated in at least 33% of PDAC cases, with the subunit, *ARID1A*, mutated in 8-15%. However, little is known about the role of *ARID1A* in the pancreas, nor about its interaction with *KRAS* (the most common mutation in PDAC). Using *in vivo* and *in vitro* systems we test the extent to which *Arid1a* and *Kras* cooperate in transformation, and what functions of *Arid1a* drive that cooperation. Methods: Using pancreas-specific expression of Cre recombinase, we deleted *Arid1a* by itself and/or activated $Kras^{G12D}$ and analyzed these mice longitudinally. We also derived mouse embryonic fibroblasts (MEFs) with the same alleles and performed RNA and ATAC-seq, and other *in vitro* analyses. Results: $Arid1a^{f/f}$ mice show progressive attrition of the acinar population and ductal expansion, with macroscopic cysts forming by 52 weeks of age. By 12 weeks, $Arid1a^{f/f}$ mice have significantly higher rates of proliferation and apoptosis than wild type controls, and this proliferative phenotype is most notable in the ductal compartment. When combined with oncogenic $Kras^{G12D}$ loss of *Arid1a* produce highly cystic pancreases that resemble human intraductal papillary mucinous neoplasm as opposed to the pancreatic intraepithelial neoplasia that predominate in the $Kras^{G12D}$ and $Kras^{G12D}$; $Arid1a^{f/+}$. In both the $Kras^{G12D}$; $Arid1a^{f/f}$ and $Kras^{G12D}$; $Arid1a^{f/+}$ cohorts there were malignancies with metastases. Using MEFs with the same alleles we have shown that the two lesions cooperate to induce replicative immortality and the ability to form foci. We used these cells to perform RNA- and ATAC-seq, which showed enrichment for AP-1 binding sites in peaks within presumed enhancers that were differentially less accessible in the $Kras^{G12D}$; $Arid1a^{f/f}$ cells compared to $Kras^{G12D}$ cells. However, intriguingly TPA (an AP-1 activator) treatment enhanced focus formation. Conclusions: *Arid1a* loss and $Kras^{G12D}$ cooperate to drive proliferation and cancer in the pancreas and *in vitro*. Initial data suggests this cooperation may be driven by altered chromatin accessibility around enhancers containing AP-1 binding sites.

93 Inhibition of PI3Kδ and blockade of VIP-R signaling pathways to enhance T cell proliferative potential and phenotype prior to CAR T manufacture

Christopher (Ronnie) Funk

Inhibition of PI3Kδ and blockade of VIP-R signaling pathways to enhance T cell proliferative potential and phenotype prior to CAR T manufacture

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Remissions of hematologic malignancy with chimeric antigen receptor (CAR) T cell therapy are associated with CAR T cell expansion kinetics, with a majority of trials associating long-term CAR T persistence with continued remission. Accordingly, expansion and persistence of a single clone (defined by CAR insertion site) comprising 94% of the total CAR T cells was sufficient to induce remission. Similarly, we reported oligoclonal expansion of T cells in a patient who experienced disease relapse following CAR T therapy (Funk et al. 2018). We hypothesized phenotype could be used to predict response and took serial measurements. In response to pancytopenic aplasia, 60% of the patient's total CD8 cells and 20% of CD4 cells lost expression of both CD27 and CD28, a phenotypic change heralding T cell senescence and a decay of CAR T numbers instead of persistence. These clinical observations suggest CAR T cell phenotype, such as expression of CD27/28, influences response to therapy.

We explored new ways to expand T cells using small molecule inhibitors of pathways known to be relevant to T cell survival and differentiation. Idelalisib is an isoform-selective inhibitor of PI3Kδ, which we hypothesized would increase numbers of viable T cells since 54% of patients who receive idelalisib develop CD8 T cell based hepatocellular injury. Additionally we hypothesized blockade of vasoactive intestinal peptide (VIP) receptors with a peptide competitive antagonist, VIPhyb, could further enhance T cell expansion, since VIP secreted by T cells promotes T cell tolerance by autocrine/paracrine mechanisms. To assess these hypotheses, healthy- and CLL-donor T cells were expanded by industry-standard methods (30 U/mL of IL-2, anti-CD3/28 beads) in translatable G-Rex culture systems. Healthy-donor T cells cultured with combination 100nM idelalisib and 30nM VIPhyb exhibit as high as 166-fold expansion over input at day 35, with 67% more live cells than control. Similarly, CLL-donor T cells cultured with 100nM idelalisib and 30nM VIPhyb exhibit 113-fold expansion at day 21, expanding 54% more cells than control. To determine an optimal dosage, T cells were expanded across a logarithmic scale of idelalisib and VIPhyb concentrations. A synergistic effect upon T cell expansion was observed, with 10 μM idelalisib, 30 nM VIPhyb combination reproducibly yielding a 2.4-fold increase in viable T over industry-standard methods at day 9. As a phenotypic correlation, cells expanded in presence of idelalisib exhibit four-fold increased frequencies of naïve and memory T cell markers, such as CD27 and CD28, over control. We hypothesize these phenotypic changes will underlie functional and mechanistic changes that improve CAR T cell function. A supported hypothesis would provide rationale toward a clinical trials that administer an FDA-approved PI3Kδ inhibitor prior to collection of T cells to assess influence upon T cell phenotype and subsequent CAR T persistence and outcomes.

94 Expression of Chimeric Antigen Receptors (CARs) in cytokine induced memory-like (ML) NK cells is a novel strategy to enhance ML NK cell immunotherapy
Margery Gang

Expression of Chimeric Antigen Receptors (CARs) in cytokine induced memory-like (ML) NK cells is a novel strategy to enhance ML NK cell immunotherapy

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Our over-arching objective is to improve cytokine-induced memory-like (ML) NK cell specificity with chimeric antigen receptors (CARs) and adapt CAR intracellular signaling domains for enhanced ML NK cell survival, expansion, and functionality. NK cells are cytotoxic innate lymphocytes that play a major role in responses against viruses and cancer cells. Because of their anti-tumor responses, NK cells are promising candidates for cancer immunotherapy, particularly for hematologic malignancies such as acute myeloid leukemia (AML). Moreover, paradigm-shifting studies have demonstrated that human NK cells display "memory-like" properties after combined IL-12, IL-15, and IL-18 cytokine-activation. ML NK cells display enhanced cytokine production and cytotoxicity after subsequent re-stimulation with various stimuli, including tumor cell lines and primary AML blasts. Although ML NK cell adoptive therapy shows promise in early phase clinical trials for treating AML, the anti-tumor responses rely on the established NK cell receptor-based recognition of AML blasts. In order to improve ML NK cell targeting with activation against a wide variety of malignancies, we are investigating CAR in ML NK cells (CAR-ML). CARs have been developed to redirect effector T cell specificity from their endogenous TCR to tumor-associated antigen. CARs utilize the antigen specificity of antibody via extracellular single chain variable fragment with T cell receptor/co-activating receptor intracellular signaling domains (CD3ζ/41BB/CD28). These CAR-T cells display enhanced tumor-specific immunity and have led to remarkable clinical responses in the context of B-cell malignancies. We hypothesize that integrating CAR antigen-specificity with ML NK cell responses will improve NK cell-based immunotherapy for AML. Indeed, αCD19-CAR-ML cells exhibit enhanced responses against NK cell-resistant CD19+ lymphomas in vitro. Here we (1) generated anti-CD33-CAR-ML NK cells and will define their functional responses against myeloid leukemia, and (2) elucidate whether CAR-ML NK cell survival and expansion can be enhanced by incorporating NK-centric cytokine receptor signaling domains. To generate CAR-ML NK cells, NK cells were isolated from normal donors and activated with IL-12/15/18 overnight, washed, and then lentiviral transduction is performed in the presence of IL-15, which is required for NK cell survival. After transduction, the cells are differentiated for 7 days and then evaluated with functional assays using CAR-relevant targets (CD33+ HL-60, CD19+ Raji) and read out IFN-γ production by flow cytometry and cytotoxicity. We have generated αCD33+ CAR-ML NK cells and studies evaluating responses against AML are ongoing. Additionally, we have designed a novel CAR incorporating the intracellular signaling components of the IL-2/15 cytokine receptor (CAR^{cyt}), which we chose for its involvement in NK cell differentiation, survival, proliferation and cytotoxicity. We have generated the αCD19-CAR^{cyt} ML NK cells and studies verifying appropriate JAK/STAT signaling in response to CD19+ Raji are ongoing. These studies will provide pre-clinical proof-of-principle for future clinical trials incorporating CAR-ML NK cell immunotherapy.

95 Training the innate immune response: How β-glucan induces trained immunity and robust anticancer responses
Anne E. Geller

Training the innate immune response: How β-glucan induces trained immunity and robust anticancer responses

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In immunology, immune responses are typically characterized as part of the innate or the adaptive immune system. The innate immune response consists of generalized and immediate defense mechanisms, while the adaptive immune system is responsible for the generation of hyper-specific clonal T and B cells that incite specialized defenses against specific pathogens. The cells of the innate immune system such as neutrophils and macrophages have not classically been thought of to possess the ability to recognize a pathogen and subsequently develop a memory response. Recently however, the idea of "trained immunity" has surfaced, whereby cells of the innate immune response have been shown to possess a type of memory to endotoxins such as LPS and bacterial derived polysaccharides such as β-glucan, a naturally occurring β-D-glucose found in fungus, yeast and bacteria. In this study we evaluate β-glucan's role and mechanism of participating in trained immunity in the setting of lung cancer. By training the immune response with β-glucan we are able to show markedly improved survival and increased immune stimulation in the setting of murine lung cancer, and further we examine the trafficking mechanism of beta-glucan within the body to understand how β -glucan asserts these effects in vivo. We show that IP injection of β-glucan leads to β-glucan trafficking to the spleen, bone marrow and pancreas. The trafficking of β-glucan to the pancreas is a novel finding and indicates that in addition to the immunogenic effects in the pancreas, β-glucan could be used as a novel delivery vehicle of delivering drugs to the pancreas in the setting of pancreatic cancer. Additionally, we show the expansion of specific myeloid populations in the bone marrow and lung as a result of β-glucan treatment, which are believed to be responsible for the enhanced immune response to cancer. Finally, we study the effects of β-glucan treatment on the expression of PD-L1 in macrophages in the tumor microenvironment, and find that β-glucan upregulates PD-L1 in numerous settings. This data indicates a potential for the combination of β-glucan with anti PD-L1 therapy to create robust anticancer responses. Together this data highlights exciting new functions of innate immune cells, which breaks the dichotomy of our current understanding of the innate and adaptive immune response. This study also shows the important therapeutic potential of β-glucan in cancer treatment, and leads to future prospects of combining β-glucan with immune therapy to treat cancer.

96 Temperature in the Hospitalized Patient
Ivayla I. Geneva

Temperature in the Hospitalized Patient

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Body temperature – now a universally accepted vital sign, had been of interest to healers and philosophers since antiquity, with deviations from normothermia being linked to clinical diagnoses. Most of the available data on human body temperature stems from measurements from healthy subjects in the outpatient setting, with much less being known about the body temperature of inpatients. To our knowledge, ours is the first study that evaluates the temperatures of all hospitalized patients at a large tertiary medical center over a long time period (1 year). Herein we present a retrospective analysis of a total of 695,107 temperature readings from 16,245 patients, ages 0 to 105 years, 50% female, with a focus on the role of measurement site, age, and gender. In our analysis, we used the average temperature (Tave) per patient and per site of measurement. The data was analyzed with EXCEL and MATLAB. Descriptive statistics, Student's T-test, and Pearson's correlation were used, where appropriate, with statistical significance set at p

97 Mathematics of cancer immunotherapy
Jason T. George

Mathematics of cancer immunotherapy

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Recent progress in immunotherapy has revolutionized modern cancer treatment. These therapies, though effective, can be quite elaborate owing in part to the complexity of the human immune system. For example, hematopoietic stem cell transplant recipients enlist an entire donor-derived allogeneic T-cell repertoire to attack a growing malignancy. Perhaps most importantly, the adaptive nature of the immune system uniquely enables this treatment approach to co-evolve alongside an evasive threat. Cancer immunotherapy, though promising, is poorly quantified and thus merits further theoretical investigation with the aim of predicting optimized treatment strategies. Here, we discuss several of our recent mathematical models developed to better understand the interaction between an evolving cancer cell population and the CD8+ T-cell repertoire. By applying our theoretical framework, we predict the likelihood of an allogeneic response given differences in host-donor minor histocompatibility antigens, explain AML age-incidence data as a result of an aging immune system, and propose evolutionary patterns in cancer progression as a result of immuno-surveillance that agree with empirical observation.

98 Differences in the tensor veli palatini between adults with and without cleft palate using high-resolution 3-dimensional magnetic resonance imaging
Thomas N. George

Differences in the tensor veli palatini between adults with and without cleft palate using high-resolution 3-dimensional magnetic resonance imaging

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Objective: To investigate the dimensions of the tensor veli palatini (TVP) muscle in adults with and without cleft palate. Design: Prospective study. Participants: There were a total of 14 adult participants, 8 noncleft and 6 with cleft palate. Methods: Analysis and comparison of the TVP muscle and surrounding structures was completed using 3D MRI data and Amira 5.5 Visualization Modeling software. TVP muscle volume, hamular process distance, mucosal thickness, TVP muscle length, and TVP muscle diameter were used for comparison between participant groups based upon previous research methods. Results: Mann-Whitney U tests revealed a significantly smaller (U 3) compared to individuals in the non–cleft palate group (median = 895.19 mm^3). The TVP muscle was also significantly shorter (U = 1.00, P = 0.003) in the cleft palate group (median = 18.04 mm) versus the non–cleft palate (median = 21.18 mm). No significant differences were noted for the other measured parameters. Conclusion: Significant differences in the TVP muscle volume and length among the cleft and noncleft participants in this study provide insight regarding the etiology of the increased incidence of otitis media with effusion (OME) seen within the cleft population. Results from this study also contribute to our understanding of the underlying anatomic differences among individuals with cleft palate.

99 Effects of developmental dieldrin exposure on neuroinflammation and α-synuclein aggregation in the mouse nigrostriatal pathway
Aysegul O. Gezer

Effects of developmental dieldrin exposure on neuroinflammation and α-synuclein aggregation in the mouse nigrostriatal pathway

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Human and animal studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of Parkin-

son's disease. Although previous work demonstrated that developmental dieldrin exposure increases neuronal susceptibility to a neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity in male C57BL/6 mice, the mechanisms driving this increased susceptibility are not well characterized. Male mice developmentally exposed to dieldrin display an enhanced response to MPTP, showing a greater increase in glial fibrillary acidic protein (GFAP) and α-synuclein (α-syn) expression. This suggests that dieldrin-induced changes in neuroinflammation and α-syn may underlie increases in neuronal susceptibility. Here, we tested the hypothesis that developmental dieldrin exposure induces changes in neuroinflammatory markers and α-syn prior to MPTP exposure. Starting at 8 weeks old, female C57BL/6 mice were exposed to 0.3 mg/kg dieldrin by feeding every 3 days, continuing throughout mating, gestation and lactation. At 12 weeks of age, both male and female pups from independent litters were sacrificed, and striatum and substantia nigra were dissected. To identify sex-specific changes in neuroinflammation or α-synuclein, both sexes were included in the analyses. We assessed markers of neuroinflammation via targeted expression assays to test if developmental exposure to dieldrin led to induction of neuroinflammatory pathways in the striatum and substantia nigra. In addition, we analyzed α-syn aggregation by western blot in non-denaturing and non-reducing conditions to test whether exposure leads to changes in α-syn species. We identified that developmental dieldrin exposure produces "sub-toxic" changes in these pathways that may underlie the differences in neuronal vulnerability. In a parallel study, we identified sex-specific DNA methylation changes in genes related to the development and maintenance of the nigrostriatal pathway. Taken together, these data suggest that developmental dieldrin exposure leads to persistent changes in phenotype that may contribute to the development of Parkinson's disease.

100 A Novel Mechanism of Targeting Ovarian Cancer Tumor Microenvironment by Dorsomorphin, Compound C

Alia Ghoneum

A Novel Mechanism of Targeting Ovarian Cancer Tumor Microenvironment by Dorsomorphin, Compound C

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Epithelial ovarian cancer (OvCa), specifically, high grade serous cancer (HGSC) is the leading cause of death from gynecologic malignancies in the USA as most patients are diagnosed at late stages. Currently, the standard care is surgical debulking followed by several cycles of cisplatin and paclitaxel. However, chemo-resistance and recurrence are encountered due to the unique metastatic pattern of HGSC in the peritoneal cavity, including the interaction of malignant cells with the cellular components of the peritoneal tumor microenvironment (TME), specifically mesothelial cells, tumor-associated macrophages (TAMs), cancer associated adipocytes (CAAs), and cancer associated fibroblasts (CAFs). Thus, there is an unmet need for OvCa treatment that not only target tumor cells but also their interactions with the peritoneal TME which provides a safe haven for resistant and recurrent disease. The PI3K-AKT-mTOR-NFκB pathway in OvCa is the most frequently altered (~70%) intracellular pathway. Several reports indicate that aggressive OvCa has a significant response to PI3K inhibitors. Our preliminary data show that Compound C (dorsomorphin, CC) not only inhibited OvCa cell proliferation and clonogenic survival, migration and matrix invasiveness, but also the reciprocal crosstalk between OvCa cells and macrophages *in vitro*. CC also inhibited p65RelA NFkB activation and nuclear localization. Importantly, CC inhibited the activation of p85 and p110α subunits of PI3K in a time and dose-dependent manner. Together, these findings promote the hypothesis that CC inhibits OvCa progression through a direct effect on the PI3K/AKT/mTOR/NFκB pathway and inhibition of OvCa-stromal cross talk. To test our hypothesis, we propose the following specific aims: Aim 1: Investigate the inhibitory role of CC on OvCa cells growth, and malignant phenotype, Aim 2: To test the hypothesis that CC inhibits cancer cell- stromal interactions, Aim 3: Determine the efficacy of CC in treatment of OvCa in using preclinical mouse models of OvCa. Successful completion of these specific aims will enhance our knowledge of the mechanisms by which CC inhibits OvCa growth and survival and their interactions with the key cellular components in the peritoneal microenvironment. We are proposing a comprehensive unbiased approach using established and primary cancerous and non-cancerous cell types in 2D and 3D multiple culture organoid system that would recapitulate the peritoneal TME and allow mechanistic studies *in vitro*. We will employ biochemical, molecular and cell biological approaches in tandem with preclinical models that model early, late, recurrent as well as chemo-resistant HGSC as well as patient-derived xenografts. In this study, our goal will be to identify the mechanisms by which CC can mitigate aggressive OvCa.

101 Helicobacter pylori genetic adaptation in response to gastric inflammation and other environmental factors

Nora J. Gilliam

Helicobacter pylori genetic adaptation in response to gastric inflammation and other environmental factors

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Helicobacter pylori infection is a significant risk factor for gastric adenocarcinoma & peptic ulcers; therefore, these bacteria are categorized by the World Health Organization in the same carcinogenic class as cigarettes. *H. pylori* colonizes the stomach in approximately 50% of the human population: most people infected with *H. pylori* remain asymptomatic while others develop gastric cancer. The risk of gastric cancer from *H. pylori* is influenced by strain-specific bacterial properties, host genetic variation, and environmental factors (including a high salt diet). In this study, we tested the hypotheses that gastric inflammation and high-salt conditions are important factors that select for specific *H. pylori*

genetic variations. To test the influence of gastric inflammation on the genetic adaption of *H. pylori*, wild-type C57BL/6 mice and interleukin-21 cytokine knockout (IL-21-/-) mice were experimentally infected with *H. pylori* input strain PMSS1 and euthanized 3 months post-infection. Gastric histological analysis showed that the infected wild-type mice developed more severe gastric inflammation than the infected IL-21-/- mice. *H. pylori* strains cultured from the mice (output strains) were tested for function of the *cag* type IV secretion system (*cag* T4SS) by measuring their ability to activate NFκB signaling in gastric epithelial cells, using a luciferase reporter assay. A loss of *cag* T4SS function was detected in all strains from wild-type mice, whereas strains from some of the IL-21-/- mice maintained T4SS activity. We also analyzed *H. pylori* genetic adaptation during long-term passage on media containing high salt concentrations and compared the resulting genetic changes with those previously identified in an analysis of *H. pylori* genetic adaptation in vivo in response to a high salt diet. Two mutations were selected in both experiments: a point-mutation (R88H) in the *fur* gene (which encodes a protein that regulates gene expression in response to variations in iron concentration) and a mutation in the *katA* gene (which encodes catalase, a protein that confers resistance to oxidative stress). These experiments help to explain how inflammation and high levels of salt act as driving forces for genetic diversification in *H. pylori*, and contribute to our understanding of why some *H. pylori*-infected individuals develop stomach cancer and others do not.

102 Autoimmune regulator gene supports early pregnancy and promotes the expression of pregnancy associated self-antigens
Eva Gillis-Buck

Autoimmune regulator gene supports early pregnancy and promotes the expression of pregnancy associated self-antigens

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Common pregnancy complications such as recurrent miscarriage and implantation failure are often associated with autoimmunity. The Autoimmune Regulator gene (*Aire*) prevents autoimmunity by promoting the expression of tissue restricted antigens (TRAs) in medullary thymic epithelial cells (mTECs), leading to clonal deletion and regulatory T cell conversion of self-reactive T cells. Similarly, extrathymic *Aire*-expressing cells (eTACs) in spleen and lymph nodes also express TRAs to further prevent immune reactivity to these antigens. Whether *Aire* is functionally involved in promoting maternal tolerance to pregnancy-associated TRAs has not been examined. And though recent studies have reported sex differences in thymic *Aire* expression in reproductive-aged mice and humans, any changes to *Aire* during pregnancy are still unknown.

We first conducted a loss-of-function experiment, using an *Aire*-diphtheria toxin receptor (DTR) transgenic mouse model to ablate maternal *Aire*-expressing cells during the first nine days of pregnancy. *Aire*DTR+DT plugged females were 6.5 times as likely to show complete embryo resorption by E9.5, compared to WT+DT dams (RR 6.5; 95% CI 1.61–26.1; pAireDTR N=28; WT N=32). *Aire*DTR+DT dams had significantly fewer FoxP3^{+} Tregs (p+ T cells (pAireKO mice are known to develop ovarian insufficiency and subsequent progesterone (P4) deficiency. However, we found no difference in serum P4 levels of *Aire*DTR+DT compared to WT+DT dams. P4 supplementation failed to prevent embryo resorption in AireDTR+DT dams or to change the alteration in thymic Tconv:Treg. Thus, *Aire* deficiency during early pregnancy leads to maternal T cell imbalance and embryo loss without ovarian insufficiency, suggesting a novel mechanism for autoimmune-mediated infertility.

We next investigated transcriptional changes to mTECs and eTACs during healthy pregnancy. We hypothesize that *Aire* promotes the expression of a unique set of pregnancy associated TRAs, which are encoded in the maternal genome, but have not been produced since the mother herself was a fetus with a placenta. We sorted GFP+MCHII+ cells from the thymus and uterus-draining lymph nodes (udLN) of virgin and E9.5 pregnant *Aire*-driven Igrp-GFP (Adig) reporter mice. Bulk RNA-sequencing found no differentially expressed genes in pregnant vs virgin mTECs, but did find 244 differentially expressed genes in pregnant vs virgin udLN eTACs (FDRAire and human patients with *AIRE* mutations and recurrent miscarriage.

103 Investigation of AIM2 loss in Bats reveals Functional Dampening of the Inflammasome Pathway
Geraldine Goh

Investigation of AIM2 loss in Bats reveals Functional Dampening of the Inflammasome Pathway

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Bats have evolved to sustain high metabolic stress during flight and are known reservoir hosts for deadly zoonotic viruses such as rabies and lyssaviruses, henipaviruses, and MERS and SARS-like coronaviruses. Due to the threat of zoonotic transmission from bats to domestic animals and humans, questions remain regarding bats' unique immune profile, apparent lack of disease and transmission of pathogens. Recent analysis of available bat genomes revealed a complete loss of the PYHIN gene family, including the human and mammalian AIM2 gene, a cytosolic dsDNA sensor capable of activating the inflammasome. Upon sensing dsDNA in the cytosol, AIM2 recruits its adaptor ASC and triggers formation of the multi-protein inflammasome complex, activating CASP1 to cleave cytokines such as pro-interleukin 1β (IL-1β) for secretion, and mediating a pro-inflammatory cell death program called pyroptosis. While the gene plays an essential role in immune responses against bacterial and viral pathogens, its over-activation can also be detrimental to the host. This is observed in enhanced expression in autoimmune diseases such as psoriasis, systemic erythematosus, and inflammatory bowel disease.

Our goal in this study was to restore AIM2 in the bat intracellular envi-

ronment and investigate if reconstitution enables efficient inflammasome activation. We first confirmed that wild-type bat immortalized kidney and primary bone marrow derived macrophages (BMDMs) indeed lack AIM2 inflammasome signaling in response to cytosolic dsDNA treatment. The AIM2 gene was reconstituted via lentiviral transduction in immortalized *P. alecto* kidney cells stably expressing ASC and primary BMDMs with endogenous ASC, and ASC-speck formation was measured by high-throughput image-based flow cytometry. As expected, AIM2 reconstitution was able to restore efficient inflammasome signaling *in vitro*, with dose-wise increase of ASC-speck formation in response to cytosolic dsDNA stimulation. This suggests that the immediate downstream components of the bat inflammasome pathway remain intact and are sufficiently evolutionarily-conserved to interact with the human AIM2 protein. Future investigations will be undertaken to measure downstream CASP1 activity, IL-1β secretion, and cell death signaling. Taken together, our data indicates that loss of AIM2 in bats results in diminished ability to respond to pattern- and danger-associated molecular patterns (PAMPs, DAMPS) in the form of cytosolic dsDNA. This study is crucial in elucidating the consequences of inflammasome-specific dampening, which may have allowed bats to evolve to protect against host DNA damage responses, yet consequentially allowing competence in their harboring of zoonotic pathogens.

104 Aerosolized Toll-like Receptor Agonists Suppress Allergic Asthma
David L. Goldblatt

Aerosolized Toll-like Receptor Agonists Suppress Allergic Asthma

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Asthma affects 300 million people worldwide and direct and associated medical costs are estimated to exceed $18b annually in the United States alone. Recent studies have linked exposure to microbial elements in the environment to a reduction in allergic asthma. We sought to determine whether activation of innate immunity by aerosolized Toll-like receptor (TLR) agonists could attenuate the development of allergic asthma in mice.

BALB/cJ mice were treated by aerosolization with 1 μM ODN M362, an agonist of the TLR9 homodimer, and 4 μM Pam2CSK4, an agonist of the TLR2/6 heterodimer, delivered together ("Pam2-ODN") at varying points around the time of sensitization to 3 different aeroallergens: ovalbumin (OVA), house dust mite (HDM), and aspergillus oryzae (Ao). The development of an asthma phenotype was assessed by quantification of leukocytes in bronchoalveolar lavage fluid (BALF) and mucous metaplasia. Quantification of T helper (T_H) subsets were assessed by flow cytometry of canonical lineage transcription factors (T-bet, GATA3, RORyt, and FoxP3). Serum immunoglobulin concentrations were assessed by standard sandwich ELISA.

Mice treated with Pam2-ODN 1 day before sensitization showed strong reduction in lung eosinophils in all 3 models. In OVA and Ao models, there was also a reduction of airway epithelium mucin content. Using the HDM model, this effect was seen when mice were treated 8 days before sensitization, but not 15 days before sensitization, or 2 days afterwards. T_H2 cells in the lungs were reduced 50% in Pam2-ODN-treated mice, without any change in T_H1, T_H17, or T_{reg} cells. Using the OVA model, total serum IgE and OVA-specific IgE were reduced, but total IgG2a was increased.

Activating innate immunity by Pam2-ODN attenuates features of allergic asthma by blocking the type 2 immune response that normally drives this disease. The inability of Pam2-ODN to have an effect after sensitization is a strong indicator that Pam2-ODN blocks the primary immune response to aeroallergens. The absence of detectable Th1 or Th17 responses suggest that Pam2-ODN is not driving an alternately polarized immune response. In previous studies of Pam2-ODN, the lung epithelium was shown to be crucial for both activation of innate immunity against pathogens and the development of allergic asthma. Taken together, Pam2-ODN may reprogram airway epithelial cells to be tolerogenic to aeroallergens and could represent a novel pathway for treatment of allergic asthma. Tolerogenic therapeutics are desperately needed to counter the rise in prevalence of allergic asthma and further studies are required to understand the precise molecular mechanism of O/P in this setting.

105 Psychological stress induces alterations in behavior and the mucosal immune system in a spontaneous mouse model of ileitis
Adrian Gomez-Nguyen

Psychological stress induces alterations in behavior and the mucosal immune system in a spontaneous mouse model of ileitis

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Patients with Crohn's disease (CD) suffer from abnormally high rates of depression and anxiety. Depression among patients with CD are higher than other debilitating chronic medical conditions, such as cancer. Behavioral co-morbidities are associated with increased rates of flares, more severe disease course, and increased rate of corticosteroid prescription. Psychological stress, even among CD patients in remission, is recognized as a risk factor for flare-ups. Despite the well-established relationship between stress and symptom relapse, a rigorous mechanistic explanation remains elusive. Here we demonstrate alterations in the behavioral profile and the mucosal immune system in the SAMP1/YitFc (SAMP1) mouse, a spontaneous model of CD-like ileitis, following exposure to acute and chronic psychological stress.

SAMP1 littermates were sex matched and divided into two groups

(n=8). The first group was subjected to restraint stress (RS) for seven days. Mice were restrained for 180 minutes per day in a 50mL conical tube with air holes drilled for adequate ventilation. Stool samples were collected each day. Subsequently, each group was subjected to behavioral testing to determine anxiety-like behavior (open field and elevated plus maze), depressive-like behavior (tail suspension), motor deficits (line crossings and rota-rod), and cognitive deficits (Y-maze). Immediately after, mice were sacrificed and tissue samples were collected for immunological analysis.

Mice subjected to RS displayed increased immobility time during tail suspension indicating a depressive-like phenotype (p

The marked difference in the MLN dendritic cell (DC) population suggests increased luminal sampling of intestinal bacteria. Determining how the microbiome affects or is affected by the altered DC population is of particular interest to us. Is the DC population altered because of the microbiome or is the DC population altering the microbiome? Our preliminary data has suggested that depressive states are associated with alterations in the microbiome. Again, whether the changes are a cause or an effect of depression is yet to be answered but is our immediate goal.

106 An ERK/hnRNPK/JUND axis regulates pancreatic β cell survival
Austin L. Good

An ERK/hnRNPK/JUND axis regulates pancreatic β cell survival

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In type 2 diabetes, oxidative stress contributes to the dysfunction and loss of pancreatic β cells. A highly conserved feature of the cellular response to stress is the regulation of mRNA translation, however, the mechanisms underlying this process in β cells are not fully understood. Here we use TRAP-seq as a means to discover novel translationally regulated genes in β cells, leading to the identification of the transcription factor JUND as translationally upregulated in islets during metabolic stress. Depletion of JUND in β cells reduces oxidative stress and apoptosis caused by high glucose and free fatty acid levels. Transcriptome assessment demonstrates that JUND regulates a cohort of genes that are commonly dysregulated during β cell dysfunction, including pro-oxidant and pro-inflammatory genes. Further, the RNA binding protein hnRNPK post-transcriptionally regulates JUND during metabolic stress in a MEK-dependent manner. Importantly, this hnRNPK/JUND axis is activated in islets from diabetic *db/db* mice and in human islets exposed to metabolic stress. Finally, hnRNPK interacts with the RNA helicase DDX3X to promote efficient translation of JUND by facilitating interaction between DDX3X and the translation pre-initiation complex. Thus, a translation-centric approach uncovered hnRNPK and JUND as stress-responsive factors in β cells that contribute to redox imbalance and apoptosis during pathophysiologically relevant stress.

108 The coronavirus macrodomain counters antiviral PARP-mediated ADP-ribosylation
Matthew E. Grunewald

The coronavirus macrodomain counters antiviral PARP-mediated ADP-ribosylation

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ADP-ribosylation is a ubiquitous post-translational addition of either monomers or polymers of ADP-ribose to target proteins by ADP-ribosyl transferases, usually by interferon (IFN)-inducible diphtheria toxin-like enzymes known as PARPs. While a few PARPs have known antiviral activities, these antiviral functions are mostly independent of ADP-ribosylation. Consequently, less is known about the antiviral effects of ADP-ribosylation. Several viral families, including the Coronaviridae, Togaviridae, and Hepeviridae, encode for macrodomain proteins that bind to and hydrolyze ADP-ribose from proteins and are critical for either replication or pathogenesis. These results suggest that macrodomains counter cellular ADP-ribosylation, but whether PARPs or other ADP-ribosylating proteins cause this modification is not clear. Here we demonstrate that PARP enzymes restricted the replication of and enhanced the IFN response to a macrodomain mutant coronavirus in primary macrophages. Specifically, knockdown of two abundantly expressed PARPs, PARP12 and PARP14, led to enhanced replication of the mutant virus. PARP14 was also important for the induction of IFN in mouse and human cells, indicating a critical role for this PARP in the regulation of innate immunity. In summary, these data demonstrate that the coronavirus macrodomain counters PARP-mediated antiviral ADP-ribosylation and illustrates a unique mechanism of viral immune evasion.

109 CellTag Indexing: a genetic barcode-based multiplexing tool for single-cell technologies
Chuner Guo

CellTag Indexing: a genetic barcode-based multiplexing tool for single-cell technologies

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Single-cell technologies have seen rapid advancements in recent years, along with new analytical challenges and opportunities. These

high-throughput assays increasingly require special consideration in experimental design, sample multiplexing, batch effect removal, and data interpretation. Here, we describe a lentiviral barcode-based multiplexing approach, 'CellTag Indexing', where we transduce and label samples that can then be pooled together for downstream application and analysis. By introducing predefined CellTag barcodes that are transcribed and readily detected, we can reliably read out barcode sequences via genomic or transcriptomic profiling, permitting the simultaneous assessment of sample identity and transcriptional state. We validate and demonstrate the utility of CellTag Indexing by sequencing multiplexed transcriptomes at a single-cell resolution. A variety of cell types are analyzed, including mouse pre-B cells, primary mouse embryonic fibroblasts, human HEK293T cells, and mouse induced endoderm progenitors (iEPs). Furthermore, we establish CellTag Indexing as a valuable tool for multiplexing and competitive lineage tracing in a transplantation experiment of iEP engraftment in a mouse model of colonic epithelial injury. We present CellTag Indexing as a broadly applicable genetic multiplexing tool that is complementary with existing single-cell RNA-sequencing and multiplexing strategies.

110 Elucidation of the genetic architecture of communicating hydrocephalus
Andrew T. Hale

Elucidation of the genetic architecture of communicating hydrocephalus

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Communicating hydrocephalus (CHP) pathophysiology is characterized by abnormal accumulation of cerebrospinal fluid (CSF) and subsequent elevations in intracranial pressure causing impaired neurodevelopment and morbidity. Proposed pathophysiological mechanisms of CHP include impaired development of the neural stem cell niche, abnormal ciliation of CSF-producing ependymal cells, and dysfunction of CSF absorption and/or secretion. While over 100 candidate genes have been implicated in CHP pathogenesis as CHP is a component of a wide array of Mendelian diseases, the underlying genetic basis of the disease is not known. Thus, using genotyping data linked directly to the electronic health record (BioVU), we perform the largest human genetic study of communicating hydrocephalus (CHP). We apply PrediXcan, which imputes the genetically-determined component of gene expression using common-variant single nucleotide polymorphism (SNP) data from and a reference transcriptome derived from 44 unique tissues in GTEx, to explore tissue-specific genes implicated in CHP. We identify a potentially causal gene, maelstrom (MAEL, a critical regulator of DNA methylation and transposon activity), with decreased expression across multiple neurological tissues akin to Mendelian loss of function, as a genome-wide predictor of CHP. We then employ an exome scan in 29,713 patients and identify rare variants in MAEL and additional differentially expressed genes associated with CHP. Analysis of a rare-variant in transmembrane protein 50B (TMEM50B), one of the top differentially-expressed genes, which overlaps an enhancer and affects binding of a transcription factor, TTF1, to the promoter of aquaporin 1 (AQP1), provides evidence for the long-hypothesized, but heretofore unproven, mechanistic basis for aquaporin dysregulation in CHP. These genetic data are then used to construct a genome-wide genetic risk score for CHP, which is more predictive than rare monogenic forms of the disease. Based on these findings, we provide the components of a novel targeted genotyping panel, based on common regulatory variants' contribution to genetically-determined gene expression, that can be used to stratify a patient's germline-genetic risk of developing CHP. Next, we isolated cerebrospinal fluid (CSF) from patients undergoing permanent CSF diversion for CHP and perform unbiased proteomic analysis, recapitulating some of the most differentially-expressed genes identified by PrediXcan. Lastly, using the Synthetic Derivative, a deidentified electronic health record containing 1,944,991 patients, we determine the epidemiological impact of CHP on other neurological diseases, and provide evidence for the top differentially-expressed genes conferring a shared genetic risk for other comorbid conditions associated with CHP. Our findings provide convergent evidence of the importance of tissue-specific pathways in the pathophysiology of CHP, identify novel molecular mechanisms of CHP, and provide the components of a novel genome-wide genetic test for elucidating CHP risk.

111 Defining the role of CDK4/6 amplification in resistance to EGFR tyrosine kinase inhibitors in patients with EGFR-mutant lung adenocarcinoma
Patrick R. Halliday

Defining the role of *CDK4/6* amplification in resistance to EGFR tyrosine kinase inhibitors in patients with EGFR-mutant lung adenocarcinoma

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Lung cancer remains the leading cause of cancer-related mortality worldwide. Genetic profiling of non-small cell lung cancer (NSCLC) has led to the discovery of actionable oncogenic driver alterations, which has revolutionized treatment for this disease. Despite these advances, responses to molecular targeted therapies in NSCLC are nearly always incomplete and transient. A recent genomic analysis of 1,122 lung cancer cell-free DNA specimens by Blakely et al. revealed the presence of co-occurring mutations with oncogenic potential in most cases of epidermal growth factor receptor (EGFR)-mutant NSCLC, suggesting that concurrent genetic mutations may play a causal role in disease persistence and treatment failure. In a group of approximately 100 patients from this cohort, copy number gains of *cyclin-dependent*

kinase 4 (*CDK4*) and *cyclin-dependent kinase 6* (*CDK6*) were clinically correlated with decreased response rate to EGFR tyrosine kinase inhibitor (TKI) treatment, and significantly reduced progression free survival (PFS) compared to patients in which these alterations were not detected. These data suggest that copy-number gains of *CDK4* or *CDK6* may serve as predictive biomarkers for patients who are less likely to respond to EGFR TKIs.

To better characterize the clinical and biological significance of copy-number gains in *CDK4* or *CDK6* directly in patient tumor specimens, we determined the frequency *CDK4* or *CDK6* amplification in a cohort of EGFR-mutant lung adenocarcinomas, and will compare it to a cohort of patients without detectable *CDK4* or *CDK6* amplification. We will compare objective response rate (ORR), overall survival (OS), and PFS between these two patient populations. To date, 248 UCSF lung cancer patients have undergone sequencing of tumor specimens with the Foundation Medicine assay. Among 64 EGFR-mutant patients, the frequency of coincident *CDK4* or *CDK6* amplification is 20.3% (13/64), which is congruent with our preliminary data from a smaller cohort. Additionally, whole genome and exome sequencing is underway for a cohort of 51 EGFR-mutant patients whose tumor specimens were collected prior to treatment with the EGFR TKI, osimertinib. We will determine whether copy number gains of *CDK4* or *CDK6* is predictive of poor clinical outcome, as measured by ORR (primary endpoint), PFS, and OS (secondary endpoints) in this cohort. These pre-treatment genetic data will also allow us to describe any other tumor genetic changes that underlying innate treatment resistance or disease persistence and early progression among patients undergoing EGFR TKI treatment.

112 3D analysis of neuronal circuitry of the mouse pancreas
Rollie Hampton

3D analysis of neuronal circuitry of the mouse pancreas

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Diabetes mellitus is a collection of metabolic disorders characterized by aberrant functioning of the endocrine pancreas leading to chronic hyperglycemia. In the US, diabetes affects one in every nine individuals, and an additional 84.1 million Americans are at risk as having pre-diabetes. Thus, it is imperative that we continue to elucidate the pathologies underlying diabetes so that more effective therapies can be developed.

Islets of Langerhans are a collection of cells within the pancreas that function to secrete insulin, glucagon, somatostatin, and pancreatic polypeptide into the systemic circulation based on physiological cues, with the ultimate goal of maintaining nutrient homeostasis. However, the nervous system also plays a critical role in pancreatic function, and thus, metabolic homeostasis. The relationship between the CNS and the pancreas was first noted in 1855 by Claude Bernard, where mechanical stimulation of the brain stem resulted in glucose dysregulation. More recent studies by Ahrén B., et al., showed that parasympathetic stimulation of the vagus nerve resulted in muscarinic-dependent stimulation of insulin secretion. These data were complimented by the studies of Frohman L., et al., where truncal vagotomy resulted in the potentiation of glucose-induced insulin secretion. Given the established relationship between the nervous system and normal pancreatic physiology, it is important to consider neuronal signals to the pancreas in the pathology of diabetes.

However, the role of neuronal inputs into the pathogenesis and progression of diabetes mellitus remains unknown. In fact, the precise innervation patterns of the human pancreas, and its relationship to the Islets of Langerhans have yet to be fully elucidated. This is largely due to the limitations of traditional two-dimensional histology and immunohistochemistry techniques. The recent development of three-dimensional, whole mount immunolabeling of large cleared samples has overcome these limitations and now allows the ability to create detailed maps of pancreatic innervation and to visualize the structural relationship between neuronal populations and the islets of Langerhans.

The objective of this study is to identify, characterize, and quantify the parasympathetic, sympathetic, and sensory innervation of mouse and human pancreatic tissue. We have successfully constructed 3D images of cleared pancreatic tissue that have been immunolabeled for endocrine markers (insulin, somatostatin, glucagon, and pancreatic polypeptide), pan-neuronal markers (synapsin and neurofilament protein 200), sympathetic markers (tyrosine hydroxylase), and parasympathetic markers (vesicular acetylcholine transporter). Our 3D reconstructed images allow us to map the number, size, and distribution of islets throughout the whole pancreas and quantify innervation density of endocrine vs exocrine pancreatic tissue.

Further investigation will focus on enhancing our current understanding of the neuronal populations in healthy mice, and elucidating the innervation patterns seen in murine models of Type 1 diabetes, such as non-obese diabetic (NOD) and Streptozotocin (STZ)- induced diabetes, and human disease.

113 Optimization of human cancer cell xenografts into zebrafish larvae for high-throughput drug screening
Meghan G. Haney

Optimization of human cancer cell xenografts into zebrafish larvae for high-throughput drug screening

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The use of zebrafish in cancer xenograft models has grown rapidly with recent preliminary results showing that some zebrafish xenograft models can correctly predict which therapies a person's cancer will respond to in as little as four days. This growth is primarily due to the fact that this model takes advantage of the ease of in vivo imaging and the high-throughput screening capabilities that zebrafish have to offer compared to the more traditional mouse xenograft models. However, researchers have yet to come to a consensus on a standardized procedure for utilizing zebrafish to xenograft human cells. This study aims to optimize a zebrafish xenografting protocol for various human cancers

with the intention of performing high-throughput drug screening.

Since zebrafish are normally grown at 28°C and human cells at 37°C, we first had to test the survival of both the fish at elevated temperatures and the xenografted cells at lower temperatures, finding that both were able to thrive at 34°C. We then fluorescently labelled human leukemia, breast, lung, colon, and brain cancer cell lines with Vybrant DiI cell staining dye and injected them into 2-day-post-fertilization zebrafish larvae. We tested injections with 4 different cell numbers and seven different anatomical injection sites reported previously in zebrafish xenograft models to find the cell number and site with the highest engraftment rate, best survival and most efficient injection time. After determining the optimal injection site and cell number, we performed RNAseq to compare the expression profile of cells xenografted into zebrafish versus those either grown in culture or xenografted into mice. We are awaiting the results of this data, but anticipate that the RNA expression will align more closely with the mouse xenograft models.

In addition, we are currently in the process of determining the extent to which the injection site affects chemotherapy response on human cells implanted into zebrafish. We are preforming a high-throughput drug screen on human lung cancer, breast cancer, and leukemia cells implanted into zebrafish to provide proof-of-principle that these methods are useful in identifying novel anti-cancer compounds. In addition, this method of rapid drug screening may be useful in the future for informing clinicians about which therapies a patients' cancer would respond to in a matter of days, allowing for better clinical decision making and more efficient stratification of patients into clinical trials. In total, this work will establish standard operating procedures for the use of xenografts in zebrafish, providing new opportunities in personalized medicine and drug discovery.

114 Mechanisms of cell death in the inherited bone marrow failure syndromes Schwachman Diamond Syndrome and Pearson Syndrome
Kathleen Hanlon

Mechanisms of cell death in the inherited bone marrow failure syndromes Shwachman Diamond Syndrome and Pearson Syndrome

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The inherited bone marrow failure syndromes consist of a number of rare diseases in which there is ineffective hematopoiesis by the bone marrow. Two of the inherited bone marrow syndromes with defined genetic mutations are Shwachman Diamond Syndrome (SDS) and Pearson Syndrome. Over 90% of SDS cases result from a mutation in the *SBDS* gene, which is involved in ribosome biogenesis, while Pearson Syndrome results from large deletions in mitochondrial DNA. These syndromes share several distinct features, including early onset of severe anemia and bone marrow failure. Hematopoiesis requires a balance between cell death, proliferation, and survival, and little is known about how cell death is regulated in these rare syndromes. Two major modes of cell death which are vital for hematopoietic homeostasis are apoptosis, an immune-silent process, and necroptosis, an inflammatory process. We developed a novel multi-color high-throughput live cell imaging platform to monitor the kinetics and transitional phases of cell death using automated custom-scripted image-processing software. In this study, we apply the platform to study cell death pathways in primary fibroblast cell lines from healthy subjects and patients with SDS and Pearson Syndrome. Our data suggest that primary fibroblasts from patients with SDS and Pearson Syndrome have increased cell death at steady state and in response to apoptotic stimuli. We have also demonstrated that this platform is widely applicable to investigate cell death in other cell types. Our current study provides a better understanding of cell death mechanisms and may help identify novel therapeutic approaches to modulate cell survival.

115 T cell vaccination prevents viral chronicity in a novel rat model of hepatitis C-related virus infection
Alex S. Hartlage

T cell vaccination prevents viral chronicity in a novel rat model of hepatitis C-related virus infection

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Chronic hepatitis C virus (HCV; human hepacivirus) infection affects 71 million people worldwide and is a major cause of liver-specific morbidity and mortality. Despite dramatic advances in antivirals, a vaccine to prevent chronic HCV infection and associated liver disease is not yet available. A principal reason for this delay is the lack of an appropriate small animal model for testing vaccination concepts and mechanisms of immune control. Recently, we developed a novel rat model of hepacivirus infection that recapitulates key features of human HCV infection, including spontaneous T cell subversion and chronic viral persistence. Here, we used this new surrogate model to test T cell vaccination as a strategy to prevent immune failure and persistent liver infection. Single immunization of rats with a recombinant human adenovirus serotype 5 vector encoding hepacivirus non-structural proteins (NS3-5B) primed functional CD4 and CD8 T cell responses against a broad range of viral epitopes. Clearance of infection occurred rapidly (

116 Mitochondrial Malate Dehydrogenase (MDH2) Regulates Macrophage Alternative Activation during Pulmonary Fibrosis Development
Chao He

Mitochondrial Malate Dehydrogenase (MDH2) Regulates Macrophage Alternative Activation during Pulmonary Fibrosis Development

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RATIONALE: Alternatively activated macrophages promote pulmonary fibrosis development with increased ROS production and unbalanced redox couples. The NAD+/NADH redox couple is known to regulate cell metabolism. An unbalanced NAD+/NADH ratio has been shown to be a hallmark of alternatively activated macrophages due to changes in cell metabolic patterns and is implicated several disease conditions such as cirrhosis and neurodegenerative diseases. Two malate dehydrogenases are important in the maintenance of cellular NAD+ and NADH balance. Cytosolic malate dehydrogenase (MDH1) is responsible for transferring NAD+ into mitochondria, and mitochondrial malate dehydrogenase (MDH2) is a key enzyme in the Krebs cycle which generates NADH using NAD+ as the substrate. Here we found that MDH2 is downregulated in lung macrophages from fibrotic subjects. We hypothesize that MDH2 regulates macrophage alternative activation via modulating NAD+/NADH balance. RESULTS: We found that MDH2 was downregulated in lung macrophages from fibrotic subjects (asbestosis and IPF) compared with normal subjects. Similarly, MDH2 was downregulated in lung macrophages from mice exposed to either chrysotile asbestos or bleomycin compared with control mice. On the contrary, MDH1 level remains unchanged in human subjects with fibrotic lung diseases and mice after asbestos exposure. MDH2 activity was reduced in lung macrophages treated with asbestos. Silencing MDH2 in macrophages increases pro-fibrotic gene, such as TGF-β1. Lung macrophages from asbestosis patients, which are known to have an alternatively activated phenotype, have reduced whole cell NADH/NAD+ ratio compared with normal subjects. The NADH/NAD+ ratio, however, was unchanged in isolated cytosolic compartment, suggesting the ratio changes are independent of glycolysis and cytosolic malate dehydrogenase (MDH1) and is related to mitochondria. CONCLUSIONS: These observations suggest a critical role for mitochondrial malate dehydrogenase (MDH2)-mediated pro-fibrotic activation of macrophages via modulation of mitochondrial NAD+ and NADH level in pulmonary fibrosis. Research Funding Source: This work was supported, in whole or in part, by National Institutes of Health Grants ES015981-11, 5T32HL105346, and a VA Merit Review Grant I01CX001715.

117 Detecting delirium: A systematic review of identification measures
Benjamin K.I. Helfand

Detecting delirium: A systematic review of identification measures

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Delirium, an acute syndrome characterized by inattention and cognitive dysfunction, affecting 3 million patients with over $160 billion in annual healthcare expenditures in the United States alone. Despite its importance, delirium is often unrecognized. One major problem in recognition of delirium is that there is no single agreed upon instrument for identification. The goal of this study is to determine the 4-5 most commonly used or well-validated instruments for delirium identification through a systematic review of systematic reviews of the published literature, with standardized quality rating criteria.

We searched six different databases (CINAHL, Cochrane, EMBASE, PsycINFO, PubMed, and Web of Science) to find a total of 2,162 articles. After removing duplicates and non-English articles, we reviewed 1,113 unique articles. Inclusion criteria were: systematic review, meta-analysis, or review article; delirium as the primary outcome, and discussing at least two delirium identification instruments. Exclusion criteria were: alcohol-related delirium (delirium tremens) studies; studies exclusively in pediatric populations; studies using animal populations; non-English language articles; commentaries, letters, editorials, conference abstracts, journal article that used primary data collection, any article that does not indicate they used a literature review of some kind; or only a single instrument reviewed.

After applying our inclusion and exclusion criteria, we found 153 eligible articles, which yielded a total of 48 different delirium identification instruments. At this stage, we elected to exclude instruments used strictly in the intensive care unit (ICU), which lowered the total to 45 instruments. From this list, we searched Google Scholar and Scopus to rank our list by citation count. The top 5 instruments by citation count were the confusion assessment method (CAM), the delirium rating scale (DRS), and the memorial delirium assessment scale (MDAS), the organic brain scale (OBS), and the Neelon and Champagne confusion scale (NEECHAM).

Our next steps will include rating measures on the following: internal consistency, reliability, measurement error, content validity (including face validity), construct validity, and criterion validity. We will collect information on the intended study population (e.g., emergency department, medical wards), level of training required to administer the instrument, number of questions, and time for administration. We will use these criteria to select our final list of the 4-5 instruments that are most commonly used or well-validated.

Once selected, we will statistically harmonize these measures using item response theory to put them on the same metric. These steps will allow the direct comparison of study results (e.g., delirium rates) across populations, and also facilitate quantitative meta-analysis and synthesis of study results, which is essential for the development of clinical guidelines and establishment of clinical practice standards. Clinically, development of a unified delirium measure would greatly advance identification of delirium across settings.

118 Late Life Acarbose or Rapamycin Treatment Ameliorates Age Related Declines in Physical Function in a Genetically Heterogenous Mouse Model
Jonathan J. Herrera

Late Life Acarbose or Rapamycin Treatment Ameliorates Age Related Declines in Physical Function in a Genetically Heterogenous Mouse Model

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The aging population is growing at an unprecedented rate. Aging is a risk factor for all major causes of death including heart disease and cancer. Furthermore, the incidence of multimorbidity, or the occurrence of 3 or more disease conditions, increases dramatically with age, which can negatively impact both longevity and overall health. It would be of great value to develop interventions that delay or reverse the aging process and thus target age related diseases and conditions collectively as a group, and initiation of effective treatments later in life would be of particular value. Acarbose (ACA), an oral diabetic medication, and Rapamycin (RAPA), an immunosuppressive agent, are two FDA approved agents that have demonstrated benefits in life extension and health in mice when treatment is started early in life (~4-9 months). Although treatment with these drugs started at 20 months of age can extend lifespan, is was unknown whether late life treatment confers health benefits. Female (F) and male (M) UMHET3 mice (n=11-37/group) were randomized to a diet with ACA or Rapamycin beginning at 4 months (ACA Early, RAPA Early) or 16 months of age (ACA Late, RAPA Late), or to a control diet throughout the experimental duration [(Young Control (YC; 4-6 months) or Old Control (OC; 22 months)]. Mice underwent physical function testing and then were sacrificed for pathologic and biochemical tissue analyses. Mean Fall latency on a continuously accelerating rotarod (0.1 RPM/sec) declined with age (YC_F: 142.9s ±60.2 vs. OC_F: 67.2s ±37.4, YC_M: 132.7 ±53.6 s vs. OC_M: 56.5s ±31.1; p

119 A growth model of neuroendocrine tumor surrogates and the efficacy of a novel somatostatin-receptor guided antibody-drug conjugate: perspectives on clinical response?
Brendon Herring

A growth model of neuroendocrine tumor surrogates and the efficacy of a novel somatostatin-receptor guided antibody-drug conjugate: perspectives on clinical response?

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Background: Patient-derived xenografts (PDXs) are invaluable tools for testing personalized therapeutics on tumors prior to their administration to patients. However, as PDX models for neuroendocrine tumors (NETs) are largely lacking, we have developed a three-dimensional (3D) flow-perfusion polydimethylsiloxane (PDMS) bioreactor model for the purpose of culturing tumor surrogates from patient-derived NET samples. This work evaluates the length of time that surrogates were successfully cultured ex vivo, and the response of surrogates to a novel antibody-drug conjugate (ADC).

Methods: 18 Patient-derived NET samples (G1 n=7, G2 n=7, GX n=4) were implanted into bioreactors, and cultured. Surrogates were incubated with the fluorescent dye IR-783 before fluorescence imaging with an In Vivo Imaging System (IVIS). Growth was defined as increased radiant efficiency on fluorescence imaging. Further, a G2 pancreatic NET sample was implanted into four bioreactors. Two surrogates were treated with ADC comprised of the potent anti-mitotic Monomethyl auristatin E, linked to an antibody to somatostatin receptor 2 (SSTR2), a NET-specific target on the cell membrane. Growth rate/viability and response to ADC treatment were assessed by incubating surrogates with IR-783 and the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay (Promega) respectively, prior to daily IVIS imaging over six days. Histologic sections of the original sample were stained to assess SSTR2 expression. Surrogates derived from a NET xenograft (BON-1 cells) were likewise evaluated.

Results: The mean duration of surrogate growth was 33.5 days. Of note, no statistically significant difference existed in surrogate growth for primary gastroenteropancreatic NETs vs. metastases ($t = -0.12$, $df = 14$, $p = 0.906$). Patient-derived NET bioreactors treated with ADC exhibited much higher degrees of apoptosis (13-fold, 9-fold) and necrosis (2.5-fold, 1.6-fold). Similarly, treated BON-1 surrogates exhibited less proliferation (1.2-fold, 1.9-fold) and higher apoptosis (1.5-fold, 1.1-fold) than controls. In all cases, response to ADC treatment correlated with SSTR2 positivity.

Conclusions: Patient-derived NET surrogates can be reliably cultured within the bioreactor system for up to 33 days, regardless of metastatic status. The bioreactor model can be used to evaluate the efficacy of antibody-guided molecular chemotherapy ex vivo and may be particularly useful for predicting clinical responses in patients not eligible for clinical trials due to deteriorating health.

120 Microbial killing activity of polymorphonuclear myeloid-derived suppressor cells isolated from tumor-bearing dogs
Sabina I. Hlavaty

Microbial killing activity of polymorphonuclear myeloid-derived suppressor cells isolated from tumor-bearing dogs

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Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are implicated in the progression and outcomes of a variety of diseases, from cancer to autoimmunity. Higher MDSC frequencies in human cancer patients correlate with a worse prognosis, underscoring the importance of better understanding the function of these cells. Dogs develop spontaneous tumors that resemble human cancer; those with a higher tumor burden have higher frequencies of PMN-MDSCs in peripheral

blood. Our previous work has identified differentially expressed genes encoding three antimicrobial peptides (AMPs) in PMN-MDSCs isolated from dogs, mice, and human patients with cancer – cathelicidin (CAMP), lipocalin 2 (LCN2), and lactoferrin (LTF). We therefore hypothesized that PMN-MDSCs in dogs with cancer have hitherto unrecognized antimicrobial activity. First, we validated our RNA-sequencing results using real time-quantitative polymerase chain reaction (RT-qPCR). The fold change (FC) of *CAMP* (log_2FC = 7.0), *LCN2* (log_2FC = 1.9), and *LTF* (log_2FC = 1.2) confirmed more abundant expression of these transcripts in PMN-MDSCs compared to neutrophils (PMNs) in dogs with cancer. Microbial killing activity was assessed by quantitating bacterial growth on Luria-Bertani (LB) agar plates after co-incubation of bacteria with canine cells for 45 minutes. In preliminary experiments, the geometric mean (GM) number of colony-forming units (CFU) for *Escherichia coli* alone was 4 x 10^6, compared to 0.3 x 10^6 for *E. coli* exposed to a positive control population of PMNs derived from healthy dogs (n = 4, p = 0.036; paired *t* test). The same experiment was repeated with *Staphylococcus spp.*, using *S. aureus* and *S. pseudintermedius*. The GM CFU for *Staphylococcus spp.* alone was 4 x 10^6, compared to 1.1 x 10^6 when cultured with PMNs from healthy dogs (n = 3, p = 0.017; paired *t* test). *E. coli* were then exposed to healthy PMNs or T cells (control cells), or PMNs and PMN-MDSCs from tumor-bearing dogs. The GM CFU for *E. coli* alone was 6.7 x 10^6, compared to 7.5 x 10^6 following co-incubation with T cells, 0.2 x 10^6 with healthy PMNs, 1.2 x 10^6 with cancer PMNs, and 1.1 x 10^6 with PMN-MDSCs (n = 2 for each condition). Our data therefore suggest for the first time that PMN-MDSCs isolated from dogs with cancer, despite being immunosuppressive, have microbial killing activity. Future work will explore the mechanistic basis of bacterial killing, dissecting the relative contributions of AMPs, phagocytosis, and reactive oxygen species against a variety of Gram-positive and Gram-negative species. We will interrogate whether the mechanism of killing differs between cells isolated from healthy or cancer patients, given that preliminary data suggest cancer PMNs and PMN-MDSCs have 6-fold lower killing of bacteria compared to healthy PMNs. The nexus of immunosuppression and antimicrobial activity represents a novel biological paradigm in cancer.

121 The role of gut microbiome and gut epithelial barrier in Cerebral Amyloid Angiopathy
Pedram Honarpisheh

The role of gut microbiome and gut epithelial barrier in Cerebral Amyloid Angiopathy

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Among the age-associated neurodegenerative diseases (NDDs), Cerebral Amyloid Angiopathy (CAA) is an emerging cause of vascular cognitive impairment. CAA is characterized by amyloid-β(Aβ) deposition in the cerebral vasculature and is associated with disruption of blood-brain barrier (BBB), infiltration of immune cells/molecules into CNS, and increased neuroinflammation. To date, there is no definitive answer for whether the observed neuroinflammation is the *result* or the *cause* of CAA pathogenesis and/or other age-related NDDs. A state of low-grade, chronic inflammation is associated with aging ("inflammaging"), which has been linked to multiple NDDs. Furthermore, evidence suggests that dysbiosis of the gut microbiota, also seen with aging, is a modulator of the immune response to CNS-injuries. We hypothesized that gut dysbiosis occurs early in CAA pathogenesis, which may contribute to the ongoing neuroinflammation and progression of CAA. We used the Tg-SwDI ("amyloid precursor protein harboring Swedish, Dutch, and Iowa mutations") transgenic mouse model of CAA to test our hypothesis. Tg-SwDI mice develop Aβdeposition in cerebral vasculature and cognitive deficits beginning at around 4 months. Our preliminary 16S rRNA sequencing of fecal samples of CAA mice (n=127) compared to wildtype (WT) controls (n=80) show higher gut microbiome alpha- (or "within-sample") diversity in the fecal samples of CAA mice (Inverse-Simpson diversity score by Mann-Whitney U rank sum test, p=0.036). Upon visualization of beta- (or "between samples") diversity of CAA and WT controls, with weighted-UniFrac-distances by principal coordinate analysis (PCoA), we found a notable clustering effect (n=207, p=0.001, PCoA axes: 34.6% and 26.4% variations explained). When comparing relative abundance of short chain fatty acids (SCFAs) in fecal samples of symptomatic (~10 months) CAA mice and WT controls, acetate and butyrate levels were significantly higher in CAA (n=30, differential false discovery rate (FDR) ongoing increased neuroinflammation that contributes to CAA *progression*. This work is significant if follow-up studies confirm that changes in gut microbiota can be detected *before* clinical manifestations of CAA. Therapeutic strategies to reverse pathology in CAA may involve manipulation of the microbiome.

122 Endothelial cell dysfunction and impaired platelet aggregation are prominent features of acute Lassa Fever
Lucy E. Horton

Endothelial cell dysfunction and impaired platelet aggregation are prominent features of acute Lassa Fever

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Lassa Fever (LF), an acute viral hemorrhagic fever endemic to West Africa, affects about 500,000 people a year with a high case fatality rate in those that develop severe disease. We sought to evaluate the hypothesis that the hemorrhagic manifestations in LF are a problem of vascular permeability due to endothelial cell dysfunction involving the Protein C pathway and abnormal platelet aggregation. To test this hypothesis, we collected plasma from patients presenting to the Kenema Government Hospital in Sierra Leone who met clinical criteria for LF and were confirmed positive by ELISA. Plasma from 81 patients with acute LF, 17 patients with non-LF febrile illnesses and 10 healthy controls were included in the analyses of inflammatory and coagulation markers. For-

ty-one patients were tested for platelet aggregation function. Patients with acute LF had significantly elevated levels of thrombomodulin (p

123 Increased diameters along the cerebral venous draining system are associated with white matter hyperintensities, cerebrospinal amyloid beta, and cerebrospinal total tau

Alexander L. Houck

Increased diameters along the cerebral venous draining system are associated with white matter hyperintensities, cerebrospinal amyloid beta, and cerebrospinal total tau

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Small vessel cerebrovascular disease manifests primarily as white matter hyperintensities (WMH) on T2-weight magnetic resonance imaging scans (MRI), and the relationship between WMH volume and Alzheimer's disease (AD) is increasingly recognized. Although often attributed to occlusive arteriopathy, recent evidence implicates collagenosis of deep medullary venules, identified with trichrome staining, which results in vasogenic edema appearing as WMH. This can impair beta-amyloid clearance and potentially also drainage into the internal cerebral veins. Historically, post-mortem analyses have been the only methods of analyzing cerebral veins, but now MRI susceptibility weighted imaging (SWI) can be used to detect cortical veins that are often difficult to visualize on T2 or proton density (PD) images. On SWI, venous vessels appear hypointense due to the magnetic susceptibility difference between oxygenated and deoxygenated blood.

The goal of this study was twofold. First, we aimed to determine if there is an association between diameters of the large draining cerebral veins and WMH volume. Second, we examined if there is a relationship between vein diameter and AD biomarkers in the cerebrospinal fluid (CSF). We collected data from two cohorts: (1) 675 older adults without dementia, in whom high resolution MRI-SWI scans were available, and (2) 50 older adults without dementia, in whom MRI-SWI scans and CSF were available. White matter hyperintensities were quantitated in-house and CSF amyloid and tau levels were measured on Innogenetics Luminex. The diameters of three regions of the cerebral venous draining system (superior sagittal sinus, internal cerebral veins, and straight sinus origin) were measured in the axial plane, and the diameters of two regions (vein of Galen and straight sinus terminus) were measured in the sagittal plane.

We found that internal cerebral vein diameter was associated with larger WMH volume (cohort 1: Beta = 0.093, p = 0.014; cohort 2: Beta = 0.286, p = 0.029). The straight sinus origin diameter was negatively associated with CSF $A\beta_{42}$ (Beta = -0.294, p = 0.036) and positively associated with CSF total tau (Beta = 0.276, p = 0.050). Overall, our results suggest that cerebral vein caliber may relate to white matter disease and to AD pathophysiology.

124 The response regulator, VpsR, uses the small signaling molecules, c-di-GMP and phosphorylation, to drive transcription of biofilm genes in Vibrio cholerae

Meng-Lun Hsieh

The response regulator, VpsR, uses the small signaling molecules, c-di-GMP and phosphorylation, to drive transcription of biofilm genes in *Vibrio cholerae*

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Biofilms pose a serious public health concern in both the medical and industrial setting. Their formation and persistence on catheters, pacemakers, sutures, and other indwelling medical devices account for over two million nosocomial infections and 100,000 deaths annually. In the vast majority of bacterial species, the highly ubiquitous and important second messenger, cyclic dimeric guanosine monophosphate (c-di-GMP), is the central regulator of biofilm formation. More specifically, in *Vibrio cholerae,* the causative agent of the disease cholera, VpsR is the master Enhancer Binding Protein (EBP) that binds c-di-GMP to increase biofilm gene expression at P_{vpsL} *in vivo*. Unlike typical EBPs that activate RNA polymerase (RNAP) containing the alternate sigma factor, sigma54, VpsR has several different features: 1) it lacks conserved residues needed to bind to sigma54 and hydrolyze ATP; 2) it retains a highly conserved D59 residue, which is typically phosphorylated; and 3) it activates P_{vpsL} in the absence of sigma54 *in vivo*. These features all suggest a different unknown mechanism of transcription activation.

To address this mechanism, I established an *in vitro* system and have shown for the first time that c-di-GMP is sufficient to directly activate transcription with VpsR at P_{vpsL}. More specifically, I have demonstrated that c-di-GMP, VpsR, and RNAP containing the primary sigma factor, sigma70, stimulates transcription by ~7-fold *in vitro*. Unlike other regulators, which use c-di-GMP to promote oligomerization and/or increase DNA binding affinity, the presence of c-di-GMP neither affects VpsR oligomerization nor significantly changes the affinity of VpsR for P_{vpsL} DNA. Instead, $KMnO_4$ and DNase I footprinting reveal that the P_{vpsL}/sigma70-RNAP/VpsR/c-di-GMP complex forms the open transcription bubble and adopts a different conformation from that formed by P_{vpsL}/sigma70-RNAP with or without c-di-GMP or VpsR. To investigate the role of the D59 residue, I have characterized the phosphodefective D59A variant and the phosphomimic D59E variant *in vivo* and *in vitro*. Although both D59A and D59E variants dimerize and bind DNA with $K_{d(app)}$s similar to that of WT, only D59E activates transcription and form the open transcription bubble while D59A yields basal transcription. DNase I footprints of the transcription complex made with D59E reveal an activated transcription complex whereas footprints with D59A resemble those seen with RNAP alone. I speculate that both c-di-GMP and phosphorylation of VpsR are needed to generate the proper protein-DNA architecture for the formation of the active transcription complex. This represents a new paradigm for c-di-GMP-dependent transcription activation. As c-di-GMP, phosphorylation, and EBPs are widely conserved in many bacterial pathogens, our studies with VpsR will not only lead to a general understanding of how these small signals

regulate gene expression, but also provide us valuable insights in the development of new therapeutics targeting biofilm-based infections via small molecule signaling.

125 Diet modulates T cell immune responses by regulating the expression of a dominant antigen from a gut symbiont
Samantha Hsieh

Diet modulates T cell immune responses by regulating the expression of a dominant antigen from a gut symbiont

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Diet is well recognized to modulate the adaptive immune responses within the mucosa in a general fashion. Dietary changes are also known to regulate the composition of the intestinal microbiome. However, the connection between the effects of diet on adaptive responses directed towards specific intestinal microbes is unclear. We hypothesize that dietary regulation of the expression of dominant microbial antigens can control the CD4$^+$T cell immune response to these bacterial antigens. Progress in this area has been hampered by the lack of a model system in which a CD4$^+$T cell response can be examined for a specific gut symbiont. To this end, we developed a novel CD4$^+$T cell model, termed Bθ-OM, that is specific for a dominant antigen in the symbiont *Bacteroides thetaiotaomicron* (*B. theta*). *B. theta* is a prototypic gut symbiont that degrades a wide variety of dietary, host, and microbial glycans, and is a representative of a prominent genus found in most human microbiomes. Adoptively transferred Bθ-OM T cells proliferated in the colon, colon draining lymph node (cdLN), and spleen in healthy mice colonized with *B. theta* and differentiated into regulatory T (T_{reg}) and effector T (T_{eff}) cells. Depletion of *B. theta*-specific T_{regs} resulted in colitis, demonstrating that a single protein expressed by *B. theta* can drive differentiation of T_{regs} that self-regulate T_{effs} to prevent disease. We identified the *B. theta* antigen recognized by Bθ-OM T cells to be BT4295, an outer membrane protein contained in one of *B. theta's* many polysaccharide utilization loci. Interestingly, the expression of BT4295 is regulated by nutrients, with glucose being a strong catabolite repressor of BT4295 expression. Despite similar *B. theta* colonization levels as control mice, mice fed a high glucose diet had greatly reduced activation of Bθ-OM T cells in the colon and cdLN. These studies establish that the immune response to specific bacterial antigens can be modified by changes in the diet that alter the antigen expression in the microbe.

126 The DAF-7/TGFβ pathway modulates F-series prostaglandins important for sperm guidance in C. elegans
Muhan Hu

The DAF-7/TGFβ pathway modulates F-series prostaglandins important for sperm guidance in *C. elegans*

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Infertility is a multifactorial disorder that is affecting a growing number of individuals in Western countries. Many studies have investigated the impact of the environment, diet, and genetics on infertility. However, the molecular mechanisms are not well understood. One key event that is largely unexplored in fertility research is the mechanism by which sperm finds the oocyte. It is well established that oocytes of marine species secrete sperm chemoattractants. However, little is known about how sperm of internally fertilizing animals, including humans, navigate the convoluted reproductive tract. In vitro studies have provided insight into sperm behavior, suggesting sperm of internally fertilizing animals can sense and react accordingly to chemical cues, temperature gradients, and fluid flow. However, adequate in vivo models are lacking. Our lab has developed *C. elegans* as an in vivo model to study sperm guidance. The clear epidermis of *C. elegans* allows for direct visualization of labeled sperm in an intact oviduct. Using this model, we have identified a specific class of F-series prostaglandins (PGFs) that are important for guiding sperm toward the fertilization site. Prostaglandins are classically synthesized from polyunsaturated fatty acids (PUFAS) via the cyclooxygenase (COX) enzymes, but these genes are not encoded by the *C. elegans* genome. Recent data from *C. elegans* show that PGF levels are regulated by the DAF-*7*/TGFβ signaling pathway. Identification of PGFs in *Cox-1;Cox-2* knockout mice and human follicular fluid suggest this novel PG synthesis pathway may be conserved in mammals. DAF-*7*, the homolog of TGFb, is a neuroendocrine factor secreted by the *C. elegans* ASI sensory neurons in response to food and pheromone cues. It signals through the DAF-1 Type I and DAF-4 Type II TGFb receptors, which act through downstream R-SMADs DAF-8 and DAF-14 to inhibit DAF-3 Co-SMAD. In this study, we show that the sperm guidance defect seen in *daf-1* mutant is suppressed in the *daf-1;daf-3* double mutant. Further studies using mass spectrometry showed the sperm guidance phenotype correlated with the levels of PGF detected in these mutants. To further understand the mechanism by which DAF-3 affects sperm guidance and PGF metabolism, we created *daf-3* mosaic animals to identify the tissues where DAF-3 function was necessary to promote sperm guidance. We found that expression of *daf-3* in the intestine and germline is important to promote sperm guidance. Furthermore, using an in vitro biochemical reaction of worm lysates and the PGF precursor, arachidonic acid, we found that TGFβ pathway mutants can synthesize similar levels of PGFs. Together, these data suggest that the DAF-*7*/TGFβ pathway may be regulating PGF levels by modulating the transport of PGF precursors from the intestine to the oocytes, where they are converted to PGFs. Future work will focus on understanding this transport mechanism.

127 A unifying mechanism for many small molecule enhancers of oligodendrocyte formation
Zita Hubler

A unifying mechanism for many small molecule enhancers of oligodendrocyte formation

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Oligodendrocytes are glial cells which produce a lipid-rich membrane called myelin. Myelin insulates neuronal axons allowing for saltatory conduction and promotes neuronal survival. Loss of myelin-producing oligodendrocytes in the central nervous system underlies a number of neurological diseases, including multiple sclerosis. In adults, the primary source of oligodendrocytes is oligodendrocyte progenitor cells (OPCs). To discover novel therapies for demyelinating disorders, we and others have performed *in vitro* chemical-genetic screens for small molecules that enhance oligodendrocyte formation from OPCs. Our high-throughput screening hits were mechanistically diverse and their canonical targets could not be ascribed to any known oligodendrocyte biology. Surprisingly, we found that as opposed to functioning via their canonical targets, our screening hits enhance oligodendrocyte formation through a unifying off-target effect of inhibiting a narrow range of enzymes in the cholesterol biosynthesis pathway, CYP51, EBP, and TM7SF2. We have shown that selective small molecule inhibitors of CYP51, EBP, and TM7SF2 enhance differentiation of OPCs to oligodendrocytes *in vitro*. Subsequent accumulation of the 8,9-unsaturated sterol substrates of these enzymes is a key mechanistic node that promotes oligodendrocyte formation, as 8,9-unsaturated sterols are effective when supplied to oligodendrocyte progenitor cells in purified form whereas analogous sterols that lack this structural feature have no effect. Further, we have shown that this pathway is also implicated for several small molecules shown to enhance remyelination *in vivo* and were ascribed alternative mechanisms of action in the literature. Inhibitors of CYP51, EBP, and TM7SF2 enhance remyelination and lead to an accumulation of 8,9 unsaturated sterols in the brains of mice. Our work describes a unifying hypothesis for many small molecules which enhance oligodendrocyte formation and illuminates a novel pathway for therapeutic targeting.

128 Markedly increased mitochondrial respiration in the murine bladder following high fat diet: Potential new therapeutic target for bladder dysfunction

Trevor C. Hunt

Markedly increased mitochondrial respiration in the murine bladder following high fat diet: Potential new therapeutic target for bladder dysfunction

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Introduction/Objectives: Bladder dysfunction is a chronic and debilitating medical problem that affects millions of men and women. There are clear differences between male and female pelvic anatomy, however the sex dimorphisms present at the cellular level in the bladder are understudied. Mitochondrial content, respiration, and reactive oxygen species (ROS) production varies between sexes and tissue type. We have demonstrated that male mice fed a 20-week high fat diet (HFD) demonstrate decreased detrusor mitochondrial respiration, increased ROS production, and bladder dysfunction. If these processes are involved in early pathogenesis, mitochondrial-targeted antioxidants could be a promising initial treatment for male or female bladder dysfunction. This study characterizes sex differences in detrusor and mucosal mitochondrial function following 6 or 12 weeks of HFD in mice.

Methods: Male and female C57BL/6N mice (10 weeks) were fed a control (10% fat) or HFD (45% fat) for 6 or 12 weeks (n=6-8/group). Food intake, body weight, and fat composition were assessed weekly. The voiding spot assay (VSA) was used as a surrogate of bladder function. Bladders were excised, weighed, separated into mucosal and detrusor layers, and placed into an Oroboros Oxygraph-2K for high-resolution respirometry. Mitochondrial oxygen consumption was assessed following activation of complexes I, II, and IV. Mitochondrial content was measured via citrate synthase assay.

Results: Mitochondrial content was similar in mucosal and detrusor layers between sexes. Mitochondrial respiration was equivalent at complexes I and II but females had lower mucosal maximal respiration at complex IV compared to males (p

Conclusions: Short-term 6-week HFD did not change bladder physiology or mitochondrial function in males or females. Following 12 weeks of HFD, increased mitochondrial respiration at complex IV was evident in mucosal and detrusor layers of both sexes. Increased respiration at complex IV can lead to electron leak and ROS production suggesting that HFD can lead to bladder dysfunction. Targeting mitochondrial function may be a new therapeutic avenue to restore HFD-induced bladder dysfunction in both males and females.

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129 The Efficacy of MTH1 Inhibitor Karonudib on Platinum-Sensitive and Platinum-Resistant Epithelial Ovarian Cancer

Rachel Hurley

The Efficacy of MTH1 Inhibitor Karonudib on Platinum-Sensitive and Platinum-Resistant Epithelial Ovarian Cancer

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Purpose: Ovarian cancer is the most lethal of gynecologic malignancies. The prognosis is particularly poor for tumors that are not responsive to platinum therapy, highlighting a key area for drug development. MTH1 inhibitors have demonstrated notable tumor activity in a variety of cancer cell lines, yet they have not been evaluated in ovarian cancer. Here, we provide evidence for MTH1 inhibitors as a therapeutic option for both platinum-sensitive and platinum-resistant ovarian cancer, specifically in combination with platinum agents.

Experimental Design: Platinum-sensitive and platinum-resistant ovarian cancer cell lines were exposed to MTH1 inhibitors TH588 and Karonudib in a clonogenic assay to assess viability. To assess cell cycle, ROS levels, and 8-oxodGTP accumulation, flow cytometry and immunofluorescence were performed. Platinum-sensitive and -resistant ovarian cancer patient-derived xenografts established intraperitoneally were treated with diluent, Karonudib, platinum, or the combination and followed for both tumor growth response and overall survival.

Results: Karonudib demonstrated efficacy in both platinum-sensitive and platinum-resistant ovarian cancer cell lines. Moreover, treatment with Karonudib increased cellular 8-oxoguanine levels. Ovarian cancer patient derived xenografts demonstrated statistical tumor growth delay relative to control in three distinct PDX models. When given in combination with platinum, Karonudib doubled overall survival in two models and demonstrated complete survival for the duration of the study (110 days) in the third.

Conclusions: MTH1 inhibition is a potentially effective strategy for the treatment of ovarian cancer, notably when given in combination with platinum agents. Further investigation of this class of agents is warranted.

130 Diverse macrophage subpopulations drive formation of post-traumatic heterotopic ossification via tunable expression of TGFβ1
Charles Hwang

Diverse macrophage subpopulations drive formation of post-traumatic heterotopic ossification via tunable expression of TGFβ1

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Heterotopic ossification (HO) leads to bone deposition in extra-skeletal sites restricting range of motion and causing chronic pain and wounds after severe musculoskeletal polytrauma, burn, or neural injury. While HO incidence ranges from 20-80% of post-trauma patients, no therapies have proven effective in ameliorating this disease process. As HO is characterized by an intense inflammatory reaction predominated by myeloid infiltration, we sought to define the key cell populations involved and to identify pro-inflammatory effectors that might prove amenable to therapeutic intervention. To this end, we induced HO in myeloid lineage reporter mice (*LysMcre/mTmG*$^{fl/fl}$) using a standard burn/Achilles tenotomy (BT) model. Following injury, a dense infiltration of GFP+ cells is observed at the affected site by 1-week post-surgery (n=3/group). Inflammatory recruitment demonstrates a peak of myeloperoxidase (MPO) activity at day1 vs day7 (total flux: 127417 vs 22424 p/s, respectively) as detected by bioluminescent *in vivo* imaging (n = 3/group). Concordant with these changes, we observe a temporal pattern of CD11b+Ly6G- monocyte (day1: 40% vs. day7: 33% of live cells) and CD11b+Ly6G+ neutrophil (day1: 19% vs. day7: 8%) infiltration as assessed by flow cytometry (n = 4/group). Using single cell RNAseq, 4 of 14 clusters after unsupervised clustering (Seurat/R) identified transcriptomes consistent with monocyte/macrophage defining genes, e.g., *H2*-Eb1 (MHCII), *Mrc1* (CD206), *Cd163*, and *Arg1*. Differential expression of these typically M2 phenotype markers demonstrated cellular heterogeneity even within a traditionally well-defined monocyte/macrophage phenotype.

To delineate what effects these cells have on the induced cellular niche, we interrogated the functional role of these recruited macrophages and found that *in-vitro* polarized M2 cells display at least a 2.5-fold increase in secreted TGFβ1, a potent pro-osteogenic effector, relative to M1/M0 cells. Macrophage F4/80 expression and TGFβ1 were further co-localized by immunofluorescent staining, paralleling our CD68 vs. TGFβ stains in excised human HO. Given these results, we sought to identify a role for TGFβ1 in HO progression by selectively deleting the growth factor from LysM myeloid cells using *LysMcreTGFβ1*$^{fl/fl}$ mice. Remarkably, TGFβ1 targeting results in a massive reduction in intramuscular ectopic bone formation in our HO model (uCT: 0.5537 vs 0.0003 mm^3, p = 0.008, n = 5/group). Taken together, these data provide the first evidence that macrophage-derived TGFβ1 is a key player in HO progression and that therapeutic interventions designed to intercept this pro-osteogenic growth factor could prove beneficial in this tissue-destructive disorder.

131 The bronchodilator and nutraceutical ginger reduces lung inflammation in a murine asthma model
Julie Hwang

The bronchodilator and nutraceutical ginger reduces lung inflammation in a murine asthma model

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Airway smooth muscle contraction and lung inflammation are hallmarks of asthma. We have previously shown that 6-shogaol, a biologically

active component of ginger, relaxes human tracheal airway smooth muscle (ASM) ex vivo, in part by inhibiting phosphodiesterase (PDE) activity. Consistent with this, 6-shogaol augments cAMP/protein kinase A (PKA)-dependent, β2 adrenergic receptor-mediated ASM relaxation. Previous reports have suggested that ginger and 6-shogaol also have anti-inflammatory properties via unclear mechanisms. Interestingly, cAMP has several PKA-dependent anti-inflammatory effects in lymphocytes. We hypothesized that chronic ginger or 6-shogaol administration would limit in vivo lung inflammation in the murine house must mite (HDM) antigen asthma model, and that 6-shogaol would increase cAMP levels in murine CD4 lymphocytes in vitro, similar to its effect in ASM.

All studies were Institutional Animal Care and Use Committee (IACUC) approved. Allergic lung inflammation was induced in C57BL/6J mice by daily intra-nasal administration of 40 µg HDM for 10 days. The mice also received oral (gavage) ginger (40 mg/kg BID) or vehicle, or intraperitoneal (i.p.) 6-shogaol (50 µl of 6.8 mM solution BID) or vehicle during this period. Subsequently, bronchoalveolar lavage (BAL) differential cell counts and lung IL-4 concentrations were compared using ANOVA with Bonferroni post-hoc analyses. In separate experiments, naïve murine CD4 cells were exposed in vitro to 10 µM 6-shogaol, 25 µM 6-shogaol, or vehicle in the presence of 0.5 µM prostaglandin E2 (PGE_2; to induce cAMP production) for 30 minutes. Cellular cAMP concentrations, assayed by ELISA, were compared.

Oral whole ginger and i.p. 6-shogaol significantly reduced BAL cell counts (predominantly lymphocytes and eosinophils) in HDM-sensitized mice compared to controls. Ginger and 6-shogaol also decreased lung IL-4 concentration by 59% and 51%, respectively, compared to controls (p 2 exposure, consistent with PDE_4 inhibition (in pmol/ml: 49.9 ± 6.4 for 10 µM 6-shogaol, 56.7 ± 6.0 for 25 µM 6-shogaol, 2.6 ± 0.7 for vehicle; p

Both oral whole ginger and i.p. 6-shogaol, a bioactive component of ginger, reduced HDM-induced allergic lung inflammation in mice. 6-shogaol also augmented cAMP concentration in CD4 lymphocytes. Given the previously established anti-inflammatory effects of cAMP/PKA activation in lymphocytes, ginger may be mitigating HDM-induced lung inflammation via immune cell PDE_4 inhibition. This effect would be consistent with its pro-relaxant mechanism of action in ASM and previous reports demonstrating that roflumilast, a PDE_4 inhibitor, both relaxes ASM and inhibits lung inflammation in humans. Given its ability to relax ASM and ameliorate allergic lung inflammation, 6-shogaol is a promising asthma therapeutic.

133 Thioredoxin-1 is an inflammatory marker for macrophages
Christopher Y. Itoh

Thioredoxin-1 is an inflammatory marker for macrophages

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Monocyte-derived macrophages are immune cells derived from hematopoietic progenitors. They interact with a variety of environmental stimuli and carry out highly variable functions, from pathogen phagocytosis to wound healing. Macrophages have traditionally been classified as M1 or M2 based on their mode of activation; this framework corresponds to proinflammatory and anti-inflammatory states, respectively. Using canonical surface markers, we found quantitative but not qualitative differences between these two stages by flow cytometry. However, these macrophages are functionally diverse and context dependent by stimulus and cytokine production. From this data, we hypothesized that there is an altered transcriptional program underlying these distinct phenotypic states.

To test this hypothesis, human monocyte derived macrophages differentiated using granulocyte-monocyte colony stimulating factor (GMCSF) or monocyte colony stimulating factor (MCSF), which are known to respectively produce pro-inflammatory and anti-inflammatory macrophages, were analyzed by single-cell RNA sequencing. This allowed an unbiased interrogation of cellular state while accounting for potential cell-to-cell heterogeneity. Thioredoxin-1 was a highly expressed gene distinguishing GMCSF-differentiated macrophages from MCSF-differentiated macrophages. We validated the increased expression of thioredoxin 1 on the protein level by western blot. MCSF-differentiated macrophages classically activated with IFNg and LPS also showed increased expression of thioredoxin, suggesting that high thioredoxin expression is a conserved feature of an inflammatory macrophage state. In addition, high thioredoxin levels in inflammatory macrophages were sustained after anti-inflammatory polarization, suggesting that thioredoxin marks inflammatory stimulation history. This durable inflammatory state was also evident upon measurement of cytokine production following TLR stimulation. Recently, thioredoxin has been directly implicated in the production of cytokines, and ongoing work is testing the hypothesis that thioredoxin functions as a regulator of inflammatory macrophage state.

134 Temozolomide-resistant glioma cells are sensitive to chloroethylating nitrosourea compounds in combination with ATR inhibitors
Christopher Jackson

Temozolomide-resistant glioma cells are sensitive to chloroethylating nitrosourea compounds in combination with ATR inhibitors

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Glioblastoma (GBM) is the most common primary brain tumor in adults. The current standard of care consists of surgery with maximal resection followed by concurrent temozolomide (TMZ) and radiation therapy. Despite decades of research, the current 5-year survival rates for GBM range from 5-10%. Indeed, tumor recurrence occurs in almost all patients. Many of these recurrences are thought to be due to TMZ

resistance. TMZ creates O^6-methylguanine lesions that are repaired by the enzyme O^6-methylguanine-methyltransferase (MGMT). Approximately 50% of patients with glioblastoma have MGMT-deficient tumors through a methylation of the promoter region, and these patients have a more favorable response to treatment. Interestingly, genetic analyses of recurrent GBM suggest that TMZ resistance is not due to changes in *MGMT* promoter methylation status but instead to deficiencies in mismatch repair (MMR) proteins that develop during treatment. The goal of this project was to identify a therapeutic strategy to treat TMZ-resistant, MGMT-deficient glioma cells. Our previous work showed that TMZ activates the Ataxia telangiectasia and Rad3-related protein-Checkpoint Kinase 1 (ATR-Chk1) axis in MGMT-deficient cells, and combination treatment with TMZ and ATR inhibitors (ATRi) is remarkably potent in these cells. TMZ-mediated activation of the ATR-Chk1 axis in MGMT-deficient cells is dependent on full MMR protein expression. However, treatment with chloroethylating nitrosoureas (CNUs) activates the ATR-Chk1 axis independent of MMR status. Thus, we hypothesized that the combination of CNUs such as the CNUs carmustine or lomustine—drugs that have been used to treat glioma for decades—with ATRi would still be effective in *MGMT*-methylated glioma cells resistant to TMZ through MMR deficiency. To test this, we obtained two isogenic cell line pairs (U251 and LN229, both *MGMT*-methylated) differing only in expression of the MMR protein MSH2. Upon constitutive shRNA-mediated knockdown of MMR protein MSH2, U251 cells became remarkably resistant to both TMZ and TMZ in combination with ATRi. In contrast, the addition of ATRi to the CNU agents carmustine or lomustine decreased the surviving fraction by over 2 orders of magnitude relative to CNU alone. Importantly, this synergy was observed at very low doses of alkylating agent, which has implications for reducing hematological toxicity in patients. Overall, our work suggests that carmustine or lomustine could be combined with ATR inhibitors to treat patients with *MGMT*-methylated glioblastoma who develop recurrent disease. Future work will evaluate these therapeutic combinations *in vivo*.

135 Determining the mechanism for the aggregation and toxic propagation of α-synuclein and tau oligomers
Noel A. Jackson

Determining the mechanism for the aggregation and toxic propagation of α-synuclein and tau oligomers

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Parkinson's disease is characterized by the presence of α-synuclein aggregates (oligomers) within the brain. Recent studies have begun to suggest that α-synuclein oligomers act as seeds, inducing the misfolding and aggregation of tau in Parkinson's brain. These findings suggest that α-synuclein and tau synergy is key in the progression of the disease. The mechanism of spread for these oligomers is still unknown. A potential mechanism is via exosomes. Released into extracellular space by all cells, exosomes facilitate cell-to-cell intercommunication through factors, such as proteins. Our goal is to further investigate the exosome-mediated release of oligomeric α-synuclein and tau as well as the synergistic interaction between the hallmark proteins. To determine whether the spread of α-synuclein oligomers occurs by exosomal release, SH-SY5Y cells were transfected and maintained to stably express wild-type α-synuclein tagged to GFP. Similarly, HEK293T cells were transfected with an APP-containing plasmid. A 24-hour co-culture assay was conducted by exposing APP+-HEK293T cells to WT α-syn+-SH-SY5Y cells. Immunostaining was conducted to detect markers for exosomes and WT α-syn within APP+-HEK293T cells. Additionally, a tau toxicity assay was conducted by exposing WT α-syn+-SH-SY5Y to recombinant tau oligomers. Immunostaining was conducted, targeting T22 using a polyclonal tau oligomer antibody. Results show APP+-HEK293T co-cultured with WT α-syn+-SH-SY5Y cells uptake α-synuclein. Evidence of colocalization of exosomes and α-synuclein was observed, suggesting an association between exosomes and the oligomers. Furthermore, tau oligomers seeded on WT α-syn+-SH-SY5Y cells altered α-synuclein to form cytoplasmic deposits, which suggests a possible interaction between them that may potentiate toxicity and subsequent spread. Overall, these preliminary findings further support exosomes as vehicles in PD and AD pathogenesis. Furthermore, they support the interplay between hallmark proteins tau, α-synuclein, and APP in neurodegenerative conditions.

136 Optimizing Mengovirus Targeting for Oncolytic Infectious Nucleic Acid Based Viro-Therapy
Yakin Jaleta

Optimizing Mengovirus Targeting for Oncolytic Infectious Nucleic Acid Based Viro-Therapy

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Oncolytic virotherapy is the selective killing of cancer cells using viruses that naturally amplify and spread within tumors or have been engineered to do so. Clinical evaluation of various oncolytic viruses has demonstrated the feasibility of this approach for anti-cancer therapy, however the level of efficacy observed in animal models has yet to be achieved in humans. This is likely due to the immune response against the viruses in patients, since many viruses can only be evaluated in immunodeficient animals bearing human tumor xenografts. In addition, virotherapy is expensive due to the cost of making viral particles. Mengovirus, a member of the Picornaviridae family, has the potential to overcome these barriers. Due to its broad tropism it can be evaluated in immune competent animal models and can be rescued from infectious RNA transcripts (iRNA), a formulation that lacks the components recognized by the anti-Mengovirus antibodies generated following infection. In addition, by administering Mengovirus as an iRNA the cost of virotherapy can be significantly reduced. An attenuated, poly(C)-truncated strain of Mengovirus (MC24) regresses syngeneic multiple myeloma mouse tumors when delivered as virus particles or iRNA, but causes lethal toxicities. In this study, we sought to generate a safe retargeted Mengovirus without reducing the specific infectivity of the iRNA. We generated a compre-

hensive panel of retargeted Mengovirus using three different strategies; i) insertion of microRNA target sequences complementary to neuronal and cardiac-enriched microRNAs within the 5′ and 3′ non-coding regions (MC24NC), respectively; ii) attenuation by eliminating known neurovirulent factors such as the poly(C) tract (MC0) and stem-loop 1 in the 3′ non-coding region, with (5′133/208-MC24DSL1; MC24DSL1-3′133/208) and without (MC24DSL1) cardiac-enriched microRNA targets; and iii) exchange of the internal ribosomal entry site (IRES) or complete 5′ non-coding region with those of picornaviruses that do not replicate in neuronal tissues (MC24-FMDV; MC24-HRV2; MG-FMDV). Our results show that the MC24NC ameliorates toxicity of the virus, but reduces the specific infectivity of the iRNA. This was attributed to the 3′ non-coding regions microRNA insert. Similarity, IRES switching further reduced specific infectivity. In contrast, MC0 and MC24DSL1 were able to rescue as well as the MC24 from RNA. Even though most constructs resulted in variable reductions of iRNA specific infectivity, all constructs maintained cytotoxicity in producer cells. Studies to determine if this reduction translates to viral RNA and to determine the oncolytic activity and safety of these retargeted viruses in vivo are currently ongoing.

137 Identification of novel pathogenic RNA splice altering gene mutations in congenital heart disease
Min Young (Megan) Jang

Identification of novel pathogenic RNA splice altering gene mutations in congenital heart disease

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BACKGROUND: Congenital heart disease (CHD) is the most common birth defect, occurring in about 1 in 100 neonates. Though DNA sequencing has become a useful tool in identifying genetic basis of CHD, known genetic causes account for less than 20% of total CHD cases. While variants that cause frameshift, nonsense, start/stop site gain or loss, and canonical splice site alterations are categorized as being pathogenic or loss-of-function (LOF), interpreting the clinical significance of variants without known functional consequences remains a challenge. Here, we aim to improve diagnostic classification of variants of unknown significance (VUS) that may be pathogenic for CHD.

METHODS: We used a published pipeline from our lab to prioritize and test variants in their ability to alter RNA splicing. Briefly, variants underwent computational selection to yield single-nucleotide VUS in splice regions that are predicted to alter splicing ("high-likelihood VUS"). These variants then underwent in vitro analysis including Minigene construction, transfection, RNA isolation, and sequencing. Splicing outcomes were quantified for each variant and its control Minigene. P-value comparing the normalized ratio of aberrant to normal splicing was generated by two-sided Fisher's exact test. We employed this pipeline on two variant lists: 1) 2,683 *de novo* variants from whole exome sequencing (WES) of 2649 trios consisting of CHD probands and unaffected parents in the NHLBI Pediatric Cardiac Genetics Consortium (PCGC), and 2) 473 splice region variants from molecular inversion probe sequencing (MIPs) in 1473 CHD probands in the PCGC.

RESULTS: In WES-identified variants, computational filtering narrowed 2,683 *de novo* variants down to 163 high-likelihood VUS. Subsequent analysis of these 163 variants yielded 53 variants as splice-altering (p KMT2D, as well as in 3 novel candidate genes *APOA1BP, ANKRD1,* and *NOS3*.

Similarly, 473 MIPs-identified variants were filtered to yield 64 high-likelihood VUS. Of these, 23 were determined to be splice-altering (p NOTCH1, as well as 10 new candidate genes including *EYA3, CAD, UBR2, ELP3, CTR9, SSRP1, PRMTS, SIN3A, CLUH,* and *MINK1*. Combined with the previously identified 81 LOF variants, this represents a 28.3% increase in total LOF variants.

CONCLUSIONS: We identified new LOF mutations in non-canonical RNA splice sites using a Minigenes assay and increased the yield of LOF mutations of traditional sequencing methods by up to 28.3%. Further analysis of splice-altering variants in both known and unknown pathogenic genes will improve our understanding of CHD as well as rules that govern RNA splicing.

138 Synonymous but not silent: A synonymous VHL mutation confers susceptibility to pheochromocytomas in a four-generation family
Angela Jasper

Synonymous but not silent: A synonymous *VHL* mutation confers susceptibility to pheochromocytomas in a four-generation family

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Pathogenic mutations in the von Hippel-Lindau (*VHL*) gene predispose individuals to VHL disease comprising of renal cell carcinomas, pheochromocytomas (PCCs), hemangioblastomas of the central nervous system and other manifestations with clinical presentation varying remarkably. In VHL disease type 2, PCC risk is higher, with type 2C manifesting PCC only. We report on four-generation family with a history of PCCs in a pattern consistent with autosomal dominant inheritance. The proband developed a unilateral PCC at age 32. Several of her family members, including her brother, father, paternal aunt and cousin, have also developed unilateral PCCs. Whole exome sequencing of her germline DNA revealed a heterozygous, synonymous mutation (c.414A>G, p.P138P) in *VHL* exon 2. No other candidate genes were identified. Sanger sequencing showed that the mutation segregated with the PCC phenotype in the family. The variant was not observed in population databases (ExAC) whereas ClinVar had 3 entries with conflicting interpretations (2 uncertain significance, 1 likely pathogenic). Nanostring-based Pheo-

Type profiling of the proband's archival PCC was consistent with a VHL subtype. In PCC from another relative, loss of the WT allele was observed at the variant locus. PCC cDNA analysis showed absence of the full length VHL transcript and presence of the shorter transcript lacking exon 2 in contrast to other PCCs which expressed both transcripts. Leukocyte cDNA analysis of carrier and WT relatives supported this finding. *VHL* encodes a tumor suppressor with ubiquitin ligase activity for degradation of hypoxia inducible factors (HIFs). *VHL* exon 2 is critical for the HIF binding domain; predominant expression of the shorter isoform leads to elevated HIF targets associated with oncogenesis. Most genetic screening workflows exclude synonymous variants. Our findings show that synonymous variants in coding regions of VHL should be taken into consideration as they may have splicing disruptions and affect protein function. Based on our findings, the c.414A>G variant is pathogenic and carriers should undergo routine follow-up for early detection. Although this family's clinical profile suggests VHL type 2C disease, broader surveillance is recommended as the consequences of this variant are not yet fully defined.

139 Unlocking the human immune response to vaccines: The use of tonsil lymphoid organoids to model human immune responses in vitro

Lauren P. Jatt

Unlocking the human immune response to vaccines: The use of tonsil lymphoid organoids to model human immune responses in vitro

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The evidence is clear: vaccines are one of the most cost-effective investments in health and development in modern human history. However, the development of new vaccines is stymied by the high cost of animal studies and clinical trials. Furthermore, it is widely recognized that the differences between the immune systems of laboratory animals and humans limit the predictive value of animal studies when evaluating the immunogenicity of vaccine candidates. As a result, many candidate vaccines which look promising in animals have been accelerated into expensive clinical trials involving thousands of individuals, vast resources, and many years to complete—only to disappoint. Because animal models of vaccination have been unreliable, we developed a simple in vitro lymphoid organoid system using human tonsil cells to mimic human adaptive immune responses. Using this system, we show that these immune organoids are able to respond to multiple antigens (e.g. live attenuated influenza vaccine, respiratory syncytial virus fusion protein, and human immunodeficiency virus envelope protein), secrete antigen-specific antibodies into culture supernatant, and support oligoclonal B cell expansion suggestive of somatic hypermutation and affinity maturation. Additionally, we exploit the flexibility of the system to conduct depletion experiments that determine which cell types are necessary and sufficient to produce a humoral response to influenza. We show that CD45 negative stromal cells (including fibroblastic reticular cells and follicular dendritic cells) and plasmacytoid dendritic cells (pDCs) are necessary for an influenza response. Additionally, we demonstrate that naïve B cells, pDCs, CD4+ T cells, and CD45 negative stromal cells are sufficient to create an influenza-specific adaptive immune response. In the future, this system can be applied to other vaccines to enable sophisticated mechanistic studies of existing vaccines and accelerate the testing and development of novel vaccines and adjuvants.

140 The dynamics of Arc expression in neuronal networks

Yuheng Jiang

The dynamics of Arc expression in neuronal networks

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Arc/Arg3.1 (activity-regulated cytoskeleton-associated protein/activity-regulated gene 3.1) is an immediate-early gene shown to be important in long-term memory formation and has been utilised as a cellular marker of the memory engram. Several recent studies and preliminary data from our lab have also suggested that Arc is implicated in the development of Alzheimer's Disease. Arc has been shown to have numerous different functions that act at several cellular compartments in an activity-dependent manner. However, no clear timeline for the action of Arc has been established and most of the previous work has been done at the cellular level on individual neurons. Therefore, we aim to investigate the expression of Arc in neuronal networks and specifically, to determine the spatiotemporal pattern of Arc expression in response to network activity. We do so by using generic cortical and hippocampal networks growing *in vitro*, which consist of mixed populations of neurons and glia. Arc expression in a subset of neurons is reliably induced by pharmacological activation of the network, which has been demonstrated previously to result in an increase in synchronised firing of neurons and a long-lasting increase in synaptic efficacy (chemical LTP). We tracked the expression of Arc after network activation and found that Arc protein moves from the cytoplasm to the nucleus, where it remains strongly induced. Nuclear Arc has previously been shown to regulate

gene expression and direct epigenetic modifications, and the timeline we established matches well with this previously described role. Surprisingly, we also discovered a shift of Arc expression from neurons to glia, specifically after long-term network activation. One other group have previously reported astrocytic Arc *in vivo*, and our results corroborate this finding. Furthermore, we found that glial Arc expression occurs after neuronal expression has peaked, which could have implications for the mechanism of expression and functional role of Arc in these glial cells. In conclusion, we have established the spatiotemporal expression profile of Arc in neuronal networks after network level activation, and describe a distinct order of localisation, not only for subcellular locations in neurons but also for neurons and glia.

141 CaV1.2 Channel Antagonists Activate Orai Channels
Martin Johnson

$Ca_V1.2$ Channel Antagonists Activate Orai Channels

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Voltage-gated calcium (Ca^{2+}) Channel Blockers (CCBs) have been widely used to treat hypertension, angina pectoris, and cardiac arrhythmias since the 1960s. CCBs, which include Amlodipine, Nifedipine, Nimodipine, Verapamil, Diltiazem, and Bay K8644, block Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels (Cav1.2) expressed at the plasma membrane (PM) of vascular smooth muscle cells (VSMCs). However, it is unclear whether CCBs interact with other Ca^{2+} channels. The store-operated Ca^{2+} entry (SOCE) pathway mediated by the Ca^{2+} Release-Activated Ca^{2+} (CRAC) channel, is the most ubiquitous Ca^{2+} entry pathway in non-excitable cells. SOCE is upregulated in VSMCs and activates fibroproliferative gene programs during cardiovascular remodeling. CRAC channels consist of hexamers of Orai proteins and are activated by the Endoplasmic Reticulum (ER) Ca^{2+} sensing proteins, STIM. The binding of physiological agonists to phospholipase C (PLC)-coupled receptors, lead to the production of inositol-1,4,5-trisphosphate (IP_3), which in turn causes Ca^{2+} release from the ER. The subsequent depletion of ER Ca^{2+} causes STIM molecules to aggregate and translocate into PM-ER junctional spaces where they trap Orai channels and cause their activation. Here, we used several cell lines from different origins to show that CCBs activate Ca^{2+} influx across the PM, which was sensitive to SOCE blockers. Through CRISPR/Cas9 knockout and overexpression in HEK293, we show that both STIM and Orai were necessary and sufficient for CCB activated Ca^{2+} entry. CCBs were equally efficient at activating Orai1, Orai2 and Orai3 but only when either STIM1 or STIM2 are present. Using a genetically encoded Ca^{2+} indicator targeted to the ER, ER-GCaMP6, we show that CCBs do not cause detectable Ca^{2+} depletion of ER stores. However, confocal and FRET microscopy showed that CCBs were able to induce STIM and Orai co-localization into puncta in PM-ER junctions. Unlike the Orai activator 2-Aminoethoxydiphenyl borate (2-APB), which unfolds the C-terminal domain of STIM1 (STIM1-CT) to expose its STIM-Orai Activating Region (SOAR), CCBs do not activate Orai through STIM1-CT. This suggests that CCBs might act on STIM N-terminal and/or transmembrane domains. Current studies are addressing these possibilities. Our findings suggest that CCBs stimulate Ca^{2+} entry by directly causing STIM reorganization into puncta without causing ER store depletion, providing a novel mechanism for CCBs action and a novel means to activate SOCE. In light of these findings, the clinical side effects associated with the use of CCBs especially during the late stages of hypertension, which are associated with cardiovascular remodeling and upregulation of SOCE, should be reevaluated.

142 Functional genetic variants mediate their regulatory effects through alteration of transcription factor binding
Andrew D. Johnston

Functional genetic variants mediate their regulatory effects through alteration of transcription factor binding

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Functional variants in the genome are recognized by their association with local gene expression, DNA methylation, or chromatin states. DNA sequence motif analysis and chromatin immunoprecipitation studies have provided indirect support for the hypothesis that functional variants alter transcription factor (TF) binding to exert their effects. In this study, we provide formal evidence to support this model. We identified a multi-functional variant within the *TBC1D4* gene encoding a canonical NFκB binding site, and edited it using CRISPR/Cas9 to remove a NFκB binding site. We show that this reduces *TBC1D4* expression, local chromatin accessibility and binding of the p65 component of NFκB. We then used CRISPR without genomic editing to guide p65 back to the edited locus, demonstrating that this re-targeting, occurring ~182 kb from the gene promoter, is sufficient to restore the function of the locus, supporting the central role of TFs mediating the effects of functional variants.

143 3D bioprinted skin accelerated closure of full-thickness wounds in mice
Adam M. Jorgensen

3D bioprinted skin accelerated closure of full-thickness wounds in mice

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Introduction: Burn injuries represent a significant clinical burden in the United States, with 1.1 million injuries annually requiring medical attention. Advances in wound treatment and skin regeneration have revolutionized burn and scar revision surgeries. However, currently available products fail to meet the need for full thickness replacement. Bioprinting has been proposed as a method for *in vitro* fabrication of full-thickness skin with multiple cell types organized into biomimetic layers. The primary aim of this study was to determine if 3D bioprinted human skin

accelerates closure of full-thickness wounds in mice.

Materials and Methods: Adipocytes, fibroblasts, and keratinocytes were isolated from human skin and expanded *in vitro*. Cells were trypsinized and resuspended in a fibrinogen bioink at $20x10^6$ cells/mL and bioprinted in a biomimetic tri-layer skin structure using the Integrated Tissue-Organ Printer (ITOP). Bioprinted constructs were then implanted on 2.5 x 2.5cm full-thickness excisional wounds on mice. Digital planimetry was performed at each bandage change, and analyzed with ImageJ to quantify total wound closure, contraction, and epithelialization. Samples were taken for histology at weeks 1, 3, 6, and 8. Samples were stained with Hematoxylin and Eosin to determine skin regeneration and epidermal barrier formation. Statistical analysis (T-test and one-way ANOVA) were calculated using SAS.

Results and Discussion: We found a highly significant difference in time to wound closure (days) between wounds treated with bioprinted skin (M = 14.83, SD = 2.54) and wound only (M= 24.5, SD= 0.5) (n= 7 per group, p

Conclusions: We have shown that bioprinted skin accelerates full-thickness wound closure through epidermal barrier formation without increasing contraction. Histological analysis confirmed that wound closure observed with digital planimetry represented true re-epithelialization. Altogether, we propose that bioprinted skin can be used for treatment of full-thickness wounds in human patients.

144 Neuroepigenetic Regulation of Imprinted Gene Grb10
Aimee Juan

Neuroepigenetic Regulation of Imprinted Gene *Grb10*

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Imprinted genes are a subset of mammalian genes that are exclusively expressed from either the maternal or paternal chromosome. Dysregulation of imprinted gene expression is associated with various human disorders. For example, the imprinting disorder Silver-Russell syndrome (SRS) is characterized by developmental delay and stunted growth. This disorder is associated with the abnormal inheritance of two maternal copies or alleles of the imprinted gene Growth Factor Receptor Bound Protein-10 (GRB10). Lack of the paternal transcripts in the brain may result in characteristic speech and motor delay in SRS patients. Normally, non-neuronal cells express *GRB10* exclusively from the maternal allele, while the paternal allele drives *GRB10* expression only in differentiated neurons. Importantly, the maternal and paternal transcripts initiate from distinct promoter regions. While the distinct promoter regions have been defined, the precise DNA sequences that are necessary for paternal transcription are unknown. We have identified candidate paternal-specific GRB10 regulatory sequences: allele-specific DNA methylation within the imprinting control region (ICR), binding sites for the zinc-finger protein CTCF, and putative downstream enhancers. This proposal will (1) test the requirement of proper DNA methylation at the ICR for normal neuronal *Grb10* expression using DNA methyltransferase and TET1 mouse models, and (2) assess the role of ten CTCF binding sites and two enhancer sequences in controlling paternal *Grb10* expression using CRISPR-edited neurons. By analyzing these epigenetic elements in a neuronal differentiation system and mouse models, we are the first to demonstrate how *Grb10* is epigenetically regulated. These findings will elucidate the mechanisms for allele and tissue-specific gene expression in the brain. Our results may also provide insight into the molecular basis of SRS, which could prompt epigenetic etiology screening and therapeutic options.

145 The effect of productive HPV16 infection on global gene expression of cervical epithelium
Sa Do Kang

The effect of productive HPV16 infection on global gene expression of cervical epithelium

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HPV infection is the world's most common sexually transmitted infection and is responsible for most cases of cervical cancer. Transition from precancerous to cancerous stages of HPV infection is marked by a significant reduction in virus production. Most previous studies of global gene expression changes induced by HPV infection have focused on the cancerous stages of infection, and therefore, not much is known about global gene expression changes at early pre-neoplastic stages of infection when the virus establishes persistent infection and progeny virions are produced at high levels. Although two previous studies looked into global gene expression changes in early passage HPV16-immortalized human keratinocytes, they used keratinocytes derived from foreskin instead of cervix, and monolayer cell cultures that do not allow the virus to complete its replication life-cycle.

In this study we show for the first time, global gene expression changes of early stage HPV16 infection in cervical tissue using 3-dimensional organotypic raft cultures that produce high levels of progeny virions. cDNA microarray analysis showed that a total of 594 genes were upregulated and 651 genes were downregulated at least 1.5-fold with HPV16 infection. Gene ontology analysis showed that biological processes including cell cycle progression and DNA metabolism were upregulated, while skin development, immune response, and cell death were downregulated with HPV16 infection in cervical keratinocytes. Individual genes were selected for validation at the transcriptional and translational levels including UBC, which was central to the protein association network of immune response genes, and top downregulated genes *RPTN*, *SERPINB4*, *KRT23*, and *KLK8*. In particular, we identified a group of genes that are typically overexpressed in cancerous stages to be significantly downregulated in our model of precancerous infection including *KLK8* and *SERPINB4*.

Organotypic raft cultures that allow full progression of the HPV life-cycle have allowed us to identify novel gene modulations and potential therapeutic targets of early stage HPV infection in cervical tissue. Additionally, our results suggest that early stage productive infection and cancerous stages of infection are distinct disease states expressing different transcriptomes, and therefore, should be studied and treated in their own separate context.

146 Modulation of choroid plexus immuno-secretory function to restore cerebrospinal fluid homeostasis in post-infectious hydrocephalus
Jason K. Karimy

Modulation of choroid plexus immuno-secretory function to restore cerebrospinal fluid homeostasis in post-infectious hydrocephalus

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Hydrocephalus is a devastating and often fatal disease effecting patients of all ages. The standard of care, cerebrospinal fluid (CSF) shunting, is an invasive neurosurgical procedure that is prone to complications, which require multiple revision surgeries and dramatically decreases quality of life. A fundamental obstacle in developing novel therapeutics has been a relative lack in our understanding of the choroid plexus epithelium (CPe) pathophysiology associated with different etiologies of hydrocephalus. Recent data has challenged the pathophysiologic dogma by demonstrating intraventricular hemorrhage (IVH) triggers inflammation-dependent CSF hypersecretion from the CPe to cause acute post-hemorrhagic hydrocephalus (PHH), and this can be prevented by pharmacologically targeting Toll-like receptor-4 (TLR4) or SPAK kinase. Like PHH, post-infectious hydrocephalus (PIH) exhibits non-obstructive ventriculomegaly, CPe inflammation, and a positive response to endoscopic choroid plexus cauterization. LPS, the canonical TLR4 ligand, is a component of many PIH-causing bacteria. We hypothesized that PHH/PIH share a common pathogenic mechanism of TLR4-SPAK-dependent CSF hypersecretion. We developed a novel rat model of PIH via the continuous intracerebroventricular infusion of LPS. *In vivo* CSF secretion measurements and MRI imaging evaluated the impact of LPS on CSF dynamics. RNAseq and LC-MS/MS phospho-proteomics assessed changes in the CPe transcriptome/phospho-proteome in response to IVH and LPS. Immunoblotting evaluated LPS-induced changes in the functional expression of specific TLR4- and SPAK-kinase-associated molecules in the CPe. ICV-LPS infusion triggered a striking increase in CSF secretion (~3.5-fold; p300%; p450%; p

148 Evaluation of the biased kappa opioid receptor agonist nalfurafine as an adjuvant therapy for modulating morphine reward
Shane Kaski

Evaluation of the biased kappa opioid receptor agonist nalfurafine as an adjuvant therapy for modulating morphine reward

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Mu opioid receptor (MOR)-targeting analgesics are efficacious pain treatments, but notorious for their abuse potential. While co-administration of a kappa opioid receptor (KOR)-targeting agonist with a MOR-targeting analgesic can decrease or abrogate reward, KOR-targeting agonists are themselves well-known for anti-therapeutic side effects (psychotomimesis, depression, anxiety, dysphoria). Recent data suggests that some functionally selective or "biased" KOR-targeting agonists might retain the therapeutic effects of KOR activation without inducing these undesirable effects. Nalfurafine, used in Japan since 2009 for uremic pruritus, is one such functionally selective KOR-targeting agonist. Here we quantify the bias of nalfurafine and several other KOR agonists against the reference standard U50,488 and further show that nalfurafine, at a dose (0.03 mg/kg) producing spinal analgesia equivalent to 5 mg/kg of the unbiased KOR agonist U50,488, does not reduce morphine-induced conditioned place preference (CPP) in C57BL/6J mice; only at a higher dose of 0.06 mg/kg nalfurafine was morphine-induced CPP effectively eliminated. In addition, nalfurafine was observed to produce robust inhibition of both spontaneous and morphine-stimulated locomotor behavior, suggesting a persistence of sedative effects at nalfurafine doses required to reduce morphine preference. The supraspinal analgesic effect of morphine, however, was seen to be potentiated by nalfurafine (and not U50,488) co-administration. Taken together, these findings suggest that β-arrestin signaling may be required for KOR agonist-induced reductions in drug reward, but not for the increased analgesic effect seen when co-administered. Thus, adjuvant administration of G protein-biased KOR agonists may be beneficial in enhancing the therapeutic potential of MOR-targeting drugs, such as morphine.

149 Over-expression of a specific signaling lymphocyte activation molecules-associated protein epitope identifies polyfunctional virus-specific memory CD8 T cells
Aaruni Khanolkar

Over-expression of a specific signaling lymphocyte activation molecules-associated protein epitope identifies polyfunctional virus-specific memory CD8 T cells

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Signaling lymphocyte activation molecules (SLAM)-Associated Protein (SAP) is an adaptor molecule that facilitates critical effector functions in T cells, such as cytotoxicity, IFNγ and IL-2 production. SAP deficiency causes a life-threatening disorder called X-linked lymphoproliferative disease-Type 1 (XLPD-Type 1), a rare condition characterized by uncontrolled lymphoproliferation, hyper-inflammation and B cell lymphomas often in response to primary Epstein Barr virus infection and three-fourths of the affected patients fail to progress beyond the first decade of life without a bone-marrow transplant. To validate a rapid diagnostic assay for XLPD-Type 1 we examined lymphocyte subsets of 54 healthy control donors and one genotypically-confirmed case of XLPD-Type 1 by flow cytometry to define reference ranges for SAP expression. As part of this effort we encountered two healthy control subjects within this cohort that displayed a unique CD8 T cell restricted bimodal pattern of SAP expression observed only with 1C9, but not the XLP-1D12, SAP antibody clone. Interestingly, a similar pattern is also depicted, but was not formally evaluated, in a recently published study that also utilized the 1C9-SAP Ab clone to evaluate three XLPD-Type 1 patients who experienced spontaneous somatic reversion of their SAP mutation. In our study we further evaluated the effect of this unique expression pattern by examining CD8 T cell function utilizing intracellular cytokine staining, phosflow analyses and surface mobilization of CD107a (a marker of degranulation potential). We demonstrated that 1C9-hi CD8 T cells displayed a memory phenotype and superior polyfunctional effector responses. Intriguingly, Epstein Barr virus and influenza virus epitope-specific responses were localized only within the 1C9-hi CD8 T cell subset. We also observed that short-term and prolonged stimulation selectively affected the detection of this subset. Overall, these observations identify a direct link between the magnitude of 1C9-SAP epitope expression and a subset of key effector CD8 T cell responses and further diversify the concept of T cell activation-induced regulation of SAP expression.

150 Caloric restriction exacerbates the effect of the menstrual cycle on sleep
Anne E. Kim

Caloric restriction exacerbates the effect of the menstrual cycle on sleep

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Dynamic changes in reproductive hormone levels across the menstrual cycle have been hypothesized to disrupt normal sleep patterns. While some studies reported increased sleep disruption in the luteal phase, others did not. In addition, metabolic hormones such as ghrelin and cortisol are affected by energy availability, and are linked to both sleep regulation and the reproductive axis. However, studies have focused on the responses of these hormones to sleep, rather than their effects on sleep. To evaluate the effects of hormonal changes across the menstrual cycle and decreased energy availability on objective sleep measures in an in-home setting, we collected daily Actigraphic sleep data (n = 578 sleep episodes) and morning urinary reproductive hormone samples from 10 healthy, regularly-cycling women aged 18 to 28 years over the course of two menstrual cycles. Urinary concentrations of luteinizing hormone (LH), estrone-3-glucuronide (E1G), and pregnanediol-3-glucuronide (PDG) were measured. As part of a larger study, subjects completed two 5-day diet interventions (neutral versus decreased energy availability) during the early follicular phases (EFP) of separate cycles. Cycles were centered on day of ovulation and standardized to 14-day follicular and 14-day luteal phases. Sleep data were analyzed using linear mixed models by menstrual phase, diet interventions, and reproductive hormones, adjusting for weekday vs weekend. Hormonal measurements confirmed ovulation in both cycles in all subjects (age 24.5 ± 2.5, BMI 22.2 ± 2.1). There was an effect of menstrual phase on sleep efficiency (SE, $p = 0.005$), wake after sleep onset (WASO, $p = 0.04$), number of awakenings per night ($p = 0.02$), and sleep fragmentation index (SFI, $p = 0.06$), consistent with increased sleep disruption in the late luteal phase (LLP). In comparison with the EFP, SE decreased by 3.3% ($p = 0.0002$), WASO increased by 15 minutes ($p = 0.001$), and number of awakenings increased by 3.0 ($p = 0.04$) in the LLP. Decreased energy availability increased sleep disruption, as indicated by decreased SE (p

151 Krüppel-like factor 5 regulates intestinal stem cell functions and identity
Chang Kyung Kim

Krüppel-like factor 5 regulates intestinal stem cell functions and identity

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The intestinal epithelium is a highly proliferative tissue with the capacity to renew within 3 to 5 days in human. It is also resilient with the ability to regenerate the entire epithelium following low-dose irradiation via dedifferentiation of precursor cells or reserve stem cells. Maintaining homeostasis of the epithelium by intestinal stem cells (ISCs) is important for providing vital functions, such as digestion, absorption, and barrier function. Despite recent progresses regarding the functions of ISCs in normal and disease states, significant gaps remain with respect to how ISC functions are regulated. Krüppel-like factor 5 (KLF5) is a zinc-finger transcription factor expressed in proliferating cells, including Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) expressing ISCs, of the intestinal epithelium and is involved in regulation of cell proliferation and differentiation. Previously, we have shown that KLF5 is required to sustain the half-life of $LGR5^+$ ISCs. To investigate the role of KLF5 in ISC self-renewal during homeostasis and in the re-

generative response after irradiation (IR) injury, we generated *Lgr5*-EGFP-IRES-Cre^{ERT2};*Rosa*^{tdTomato} (*Lgr5*^{Ctrl}) and *Lgr5*-EGFP-IRES-Cre^{ERT2};*Rosa*^{tdTomato};*Klf5*$^{fl/fl}$ (*Lgr5*$^{\Delta Klf5}$) mice. Mice were injected with tamoxifen to induce *Klf5* deletion and lineage tracing. For injury model, mice were exposed to 12 Gy total-body γ-irradiation (TBI). During homeostasis, acute *Klf5* deletion at 3 to 9 days after tamoxifen treatment increased the proliferation rate of LGR5$^+$ cells with simultaneous loss of LGR5$^+$ cells in the crypts, suggesting failure in self-renewal. In contrast, KLF5 plays an opposing role in precursor cells. *Klf5* deletion decreased the proliferation rate of precursor cells and led to overall reduction in lineage generation, followed by subsequent loss of *Klf5*-deleted lineage crypts. Confirming this phenotype, *Klf5*-deleted LGR5$^+$ cells failed to form enteroids in 3D culture as compared to control cells with intact KLF5. Transcriptome analysis of LGR5$^+$ cells isolated from *Lgr5*^{Ctrl} and *Lgr5*$^{\Delta Klf5}$ mice revealed that *Klf5*-deleted LGR5$^+$ cells lost the expression of ISC signature genes, while upregulated genes that are highly expressed in differentiated cells. These data suggest KLF5 is necessary for maintenance of stem cell identity, and ISCs undergo precocious differentiation without *Klf5* expression. Mechanistically, we showed that KLF5 transcriptionally activates key ISC genes, such as *Lgr5, Ascl2,* and *Olfm4*. Since KLF5 is required for stem cell functions and co-expressed in regenerating cells of the intestinal epithelium post-IR injury, we next examined the role of KLF5 in regeneration post-TBI injury. TBI-exposed *Lgr5*$^{\Delta Klf5}$ mice showed increased apoptosis in LGR5-lineages at earlier time points and decreased proliferation compared to *Lgr5*^{Ctrl} mice, suggesting KLF5 plays a role in cell survival and regeneration following IR damage. Ultimately, *Klf5*-deleted cells were not able to regenerate. Taken together, these data support that KLF5 is critical for the intestinal epithelium tissue self-renewal during homeostasis and regeneration post-IR injury.

153 Neural circuitry for maternal behavior and recognizing infant distress in the mouse primary auditory cortex
Gerina Kim

Neural circuitry for maternal behavior and recognizing infant distress in the mouse primary auditory cortex

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Maternal behavior is an evolutionarily conserved trait that ensures survival of the young and passage of genes to the next generation. A mother's response to infant cries is a key feature of maternal behavior, but the neural circuitry behind this behavior is not fully understood. In mice, maternal behavior can manifest through pup retrieval. Mouse pups emit ultrasonic vocalizations when they are separated from the nest, and mothers ('dams') respond to this auditory cue by retrieving the pups back to the nest. Virgin females co-housed with dams and litters are not initially responsive to pup calls, but start behaving maternally usually after hours to days. Previous work from our lab and others identified left auditory cortex as an important locus for maternal plasticity related to infant distress and recognition of pup call sounds (Marlin et al. Nature 2015). However, little is known about the cell types and microcircuits within auditory cortex that selectively respond to (or filter out) pup calls, or are responsible for the over-representation of these calls on the left side of the maternal cortex.

Here we aim to identify and quantify activities of excitatory and inhibitory neuronal subpopulations in the mouse primary auditory cortex as inexperienced virgin mice learn to respond to pup calls and retrieve pups. Our goal is to relate statistical learning of pup call sounds at the levels of behavior and cortical plasticity as virgins learn to retrieve. Wild-type C57BL/6 virgin mice were injected with an adeno-associated viral (AAV) vector for expression of Ca^{2+} indicator GCaMP6f under the CaMKII promoter for in vivo 2-photon imaging of excitatory neurons. Parvalbumin-Cre (PV-Cre) and somatostatin-Cre (SST-Cre) knock-in mice were also injected with AAV vectors for in vivo 2-photon imaging of PV and SST interneurons. Virgins were then co-housed with a dam and were imaged to see how the auditory cortex responded to pup calls before and after virgins began retrieving pups. We also played synthetic pup calls with different temporal modulations and at different frequencies, to examine to what degree single neurons or populations had an invariant representation of pup calls across stimulus statistics. Our preliminary results show that temporal tuning curves of excitatory and inhibitory populations are mismatched in naïve virgins but become aligned in virgins as retrieval abilities emerge over cohousing.

154 Dicer inhibition sensitized medulloblastoma and colorectal cancer to chemotherapy
Sarah Kim

Dicer inhibition sensitized medulloblastoma and colorectal cancer to chemotherapy

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Medulloblastoma (MB) is a common pediatric brain cancer, and treatment consists of chemotherapy and radiation. However, radiotherapy is associated with high neurological deficits that significantly decrease the quality of life for adolescents. Therefore, this study aims to sensitize tumor cells to chemotherapy in order to eliminate the use of radiation. How did we chemosensitize MB? Our approach was to inhibit Dicer, a key enzyme for the biogenesis of microRNA.

A mouse model for MB showed that loss of one Dicer allele accelerated cancer development; whereas loss of both Dicer alleles in MB promotes cancer cell death and chemosensitivity. Why does complete loss of Dicer cause chemosensitivity in MB? Along with microRNAs biogenesis, Dicer assists in repairing DNA defects. Hence, a complete loss of Dicer creates higher DNA damages resulting in apoptosis. These findings showed that Dicer inhibition is a promising approach to treating MB.

Using high throughput screen (HTS), 1594 compounds obtained from the National Cancer Institute's Developmental Therapeutics Program

(Diversity Set V) were screened and 29 compounds yielded > 50% Dicer inhibition. Out of the 29 compounds, cell-based assay showed that 9-hydroxyphenyl-fluoron inhibited Dicer in a dose dependent manner (IC_{50} ~20 mM). Two of the compounds, redoxol and celastrol, also inhibited Dicer and decreased cell viability by 50% at 30 mM and 100 mM, respectively. In summary, MB cells treated with Dicer inhibitors showed increased cell death.

Additionally, Dicer inhibition can be expanded to other cancers that rely on DNA damages as modes of treatment. For example, colorectal cancer cells treated with Dicer inhibitors had an increased sensitivity towards etoposide, a DNA damaging chemotherapy. This study has created a new paradigm for eradicating cancer by inhibiting Dicer. This new paradigm opens ambitious doors for treating cancers that rely on DNA damaging chemotherapeutics and decreasing invasive therapies.

155 Neighborhood-level factors that contribute to Colorectal Cancer Disparities: An exploratory analysis
Uriel Kim

Neighborhood-level factors that contribute to Colorectal Cancer Disparities: An exploratory analysis

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Disparities in cancer outcomes are driven by the combination of increased incidence, later diagnosis, and poorer stage-specific survival. Both individual and contextual-level factors contribute to these disparities. For example, a person's health behavior (an individual-level factor), or local air pollution (a contextual-level factor), can both contribute to increased cancer incidence. In colorectal cancer, the contextual-level factors that contribute to disparities-in-incidence (and how these factors interact with individual-level ones) are poorly characterized. Thus, we identified neighborhoods (census tracts) with the highest burden of colorectal cancer incidence, and characterized how these neighborhoods differ from other communities at both the neighborhood and patient levels. To accomplish this aim, we linked together data from the Ohio Cancer Incidence and Surveillance System (patient-level data), the US Census (contextual-level data), and the Health Resources and Services Administration (contextual-level data). Our final analytic sample included all incidence cases of colorectal cancer in Northeast Ohio from 2010-2014. Then, we compared how census tracts in the 75th percentile or higher for colorectal cancer incidence compared to those below the 75th percentile, both in terms of neighborhood and patient characteristics. We found that high-incidence communities were more likely to have larger Black populations and have more vacant houses. Additionally, the cancer patients who live in high-incidence communities were more likely to be Black and not be married compared to those who live in lower-incidence communities. This exploratory analysis demonstrates possible relationships between colorectal cancer incidence and individual-level, contextual-level, and cross-level factors. Future multi-level modeling studies can help clarify these relationships, which will be important in informing public health efforts to reduce colorectal cancer.

156 Gene-specific inhibition of nonsense-mediated mRNA decay in cystic fibrosis
Young Jin Kim

Gene-specific inhibition of nonsense-mediated mRNA decay in cystic fibrosis

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The *W1282X* nonsense mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene causes a severe form of cystic fibrosis (CF), but current CF treatments are not adequate for patients with this mutation. The truncated CFTR-W1282X protein has residual activity, but it is expressed at a very low level, due to nonsense-mediated mRNA decay (NMD). Thus, a gene-specific NMD inhibition strategy may lead to an effective allele-specific therapy for CF. NMD requires the binding of protein complexes called exon junction complexes (EJCs) on spliced mRNA. An EJC bound downstream of a premature-termination codon (PTC) strongly enhances NMD of the target mRNA. Other studies and our unpublished data suggest that the *CFTR-W1282X* mRNA harbors multiple NMD-inducing EJCs. Previously, we showed that synthetic antisense oligonucleotides (ASOs) designed to prevent binding of multiple EJCs downstream of PTCs attenuate NMD in a gene-specific manner. These results suggested that a cocktail of ASOs could be used for certain disease-causing nonsense mutations. Using *CFTR* minigene NMD reporters, we identified lead ASOs that efficiently target individual EJCs downstream of the W1282X mutation. Combination of the lead ASOs specifically increases the expression of endogenous *CFTR* W1282X mRNA and CFTR protein in transfected human bronchial epithelial cells. These results set the stage for the development of an allele-specific therapy for CF caused by the W1282X mutation.

157 H63D HFE protects cells from α-synuclein mediated toxicity
Yunsung Kim

H63D *HFE* protects cells from α-synuclein mediated toxicity

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting more than 7.5 million people worldwide. Current treatments for PD provide symptomatic relief, but have no effects on the overall disease progression. There is a need to better understand the neurodegenerative process, including genetic influences that modify the disease, for identification of therapeutic targets and improved design of clinical trials. Pathologically, PD is characterized by the presence of α-synuclein-containing Lewy bodies in select neuronal populations, in particular the dopaminergic neurons of the substantia nigra pars compacta. Increased levels of iron and ferritin in the substantia nigra are

also consistent features of the disease; however, it is unclear how these two factors interact with each other to ultimately result in neuronal cell death. The goal of our study is to understand the effects of iron on α-synuclein protein homeostasis and cellular response to pre-formed fibrils (PFFs) in a genetic model of iron overload. Specifically, we have chosen a cellular model involving a mutation in the *HFE* gene for this study. The *HFE* gene encodes a protein that plays a role in regulation of cellular iron uptake through the transferrin receptor (TfR). Mutations in HFE can disrupt its interaction with TfR leading to iron-overload. It is noteworthy that H63D is the most common *HFE* mutation with 10.9% allele frequency in the Caucasian population and has been shown to increase brain iron content. We investigated the effects of increased intracellular iron on α-synuclein expression, clearance of α-synuclein PFFs, and PFF mediated cell toxicity. SH-SY5Y neuroblastoma cells expressing H63D *HFE* had decreased α-synuclein compared to WT *HFE* expressing cells. Treatment with PFFs also showed decreased oligomerization of α-synuclein as well as protection from PFF mediated cell death in H63D *HFE* cells. As a potential mechanism, the basal level of autophagy was assessed. Autophagy is the main protein degradation pathway associated with oligomeric α-synuclein and induction of autophagy is thought to be neuroprotective. H63D *HFE* expressing cells had increased autophagy, which supports our findings of decreased α-synuclein oligomerization and increased cell viability. Importantly, treatment with an iron chelator (deferiprone) abolished the protection from PFF mediated cell death seen in H63D *HFE* cells. Collectively, these results reveal a novel role of intracellular iron as a protective factor in α-synuclein mediated toxicity. Furthermore, because H63D *HFE* is a genotype with high allele frequency, it has the potential to modify the disease process in ways to have significant clinical implications. These data indicate the importance of considering the *HFE* genotype in PD clinical trials involving iron chelation therapy.

158 Advanced maternal age and assisted reproductive technologies impact mitochondria and genomic imprinting in mouse preimplantation embryos

Audrey J. Kindsfather

Advanced maternal age and assisted reproductive technologies impact mitochondria and genomic imprinting in mouse preimplantation embryos

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Over the last several decades, the average age of first-time mothers has risen steadily. Advanced maternal age, defined in humans as above 35 years old, is known to increase the risk of spontaneous abortion, stillbirth, preterm birth, aneuploidy, and other chromosomal abnormalities and birth defects. As a woman ages, molecular changes occur in her oocytes that can affect the ability of the oocytes to be fertilized and embryo developmental competence. These changes include oxidative stress, which is known to damage mitochondria. In addition to other cellular processes, mitochondria likely play a role in regulating epigenetic mechanisms, such as genomic imprinting. Genomic imprinting is an epigenetic phenomenon that restricts expression to predominantly one parental allele through various mechanisms including cytosine methylation. Mitochondria in preimplantation embryos provide the ATP and methyl groups necessary for maintenance of imprinted methylation as the rest of the genome is demethylated. Assisted reproductive technologies (ARTs), including superovulation (SO) and embryo culture (EC) have been shown to alter imprinted DNA methylation in both human and mouse blastocysts. Therefore, we hypothesized that ARTs and maternal age, separately and together, affect mitochondrial activity and imprinted methylation in mouse preimplantation blastocysts.

Female C57BL/6(CAST7) mice from 2 to 14 months old were split into 4 treatment groups: no ARTs, SO only, EC only, and SO+EC. Spontaneously ovulating or superovulated females were mated with C57BL/6 male mice. Blastocysts were collected at day E3.5 or 2-cell embryos were collected at day E1.5 and cultured in Whitten's medium at 37ºC, 5% CO_2, and 5% O_2 for 3 days until the blastocyst stage. All blastocysts were stained with MitoTracker Green and Red to visualize total and active mitochondrial mass, respectively, and Hoechst to stain nucleic acids. Total and active mitochondrial mass was quantified in individual inner and outer cells in each blastocyst. Imprinted methylation levels at the maternally methylated *Snrpn* and *Kcnq1ot1* and the paternally methylated *H19* were assessed with bisulfite mutagenesis and clonal sequencing.

Our data showed that both ARTs and maternal age decreased mitochondrial levels and activity preferentially in outer trophectoderm cells but not in inner embryonic cells in preimplantation blastocysts. Treatment with any ART decreased imprinted methylation on the methylated allele of *Snrpn, Kcnq1ot1,* and *H19* in blastocysts from both young and aged mothers. However, increasing maternal age with or without ARTs had no additional effect on imprinted methylation.

Collectively, these results indicate that ARTs and maternal age alter mitochondrial levels and function in blastocysts, but only ARTs affect genomic imprinting maintenance. Future studies will determine if there is a correlation between imprinted methylation and mitochondrial loss, which could indicate a possible mechanism for genomic imprinting alterations, as well as the consequences of the observed mitochondrial dysfunction on metabolites and other maternal-effect factors.

159 Dopaminergic or glutaminergic system destruction in the caudate nucleus modulates the effects of methylphenidate exposure

Nicholas King

Dopaminergic or glutaminergic system destruction in the caudate nucleus modulates the effects of methylphenidate exposure

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Methylphenidate (MPD) is the most widely prescribed psychostimulant for the treatment of attention deficit hyperactivity disorder (ADHD), and is growing in use as recreational drug or academic enhancer. MPD acts

on the motive and motor circuits to produce its effects on behavior. The caudate nucleus (CN) is known to be a part of the motive and motor circuits, hence this study focusses on the role of the CN in response to acute and chronic MPD exposure. Five groups of rats were used: control (n=8), sham CN lesion (n=8), non-specific electrolytic CN lesion (n=8), dopaminergic-specific by 6-OHDA toxin CN lesion (n=8), and glutaminergic-specific by ibotenic acid toxin CN lesion (n=8); lesions were placed bilaterally. On experimental day (ED) 1, all groups received a saline injection. On ED 2 or 3, surgeries took place and rats were allowed to recover for 4 days (ED 3-7). Rats received six daily MPD 2.5 mg/kg injections (ED 9-14), three days of washout with no injection (ED 15-17), followed by a re-challenge with MPD 2.5 mg/kg (ED 18). Locomotive activity was recorded immediately after each injection for 60 minutes by a computerized animal activity monitor, i.e. the open field assay. The electrolytic CN lesion group responded to MPD acute and chronic exposure similarly to the control and sham groups. The dopaminergic-specific 6-OHDA CN lesion group failed to respond to MPD exposure both acute and chronically. The glutaminergic-specific ibotenic acid CN lesion group responded to MPD exposure acutely but failed to respond to chronic MPD exposure. The dopaminergic system of the CN is necessary for MPD to manifest acute and chronic effects on behavior. The glutaminergic system within the CN is essential for the chronic effects of MPD. Thus, the CN plays a significant role in the expression of acute and chronic MPD exposure's effects on behavior.

160 RSV virions produced by primary airway cultures display altered attachment protein structure and function
Tiffany King

RSV virions produced by primary airway cultures display altered attachment protein structure and function

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Respiratory syncytial virus (RSV) is responsible for the majority cases of hospitalized severe bronchiolitis in children under 1 year of age. Currently, there are no available vaccines or specific antiviral treatments for children infected with this virus. Understanding the mechanisms of viral entry is important to developing vaccines and treatments against this pathogen. RSV has two glycoproteins embedded in its membrane and both are important for viral entry: fusion (F) glycoprotein and attachment (G) glycoprotein. The G glycoprotein has been shown to be essential *in vivo* and in primary cell culture but not in immortalized cells. We have reported that the G protein is larger (LgG) when produced in primary human airway epithelial (HAE) cultures: 180kDa, compared to 90kDa when produced in immortalized cells. Virus harboring LgG is >100-fold more infectious in primary cell cultures than in immortalized cells, demonstrated through virus titration and quantification of viral genomes using qRT-PCR. Here we demonstrate LgG is present in both RSV-A and RSV-B laboratory and clinical isolates. Understanding the structural modification responsible for LgG and how LgG influences infection in primary cell culture is important for targeting the G glycoprotein in vaccines and anti-viral drug development.

161 Evi1 mediates cell of origin-specific responses of AML cells to chemotherapy and targeted epigenetic therapy
Mitali Kini

Evi1 mediates cell of origin-specific responses of AML cells to chemotherapy and targeted epigenetic therapy

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Acute myeloid leukemia (AML) comprises a group of blood cancers characterized by clonal expansion of malignant hematopoietic cells with impaired myeloid maturation. Some of the most aggressive AMLs are driven by rearrangements of the mixed lineage leukemia (MLL) gene. Patients with leukemias harboring the MLL-AF9 gene rearrangement have particularly poor prognoses and rarely achieve lasting remissions. Multiple studies have demonstrated that the cell of origin of leukemic transformation influences the sensitivity of MLL-AF9 leukemias to cytotoxic chemotherapy, such that cells derived from hematopoietic stem cells (HSC) are more aggressive, chemoresistant, and exhibit higher expression of the transcription factor *Evi1* than AML cells originating from more differentiated granulocyte macrophage progenitor (GMP) cells. Transcriptional profiling revealed upregulation of p53 target genes in GMP-derived leukemias and a p53-null signature enriched in HSC-derived leukemias, suggesting a potential *Evi1*- p53 interaction in these cells. The goal of our study was to determine the role of *Evi1* in mediating the differential response to chemotherapy observed in HSC- and GMP-derived MLL-AF9 leukemias.

In order to assess drug sensitivity of these cell types *in vitro*, we treated both HSC- and GMP-derived leukemias with increasing concentrations of either inhibitors targeting the histone demethylase activity of lysine-specific demethylase 1 (LSD1) or the chemotherapeutic agent doxorubicin. Cell viability was measured after three days of culture. We found that HSC-derived leukemias exhibited greater resistance to treatment with doxorubicin or LSD1 inhibitors than GMP-derived leukemias, even though leukemia cells derived from either compartment exhibited identical steady-state growth kinetics.

Based on the observation that *Evi1*-low GMP-derived leukemias exhibited higher p53 transcriptional output, we hypothesized that *Evi1* expression modulates p53 protein stability. Consistent with this hypothesis, we observed greater p53 protein expression at rest in GMP-derived AML cells when compared to HSC-derived AMLs. Moreover, shRNA-mediated knockdown of *Evi1* in HSC-derived leukemias resulted in increased p53 protein stabilization as assessed by Western blot analysis. Conversely, ectopic overexpression of FLAG-tagged *Evi1* in NIH-3T3 mouse fibroblast cells blunted doxorubicin-induced p53 stabilization relative to empty vector controls.

Our findings suggest that the difference in *Evi1* expression observed in HSC- and GMP-derived leukemias plays an important role in cell of

origin-specific responses to chemotherapy and to targeted epigenetic therapy (i.e. LSD1 inhibitors). Furthermore, these data suggest that *Evi1* may suppress p53-dependent apoptosis by destabilizing p53 protein, thereby conferring drug resistance in *Evi1*-high HSC-derived leukemias. Future work elucidating the role of *Evi1* in modulating p53 activity will allow us to identify additional therapeutic targets for this particular subset of *EVI1*-high AML patients with poor prognoses in need of more effective treatment strategies.

162 Type-I interferon induces temporally distinct activities of two STAT1-containing transcription factor complexes
Kensei Kishimoto

Type-I interferon induces temporally distinct activities of two STAT1-containing transcription factor complexes

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Type I Interferons (IFNs) are secreted by host cells upon recognition of pathogens to activate and modulate immune and inflammatory responses. Misregulation of type I IFN signaling is associated with an increased risk of developing severe infections and with a number of autoimmune diseases. Type I IFN signaling activates a transcription factor called ISGF3, made up of STAT1, STAT2, and IRF9 proteins. STAT1 can also form a homodimer known as GAF which recognizes an entirely different binding motif. Although GAF is typically activated in response to type II IFNs, it has been reported that small amounts of GAF are also induced by type I IFN signaling. However, the mechanisms and significance of this crosstalk are unclear, and studies describing type I IFN activation of GAF have not taken into account temporal dynamics of STAT1 binding activity to DNA. To probe the temporal control of ISGF3 vs GAF activation, we performed time-resolved EMSA and ChIP-seq assays of mouse lung epithelial cells treated with type I IFN. We found that type I IFN indeed activated both ISGF3 and GAF, but with distinct temporal dynamics. GAF was activated early in the IFN response but was transient, while ISGF3 had its first peak activity at 1 hour and a second and more amplified activity at 4 hours. This biphasic activity of ISGF3 suggests a feedback loop, possibly involving a newly synthesized GAF target proteins inhibiting GAF or enhancing ISGF3. Motif analysis of STAT1 ChIP-seq binding events revealed enrichment of GAF-binding motifs among early peaks and ISGF3-binding motifs among later peaks. The majority of STAT1 binding events at early time points were also inducible by type II IFN while the majority of STAT1 occupancy at later time point were not, further suggesting that STAT1 acts and binds to genome as GAF at early and as ISGF3 at later time points. It is possible that this temporal shift from GAF to ISGF3 may play a role in shifting pro-inflammatory gene expression to antiviral one to avoid prolonged inflammation, which can lead to autoimmune phenotypes.

163 Functional testing of thousands of osteoarthritis-associated variants for regulatory activity
Jason C. Klein

Functional testing of thousands of osteoarthritis-associated variants for regulatory activity

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Genome-wide association studies (GWAS) have successfully implicated thousands of genetic loci in common human diseases. Most of the underlying signal is believed to derive from variation in non-coding regulatory sequences. To date, GWAS have identified at least 35 loci in osteoarthritis. However, we have yet to pinpoint the specific variants that underlie these associations, nor the mechanisms by which they contribute to disease risk. The current gold standard is to test all alleles in each locus one at a time for potential function, which is not scalable to thousands of alleles. Here we functionally tested 1,605 single nucleotide variants associated with osteoarthritis for regulatory activity using a massively parallel reporter assay. An MPRA involves cloning thousands of candidate regulatory sequences to a single reporter gene, transfecting them to a cell line *en masse*, and performing deep sequencing of the resulting transcripts to quantify the degree of transcriptional activation mediated by each candidate regulatory sequence. We modified the traditional MPRA in this case to make several independent measurements of each SNP in a single experiment to provide us with statistical power to differentiate between alleles. Doing so, we identified six single nucleotide polymorphisms (SNPs) with differential regulatory activity between the major and minor alleles. We show that our most significant hit, rs4730222, drives increased expression of an alternative isoform of *HBP1* in a heterozygote chondrosarcoma cell line, a CRISPR-edited osteosarcoma cell line, and in chondrocytes derived from osteoarthritis patients. HBP1 acts a repressor of Wnt signaling, which has been implicated in OA pathogenesis. We further show that the two alleles at rs4730222 show differential protein binding, suggesting that the major allele binds a transcriptional repressor. In this study, we applied a new framework to screen thousands of putative variants for regulatory activity in order to prioritize ones important in OA pathogenesis. In doing so, we provide further support for a role of Wnt signaling in OA.

164 Structural and functional insights into beta-arrestin coupling to muscarinic acetylcholine receptor M2
Alissa L.W. Kleinhenz

Structural and functional insights into beta-arrestin coupling to muscarinic acetylcholine receptor M2

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G-protein coupled receptors (GPCRs) are the most abundant receptor family in the human body and regulate numerous physiological processes by transducing extracellular signals into cellular responses through a highly conserved mechanism. Binding of endogenous ligand (i.e hormones) to the extracellular orthosteric pocket induces conformational changes within the seven transmembrane helices, which enable intracellular coupling and activation of transducer G-proteins to execute specific cell signaling pathways. Subsequently, phosphorylation of the activated GPCR C-terminus and/or intracellular loops leads to biphasic recruitment of β-arrestin: β-arrestin first binds the phosphorylated C-terminal tail, then subsequently engages the transmembrane receptor core. This β-arrestin recruitment terminates, or desensitizes, G-protein signaling by sterically occluding the G-protein binding site and inducing receptor internalization. Importantly, β-arrestin can direct its own downstream signaling pathways independent from those mediated by G-proteins. Despite the publication of at least a dozen high resolution GPCR-G-protein complex structures, high resolution structures of GPCR-β-arrestin complexes have not yet been reported. This is most likely due to the inherent difficulty in homogenously phosphorylating GPCRs *in cellulo*, and the low-affinity interactions between β-arrestin and receptors. To circumvent these obstacles, we developed a technology which allows us to form robust GPCR-β-arrestin complexes *in vitro* using a sortase enzyme system to ligate a synthetic phosphopeptide onto the C-terminal tails of GPCRs. We screened a variety of such phosphorylated GPCRs for coupling to β-arrestin, and identified the muscarinic acetylcholine receptor M2 (M2R) as the most promising candidate for structure determination via cryo-electron microscopy (cryo-EM). Whereas existing cryo-EM structures of GPCR-G-protein complexes were determined in detergent, we found that reconstituting M2R into synthetic model membrane systems, i.e. nanodiscs, is required to enable β-arrestin coupling. Inclusion of M2R's third intracellular loop (ICL3) and addition of an antibody fragment to stabilize the interaction of β-arrestin with M2R's synthetic phosphopeptide tail further increases coupling. These complexes in nanodiscs have yielded a preliminary, low-resolution cryo-EM structure revealing previously unseen contacts between β-arrestin and the lipid bilayer. As we continue to optimize our complexes to achieve a high resolution structure, we will compare our findings with existing GPCR-G-protein structures to define the conformational differences in receptors that underpin coupling to G-proteins versus β-arrestin. This information will lead to an understanding of the important phenomenon of "biased" signaling, a process whereby some molecules can preferentially stimulate signaling via either G proteins or β-arrestins. Such molecules offer the possibility of developing novel GPCR drugs with improved efficacy and reduced side effects.

165 The IRF8-osteopontin-CD44 axis functions as an immune checkpoint to control CD8+ T cell activation and tumor immune evasion

John D. Klement

The IRF8-osteopontin-CD44 axis functions as an immune checkpoint to control CD8+ T cell activation and tumor immune evasion

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Despite breakthroughs in immune checkpoint inhibitor (ICI) immunotherapy, not all human cancers respond to ICI immunotherapy and only fraction of patients with responsive tumors have a durable response to current ICI immunotherapy. This clinical conundrum suggests that additional immune checkpoints may exist, particularly in cancers resistant to current ICI immunotherapy, such as colorectal cancer. We report here that interferon regulatory factor 8 (IRF8) deficiency led to impairment of cytotoxic T lymphocyte (CTL) activation in a peptide vaccine model and allowed allograft transplant tumor tolerance. These effects were associated with upregulation of the CTL surface marker CD44. However, analysis of chimeric mice with competitive reconstitution of wild type and IRF8 KO bone marrow cells as well as mice with IRF8 deficiency only in T cells indicated that IRF8 plays no intrinsic role in CTL activation. Instead, IRF8 functioned as a repressor of osteopontin (OPN), the physiological ligand for CD44 on T cells, in CD11b+Ly6CloLy6G+ myeloid cells and OPN acted as a potent T cell suppressor. In vitro stimulation of CTLs in the presence of OPN resulted in decreased expression of activation markers CD69 and CD25 and inhibited proliferation and interferon gamma (IFNg) secretion. Accordingly, blockade of CD44 enhanced in vitro CTL responses to OPN-secreting colorectal cancer cell lines. Expression of OPN was found to be upregulated in both myeloid cells and colon epithelial cells following silencing of IRF8 expression. IRF8 bound to the Spp1 promoter, which encodes OPN, to repress OPN expression in colon epithelial cells. Correspondingly, human colon carcinoma cells exhibited decreased IRF8 and increased OPN expression. These increased OPN levels inhibited human PBMC proliferation and IFNg secretion in a dose-dependent manner at concentrations found in colorectal cancer patients. The elevated expression of OPN in human colon carcinoma was correlated with decreased patient survival. Our data indicates that myeloid and tumor cell-expressed OPN acts as a novel immune checkpoint to suppress T cell activation and confer host tumor immune tolerance. Blockade of this checkpoint may expand the pool of patients who may benefit from ICI immunotherapy

166 Are there differences in emergency department length of stay and throughput between males and females? Preliminary results of the sex equity in emergency departments (SEED) study group

Catherine G. Knier

Are there differences in emergency department length of stay and throughput between males and females? Preliminary results of the sex equity in emergency departments (SEED) study group

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Sex and gender disparities exist in healthcare, including getting needed care, receiving high risk medications, and receiving potentially harmful medications, as outlined by the Centers for Medicare and Medicaid services. Current knowledge of gender disparities in emergency departments (ED) is limited by analysis of individual chief complaints or diagnoses instead of all comers. For example, women presenting with abdominal pain wait longer for analgesic administration than men; women are less likely to receive thrombolysis following STEMI or ischemic stroke; and female sex is associated with longer time to CT and appendicitis diagnosis. At a disease level there is more chance of differences being attributable to gender-specific tests such as pelvic exam or pregnancy testing. We aim to determine if females presenting to the ED have longer overall length of stay (LOS) than males and understand other factors such as age, race, chief complaint (CC), body mass index (BMI), and insurance status that may contribute to differences.

This is a retrospective study approved by the institutional review board of all adult visits to a quaternary academic ED between July 2015 and July 2016. Data is gathered from the electronic medical record and throughput markers such as arrival to the ED, time moved to a treatment room, time seen by a provider, time a disposition is determined, and departure time from the department are harvested along with demographic data. Normally distributed data are presented as means with range and standard deviation reported, whereas non-normal data are presented as medians. Moods median test is used to compare medians. Multivariable analyses adjusting for age, race, chief complaint, BMI, and insurance status will be explored for potential role in disparities.

During the study period, the ED had 65,533 adult patient visits of which 34,105 were female (52%) and 31,428 were male (48%) with an average age of 53 (min 18, max 104, SD 21). The median LOS was 229 minutes (IQR 153, 323), for females 236 (IQR 159, 329) and for males 222 (IQR 147, 317). Men spent 3 minutes less in the waiting room (p

These results highlight that differences exist between LOS and throughput of males versus females in the emergency department. Although there may be differences in testing and differential diagnoses and treatments between the sexes, the difference in the waiting room time and time to being seen by a provider are harder to account for at this time. This author group believes identifying biases can help eliminate unintended differences and advance toward health equity.

167 Shortened ex vivo expansion of Th17 cells enhances anti-tumor immunity

Hannah M. Knochelmann

Shortened *ex vivo* expansion of Th17 cells enhances anti-tumor immunity

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Adoptive T cell transfer therapy mediates potent immunity in patients with bulky metastatic malignancies but proves difficult to translate clinically due to cost, time, and labor required to generate personalized T cell products. Though several CAR-T cell preparations were recently FDA approved, patients indicated for these therapies are at risk of insurance coverage denial due to the expense of T cell manufacturing. As a result, methods of reducing production costs by generating T cells with potent antitumor properties more quickly are in high demand. We proposed a method of shortened *ex vivo* expansion using Th17 cells to treat melanoma using the TRP-1 transgenic mouse model in which $CD4^+$ T cells express a TCR specific for TRP-1 antigen on melanoma. Naïve $CD4^+$ T cells were polarized to secrete IL-17 and infused into mice with B16F10 melanoma. By studying antitumor efficacy of cells kinetically over *ex vivo* expansion, we found that Th17 cells expanded only four days can eradicate tumors even when only few cells (~200K) are infused into the animal. These day-4 cells mediate more potent antitumor responses than greater numbers (>25X more) of Th17 cells expanded up to two weeks. In contrast to long-term expanded cells, day-4 Th17 cells 1) express peak levels of IL-2Ra and costimulatory molecules (CD28, OX40, ICOS), 2) persist at greater fold once infused in the animal, 3) induce significantly increased production of IL-6, IL-17, and GM-CSF within the tumor-bearing host, and 4) provide long-lived protection against tumor recurrence. Our findings indicate that a brief, four-day expansion protocol generates highly activated Th17 cells which induce a robust inflammatory response within the host. Despite lower yield versus long-term expansion, four-day expansion of Th17 cells can augment efficacy, reduce expense, and improve accessibility of adoptive cell therapy to patients clinically.

168 Islet Protecting Insulin Releasing Compound (IPIRC): a dynamic approach to the standard treatment of type 1 diabetes mellitus

William J. Koch

Islet Protecting Insulin Releasing Compound (IPIRC): a dynamic approach to the standard treatment of type 1 diabetes mellitus

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According to the Centers for Disease Control and Prevention (CDC), 30 million Americans have diabetes, of which about 5% have type 1 diabetes mellitus (T1DM). The pathogenesis of T1DM is the result of a surge in cell signaling molecules, known as cytokines, which induce immune-mediated cell death of beta cells within pancreatic islets. Upon diagnosis of T1DM, the immune system has already destroyed an estimated 70-90% of insulin-secreting beta cells. We have discovered a small molecule termed Islet Protecting Insulin Releasing Compound (IPIRC) with the potential to treat T1DM by protecting pancreatic islets from immune-mediated destruction and restoring insulin secretion.

We investigated the protective effects of IPIRC against several pro-inflammatory cytokines. Mouse islets were treated overnight with 5ng/mL IL-1β and 10ng/mL TNF-α in combination. IPIRC strongly protected against cell death measured by propidium iodide and annexinV fluorescence for IL-1β + TNF-α. Additionally, IPIRC exerts similar protection against cell death induced by the combination of cytokines on human islets.

During overnight IPIRC treatment in 11mM glucose, we observed a maximal 5-fold increase in insulin release with 200μM IPIRC (EC50: 54+/-36μM). Significant stimulatory effects of IPIRC on insulin secretion were observed out to 6-days, in vitro. We also show that the sulfonylurea tolbutamide, a potent insulin secretagogue, caused a large reversible increase in intracellular calcium, whereas IPIRC caused a small reversible decrease, indicating that the mechanism of IPIRC is novel and different than any known sulfonylurea.

Our results indicate that IPIRC has the potential to protect islets from cytokine-mediated cell death and enhance insulin secretion. Protecting beta cells and enhancing insulin secretion synergistically are significant in the treatment of T1DM. Collectively, these finds suggest a novel therapeutic for the treatment of immune-mediated diabetes. Donor human islet studies are ongoing, as well as, in vivo mouse studies. The exact mechanism(s) of this dual-acting compound requires further study.

169 Bilateral total hip arthroplasties performed at a rural critical access hospital; results and analysis of reimbursement

Christian J. Konopka

Bilateral total hip arthroplasties performed at a rural critical access hospital; results and analysis of reimbursement

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Introduction: Despite the prevalence of bilateral hip arthritis and benefits of one-stage bilateral total hip arthroplasty (BTHA), it remains an uncommonly performed procedure. Current reimbursement models, especially Medicare, disincentivize this procedure due to disproportionately low reimbursement. Critical access hospitals (CAH) are reimbursed at cost plus 1% by Medicare. We hypothesized that with the CAH payment model, it is financially feasible for hospitals to perform BTHA.

Methods: A retrospective chart review was performed on 30 patients who had one-stage, direct anterior bilateral THA by a single surgeon in a rural critical access hospital. The charts were examined for patient and surgical factors, clinical outcomes, and financial data.

Results: Twenty-three women and 7 men underwent one-stage BTHA. The mean age was 66. Average follow up was 2.1 ± 1.5 years. Average total surgical time was 158 min ± 22 min. Mean estimated blood loss was 572 ± 199 cc, and 33% of patients required transfusion. There were no readmissions or periprosthetic infections. One patient had an outpatient procedure for secondary wound closure in the absence of deep periprosthetic infection. Fifty-two percent of patients were discharged to home, while the remainder were discharged to swing bed. For BTHA, average revenue was $16,628 for Medicare vs average expense of $41,655, leading to net loss of $25,027. After cost reconciliation under the CAH reimbursement model, average profit per Medicare case was $417. Reimbursement based on DRG would have been $21,483, leading to average loss of $20,172. Considering all insurers, average profit for BTHA was $5,119 vs $7,769 for unilateral THA.

Conclusion: This study demonstrates that one-stage BTHA is safe and effective. Under current the Medicare reimbursement model, this procedure leads to a substantial financial loss, possibly discouraging its use. A revised Medicare payment model may lead to increased use of this safe and effective procedure.

170 A dynamic redox pathway for CaMKII activation and cardioprotection

Klitos Konstantinidis

A dynamic redox pathway for CaMKII activation and cardioprotection

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Excessive activation of the Ca^{2+} and Calmodulin (CaM)-dependent protein kinase II (CaMKII) leads to heart failure and arrhythmias. Understanding pathways governing CaMKII activation, and developing CaMKII inhibitor drugs are goals for producing new cardiovascular therapeutics. While CaMKII is initially activated by CaM binding, it is unknown if CaM binding to CaMKII is a biologically regulated process. CaMKII activity is responsive to methionine oxidation, so we screened purified CaMKII using mass spectroscopy in the presence of MICAL1, a methionine oxidase, and MSRB, a methionine reductase. Although actin was the only known substrate for MICAL1, we identified methionine 308 (M308) in the CaM binding domain as a site for MICAL1 oxidation, and MSRB reduction. We combined direct measurements and computational modeling of CaM binding to WT CaMKII, M308 oxidized CaMKII, and various M308 mutant peptides. These studies showed that M308 oxidation or replacement by valine (M308V) markedly decreased CaM binding to CaMKII, and CaMKII activation. We found that mice lacking MICAL1 have increased levels of active, T287 autophosphorylated CaMKII at baseline in the heart, suggesting MICAL1 is a molecular brake to constraint basal CaMKII activity by M308 oxidation. Compared to WT littermate controls, MICAL1 knockout mice exhibited significantly increased mortality after transaortic constriction surgery (TAC), a pathological stress known to activate CaMKII. Using functional assays, we screened various MICAL1 mutants and identified a MICAL1 mutant (R116H) that can discriminate between actin and CaMKII; MICAL1 R116H does not oxidize actin, but maintains its ability to oxidize CaMKII. To test whether the increased mortality we observed in the MICAL1 knockout mice after TAC was due to loss of actin oxidation or loss of CaMKII oxidation we developed MICAL1 knockin mice (R116H). R116H mice had significantly lower mortality compared to MICAL1 knock out mice after TAC, suggesting that loss of CaMKII M308 oxidation by MICAL1, and not actin, is responsible for the high mortality rate seen in the MICAL1 knockout mice after stress. To test whether CaMKII oxidation by MICAL1 at M308 plays a role in human disease, and whether this pathway can be targeted therapeutically, we introduced M308V into human induced pluripotent stem cells (hiPSCs) derived cardiomyocytes from patients with CPVT (catecholaminergic polymorphic ventricular tachycardia), a genetic arrhythmia known to be suppressed by CaMKII inhibition. The cardiac hiPSCs harboring a validated CPVT human mutation together with M308V were resistant to a CPVT cellular arrhythmia phenotype, in contrast to cardiac hiPSCs with the CPVT mutation only. These data point to a previously unrecognized pathway for methionine oxidation and reduction to dynamically regulate CaMKII activation in vivo.

171 CREBRF regulates cardiomyocyte bioenergetics and survival

Aneta Kowalski

CREBRF regulates cardiomyocyte bioenergetics and survival

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Cardiovascular diseases are among the most common and devastating conditions of the modern era. A better understanding of how the heart adapts to cellular stress could improve prevention and treatment of these increasingly prevalent disorders. A GWAS in Samoans has recently identified a variant in a putative cellular stress/energy sensor ($CREBRF^{R457Q}$) linked to metabolic phenotypes in humans. Furthermore, the Drosophila homolog of CREBRF has recently been implicated in cellular bioenergetics and survival in response to nutritional stress downstream of TORC1, a cellular energy sensor known to play a critical role in cardiomyocyte metabolism and function. Despite the significance of these discoveries, CREBRF and its metabolic-risk variant remain poorly characterized. The overall objective of this project is to understand how CREBRF and its metabolic risk variant influence cardiac/cardiomyocyte metabolism/function and, ultimately, cardiovascular risk. The central hypothesis of this project is that CREBRF and its risk variant differentially regulate cardiomyocyte bioenergetics and survival via adaptive transcriptional responses to cellular stress downstream of the cellular energy sensor TORC1. To test this hypothesis, we 1) characterized the expression and regulation of CREBRF in cardiomyocytes and murine heart under conditions of low and high nutritional stress / TORC1 activity, and 2) determined if CREBRF is necessary and/or sufficient to mediate cardiomyocyte bioenergetics and survival under these conditions using knockdown/out/in and overexpression approaches. Our results demonstrate that CREBRF is expressed and regulated (induced by nutritional stress / TORC1 inhibition, suppressed by insulin / mTORC1 activation) in H9c2 immortalized embryonic rat ventricular myoblasts and differentiated cardiomyocytes, primary neonatal murine cardiomyocytes, and murine hearts. We further demonstrate that CREBRF is required for cardiomyocyte survival, mitochondrial function, and metabolic flexibility (ability to switch between carbohydrate and lipids as energy substrates) in the above cell models. Finally, we demonstrate that CREBRF is induced in murine hearts in response to cardiac ischemia (due to transverse aortic constriction, TAC) in a diet-dependent manner. Ongoing studies are focused on systematically dissecting the pathways surrounding CREBRF that mediate these effects and to clarify the specific pathways differentially affected by the CREBRF risk variant. Taken together, these data support the hypothesis that CREBRF regulates cardiomyocyte bioenergetics and survival in response to cellular stress downstream of TORC1. The impact of these studies, in combination with our ongoing work in human carriers of the CREBRF variant, is that understanding the contribution of CREBRF to cardiac/cardiomyocyte metabolism and function may improve diagnosis and therapy of cardiometabolic disease.

172 Patient Derived Colorectal Cancer Spheroids for Single Cell Characterization of Intratumor Heterogenity in Response to EGFR Inhibition

Jeremy D. Kratz

Patient Derived Colorectal Cancer Spheroids for Single Cell Characterization of Intratumor Heterogenity in Response to EGFR Inhibition

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Background: Colorectal cancer (CRC) remains the second leading cause of cancer-related mortality for which novel treatment strategies are needed to improve survival and understand mechanisms of therapeutic resistance. Current management includes chemotherapy and targeted agents such as epidermal growth factor receptor inhibitors (EGFRi). Targeting strategies specific to EGFRi have included molecular profiling and primary sidedness in predicting clinical outcomes. We recently reported disease bulk as an independent marker of clinical outcomes for EGFRi suggesting intra-tumor heterogeneity as a likely mechanism of resistance. Clinical tools are needed to track EGFR resistance to characterize the mechanisms of therapeutic resistance and further to tune novel therapeutic strategies. Our group has recently demonstrated that patient-derived organotypic cancer spheroids (PDOCS) and optical metabolic imaging (OMI) can predict *in vivo* chemotherapy response.

Methods: PDOCS were generated from patients with mCRC at time of molecular profiling. Following culture maturation, PDOCS were treated with physiologic doses of EGFRi panitumumab. Response was evaluated by change in sphere diameter and OMI to exploit intrinsic autofluorescence of NAD(P)H and FAD at spheroid and single-cell levels. Effect size was calculated using Glass's delta (GΔ) defined as differences in means between treatment groups normalized to control standard deviation with comparison to predetermined sensitivity thresholds.

Results: PDOCS from patients with mCRC were generated from tissue biopsies, surgical specimens, and malignant effusions (n=38). Mutational profiles were stratified by RAS status from next-generation sequencing. Eight PDOCS were evaluable for experimental and clinical response. KRAS mutation predicted primary resistance to EGFRi with no difference in diameter (GΔ=-0.01) or single cell response by OMI (GΔ=0.02). RAS wild-type PDOCS had significant response with decreased diameter with EGFRi (P

Conclusions: PDOCS predict response to EGFRi in these preliminary investigations. Diameter and OMI analyses provide complementary information for the characterization of line-specific sensitivity. Further studies are warranted to characterize the molecular profiles underlying early observations of intratumor heterogeneity. Prospective investigation is needed to understand the predictive role of this technique in targeted therapeutic response and mechanistic studies to understand both primary and secondary resistance.

174 Single-cell genomic analysis of pulmonary fibrosis phenotypes

Jonathan A. Kropski

Single-cell genomic analysis of pulmonary fibrosis phenotypes

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Despite years of research, an integrated understanding of the fundamental mechanisms driving the pathogenesis of pulmonary fibrosis has remained elusive. Numerous histopathologic patterns of pulmonary fibrosis have been identified in association with different patterns of risk factors, but to date it remains unclear as to what mechanisms are shared across different forms of pulmonary fibrosis and which drive distinct pathologies and outcomes. Our objective was to utilize single-cell genomic technologies to determine both the conserved and distinct mechanisms driving pulmonary fibrosis phenotypes. At the time of lung transplantation, single-cell suspensions were generated from the lung parenchyma of pulmonary fibrosis patients and from declined donor lungs (controls). Unsorted single-cell suspensions and CD45 depleted fractions were used for single-cell RNA-sequencing (scRNA-seq). scRNA-seq library preparation was performed using the 10X Genomics Chromium platform, and sequencing was performed on an Illumina hiSeq4000 or Novaseq. Following alignment, demultiplexing was performed using Cell Ranger. Graph-based clustering and scRNA-seq analysis was performed using the Seurat package in R. Developmental lineage reconstruction was performing using Monocle and p-Creode. Localization analyses were performed by multiplex fluorescence immunohistochemistry or RNA-scope and quantified by histocytometry. Diagnoses were assigned based on clinical interpretation of explant pathology according to consensus criteria. Joint graph-based clustering and canonical correlation analysis of scRNA-seq profiles from >40,000 cells from control (n=9), IPF (n=8), chronic hypersensitivity pneumonitis (cHP, n=4), and nonspecific interstitial pneumonia (NSIP, n=3) identified 21 distinct clusters, representing the major known subtypes in the lung, as well as numerous intermediate/transitional cell types and/or states. Within most cell clusters, hundreds of differentially expressed genes were identified. Strikingly, across pulmonary fibrosis phenotypes, collagen and ECM gene expression was highly enriched in *ACTA2*^{low}, *PDGFRα*$^{+}$ fibroblasts, while collagen and ECM gene expression were

lower in *ACTA2^{hi}* myofibroblasts; few collagen-expressing inflammatory or epithelial cells were identified. A progression of cell states expressing alveolar type 1 (AT1) and type 2 (AT2) markers were identified both fibrotic and control lungs characterized by a signature of interferon response in AT1 cells. Compared to NSIP and cHP, IPF epithelial cells demonstrated increased senescence markers. Unsupervised clustering analyses multiple distinct pulmonary fibrosis endotypes based on cell-type specific gene expression programs. These data together provide unprecedented insights into the shared and divergent pathologic gene expression programs across pulmonary fibrosis phenotypes, and represent the first attempt to classify pulmonary fibrosis phenotypes based on molecular profiles.

175 Developing zebrafish models to study the link between SoxC transcription factors and CHARGE syndrome
Laura A. Krueger

Developing zebrafish models to study the link between SoxC transcription factors and CHARGE syndrome

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CHARGE syndrome (coloboma, heart defects, choanal atresia, growth retardation, genital abnormalities, and ear abnormalities) is a complex congenital genetic disorder resulting in severe defects in multiple organ systems with an occurrence of 1:8,000-10,000 live births. Mutations in chromodomain helicase binding protein 7 (*CHD7*) and defects in neural crest cell development and migration have been implicated in the pathogenesis of CHARGE syndrome, however the mechanisms underlying the ocular birth defects observed in CHARGE patients have not been identified. Our laboratory studies the development of the vertebrate visual system using zebrafish (*Danio rerio*). Previous work from our lab has shown that knockdown of *Sox11*, a member of the SoxC family of transcription factors, in zebrafish results in microphthalmia, coloboma, brain, trunk, and heart defects, all phenotypes observed in CHARGE syndrome. Furthermore, a duplication of *Sox11* has been identified in a patient clinically diagnosed with CHARGE syndrome, and *CHD7* has been shown to directly interact with *Sox11* and *Sox4* in neural stem cells. Taken together, these data strongly suggest that loss of SoxC expression contributes to the ocular and other phenotypes observed in Chd7-associated CHARGE syndrome. In this study, we begin to further investigate the role that *Sox11* plays in the phenotypes seen in CHARGE syndrome by generating *Sox11*-mutant zebrafish using the CRISPR-Cas system. Zebrafish have two co–orthologs of *SOX11*, *sox11a* and *sox11b*. CRISPR target sites were chosen to disrupt the high mobility group (HMG) DNA-binding domain and the transactivation domain of *sox11a* and *sox11b*. Corresponding single strand guide RNAs (sgRNAs) were generated and microinjected with Cas9 protein into fertilized zebrafish embryos at the one-cell stage. Founder lines for *sox11a* and *sox11b* have been generated resulting in large deletions leading to frame shifts removing the HMG DNA-binding and transactivation domain. These founders are currently being bred to form a first generation. The resulting *Sox11* mutant lines will be characterized for phenotypes related to CHARGE syndrome and will be compared to an established *CHD7* mutant line. We will also characterize the role of *Sox11* in neural crest cell development and migration by crossing the *Sox10:RFP* transgenic line (which fluorescently labels neural crest cells) with the *Sox11* mutant lines and performing live imaging of neural crest cell dynamics. These experiments will provide a better understanding of the potential role of *Sox11* in the pathogenesis of CHARGE syndrome.

176 The mediating role of pain and function in the association between stiffness and quality of life
Yu Heng Kwan

The mediating role of pain and function in the association between stiffness and quality of life

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The pathways linking stiffness to quality of life (QoL) remains unclear. Therefore, we aimed to examine the role of pain and function in linking stiffness and QoL in patients with axSpA. We used cross-sectional data from a registry from a tertiary referral centre to assess patients on stiffness, pain and function on QoL. Path analysis was used to analyse the associations between these domains, pursuing four hypotheses: H_1 – More stiffness is associated with poor QoL; H_2 – More pain and decreased function are associated with poor QoL; H_3 – More stiffness is associated with more pain and decreased function; H_4 – The linkage between stiffness and QoL is mediated by function and pain. Data from 221 patients (Mean age 38.5, 79.0% males and 83.1% Chinese) were analyzed. Our mediation model achieved good fit. Results supported all 4 hypotheses (p

177 A novel role for a long noncoding RNA in airway differentiation during allergic asthma
Grace J. Kwon

A novel role for a long noncoding RNA in airway differentiation during allergic asthma

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Allergic asthma is characterized by airway hyperresponsiveness to a type 2 adaptive immune response. Immune cells release type 2 cytokines, such as IL-4 and IL-13, which drive airway inflammation. Airway epithelial cells are essential in orchestrating this immune response and undergo distinct morphological changes following type 2 cytokine exposure. However, the molecular pathways regulating these alterations

are not fully understood. Long noncoding RNAs (LncRNAs) are defined as non-protein-coding transcripts longer than 200 nucleotides with an increasingly diverse set of functions and are generally more cell-specific than protein-coding transcripts. Despite evidence of their function in regulating the immune response, few lncRNAs have been functionally identified in airway epithelial cells, leading us to speculate their potential role in airway immunity. We cultured primary human bronchial epithelial cells (HBECs) under air-liquid interface (ALI) conditions and performed RNA sequencing from eight individual donors following IL-13 stimulation. The most significantly induced lncRNA was *WFDC21P*, a lncRNA previously reported to modulate STAT3 dephosphorylation in dendritic cells, but whose function is unknown in airway epithelium. *WFDC21P* is cytoplasmic and highly expressed relative to previously identified lncRNAs in the airway. While IL-13 induced *WFDC21P* expression, IL-6 stimulation (which activates STAT3) dampened this induction in ALI-cultured primary HBECs chronically stimulated with IL-13 over 2 weeks. IL-13 is a critical mediator of differentiation in airway epithelial cells, and we hypothesized *WFDC21P* may mediate differentiation via regulating STAT phosphorylation, similar to dendritic cells. Knockdown via short-hairpin-mediated RNA of *WFDC21P* in an immortalized bronchial epithelial cell line resulted in increased STAT3 signaling, contrary to its effect in dendritic cells. Furthermore, knockdown of *WFDC21P* in primary HBECs under ALI conditions resulted in morphological disturbances and defective cilia development, as shown by an absence of beating cilia in culture via time lapse and lack of *FOXJ1* expression via quantitative PCR. Our studies reveal a novel role for *WFDC21P* in airway epithelium and type 2 immunity. Current and future studies will further characterize the regulation of *WFDC21P* and its effects on the airway epithelium, in addition to downstream pathways affected by loss of *WFDC21P* following IL-13 exposure via RNA-sequencing. The results of these studies will identify and establish a novel role for a lncRNA in airway differentiation and lead to a better understanding of the mechanisms that govern allergic asthma and airway homeostasis.

178 Identification of novel sarcomere interactions using proximity-labeling BioID technique
Feria A. Ladha

Identification of novel sarcomere interactions using proximity-labeling BioID technique

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Mutations in components of the sarcomere, the contractile unit of cardiomyocytes, are a leading cause of genetic cardiomyopathies, such as dilated cardiomyopathy (DCM), which is an important contributor to heart failure burden. Using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), our work has previously shown that DCM-causing mutations in titin, a major structural and functional component of the sarcomere, lead to diminished force production and impaired sarcomerogenesis. A classic model of sarcomerogenesis suggests that sarcomere assembly begins with premyofibrils containing beaded Z-disks composed of alpha-actinin, actin, and non-muscle myosin, with further assembly marked by addition of muscle myosin and titin. Once assembled, sarcomeres exhibit linear Z-disks and distinct protein markers. We are interested in understanding this stepwise process by probing sarcomere protein-protein interactions, with the objective of identifying novel developmental mediators and structural components of the sarcomere. More specifically, we would like to identify proteins that interact or localize near Titin at the M-line of the sarcomere. To do this, we have combined CRISPR/Cas9 genome-editing with BioID proximity-labeling to produce isogenic iPSC-CMs that express Titin fused with BirA, a promiscuous biotin ligase that biotinylates vicinal proteins. In addition to identifying novel interactions, we will also study changes in interactions in Titin truncated mutations. We have also generated a sarcomere-deficient iPSC-CM model that can readily reform sarcomeres on-demand, which we will use to further understand stage-specific interactions of sarcomere structure and development. Our results will not only provide novel insights into human sarcomere biology, but may also uncover novel targets for heart failure drug development.

179 Glioblastoma-derived interleukin-6 promotes immunosuppression and tumor progression through induction of programmed death-ligand 1 on circulating myeloid cells
Jonathan B. Lamano

Glioblastoma-derived interleukin-6 promotes immunosuppression and tumor progression through induction of programmed death-ligand 1 on circulating myeloid cells

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Glioblastoma (GBM) represents the most common central nervous system malignancy in adults and remains fatal with 5-year survival rates

Previously, we observed that elevated myeloid PD-L1 expression is not limited to the tumor microenvironment, but also extends to the systemic circulation of GBM patients. Moreover, we demonstrated that PD-L1 expression on circulating myeloid cells is associated with poor immunotherapeutic vaccine efficacy and worse survival. While GBM-derived

factors were found to induce PD-L1 expression on myeloid cells, the identity of the factors remained unknown. Thus, the goal of the current study was to identify GBM-derived factors driving myeloid PD-L1 expression as potential targets for reducing myeloid-associated immunosuppression in GBM.

To identify GBM-derived PD-L1 inducing factors, GBM conditioned media (GCM) collected from patient-derived GBM cell cultures was used to stimulate naïve myeloid cells. Myeloid PD-L1 induction was characterized via flow cytometry, resulting in the classification of high and low PD-L1 inducing GCM samples. Cytokine expression across GCM samples was assessed through multiplexed cytokine array, identifying interleukin-6 (IL-6) as a PD-L1 inducing factor. Treatment of myeloid cells with antibodies targeting the IL-6 receptor (tocilizumab) or IL-6 (siltuximab) blocked PD-L1 induction by GCM. Moreover, myeloid cell treatment with tocilizumab or siltuximab rescued T cells from undergoing apoptosis and anergy when exposed to GCM stimulated myeloid cells. Mechanistically, IL-6 promoted PD-L1 induction was dependent on STAT3 signaling. Clinically, the association between IL-6 and myeloid PD-L1 was investigated utilizing GBM patient samples, which demonstrated a correlation between IL-6 and increased myeloid PD-L1 expression in both the tumor microenvironment and peripheral circulation. Furthermore, increased IL-6 expression correlated with worse survival outcomes.

To determine the translational relevance of IL-6 targeted therapy, the murine GL261 glioma model was investigated in vivo. Utilizing both CRISPR/Cas9 mediated IL-6 knock-out and IL-6 targeted antibody treatment, we observed reduced myeloid cell PD-L1 expression, decreased tumor growth, and improved survival that was $CD8^+$ T cell dependent. IL-6 blockade was associated with increased T cell activation and synergized with PD-1 targeted immunotherapy to improve survival. Ultimately, these results suggest that GBM-derived IL-6 induces peripheral myeloid PD-L1 expression which contributes to systemic immunosuppression and that targeting IL-6 may improve immunotherapeutic approaches for GBM.

180 Engineering a staphylococcal biosensor via quorum-sensing system

Peter J. Larson

Engineering a staphylococcal biosensor via quorum-sensing system

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Staphylococcus aureus is a major cause of skin and soft tissue infections in both healthcare and community settings. Methicillin-Resistant *S. aureus* (MRSA) has been flagged a "serious threat" by the CDC. The effectiveness of current antibiotic treatment options against MRSA has been declining, and despite treatment, MRSA colonization can persist for years. Here, we pursue the development of an engineered *S. epidermidis* probiotic biosensor, that can colonize the skin and detect *S. aureus,* with the goal of eliminating the pathogen with bacteriocin production while minimizing damage to the surrounding microbiota.

We assembled a modified *S. aureus* quorum-sensing circuit in a shuttle vector by PCR amplifying the promoter and auto-inducer peptide sensor genes from MRSA USA300 and cloning in a GFP output gene. We transduced the vector into *S. epidermidis* and monitored fluorescence induction in the presence of supernatant from *S. aureus* strains. Additionally, to validate the ability of *S. epidermidis* as a probiotic to colonize human skin and compete against local microflora, we developed a novel assay using a living human skin equivalent. We assembled synthetic skin communities to simulate the microflora composition of common skin sites and colonized them onto human skin organoids. After two days, we challenged those communities with a probiotic dose of *S. epidermidis*. At four days, the microbial load and composition on the organoids were determined by CFU counts, QPCR, and 16S sequencing.

Our *S. epidermidis* biosensor exhibited a significant increase in GFP fluorescence per OD620 when incubated with supernatant from MRSA USA300 (Fold change 3.07 +/- 0.35, p=7.38e-7), MRSA Newman (Fold change 3.10 +/- 0.24, p=3.60 e-8) and S. aureus RN4220 (Fold change 2.94 +/- 0.21, p=1.97 e-8). Furthermore, *S. epidermidis* exhibited robust colonization of synthetic skin communities characterized by either high *Propionibacterium* (3.5e5 (1.8e5) CFU/cm^2) or high *Staphylococcus* (1.3e5 (1.2e5) CFU/cm^2) on human skin organoids.

We have prototyped a plasmid-based *S. epidermidis* probiotic biosensor with inducible GFP expression by *in vitro* exposure to clinically relevant MRSA strains. We have demonstrated ability of *S. epidermidis* to effectively compete with normal skin flora. We will next modify the biosensor to produce MRSAcidal bacteriocins, and test its ability to compete in a wide range of skin conditions and microbiota. This "detect and destroy" probiotic biosensor will provide new therapeutic approaches to both remediate and prevent MRSA colonization and infection.

181 Circumstantial evidence for Epstein-Barr virus in the pathogenesis of chronic lymphocytic leukemia

Viktoryia Laurynenka

Circumstantial evidence for Epstein-Barr virus in the pathogenesis of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) remains the most prevalent form of leukemia in western countries. The incidence of CLL increases with age. The early clinical course of CLL can be asymptomatic, but it is generally incurable and ~5,000 people die from CLL each year. Epstein-Barr virus (EBV) is thought to contribute to the highly malignant Richter transformation that occurs in many CLL cases.

We applied a recently developed strategy (Nat Genet 50:699, 2018) to determine whether or not the binding of EBV transcription factors (TFs)

was concentrated at the 84 risk loci for CLL in the germline DNA, all with p-8, as curated from published genome wide association studies (GWASs). We evaluated 52 virally encoded TFs by ChIP-seq (chromatin immunoprecipitation with DNA sequencing) datasets and complemented this analysis with the results from 1535 human TF ChIP-seq datasets.

We found that Epstein-Barr nuclear antigen leader protein (EBNALP), EBNA3C and EBNA2 and were concentrated in the CLL loci by a 3.71-fold, 3.66-fold and 3.49-fold enrichment with substantially more intersections than expected by chance, $p=4.89*10^{-19}$, $p=2.74*10^{-11}$ and $p=1.07*10^{-8}$, respectively. Interestingly, a set of human TFs (n=40) were also found to be concentrated in the CLL risk loci at p-6 for the 1535 ChIP-seq datasets tested, which included HMGN1, STAT5A, PAX5, SPI1, POLR2A, NFATC1, PML, NOTCH1, NFATC2, RUNX3, SP1 and others. The viral and human TFs cluster together in an optimal subset of approximately 15 of the 84 known loci in CLL. Eighty percent of the associated viral and human TF ChIP-seq datasets were collected from EBV transformed B cell lines in the Latency III program of viral expression, for which EBNALP, EBNA3C and EBNA2 are viral gene products. Therefore, these results nominate EBV for a role in the pathogenesis of CLL by a mechanism operating in transformed B cells through the EBV Latency III program of viral expression.

182 Reversing major histocompatibility complex class I downregulation in cancer
Patrick Lee

Reversing major histocompatibility complex class I downregulation in cancer

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Loss of major histocompatibility complex class I (MHC I) is an important mechanism by which cancer cells evade immune surveillance. Decreased MHC I expression has been reported in 16% to 80% of lesions across different cancers, and it often correlates with worse prognosis and decreased response to T-cell based immunotherapies. Loss of MHC I can occur when one or more components of the class I antigen presentation machinery (APM) are dysfunctional. These lesions can be either irreversible (somatic mutations, loss of heterozygosity) or reversible (transcriptional or posttranscriptional downregulation). While reversible MHC I loss has the potential to be pharmacologically restored, there are no available targeted drugs for clinical use, and effective in vitro drugs such as interferon-gamma (IFN-γ), histone deacetylase inhibitors, or demethylating agents have significant side effects or lack specificity. Moreover, the regulatory network controlling MHC I expression in non-immune cells remains poorly understood. We aim to identify specific, druggable targets that govern MHC I expression. Pharmacologic modulation of these targets to restore MHC I on cancer cells has the potential to synergize with existing T-cell based immunotherapies, such as checkpoint blockade or peptide vaccines. To study reversible MHC I loss, we have chosen Merkel cell carcinoma (MCC) as a model system. MCC is a rare but aggressive neuroendocrine skin cancer, 80% of which is caused by the Merkel cell polyoma virus and 20% by ultraviolet sun damage. Importantly, MHC I downregulation is prevalent and occurs in 84% of MCC tumors. We have generated and characterized a series of patient-derived MCC cell lines, which have absent MHC I expression that is reversible with IFN-γ stimulation. With these lines, we are conducting paired genome-wide CRISPR-knockout and open reading frame (ORF) gain-of-function screens to identify genes whose alteration can increase MHC I expression. Identification of such targets has the potential to uncover mechanisms of MHC I regulation in MCC and other cancers.

183 Novel chemotherapy stable subpopulations are conserved across multiple Small Cell Lung Carcinoma Patient Derived Xenograft Models
Jonathan M. Lehman

Novel chemotherapy stable subpopulations are conserved across multiple Small Cell Lung Carcinoma Patient Derived Xenograft Models

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Introduction: Small cell lung cancer (SCLC) is an aggressive neuroendocrine carcinoma of the lung responsible for up to 25% of lung cancer deaths and the 6th leading cause of cancer death. SCLC initially responds well to chemotherapy, but inevitably recurs even after initial complete responses. The etiology of this relapse is likely secondary to tumor heterogeneity and/or chemotherapy resistance subpopulations reconstituting tumor. Mass cytometry uses metal labeled antibodies to profile expression and phosphorylation of multiple proteins in a single cell and offers the opportunity to identify new subpopulations as targets for novel therapies in SCLC. Methods: Nude mice with SCLC patient derived xenografts (PDXs) were treated with a single cycle of carboplatin/etoposide or saline injection. PDX samples were stained with a 26-30 marker panel and an intercalator dye to identify nucleated cells. This panel measured phospho-signaling, neuroendocrine, immune, and mesenchymal cell markers, and functional markers including ki67 and cleaved caspase 3. Mouse cells, including leukocytes, were excluded using mouse MHC1 gating and Histone H3 was used to identify nucleated cells. Single cell protein expression and phosphorylation was analyzed using viSNE, manual gating, as well as unsupervised clustering approaches with SPADE to identify subpopulations with neuroendocrine and non-neuroendocrine features. Results: Patient derived Xenograft (PDX) tumors across 4 distinct models including models with and without a single cycle of chemotherapy treatment contained viable tumor and stromal cells suitable for cryopreservation and mass cytometry. Chemotherapy treated tumors had dramatic changes in subpopulation distribution compared to matched mock treated tumor. This included enrichment in EPCAM+, CD24+, CD44- progenitor like subpopulations.

Similar patterns of population shift were observed in multiple models. Of note, chemotherapy stable subpopulations were conserved across 5 different PDX models including SOX2+ and Oct ¾+ tumor populations. These subpopulations sorted similarly in multidimensional space in multiple PDX models suggesting conserved origins. Conclusions: Mass cytometry was able to identify multiple Nueroendocrine and non-neuroendocrine cell populations from SCLC PDXs and characterize their signaling include rare subpopulations with stem like signaling factors of interest. Chemotherapy treated PDX had differential subpopulation distribution with enrichment of progenitor like cells with chemotherapy treatment similar to previous work in mouse genetic SCLC. However, rare conserved chemotherapy stable subpopulations enriched in stem-like signaling factors were identified across 5 PDX models including those with and without chemotherapy treatment. This work raises the possibility that chemotherapy stable subpopulations may contribute to progenitor populations which lead to relapse.

184 The effect of energy deprivation on the responses of metabolic and stress hormones to meals
Helen F. Leka

The effect of energy deprivation on the responses of metabolic and stress hormones to meals

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Energy deprivation has been shown to reduce concentrations of leptin, insulin, and glucose, while increasing growth hormone (GH) levels; however, their responses to meals have not been investigated. Acute meal-related hormone changes are important in energy metabolism and are thus important in understanding the mechanisms whereby energy balance is maintained with reduced intake. To investigate the daytime changes in metabolic and stress hormones in response to acute energy deprivation, we measured serum concentrations of leptin, insulin, glucose, GH, and cortisol in the early follicular phase of the menstrual cycle. Subjects were regularly-cycling, sedentary, young women who completed two diet interventions based on habitual energy intake and lean body mass (LBM) in separate menstrual cycles: neutral energy availability (NEA, 45 kcal/kg LBM*d) and decreased energy availability (DEA, 20 kcal/kg LBM*d). Blood was sampled over eight hours starting at 0700 h on the fifth day of each intervention. Scheduled breakfast and lunch were administered according to the assigned caloric intake while a snack based on NEA was provided in the afternoon. Leptin, insulin, glucose, and GH were measured at 10-min intervals while cortisol was measured at 30-min intervals. Paired Student's t-tests and repeated measures analysis of variance were used to compare values between NEA and DEA. In eleven women (age 24.1 ± 2.1) with paired studies, caloric restriction did not result in changes in body mass index (BMI, NEA 22.5 ± 0.7 vs DEA 22.1 ± 0.6) or % fat (NEA 26.6 ± 1.5 vs DEA 27.5 ± 1.5). Despite the lack of change in glucose during energy deprivation, there was a reduction in the total concentrations of leptin and insulin, as well as the insulin-glucose ratio (all p

186 Examining the role of Dyrk1a in the development and function of inhibitory neurons
Rachel V. Levy

Examining the role of Dyrk1a in the development and function of inhibitory neurons

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Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1a) has a crucial role in brain development, and studies have revealed links to Down Syndrome (DS) and autism spectrum disorders (ASDs). The purpose of this study was to determine whether deletion of Dyrk1a alters the number and distribution of parvalbumin (PV) neurons in the cortex and innervation of neurons by PV neurons. The overall goal was to reveal how this genetic mutation plays a role in the development of ASD. Cre-lox technology was used to produce the genetically mutated mice carrying heterozygous deletion of Dyrk1a in inhibitory neurons. Perfusion was performed on both mutant and control mice at 8 weeks old. After perfusion, the mice were dissected, and their brains were sectioned and treated with immunofluorescent staining for PV and GAD67. They were then analyzed through fluorescent and confocal microscopy, with a focus on the cerebral cortex. Data analysis showed that Dyrk1a mutation disrupts the development of PV neurons. These trends were present in the density and size of PV neurons, as well as in the distribution of synaptic terminals. This study could serve as a base for future research into ASD in humans, including potential for treatment and preventative measures.

187 Selective attention deficits and HIV-associated neurocognitive disorder: roles of aging and cART
Brandon J. Lew

Selective attention deficits and HIV-associated neurocognitive disorder: roles of aging and cART

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Introduction: HIV-associated neurocognitive disorder (HAND) affects up to 60% of individuals living with HIV. Selective attention dysfunction has specifically been associated with neural aberrations related to HAND. NeuroHIV has also been tied to premature aging, however few

studies have examined the relationship between premature aging and selective attention dysfunction. Additionally, the impact of combined antiretroviral treatment (cART) on selective attention and HAND is also unclear. Updated US treatment guidelines recommend integrase strand transfer inhibitor (INSTI) based therapy as the first-line for HIV. However, recent studies have raised concerns about neuropsychiatric-related adverse effects of INSTIs. Therefore, the current study examines the effects of HAND, aging, and cART therapy on selective attention.

Methods: 77 participants with HIV were compared to 93 uninfected and cognitively unimpaired controls. Participants completed a battery of neuropsychological tests which were used to diagnose HAND. Participants then completed an arrow-based flanker task to examine selective attention function. Mixed-model ANOVA was used to examine reaction time on the task, using condition as a within-subjects factor, group (HIV, HAND, control) as a between-subjects factor, and age as a covariate of interest. Additionally, cART was examined in HIV-infected participants using a mixed-model ANOVA to examine reaction time, using condition as a within-subjects factor, current INSTI therapy as a between-subjects factor, and age as a covariate.

Results: Out of the 77 participants with HIV, 28 participants were found to have HAND. An ANOVA indicated a significant three-way interaction of condition by group by age on reaction time (p=.017). Probing this interaction showed a significant condition by group interaction such that participants with HAND had the largest flanker effect (selective attention deficit). Additionally, controls showed a significant condition by age interaction such that older age was associated with larger flanker effects. This association with age was not present in HIV-positive participants. Simple main effects of condition, group, and age were all significant such that the incongruent condition, HIV and HAND, and older age were associated with longer reaction times (all p Discussion: Our results indicate that selective attention deficits related to HAND also change as a function of aging and cART. The association between selective attention performance and aging seen in control participants was not seen in participants with HIV, which may be a sign of premature aging. Additionally, current therapy with an INSTI based regimen may be associated with selective attention deficits above and beyond age. However, it is important to note that INSTI based treatment was not associated with HAND status in our sample. Further study is therefore needed to investigate this relationship.

188 Benefits of Antifungal Therapy in Asthma Patients with Airway Mycosis: A Retrospective Cohort Analysis
Evan Li

Benefits of Antifungal Therapy in Asthma Patients with Airway Mycosis: A Retrospective Cohort Analysis

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Introduction: Fungal airway infection (airway mycosis) is increasingly recognized as a cause of asthma and related disorders. However, prior controlled studies of patients treated with antifungal antibiotics have produced conflicting results. Our objective is to measure the effect of antifungal therapy in moderate to severe adult asthmatics with positive fungal sputum cultures in a single center referral-based academic practice.

Methods: We retrospectively evaluated 41 patients with asthma and culture-proven airway mycosis treated with either terbinafine, fluconazole, itraconazole, voriconazole, or posaconazole for 4 to >12 weeks together with standard bronchodilator and anti-inflammatory agents. Asthma control (1 = very poorly controlled; 2 = not well controlled; and 3 = well controlled), peak expiratory flow rates (PEFR), serum total IgE, and absolute blood eosinophil counts before and after antifungal therapy were assessed. In comparison, we also studied nine patients with airway mycosis and moderate to severe asthma who received standard therapy but no antifungals.

Results: Treatment with azole-based and allylamine antifungals was associated with improved asthma control (mean change in asthma control 1.72-2.25; p = 0.004), increased PEFR (69.4% predicted to 79.3% predicted, p = 0.0011) and markedly reduced serum IgE levels (1,075 kU/L to 463 kU/L, p = 0.0005) and blood eosinophil counts (Mean absolute count 530-275, p = 0.0095). Reduction in symptoms, medication use, and relapse rates decreased as duration of therapy increased. Asthmatics on standard therapy who did not receive antifungals showed no improvement in asthma symptoms or PEFR. Antifungals were usually well tolerated, but discontinuation (12.2%) and relapse (50%) rates were relatively high.

Conclusion: Antifungals help control symptoms in a subset of asthmatics with culture-proven airway mycosis. Additional randomized clinical trials are warranted to extend and validate these findings.

189 TIP60-dependent histone acetylation promotes DNA repair by homologous recombination
Mischa Li

TIP60-dependent histone acetylation promotes DNA repair by homologous recombination

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Proper and timely repair of DNA double-strand breaks (DSBs) is critical for preservation of genome integrity. While DSBs are repaired through a balance of canonical nonhomologous end-joining (NHEJ) and homolo-

gous recombination (HR) pathways, this balance is frequently disrupted in HR-deficient cancer cells, causing misuse of NHEJ that destabilizes the genome. Understanding the balance between HR and NHEJ, and the factors that could modulate this balance, informs our understanding of genome integrity, tumorigenesis, and response to clinical therapy. The HR-NHEJ balance is regulated by several factors, including cell cycle-dependent phosphorylation events and chromatin remodeling. Our group and others previously published that the histone acetyltransferase Tat-Interacting Protein 60kDA (TIP60) limits the accumulation of p53-Binding Protein 1 (53BP1) at DSBs to promote the loading of Breast Cancer Type 1 Susceptibility Protein (BRCA1) and repair by HR, but the mechanism was unclear. Here we report that *Tip60* conditional knockout causes meiotic HR defects in a BRCA1-independent manner, that TIP60 pro-HR activity is regulated by cell cycle-dependent phosphorylation, and that its acetylation of histones H4 and H2A.Z suppresses 53BP1 occupancy at DSBs. This body of work sheds light on the specific mechanisms by which this multi-faceted acetyltransferase directs DSB repair pathway choice.

190 Neuronal substrates of group competitive foraging in male mice

Songjun William Li

Neuronal substrates of group competitive foraging in male mice

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Group social interactions play a prominent role in both human and animal behavior, and competitive foraging among conspecifics is an especially significant form of social interaction due to its prevalence in nature and its importance in determining survival and reproductive outcomes. Previous studies have revealed features of competitive foraging behavior that are common to most species, as motivated individuals pursue limited resources from the same food source area with simultaneous access. However, despite the importance of interactive social behavior and its dysfunction, its neuronal underpinnings are poorly understood. In this study, we developed a novel behavioral assay to observe the influences of social dominance hierarchies on the competitive foraging behavior, which offers a versatile method to ordinally quantify competitive success among larger groups of animals. We also recorded single-unit neuronal activity within the dorsal medial prefrontal cortex (dmPFC) in male wild-type mice while they performed the task. Consistent with prior studies that characterized the tendency of dominant animals to tend to monopolize food more effectively than submissive counterparts, our behavioral data revealed that greater social dominance directly correlated with greater competitive success. Thus, these results demonstrated a relationship between dominance and competitive success that extends across a social group of familiar mice in a higher-order group setting. Neuronally, we found a subset of neurons in the dmPFC that selectively encoded the animals' hierarchical rank, the order in which they accessed the reward zone, and the reward amount. It is notable that individual dmPFC neurons differed in activity based on the subject's relative rank regardless of others' identity, while other neurons responded selectively to competitive success only before the recorded animal entered the reward zone - suggesting that dmPFC neurons may predict competitive outcomes based on information about competitors. This research provides insight into the social and neurobiological mechanics of dominance, competition, and success, allowing us to better understand group competitive behavior.

191 A three-pronged mechanism of hydroxychloroquine against Zika virus vertical transmission

Brooke Liang

A three-pronged mechanism of hydroxychloroquine against Zika virus vertical transmission

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Zika virus (ZIKV) is a mosquito-transmitted flavivirus that became a major global health threat when infections during pregnancy were linked to microcephaly, intrauterine growth restriction, and fetal demise. Mouse models developed by our group demonstrated that the route of maternal-fetal transmission of ZIKV is trans-placental. We further demonstrated that ZIKV co-opts placental autophagy for its own replicative advantage, and that inhibition of autophagy with hydroxychloroquine (HCQ) attenuates ZIKV placental infection and ameliorates adverse fetal outcomes. HCQ is a promising candidate for prevention of congenital Zika syndrome as it is already given to pregnant women for suppression of rheumatic diseases such as systemic lupus erythematosus.

To develop the use of HCQ as a therapeutic intervention, we sought to determine the molecular mechanism of action of HCQ against ZIKV and to noninvasively determine dosage and timing of HCQ administration during pregnancy.

The NS2B-NS3 protease encoded by ZIKV plays an essential role in ZIKV pathogenesis as it cleaves the ZIKV polypeptide into functional proteins. We performed molecular docking and molecular dynamics simulations that predicted considerable affinity between HCQ and the active site of the NS2B-NS3 protease, with a docking score of –10.725 kcal/mol. In vitro enzymatic assays further demonstrated that HCQ competitively inhibits proteolytic activity of the NS2B-NS3 protease with an inhibition constant of 92.34 ± 11.91 μM. Therefore, HCQ may inhibit ZIKV pathogenesis by competitively binding the active site of the NS2B-NS3 protease and blocking its normal function.

In vivo magnetic resonance imaging (MRI) is an established technique for the study of placental function in real time. In our study, pregnant wild-type mice were infected with ZIKV and then treated with HCQ daily

for 5 or 9 days post-infection. Mice were imaged at multiple timepoints and sacrificed at 9 days post-infection for tissue collection and viral titering. T1 and T2* maps were acquired and R_1 values (which are proportional to tissue oxygenation) and R_{2^*} values (which are proportional to deoxyhemoglobin concentration) were determined for all placentas. Our data showed that (1) HCQ treatment alone did not adversely affect the fetus, (2) HCQ treatment improved placental oxygenation, and (3) HCQ treatment administered throughout pregnancy showed maximal benefit to the fetus.

Altogether, we demonstrated that HCQ safely and effectively mitigates vertical transmission and ZIKV-associated adverse fetal effects through (1) inhibition of placental autophagy, (2) competitive inhibition of the ZIKV NS2B-NS3 protease, and (3) ameliorating fetal growth restriction through enhancement of placental oxygenation.

192 Identification of age-dependent IgA production by bladder tertiary lymphoid follicles in mice and women
Marianne M. Ligon

Identification of age-dependent IgA production by bladder tertiary lymphoid follicles in mice and women

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Immunosenescence encompasses changes to the immune system with advanced age that lead to defects in immunity to infection and a predisposition for chronic inflammatory disease. Urinary tract infections (UTIs) are the second most common infections among the elderly, and women over age 55 have the highest rates of recurrent UTIs (rUTIs). The bladder has specialized microbial defense mechanisms that include the water-impermeable urothelium and resident immune cells, predominantly macrophages. However, little is known about how immune responses in the bladder change with age and if immunosenescence contributes to the increased risk of UTIs and rUTIs. We hypothesized that aging would alter bladder immune responses to promote chronic inflammation and recurrence of infection.

To test this hypothesis, we compared the immune cell compartment in the bladders of young adult mice (3 month old) and aged mice (18 month old, approximately 60 year old human equivalent). We found that, independent of infection, aged bladders contained significantly more CD45+ immune cells with higher frequencies of CD4+ T cells, CD8+ T cells, and B cells than young bladders. Histologically, we localized these cells to dense lymphoid aggregates with distinct, segregated T and B cell zones, a follicular dendritic cell network, and high endothelial venules. This organization is characteristic of tertiary lymphoid follicles, which resemble secondary lymphoid tissues (e.g. lymph nodes) but form ectopically at sites of chronic inflammation. Thus, we named the structures we found bladder tertiary lymphoid follicles (BLTFs). Using RNA-seq, we found that aged bladders had high upregulation of TNF, lymphotoxin, homeostatic lymphoid chemokines, and immunoglobulins. We next found that urinary IgA concentrations increased throughout the lifespan concurrent with BTLF development and that aged bladders produced high levels of IgA when cultured *ex vivo*. Aged bladders also had increased permeability to FITC-dextran, indicating that urothelial integrity was compromised with age. Finally, we identified older adult rUTIs patients with cystoscopic findings of a nodular, cobblestone appearance termed *cystitis cystica*. Biopsies of these nodules showed striking similarity to BTLFs observed in aging mice.

Together, our findings suggest that (1) mouse bladders develop BTLFs as a function of age; (2) BTLFs promote the production of IgA from locally-generated plasma cells; (3) increased urothelial permeability with age may stimulate chronic inflammation; and (4) similar BTLFs are found in rUTI patients with *cystitis cystica*. This work demonstrates that there are significant changes to the immune environment in the bladder during aging that may play a role in responses and susceptibility to UTIs in the elderly.

193 Renal Cell Carcinoma Exosomes Regulate Tumor Immunity
Aaron R. Lim

Renal Cell Carcinoma Exosomes Regulate Tumor Immunity

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Renal cell carcinoma (RCC) accounts for 4% of new cancer diagnoses in the United States every year. Although anti-programmed death-1 (PD-1) immunotherapy was recently approved to treat metastatic RCC, only 20% of patients respond to this potentially life-saving treatment. We previously demonstrated that RCC has the highest infiltration of cytotoxic CD8+ T lymphocytes of all solid tumors in The Cancer Genome Atlas. However, CD8+ tumor-infiltrating lymphocytes (TILs) from freshly resected RCC patient tumors have elevated expression of PD-1 and are functionally exhausted. Thus, understanding how RCC evades our immune system is crucial to improve immunotherapy efficacy. One potential mechanism by which tumors can evade the immune system is the release of exosomes. These nanosized extracellular vesicles secreted by most tissues are gaining considerable attention in cancer biology for their roles as intercellular communicators and biomarkers. Studies of plasma exosomes from cancer patients revealed protein cargo that are known to suppress the immune system, such as programmed death-ligand 1 (PD-L1) and TNF-related apoptosis-inducing ligand (TRAIL). In addition, exosomes containing PD-L1 have been found to suppress T cells in melanoma and glioblastoma. However, the role of exosomes in RCC has been understudied. We hypothesized that RCC secretes exo-

somes containing PD-L1 and TRAIL to suppress the function of TILs and thus create an immunosuppressive environment. To test this hypothesis, we used differential ultracentrifugation and density gradient separation to isolate and purify exosomes from the culture media of human RCC cell lines. Using nanoparticle tracking analysis and transmission electron microscopy, we confirmed that RCC secretes vesicles consistent with exosome size (~100nm) and morphology. In addition, RCC secretes significantly more exosomes compared to normal kidney cells. Furthermore, with western blot, we found that RCC exosomes contain both immunosuppressive proteins PD-L1 and TRAIL. Finally, treating human CD8+ T cells with RCC-derived exosomes decreases T cell activation as measured by flow cytometry. Taken together, our results indicate that RCC releases exosomes with immunosuppressive cargo, such as PD-L1 and TRAIL, that can suppress tumor immunity.

194 Management of analgesia and anesthesia for patients undergoing Total Hip Arthroplasty in a University Hospital System

Amy Liu

Management of analgesia and anesthesia for patients undergoing Total Hip Arthroplasty in a University Hospital System

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Background: Enhanced Recovery after Surgery (ERAS) protocols are commonly implemented to improve patient outcomes after surgery, among other goals. While most ERAS protocols have been applied and studied mainly in major abdominal surgeries, ERAS protocols in recent years have also been implemented in orthopedic surgeries, including total knee and total hip arthroplasties. In general, ERAS protocols emphasize enhanced pain management with minimal use of opioids, early mobility, and rapid recovery, with the application of peripheral nerve blocks for improved pain control. However, the clinical impact of peripheral nerve blocks and optimal peripheral nerve block techniques has not been well-studied in the setting of orthopedic surgeries.

We investigated the relationship between different ERAS protocols throughout different University of Pittsburgh Medical Center (UPMC) hospitals on opioid usage after total hip arthroplasty. Namely, we compared different anesthesia types (general, spinal, with and without the addition of nerve block) and subsequent levels of post-operative opioid consumption (oral morphine equivalents, or OMEs) during hospitalization. We hypothesized that the addition of peripheral nerve blocks into ERAS protocols will lead to improved pain control as measured by decreased levels OMEs postoperatively.

Methods: Data was collected as part of a retrospective study of 848 patients who had undergone total hip arthroplasty at UPMC between August 2016 and December 2017. Data include demographic information (e.g. gender, age) and measures of clinical outcome (including opioid usage, pain scores, and length of stay). Analyses were performed using R. Multiple linear regression models were developed for PODs (post-operative days) 0-5 studying the effect of different anesthesia types on the outcome, OME level. Statistical significance was achieved when p

Results and Conclusions: A total of 848 total patients were studied, after 48 cases were excluded due to being repeated surgeries on the same patients. The mean patient age was 64.9 years, divided nearly equally between males and females (49.8%), who were of predominantly Caucasian race (92.6%). Regression models showed type of anesthesia to be a significant factor in opioid requirement, showing spinal anesthesia with or without peripheral nerve block to be superior to general anesthesia in decreasing opioid requirement. Additionally, younger age was consistently shown to be significantly related to more opioid usage postoperatively. Other significant factors in certain models included race, consumption of morphine prior to surgery, sex, and duration of surgery.

Limitations include the retrospective nature of this study and unknown pain status after discharge. Further steps, such as studying pain scores directly rather than OMEs, are needed to corroborate our findings.

195 An Immunogenomics Approach to Neoantigen Identification in Preclinical Models of Glioblastoma

Connor J. Liu

An Immunogenomics Approach to Neoantigen Identification in Preclinical Models of Glioblastoma

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Despite the recent clinical success using checkpoint-blockade inhibitors to treat various solid tumors, efficacy against malignancies of the central nervous system (CNS) has not yet been demonstrated. Glioblastoma (GBM), which is the most common and lethal malignancy of the CNS, remains a terminal diagnosis, with current standard-of-care surgical resection plus radiation and chemotherapy extending median survival to just 15 months. However, the effectiveness of checkpoint blockade therapy against secondary brain metastases provides strong evidence of these drugs' therapeutic activity within the CNS. Successful treatment with checkpoint blockade depends on robust immune recognition and response to tumor-specific protein coding mutations, termed *neoantigens*. Thus, the identification and validation of GBM neoantigens as the immunodominant targets mediating therapeutic responses to checkpoint-blockade remains a critical step towards their clinical utility.

To address this, we utilized a preclinical framework enabling high dimensional tumor profiling and characterization of neoantigen specific immune responses within the murine CNS. We observe that the CT2A glioma model exhibits a highly aggressive and checkpoint blockade resistant phenotype *in vivo*, while GL261 remains sensitive to anti-PD-L1 therapy. Given these distinct phenotypic differences, we profiled the tumor infiltrating lymphocyte (TIL) populations using flow cytometry anal-

ysis and demonstrate the presence of functionally suppressed effector T-cell populations in CT2A. To identify endogenous H2-Kb and H2-Db restricted neoantigens, we applied DNA whole exome sequencing, RNA sequencing, and neoantigen prediction analysis, revealing 512 and 434 predicted neoantigens in GL261 and CT2A respectively. Screening of top ranking neoantigen candidates by IFNɣ ELISPOT demonstrated the presence of $CD8^+$ TIL reactive against three predicted CT2A neoantigens. Using the previously identified mutant Imp3 neoantigen, synthetic long peptide vaccination increased the abundance of Imp3 specific CD8+ TIL in tumor bearing mice and conferred protection against intracranial GL261. Neoantigen vaccination combined with anti-PD-L1 therapy conferred survival advantage in mice bearing intracranial CT2A, whereas neither monotherapy was effective alone. By determining the immunogenicity of endogenous murine GBM neoantigens, these studies provide the necessary immunological tools to further study anti-GBM immunity both systemically as well as within the CNS. Furthermore, our results demonstrating the therapeutic synergy between checkpoint blockade therapy and neoantigen vaccination in treatment-resistant murine glioma, provide preclinical evidence to guide future GBM immunotherapy clinical trials.

196 Wnt-dependent lncRNAs in RNF43-mutant pancreatic cancer

Shiyang Liu

Wnt-dependent lncRNAs in *RNF43*-mutant pancreatic cancer

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Long non-coding RNAs (lncRNAs) have been recognized to play key roles in the pathogenesis of various types of cancers. They are essential regulators of important signaling pathways, such as p53 and Notch. However, less is known about lncRNAs in the Wnt signaling pathway, which has crucial functions for embryonic development and tissue regeneration. Dysregulation of Wnt signaling can lead to different types of cancers. One example is *RNF43*-mutant pancreatic ductal adenocarcinoma (PDAC), which is dependent on Wnt signaling for proliferation.

To discover Wnt-dependent lncRNAs in *RNF43*-mutant pancreatic cancer, we developed a computational pipeline to *de novo* reconstruct the transcriptome of an *RNF43*-mutant PDAC tumor from a mouse xenograft model treated with a Wnt inhibitor. In total, we identified 3633 lncRNAs, of which 1503 annotated and novel lncRNAs were significantly differentially expressed upon Wnt inhibition *in vivo*. Compared to the same cancer cells treated in tissue culture, the *in vivo* model identified 2.5-fold more Wnt-regulated lncRNAs, and majority of them were upregulated in the orthotopic tumor than *in vitro* tissue culture. A subset of Wnt-dependent lncRNAs and their nearest protein coding genes had a significant positive correlation, indicating they may be co-regulated or they might regulate each other *in-cis*. The Wnt-dependent lncRNAs were clustered with protein coding genes into 63 different clusters based on their time-based expression profiles. The clusters are enriched for functionally relevant biological processes, such as negative regulation of developmental process and epithelial to mesenchymal transition. To further understand the role of Wnt-dependent lncRNAs in pancreatic cancer, we performed a CRISPRi screening that targets 1503 Wnt-dependent lncRNAs. We found 19 lncRNA loci that affect cancer cell growth, and 15 loci that could modulate cancer cell sensitivity to the Wnt-inhibitor.

Overall, our study provides an annotated catalogue of Wnt-dependent lncRNAs in *RNF43*-mutant pancreatic cancer, which will be used to understand the function and mechanism of important Wnt-dependent lncRNAs involved in the pathogenesis of pancreatic cancer.

198 Surgically-based treatments and associated outcomes of acral lentiginous melanoma: A systematic review

Marissa B. Lobl

Surgically-based treatments and associated outcomes of acral lentiginous melanoma: A systematic review

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Background: Acral lentiginous melanoma (ALM) is a variant of cutaneous malignant melanoma that develops on the acral surfaces of the body such as the palms and soles. It is more common in patients with darker skin and is associated with disproportionally high morbidity and mortality. The reason for these poor outcomes is multifactorial and may be due in part to the anatomic location, delays in diagnosis, inherently aggressive nature of the tumor, disparities in access to treatment, or the lack of standardized treatment regimens. Surgery is the mainstay treatment for ALM, although new adjuvant and immunotherapies are becoming part of a comprehensive treatment plan for patients with melanoma.

Objectives: To perform a systematic review evaluating surgically-based treatments and corresponding survival outcomes in ALM in order aid physicians in effective management of this aggressive skin cancer.

Methods: A literature search was conducted in accordance with PRISMA guidelines using MeSH terms in MEDLINE via PubMed and key terms in EMBASE. The search yielded 209 articles. Duplicates, case-reports, and manuscripts unavailable in English were excluded from full-text review. After evaluating full-text articles for strength of evidence and relevance to our study, 22 manuscripts remained for inclusion in the qualitative review. Strength of evidence was evaluated by the number of patients included (10 or more required). Exclusion criteria included: (1) descriptive studies without any novel patient data (i.e. review articles) (2) the melanoma type was not ALM, and (3) the topic was not surgical management. All articles included had level IV evidence, as assessed using guidelines by Ackley et al (2008).

Results: Wide local excision (WLE) was associated with the best outcomes when combined with isolated limb perfusion and lymph node

treatment, resulting in an approximate survival of 70-75% at five years. Mohs Micrographic Surgery (MMS) is a newer technique showing great promise for treating ALM, already demonstrating survival rates above 80% after 5 years. Newer adjuvant therapies such as interferon therapy may be useful in cases where ALM does not respond to traditional chemo- or immunotherapies, and when surgery alone is not successful in excising all of the tumor.

Discussion/Conclusion: The surgical management of ALM has been evolving with the advent of new techniques and adjuvant therapies. This review aims to provide an overview of the past, current, and future surgical management techniques utilized in patients with ALM in order to help standardize protocols and improve outcomes.

199 Bilevel spectral analysis reveals narrowband macroperiodic oscillations in the EEG of young children following acquired brain injury

Maren E. Loe

Bilevel spectral analysis reveals narrowband macroperiodic oscillations in the EEG of young children following acquired brain injury

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Introduction: Two time-scale activation patterns commonly observed in clinical EEG include burst suppression and *tracé alternant*, in which the EEG alternates between bursts of fast, high-voltage activity, interspersed with periods of relative quiescence. Recently, we observed a two-time scale pattern, termed macroperiodic oscillations (MOs), in young children following acquired brain injury that differs from these canonical patterns. When observed, MOs often preceded the appearance of recurrent seizures and status epilepticus (SE). Here, we introduce an analysis approach involving two levels of time-frequency decomposition in order to systematically characterize MOs in terms of their spectral and spatial distribution.

Methods: From October 2015 to February 2018 we identified n=16 subjects in either the neonatal, cardiac or pediatric intensive care units (ICUs) whose EEGs exhibited slow cycling. We performed a bilevel spectral analysis on these recordings. In the first level of analysis, a time-series of 2-15Hz band-limited power is extracted for each channel using sliding window, multi-taper spectral estimation. The second level of analysis involves performing time-frequency analysis and dimensionality reduction on these power envelope signals, thus revealing slow, harmonic modulatory processes that gate underlying high-frequency EEG activity.

Results: Our bilevel spectral analysis reveals slow modulation at 0.005 - 0.009Hz, a much slower frequency than is typically associated with burst suppression or *tracé alternant*. In contrast to these classical patterns, these MOs are narrowband (highly regular periodicity) and well-defined on the second-level spectrograms. Bouts of MOs last between 10 and 30 minutes and manifest heterogeneously across the scalp. Nested within the 'up' phase of each MO is high-frequency spectral content consistent with continuous background EEG.

Conclusion: MOs are a two-time scale EEG pattern with spatiotemporal characteristics that deviate from other similar patterns observed in critically ill patients. Bilevel spectral analysis can quantify MOs, revealing their spectral and spatial profiles and their unusually slow, narrowband modulatory dynamics. This pattern may thus represent a novel EEG biomarker for impending SE and recalcitrant seizures.

200 Examining the Intramolecular Interactions of MARCKS (Myristoylated Alanine-Rich C Kinase Substrate), an Actin-filament Crosslinking Protein, and the Effect of its Inhibition on Cell Morphology and Pro-inflammatory Cytokine Secretion

Brian C. Longbottom

Examining the Intramolecular Interactions of MARCKS (Myristoylated Alanine-Rich C Kinase Substrate), an Actin-filament Crosslinking Protein, and the Effect of its Inhibition on Cell Morphology and Pro-inflammatory Cytokine Secretion

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One of the goals of treating chronic inflammatory disorders such as rheumatoid arthritis (RA) is to reduce inflammation, and this may be accomplished via anti-cytokine therapies, or "biologicals," such as blocking TNF or IL-1. With this project, instead of targeting pro-inflammatory cytokines already released by macrophages, I seek to target and inhibit a protein implicated upstream in the cytokine secretion pathway, such as MARCKS, which should hypothetically eliminate the need to target the massive efflux of pro-inflammatory cytokines (i.e. TNFα, IL-6, IL-1 and IL-12), as biologicals do.

The literature has shown MARCKS to be a natively unfolded protein, but I aim to demonstrate that the phosphorylation site domain (PSD) peptide (amino acids 166-190 of MARCKS) directly binds to full length MARCKS through the MARCKS' autoinhibitory domain (AID) corresponding to residues 36-146. Furthermore, I aim to express the AID peptide in a murine macrophage cell model and examine the effect on the secretory pathway of pro-inflammatory cytokines.

To do this, a BL21 *E. coli* cell line, bacterial expression vector pET12a (4674bp), mammalian expression vector pCMV6-AC-mGFP (6631bp) and a standard cloning vector pMA containing the sequence of interest corresponding to the hypothesized AID of MARCKS are being used to express the recombinant MARCKS AID sequence *in vitro*. Direct binding assays with rhodamine labeled PSD peptide and AID peptide will be conducted to test that the hypothesized association and loss of functionality in PSD occurs from AID binding. It is expected that the MARCKS AID peptide binds with greater affinity to PSD than a random equivalent peptide.

Mouse monocyte macrophage RAW264.7 cell line is being used for transient transfection and expression in a eukaryotic cell model. Enzyme-linked immunosorbent assay (ELISA) will detect the amount of pro-inflammatory cytokines secreted by the AID-transfected macrophages compared to mock-transfected macrophages. Fluorescent microscopy will elucidate the effect of GFP-tagged AID peptide on the regulation of the actin cytoskeleton. The murine macrophages are expected to reveal an abnormal morphology of the actin cytoskeleton and a reduction in secretion of pro-inflammatory cytokines such as TNFα, IL-6 and IL-12.

201 Folded retina observed in adult mice lacking Maturin
Christine Ly

Folded retina observed in adult mice lacking *Maturin*

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During retinal development, a pool of progenitor cells divides to generate daughter cells that eventually differentiate into the seven retinal cell types, including horizontal cells (HCs) and retinal ganglion cells (RGCs). Mechanisms preventing these newly born cells from reentering the cell cycle remain unknown. Our previous work in *Xenopus laevis* identified *Maturin* (*Mturn*) in a screen for genes required for normal eye formation. *Mturn* knockdown in the neural plate increases cellular proliferation, while overexpression drives neural differentiation. During central nervous system development, Maturin is expressed most strongly in differentiating neurons. This expression pattern, as well as the Maturin sequence, is highly conserved in vertebrates. To determine if Maturin is required for normal mammalian retinogenesis, we collected, sectioned, and stained wild-type (WT) and Maturin null (*Mturn*$^{-/-}$) mouse retinas with cell-type specific markers to detect differentiated retinal cell types. The location and average number of each cell type were determined. *Mturn*$^{-/-}$ mice were also intraperitoneally injected with 5-ethynyl-2′-deoxyuridine (EdU) from P11 to P17 to detect S-phase cells in the postmitotic retina.

We detected MTURN in differentiated HCs and RGCs. At embryonic age 14.5, Maturin transcript, but not protein, is detected. In adult mice, we found *Mturn*$^{-/-}$retinas to be 25% longer than WT retinas. In mild cases, *Mturn*$^{-/-}$ retinas had localized thickening of the retinal layers. In severe cases, we observed buckling of the retina to form multiple folds that resulted in detachment from the retinal pigment epithelium. The folded retina contains all the differentiated retinal cell types positioned in the expected layers. While we did not observe a significant difference in the individual number of most retinal cell types, we did detect a significant reduction in the number of Lim1^{+} HCs in *Mturn*$^{-/-}$ retinas relative to controls. Furthermore, despite all retinal cells being postmitotic by P10, we identified EdU^{+}, Lim1^{+} cells in *Mturn*$^{-/-}$ retinas.

Although multiple possible cellular mechanisms could explain the folded retinal phenotype observed in *Mturn*$^{-/-}$ mice, our results suggest that hyperplasia is the most likely mechanism. If we observe a significant increase in cell number, then Maturin loss could result in: 1) a change in cell fate (non-retinal cells are converted to retinal cells), 2) a reduction in retinal cell death, and/or 3) the generation of additional retinal cells, either during development or in the adult retina. If cell number is unaltered in *Mturn*$^{-/-}$ retinas, then Maturin is required for retinal development independent of proliferation. Future experiments will distinguish between these possibilities.

202 Etv2-miR-130a-Mier1 cascade regulates the hemato-endothelial lineages and the epigenetic landscape during embryogenesis
Daniel V. Ly

Etv2-miR-130a-Mier1 cascade regulates the hematoendothelial lineages and the epigenetic landscape during embryogenesis

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Etv2 (Ets-family transcription factor) marks the earliest endothelial progenitors during development. Previously, we identified miR-130a as a direct downstream target of Etv2 and demonstrated its role in the segregation of bipotent hemato- endothelial progenitors toward the endothelial lineage. Here, we propose that miR-130a controls epigenetic status by regulating the expression of several histone modifying genes. Gene ontology enrichment analysis within the protein class category demonstrated that miR-130a has a functional role in the regulation of DNA-binding as well as histone-binding factors. Further, our bioinformatics analysis demonstrated that miR-130a has conserved binding sites in the 3′UTR of several genes including Mesoderm inducing early response 1 (*Mier1*). Loss-of- function experiments using the *miR-130a-/-* ES cells showed that the levels of *Mier1* were upregulated in differentiating embryoid bodies (EBs), whereas, doxycycline mediated over-expression of miR-130a resulted in the reduction of its levels. HAT assay using a *miR-130a-/-* ES/EB system revealed increased histone acetylation as compared to the control EBs. These findings suggest a critical role of miR-130a in the regulation of chromatin modifications during ES/EB differentiation.

203 Understanding Patient-derived resistance mutations and how they affect the process of imatinib binding to Abl kinase
Agatha Lyczek

Understanding Patient-derived resistance mutations and how they affect the process of imatinib binding to Abl kinase

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Protein kinase inhibitors are potent anti-cancer therapeutics. However, many patients develop resistance against these inhibitors. For example, the Bcr-Abl kinase inhibitor imatinib decreases mortality for Chronic Myeloid Leukemia (CML) by 80%, but 22-41% of patients acquire resistance to imatinib during treatment. The majority of relapsed patients harbor mutations in the Bcr-Abl kinase domain (KD), where more than 90 different mutations have been identified. Many of these patient-derived resistance mutations show no change in equilibrium affinity for imatinib towards Abl kinase and are therefore expected to alter the imatinib binding process by other mechanisms. Mutations that affect the binding kinetics of imatinib by binding and dissociating rapidly may retain a high affinity for imatinib under equilibrium conditions. However, in the non-equilibrium environment of the cell or human body, mutations that increase the drug dissociation rate from its target can presumably confer resistance by reducing drug residence time. Not surprisingly, the concept of drug residence time has emerged as a superior predictor of cellular drug efficacy. Recent structure and dynamics experiments have shown that association and dissociation rates of drugs to their target kinases can be limited by the accessibility of the binding site, highlighting the importance of studying the process of drug binding to proteins. Additionally, our recent simulations of imatinib binding to Abl have revealed that Abl kinase accesses a transient conformational state where it becomes partially unfolded. Presumably, mutations that destabilize Abl kinase, mimicking the partial unfolding that we observe in our simulations, would increase association/dissociation rates of imatinib and potentially confer drug resistance. Furthermore, chaperone machinery (hsp90/cdc37) in the cell is able to buffer structurally destabilizing mutations, including Bcr-Abl mutants, assisting cancer cells by decreasing the availability of the kinase to be degraded. We have identified patient-derived resistance mutations that show no change in equilibrium affinity for imatinib, but rather affect the stability of the protein. Taken together, *we hypothesize that these clinical mutations in Abl kinase domain can cause imatinib resistance by increasing the dissociation rate and by tightening the interaction between Abl kinase and Hsp90*. This hypothesis will be tested through the design of specific resistance mutations on Abl kinase and determination of the resulting changes in ligand binding rates and affinities determined by stopped-flow kinetics, surface plasmon resonance (SPR), fluorescence spectroscopy, NMR, and isothermal titration calorimetry (ITC) experiments. *The results of this project will provide insights into the mechanism of "kinetic" resistance mutations. On a broader level, this study will show how ligands find their binding sites on proteins, leading to new strategies in drug design.* Additionally, we will gain a better understanding of how rates of conformational changes in protein kinases relate to their function and regulation.

204 Intracellular Ca2+ Homeostasis and Nuclear Export Mediate Exit from Naïve Pluripotency
Matthew S. MacDougall

Intracellular Ca^{2+} Homeostasis and Nuclear Export Mediate Exit from Naïve Pluripotency

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The progression through states of pluripotency is required for cells in early mammalian embryos transition away from a heightened self-renewal and toward competency for lineage specification. Identifying gene products mediating transitions through pluripotent states is needed to better understand pluripotency. Here we use a CRISPR mutagenesis screen in mouse embryonic stem cells (ESC) to identify essential roles for nuclear export by RanBP3 and for intracellular Ca^{2+} homeostasis during the exit from naïve pluripotency. Mutation of a plasma membrane Ca^{2+} pump encoded by Atp2b1 increased intracellular Ca^{2+}, which must be decreased for ESC to rapidly exit from naïve pluripotency. Persistent self-renewal of Atp2b1$^{-/-}$ Tcf7l1$^{-/-}$ ESC in chemically defined media without exogenous inhibitors or LIF cytokine indicates a central role for Ca^{2+} dynamics in pluripotency. The identification of this new role for intracellular Ca^{2+} homeostasis provides new perspective on how mechanisms (Wnt/β-catenin, LIF/Jak/Stat, intracellular Ca^{2+}) for maintenance of a naïve state are related to those activated during embryo implantation in the uterus.

205 Acute intermittent hypoxia and basal thermal pain sensitivity
Taylor A.M. Maderazo

Acute intermittent hypoxia and basal thermal pain sensitivity

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Acute intermittent hypoxia (AIH) is a novel therapy that induces neuroplasticity via increased synthesis of brain-derived neurotropic factor (BDNF), which can lead to somatic benefits following a spinal cord injury such as functional motor gains and enhanced respiratory motor control. Although its effects on motor output are well-documented, its effects on sensory input and pain have yet to be studied.

To explore AIH's effects on sensory function, we measured basal pain sensitivity in response to a thermal stimulus following an AIH treatment. Quantitative sensory testing of healthy subjects (n = 13) was performed before and immediately after receiving fluctuating oxygenation (FLO) and continued for 60-minutes post-treatment in 10 - minute intervals. The four separate hypoxia treatments included varying ratios of hypoxia (9% - 13% O_2) to hyperoxia (40% O_2): 15 bouts of 1:1, 8 bouts of 2:1, 15 bouts of 2:1, and a sham (normoxia). Oxygen saturation was continuously monitored throughout the treatment, ranging from 80%-100% spO_2.

Thermal pain thresholds decreased immediately post-FLO then recovered over the next hour. The only statistically significant effects, however, were noted for 15 bouts of 1:1 and 8 bouts of 2:1, ($p = .030$ and $p = .032$ respectively) . No significant effects were observed for the sham or 15 bouts of 2:1 treatment.

In conclusion, acute intermittent hypoxia immediately increased pain

sensitivity to thermal stimuli in healthy subjects suggesting acute plasticity in sensory function. Further investigation is required to confirm this observation since this is merely an exploratory overview of the novel modality. If acute intermittent hypoxia can potentially increase sensitivity in healthy subjects, then its effects may eventually be used to modulate current therapies targeting pain relief.

206 Assessing the fatality rate in Congenital Zika Syndrome since the 2015 Zika outbreak
Jessika Maia

Assessing the fatality rate in Congenital Zika Syndrome since the 2015 Zika outbreak

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Background: Many studies have demonstrated a causal link between Zika virus (ZIKV) infection, microcephaly (MCP) and other congenital abnormalities (CA). This study aimed to determine the perinatal case fatality rate in cases of Congenital Zika Syndrome (CZS) in the Rio Grande do Norte State (RN), a Brazilian Northeast State highly impacted by the Zika virus outbreak.

Methods: A cross-sectional study was conducted using data obtained through the State Health Department (SHD) for cases of MCP and CA in Rio Grande do Norte from April 2015 to December 31, 2017. Definition of perinatal period: commences at 22 completed weeks (154 days) of gestation and ends seven completed days after birth. Perinatal case fatality rate is defined as the number of deaths as a fraction of the number of sick persons with a specific disease (×100).

Results: During the study period, there were 519 cases of MCP and others CA notified in RN, of which 150 were confirmed and 126 remain under investigation. The remaining 243 cases have been ruled out by presenting normal exams or due to presenting microcephaly by non-infectious causes. Of the total confirmed cases, 30.0% (45/150) died after birth or during pregnancy. 64.4% (29/45) of confirmed deaths had ZIKV infection during pregnancy and 4.4% (02/45) had a positive TORCH blood test. The deaths related to Zika were confirmed using either clinical/epidemiological/radiological (presence of typical and indicative alterations of congenital ZIKV infection) or clinical/epidemiological/serological (RT-PCR and/or IgM/IgG antibodies against ZIKV). 11 cases remain under investigation and 5 were ruled out.

Conclusion: This study highlights a high rate of perinatal lethality (64.4%) in cases of CZS. Despite the growing number of CZS cases, the real incidence and prevalence might be higher due to the underreporting and lack of resources for confirmatory diagnostic tests (laboratory and imaging). Due to the high rate of lethality, our findings predict an increase in the infant mortality rate in areas endemic for arboviruses. Because the severe neurological complications caused by CZS, it is likely to pose a substantial burden on public spending on health care. This study may be used to better describe the congenital Zika syndrome, its prognosis and natural history.

207 Computational modeling of tissue-selective liver ablation using focused ultrasound
Lauren Mancia

Computational modeling of tissue-selective liver ablation using focused ultrasound

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Introduction: Histotripsy is a non-thermal focused ultrasound procedure that uses targeted groups of microscopic cavities or 'bubbles' to homogenize soft tissue into acellular debris. Experimental studies of histotripsy have shown that tissues with higher Ultimate Tensile Stress (UTS) are more resistant to cavitation damage. In particular, blood vessels and gallbladder tissue have higher UTS values than liver and are observed to be more resistant to histotripsy erosion. This difference in damage susceptibility has been suggested as a basis for localized liver tumor treatments that spare these critical structures.

Objective: This study aims to quantify mechanical stress produced by cavitation bubbles, which is a likely mechanism for tissue damage in histotripsy. We then compare calculated stress fields to the UTS of liver, gallbladder, and blood vessel to determine the spatial extent of stresses high enough to rupture cells in each tissue.

Methods: A computational approach is used to quantify stress fields because the stresses developed within microns of cavitation bubbles are too localized and transient to measure. We model bubble dynamics in response to a histotripsy waveform in model tissues. Our computational model simulates the dynamics of a single bubble in a homogenous medium with viscoelastic properties representative of each tissue. Radial bubble dynamics are described by the Keller Miksis equation, and we use a Kelvin-Voigt-based viscoelastic constitutive equation to calculate the stress fields produced in each model tissue.

Results: The distinct viscoelastic properties of each tissue affected the magnitudes of stress developed at all distances from the bubble. Stresses capable of tissue rupture achieved a greater spatial extent in liver than in blood vessel and gallbladder. We also found that high stresses are localized to the bubble wall and decrease by at least two orders of magnitude within 50 microns from the wall of a ~300 micron bubble.

Conclusions: Simulation results support the hypothesis that differential tissue responses could be used to design liver tumor treatments that spare critical structures such as large blood vessels and gallbladder.

Our finding that stresses are highly localized to the bubble wall is consistent with experiments demonstrating sharp boundaries of the histotripsy ablation zone.

Conflict-of-interest disclosure: E.V. and Z.X. have financial interests and/or other relationship with HistoSonics Inc.

208 Targeting type III interferons promotes recovery during CNS autoimmune disease
Sindhu Manivasagam

Targeting type III interferons promotes recovery during CNS autoimmune disease

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Multiple sclerosis (MS) is a chronic autoimmune, demyelinating disease that affects 2.5 million people worldwide. MS is characterized by pathologic infiltration of lymphocytes and macrophages into the central nervous system (CNS) that leads to demyelination and axonal injury. Extent of axonal injury is strongly correlated with disease progression and permanent disability in MS patients. Currently available treatments for MS have varying efficacy, do not impact transition to progressive disease, and do not reverse disability. Here, we show that type III interferons may play a role in progression of CNS autoimmune diseases, such as MS, by promoting inflammation and axonal injury.

Type III interferons, consisting of interferon lambda (IFNλ), are a relatively new member of the interferon (IFN) family of proteins and are closely related to type I IFN. As IFNλ has not been widely studied outside of viral models, it is not known whether its immunomodulatory properties impact other inflammatory diseases, including MS. In preliminary studies, we found that IFNλ signaling impacts recovery in mice with experimental autoimmune encephalomyelitis (EAE), a well-established murine model for MS. Mice with targeted deletion of the IFNλ receptor (*Ifnlr1*$^{-/-}$) demonstrated improved clinical recovery from EAE compared to wildtype (WT) animals. This recovery was linked to resolution of inflammation and prevention of axonal injury. *Ifnlr1*$^{-/-}$ mice exhibited decreased recruitment and activation of endogenous host T cells and a subsequent decrease in inflammatory T cell cytokine (IFNg and GMCSF) production within the CNS. Furthermore, targeting IFNλ signaling using neutralizing antibodies resulted in similar improvements in clinical disease score and axonal damage compared to control antibody treatment. Finally, in human spinal cord tissue, we found increased levels of IFNλ in lesions of secondary progressive MS patients compared to relapsing remitting MS patients. These data suggest that IFNλ may promote disease progression during CNS autoimmunity and be a novel therapeutic target in MS patients.

209 Sensitizing tumors to oncolytic virotherapy by targeted inhibition of tumor innate immunity with Mengovirus replicons
Justin W. Maroun

Sensitizing tumors to oncolytic virotherapy by targeted inhibition of tumor innate immunity with Mengovirus replicons

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Oncolytic viruses are designed to specifically infect and kill tumors. However, clinical trials are failing to demonstrate the expected efficacy in most patients. One possible explanation could be active antiviral pathways in tumor cells and stroma restrict viral replication and spread *in vivo*. For safety reasons, oncolytic viruses in clinical development are attenuated and have a diminished capacity to counter host immune responses and are very sensitive to interferon mediated immune responses. We have demonstrated that certain human and mouse tumor cell lines are capable of initiating and responding to interferon based antiviral signaling that restricts several oncolytic virus infections. In order to overcome innate immunity, we have developed a viral replicon capable of expressing interferon antagonists. The replicon is based on an oncolytic picornavirus, Mengovirus, that can be detargeted from its natural tissue tropism while retaining the ability to infect various human and mouse tumor types. The replicon was generated by deleting a portion of the viral genome that encodes the viral capsid and adding the innate immune antagonist transgenes. The replicon can express a transgene through limited passages with a parental virus, but cannot recombine with the parental virus due to genomic size restraints imposed by the rigid capsid providing two layers of safety to the system. We have encoded a panel of interferon and innate immune antagonists within the replicon and have successfully limited the induction of interferon *in vitro*. We have discovered that a Mengovirus replicon expressing wild type Measles P protein can reduce IFNβ induction and completely prevent IFNα induction after infection of tumor cells *in vitro*. Our system has the unique ability to safely arm picornaviruses with interferon antagonists, which can enhance viral mediated oncolysis by blunting tumor innate immune responses.

210 Mechanisms underlying permanence or remittance of KATP-induced neonatal diabetes
William H. McAllister

Mechanisms underlying permanence or remittance of K_{ATP}-induced neonatal diabetes

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Gain of function (GOF) mutations in the ATP-sensitive potassium (K_{ATP}) channels cause human neonatal diabetes mellitus (NDM) due to disrup-

tion of glucose-dependent insulin secretion. In humans, the disease outcome may vary from transient to permanent NDM. Inducible K_{ATP}-GOF mice reiterate the features of human NDM as well as the variations in disease outcome after a short period (5 days) of sulfonylurea therapy at disease onset. While some mice, as expected, remain severely diabetic after treatment ended (non-remitters), others showed relatively normalized blood glucose levels (remitters). Previous studies on K_{ATP}-GOF remitter and non-remitter mice showed similar serum insulin, glucagon, leptin, and GLP-1 levels during and soon after the sulfonylurea treatment ended. Differences in insulin sensitivity between remitter and non-remitter mice were seen only long after treatment ended, suggesting that these changes are a consequence, and not a cause, of remittance. Importantly, however, inflammatory cytokines, IL-6 and TNF-alpha, were significantly elevated in non-remitters compared to remitter mice before (Day 0) and during diabetes induction (Day 5 and 14).

To further exam the possibility of differential levels of basal insulin secretion and the role of inflammatory cytokines as driving factors in NDM remittance, fasted and fed blood samples from K_{ATP}-GOF mice treated with the sulfonylurea glibenclamide for 5 days were taken at days 0, 5, 14, and 35 to measure plasma insulin (ELISA) and multiple cytokines (Immunology Multiplex Assay).

Basal insulin levels at day 0, 5, 14, and 35 revealed no significant differences between groups. All groups followed the same trend of increased basal plasma insulin during glibenclamide treatment and subsequent decline after treatment ended. Cytokines involved in NF-κB pathway revealed contrasting trends than those observed for IL-6 and TNF-alpha. IP-10 levels were increased only in remitter mice at Day 0 and 5 compared to control mice (p

Despite the differences observed in several inflammatory cytokines between remitter and non-remitter mice, preliminary data demonstrating increased remittance rates in K_{ATP}-GOF mice co-treated with the anti-inflammatory agent meloxicam suggests that certain cytokines play a crucial role in NDM progression. These results prompt us to further explore the role of inflammation and inflammatory cytokines as a mechanism underlying the remittance of NDM.

211 Appropriately timed histone deacetylase inhibition empowers T cell-mediated immunity to reject established breast tumors in pre-clinical models

Tyler R. McCaw

Appropriately timed histone deacetylase inhibition empowers T cell-mediated immunity to reject established breast tumors in pre-clinical models

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Malignant cells harbor an imbalance in histone acetyltransferase and histone deacetylase (HDAC) activity, contributing to altered epigenetic regulation and increased tumor cell fitness. HDAC inhibitors disrupt this balance to impact both cellular transcription and protein function, reducing fitness and survival of tumor cells. Despite their ability to impair proliferation and render tumors more immunogenic, the effects of HDAC inhibition on tumor-infiltrating T cells have been controversial. These agents might impede T cell activation, an epigenetic event, and thereby prevent T cells from acquiring cytotoxic capacity. This led us to hypothesize that appropriately timed HDAC inhibition could mitigate negative effects on T cell activation, while increasing tumor cell immunogenicity and boosting cytotoxicity of tumor-infiltrating T cells.

To test this, we first treated two murine breast cancer models, TS/A and 4T1, daily with the class I specific HDAC inhibitor entinostat starting at various times. We found that simply adjusting timing of HDAC inhibition relative to T cell activation could abolish anti-tumor effects or lead to rejection of established tumors in 40% of mice. Impairment of tumor growth was absolutely dependent on adaptive immunity, specifically CD8 T cells and IFNγ production. Indeed, single-cell RNA-sequencing and protein-level analysis by flow cytometry both showed that CD8 T cell production of effector cytokines was dramatically increased, even at later time points. Upregulation of cytotoxic function was paralleled by significant changes in CD8 T cell transcription factor profiles that suggest entinostat treatment can impede progression of the T cell exhaustion program. Additionally, treatment of tumor-bearing mice with entinostat turned on many components of an IFNγ signature recently reported to identify patients that will respond to anti-PD1. Although TS/A tumors do not respond to anti-PD1 monotherapy, treating tumor-bearing mice with entinostat then anti-PD1 at the right times led to tumor rejection in the majority of mice.

Collectively, our data shows that HDAC inhibition using entinostat can lead to rejection of established breast tumors in mouse models but only when given at precisely the right time—after T cell activation and expansion but before development of T cell exhaustion. Appropriate timing of HDAC inhibition can also sensitize tumors to anti-PD1 therapy and lead to consistent rejections. However, haphazard timing of HDAC inhibition may actually nullify benefits of checkpoint blockade, reaffirming the need to emphasize mechanisms when designing combinatorial strategies.

212 Compound library analysis identifies a combination of small molecules that increase the terminal differentiation of human induced pluripotent stem cells into myotubes and their maturation

Holly E. McKee

Compound library analysis identifies a combination of small molecules that increase the terminal differentiation of human induced pluripotent stem cells into myotubes and their maturation

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Patient-specific pluripotent stem cell skeletal muscle derivatives represent an attractive potential alternative for in vitro disease modeling without the requirement of muscle biopsy. The technology of reprogramming somatic cells into induced pluripotent stem (iPS) cells offers tremendous potential for the generation of large amounts of lineage-committed cells for disease modeling, as well as for other applications, including drug screening. Nevertheless, the overall embryonic nature of iPS cell-derivatives (across lineages) stands as a barrier for reliable disease modeling studies. Several signaling pathways have been reported to affect myoblast fusion and their differentiation into myotubes. Thus, screening for the effect of different signaling pathways and epigenetic modifiers is a promising approach to further promote the differentiation of pluripotent stem cells into myotubes and their subsequent maturation. Following an initial screening using a small molecule compound library, six compounds were selected based on initial improvement of differentiation efficiency of iPS cell-derived myogenic progenitors into myotubes, as identified through the quantification of all myosin heavy chain (MHC) isoforms by immunofluorescent staining. Of note, our results revealed that the combined exposure of the selected compounds during differentiation promotes a significant increase in fusion index (2-fold). More importantly, there was a 100-fold increase in the expression of the neonatal isoform of MHC(MHC-neo) in compound-treated myotubes in comparison to untreated controls, indicating this compound combination induced maturation of IPSC-derived myotubes.

213 Evaluation of oligodendrocyte progenitor cells near the lesion site after spinal cord transection and olfactory ensheathing cells or fibroblast transplantation
Mahlet A. Mekonnen

Evaluation of oligodendrocyte progenitor cells near the lesion site after spinal cord transection and olfactory ensheathing cells or fibroblast transplantation

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Following a complete spinal cord transection, the nervous system forms a glial scar to protect the intact tissue remaining after the injury. The scar, however, contains components that can be inhibitory to axon outgrowth such as oligodendrocyte progenitor cells (OPCs). While axon regrowth is required at the glial scar, the axons further from the injury site often become demyelinated and damaged. In this area, OPCs can differentiate into myelinating oligodendrocytes and have a positive protective effect on axonal function. Previously we showed that there are fewer OPCs associated with the glial scar border in completely transected rats that received olfactory ensheathing cells (OECs) transplantations compared to control fibroblast (FB)-treated rats at 2 and 8 weeks post-injury. This was true in rats with transplanted cells by 8 weeks and in those treated with cyclosporine A (CSA) immunosuppression to enhance transplant survival. The current study asks if the number of OPCs 1 mm rostral and caudal to the injury site varies between OECs- and FB-transplanted spinal rats with and without CSA immunosuppression in order to determine if differences persist in areas where axon myelination is more important than regrowth. OPCs were identified by glutathione s-transferase pi (GST-pi) immunoreactivity in the cell nucleus and were counted in a sample area 1 mm into the two stumps. We found that the number of OPCs within the spinal stumps did not differ between transplant treatments at either 2 or 8 weeks. The number of OPCs at the scar border in OECs-treated rats is similar to numbers 1 mm further into the stumps in rats that receive either OECs- or FB-transplants. Together, these findings suggest that OECs transplantation reduces the number of growth-inhibitory OPCs at the scar border compared to FB-treated rats, but does not affect the number of OPCs deeper into the rostral and caudal stumps. Further analyses are being conducted to determine if these numbers are similar to those in uninjured rats. Additional studies are also looking at the percentage of myelinated axons and the relationship between the OPCs and mature oligodendrocytes in long-term (5-6 months post-injury) spinal rats that received either OECs- or FB-transplantation.

214 Exploring the mechanisms of motor rehabilitation-induced recovery after white matter stroke
Tatiana G. Mengistu

Exploring the mechanisms of motor rehabilitation-induced recovery after white matter stroke

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Subcortical white matter stroke (WMS) accounts for 25% all stroke subtypes. White matter is composed of axons that relay brain signals and oligodendrocytes, which produce myelin. Myelin is a multi-lamellar extension of oligodendrocyte membrane that wraps around the axon and provides it with both electrical insulation and metabolic support. WMS is characterized by the formation of white matter lesions, which results in oligodendrocyte death and myelin degeneration. Loss of myelin results in axon degeneration and functional impairment. Previous work in the Carmichael lab demonstrates that after WMS, there is a significant increase in proliferation of oligodendrocyte precursor cells (OPCs), a resident stem cell that functions in part to differentiate into mature oligodendrocytes during development and in adulthood. However, despite proliferation, these OPCs fail to mature into oligodendrocytes following WMS. Recent work in our lab suggests that subjecting mice to motor rehabilitation, such as skilled reach, enhances recovery by promoting both OPC proliferation and their maturation into myelinating oligodendrocytes. The goal of this project is to build upon these initial findings by identifying molecular markers of myelin and oligodendrocyte recovery following motor rehabilitation. Understanding the mechanisms that drive recovery and re-myelination in mice after WMS will enable us to better understand the pathology of WMS and develop novel therapeutic strategies for recovery. Supported by AHA grant 14BFSC17760005.

215 APSA Undergraduate Mentorship Program: Evaluating efficacy toward increasing the number and diversity of physician-scientists in training
Emily A. Minor

APSA Undergraduate Mentorship Program: Evaluating efficacy toward increasing the number and diversity of physician-scientists in training

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The number of students entering the physician-scientist workforce (PSW) is insufficient to maintain the field. In an effort to address this problem, the American Physician Scientists Association (APSA) created the Undergraduate Mentorship Program, which seeks to increase both the number and diversity of young investigators entering the PSW. This program pairs medical students or dual-degree (MD-PhD or DO-PhD) trainees with undergraduate students who are interested in pursuing careers as physician-scientists. Special emphasis is placed on recruiting women and students who are underrepresented in medicine. Monthly prompts facilitate a dialogue between mentees and mentors, which opens the door for individualized advice and guidance. In order to evaluate the efficacy of this program, we are conducting a longitudinal study of the 2018-2019 mentorship program cohort in the form of multiple surveys. Mentees completed a pre-survey prior to the start of the program and will complete a post-survey at the end of the spring semester, while mentors will only complete a post-survey. The surveys were created and distributed using REDCap, which will enable us to track pre/post responses and evaluate mentor/mentee pairs. We are particularly interested in the diversity of participants in the program, the usefulness of the monthly prompts, how frequently mentorship pairs interacted, and whether the program increases the chance that mentees will go on to pursue a career as a physician-scientist. There are 318 mentees and 275 mentors enrolled in the mentorship program this year. Data from the pre-survey indicates that 73% of the mentees are women and 42% are underrepresented in medicine. The majority of respondents (82%) say they are likely or very likely to pursue a career as a physician-scientist and 86% of them think that participating in the mentorship program will help make them more competitive graduate school applicants. Post-surveys will be sent out at the conclusion of the spring semester, which will enable us to further evaluate the efficacy of the program and determine where any improvements can be made.

216 Development of a novel iPSC-derived intestinal organoid differentiation platform to model Crohn's Disease (CD)
Aditya Mithal

Development of a novel iPSC-derived intestinal organoid differentiation platform to model Crohn's Disease (CD)

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Efficient generation of iPSC-derived HIOs would greatly enhance our capability to develop *in vitro* models for a variety of gastrointestinal diseases, including CD. Currently published protocols include serum-based differentiations leading to the inconsistent generation of mesenchyme-supported HIOs. However, a more robust mesenchyme-free system would be most effective for modeling diseases impacting the GI epithelium, such as CD. HiPSCs were differentiated into definitive endoderm, followed by dual inhibition of TGFβ and BMP to prime the formation of lineages throughout the developing gut tube. CDX2 kinetics were tracked throughout differentiation using a novel CDX2-eGFP iPSC reporter cell line. At day 15, FACS sorting enables the exclusion of NKX2-1 positive anterior foregut lineages while selecting for CDX2 positive distal lineages. These organoids can be additionally patterned by FGF7 signaling towards a distal colonic phenotype. CDX2 expression was primarily driven by Wnt signaling. Sorting for hindgut progenitors ultimately generated a robust population of CDX2 positive mesenchyme-free HIOs that co-expressed a variety of specific markers of intestinal epithelium. Here, we report a novel directed differentiation protocol for the generation of mesenchyme-free HIOs that can be primed towards more colonic or proximal intestinal lineages, furthering our ability to model diseases affecting the epithelium of the gastrointestinal tract.

217 Protein-protein interaction network analysis as a guide for precision engineering of oncolytic virotherapies against glioblastoma
Dileep D. Monie

Protein-protein interaction network analysis as a guide for precision engineering of oncolytic virotherapies against glioblastoma

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Gliomas, including glioblastoma multiforme (GBM), account for 80% of malignant brain tumors. Treatment is limited to surgical resection, radiation, and chemotherapy. Recently developed immunotherapies promise to improve the prognosis for GBM. Oncolytic virotherapy (OV) is an intervention that has shown early promise in clinical trials for other cancers. OV debulks, targets local and metastatic cancer cells with high specificity, and may offer immune-mediated protection against tumor recurrence. Like other immunotherapies, however, OV is hindered by tumor distribution kinetics and safety concerns such as promiscuous tropism and tumor lysis syndrome. Synthetic biology approaches may facilitate engineering of OV to overcome these obstacles and offer precision antitumor efficacy for an individual patient. Our primary goals are to decode gene regulatory networks that influence the efficacy of OV

and use this analysis to suggest improved designs. In this present study, we use NetDecoder to elucidate protein-protein interaction networks in microarray data from LN229 human GBM cells treated with herpes simplex virus type 1 (HSV-1) OV. These cells have inducible expression of the OV-inhibitory extracellular matrix protein cysteine rich 61 (CCN1). Our transcriptome analyses prioritize human genes that are differentially expressed between CCN1-induced and uninduced control cell phenotypes. This NetDecoder analysis yielded a number of high impact genes, notable for their differential edge flows, organized in a prioritized subnetwork. Our results indicate 39 nodes that may influence susceptibility of CCN1-expressing GBM to OV. Of these, a router (IKBKE) and a sink (YBX1) have been implicated in GBM pathogenesis. Furthermore, category enrichment suggests that measles virus may be more effective in these types of tumors. Our results suggest that network analysis unravels protein-protein interactions in GBM development and progression. By better understanding these networks, targeted therapies can be developed to improve outcomes for patients.

218 Tumor-derived exosomes polarize macrophages to upregulate PD-L1 through metabolic reprogramming in a pre-metastatic niche
Samantha Morrissey

Tumor-derived exosomes polarize macrophages to upregulate PD-L1 through metabolic reprogramming in a pre-metastatic niche

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The seed and soil hypothesis, a long-standing paradigm in cancer, states that cells from primary tumors seed distant soils, or tissues, that have a favorable microenvironment. However, the question remains as to what creates a favorable pre-metastatic niche precipitating the arrival of metastatic tumor cells. Furthermore, current immunotherapies, while successful, have a limited window for progression free survival before active disease returns. The purpose of this study is to determine if tumor derived nanoparticles, namely exosomes (TDE), drive tumor metastasis by polarizing macrophages in a distant pre-metastatic niche towards an immunosuppressive phenotype capable of antagonizing tumor immunotherapies resulting in progressive metastatic disease.

The TDE were isolated from the supernatants of murine Lewis Lung Carcinoma cells or human A549 adenocarcinoma cells and control exosomes (CE) were isolated from normal lung epithelial cells of both mouse and human. Murine macrophages and human CD14+ monocytes were treated for 16 hours with TDE prior to analysis. Metabolic function was assessed by real-time PCR and Seahorse analyses.

TDE but not CE stimulation differentially upregulates PD-L1 on murine macrophages and $CD14^+$ human monocytes capable of inhibiting T cell proliferation and effector function, an effect rescued by PD-1 antibody. The upregulation of PD-L1 on macrophages induced by TDE occurs through TLR2/MyD88 signaling and the downstream NF-κb pathway. Furthermore, induction of immunosuppressive macrophages by TDE appears to be through metabolic reprogramming as revealed by increased glycolytic rate (ECAR) and lactate production, as well as increased Arginase-1 expression. Inhibition of glucose uptake by 2-DG significantly downregulates PD-L1 expression on polarized macrophages. In vivo studies demonstrate that lung macrophages have increased PD-L1 expression despite the absence of tumor metastases.

Both mouse and human TDE are capable of polarizing macrophages towards an immunosuppressive phenotype characterized by increased PD-L1 expression. These effects are dependent on the TLR2 pathway and metabolic reprogramming. These findings suggest TDE released from primary tumors could prime myeloid cells in distant tissues to establish an immunosuppressive microenvironment favorable for tumor metastasis.

219 Stent fracture in congenital heart disease: a retrospective review
Bryan P. Mosher

Stent fracture in congenital heart disease: a retrospective review

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Stent implantation in the pediatric population is facilitated by the availability of low-profile stents that are deliverable through small delivery sheaths. However these smaller stents cannot be dilated to match an adult vessel size. Several *in vitro* studies demonstrate that small- and medium-size stents can be fractured using ultra-high-pressure balloons. Recently, an *in vivo* model of stent fracture (i.e., "unzipping") confirmed the feasibility of intentional fracture of several different stents in pigs. Five intentional longitudinal stent fractures were reported in humans using high-pressure balloons without immediate adverse events. *In situ* spontaneous stent fracture in humans is not uncommon and has been reported in up to 21% patients, with resultant obstruction in 80% (of which 39% were considered severe). Some fractures can cause stent collapse, hemodynamic compromise, and embolization of stent fragments, requiring additional intervention in 75% of cases. In a large report of the spontaneous fracture of 3,650 stents, there was a 42% incidence of in-stent restenosis and 4.6% incidence of thrombosis. Furthermore, in a study demonstrating the feasibility of intentional stent fracture in humans, there was a significant incidence of complications (15%), including embolization of stent fragments, unstable stent fracture, vascular tear, non-obstructive intimal tear, and aorto-pulmonary window. All complications except embolization were prevented by pre-stenting (Bratincsak et al., 2017). Retrospective chart review was performed of all pediatric patients who underwent cardiac stent placement and then later had the stent balloon dilated or re-stented from Jan 2005 to September 2018. All data was collected from electronic medical records from the Rady Children's Hospital EPIC, Chartmax, Pedcath systems. Peri-procedural variables including indication for catheterization, nature of indication, interval since stent placement, indication for stent, pre- and post-oxygen saturation, type, size and number of stents, procedure, access, addition-

al procedures, immediate complications and immediate re-interventions were obtained. Additional post-procedural variables including subsequent re-intervention, current status, mortality, etiology of death were also collected. Our study indicates the potential benefits of pre-stenting at the time of intentional pre-existing stent fracture compared to simple stent fracture. We hope these findings guide vessel angioplasty or stenting in the pediatric population.

220 Joint assay of single cell RNA-seq and transcription factor binding
Arnav Moudgil

Joint assay of single cell RNA-seq and transcription factor binding

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Single cell RNA-seq (scRNA-seq) identifies cell types in heterogeneous mixtures and complex tissues but cannot explain the underlying regulation driving specific cell states. To better understand mechanism, multi-modal single cell techniques have emerged to connect transcriptome to other genomic data, such as genotype, CpG methylation, and chromatin accessibility. There is no method, however, that can link scRNA-seq to transcription factor (TF) binding in those same cells. Here we report the development of self-reporting transposons (SRTs) and their subsequent use in single cell calling cards (scCC), a novel assay for the simultaneous identification of transcriptome and TF binding in single cells. We show that the genomic locations of SRTs can be mapped using either cellular RNA or DNA, but transposon recovery is more efficient with RNA. Next, we use the *piggyBac* transposase, which has a strong affinity for the bromodomain protein Brd4, to identify Brd4 binding sites solely from SRT localization. We then present a method to recover SRTs from scRNA libraries (scCC), which leads to concomitant labeling of cell types and identification of TF binding sites within those populations. Using scCC, we map Brd4 binding *in vitro* and *in vivo* in the mouse cortex. Finally, we show that fusing *piggyBac* to the TF SP1 redirects insertions to SP1 binding sites, thus demonstrating that this method can be used to study potentially any TF *in situ*.

221 Congenital Zika Syndrome: a relation between neurological and radiological findings
Nilson N. Mendes Neto

Congenital Zika Syndrome: a relation between neurological and radiological findings

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Background: Although Zika virus (ZIKV) infection causes a broad spectrum of congenital neurological disorders, radiographic correlates of clinical outcome are lacking. During 2015-2016 ZIKV outbreak we faced a high incidence of microcephaly (MCP) in Rio Grande do Norte State (RN), located in northeast of Brazil. Among all regions, the northeast was the most affected by ZIKV. We aimed to identify distinct CT brain scan findings associated with congenital ZIKV infection and correlate them with neuro-clinical disorders in babies with MCP. Their mothers had exanthematous diseases (ED) compatible with ZIKV infection during their pregnancy.

Methods: Medical evaluation was performed on 38 babies with MCP, up to 17 months old, followed at a center for child rehabilitation in RN. All subjects underwent CT brain scan. Cohort enrollment occurred with subjects born between January 2015 and May 2016.

Results: 38 babies with MCP underwent head CT. 68.5% were male, 31.5% were female. The main clinical presentations were spasticity, irritability and seizure. On CT, all subjects had brain volume reduction. Intracranial calcification (IC) was observed in all of the subjects who presented with irritability and seizures (n = 27) and in 83.3% of subjects with spasticity. Lissencephaly was seen in 80% of subjects with irritability, 75% of subjects with seizures and 50% with spasticity. Ventricular dilatation was seen in 19 subjects, all of whom had spasticity, 60% who presented with irritability and 50% who presented with seizures.

Conclusion: These new data from a relatively large study, demonstrate that neuroradiographical findings are associated with clinical syndromes in affected neonates. IC was the most prevalent CT scan finding (after reduction in the brain volume). It seems to be the most common radiological finding related to neuro-clinical disorders in ZIKV infection. This study may be used to better describe the congenital Zika syndrome, its clinical/radiological outcomes and natural history.

222 Evaluation of secondary diagnostic codes on expert system effectiveness in neurosurgery
Daniel Naftalovich

Evaluation of secondary diagnostic codes on expert system effectiveness in neurosurgery

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An expert system prototype is presented which aims to predict procedure choice based on diagnostic information in a neurosurgery context. The model input is in the form of standardized diagnostic codes from the International Classification of Diseases (ICD-10) and the model output is in the form of Current Procedural Terminology codes (CPT). This

input-output modeling approach and use of standardized coding terminology presents an abstraction and simplification of clinical decision making. In this study a model is trained via machine learning algorithms, and particular attention is addressed to the effect of including additional diagnostic codes into the model inputs.

223 Bionanosensors for in vivo monitoring of chemotherapeutic drug delivery and treatment efficacy
Freddy T. Nguyen

Bionanosensors for *in vivo* monitoring of chemotherapeutic drug delivery and treatment efficacy

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Brain tumors are the most common form of pediatric solid tumors affecting ~20% of all pediatric cancers. Pediatric gliomas make up approximately 8-12% of pediatric central nervous system tumors. High grade gliomas are highly aggressive and malignant tumors with 5-year survival rates between 15 to 35%. Current management follows a multipronged approach that include surgery, radiation, and chemotherapy. Surgery is used for tumor debulking followed by radiation and chemotherapy. There is a pressing need for a platform technology to provide precision chemotherapy screening, drug delivery detection, and real time therapy efficacy monitoring to increase survival rates, reduce adverse effects, and lower overall costs. Current standard of care utilizes imaging to assess tumor response to therapeutic interventions and to monitor for cancer recurrence. Endogenous H_2O_2 and NO are involved in numerous signaling pathways that contribute to the initiation, progression, metastasis, and regression of cancer. Being able to measure the real-time *in* vivo concentrations of NO and H_2O_2 during tumor initiation, progression, and regression is crucial to better understand their dual roles in cancer metabolism and response to chemotherapy. We recently developed a series of near-infrared fluorescent probes for NO and H_2O_2 using single-walled carbon nanotubes (SWNT) that have been demonstrated *in vitro* with no signs of photobleaching, and *in vivo* with no decrease in sensor performance or signs of inflammation for 400+ days. The near-infrared fluorescence emission allows for deep tissue penetration. We also present recently developed SWNT bionanosensors for major chemotherapeutic drugs in glioblastomas (5-Amino-4-imidazolecarboxamide (AIC), an inactive byproduct of degradation of temozolomide; irinotecan; cisplatin; and lomustine). During degradation of 50 μM TMZ into AIC and diazomethane, DNA-SWNT show a 50% fluorescence increase with no significant solvatochromic shift and most sensitive to concentrations between 5 and 500 μM. Molecules similar in structure to AIC were tested demonstrating our bionanosensor's high specificity to AIC achieved using the corona phase molecular recognition (CoPhMoRe) technique developed at MIT. A sensor for irinotecan was also developed that demonstrated a strong fluorescence quenching and red-shift of the fluorescence emission peaks across multiple SWNT chiralities when tested with 50 μM of irinotecan. The sensors for cisplatin and lomustine both demonstrated 20% fluorescence quenching at concentrations of 50 μM of cisplatin and lomustine, individually. With this dynamic and real-time information from the previously described sensors and others currently under development, we can begin to build a multiplexed *in vivo* assay that can provide information about the local delivery and diffusion of chemotherapeutics and the tumor therapy response directly in the complex heterogeneous tumor microenvironment on the time scale of hours or days as opposed to the weeks or months currently needed to see measurable size changes on CT or MRI.

224 Metabolic cooperation between cancer cells and tumor-associated macrophages: The role of MIF and mitochondrial lactate metabolism
Jordan Noe

Metabolic cooperation between cancer cells and tumor-associated macrophages: The role of MIF and mitochondrial lactate metabolism

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Polarization of tumor-associated macrophages (TAMs) to an M2-phenotype increases tumor malignancy by promoting angiogenesis, metastasis, and resistance to immunotherapies. As cancer cells exhibiting the "Warburg effect" produce copious amounts of lactate that enhances M2 polarization, we have been interested in delineating the mechanistic effectors of lactate-enhanced M2-TAM polarization. Here we report that macrophages can maintain M2 polarization in low glucose/high lactate conditions, which is similar to the metabolic conditions within the tumor microenvironment. Mechanistically, lactate is metabolized to pyruvate and taken up within the mitochondrial to produce substrates needed for epigenetic modifications and expression of M2-associated gene products. As a result, inhibition of mitochondrial pyruvate uptake blocks M2 polarization and the immunosuppressive capacity of macrophages. Finally, we show that the metabolic reprogramming in M2 macrophages, which is needed for enhanced mitochondrial metabolism, is dependent on the expression of the cytokine macrophage migration inhibitory factor (MIF). Macrophages that are deficient in MIF exhibit reduced mitochondrial metabolism and M2 polarization. This effect is due to MIF's ability to regulate CSN5 activity as a small molecule that "phenocopies" CSN5 activity rescues the loss of M2 polarization in MIF-deficient macrophages. Altogether, this investigation shows that mitochondrial pyruvate metabolism is a previously unidentified metabolic requirement of M2 polarization and that MIF is a critical regulator of mitochondrial metabolism, as well as determining the downstream mechanistic contributions of MIF in M2 polarization. We consider that targeting MIF or macrophage-dependent lactate metabolism could be a viable therapeutic strategy in combination with currently available immunotherapies.

225 The application of machine-learning to predict gastroenteritis in patients
Brian Nohomovich

The application of machine-learning to predict gastroenteritis in patients

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Gastroenteritis can be devastating to young children and is a major cause of complications worldwide. In the United States, there are 179 million cases of acute gastroenteritis each year, which results in high rates of mortality and morbidity. We have previously identified the microbiome profiles of patients with gastroenteritis, recovering from gastroenteritis, and their healthy family members. Machine-learning is a powerful tool currently being used for facial recognition, signature identification, and self-driving cars; however, it has not been extensively utilized in trying to classify gastroenteritis based on the type of data we have collected. Here we use the above dataset to train a machine-learning model that can identify the health state of a patient given their intestinal microbial profile. 270 intestinal microbiomes were analyzed, consisting of 79 confirmed cases, 66 samples of recovered patients, and 125 healthy family member controls. A microbiome pipeline with a center-log ratio transformation was generated to identify the taxa present and normalize the data. A random forest classifier was employed to predict the health status of a sample given the microbiome profile. Initially, the model was built by splitting the data into training and testing sets. Then, two assessments were undertaken: case vs healthy family members and case vs follow-up states. Confusion matrices were generated to visualize the accuracy, specificity and sensitivity of the model. The initial model identified the health state of a given microbiome profile accurately more than 80% of the time. We found that the relative abundance of taxa such as Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Viruses could classify the results into one of the above health states with 83% accuracy. Incorporating clinical data such as bloody diarrhea, diarrhea, fever, hospitalization into the model improved the accuracy to 92%. Overall, precision reached 93%. These findings are in congruence with our previous reports in identifying the taxa important in gastroenteritis. However, these data extend our knowledge of gastroenteritis by applying clinical data with a supervised learning approach to build a model that a) has a high-degree of accuracy when coupling clinical data with microbiome profiles and b) can be easily used in clinical practice. Future work will focus on revising the model to improve the accuracy and applicability to clinical settings, including revising the model to reflect PCR results using a Jaccard distance matrix with binary microbiome profiles. This work could improve the diagnosis time of gastroenteritis and, as a result, expedite treatment.

226 Behavioral sex differences and histopathological Progression of Alzheimer's Disease in septic Alzheimer's Disease transgenic mice
Divine C. Nwafor

Behavioral sex differences and histopathological Progression of Alzheimer's Disease in septic Alzheimer's Disease transgenic mice

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Alzheimer's disease (AD) is the most common neurodegenerative disease associated with aging and is also one of the leading causes of morbidity and mortality in the elderly. It is characterized by progressive memory loss accompanied by post-mortem histopathological findings of neurofibrillary tangles, amyloid plaques, and a reduction in acetylcholine (ACh) levels within the basal forebrain. Moreover, recent studies suggest a strong link between AD and systemic infection, and interestingly, one of the leading causes of death in AD patients is sepsis. Although sex differences are clearly established in AD, few studies have examined the link between sex differences and infection in AD. Therefore, we hypothesized that sepsis would exacerbate neurodegeneration and neuroinflammation in female AD transgenic mice compared to age-matched male mice. We employed the *APPSwDI/Nos2*$^{-/-}$(CVN-AD) mouse model of AD and an experimental model of sepsis, cecal ligation and puncture (CLP), to investigate this hypothesis. Cognitive, neuroinflammatory, and histopathological outcomes were compared between CVN-AD^{sham} and CVN-AD^{CLP} for both sexes (8-10 months old). After induction of experimental sepsis all mice were monitored for 21 days, followed by an assessment of neuropathological outcomes. Sickness behavior was assessed with a murine modified sepsis score (MMSS) coupled with testing to evaluate several behavioral domains including: spatial learning and memory (two-day radial arm water maze), nociception (hot plate), locomotion (open field), and depression (forced swim test). Our results revealed a significantly lower survival (*p*CLP (27% survival) and female CVN-AD^{CLP} (48% survival) compared to female CVN-AD^{sham} (100% survival) and male CVN-AD^{sham} (100% survival). The sickness score in the first 6 days was also significantly higher (*p*CLP mice (male and female) compared to CVN-AD^{sham} mice (male and female). Hot plate testing revealed a significantly longer (p=0.03) latency to nociception in female CVN-AD^{CLP} compared to the male CVN-AD^{CLP} mice. In addition, the total number of nociceptive behaviors was significantly lower (p=0.001) in female CVN-AD^{CLP} mice compared to male CVN-AD^{CLP} mice. Open field locomotor assessment of horizontal (p=0.001) and rear (p=0.0002) movements revealed a significant treatment effect between the CVN-AD^{sham} and CVN-AD^{CLP}; however, no sex differences were observed. At day 21, brains and spinal cords were harvested for immunohistochemistry. Ongoing studies will address the interaction between sex, AD, and sepsis on beta-amyloid deposition, blood-brain barrier disruption, neuronal loss, and cholinergic neurodegeneration. Taken together, these findings suggest that biological sex may interact with sepsis to stimulate the trajectory of cognitive decline and disease severity in human AD patients.

228 Characterization of tumoral immunity in triple-negative breast cancer in African American compared to non-African American patients

Tess O'Meara

Characterization of tumoral immunity in triple-negative breast cancer in African American compared to non-African American patients

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Background: African American (AA) patients with triple-negative breast cancer (TNBC) are less likely to achieve pathologic complete response (pCR) following chemotherapy compared to non-AA patients, even after adjusting for clinical stage at presentation and socio-economic variables. The abundance and composition of immune cells in the tumor microenvironment are powerful prognostic factors in TNBC. Therefore, we hypothesize that the microenvironment of AA TNBC may be different than that of non-AA. We harnessed publicly available data from The Cancer Genome Atlas (TCGA) and clinical samples from Yale Pathology to measure immune-related RNA and protein expression, respectively, in the tumor microenvironment of AA and non-AA cases.

Methods: RNA-seq expression data were obtained from TCGA for n=58 AA and n=114 non-AA TNBC cases. N=43 AA and n=43 non-AA TNBC samples, matched by diagnosis date, were selected from the Yale Pathology archives. Using RNA-seq data, the expression of 15 previously reported immune metagenes, representing various immune cell functions and predictions of response to immune checkpoint blockade (ICB), were calculated. CIBERSORT deconvolution was performed to estimate the proportions of 22 immune cell sub-populations. Using clinical samples, CD8, CD68, and PD-L1 protein expression was measured in both the tumor and stromal compartments in whole slides from formalin fixed paraffin embedded (FFPE) tissues using multiplexed quantitative immunofluorescence (QIF). The average of each marker expression was calculated in all Fields of View (FOV) as well as the top 10% of brightest FOV on each slide (i.e. hotspot).

Results: Immune metagene analysis demonstrated marginal immune attenuation in AA TNBC relative to Caucasian TNBC that did not reach statistical significance. Gene signatures predicting response to ICB did not differ significantly between race groups. CIBERSORT deconvolution estimated a higher proportion of CD4+ resting T-cells in non-AA compared to AA TNBC (p=0.014). CD8+ cytotoxic T-cells, as measured by QIF, were also more abundant in non-AA compared to AA cases, specifically when hotspots were measured within the tumor compartment (p=0.017). The frequency of macrophages, as assessed by the expression of CD68 on QIF, was significantly higher in AA compared to non-AA cases. This difference was present when all FOVs were measured (mean AA=3627au vs. mean non-AA=2273au; p=0.005), when hotspot FOVs were measured (mean AA=6371au vs. mean non-AA=4858au; p=0.041), and when tumor and stromal sub-compartments were assessed.

Conclusions: The significantly higher CD68 and lower CD8 expression in AA compared to non-AA TNBC might contribute to a more attenuated immune microenvironment. Ongoing experiments aim to characterize the macrophage polarity and cytokine milieu of these samples, as well as to perform RNA sequencing on clinical cases to explore more nuanced racial differences in immune gene expression.

229 Induction of Totipotent-like Cells by Defined Factors in Culture

Sanders Oh

Induction of Totipotent-like Cells by Defined Factors in Culture

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Totipotent cells can be created by nuclear transfer into mature oocytes, but the identities of maternal factors that induce this reprogramming remain a mystery. Here, we demonstrate the induction of totipotency in pluripotent stem cells by introducing Hist1h2aa, H3f3b, H1foo, p-Npm2, Zscan4d, and Ubtfl1. We observed dose-dependent increases of murine endogenous retrovirus-like transposons, typically expressed in 1- and 2-cell stage embryos. Adding p150 siRNA and trichostatin A further increased this expression. These cells, which we designated iTLCs (induced totipotent-like cells), showed increased expression of totipotent genes and downregulation of pluripotent and differentiation genes, indicating a shift towards the totipotent state. iTLCs displayed enlarged nuclei, a unique characteristic of zygotic genome activation (ZGA), as well as telomere lengthening, and they survived in totipotent-specific culture media. There was no evidence of malignant transformation as indicated by normal karyotypes, cell death in nutrient-deprived condition, and cell density-dependent inhibition of proliferation. iTLCs demonstrated expanded cell fate potential by expressing markers for both embryonic and extraembryonic lineages, and they were capable of differentiating into all three lineages of the pre-implantation embryo. RNAseq data showed remarkable similarities between iTLCs and totipotent cells. In particular, early ZGA genes were strongly upregulated in iTLCs, indicating an active totipotent state. When reprogrammed cells were cultured for an extended period, we observed cells morphologically resembling various stages of early embryogenesis. These data suggest that pluripotent stem cells can be reprogrammed towards a totipotent state using defined factors without the need for oocytes.

230 Classification of Psychosis Diagnoses using Multisite Resting State Functional Connectivity Data from BSNIP1 Study

Victoria T. Okuneye

Classification of Psychosis Diagnoses using Multisite Resting State Functional Connectivity Data from BSNIP1 Study

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Classification based on multisite MRI data presents a significant challenge due to high variance between scanners, but is a worthwhile endeavor as the potential utility of MRI as a clinical diagnostic or prognostic tool will need to be scanner independent. Here, we examined diagnostic classification based on resting state connectivity in a large multisite-sample of psychotic subjects - schizophrenia (SCZ), schizoaffective disorder (SAD), bipolar disorder with psychotic features (BP)- and healthy controls (HC). In addition, we compare the classification discrimination with BSNIP biotypes – clinical sub-groups based on independent cognitive and neurophysiological biomarkers. Quality rs-fMRI data was obtained from 124 SCZ, 103 SAD, 116 BP and 187 healthy controls recruited as part of the multisite Bipolar & Schizophrenia Network on Intermediate Phenotypes (BSNIP1) study. Correlation matrices for all regions were calculated along with partial and tangent correlation matrices. Multiclass and binary support vector classifiers (SVC) were trained on the balanced datasets containing, rs-fMRI partial correlation matrices to evaluate prediction accuracy for both traditional and biotype diagnoses. Important trends can be observed in the relative performance of support vector classifiers. Highest binary discrimination from healthy controls is for SCZ with accuracy decreasing from SAD to BP. Concurrently binary discrimination between SCZ and BP is greatest amongst the clinical diagnoses, a with relatively weak discrimination compared to chance for SAD to both SZP and BP. Four class multiclass discrimination between all four groups and three-way multiclass discrimination for only the psychotic subjects is moderately above chance. Our findings show above chance classification accuracy for psychotic diagnoses based on rs-fMRI for both traditional and biotype based subgroups and are comparable to recent machine learning classification effort of multi-site psychosis data. Though multi-site classification performance is still below a reasonable standard for clinical utility. While further turning of parameters may marginally increase classification, literature suggests alternative factors such as preprocessing steps, brain parcellation and subsequent feature selection can also play significant roles in classification performance.

231 Regulation of Intestinal Epithelial Cell Inflammatory Response by TGF-β Growth Factor Acting Through Transcription Factor SMAD-4
Simbiat Olayiwola

Regulation of Intestinal Epithelial Cell Inflammatory Response by TGF-β Growth Factor Acting Through Transcription Factor SMAD-4

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Our research aims to further uncover the mechanism of cancer formation in the context of inflammation, the significance of inflammatory regulation, its key molecular factors, and the ultimate prevention of inflammatory associated carcinogenesis in humans. Evidence suggests that Inflammatory Bowel Disease (IBD)- induced colorectal cancer is triggered by both microbiome and host-dependent pro-inflammatory mechanisms. In mice, genetic ablation of *Smad4* in colon epithelium increases the expression of pro-inflammatory genes and colitis-associated adenocarcinoma (CAC) with morphology similar to human ulcerative colitis-associated colorectal-cancer (UCAC). Further, SMAD4 loss in human UCAC is more prevalent (48%) than in sporadic cancer (19%). In both mouse normal colonocytes and TGF-β-responsive human CRC cell lines, TGF-β results in downstream activation of R-SMADs/SMAD4 and the suppression of pro-inflammatory gene expression. Our aim is to understand how SMAD-mediated regulation of inflammation contributes to carcinogenesis. We hypothesize that TGF-β/SMAD4 reduces tumor incidence by suppressing inflammation-associated gene expression in colonic epithelial cells, including chemokines that are up-regulated by NF-κB pro-inflammatory gene regulation. Preliminary results show that *in vitro,* in immortalized mouse colonocytes (IMCs), 2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide (TPCA), an inhibitor of IκB kinase (IKK)-mediated NF-κB activation, is sufficient to suppress TNF-α induction of CCL20 and CXCL5 chemokines. Importantly, TGF-β appears to synergize with TPCA to inhibit chemokine gene expression. Therefore, the mechanism by which SMAD4 regulates gene expression appears to intersect with NF-κB for specific gene targets. Further, 3-months treatment with daily intraperitoneal (IP) injection of TPCA in SMAD4 deficient mice did not prevent tumor formation as compared to vehicle-treated mice. These findings suggest that the loss of the tumor suppressor role of SMAD4 may be independent of NF-κB. Ongoing studies will further determine whether inhibition of STAT3 or NF-κB or reduction of colonic bacterial populations reduces tumor incident after induction of colonic injury in the *Smad4*-deficient mouse model. Further studies seek to understand more about the mechanisms by which SMAD4 regulates inflammation and colitis-associated carcinogenesis in response to microbiome and host-dependent inflammation in the colon.

232 CX3CR1+ macrophages: regulators of tertiary lymphoid organs in an infectious inflammatory bowel disease model
Maryknoll Palisoc

CX_3CR1+ macrophages: regulators of tertiary lymphoid organs in an infectious inflammatory bowel disease model

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Inflammatory bowel disease (IBD) is a group of idiopathic conditions characterized by chronic inflammation of the gastrointestinal tract. Persistent inflammation in IBD causes changes in tissue morphology, which includes the development of tertiary lymphoid organs (TLOs) containing aggregates of lymphocytes and high endothelial venules (HEVs). TLOs

exacerbate tissue injury by recruiting naïve and central memory T lymphocytes to the site of inflammation. Unfortunately, the cellular mechanism involved in TLO formation and function remains unclear. This study shows that CX_3CR1+ macrophages play an important role in TLOs in an established murine colitis model.

To model mucosal inflammation observed in IBD, streptomycin-pretreated CX_3CR1-GFP mice were infected with *Salmonella typhimurium*. Colons were harvested and used for immunostaining, gene expression, and immunosorbent assays. The number and size of B220+ B cell aggregates in the colonic mucosa increased within 10 days after infection compared to uninfected mice. The aggregates were confirmed to be tertiary lymphoid follicles as they developed around MAdCAM-1+ high endothelial venules (HEVs) and contained CX_3CR1+ macrophages, B220+ B cells, and CD4+ T cells. Isolated TLO B cells significantly increased the expression of isotype switching genes, *Aicda, aGT,* and *PST* compared to non-TLO B cells (*p*

To confirm if TLOs were the main site of *Salmonella*-specific IgA response, B cells from TLOs, Peyer's patches, and non-germinal center (GC) mucosa were re-stimulated ex vivo via CD40 ligand. Although not significant, TLOs provided the highest number of B cells expressing *Salmonella*-specific IgA (~30 per 10^5 cells) compared to Peyer's patches (~10 per 10^5 cells) and non-GC B cells (~7 per 10^5 cells).

Finally, detection of CX_3CR1+ macrophages in TLOs prompted the investigation of their role using mice depleted of intestinal macrophage (ΔMΦ), which were subsequently infected with *Salmonella typhimurium*. ΔMΦ mice demonstrated significantly decreased number and size of TLOs post-infection (*p*3CR1-GFP mice. *Salmonella*-specific IgA production was also significantly diminished in ΔMΦ mice colonic mucosa (*p*

In conclusion, this study underscores the importance of CX_3CR1+ macrophages in the development and function of tertiary lymphoid organs in inflamed colonic mucosa, which is an important hallmark of IBD. Repeating the experiments above using mice depleted of CX_3CR1+ macrophage only is necessary to show their precise importance in IBD. Lastly, studying a non-infectious IBD models such as dextran sulfate sodium and trinitrobenzene sulfonic acid colitis is crucial to determine if CX_3CR1+ macrophages will demonstrate similar effects in the absence of an active intestinal infection. Nevertheless, despite these limitations, our study demonstrates that a subset of macrophages play an important role in the pathogenesis of IBD as supported by the presence of CX_3CR1+ macrophages in TLOs with IgA-secreting plasma cells and the decrease of TLO development and IgA expression in ΔMΦ mice.

233 Investigating the effects of Vibrio cholerae cholera toxin on intestinal stem cell differentiation and proliferation using human intestinal enteroids

Heidi S. Park

Investigating the effects of *Vibrio cholerae* cholera toxin on intestinal stem cell differentiation and proliferation using human intestinal enteroids

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Cholera is an acute gastrointestinal illness caused by ingestion of food or water contaminated by the Gram Negative bacterium *Vibrio cholerae*. It is estimated that 3 to 5 million people are infected by *V. cholerae* annually, resulting in over 100,000 deaths globally. Although oral cholera vaccines are available, they confer only around 60% efficacy in adults and even less in children. Better vaccine development and alternative approaches to control cholera are needed. Studies show that *V. cholerae* preferentially colonizes intestinal crypts, where intestinal stem cells (ISCs) are located. This suggests that the bacteria may target ISCs. Indeed, studies show that the *V. cholerae* virulence factor cholera toxin (CTX) suppresses intestinal stem cell division and proliferation in fruit flies. However, it is unclear whether CTX has the same effects in the human intestine. Therefore, we have used human intestinal enteroids to investigate the effects of CTX on intestinal stem cell differentiation and proliferation. Enteroids are 3D spheroid cultures derived from human intestinal stem cells. They recapitulate normal intestinal physiology and can be differentiated into many of the cells that are found in the mature intestinal epithelium, making them a realistic and tractable model to study host-pathogen interactions. By furthering our understanding of *V. cholerae* pathogenesis, we hope to identify a novel molecular pathway for developing new cholera therapeutics and vaccines.

234 Characterizing human target cell infection by three geographically distinct isolates of Mayaro virus

Aum Patel

Characterizing human target cell infection by three geographically distinct isolates of Mayaro virus

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Background: Mayaro virus (genus *Alphavirus,* family *Togaviridae*) is an emerging arthropod-borne virus transmitted by *Haemagogus* mosquitoes in sylvatic regions of Central and South America. Like Chikungunya virus, Mayaro virus (MAYV) infection leads to fever, maculopapular rash, and arthralgia. Limited data exist pertaining to regional differences in MAYV in vitro infectivity in human cells. Here we describe viral kinetics, cytopathic effects, and human target cell susceptibility to three geographically distinct MAYV isolates represented genotypes D and L (Uruma, Peru and Brazil).

Methods: MAYV susceptibility of key human target cells (human dermal fibroblasts, human embryo kidney cells (HEK293), monocytes and skeletal muscle satellite cells) as well as Vero E6 cells was visualized using immunofluorescence confocal microscopy, and quantified by flow cytometry at 0, 24, 48 and 72h post infection (p.i.). Viral kinetics were determined for each cell line from 0h to 72h p.i. at MOI=1, followed by viral plaque assays in Vero E6 cells to determine viral titers. Cytopathic effect was observed and compared across viral isolates and cell lines

by staining with crystal violet.

Results: Immunofluorescence and flow cytometry revealed that human dermal fibroblasts, skeletal muscle satellite cells and Vero E6 cells were all susceptible to each MAYV isolate, though to differing degrees (MAYV-Uruma > MAYV-Peru > MAYV-Brazil). HEK293 became infected at significantly lower rates, and monocytes were nearly refractory to infection. Viral replication kinetics assays revealed that peak viral titers occurred for all three viral isolates around 24h p.i, reaching $1x10^8$ pfu/ml. MAYV-Uruma reached this peak the fastest, followed by MAYV-Peru and then MAYV-Brazil. Crystal violet staining also demonstrated lower viral pathogenesis with greater cell survival and decreased cell apoptosis for MAYV-Uruma, Peru, and Brazil, respectively.

Conclusions: These results indicate that MAYV can infect human dermal fibroblasts, which are abundant at the initial site of exposure. Further, skeletal muscle satellite cells are quite susceptible to MAYV, in keeping with clinical symptoms associated with this virus. Some differences in infectivity are apparent across different MAYV isolates and may contribute to variable virulence and pathogenicity. These findings advance our understanding of MAYV infection of human target cells and provide some initial data with regards to MAYV phenotypic variation according to geography.

235 Comparing Documentation and Progression of Patients' Mobility in the Cardiac ICU after Improving the Mobility Protocol
Chirag Patel

Comparing Documentation and Progression of Patients' Mobility in the Cardiac ICU after Improving the Mobility Protocol

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Background: Mobility is an essential part to a patient's recovery in the Cardiac Intensive Care Unit (CICU). Patient mobility leads to decreased morbidity and mortality. Vidant Medical Center uses a protocol known as Greenville Early Mobility Scale (GEMS) to progress a patient's mobility. A previous Plan-Do-Study-Act (PDSA) cycle in the Cardiac ICU found that the GEMS protocol was able to identify a patient's ability to mobilize but was unclear on the exercises the patients should be performing; thus, the ICU Mobility Protocol: VMC Early (IMPROVE) movement program was created to remedy this issue and increase documentation of patient's mobility in the Cardiac ICU. The project recently completed its fifth PDSA cycle which focused on gathering data to determine how effective the IMPROVE movement program was in increasing documentation and progression on the GEMS.

Methods: A retrospective analysis was performed comparing 26 patients from July 2017 and July 2018. The number of times activity was documented and the total GEMS level progression was collected for each patient. A Two Sample T-test Assuming Equal Variances was conducted between the July 2017 and July 2018 data to determine statistical significance.

Results: The number of times activity was documented was 58 in July 2018 and 38 in July 2017; the p-value comparing this data was 0.13. The total GEMS levels progression was 26 in July 2018 and 21 in July 2017; the p-value comparing this data was 0.46.

Discussion/Conclusions: Although there is a trend towards a benefit with the protocol on documentation and progression of mobility in the CICU, further PDSA cycles must be implemented to perfect the protocol and obtain significant results. Moving forward, after obtaining feedback from nurses, our next PDSA cycle will focus continuing unit education and a training session on the protocol. We also plan to improve the EHR interface to make it easier to document patient mobility over time. We believe these two changes could increase compliance with the protocol; we will implement these changes in our sixth and seventh PDSA cycle.

236 Determining the distal effects of gut microbiota on tumor microenvironment, cancer progression, and checkpoint blockade efficacy
Evan Patel

Determining the distal effects of gut microbiota on tumor microenvironment, cancer progression, and checkpoint blockade efficacy

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Despite impressive efficacy of immune checkpoint blockade in certain solid malignancies, there is poor consistency across many cancers. There is discrepancy between patients with remarkably similar histologic/genetic disease, and some patients demonstrate inexplicable recrudescence after several months despite lifelong remission in others. Clinicians have so far relied on biomarkers demonstrating some utility (e.g. programmed death-ligand 1 [PD-L1] staining) as well as disease states that are especially responsive (e.g. mismatch repair [MMR] deficient). However, data show a poor overlap between many such ostensibly related disease attributes. As already suspected in bone marrow transplant patients, the diversity and specific quality of the gut microbiota is heavily implicated in this form of cancer immunotherapy. Multiple research and clinical groups have recently demonstrated the phenomenon of differential therapeutic response after modulation of the microbiota. There is some consensus that either gnotobiotic conditions or imposed antibiotic treatment abrogates baseline therapy responsiveness (corroborated by retrospective analysis of patient cohorts also receiving antibiotics). However, there is significant discrepancy with each group's metagenomic analysis as to what the putative bacterial organisms are. Several groups have also taken stool from responding and non-responding patients, and upon fecal matter transfer into mice, have recapitulated therapeutic efficacy or lack thereof. Although the above work has highlighted this exciting phenomenon, there is no consensus on the various mechanisms so far proposed. Furthermore, there is also no consensus as to which microbes produce the most dramatic phenotype in this setting.

This research project aims to initiate focal autochthonous lung tumors via the Kras-p53 (KP) transgenic model with physiologic growth kinetics, immune microenvironment, and mutational burden. In modern iterations, lung tissue in a KP transgenic animal is intratracheally inoculated with an adenovirus or lentivirus, delivering Cre recombinase to induce expression of the K-ras mutant allele and to induce loss of p53. While KP transgenic animals enable genetically defined autochthonous tumors, previous work has shown that such tumors are not particularly immunogenic. Therefore, we will utilize the same viral vectors to not only deliver Cre to the lung parenchyma but to also deliver Cas9 and a guide RNA to knock out the MMR protein Msh2. We will then quantitatively screen tumor growth kinetics (via bioluminescence and MRI) during checkpoint blockade therapy across gut microbiota manipulations (broad antibiotics, mono- colonization, consortia-colonization). After establishing a robust model, we aim to characterize the mechanism of microbiota-dependent modulation of checkpoint blockade. While the primary site of biology is unclear, proposed mechanisms include augmented dendritic cell function, improved CD8 priming in draining nodes, molecular mimicry of epitopes, or decreased regulatory T cells. To characterize this phenomenon, the lung tumor microenvironment will be analyzed with conventional phenotyping of the immune cells by flow cytometry, whole-transcriptome analysis, and ultimately single-cell RNA sequencing.

238 A role of isolevuglandin-adducts in systemic lupus erythematosus-associated hypertension and inflammation
David M. Patrick

A role of isolevuglandin-adducts in systemic lupus erythematosus-associated hypertension and inflammation

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Immune activation contributes to hypertension and its attendant end-organ damage. The formation of reactive oxygen species (ROS) within antigen presenting cells, such as dendritic cells (DCs) and monocytes, contributes to hypertension. Our group showed that oxidation products of arachidonic and other fatty acids, termed isolevuglandins (IsoLG) lead to formation of protein adducts that are immunogenic. IsoLG modified peptides are presented as neoantigens and can activate a subset of T cells, resulting in tissue inflammation and hypertension. Importantly, adoptive transfer of DCs from hypertensive mice prime hypertension to subpressor doses of angiotensin II, and this can be prevented if the donor mice are treated with the isoLG scavenger 2-hydroxybenzylamine (2-HOBA). 2-HOBA also lowers blood pressure in several hypertensive models. Hypertension, vascular and renal inflammation are characteristics of systemic lupus erythematosus (SLE), a multisystem autoimmune disease that is complex and poorly understood. We demonstrate that isoLG-adducted peptides are markedly enriched in monocytes from patients with SLE compared to matched healthy controls. We also demonstrate isoLG enrichment in monocytes and plasma cells from *B6.SLE123* mice, a mouse model of SLE, when compared to wild-type *C57BL/6* mice. Scavenging of isoLG with 2-HOBA reduces blood pressure, improves renal function, and reduces anti-nuclear-antibody titers in a mouse model of SLE. Moreover, 2-HOBA reduces bone marrow plasma cells and improves endothelium-independent vasodilation in isolated mesenteric vessels. We also demonstrate that patients with essential hypertension or SLE generate IgG antibodies against isoLG-adducted protein, further demonstrating the immunogenicity of isoLG adducts. Finally, we have identified specific isoLG modified peptides in monocytes from SLE patients by mass spectrometry. These findings support a role of isoLG-adducts in the genesis and maintenance of autoimmunity and its associated cardiovascular complications.

239 Mucus Matters: Ferrets Demonstrate Restrictive Lung Physiology, Sustained Fibrosis, Mucociliary Decrement in Airways, and Aberrant Repair Following Bleomycin-Induced Pulmonary Fibrosis
Jacelyn E. Peabody

Mucus Matters: Ferrets Demonstrate Restrictive Lung Physiology, Sustained Fibrosis, Mucociliary Decrement in Airways, and Aberrant Repair Following Bleomycin-Induced Pulmonary Fibrosis

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Rationale: A gain-of-function promoter variant for MUC5B is a strong risk-factor for the development of idiopathic pulmonary fibrosis (IPF); yet, the role of MUC5B mucin in IPF pathogenesis is unknown. Ferrets, unlike mice, have submucosal glands and human-like distribution of MUC5B in the lung. We hypothesize the mucus microenvironment matters for the initiation and propagation of fibrosis in IPF, and developed a novel bleomycin(BL)-exposed ferret model to test whether it exhibits injury-repair patterns more akin to human IPF pathophysiology.

Methods: BL (5U/kg) or saline-control(SCt) was administered via intratracheal microspray to wild-type (WT) ferrets. Fibrosis was assessed with μCT, hydroxyproline (Hyp), and histology. Respiratory system mechanics (inspiratory capacity(IC), compliance(Crs), and elastance(Ers)) were measured by forced-oscillation-technique using the FlexiventFX6. Muc5B, alpha-smooth-muscle-actin(aSMA), acetylated tubulin(AT) and club-cell-secretory-protein(CCSP) expression were assessed by immunohistochemistry (IHC) and immunofluorescence (IF). Mucociliary transport (MCT) rate and ciliary beat frequency (CBF) were assessed

ex-vivo using micro-optical coherence tomography (μOCT).

Results: Apparent at 2wks, ground-glass opacities persisted through 6wks on μCT. Volumetric, threshold-based μCT analysis revealed increased fibrosis in BL lungs (increased 18.1±2.2% vs -0.8±0.8% in SCt, P2O BL vs 4.3±0.35 mL/cmH_2O SCt, N=7/group, P=0.026), and increased Ers (0.27±0.05 cmH_2O/mL BL vs 0.23±0.02 cmH_2O/mL SCt, N=7/group, P=0.026), demonstrating restrictive lung physiology. IF revealed collagen-rich matrices, scattered myofibroblasts, aSMA$^+$ fibroblastic foci (FF), and diffuse expression of Muc5b in areas of severe interstitial fibrosis. BL-ferrets had abnormal expression of mucin-rich proximal airway markers in cystic distal airspaces and aberrant AT+ ciliation of CCSP+ epithelium, akin to MUC5B positive honeycomb change and bronchiolization of the distal lung. μOCT demonstrated reduced CBF in the bronchi at 3wks and 6wks post-BL exposure (mean decrement -2.4 and -1.8 Hz BL vs. SCt, P

Conclusion: BL-exposed ferrets exhibit features of IPF not found in rodent models and may be related to human-like Muc5b expression: FF, mucin-rich honeycomb cysts, bronchiolized distal airspaces, and sustained fibrosis associated with aberrant lung and mucociliary physiology. Our ongoing studies are investigating fibrosis development and mucociliary physiology contemporaneously in BL-exposed ferrets with genetic and pharmacologic modulation of Muc5b expression to elucidate the role of Muc5b microenvironments in pathogenesis of fibrosis and dysregulated repair.

240 Novel role for DAXX in survival of breast cancer stem cells by Endocrine therapy
Daniel S. Peiffer

Novel role for DAXX in survival of breast cancer stem cells by Endocrine therapy

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Background: Endocrine therapy (ET)-associated resistance and tumor recurrence remain clinical challenges for women with estrogen receptor-positive (ER+), metastatic breast cancer. Breast tumors are inherently heterogeneous and a subset of multipotent cells within the tumor referred to as breast cancer stem cells (BCSCs) are thought to be responsible for treatment resistance. BCSCs require Notch signaling and determining mechanisms that contribute to Notch activation may elucidate a new therapeutic target. Through an unbiased gene expression approach, DAXX was identified to be a novel Notch target gene, whose expression inversely correlated with Notch inhibition in a human pre-surgical biomarker trial of ER+ breast cancer patients (ClinTrials.gov NCT00756717). High DAXX expression is prognostic for longer recurrence free survival. Findings from the current study demonstrate for the first time that stabilization of the DAXX protein is dependent on activation of ERa by estrogen. Based on these findings, we hypothesize that targeting ERa by ET depletes DAXX, resulting in Notch activation and enrichment of BCSCs.

Methods: A panel of ER+ breast cancer cells were treated with physiologic levels of 17β-estradiol (E_2) at 5nM or deprived of estrogen, mimicking the use of an aromatase inhibitor. Expression of Notch4 and other BCSC-associated genes were quantified by real-time PCR. BCSC survival was assessed by the mammosphere forming assay. To test if ET increases BCSC by depleting DAXX, DAXX was knocked down with DAXX-specific siRNA under ET and 5nM E_2 conditions. To assess the role of DAXX in tumor initiating potential, an extreme limiting dilution assay was conducted using ER+ cells injected into mammary fat pads of female mice. As DAXX is known to function as a transcriptional repressor, nuclear levels of DAXX protein were assessed by cell fractionation.

Results: Activation ERa increases DAXX protein expression and nuclear localization, but potently inhibits BCSCs and stem cell associated gene transcripts. Antagonizing ERa by ET decreases DAXX protein levels, but conversely increases survival of BCSCs and expression of BCSC-associated genes in three ER+ breast cancer cell lines. Depletion of DAXX mimics the phenotype of ET, thus resulting in increased BCSCs and stem-cell gene transcripts to levels. In agreement, *in vivo* xenograft tumor initiating studies showed that ER+ breast cancer cells-depleted of DAXX have a higher estimated stem cell frequency, increased tumor burden, and significantly shorter rate of tumor onset compared to DAXX-expressing cells.

Conclusions: E_2-mediated activation of ERα increases nuclear DAXX protein levels to repress transcription of BCSC-associated genes, survival of BCSCs, and tumor initiation potential. Importantly, ET depletes DAXX, relieving repression of BCSC-associated genes, promoting BCSC-survival, and potentially facilitating breast cancer recurrence. Thus, new DAXX-promoting therapeutic strategies during ET treatment may prevent induction of BCSC gene expression and enrichment of BCSCs, improving therapeutic outcomes for women with ER+, metastatic breast cancer.

241 The Glucocorticoid Receptor Is Essential For TGFβ And p38 MAPK Mediated Cancer Phenotypes In Triple Negative Breast Cancer
Carlos J. Perez Kerkvliet

The Glucocorticoid Receptor Is Essential For TGFβ And p38 MAPK Mediated Cancer Phenotypes In Triple Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is the most metastatic and deadliest breast cancer (BC) subtype, accounting for 20-30% of all BCs. There is a critical need to identify molecular targets that could be exploited

as new biomarkers of TNBC prognosis and for improving therapies. Although TNBC lacks estrogen and progesterone receptors, 15-40% of TNBC patients express the glucocorticoid receptor (GR). Women with TNBC that express high levels of GR have poor outcomes. We hypothesize that GR is a key mediator of advanced cancer phenotypes in TNBC. Specifically, we propose that GR acts as a "sensor" for stress signaling pathways commonly activated by soluble factors that are abundant within the tumor microenvironment (TME). Using TNBC models, we showed previously that GR is phosphorylated on Ser134 by p38 MAPK in response to cellular stress stimuli such as hypoxia. Herein, we show that pS134-GR is elevated in TNBC tumor tissue samples relative to non-TNBC tissues. In vitro studies in TNBC models demonstrate that GR Ser134 phosphorylation is promoted by cytokines (TGFb), and growth factors (HGF) and occurs in the absence of GR ligands such as Dexamethasone or cortisol. In response to stress signaling inputs, studies with kinase inhibitors confirmed that p38 is required for GR Ser134 (pS134-GR) phosphorylation. To evaluate the functional significance of pS134-GR, we created CRISPR models of MDA-MB-231 TNBC cells expressing either wt GR or phospho-mutant GR that cannot be phosphorylated at Ser134 (S134A). RNAseq studies were performed to identify pSer134-GR target genes in TNBC models. Our transcriptome data demonstrated a requirement for pS134 GR in the expression of gene sets associated with TGFβ and p38 MAPK signaling. Pathway analysis revealed that pS134 GR target genes primarily regulate cancer cell migration. *In vitro* assays revealed that pS134-GR is essential for inducing cell migration and anchorage-independent growth in TNBC cells, even in the absence of exogenous GR ligands. Furthermore, using co-IP assays, we identified that upon phosphorylation at Ser134, GR interacts with the scaffolding protein 14-3-3z. Like pS134-GR, 14-3-3z is highly expressed in TNBC when compared to non-TNBC patients. We observed co-recruitment of both pS134 GR and 14-3-3z to known pS134-GR target genes (i.e. PTK6) in TNBC cells. These data prompted us to test the requirement for 14-3-3z in GR-mediated phenotypes. Short hairpin RNA knockdown experiments demonstrated that expression of 14-3-3z is required for serum-induced TNBC cell migration. We conclude that the pS134-GR/14-3-3z complex is a key "sensor" of local stress signals within the TME (TGFb) and a potent mediator of cell migration in TNBC models. Further studies are aimed at exploring pS134 GR as a biomarker and therapeutic target in TNBC.

242 Many-body chromatin interactions in super-enhancer TADs

Alan Perez-Rathke

Many-body chromatin interactions in super-enhancer TADs

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Chromatin interactions are thought to be important for gene regulation via enhancer-promoter looping as well as for critical functions such as cellular specialization. There is now emerging evidence that many-body (>2) chromatin interactions may be an important feature of super-enhancer (SE) regions - for example, condensing the SE region into a cohesive transcriptional apparatus. Chromosome conformation capture techniques such as Hi-C have greatly contributed to our understanding of the chromatin folding landscape. However, Hi-C has limitations as it only captures pairwise chromatin interactions and the interaction frequencies mostly represent population averages. Therefore, it is generally not possible to directly infer the existence of significant many-body chromatin interactions. With the goal of solving these problems, we have developed a computational model which utilizes physical properties of chromatin folding (e.g. nuclear confinement and self-avoidance) as well the experimental Hi-C data to reconstruct the corresponding ensemble of 3-D polymers. We deeply sample from a Bayesian generative model to infer the existence of significant many-body chromatin interactions in topologically associating domains (TADs) bounding SE regions. Specifically, we investigate: i) the prevalence of significant many-body chromatin interactions beyond random polymer folding; ii) the extent of enrichment of many-body interactions in SE regions; iii) which epigenetic markers are predictive of many-body interactions. Our analysis is performed on GM12878 and K562 cell lines at 5 KB resolution.

243 Restoration of cardiac function in a human troponin T knockout model following lentiviral transgene delivery

Anthony M. Pettinato

Restoration of cardiac function in a human troponin T knockout model following lentiviral transgene delivery

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Mutations in components of the sarcomere, the contractile unit of cardiomyocytes, are a leading cause of genetic cardiomyopathies, including dilated (DCM) and hypertrophic (HCM) cardiomyopathy. These inherited myocardial diseases are significant contributors to heart failure burden and result from abnormal sarcomere content and cardiomyocyte morphology. However, the exact mechanism of how perturbed sarcomere biology leads to cardiomyocyte and myocardial pathology is unknown, due in part to models that lack scalability and translational relevance, such as mouse models and skeletal muscle cells, which have divergent sarcomere gene components. Fortunately, modern advances have produced human induced-pluripotent stem cell (iPSC) models that can be propagated at-scale to generate iPSC-derived cardiomyocytes (iPSC-CMs), which have been used to model normal and pathological cardiac physiology. As such, we believe that this *in vitro* model offers a robust platform for interrogating human sarcomere and cardiomyocyte biology. To test this, we are focusing on human *TNNT2*, the gene encoding cardiac troponin T (cTnT), a key component of the troponin-tropomyosin complex that controls thin filament regulation of calcium-mediated cardiac contraction. *TNNT2* mutations are estimated to account

for 5-30% of HCM, and are also a frequent cause of DCM. We have used CRISPR/Cas9 to engineer a homozygous *TNNT2* knockout human iPSC line (*TNNT2-/-*) for the production of *TNNT2-/-* iPSC-CMs. These *TNNT2-/-* iPSC-CMs express no cardiac troponin T, do not form sarcomeres, and produce no contractile force. Upon lentiviral transduction with wildtype *TNNT2*, cTnT expression, sarcomere structure, and contractile function are restored. This unique sarcomere "switch" paves the way for novel interrogation of the sarcomere and its importance to human cardiomyocyte biology. We will further characterize these sarcomere-deficient *TNNT2-/-* iPSC-CMs by assessing changes in transcriptomics (e.g. RNA-Seq), cell cycle regulation, and protein-protein interactions following introduction of transgenic *TNNT2*. This will provide fundamental understanding of the multi-dimensional role of the sarcomere and *TNNT2* in cardiomyocyte development and function, which could generate insights into the mechanism of cardiomyopathy development.

244 Heat shock protein-90 inhibition reverts IL-13- and IL-17-induced airway goblet cell metaplasia
Alejandro A. Pezzulo

Heat shock protein-90 inhibition reverts IL-13- and IL-17-induced airway goblet cell metaplasia

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Goblet cell metaplasia is a disabling hallmark of chronic bronchitis, T_h2-low asthma, and cystic fibrosis. There are no curative treatments are available for most diseases with chronic goblet cell metaplasia. To identify novel therapeutic targets for goblet cell metaplasia, we studied the transcriptional response profile of asthmatic airway epithelia in vivo and IL-13-exposed primary human airway epithelia in vitro. A perturbation-response profile connectivity approach identified geldanamycin, an inhibitor of heat shock protein-90 (HSP90), as a candidate therapeutic target. Exposure to IL13 induced upregulation of HSP90 protein in most apical surface human airway epithelial cells. Our experiments confirmed geldanamycin and other HSP90 inhibitors prevented IL-13-induced goblet cell metaplasia in vitro and in vivo. Geldanamycin also reverted established goblet cell metaplasia. Geldanamycin did not induce overt goblet cell death, did not solely block mucin synthesis, and did not block IL-13 receptor-proximal signaling. Geldanamycin affected the transcriptome of airway cells when co-exposed to IL-13 but not when exposed to vehicle. We performed signaling assays for various known HSP90 clients. We found that geldanamycin blocked signaling steps shared with inflammatory triggers other than IL-13, including IL-17 and TNFα. We predicted that geldanamycin would revert IL-17-induced goblet cell metaplasia; a prediction our experiments confirmed. Our findings suggest that persistent airway goblet cell metaplasia requires HSP90 activity and that HSP90 inhibitors will revert goblet cell metaplasia despite active upstream inflammatory signaling. We speculate that HSP90 modulates a cell identity switch between ciliated or secretory and goblet cells. Moreover, HSP90 inhibitors may be a therapeutic option for airway diseases with goblet cell metaplasia of unknown mechanism.

245 PD-ligands diverged in placental mammals and dimerize via transmembrane domains
Elliot A. Philips

PD-ligands diverged in placental mammals and dimerize via transmembrane domains

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PD-1 is an inhibitory receptor on T lymphocytes that is critical for modulating adaptive immunity and has been exploited for cancer immunotherapy. PD-L1 and PD-L2 are ligands for PD-1, the former ubiquitously expressed in inflamed tissues and the latter restricted to antigen presenting cells (APCs), suggesting non-redundant function. We show that the 3-fold affinity advantage of PD-L2 is the consequence of two opposing features, a $W110_{PD\text{-}L2}$ 'elbow' and a C-D region 'latch,' that evolved simultaneously with the emergence of placental mammals, but alone do not mediate differential function. We found that PD-L1 and PD-L2 homo- and heterodimerize in *cis*, that this dimerization is mediated by their transmembrane domains, and that constructs mimicking the heterodimeric form are more active than either homodimer. We conclude that it is the propensity of PD-ligands to heterodimerize that mediates the unique immune checkpoint requirements of APCs.

246 Peer mentoring program for first and second year medical students in an MD-PhD program
Andrew J. Phillips

Peer mentoring program for first and second year medical students in an MD-PhD program

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During the 2017-2018 academic year, we created a peer mentoring program for first and second year medical students in the University of Nebraska Medical Center's (UNMC) MD-PhD Scholars Program. This program serves several purposes: to help incoming MD-PhD students with the transition to medical school, to train current MD-PhD students

in teaching, leadership and communication skills, and to build relationships and a sense of community within the program.

We designed this program to be student-led and student-run. Its members include student leaders, peer mentors, and incoming MD-PhD students. Student leaders who have completed the pre-clinical training serve as experienced individuals who monitor the programs progress and success, offering suggestions and feedback to peer mentors. Peer mentors meet directly with incoming students on a regular basis. This hierarchical structure provides students involved in the mentoring program with direct ownership over its success.

Mentoring takes place on a weekly basis, with opportunities to meet with peer mentors outside of the scheduled time. Two student mentors rotated weeks, allowing the mentees to get different perspectives. Each session began with the opportunity to ask questions about class content. Then mentors would discuss high-yield or challenging topics using comprehensive images, figures or text to help tie ideas together, Step 1 review material to emphasize importance, and finally, questions to practice test-taking skills and assess understanding of concepts. While structure exists within this program, a great deal of autonomy is given to mentors, allowing them to create their own schedules and to adapt their teaching style in order to best meet the needs of different learning styles. Student leaders are in regular contact with MD-PhD program directors and peer mentors, which has allowed the program to evolve and develop significantly.

During its first year, both group and one-on-one meetings were held. Group meetings occurred more frequently at the beginning of the year, allowing students to benefit from each other's questions and approaches to remembering key concepts and details. As students became more comfortable with medical school and what was needed from them, weekly meetings became more targeted in a one-on-one environment. Overall, this program promotes several levels of student career development by enhancing understanding of medicine, mentoring, and leadership. We intend for this program to continue to develop and evolve for years to come. Here, we present the idea of an MD-PhD mentoring program in which peer mentors work with M1 and M2 students to make the transition to medical school and preparation for USMLE Step 1 a smoother process.

247 Optogenetic activation of mouse airway parasympathetic neurons to provoke bronchoconstriction
Alexandra B. Pincus

Optogenetic activation of mouse airway parasympathetic neurons to provoke bronchoconstriction

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Background: Parasympathetic nerves innervate airway smooth muscle and cause bronchoconstriction. Studies of these important nerves, and of disease-related changes in their function, have been limited by methodologies that are unable to select for specific subpopulations of neurons to be activated. While optogenetics approaches could overcome this problem, until recently this has been largely limited to the central nervous system.

Aim: Develop a tool for stimulating airway parasympathetic neurons in vivo, and test whether these nerves are more reactive after acute allergen challenge.

Methods: Transgenic mice with the light-activated cation channel channelrhodopsin 2 expressed under a choline acetyltransferase promoter were used to selectively stimulate acetylcholine-producing neurons. We used laser-scanning confocal microscopy to verify the localization of these channels to airway parasympathetic nerve ganglia. Before nerve stimulation, some mice were treated with house dust mite, a common household allergen. To measure bronchoconstriction, mice were anesthetized and mechanically ventilated, and their airway pressures were monitored continuously while an LED 460nm light source was positioned ventral to the trachea. Pulse trains of light of varying frequency and duration were used to activate the airway nerves. Some mice were given physostigmine to inhibit acetylcholine breakdown and guanethidine to deplete catecholamines.

Results: Light stimulation of transgenic mice caused bronchoconstriction. Physostigmine enhanced the airway response to light, while bronchoconstriction was blocked by atropine, confirming that acetylcholine release was responsible. Optimal parameters for light stimulation of airway parasympathetic neurons were found to be 20Hz and at least 20 seconds of duration. No effect was seen with guanethidine alone. Mice treated with house dust mite had an increased bronchoconstriction response to light.

Conclusions: We have shown for the first time that post-ganglionic parasympathetic neurons can be activated optogenetically. We have shown that an increased response to acute allergen challenge is at least partially mediated by airway parasympathetic nerves, and we are equipped to investigate this further in the future. This method also has the potential to be used to investigate the function of other neuronal phenotypes in the airways, including peptidergic and nitrergic, by expressing the channelrhodopsin in appropriate neurons.

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248 Targeting METTL3 in Neuroblastoma
Monica M. Pomaville

Targeting METTL3 in Neuroblastoma

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Neuroblastoma (NB), a malignancy of the developing sympathetic nervous system, is the most common extracranial solid tumor in children and has a survival rate of 6-methyladenosine (m^6A) position. m^6A modifications serve to regulate posttranscriptional expression of proteins by regulating RNA stability, mRNA biogenesis, and decay to influence a

diverse set of biological processes. METTL3 is highly expressed in cancerous tissue compared to normal tissue, and METTL3 has specifically been found to enhance translation in lung cancer. Analysis of publicly available microarray data revealed that METTL3 mRNA expression level is elevated in a subset of patients with NB and that high METTL3 expression significantly correlates with poor patient survival. Therefore, we hypothesize that METTL3 regulates translation in NB to affect protein expression of NB drivers such as MYCN and ALK.

Our preliminary studies demonstrated that shRNA-mediated knockdown of METTL3 in MYCN-amplified NB cells revealed a significant decrease in cell viability. Subsequently, we mapped m^6A methylation on NB cell lines using methylated RNA immunoprecipitation sequencing (MeRIP-Seq) and identified MYCN and ALK as two of the top heavily methylated genes with m6A modifications. Protein expression, but not mRNA expression, of MYCN and ALK were diminished upon METTL3 knockdown, consistent with the role of METTL3 in translation. Depletion of MYCN protein expression using a dox-repressible system led to decreased METTL3 mRNA expression, implying an additional role for MYCN regulation of METTL3 mRNA expression.

These results implicate METTL3 as a crucial component in regulating viability of MYCN-amplified NB cells by its effect on protein expression of key oncogenes. Further research will be conducted to understand the molecular function of METTL3 and its significance in regulating expression of oncogenic drivers in NB.

249 Comprehensive metagenomic characterization of the cancer microbiome and its clinical applications
Gregory D. Poore

Comprehensive metagenomic characterization of the cancer microbiome and its clinical applications

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Cancer has long been heralded as a disease of the human genome. Recent literature, however, has begun to broaden that view, suggesting that the development, progression, and treatment resistance of solid and hematologic malignancies may indeed be influenced by its microbial environment. In order to systematically characterize this 'cancer microbiome,' we re-examined The Cancer Genome Atlas (TCGA) compendium of treatment-naive, whole genome sequencing and transcriptomic sequencing datasets for microbial reads. This included 18,116 samples across 10,481 patients and 33 tumor types. Briefly, we mapped all non-human reads against ~54,000 viral and bacterial metagenomes using the Kraken algorithm. Direct alignments with reads from four cancer types selected for prior microbial signatures or viral-mediated carcinogenesis (cervical, stomach, ovarian, and non-small cell lung cancers) confirmed the accuracy of this method for identifying tumor-related microbes. Cognizant of technical variation among sequencing centers, platforms, and experimental designs, we developed a secondary pipeline to quantify and remove batch effects while simultaneously increasing the signal attributed to biological variables. Using this normalized dataset, we persuasively demonstrated the validity of our putative cancer microbiome profiles using (i) known clinical metadata (e.g. prior hepatitis B infection in liver cancer patients), (ii) alternative microbial detection pipelines (i.e. *de novo* assembly methods, PathSeq algorithm), and (iii) data from the NIH's Integrative Human Microbiome Project. Finally, we trained machine learning models on these microbiome profiles to determine if we could distinguish between treatment-naive primary tumors and adjacent normal solid tissue as well as clinically relevant variables (e.g. tumor stage) within and across, each cancer type. Notably, we report high discrimination in paired tumor-versus-normal comparisons (Avg. AUROC=0.94, n=19 cancer-types) and primary tumor one-cancer-type-versus-all-others (Avg. AUROC=0.97, n=32 cancer-types) solely using normalized microbial abundances. These results pave the way for broadly characterizing and exploiting cancer's 'second' genome, enabling microbially-based diagnostics, prognostics, and therapeutics to improve patient outcomes.

Disclosure(s): GDP and RK jointly filed U.S. Provisional Patent Application Serial No. 62/754,696 using this work.

250 CEP290 localization in the rod connecting cilium of CEP290rd16 mice with fluorescence nanoscopy
Valencia L. Potter

CEP290 localization in the rod connecting cilium of *CEP290*^{rd16} mice with fluorescence nanoscopy

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Mutations in *CEP290* account for roughly 25% of cases of Leber Congenital Amaurosis (LCA), a disease resulting in blindness in the first year of life. CEP290 has been proposed to function in a structural role in primary cilia, linking the microtubule doublets to the ciliary membrane; however, its actual function in the photoreceptor connecting cilium (CC) and link to disease is unclear. In this study, we used the *CEP290*^{rd16} mutant mouse, an animal model for LCA. The *CEP290*^{rd16} allele encodes a CEP290 protein with an in-frame deletion of the microtubule binding domain. We hypothesized that CEP290 and CEP290 interacting partner NPHP5 mislocalize in *CEP290*^{rd16}photoreceptors and that this mislocalization contributes to the rapid retinal degeneration in the *CEP290*^{rd16} animal. To obtain sub-diffraction resolution images, we used Structured Illuminated Microscopy (SIM) and Stochastic Optical Re-

construction Microscopy (STORM) in age-matched wild-type and mutant mice. In order to localize CEP290 prior to retinal degeneration, retinas from 10-day old mice were immunostained with antibodies for CEP290 and antibodies to other CC proteins. In wild-type rod cilia, we found that CEP290 and NPHP5 localize throughout the length of the CC and between the microtubule doublets of the axoneme and the ciliary membrane. Prior to retinal degeneration in *CEP290*^{rd16}animals, CEP290 and NPHP5 exhibited normal localization. Our results indicate that the mutant CEP290 does not prevent formation of the CC or the localization of proteins within the CC, and that the retinal degeneration in *CEP290*^{rd16} animals is not due to CEP290 mislocalization. Thus, degeneration must be due to the affected function or mislocalization of other proteins.

251 Inhibition of pilus assembly by the small molecule nitazoxanide
John J. Psonis

Inhibition of pilus assembly by the small molecule nitazoxanide

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Uropathogenic *Escherichia Coli* (UPEC) is the primary causative agent of urinary tract infections (UTIs), which afflict more than 50% of women and 15% of men. Critical to the establishment of UTIs by UPEC are pili. The pili (or fimbriae) expressed by UPEC are virulence-associated surface structures that are assembled by the chaperone/usher (CU) pathway and mediate bacterial adhesion to host cells. We have discovered that the small molecule nitazoxanide (NTZ) inhibits pilus biogenesis in UPEC by interfering with proper maturation of the usher protein in the bacterial outer membrane (OM). The usher is required for assembly of the pilus fiber and secretion of the fiber across the OM to the cell surface. No small molecule compound other than NTZ has been reported to inhibit the assembly of the usher or any other β-barrel protein for that matter. The usher is folded and inserted into the OM by the ß-barrel assembly machinery complex (Bam), which in *E. coli* consists of five proteins, BamA-E. We have shown that the inhibitory effect of NTZ on usher folding into the OM is dependent on BamB and BamE. Moreover, the sensitivity of OM usher levels to the effect of NTZ is dependent on the levels of Bam complex expression, suggesting a possible mechanism by which NTZ inhibits pilus biogenesis. Using saturation transfer difference (STD)-NMR spectroscopy, we have generated evidence for the direct binding of NTZ to both the complete Bam complex and to the central BamA component alone. These experiments identified the nitrothiazole ring of NTZ as directly involved in the drug-target interaction. Based on this analysis, we are screening NTZ analogs and have identified compounds that contain the nitrothiazole ring and exhibit more potent activity against the usher compared to NTZ. In contrast, compounds lacking the nitrothiazole ring have no appreciable effect on usher levels. To identify the specific binding site of NTZ, we are pursuing a genetic screen to isolate bacterial mutants that are resistant to NTZ. Using fluorescence-activated cell sorting, we have isolated mutagenized bacterial cells that maintain high OM usher levels in the presence of NTZ. Whole genome sequencing of these resistant mutants will help to identify genes involved in usher folding and NTZ activity, and possibly the specific binding site of the drug. We are also using a murine model of ascending urinary tract infection to evaluate the efficacy of nitazoxanide against *E. coli* pathogenesis in the urinary tract. These studies will help to identify the mechanism of action of NTZ, and will facilitate the design of new therapeutic agents that target bacterial adhesion. In addition, these studies will reveal new details by which proteins such as the usher fold in the OM.

252 BLAzER: A versatile and efficient workflow for analyzing PET neuroimaging data in Alzheimer's disease
Fabio Raman

BLAzER: A versatile and efficient workflow for analyzing PET neuroimaging data in Alzheimer's disease

Fabio Raman[1,2], Sameera Grandhi[1,2], Chad Murchison[2,3], Richard Kennedy[2,3], Susan Landau[4], Erik D. Roberson[2,5,6], Jon McConathy[1,2], and Alzheimer's Disease Neuroimaging Initiative

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Objective: The semi-automated Biomarker Localization, Analysis, Visualization, Extraction, and Registration (BLAzER) workflow allows for rapid evaluation of amyloid– and tau–PET images, combining a set of features well-suited for both clinical and research workflow. The purpose of the study was to assess BLAzER for regional brain PET quantification using participants with different cognitive statuses from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. Additionally, we determined whether different segmentation inputs, FreeSurfer and Neuroreader, can be used and provide similar results with our workflow.

Methods: 127 amyloid–PET and 55 tau–PET studies along with corresponding volumetric MRI were selected from ADNI. The BLAzER workflow begins with segmentation of MR images by FreeSurfer v6.0.0 (Boston, MA) or Neuroreader (Horsens, Denmark). Segmented output files along with source MR and PET scans are then visualized and quantified using an automated workflow on FDA-approved software (MIM v6.6.13, Cleveland, OH), enabling quality control to ensure optimal registration.

Results: For efficiency, Neuroreader was faster than FreeSurfer on a per case basis (15 min/case vs. 12 hours/case) yet slower for total processing time for batches of studies (45.5 vs. 12 hours) due to parallelizing on FreeSurfer. For reproducibility, all amyloid– and tau–PET showed strong agreement between operators (ICC > 0.97). For global accuracy, BLAzER showed strong agreement with ADNI for amyloid–PET (r=0.9922, p 0.97, p

Conclusions: BLAzER provides an efficient workflow for regional brain PET quantification. FDA-approved components and the ability to visu-

alize registration reduces barriers between research and clinical applications.

253 Characterizing the role of cancer-associated fibroblasts in anti-melanoma immunity
Julie Y. Ramseier

Characterizing the role of cancer-associated fibroblasts in anti-melanoma immunity

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The complex interplay between tumor cells and surrounding host tissue strongly influences tumor initiation and progression. Cancer-associated fibroblasts (CAFs) are ubiquitous in the tumor microenvironment, yet their precise role in regulating tumor growth and anti-tumor immunity remains unclear. Although many reports have implicated CAFs as tumor-promoting due to their pro-tumorigenic secretome, recent studies have also suggested that CAFs may regulate anti-tumor immunity, highlighting their emerging role as complex mediators of cancer progression.

We investigated the properties and functions of CAFs in anti-melanoma immunity using the previously described YUMMER1.7 model, a neoantigen-rich melanoma cell line syngeneic to C57BL/6 mice with human-relevant driver mutations (*Braf*^{V600E}, *Pten*$^{-/-}$, *Cdkn2a*$^{-/-}$). YUMMER1.7 tumors were grafted into C57BL/6 mice and harvested 22 days after injection. The tumor's invasive margin was dissected from the tumor core and single-cell RNA sequencing was performed on each fraction. While fibroblasts were found to be abundant at the tumor's immune-infiltrated invasive margin, few fibroblasts were recovered from the center of the tumors, a spatial finding that was also observed in immunohistochemical analysis of fibroblasts in YUMMER1.7 tumors.

To better understand the temporal context of CAF localization within immunosuppressive and immune checkpoint inhibitor-treated tumor microenvironments, mice with YUMMER1.7 tumors were treated with anti-CTLA-4 plus anti-PD-1 and compared to control, untreated mice. Tumors were harvested every two days following tumor implantation and treatment. Immunohistochemistry revealed that at early time points, CAFs are evenly distributed throughout the tumor. However, by day 9 post-engraftment, CAFs are excluded from the center of the untreated tumors and remain concentrated at the tumor periphery as the tumor escapes immune surveillance and continues to grow. In checkpoint inhibitor-treated tumors, we observed a CAF proliferation that corresponded with the height of the anti-tumor immune response (day 13 post-injection), which formed infiltrative cords and bands of CAFs throughout the center of the tumor.

These preliminary results suggest that effective anti-tumor immune responses induced by immunotherapy drastically alter fibroblast abundance and distribution, which we hypothesize could be due to crosstalk between fibroblasts and key players of anti-tumor immunity. Ongoing studies are probing the functional role of fibroblasts in the anti-melanoma immune response and determining the signaling mechanisms that may mediate the interaction of CAFs with other cell populations in the microenvironment, which will expand our understanding of the functions of CAFs in melanoma.

254 APC-β-catenin-TCF Signaling Silences the Intestinal Guanylin-GUCY2C Tumor Suppressor Axis
Jeffrey A. Rappaport

APC-β-catenin-TCF Signaling Silences the Intestinal Guanylin-GUCY2C Tumor Suppressor Axis

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Colorectal cancer is the fourth most common cancer and the second leading cause of cancer death in the United States. In >90% of sporadic colorectal tumors, transformation begins with mutations in APC or its degradation target β-catenin, producing a gain-of-function in TCF-dependent nuclear transcription that drives tumorigenesis. However, mechanisms coupling these mutations to tumorigenesis continue to be refined. GUCY2C is the membrane-bound guanylate cyclase expressed by intestinal epithelial cells, and serves as the receptor for the hormone, guanylin, secreted by the colorectal epithelium. The guanylin-GUCY2C signaling axis contributes to intestinal homeostasis by regulating the continuous regenerative cycles of proliferation, migration, and differentiation that maintain intestinal epithelial architecture. GUCY2C signaling inhibits proliferation by decreasing β-catenin and its transcriptional targets, such as cyclin D1 and MYC. Conversely, silencing GUCY2C produces crypt hyperplasia, DNA damage, cell cycle acceleration, and metabolic reprogramming to a Warburg glycolytic phenotype characteristic of transformed tissue. Interestingly, guanylin, but not GUCY2C, is among the most commonly lost gene products in colorectal cancer in humans and mice. Furthermore, GUCY2C agonists reduce epithelial transformation in genetic, carcinogen, and inflammatory mouse models of intestinal tumorigenesis. Together, these observations suggest a pathophysiological model in which transformation reduces guanylin expression, silencing GUCY2C signaling and driving tumorigenesis.

In the present studies, we tested the hypothesis that APC-β-catenin signaling suppresses guanylin via TCF-dependent transcriptional regulation. We observed GUCY2C retention, and guanylin mRNA and protein elimination at the earliest stages of tumorigenesis in human samples of sporadic and hereditary colorectal cancers. Further, intestine-specific biallelic APC inactivation in mice led to guanylin loss and silencing of GUCY2C signaling. We directly tested the individual roles of APC, β-catenin, and TCF4 in the regulation of guanylin expression using human colon cancer cell models harboring mutant APC or β-catenin. These cells were devoid of guanylin at baseline, and normalization of APC-β-catenin signaling with wild type APC, β-catenin shRNA, or a dominant negative isoform of TCF4 restored guanylin expression. Furthermore, metabolic labeling of newly synthesized RNA transcripts in these cells revealed reconstitution of nascent guanylin mRNA synthe-

sis, suggesting that dysregulated APC-β-catenin signaling suppresses guanylin hormone expression as part of its canonical reprogramming of nuclear transcription. Together, these findings suggest a mechanistic basis for guanylin hormone loss early in transformation, silencing GUCY2C signaling, and lifting a block on tumorigenesis. Critically, this novel step in tumor formation represents a therapeutic opportunity where reconstitution of GUCY2C signaling with oral agonists could replace lost guanylin. Linaclotide (Linzess™) and plecanatide (Trulance™) are FDA-approved oral GUCY2C agonists that could be leveraged for hormone replacement therapy, transforming colorectal cancer from an irreversible disease of genetic mutation, to a reversible syndrome of hormone insufficiency.

255 Hyperoxia-induced soluble Guanylyl Cyclase (sGC) dysfunction in developing airway involves the Calcium Sensing Receptor (CaSR)
Jovanka Ravix

Hyperoxia-induced soluble Guanylyl Cyclase (sGC) dysfunction in developing airway involves the Calcium Sensing Receptor (CaSR)

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Background: Hyperoxia with/without respiratory support is a vital intervention following premature birth. However, early oxygen exposure leads to subsequent airway hyperreactivity, remodeling (proliferation, fibrosis) and asthma; necessitating understanding of mechanisms that promote contractility/remodeling, vs. bronchodilation/anti-remodeling. Here, airway smooth muscle (ASM) is a key cell type. In adult ASM, we previously found the extracellular calcium sensing receptor (CaSR) is important. Given higher Ca^{2+} in developing lung, studies suggest CaSR in fetal ASM (fASM) is important, and enhanced by hyperoxia. Conversely, we find that the bronchodilatory NO-sGC-cGMP axis in adults is dysfunctional in prematurity and with oxygen, and thus direct sGC activation may be beneficial (e.g. cinaciguat, BAY58). In this study, we tested the hypothesis that CaSR and sGC dysfunction are linked in enhanced contractility and remodeling.

Methods: Human fASM cells were isolated from canalicular stage (18–22 week gestation) lung tissue following fetal demise (StemCell Express; Mayo IRB exempt). Cells were exposed to 21% O_2 vs. 50% O_2 (hyperoxia) for 48h with/without heme-independent sGC activator BAY58 or with/without CaSR antagonist NPS2143 (10uM). Expression of CaSR and sGC isoforms, and changes in cGMP, p-VASP-Ser 239 (PKG target) and extracellular matrix deposition were assessed. Extracellular Ca^{2+} was altered (0, 0.5, 1 or 2 mM) with/without CaSR agonist R568 (10 uM) vs. antagonist NPS2143 (10uM), and concurrent presence of BAY58. $[Ca^{2+}]_i$ responses to 10uM histamine were recorded in fura-2AM loaded cells.

Results: Hyperoxia increased fASM expression of CaSR but not sGC isoforms. Both NPS2143 and BAY58 individually suppressed hyperoxia effects on collagen deposition and $[Ca^{2+}]_i$ responses to histamine. $[Ca^{2+}]_i$ responses to agonist increased with extracellular Ca^{2+}: exacerbated with hyperoxia or R568, but suppressed by NPS2143. In the presence of R568, BAY58 was without effect but in the presence of NPS2143, BAY58 more potently reduced $[Ca^{2+}]_i$ than by itself. Neither NPS nor R568 altered expression of sGC isoforms.

Conclusion: In developing human ASM, CaSR regulates $[Ca^{2+}]_i$, with enhanced effects in hyperoxia. sGC effects on fASM are blunted by CaSR, with greater impact in hyperoxia. These novel data point to the potential for concurrent application of CaSR antagonists with sGC activators to overcome effects of hyperoxia in developing airways.

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256 Tumor "faces" improve digital pathology deep learning
Rishi R. Rawat

Tumor "faces" improve digital pathology deep learning

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Inspired by facial recognition, we define an analogous problem, tissue recognition, in digital pathology. In tissue recognition, the objective is to learn distinctive histologic features that can identify or cluster tissues from the same patient. Unlike supervised classification problems in pathology, tissue matching can be trained in an unsupervised manner, making it an attractive way to learn from vast troves of unlabeled hematoxylin and eosin (H&E) stained pathology images. However, bigger data doesn't always lead to better accuracy, as we discovered in our early tissue matching experiments. In fact, it wasn't until we added a style-transfer approach to explicitly normalize and remove batch effects that we saw the impact of training on very large histology datasets. Incorporating these observations, our final neural network learned histologic features that could match tissues to patients with 93% accuracy (n=104 patients, baseline accuracy

257 Characterizing dynamic functional connectivity changes following a physiological stressor in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Gulf War Illness
Rakib U. Rayhan

Characterizing dynamic functional connectivity changes following a physiological stressor in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Gulf War Illness

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Gulf War Illness (GWI) are phenomenological disease states with similar phenotypes characterized by chronic widespread pain, fatigue, and dyscognition. A shared syndromic feature of both patient populations is post-exertional malaise (PEM). PEM is defined as an exacerbation of baseline symptoms following a physically taxing or cognitively demanding activity. We previously reported a novel paradigm that modeled this hallmark symptom by utilizing fMRI scans taken before and after sub-maximal exercise. Prior studies analyzing resting state scans have led to inconsistent results of both increased and decreased functional connectivity in ME/CFS and GWI. This may be due to the methodologies used for analysis, as functional connectivity indices were averaged over the entire duration of scanning sessions. Recently, resting-state fMRI experiments have reported meaningful changes in correlational patterns that occur within one session. This dynamic behavior of functional connectivity has not been explored in ME/CFS or GWI. Dynamic functional connectivity (dFC) was assessed using the resting states scans acquired before and after exercise. The functional brain data was decomposed into components. Subsequent analysis then computed changes within session using sliding window analysis (40 s in length). Analysis of data revealed ME/CFS and GWI subjects had altered dFC in the Default Mode Network (DMN) compared to controls. Exercise-induced alterations of dFC within large scale neural networks provides further evidence of PEM in ME/CFS and GWI. While important differences have been identified, future studies should verify these findings. Taken together, our results expand on the limited knowledge regarding the effects of PEM on cognition in ME/CFS and GWI, and strongly suggests the use of dFC analyses to better account for changes in resting state functional connectivity.

258 The role of the nigrostriatal dopamine pathway in the learning and performance of complex sequential movements and their associated vigor
Tori Riccelli

The role of the nigrostriatal dopamine pathway in the learning and performance of complex sequential movements and their associated vigor

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The basal ganglia (BG) are a collection of evolutionarily conserved brain nuclei critical for diverse aspects of voluntary, purposive movement. Conflicting clinical studies show that pathological disruption of BG circuitry, such as that found in Parkinson's disease (PD), differentially impairs motor sequence learning, or performance, possibly reflecting differences in pathology or evaluation. Genetic mouse models are particularly useful for specific, reproducible perturbation of BG function as well as selective manipulation and monitoring of neuronal populations in behaving animals. However, current behavioral tasks to study the acquisition and performance of motor sequences often confound aspects of performance (regulation of kinematics) with learning (acquisition of a movement sequence representation). Here we develop a task for mice that can dissociate these aspects of a flexible motor skill while remaining tractable for recording and functional perturbation. Briefly, we developed a behavioral apparatus ("climbing wall assay"; CWA) consisting of configurable, touch-sensitive rungs allowing for unique spacing sequences that mice must traverse to obtain a liquid reward. The tilt of the CWA can be changed thereby dissociating kinematics from the specific sequence of rung positions. Performance can be accurately tracked using deep learning to track individual body parts producing 3D trajectories. We first confirmed that wild type mice exhibit classic indicators of motor skill learning on the CWA. Measurements of motor skill learning include both measurements over the whole sequence, and measurements of individual limb trajectories. With training, the average speed of sequence completion significantly increased while time spent on individual sensors decreased to approximately 500ms in expert mice. Mice also made fewer gross errors with training and overlapping motor programs became evident. For individual limb trajectories, average speed increased while error and variance decreased, indicating that trajectories were becoming more stereotyped. Finally, after a minor sequence change, mice made more gross errors than on the previously learnt sequence, indicating that learning was sequence specific. Overall, we find that in fewer than 300 trials of our task mice dramatically increase the speed with which they can traverse the rungs while simultaneously increasing the accuracy of individual limb trajectories. In ongoing experiments, we are selectively removing dopaminergic inputs to the dorsal striatum with pharmacological, cell-type specific lesions. In addition, we have developed hardware to allow extracellular recording and imaging on the CWA without impeding precise motor control. These methods will allow us to examine population activity of identified BG cell populations during the acquisition and performance of movement sequences. In summary, we describe a novel apparatus that dissociates the learning and performance of sequential movements, allowing us to more accurately determine the role of the dorsal striatum and dopaminergic neurons in the performance of complex motor sequences.

259 Cellular expression profile of the polymeric immunoglobulin receptor in human lungs
Bradley W. Richmond

Cellular expression profile of the polymeric immunoglobulin receptor in human lungs

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The polymeric immunoglobulin receptor (pIgR) maintains homeostasis in small airways by facilitating transcytosis of polymeric immunoglob-

ulin A across the airway epithelium. Reduced pIgR is common in the small airways of patients with chronic obstructive pulmonary disease (COPD) and preclinical models directly implicate loss of pIgR in COPD pathogenesis. However, the cellular expression profile of *PIGR* remains poorly defined. To address this, we quantified *PIGR* mRNA by RNA in situ hybridization (ISH) and single-cell RNA sequencing (scRNA-seq) using lung tissue from non-diseased deceased organ donors whose lungs were declined for transplantation (n=3). For RNA-ISH, probes specific for *PIGR* mRNA were analyzed in 5 micrometer distal lung sections alongside probes for *FOXJ1* (a marker of multiciliated cells, MCCs) and *SCGB1A1* (a marker of Club cells) using HALO image analysis software (Indica Labs). scRNA-seq was performed on single-cell suspensions generated from peripheral portions of donor lungs using the 10X Genomics Chromium platform and analyzed using the Seurat package in R. To explore factors influencing *PIGR* expression in vitro, we measured *PIGR* mRNA by RT-qPCR in HBEC3-KT cells at varying levels of confluency and in submerged and air-liquid interface (ALI) culture. In addition, we cultured primary murine tracheal epithelial cells (MTECs) from wild-type C57Bl6 mice in ALI culture with and without DAPT, an inhibitor of the Notch pathway and secretory cell differentiation. RNA-ISH showed minimal overlap between probes specific for *FOXJ1* and *SCGB1A1* mRNA in human lung sections, suggesting these canonical markers of multiciliated and Club cells label distinct cell populations at the mRNA level. *PIGR* mRNA expression correlated with *SCGB1A1* expression but not *FOXJ1* mRNA expression, although on a per-cell basis some *FOXJ1*$^{+}$ cells expressed low levels of *PIGR*. Additionally, a substantial number of cells expressed *PIGR* mRNA but not *FOXJ1*, *SCGB1A1*, or *MUC5AC* (a marker of goblet cells). scRNA-seq demonstrated *PIGR* was most highly expressed by *SCGB1A1hi* and *MUC5B*-expressing cells, in addition to a subset of *FOXJ1*$^{+}$ multiciliated cells and type II alveolar epithelial cells (AECs). In HBEC3-KT cells, *PIGR* was only highly expressed at 100% confluency and correlated closely with expression of *SCGB1A1* and *MUC5AC* but not *FOXJ1*. At 100% confluency, *PIGR* was highly expressed in both submerged and ALI culture. In primary MTECs, treatment with DAPT significantly reduced pIgR expression. Together, these in vitro studies suggest *PIGR* is highly expressed in culture conditions that favor secretory cell differentiation. In summary, we found that in non-diseased human lung tissue *PIGR* is expressed by secretory cells, type II AECs, and some MCCs. In vitro experiments suggest that secretory cells are particularly important for pIgR expression in the airway epithelium. Future studies should analyze pIgR protein expression and determine how the cellular expression profile of pIgR is altered in diseases such as COPD.

261 Identifying chemoreceptors modulating respiratory behavior in Drosophila melanogaster
Douglas Rioux

Identifying chemoreceptors modulating respiratory behavior in *Drosophila melanogaster*

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In humans, breathing is a highly regulated and coordinated behavior for gas exchange. Research into nervous system control of breathing in mammals has revealed principles of organization and function, such as the existence of a central pattern generator influenced by feedback. However, due to the complexity of the mammalian nervous system, several fundamental questions remain concerning the mechanisms by which the pattern of breathing is generated, feedback is incorporated, and coordination with other behaviors is established. With its simpler nervous system and powerful genetic toolkit, the fruit fly, *Drosophila melanogaster,* provides a model system for the study of how nervous systems control respiratory behaviors. Like mammals, terrestrial insects control gas exchange between the atmosphere and their internal environment. To do so, they regulate the opening of valves in their exoskeleton, called spiracles, in a manner that is correlated with activity level and is sensitive to internal levels of oxygen and carbon dioxide.

To make inroads into the nervous system control of gas exchange in the fly, we seek first to identify the sensory input to the system: the receptors and neurons that provide feedback to the motor control system by sensing internal oxygen and carbon dioxide levels. To do so, we established a quantitative readout of gas exchange using flow-through respirometry, which measures carbon dioxide output from flies as a correlate of spiracle opening. With this assay, we have observed graded carbon dioxide output from flies presented with increasing levels of hypoxia, consistent with the hypothesis feedback from oxygen sensors. Screens of established and new genetic mutants for an oxygen chemoreceptor using this assay are underway, with special interest in the atypical soluble guanylyl cyclases implicated in sensing oxygen in *D. melanogaster* larvae and other species. Additional work to identify the muscles and motor neurons controlling spiracle movement is also in progress, with an eye towards obtaining electrophysiological measurements of spiracle control as a more direct method of measuring respiratory behavior. Once we have identified potential receptors, we can then exploit the genetics of the fruit fly to identify the neurons expressing them, perform perturbation experiments, and begin to elucidate downstream elements of the circuit. Ultimately, testing hypotheses of the control of respiratory behaviors in the fruit fly may demonstrate a framework for how sensory feedback regulates motor outputs.

262 Frequency and Genetic Diversity of Antibiotic Resistant Campylobacter jejuni in Michigan
Jose A. Rodrigues

Frequency and Genetic Diversity of Antibiotic Resistant *Campylobacter jejuni* in Michigan

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Campylobacter jejuni (C. jejuni) is a gram-negative bacterium and the leading cause of bacterial gastroenteritis in the world. Campylobacter jejuni infections are generally self-limited, however antibiotic resistant infections have been attributed to increased duration of hospitalization. The Centers for Disease Control and Prevention (CDC) has classified an-

tibiotic resistant C. jejuni as a serious threat and estimated that 31,000 infections, 13,000 hospitalizations and 120 deaths are attributed to antibiotic resistant C. jejuni annually. Campylobacter spp. recovered from patients at 4 different hospitals in Michigan between 2011 and 2014 were isolated, speciated, sequenced and examined for susceptibility to 9 antibiotics using microbroth dilution. Among all 214 isolates, 44.9% were resistant to at least one antibiotic, 16.4% were resistant to two antibiotics and 1.9 % were multi-drug resistant to 3 or more antibiotics. Whole-genome sequencing was done on a subset of 149 strains; multi-locus sequence typing loci as well as common resistance and virulence genes were extracted from the genomes. The phylogeny based on MLST data identified three distinct clusters of C. jejuni. Importantly, one cluster was comprised of 3 of the 4 multi-drug resistant strains, suggesting the dissemination of closely related resistant genotypes. Future work will link the bacterial diversity and epidemiological variables to the resistance phenotypes to elucidate relationships between virulence, severity of disease, and patient outcomes.

263 ERK-mediated repression of peroxisome proliferator-activated receptor-alpha (PPARα) promotes fatty liver disease

Jessica M. Rodriguez

ERK-mediated repression of peroxisome proliferator-activated receptor-alpha (PPARα) promotes fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis resulting in inflammation, insulin resistance, and fibrosis leading to nonalcoholic steatohepatitis (NASH). With childhood obesity on the rise, approximately 34% of overweight children are affected by NAFLD. At present, there is no approved drug for the treatment of NASH due to poor understanding of the disease pathogenesis. NAFLD occurs due to an imbalance between the fatty acid import/synthesis and export/catabolism in the liver.

In obesity and NASH, hypoxic signaling is chronically activated. Our study showed that chronic activation of hypoxia signaling decreased genes involved in fatty acid β-oxidation. Hypoxia signaling is mediated by hypoxia inducible factor (HIF)-1α and HIF-2α. HIF-mediated activation of ERK decreased genes involved in fatty acid β-oxidation through repression of the nuclear transcription factor peroxisome proliferator-activated receptor alpha (PPARα). Inhibition of ERK ameliorated hepatic steatosis in primary hepatocytes from a genetic model of spontaneous steatosis and in primary hepatocytes loaded with free fatty acids. Moreover, inhibition of ERK potentiated ligand-dependent PPARα activation. PPARα is the critical regulator of fatty acid β-oxidation during fasting. During refeeding state, β-oxidation decreases through mechanisms that are unclear. Our data shows that ERK plays a critical role in hepatic lipid homeostasis by repressing postprandial PPARα activity. Further investigation is underway to determine the mechanism of PPARα repression by ERK with an overarching goal of identifying novel therapeutic targets in the treatment of NAFLD and NASH.

264 Posttranslational modification and biochemical properties of Plasmodium falciparum hexokinase

Abu B. Rogers

Posttranslational modification and biochemical properties of *Plasmodium falciparum* hexokinase

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Development of drug resistance in *Plasmodium falciparum*, the most pathogenic malarial parasite, necessitates the search for selective and specific inhibitors of novel drug targets. *Plasmodium falciparum* hexokinase (PfHK), the enzyme responsible for the conversion of glucose to glucose-6-phosphate in glycolysis, is a promising target because the pathogenic red blood stages depend solely on glycolysis for ATP production. However, the mechanism by which the parasite regulates its glycolytic flux to compensate for its rapid growth and multiplication remains unclear. Previous studies have shown that PfHK undergoes S-glutathionylation, a posttranslational modification that adds glutathione to cysteine residues. S-glutathionylation reportedly reduces enzyme's activity and regulates oxidative stress. This suggests that the parasite utilizes S-*Glutathionylation* in PfHK to regulate glycolytic flux to maintain the ATP-ADP ratio, as the demands for ATP changes. Using immunoprecipitation, kinetic assays, and proteomic analysis of PfHK, we seek to identify the post-translational modifications. The generation of PfHK antisera has enabled us to characterize its biochemical properties, *in vivo*. Current data show that native PfHK is a tetramer, with biochemical properties similar to recombinant PfHK. In addition, the presence of a reducing agent increases the activity of PfHK lysates. This supports our hypothesis that the cysteine residue in the monomeric form engages in disulfide linkage. Determining the role of S-glutathionylation and characterizing other PTMs in the different stages of the *P. falciparum* could elucidate new therapeutic avenues, which would support our continued efforts to develop PfHK inhibitors as antimalarial therapeutics.

265 Engineered human cardiac microtissues to study dilated cardiomyopathy genetic and allelic heterogeneity

Robert Romano

Engineered human cardiac microtissues to study dilated cardiomyopathy genetic and allelic heterogeneity

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Heart failure (HF) is an epidemic that affects five million patients in the United States and has a similar mortality rate to cancer (~50% in 5

years). Dilated cardiomyopathy (DCM), a predominant form of HF, is a genetic condition that affects 1:250 individuals. DCM is associated with high morbidity and mortality, and is characterized by heart chamber enlargement and impaired contraction. DCM is frequently caused by inheritance of autosomal dominant mutations in sarcomere genes that encode for protein components of the contractile unit of the cardiomyocyte. Currently, it is not known how mutations in distinct sarcomere genes lead to DCM and whether mutation localization, such as mutation in distinct structural domains within the same protein, modifies disease severity, treatment response and DCM pathogenesis. For example, truncation mutations in the giant sarcomere gene titin (*TTN*) are the most common mutations identified in DCM patients, but surprisingly have also been found in the apparently normal population without cardiac disease. In addition, missense mutations in the cardiac beta-myosin heavy chain gene (*MYH7*) are also a cause of DCM, but have been associated with higher DCM penetrance compared to *TTN* mutations. Here, we hypothesize that the role of DCM genetic and allelic heterogeneity can be identified by engineering human cardiac microtissues differentiated from induced pluripotent stem (hPS) cells that have been genetically modified by CRISPR/Cas9 technology to contain human DCM sarcomere mutations in *TTN* and *MYH7*. Through a combination of cardiac microtissue physiological assays including contractility, calcium handling and structural analyses, as well as RNA sequencing and cell signaling assays, we aim to uncover new insights into DCM pathogenesis in a biomimetic three-dimensional context. Using CRISPR/Cas9 technology applied to hPS cells, we have engineered an isogenic *TTN* truncation mutation and two *MYH7* mutations located within the actin-binding and ATPase domains that are most enriched for pathogenic cardiomyopathy mutations. Our experience with generating sarcomere mutations has revealed preliminary insights into mechanisms of homologous recombination at the *MYH7* locus. Moreover, we have performed a comparative analysis of genome editing methods to identify an optimized strategy to introduce patient-specific missense mutations that have been previously technically challenging. Finally, with these novel human cell models in hand, we can now generate cardiac microtissues with DCM-associated *TTN* and *MYH7* mutations to elucidate the role of genetic and allelic heterogeneity in DCM and treatment responses within a human *in vitro* model system. Insights from this study will enhance our understanding of DCM pathogenesis, ultimately to inform more precise treatment strategies for patients with heart failure.

266 Local perturbations in cortical excitability propagate along specific resting state functional connectivity networks

Zachary P. Rosenthal

Local perturbations in cortical excitability propagate along specific resting state functional connectivity networks

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The balance of excitation and inhibition in brain circuitry has been shown to play a key role in mesoscale network function, plasticity, and injury/repair processes in human disease. For example, imbalanced excitability is known to hinder clinical outcomes after stroke, traumatic brain injury, and seizure, however it remains unclear how focal changes in excitability affect global brain network activity and function. In this study we investigate how manipulating inhibitory circuitry (specifically parvalbumin inhibitory interneurons in the mouse whisker barrel sensory cortex) more broadly affects cortical network dynamics and behavior. We aim to understand how local E/I imbalance in the somatosensory cortex impacts 1) patterns of spontaneous activity in the brain at rest (e.g. resting state functional connectivity), 2) excitability of the cortex in response to sensory stimulation, and 3) sensorimotor behavioral functions corresponding to both balanced and imbalanced networks. To answer these questions, we use chemogenetics to bidirectionally manipulate parvalbumin interneuron firing rates, combined with widefield optical neuroimaging of both hemodynamics and neural calcium dynamics in the cortex of awake animals. We reveal that runaway excitability in the somatosensory cortex can propagate across the brain to distant functional connected brain networks and differentially enhance or weaken mesoscale synchrony depending on anatomical microcircuit wiring. In addition, we demonstrate that chemogenetic manipulation of parvalbumin interneurons leads to dramatic plasticity in functional connectivity, suggesting a potential target for therapy for neurologic diseases that disrupt healthy patterns of network synchrony across the brain. These experiments will lay the groundwork for future studies investigating how precise manipulation of excitability in cortical microcircuits can enhance plasticity and recovery after injury.

267 Dual Inhibition of Phospholipase D1 and D2 reduces metastasis and tumor growth by limiting metabolic flexibility

Eric Roth

Dual Inhibition of Phospholipase D1 and D2 reduces metastasis and tumor growth by limiting metabolic flexibility

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Breast cancer cells must adapt with and manipulate the environment to grow and metastasize. The two classic isoforms of phospholipase D (PLD) each regulate multiple oncogenic traits such as inducing angiogenesis and sustaining proliferative signaling. PLD1 and PLD2 do so by hydrolyzing abundant phosphatidyl choline to produce a short lived second signaling lipid, phosphatidic acid (PA). Although differences in localization of activity produce isoform specific effects, some phenotypes are more pronounced or only occur when both isoforms are inhibited. This possible compensatory mechanism would suggest the anti-tumor effects of inhibiting PLD reported by previous studies

might be improved by dual inhibition. Double knockout of PLD1 and PLD2 in MMTV-PyMT mice results in a delay of palpable tumor formation, reduces tumor growth as measured by calipers and at dissection, and fewer lung metastases. This attenuation was found to be caused in part by a restriction of metabolic flexibility. Such aberrations were probed with the Seahorse cellular efflux system, combined with nutrient deprived conditions. Our findings show PLD inhibition limits metabolic flexibility, shifting cells to a glycolytic dependency. This may provide an opportunity for therapies combining PLD and metabolic inhibition to further slow tumor growth, minimize metastasis, and kill cancer cells by targeting energetic dependencies.

268 Stereotactic navigation using 7T MRI for deep brain stimulation surgery
Aaron E. Rusheen

Stereotactic navigation using 7T MRI for deep brain stimulation surgery

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Use of magnetic resonance imaging (MRI) is critical to stereotactic and functional neurosurgery for navigation and accurate targeting of specific neurologic structures. This work is focused on deep brain stimulation (DBS) where targets such as the subthalamic nucleus (STN) are difficult to visualize with 3T MRI. Because these targets are not directly visible, indirect targeting methods and difficult intra-operative microelectrode recordings are necessary for placement of DBS electrodes. Here, we test the hypothesis that the increased static magnetic field strength (B_0) of 7T MRI, which increases the SNR and contrast-to-noise ratio, can resolve the STN and provide the necessary detail to improve neuronavigation. To utilize 7T MRI, a new skull-contoured and skull-mounted localizer was designed and 3D printed to be accommodated in the reduced bore of a Siemens™ 7T MRI and related transmit-receive RF coil. The novel localizer was affixed with point fiducials and tested on human cadaveric head specimens for image co-registration in both clinical 7T (Terra, Siemens™) and 3T (Prisma, Siemens™) MR scanners using FGATIR, MP2RAGE, MPRAGE, T2 Axial, and 3D FSE pulse sequences. 3D Slicer software was used for image co-registration and the IGT module was used for targeting. Image co-registration was achieved with our localizer and an average fiducial registration error (FRE), a marker of distortion, of 5.7 mm was found across pulse sequences using a rigid transformation. While both the FGATIR and MP2RAGE sequences offered improved image resolution for STN identification, the FGATIR sequence had reduced FRE and better fiducial localization comparatively. In addition, the IGT module was successfully able to generate X, Y, and Z coordinates for targeting. These findings demonstrate that co-registration and neuronavigation was successful with our new localizer for 7T clinical applications and that 7T MR offered enhanced spatial and contrast resolution, which may permit direct targeting of the STN.

269 CD103+ dendritic cells are not required for gut commensal-specific peripheral Treg cell differentiation
Emilie V. Russler-Germain

CD103$^+$ dendritic cells are not required for gut commensal-specific peripheral Treg cell differentiation

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Peripheral differentiation of regulatory T (pTreg) cells in response to food and commensal bacterial antigens is necessary to prevent intestinal inflammation, and is commonly thought to be mediated by a distinct subset of intestinal dendritic cells (DCs) characterized by CD103 expression. However, the roles of different DC subsets in the in vivo induction of commensal-specific pTreg cells have not been formally established. Unexpectedly, we found that all subsets of colonic migratory DCs, not just the CD103$^+$ subset, carry colonic *Helicobacter spp.* antigens and activate naïve *Helicobacter*-specific T cells ex vivo. Loss of CD103$^+$ DCs results in altered *Helicobacter* antigen presentation in vivo, but surprisingly *Helicobacter*-specific T cells are still able to differentiate into pTreg cells. These data indicate that CD103$^-$ DCs are capable of inducing tolerogenic pTreg cells and imply that for at least two gut commensal bacterial antigens, a specific "tolerogenic" DC subset is not the primary driver of pTreg cell differentiation.

270 Reprogramming adult-born dentate granule cells to generate inhibitory interneurons to treat temporal lobe epilepsy
Bryan E. Ryba

Reprogramming adult-born dentate granule cells to generate inhibitory interneurons to treat temporal lobe epilepsy

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Mesial temporal lobe epilepsy (mTLE) is the most common form of epilepsy in adults, and yet more than 40% of patients with mTLE fail medical therapy. mTLE is characterized by seizure activity and pathology within the medial temporal limbic regions, including the hippocampal formation. Aberrant hippocampal neurogenesis, which results in dramatic alterations in the migration and wiring of immature adult-born dentate granule cells (DGCs), is thought to be a major contributor to hippocampal hyperexcitability and epileptogenesis in mTLE. Strategies involving ablation of immature DGCs or introduction of inhibitory GABAergic interneurons to the dentate gyrus (DG) via fetal stem cell grafts miti-

gate seizures in rodent models of mTLE, but have significant limitations. Recent attempts at in vivo reprogramming of native cells in the brain to overcome these issues, moreover, have suffered from a lack of target selectivity and an inability to control cell fates with sufficient precision.

We proposed to use retrovirus to selectively target adult-born DGCs in vivo and induce overexpression of a complement of multipotency-promoting and inhibitory neuronal transcription factors, reprogramming these cells into GABAergic inhibitory interneurons and achieving measurable decreases in seizure frequency in a rodent model of mTLE. We hypothesized that by using retrovirus to transform a well-defined population of native adult-born cells, we would be able to implement a less severe reprogramming strategy than previous efforts at in vivo reprogramming.

Multiple retroviral plasmid constructs were designed to express various combinations of early neural developmental regulators and multipotency-promoting factors, such as SOX2. Others were designed to express various combinations of inhibitory neuronal transcription factors thought to be important for specifying interneuron fate in the medial ganglionic eminence of the developing brain, such as DLX5. Constructs were packaged into retrovirus via an optimized polyethylenimine-based transfection method, coupled with concentration via ultracentrifugation. Pilot experiments involving stereotactic retroviral injections into the dorsal DG of wild-type C57BL/6 mice determined that large construct size was limiting viral titer, necessitating a redesign of all constructs. In vitro infection of mouse neural progenitor cells with different combinations of redesigned viruses, followed by immunohistochemical staining for inhibitory interneuron markers to identify promising reprogramming vectors, are underway. Stereotactic injections of promising viral vectors into the dorsal DG of wild-type mice, followed by brain harvest, fixation, slicing, and immunohistochemical techniques, are concurrently underway. We anticipate that our optimized retroviral design, packaging, and screening protocols will allow for rapid assessment of the reprogramming capacity of many combinations of inhibitory/multipotency-promoting factors, both in vitro and in vivo.

272 Tumor microenvironment mimetic culture aids isolation, expansion, and potency of tumor stromal progenitors from primary lung cancer resections
Douglas O. Saforo

Tumor microenvironment mimetic culture aids isolation, expansion, and potency of tumor stromal progenitors from primary lung cancer resections

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The tumor microenvironment (TME) is a complex ecosystem of tumor cells, activated fibroblasts, stem cells, and the cytokines and extracellular matrix (ECM) they produce. Cancer associated fibroblasts (CAFs) and their contribution to the TME are important in tumor progression. CAFs are hypothesized to arise from multiple progenitor cell types, including multipotent mesenchymal stem cells. Isolation of TME stroma from patients is complicated by limited availability of biopsy material and cell stress incurred during adaptation to atmospheric oxygen (20% O_2) in cell culture, limiting pre-clinical studies of sensitive tumor stromal progenitors and tumor-stromal interactions.

We hypothesized that an *in vitro* environment that mimics the physiological microenvironment of the human lung will improve isolation and expansion of tumor stromal progenitors.

We used single cell suspensions of patient primary lung tumor resections and cultivated them on plastic in normoxia (2DN, 20% O_2) or ECM in physiological hypoxia (3DH, 5% O_2). Patient tumor derived cell lines were characterized by western blot and FACS. Clonal ability was measured by colony forming assay. Stem and potency marker expression were assessed by immunofluorescence and qRT-PCR. Stromal cell lines were grown with A549 lung adenocarcinoma cells *in vitro* and subcutaneously injected into mice to assess tumor-stromal interactions. Statistical analysis was performed using one-way ANOVA with Tukey's or Sidak's multiple comparison test where appropriate.

We found that combinatorial 3DH environment increased expansion and clonal ability (pin vitro and metastasis *in vivo*.

Using an *in vitro* system that mimics the tumor microenvironment, we easily isolated and rapidly expanded stromal progenitors from patient lung tumor resections without complex sorting methods or growth supplements. These progenitors retained expression of pluripotency markers, secreted factors associated with cancer progression, and enhanced tumor cell growth and metastasis. An understanding of the biology of these progenitor cell populations in a TME-like environment may advance our ability to target these cells therapeutically and limit their effects on promoting cancer metastasis.

274 African spiny mouse (Acomys) regeneration following acute, chronic, and volumetric muscle injury
Aaron Gabriel Sandoval

African spiny mouse *(Acomys)* regeneration following acute, chronic, and volumetric muscle injury

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Regeneration is the perfect regrowth and repair of damaged tissue. Essentially, regeneration is nature's ultimate solution to wound healing. Although several animal models for regeneration exist, the African spiny mouse (*Acomys*) is the only known mammal in the world capable of scar-free skin regeneration as an adult. Discovered in 2012, the regenerative capabilities of *Acomys* are being further studied by comparing it to a normal mouse (*Mus*).

To compare ear regeneration in the two species, a hole was punched in the ears of each of the mice. Microscopic analysis of the healing ears over the course of several days revealed that *Mus* produced large amounts of collagen scarring, while *Acomys* was able to regenerate the hair, adipocytes, cartilage, and, most interestingly, skeletal muscle that

make up the ear.

Intrigued by the de novo regeneration of skeletal muscle observed in *Acomys*, we sought to further characterize *Acomys*'s ability to regenerate other types of skeletal muscle. To do so, the Tibialis Anterior (TA) leg muscles of both species were injected with cardiotoxin, a snake venom derivative that damages the muscle. It was found that regeneration is present in both but occurs much faster in *Acomys*. We immunostained for collagen XII, which is wound-induced and not present in regular, healthy muscle. Its presence in *Mus* indicated substantial scarring, whereas no such evidence of fibrosis was present in *Acomys*.

Next, we sought to determine the extent to which *Acomys* is able to regenerate in response to repeated injury. After the initial injection, the mice were given 3 weeks to heal and then were injected again. This was repeated for a total of 5 injection-healing cycles. Amazingly, even after chronic insult *Acomys* was still able to regenerate its muscle perfectly. However, *Mus* showed an intriguing result: adipocytes within the muscle. Although initially surprising, the abundance of fat cells in the *Mus* muscle is reminiscent of Duchenne muscular dystrophy. Further study of *Acomys* could give helpful insight into preventing the disease in humans.

We then looked to see whether *Acomys* could recover from volumetric muscle loss (VML) in which an entire portion of the muscle is removed. VML injuries are common in gun shot or car accident victims. Most modern therapies for these injuries are aimed at merely strengthening the remaining muscle. To simulate VML, hole punches were made in the TA muscles of the mice. Preliminary data suggests that although the regeneration is not perfect, *Acomys* shows improved regeneration compared to *Mus* following VML injury.

The results of continued study of *Acomys* could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the entire healthcare field by allowing for the regeneration of muscle and other types of tissue.

275 Antagonism of STAT1 by Nipah virus P gene products modulates disease course but not lethal outcome in the ferret model

Benjamin A. Satterfield

Antagonism of STAT1 by Nipah virus P gene products modulates disease course but not lethal outcome in the ferret model

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Nipah virus (NiV) is a highly pathogenic zoonotic agent in the family *Paramyxoviridae* that has been associated with very high case fatality rates. Previous protein over-expression studies have shown that various mutations to the common N-terminal STAT1-binding motif of the NiV P, V, and W proteins affected the ability of these proteins to bind STAT1 thus reducing their ability to inhibit type-I IFN signaling through the JAK/STAT pathway, but due to differing techniques it was unclear which amino acids were most important in this interaction or what impact this had on pathogenesis *in vivo*. We compared all previously described mutations in parallel and found the amino acid mutation Y116E demonstrated the greatest reduction in binding to STAT1 and the greatest reduction in interferon antagonism. A similar reduction in binding and activity was seen for a deletion of twenty amino acids constituting the described STAT1-binding domain. To investigate the role of the NiV P/V/W STAT1-binding motif in NiV-mediated disease, we produced recombinant (r)NiVs with complete deletion of the STAT1-binding motif or the Y116E mutation for ferret challenge studies ($rNiV_M$-$STAT1^{blind}$). Despite the reduction in the ability to inhibit IFN signaling through the JAK/STAT pathway, ferrets challenged with these $rNiV_M$-$STAT1^{blind}$ mutants had a lethal, albeit altered, NiV-mediated disease course. These data, together with our previously published data, suggest that the major role of the NiV P gene products in NiV-mediated disease in the ferret model are likely to be in the inhibition of viral recognition/innate immune signaling induction with a minor role for inhibition of IFN signaling.

276 Ground Level Falls in Patients Aged 65 and Older Treated in a Rural Level I Trauma Center: Implications For Targeting High-risk Individuals in a Falls Prevention Strategy

Seth Saylors

Ground Level Falls in Patients Aged 65 and Older Treated in a Rural Level I Trauma Center: Implications For Targeting High-risk Individuals in a Falls Prevention Strategy

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Background: Falls are the leading cause of doctor and ED visits, hospital and nursing home admissions, and accidental death in people 65 years and older. Previous studies have shown the efficacy of a structured interdisciplinary approach, including occupational therapy assessment, to reduce the occurrence of falls in the geriatric population.

Objective: This study aims to describe the current geriatric population seen for ground level falls and to identify potential targets for a future falls prevention strategy.

Methods: This retrospective chart review analyzed the demographic and clinical characteristics of 774 patients aged 65 and older that were seen and treated for ground level falls during 2015.

Results: Most of the patients in this study fell in their own homes (59.5%) and lived in Pitt county (32%) with the next most common counties being Beaufort and Lenoir (6.9% each). Most patients had previous comorbidities and on average were taking 6.5 medications prior to their fall. Patients were predominantly white (84.7%) and more frequently female (63.5%). 24.8% of patients seen had a history of previous falls

within the previous 12 months. The most common risk factors for falls that were observed in this study were patients taking ≥4 medications (82.7%), patients with history of arthritis (36.5%), patients with history of stroke/CVA (23.7%), and patients with impaired cognition (14.1%). Of the medications shown to have the strongest links to an increased risk of falling, the most prevalent medications being taken prior to falling were selective serotonin-reuptake inhibitors (28.7%), benzodiazepines (28.2%), anticonvulsants (21.5%), antidepressants (19.5%), and neuroleptic agents (12.4%). There was no significant difference in ICU or hospital length of stay in patients who received a PT consult (ICU LOS: p=0.531; Hospital LOS: p=0.96). Significant predictors of a repeat fall for patients included age (OR=1.036) and prior admission in the past 30 days (OR=6.919).

Conclusions: Current results show that a majority of the geriatric patients being treated for ground level falls are falling in their own homes. These results also suggest that a significant portion of these patients have predisposing factors known to increase risk of subsequent falls, including the use of 4 or more prescription medications. Further analysis will use these observed characteristics to implement a targeted fall prevention strategy. Success will be based on the reduction in overall occurrence of ground level falls and associated morbidity and mortality.

277 Intact islets and dispersed beta-cells show distinct differences in glucose-stimulated calcium oscillations: a case for glucose-sensitive vs. glucose-modulated beta-cells
Rachel T. Scarl

Intact islets and dispersed beta-cells show distinct differences in glucose-stimulated calcium oscillations: a case for glucose-sensitive vs. glucose-modulated beta-cells

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Pancreatic islets produce pulses of insulin and other hormones that maintain normal glucose homeostasis and prevent diseases like type 2 diabetes. Beta-cells specifically possess exquisite glucose sensing capacities allowing for precise changes in pulsatile insulin secretion in response to small changes in glucose. In recent years, evidence of heterogeneity among beta-cells has emerged in which some cells respond to rising glucose concentrations more efficiently than others, and some labs have demonstrated beta-cell "hubs" which act to orchestrate consensual and precise insulin release. This suggests that communication throughout the islet, possibly through gap junctions, is critical for successful insulin regulation When communication among these cells is disrupted, this precise glucose sensing falters giving a wide variety of responses from individual beta-cells.

In this study, we isolated mouse islets and dispersed individual islet cells onto glass coverslips. Using fura-2AM, we measured intracellular calcium patterns in 2-mM-steps from 8-to-12mM glucose and also 6-mM-steps between 0 and 16mM glucose to systematically compare glucose sensing among intact islets and dispersed islet cells derived from the same mouse pancreas.

Intact islets were uniformly quiescent below 6mM glucose and active above 8mM glucose. We confirmed that dispersed beta-cells displayed a much broader activation range of 2mM to 10mM glucose. Islets maintained 4-to-5-min oscillatory periods, whereas beta-cells maintained 7-10-min periods. Islets invariably increased the oscillatory plateau fraction for each 2mM glucose increase, whereas beta-cells produced either a similar pattern as islets (32%) or oscillations with no modulation of period or plateau fraction across glucose steps (36%). The remaining 32% of islet cells did not fall into either category due to inactivity, activity in only one glucose concentration, or activity consistent with alpha-cells or delta-cells.

We have demonstrated that dispersed beta-cells display two glucose-sensing subtypes: beta-cells that modulate their activity in response to small glucose changes and beta-cells that display no modulation despite large glucose shifts. Stem cells demonstrating this glucose-modulated response, in addition to producing insulin, should be viewed as ideal targets for the development of therapeutics for patients suffering from beta-cell failure and type 2 diabetes.

278 Role of TOX1 and STAT3 pathways in the pathogenesis of cutaneous T-cell lymphoma
Angelina M. Seffens

Role of TOX1 and STAT3 pathways in the pathogenesis of cutaneous T-cell lymphoma

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Sézary Syndrome (SS) and Mycosis Fungoides (MF) are the most common clinical variants of cutaneous T-cell lymphoma (CTCL), a group of lymphomas characterized by the accumulation of malignant T cells in the skin. The molecular and cellular etiologies of this neoplasm have remained elusive, and diagnostic, prognostic markers and therapeutic targets are lacking. Thymocyte selection associated high mobility group box 1 (*Tox1*), a transcription factor that is required to establish the CD4+ lineage, has been shown to be overexpressed in malignant cells found in the skin and blood of patients with CTCL. Knockdown of *Tox1* results in decreased malignant cell viability, while treatment with FDA-approved HDAC inhibitors results in normalization of *Tox1* expression in patient-derived cell lines. Another gene which is consistently overexpressed in patient samples is signal transducers and activators of transcription3 (*Stat3*), a transcription factor critical for the differentiation of Th17 and follicular helper T cells. Treatment of CTCL cell lines with a

STAT3 inhibitor leads to decreased cell number and increased apoptosis, demonstrating that this pathway is also important for malignant cell survival. Importantly, the Koralov lab has developed a mouse model which constitutively expresses a hyperactive STAT3 allele, STAT3C, that recapitulates several key features of MF.

To evaluate the contribution of TOX1 overexpression to CTCL pathogenesis, we have introduced *Tox1*cDNA downstream of a floxed stop cassette into the ubiquitously expressed Rosa26 locus of C57Bl/6J ES cells. We have screened the ES clones to validate the presence of the correctly targeted allele. Furthermore, we have treated targeted ES clones with a transducible Cre protein (TAT-Cre) to demonstrate appropriate deletion of the floxed stop cassette and subsequent expression of *Tox1* cDNA. We have now generated R26Tox1$^{stop^{fl}}$mice using tetraploid complementation to generate 100% ES cell derived animals. The newly generated animals will be crossed to CD4Cre and CD4Cre STAT3C-$^{stop^{fl}}$strains, thus enabling us to study the contribution of TOX1 overexpression to T cell lymphomagenesis and giving us an opportunity to examine synergy between TOX1 overexpression and hyperactive JAK/STAT signaling in CTCL pathogenesis. We hope that the newly generated mice will pave the way to a better understanding of this enigmatic malignancy and allow us to develop a relevant small animal model of this disease. The conditional gene targeted *Tox1*allele should also prove useful for analysis of the role of this protein in early hematopoiesis and other biological processes.

279 Development of a microfluidic platform as an ex vivo model of the bone marrow microenvironment in metastatic prostate cancer
Nan Sethakorn

Development of a microfluidic platform as an *ex vivo* model of the bone marrow microenvironment in metastatic prostate cancer

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Background: Bone metastases contribute to major morbidity and mortality of several primary malignancies, and often result in pathologic fractures leading to catastrophic events such as cord compression and disability. Metastatic prostate cancer is a lethal disease, and represents the second most common cause of cancer related deaths in the US. Prostate cancer in particular demonstrates bone tropism and therefore represents a useful model to evaluate the interactions between metastases and microenvironment. We have previously utilized a configurable model called STACKS to evaluate the effect of prostate and stromal compartments on expression of tumor promoting factors in macrophages. We propose to develop this platform as an *ex vivo* model of the bone marrow microenvironment in order to evaluate the complex interactions between various cell types residing in the bone marrow niche. Methods: The STACKS is a microfluidic platform that consists of modular co-culture wells stacked in a vertical array, which facilitates the use of small volumes and amounts of primary cells. This approach allows the passage of paracrine factors between layers, however each compartment can be isolated for downstream analysis. Bone marrow aspirates and core biopsies were obtained from patients through the biomarker study protocol at the University of Wisconsin. Mononuclear cells were isolated from aspirates. Stromal cells were isolated by digestion of the core biopsies and then embedded in 3D culture in Matrigel. Populations were plated in separate wells of the STACKS co-culture platform. Results: We report that primary cells isolated from human blood and bone marrow biopsies were able to be propagated both individually and in co-culture in the STACKS model. Individual cell populations were treated prior to co-culture in order to assess the effect of differentiation into polarized subtypes on prostate cancer cell phenotype. Preliminary results with tumor cell lines cultured with macrophage populations identify secreted factors that can promote tumor cell proliferation, including expression of IL-1B. Discussion: We have demonstrated that the STACKS model can be used to co-culture multiple cell types. Its configurable design allows fine temporal and spatial control over the combinations of cell types used in individual assays, as well as the ability to isolate particular compartments for detailed downstream analysis. Isolated compartments remain intact, allowing for assessment of gene expression, protein expression, imaging, and functional assays. This model is highly adaptable, and thus can be used to recreate multiple microenvironments of interest. Future directions include the use of this co-culture model to study the effect of specific microenvironment cell populations on the growth and proliferation of prostate cancer cells and identify factors that promote bone metastasis. Ultimately, this approach can be leveraged as a drug-discovery platform that recapitulates the complex effects of the microenvironment on cancer cell behavior.

280 A comprehensive analysis of transposable element-derived cryptic promoters in cancer and evaluation of therapeutic potential
Nakul M. Shah

A comprehensive analysis of transposable element-derived cryptic promoters in cancer and evaluation of therapeutic potential

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Transposable elements (TEs) represent close to half of the genome, but they are generally disregarded in cancer genomic studies due to their silencing in somatic cells. Recently, studies have uncovered examples of TEs being activated as alternative promoters of oncogenes; however, a comprehensive analysis of the prevalence and impact of this phenomenon has yet to be performed. Here, we show that the activation of TE-derived cryptic promoters is an important mechanism by which oncogenes are activated during tumorigenesis. In addition, the chimeric protein products of these transcripts have the potential serve as a novel source of tumor-specific antigens.

To detect these events, we developed a stringent computational framework to predict novel transcription start sites using RNA-sequencing

data that originated from TEs. Using this framework, we analyzed 7,769 tumors and 625 normal datasets from 15 TCGA cancer types, identifying 260 TE cryptic promoter activation events involving 174 oncogenes across 3,554 tumors. We report widespread, TE-derived cryptic promoters that boost oncogene transcription in all cancer types. Furthermore, we interrogated the *AluJb-LIN28B* candidate: the genetic deletion of the TE eliminated oncogene expression, while dynamic DNA methylation-controlled promoter activity, illustrating the necessity and sufficiency of a TE for oncogene activation.

Next, we evaluated the potential of these transcripts to generate tumor-specific antigens. We processed RNA-sequencing data of three glioblastoma cell lines and identified TE cryptic promoter activation events. We then computationally assessed the coding potential and reading frames from the transcripts generated from these events and found that they were predicted to produce novel peptides. Mass spectrometry analysis of whole lysate and MHC-pulldown peptidomes revealed that multiple novel antigens from these transcripts were not only present in the cell, but also presented by MHC molecules. These preliminary results indicate that these protein products have the potential to be targeted with immunotherapy.

Our results showcase the high prevalence of TE-derived promoter activation in cancer and suggest multiple avenues by which this phenomenon can be targeted therapeutically. In addition, we have developed computational tools to identify these events with high specificity and predict targetable antigens. Currently, we are in the process of validating the existence and antigenicity of additional candidates and evaluating their therapeutic potential.

281 Oncogenic KRASG12D regulates extracellular redox status in PDAC via TLR4/OLR1-p38-NFkB axis
Sagar Shah

Oncogenic $KRAS^{G12D}$ regulates extracellular redox status in PDAC via TLR4/OLR1-p38-NFkB axis

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease with universal resistance to conventional cancer therapies. Over 95% of these tumors exhibit oncogenic $KRAS^{G12D}$ mutations necessary for induction, growth, and maintenance. Recent studies of genetic aberrations dictating immune composition in other cancers indicate a need to explore putative pathways by which oncogenic $KRAS^{G12D}$ suppresses antitumor immune activity in PDAC. Here, utilizing an inducible Kras* PDAC model (iKras*; p48-Cre;LSL-rtTA;tet-O-$Kras^{G12D}$;LSL-$TP53^{+/-}$) we explore whether and how Kras* signaling shapes PDAC's TME, and concomitantly, whether tumor-induced leukocytes (TILs) directly augment PDAC's growth and aggressiveness. Preliminary immune profiling of the TME in our Kras* GEMM model via time of flight mass spectrometry (cyTOF/SINE) shows a preponderance of myeloid-derived suppressor cells (MDSCs, or M2 macrophages; 46.4%) and tumor-infiltrating macrophages (TAMs; 10.0%). Because these cells are known to secrete cytokines that attenuate humoral and cytotoxic immunity, we audited in vitro microarray expression patterns of ~650 mouse cytokine network genes in Kras* "on" versus Kras* "off" cell lines established from an autochthonous iKras* tumor. Among the 15 most upregulated cytokine network genes were *Olr1* and *Tlr4*, which encode redox-sensitive receptors that bind oxidized LDL (oxLDL) to preferentially regulate oxidative stress in the TME. These data were consistent with transcriptomic profiles showing *Olr1* (8.295-fold increase; $p=2.5^{-16}$) and *Tlr4* (2.216-fold increase; $p=7.12^{-5}$) overexpression in human PDAC samples compared to benign epithelium. These differentially expressed genes were then analyzed using Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA), which identified gene set enrichment of the redox-sensitive MKK3/6-p38-NFkB signaling axis. Through selective induction and extinction of Kras* signaling, we are now validating correlational changes in expression of these targets via qPCR, ELISA, and Luminex assays, after which I will determine how selectively induced Kras* cells respond to oxLDL treatment and measure phosphorylated p38, ERK1/2, and JNK in Kras* "on," "off," and oxLDL-supplemented Kras* "off" cells. These experiments will help us determine which specific pathways TLR4 and OLR4 act through in PDAC cells and whether $KRAS^{G12D}$ PDAC relies upon these pathways to exert oxidative control over the TME. Investigation of these targets via gene editing in syngeneic iKras-derived cell transplant models will follow. These experiments may better clarify the role of $KRAS^{G12D}$ signaling in modulation of PDAC's immunosuppressive TME.

282 Tumor-associated neutrophils promote a stem-cell phenotype in Glioblastoma cells via an Osteopontin-CD44 dependent manner
Sumedh S. Shah

Tumor-associated neutrophils promote a stem-cell phenotype in Glioblastoma cells via an Osteopontin-CD44 dependent manner

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Glioblastoma (GBM) is the most common and aggressive form of primary brain cancer, and despite optimized treatments, its expected median survival remains under two years. Several groups have demonstrated that GBMs contain self-renewing, tumorigenic cells known as GBM stem-like cells (GSLCs). GSLCs are implicated in tumor recurrence due to their resistance to conventional therapy, thus representing a potential avenue for therapeutic intervention. Given the recent emphasis into interaction between cancer and their microenvironment, further elucidating the complex interplay between the microenvironment and GBM cells may unlock novel targets and augment current cancer therapy.

One secreted factor identified in the GBM microenvironment is osteopontin (OPN), also known as SPP1. OPN binds to the cell-surface

receptor, CD44, and when activated, the CD44 intracellular domain is a strong mitogenic signal. Upon interrogating RNA-sequence data from an open-access GBM patient database, we found high SPP1 expression was negatively correlated with patient outcome. Therefore, we sought to identify the mechanisms of OPN-CD44 interactions necessary to promoting the stem-cell phenotype and determine the source of OPN in the GBM microenvironment.

We utilized three GBM cell lines, GBM6 (Mayo Clinic), the Denver Brain Tumor Research Group (DBTRG) line, and G55 (UCSF), and isolated the $CD133^+$ stem population in each via flow sorting. GSLCs were further characterized through qPCR for stem gene expression of NANOG, SOX2, and OCT4. CD44 expression was confirmed through immunofluorescence. We utilized Western blot analysis to determine whether exposure to soluble OPN (500ng/mL) stimulates oncogenic pathways and found increased phosphorylation of AKT and mitogen activated protein kinase (MAPK), indicating OPN-CD44 binding activates pro-tumor signaling. Similarly, cotreatment with OPN led to the increased resistance of GSLCs to supratherapeutic doses of temozolomide (50 – 500μM)—determined by MTS Assay—and promoted statistically increased sphere formation (G55; 5.1 versus 6.6 spheres, p =0.009; GBM6, 0.73 versus 1.6 spheres, p = 0.011) in the sphere-forming assay, which is used as an indication of stemness.

Through these initial experiments, we showed that OPN binding to CD44 leads to maintenance of GSLC phenotype and confers resistance to temozolomide. To determine the origin of OPN in the tumor microenvironment, we have begun to investigate the role of infiltrating immune cells and have produced preliminary data suggesting that tumor-associated neutrophils (TANs) display increased OPN gene expression when exposed to tumor-conditioned media. Our future experiments look to isolate TANs from patient samples, determine whether TANs produce quantifiable amounts of OPN, and reproduce our aforementioned results through TAN-produced OPN.

283 Jumonji-C Histone Demethylases are Cellular Iron Sensors that Control mTORC1

Jason S. Shapiro

Jumonji-C Histone Demethylases are Cellular Iron Sensors that Control mTORC1

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Glucose and amino acids are essential sources of carbon and nitrogen that form the building blocks of proteins, nucleotides and membranes. Less often considered is the role of molecular iron, an essential nutrient required for life-sustaining processes including oxidative metabolism, DNA synthesis and translation. Still, the fundamental mechanisms by which cells simultaneous sense the levels of multiple essential nutrients and integrate these signals into a cohesive output are unclear. We have shown that prolonged iron deprivation activates an mTOR-dependent adaptive program, which is critical for iron conservation and cell survival. However, how mTOR activity is regulated by iron levels is unknown. Here, we discover a novel iron sensing mechanism that responds to physiologic changes in cellular iron levels and controls mTOR activity through epigenetic silencing of critical genes at multiple levels in the mTOR pathway. We have demonstrated this regulation both *in-vitro* and in multiple vital organs from animal models of iron deficiency. Specifically, Jumonji-C domain containing histone-demethylases require direct binding of molecular iron for enzymatic function and iron deficiency results in global histone hyper-methylation and genome-wide changes in transcription. Among these changes, silencing of both the leucine transporter *LAT3* and obligatory mTORC1 cofactor *RAPTOR* are responsible for mTORC1 inactivation when iron starvation lasts longer than 12 hours. Furthermore, we show that traditional nutrient sensing by mTOR, such as activation by growth factors and amino acids, is dependent on having sufficient levels of iron. This novel regulation of mTORC1 is independent of currently known regulators including TSC1/2, AMPK, REDD1, PROTOR and DEPTOR. The delayed regulation of mTORC1 activity by iron deficiency allows the cell to maintain normal responsivity to the levels of other essential nutrients during transient changes in iron availability. In conclusion, we are the first to describe a novel mechanism in which regulation of mTORC1 activity through transcriptional control of *RAPTOR* by iron-containing Jumonji-C domain containing histone demethylases allows for the integration of fast-acting nutrient sensors, such as growth factors and amino acids which mediate mTORC1 activity through protein-protein interaction on the time-scale of minutes, with long-term regulation by iron levels. This work also bears relevance for patients with chronic iron deficiency by implicating unexplored pathways potentially involved in diseases of iron deficiency.

284 Androgen receptor expression and subcellular localization on circulating tumor cells in a Phase I trial of anti-androgen bicalutamide with CDK4/6 inhibitor ribociclib in metastatic androgen receptor-positive triple negative breast cancer

Marina N. Sharifi

Androgen receptor expression and subcellular localization on circulating tumor cells in a Phase I trial of anti-androgen bicalutamide with CDK4/6 inhibitor ribociclib in metastatic androgen receptor-positive triple negative breast cancer

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Whereas advances in targeted therapy have dramatically improved outcomes for hormone receptor positive and HER2 positive breast cancer over the last 20 years, triple negative breast cancer (TNBC) remains a challenging clinical entity with poor prognosis, particularly in the metastatic setting. TNBC is increasingly understood to be biologically heterogeneous, encompassing multiple molecular subtypes with widely variable clinical behavior. A luminal androgen receptor (LAR) subtype has been identified that is dependent on androgen receptor signaling with the androgen receptor (AR) expressed in approximately 50% of patients with TNBCs that can extend across both LAR and non-LAR subtypes. This has led to increasing interest in evaluating anti-androgen therapy as a targeted treatment approach in TNBC, leveraging FDA-approved anti-androgen therapies developed for and utilized in men with prostate cancer.

Given this broad expression pattern, there is a critical need to identify patients more likely to benefit from AR targeted therapies. To address this need for new biomarkers, we have developed a microfluidic platform to isolate and analyze circulating tumor cells (CTCs) from patients with advanced cancer. This platform is known as VERSA (Versatile Exclusion-based Rare Sample Analysis) platform and integrates cell capture with downstream molecular analysis of protein, gene expression and genomic signatures. We report the development of AR protein analysis in CTCs from patients with TNBC. After pre-clinical validation using TNBC and prostate cell lines, we confirm that CTCs from patients with TNBC express the AR. We have further developed assays to evaluate gene expression signatures of AR activity and AR splice variants.

We are now performing a prospective evaluation of CTCs from patients with AR+ TNBC as part of a phase I/II trial evaluating the safety and clinical activity of the combination of the anti-androgen bicalutamide and the selective CDK4/6 inhibitor ribociclib in metastatic AR+TNBC. Circulating tumor cells (CTCs) are collected serially from enrolled patients prior to treatment, after two cycles of combined anti-androgen/CDK inhibition, and upon disease progression and analyzed using the VERSA platform. We report initial results including androgen receptor expression, localization, and downstream target gene expression.

285 Donor Lymphocyte Infusion for Chronic Lymphocytic Leukemia Following Allogeneic Bone Marrow Transplantation

Kevin G. Shim

Donor Lymphocyte Infusion for Chronic Lymphocytic Leukemia Following Allogeneic Bone Marrow Transplantation

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Donor Lymphocyte Infusion (DLI) has demonstrated treatment efficacy for numerous hematological malignancies through a putative mechanism of graft vs. leukemia effect (GVL). We document the treatment strategy and clinical outcomes of 15 patients with Chronic Lymphocytic Leukemia (CLL) treated with DLI after allogeneic bone marrow transplant (allo-BMT) at a single institution. The majority (11/15) patients were treated with therapeutic DLI after evidence of CLL progression. 11/15 patients also achieved a best outcome of either stable disease or a response to treatment. However, 5/11 of those patients experiencing a response ultimately had relapse or progression of disease. Only 2/15 patients developed acute GVHD in the 100 days following DLI. Median overall survival for the entire cohort was 38 months. These data contribute to the body of knowledge available on DLI outcomes and support the consideration of further investigation and clinical trials to characterize this potentially curative treatment.

287 Vascularized Composite Allografts Perfused with a Complement Inhibitor are Protected from Brain Death Induced and Ischemia Reperfusion Injuries

Mohamad Mahdi Sleiman

Vascularized Composite Allografts Perfused with a Complement Inhibitor are Protected from Brain Death Induced and Ischemia Reperfusion Injuries

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Following severe facial injury or limb loss, transplantation is an accepted surgical approach for replacement, and transplantation of composite tissue is required. Reconstructive surgery involving vascularized composite allografts (VCA) is an effective treatment for patients with severe disfigurement or limb loss. However, VCA transplantation generates a strong immunological response and requires aggressive and life-long immunosuppression. Since VCA is usually performed in the context of non-life threatening defects, a major concern is the toxicity of immunosuppressive drugs. We have been investigating mechanisms of VCA rejection with the goal of developing complement inhibitory approaches that will minimize the need for immunosuppression. Brain death and ischemia reperfusion are two unavoidable sources of acute graft injury, and both are linked to complement activation and worse graft outcomes. In an experimental paradigm incorporating the continuum of brain death and ischemia/reperfusion, we investigated the effect of pre-transplant graft treatment with CR2-Crry, a C3d-targeted complement inhibitor. Vascularized composite allografts were procured from brain dead or living donor BALB/c mice, perfused with UW solution containing CR2-Crry, and stored on at 4°C for 6 hours before heterotopic transplantation into C57BL/6 recipients. To assess binding of CR2-Crry to grafts pre-Tx and its kinetics post-Tx, fluorescently labeled CR2-Crry was tracked using live animal fluorescence tomography imaging. CR2-Crry bound and persisted at significantly higher levels in grafts from brain dead donors compared to grafts from living donors, as measured pre-transplant and at 6 and 24 hours post-transplant. These data are consistent with higher levels of C3d deposition in brain dead vs. living donor grafts. Acute (48 hour) injury and immune cell infiltration was significantly higher in grafted muscle and skin from brain dead do-

nors compared to living donors. However, CR2-Crry treatment resulted in a significant reduction in acute injury in both brain dead and living donor grafts. Importantly, there was an equivalent level of protection in grafts from both brain dead and living donors, implying that the higher levels of CR2-Crry bound in brain dead donor grafts protects them from their otherwise worse outcomes. Additionally, CR2-Crry treatment significantly improved survival of allografts from both brain dead and living donors.

288 Hyperacylation and global chromatin decompaction are consequences of metabolic poisoning due to mitochondrial electron chain dysfunction
John Smestad

Hyperacylation and global chromatin decompaction are consequences of metabolic poisoning due to mitochondrial electron chain dysfunction

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Mitochondrial electron transport chain dysfunction has emerged as a surprisingly common pathologic mechanism mediating observed molecular phenotypes in diseases ranging from cancer to diabetes. In many cases, the mechanisms by which electron transport chain dysfunction causes disease are not clear, although several intriguing mechanisms have been identified that directly connect mitochondrial metabolism to important biological regulatory processes, including epigenetic regulation of gene expression. Here, we report that, in the course our characterization of a mouse embryonic fibroblast cell culture model of electron transport chain complex II, succinate dehydeogenase (SDH), we discover that SDH-loss results in dramatic up-regulation of the cellular NADH/NAD+ ratio, with inhibitory effects upon sirtuin deacylase enzyme activities. We observe a corresponding hyperacylation phenotype affecting multiple acyl post-translational modifications, including acetyllysine, propionyllysine, butyryllysine, succinyllysine, and malonyllysine. These hyperacylation effects are observed in multiple subcellular compartments, including histone proteins in the nucleus. We further characterize bulk chromatin decompaction as a consequence of hyperacylation in SDH-loss context, additionally demonstrating that chemical acylation of isolated cell nuclei is capable of directly eliciting similar chromatin decompaction effects. Chromation decompaction in SDH-loss is additionally found to correlate with an increase in ectopic expression of tissue-specific genes that are normally silenced via epigenetic sequestration. Finally, we leverage sequencing-based technologies to characterize the impacts of bulk chromatin hyperacylation upon bulk organization of the 3D genome. Collectively, these data suggest a previously unknown mechanism by which mitochondrial electron chain dysfunction is capable of regulating bulk chromatin compaction state.

289 Enhancing the function of CD16A in natural killer cells to improve tumor cell killing
Kristin M. Snyder

Enhancing the function of CD16A in natural killer cells to improve tumor cell killing

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Natural killer (NK) cells are a component of the innate immune system. Functioning as cytotoxic lymphocytes, NK cells rapidly kill virally infected cells and tumor cells without prior sensitization. A key anti-neoplastic function of NK cells is their ability to kill tumor cells via antibody-dependent cell-mediated cytotoxicity (ADCC), which is exclusively mediated by the IgG Fcγ receptor CD16A (FcγRIIIA). Fc engagement by CD16A results in a series of downstream signaling cascades that induce the release of cytoplasmic granules containing perforin and granzyme leading to tumor cell lysis. Many clinically successful therapeutic monoclonal antibodies (mAbs) utilize ADCC as a mechanism of action. However, CD16A binds IgG with low affinity and is also rapidly cleaved by a proteolytic process from the cell surface upon activation, thereby limiting the efficacy of therapeutic mAbs. We have created NK cells expressing engineered FcγRs in order to enhance NK cell binding to tumor-targeting therapeutic mAbs and subsequent ADCC. Here, we investigated CD64/16A, a chimeric receptor consisting of the extracellular region of CD64 (FcγR1), the highest affinity IgG FcγR, and the transmembrane and intracellular regions of CD16A, which associate with the signaling molecules FcRγ and CD3ζ. We expressed CD64/16A in the human NK cell line NK92, a cell line that lacks expression of endogenous FcγRs but mediates ADCC upon expression of CD16A, and in induced pluripotent stem cells which were differentiated into primary NK (iNK) cells. We determined that CD64/16A was functional *in vitro* and facilitated ADCC and the production of cytokines, and did not undergo rapid downregulation in expression upon cell activation. In addition, as ADCC requires intercellular adhesion and stable conjugate formation between the NK cell and its target cell, we developed a two color flow cytometry conjugation assay and have shown that CD64/16A NK92 cells facilitated conjugation to antibody-opsonized target cells. *In vitro* experiments lack the influence of the tumor microenvironment and are unable to recapitulate all aspects of *in vivo* ADCC. We are currently exploring an *in vivo* model using NOD-scid IL2Rγnull (NSG) immunocompromised mice engrafted with SKOV-3 HER2$^+$ ovarian cancer cells expressing firefly luciferase to determine if NK92 or iNK cells expressing CD64/16A have ADCC potential *in vivo*. Taken together, our findings suggest that CD64/16A could be utilized by engineered NK cells, leading to the formation of an "off-the-shelf" cellular therapy that can be combined with therapeutic mAbs for the treatment of various tumor types.

290 ahr2, but not ahr1a or ahr1b, is required for craniofacial and fin development and TCDD-dependent cardiotoxicity in zebrafish
Jaclyn P. Souder

ahr2, but not *ahr1a* or *ahr1b*, is required for craniofacial and fin development and TCDD-dependent cardiotoxicity in zebrafish

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that binds environmental toxins and regulates gene expression. AHR also regulates developmental processes, like craniofacial development and hematopoiesis, in the absence of environmental exposures. Zebrafish are an established model for toxicology studies due to their high fecundity, ease of chemical exposure, and transparency throughout embryonic development. However, while humans have a single AHR, zebrafish have three paralogues of AHR: *ahr1a, ahr1b* and *ahr2*. To better utilize the zebrafish model, we investigated the role of each of these paralogues in the endogenous and environmentally-induced functions of AHR. Adult zebrafish with mutations in *ahr2* exhibit fin and craniofacial defects. However, the degree to which *ahr1a* and *ahr1b* influence *ahr2* signaling and contribute to fin and craniofacial development are not known. We compared morphology of adult *ahr2* mutants and *ahr1a/ahr1b* single and double mutant zebrafish. We found that *ahr1a/ahr1b* single and double mutants were morphologically normal while *ahr2* mutant zebrafish demonstrated fin and craniofacial malformations. At 5 days post fertilization, both *ahr1a/ahr1b* and *ahr2* mutant larvae were normal, suggesting that adult phenotypes are due to defects in maturation or maintenance. Next, we analyzed the function of zebrafish AHRs activated by environmental ligands. The prototypical AHR ligand, TCDD, induces toxicity in humans and rodents via AHR and causes cardiotoxicity in zebrafish embryos. It has been shown that embryos with mutations in *ahr2* are resistant to TCDD toxicity, yet it is unclear whether *ahr1* receptors are involved in TCDD toxicity. Further, though AHR was shown to interact with estrogen receptor alpha following TCDD treatment, it is not known whether this interaction is constitutive or context-dependent. To determine whether estrogen receptors and *ahr1* genes are required for TCDD toxicity, we used genetic and pharmacologic techniques to analyze TCDD-dependent cardiotoxicity in estrogen receptor and *ahr* mutant embryos. We found that embryos with mutations in *ahr1a/ahr1b* or estrogen receptor genes are susceptible to TCDD toxicity while *ahr2* mutant embryos are TCDD-resistant. Moreover, pharmacologic blockade of nuclear estrogen receptors failed to prevent TCDD toxicity. These findings suggest that *ahr1* genes do not have overlapping functions with *ahr2* in fin and craniofacial development or TCDD-dependent toxicity, and that estrogen receptors are not constitutive partners of *ahr2*. Future studies using the zebrafish model to study AHR signaling should focus on *ahr2* as the primary functional paralogue of the human AHR.

291 Virulence-associated evasion of the innate immune protein siderocalin
Lindsey K. Steinberg

Virulence-associated evasion of the innate immune protein siderocalin

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Urinary tract infections (UTIs) are among the most common bacterial infections in humans and are becoming increasingly antibiotic resistant. Siderocalin (SCN), an antibacterial innate immune protein, appears in the urine of patients with UTIs caused by enterobacteria. SCN interferes with bacterial uptake of iron, an essential nutrient. Two mechanisms have been proposed for SCN: 1) competitive binding of bacterial siderophores (small molecule iron chelators), preventing their use by bacteria; 2) competitive binding of bioavailable iron mediated by iron-binding urinary metabolites likely derived from diet and intestinal microbiome metabolism. It is unclear how the siderophores produced by uropathogenic *E. coli* are able to resist both mechanisms of action. We developed a bacterial culture assay that can model either mode of SCN function to observe siderophore-mediated evasion of SCN and probe the impact of the metabolite environment on this system. We monitored intrinsic fluorescence quenching in purified SCN to detect physical interactions with iron-bound bacterial siderophores or urinary metabolites. Our results suggest that the mode of SCN activity is dependent on the host urinary environment. Evolved, virulence-associated siderophore modifications may permit uropathogens to evade SCN through two different modes of action. The apparent structural rules guiding siderophore modification in urinary pathogens suggest that both mechanisms of SCN action may have driven uropathogen evolution. New UTI prophylactic and treatment strategies may be able to interfere with key aspects of these interactions without the use of traditional antibiotics.

292 Optimizing CRISPR gene editing tools for potential use in clinical trials
Marta Stevanovic

Optimizing CRISPR gene editing tools for potential use in clinical trials

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CRISPR/Cas9 gene editing is a promising tool to treat hereditary retinal dystrophies. The CRISPR/Cas9 system consists of guide RNA (gRNA), which is complementary to a target DNA sequence and allows for sequence-specific binding of the Cas9 protein. "Active" Cas9 can bind to and cut the target region while the "deactivated" form inhibits transcription. CRISPR/Cas9 can be delivered into cells as a transgene that

consists of a polymerase II promoter (e.g. CMV) driving Cas9 expression and a polymerase III promoter (e.g. U6) driving gRNA expression.

Before CRISPR/Cas9 is used in vivo, several components of the system could be optimized. For example, the U6.gRNA may experience transcriptional interference from the nearby Cas9 coding sequence due to its location and orientation in the transgene. Improving transcriptional efficiency would increase the amount of both the Cas9 and gRNA elements.

In this study, we reverse the orientation of the U6.gRNA in the deactivated *S. aureus* (dSaCas9) construct and assess the effect of this change on transcriptional efficiency.

A transgene containing dSaCas9, U6.gRNA, and the transcription repressor Krüppel associated box (KRAB) domain was created (from Plasmids #106219 and 61591, Addgene). Multiple gRNA variants targeting green fluorescence protein (GFP) in HEK293 cells were inserted into the construct downstream of the U6 promoter. The orientation of the U6.gRNA was then reversed to read from the complementary strand. The ability of the plasmids with the "forward" and "reversed" U6.gRNA regions to reduce GFP expression in HEK293 cells was tested and compared. GFP expression was evaluated using lysate fluorescence and quantitative polymerase chain reaction (QPCR). QPCR was also used to detect and compare expression of dSaCas9 and gRNA levels.

293 xCT and glutamate metabolism regulates Th17 and Treg cells
Ayaka Sugiura

xCT and glutamate metabolism regulates Th17 and Treg cells

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Inflammatory diseases are characterized by an imbalance between pro-inflammatory effector T (Teff) cells and anti-inflammatory regulatory T (Treg) cells that leads to dysregulated immune responses. Many currently available therapies broadly target the immune compartment, including the protective Treg cells. Thus, selectively targeting the specific T cell subset that contributes to disease may provide a new avenue for development of improved immunotherapies. Previously, our lab has shown that Teff and Treg cells can be distinguished by their reliance on distinct metabolic programs. Teff cells are characterized by a highly anabolic metabolic program driven by mTORC1 that relies heavily on increased levels of glucose and amino acid uptake to maintain aerobic glycolysis for proliferation and effector function. In contrast, Treg cells are more catabolic and can function with lower rates of nutrient uptake. This makes nutrient transporters attractive targets for immunomodulation. Current data suggest that Th17 cells are highly dependent on glutamate metabolism, and that depletion or excess of intracellular glutamate levels can lead to dysfunction. In contrast, excess glutamate as induced by interference with the amino acid transporter xCT, which exports glutamate in exchange for cystine, appears to enhance the suppressive activity of Treg cells. Excess glutamate may be exerting its effects through multiple metabolic pathways that can alter intracellular signaling and T cell function. This includes synthesizing glutathione to buffer reactive oxygen species, synthesizing nonessential amino acids, donating and accepting nitrogen groups in transamination reactions, replenishing intermediates of the TCA cycle through anaplerosis, altering NAD(P)+/NAD(P)H ratios through conversion to downstream metabolites, as well as regulating the activity of multiple epigenetic modulators. Given potential differential dependencies of Th17 and Treg cells on these processes, xCT transporter blockade may provide a viable method for restoring immunological homeostasis in Th17-driven inflammatory diseases.

294 NFS1 Undergoes Positive Selection in Lung Tumours and Protects Cells from Ferroptosis
Vladislav O. Sviderskiy

NFS1 Undergoes Positive Selection in Lung Tumours and Protects Cells from Ferroptosis

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Cancer cells experience substantially different nutrient concentrations when grown in culture versus in vivo, necessitating a broader understanding of how tumor nutrient microenvironment impacts metabolic dependencies. For this purpose, we performed parallel *in vitro* and *in vivo* genetic screens and identified oxygen levels as a major driver of differential gene essentiality between *in vitro* model systems and *in vivo* tumors. Most strikingly, we find that suppression of the iron-sulfur cluster (ISC) biosynthetic enzyme NFS1 sensitizes tumor cells to elevated oxygen levels and ferroptosis.

Iron-sulfur clusters are cell essential cofactors that enable their associated proteins to support critical biological processes including energy metabolism, iron homeostasis, and DNA synthesis and repair. Due to the importance of these cofactors, abnormalities in the pathway have been implicated in human diseases such as Friedreich's ataxia and sideroblastic anemia. However, a link with cancer had not been previously described. We find that at elevated oxygen levels, such as those found in the lung, tumor cells require high levels of NFS1 to replenish ISCs damaged by molecular oxygen. In accordance, the genomic locus of NFS1 is amplified in a subset of lung tumors, and staining of human tissues demonstrated high expression of NFS1 particularly in well-differentiated lung adenocarcinomas. Moreover, suppression of NFS1 in mouse xenograft models prevented metastatic or primary lung tumor growth, but not growth in low oxygen environments such as the mammary fat pad. Altogether, these results demonstrate a requirement for ISC biosynthesis during early tumorigenesis in specifically high oxygen tension environments like the lung.

In addition to sensitizing cancer cells to elevated oxygen levels, we find that NFS1 suppression makes tumor cells susceptible to ferroptosis,

an iron catalyzed cell death that leads to accumulation of lipid peroxide species. At low levels of ISC biosynthesis, cells activate the iron starvation response, leading to an iron-overloaded state that is primed for superoxide production through Fenton chemistry. Hence, treatment of NFS1 suppressed cells with oxidants, such as inhibitors of cysteine transport, triggered ferroptosis *in vitro* while slowing tumor growth in xenograft mouse models.

Collectively, these data demonstrate that suppression of ISC biosynthesis can inhibit proliferation of tumors in the high oxygen environment of the lung, while also leaving the tantalizing possibility that tumor cells can be tricked into undergoing ferroptosis by combining induced iron uptake with an oxidant treatment.

295 Myeloid Krüppel-like factor 2 transcriptionally regulates a vascular remodeling program critical in protecting against occlusive disease
David R. Sweet

Myeloid Krüppel-like factor 2 transcriptionally regulates a vascular remodeling program critical in protecting against occlusive disease

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In response to vascular occlusion, collateral vessels are able to remodel to bypass the occlusion, a process termed arteriogenesis. Previous studies from our group have identified Krüppel-like factor 2 (KLF2) as a transcriptional regulator of myeloid cell activation, a process critical for effective arteriogenesis. Accumulating evidence implicates myeloid cells as key mediators of vascular remodeling, however nodal transcriptional regulators of arteriogenesis remain unclear. In this study, we use a myeloid-specific KLF2 knockout mouse model (K2KO) to establish that loss of myeloid KLF2 protects mice from hindlimb ischemia (HLI) via enhanced remodeling of collateral vessels. Although reports from the past decade have implicated alternatively activated, anti-inflammatory macrophages in proper arteriogenesis, it is widely understood that inflammation is critical to vascular remodeling. Here, we demonstrate that loss of myeloid KLF2 is associated with heightened inflammation at the site of collaterization that correlates with enhanced arteriogenesis and perfusion. Additionally, K2KO macrophages exhibit enrichment in genesets associated with extracellular matrix remodeling including those for the matrix metalloproteinases (MMPs), which are critical for vascular remodeling processes. Finally, because K2KO mice demonstrate protection against peripheral vascular occlusion, we sought to determine if myeloid KLF2 plays an important role in the response to transaortic constriction (TAC), a model of non-ischemic heart failure. Remarkably, K2KO mice have improved cardiac performance, increased arteriolar vascularization, and reduced fibrosis after TAC. Together, these data demonstrate that loss of KLF2 enhances macrophage-mediated vascular remodeling, in part through enhanced inflammatory activation and the activation of matrix remodeling transcriptional programs. Additional studies will look into the relative role of KLF2-regulated MMP activity on arteriogenesis, possibly identifying a means to target peripheral artery disease and other vasoocclusive events.

296 Designing more physiological in vitro models of vascular wall with 3D co-culture and force-generating bioreactors
Christopher B. Sylvester

Designing more physiological *in vitro* models of vascular wall with 3D co-culture and force-generating bioreactors

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Radiation exposure, such as that used in cancer treatments, is associated with accelerated cardiovascular disease (CVD), especially atherosclerosis. Epidemiological data show that radiation can increase plaque vulnerability and chance of rupture, but the mechanisms behind this acceleration are not understood. Further, mechanics classically play a role in atherosclerotic progression. The study of radiation and mechanics together may help to improve long-term cardiovascular outcomes in cancer patients, but it is difficult to control for the contributions of radiation and mechanics together. More complex *in vitro* models that more accurately recapitulate physiological and pathological stimuli while maintaining the ease, scale, and reproducibility of cell culture could overcome these barriers to the study of the pathophysiology of and interventions for radiation-induced CVD (RICVD). To address this issue, a three-dimensional co-culture system was constructed and validated to model atherosclerotic plaques using cross-linkable poly(ethylene glycol) (PEG) functionalized with the bioactive peptides to allow for cellular adhesion and remodeling. Vascular smooth muscle cells (VSMC) were encapsulated within the hydrogels, and endothelial cells (EC) were seeded on top to create an appropriately oriented, 3D cell culture. Both cell types expressed phenotypical markers (CD31 and von Willebrand factor for EC and alpha-smooth muscle actin and myosin heavy chain for VSMC), and EC did not express detectable alpha-smooth muscle actin. EC attached and formed a confluent monolayer with cobble-stone like morphology on the free surface of the hydrogel. Confocal microscopy showed that EC remained on the surface of the gel and did not infiltrate into the hydrogels. VSMC attached to peptide sequences within the hydrogels and began to display elongated morphologies by day 2 of culture. To test the responses of the hydrogel co-culture, samples were irradiated with 0 or 2 Gy of ^{137}Cs γ-rays. Confocal microscopy showed expression of the inflammatory markers VCAM1 and ICAM1 in the EC layer. Further, both EC and VSM showed increased DNA breaks after irradiation. In conclusion, a hydrogel-based co-culture model of atherosclerotic plaque was successfully created in a manner mimicking the spatial orientation of stable atherosclerotic plaques that might be caused to progress and destabilize by thoracic radiation. After irradiation, the hydrogel co-cultures replicated some of the key

features of radiation-induced atherosclerosis such as inflammation and DNA damage. Future work with the model developed will focus on incorporating it into force-generating bioreactors to study how altered mechanics and radiation can intersect to increase plaque vulnerability and rupture in patients treated with thoracic radiation.

298 A microRNA Reporter Assay Characterizing mRNA Targets of Imprinted microRNAs

Overbeck Christian Takou Mbah

A microRNA Reporter Assay Characterizing mRNA Targets of Imprinted microRNAs

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MicroRNAs (miRNAs) are short non-coding transcripts which degrade or transcriptionally repress mRNA targets by binding to the 3'-untranslated regions (3'UTR). In particular, the miR-379/410 gene cluster contains 38 imprinted miRNAs expressed in the brain which are only transcribed from the maternal chromosome. Deletion of the miR-379/410 cluster in mice confers a partial perinatal lethal phenotype wherein a significant proportion of newborn pups die after birth due to liver-related issues, and the surviving ones present with anxiety-related behaviors. The Sharp Laboratory has previously designed a tool to characterize mRNA targets of miRNAs through miRNA-mediated degradation of 3'UTRs, and I have adapted the system to assess predicted mRNA targets of the miR-379/410 cluster. We hypothesized that predicted mRNA targets with neuronal function mediate miRNA activity of the miR-379/410. First, I selected seven computationally predicted mRNA targets of miR-379/410 with important neuronal functions, including genes responsible for the expression of several ion channels and neurotransmitters. I then cloned the 3'UTRs of these selected mRNA targets into fluorescent bidirectional reporter that expresses mCherry and cerulean. I transfected the miRNA reporters into Embryonic Stem Cells (ESCs) that were subsequently differentiated into neurons to assay fluorescent expression through fluorescence-activated cell sorting (FACS). To validate the reporter system, I transfected a positive control miRNA reporter containing miRNA binding sites for miR-182, which has been shown to cause mCherry downregulation in the past. In support of my hypothesis, I observed a decrease in expression of mCherry compared to cerulean in neurons in experimental conditions which suggests mCherry-containing 3'UTR targets are regulated by miR-379/410. These results demonstrate the utility of the miRNA reporter assay system for interrogating the importance of the 3'UTR of mRNA targets. My findings will provide a system to further investigate the neuronal importance of imprinted miRNAs in understanding anxiety-related disorders associated with these imprinted genes.

299 Human monoclonal antibody ZKA190 inhibits antibody-dependent enhancement of Zika virus mediated by cross-reactive dengue antibody DV62.5

Ter Yong Tan

Human monoclonal antibody ZKA190 inhibits antibody-dependent enhancement of Zika virus mediated by cross-reactive dengue antibody DV62.5

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Zika virus (ZIKV) infection is a clinically important emerging infectious disease with no approved therapeutic or vaccine. Although ZIKV infection typically causes a mild self-limiting disease, severe neurological complications such as Guillain-Barré syndrome and congenital Zika syndrome may arise. The ZIKV genome codes for three structural (Envelope, E; Precursor-membrane, prM/M; Capsid, C) and seven non-structural proteins. The three structural proteins assemble ZIKV at the endoplasmic reticulum first as immature virions which then subsequently undergo maturation during transport through the trans-Golgi network. Due to inefficiency of the maturation process, a heterogeneous mix of mature and immature ZIKV are released.

Dengue virus (DENV), another flavivirus, is closely related to ZIKV. As both DENV and ZIKV share the same arboviral vector, ZIKV often co-circulate in dengue endemic regions. There are four serotypes of DENV (DENV1-4). Primary infection with any DENV serotype confers lifelong immunity to the infecting serotype but only transient protection against the other three. A secondary DENV infection with a heterologous DENV is associated with greater risk of severe dengue disease. It is thought that severe dengue is attributed in part to the phenomenon called antibody-dependence enhancement (ADE) where cross-reactive antibodies generated during a primary DENV infection binds to the heterologous DENV during secondary infection and facilitate infection through the Fcɣ receptor. Studies on dengue immune sera showed that a significant proportion of DENV-induced antibodies could cross-react with ZIKV. Furthermore, these cross-reactive DENV antibodies were found to enhance ZIKV infection through ADE both in vitro and in vivo. In light of these findings, there is growing concern that a prior DENV infection may prime naïve individuals to develop more severe ZIKV infection. As ADE is a potential contributor of severe disease, any antibody-based therapeutic against ZIKV should ideally neutralize not only the virus but also inhibit ADE so as to achieve maximal therapeutic potential. In addition, given that both mature and immature ZIKV could potentially undergo ADE, identifying a neutralizing antibody that could block ADE of ZIKV at all maturation states would be most ideal.

Recently, Wang and colleagues showed that the hMAb ZKA190 can inhibit mature ZIKV infection. However, the effect of ZKA190 on immature ZIKV is unknown. In this study, we show that the anti-ZIKV E protein antibody ZKA190 can inhibit ADE of immature ZIKV mediated by the cross-reactive DENV antibody DV62.5. Further, we used Cryo-electron microscopy to investigate the structural interaction between the immature ZIKV and the enhancing antibody DV62.5 or the neutralizing antibody ZKA190. We will also investigate the underlying neutralizing mechanism of ZKA190 to ascertain whether this hMAb interfere with ADE at the internalization and/or endosomal membrane fusion step of infection.

300 Clinical Data Mining through Guided Simulation: a model-based reinforcement learning framework for coupling Clinical Decision Support Systems with automated data-mining
Fengyi Tang

Clinical Data Mining through Guided Simulation: a model-based reinforcement learning framework for coupling Clinical Decision Support Systems with automated data-mining

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In recent years, data science has emerged as a popular research direction in clinical and translational medicine. Of particular interest are Clinical Decision Support Systems (CDSS), which include statistical modeling of a wide variety of clinical tasks such as disease progression, unplanned readmissions, mortality risks, length of stay costs and treatment recommendations. Traditionally, predictive modeling of clinical tasks are formulated as *supervised learning*, whereby label data from physician notes, electronic health records (EHR) and billing summaries are used to guide the modeling process. In practice, however, we often find the provision of gold-standard labels from clinical data sources to be noisy and incomplete. Clinically measurements such as blood tests and imaging are sampled at sparse intervals, and small fluctuations in certain features frequently lead to unstable performance in the resulting models.

In this work, we introduce a more robust approach for clinical decision support by formulating the learning tasks as Partially Observable Markov Decision Processes (POMDPs). Specifically, we treat the CDSS predictive models as a *Simulation Environment* (SE) under which *reinforcement learning* can be applied to emulate the data mining process. The SE is learned by supervised learning to model patient trajectories (i.e. disease progression, physiologic response to drugs etc.). Using this SE, a *reinforcement learning agent (RL Agent)* is trained to produce individualized *intervention strategies* (i.e. ordering of labs and medications) based on simulated trajectories of potential outcomes. While the SE tries to model the clinical process of interest, the RL agent tries to adversarially produce intervention strategies which exploit overtly optimistic predictions of the SE model. The result is a feedback cycle of refining the vulnerable parts of the prediction models and identifying the right intervention strategy to diagnose future patients.

We evaluate our framework based on retrospective study of clinical tasks under two different settings: (1) EHR data mining for inpatient CDSS, and (2) dialogue strategy identification for outpatient MCI screening. Currently, our experiments show that the RL agent identifies more efficient conversational strategies for MCI screening (2) compared to trained medical interviewers. We also introduce an evaluation strategy which accounts for the imperfections of the simulation and RL systems, providing an expectation over the lower bound performance of our proposed system.

301 Single cell RNA-sequencing reveals fibroblast reprogramming during successful melanoma immunotherapy
Durga Thakral

Single cell RNA-sequencing reveals fibroblast reprogramming during successful melanoma immunotherapy

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Though immunotherapy has drastically transformed the treatment of melanoma, many patients do not respond and many who do often relapse and eventually succumb to the disease. Many studies have profiled cell states across the immune compartment in melanoma in the context of immunotherapy. However, an integrated and comprehensive understanding of response to therapy requires incorporation of all components of the tumor microenvironment, including a defined role of the non-immune components. We present an in-depth characterization of the immune heterogeneity of melanomas in the YUMMER1.7 mouse model at single-cell resolution and, for the first time, begin to spatially connect single-cell findings with the complex stroma of the native and immunotherapy-treated melanoma microenvironment. Analysis of transcriptomic changes after immunotherapy demonstrate fibroblast reprogramming from a growth and survival, pro-tumor state to a pro-inflammatory, T cell-recruiting state. These results implicate fibroblasts as important immune modulators in melanoma and as underexplored and promising therapeutic targets for influencing immune infiltration, immunotherapy success, and improved outcomes in the treatment of melanoma.

302 Candida colonization is associated with vaginal community state type in women of reproductive age
Brett A. Tortelli

Candida colonization is associated with vaginal community state type in women of reproductive age

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The composition of the vaginal microbiome has been associated with reproductive health and disease. 16S ribosomal gene profiling has provided insight into the bacterial composition of the vaginal microbiome and lead to community state type (CST) classifications. *Candida* is a common member of the vaginal microbial community and can frequently colonize asymptomatically. However, different relationships between vaginal *Candida* and bacteria have been reported. Determining the relationship between Candida and bacteria in the vagina remains important to understanding the role of vaginal microbiota in reproductive health.

We conducted a cross-sectional study of nonpregnant women of reproductive age from the St. Louis area. Vaginal swabs were used to characterize bacterial communities and *Candida* colonization. Sequencing of the V4 region of the 16S ribosomal gene was used to characterize CSTs by virtue of the dominant *Lactobacillus* species present (*L. crispatus*-CST1, *L. gasseri*-CST2, *L. iners*-CST3, *L. jensenii*-CST5). We defined dominance as 50% of the community or greater. Subjects without dominance by a single *Lactobacillus* species were classified as CST4. A *Candida*-specific quantitative PCR (qPCR) targeting the ITS1 region of the genome was used to assess *Candida* colonization. Generalized linear models were employed to evaluate associations between CSTs, sociodemographic and risk characteristics and vaginal *Candida* colonization. Cell free supernatants from *L. crispatus* and *L. iners* cultures were characterized and their potential to inhibit *Candida* growth *in vitro* was evaluated.

Of the 255 women in our analysis, an approximately equal number of black (47%) and white (53%) women were evaluated and forty-two (16%) were vaginally colonized with *Candida*. Three CSTs were well represented among our cohort: CST1 (20%), CST3 (39%) and CST4 (38%). CST was associated with both race and *Candida* colonization. However, women hosting CST3 were more likely to be colonized than women hosting non-*L. iners* dominated CSTs after accounting for race ($p = 0.045$). Women hosting CST1 were significantly less likely to harbor *Candida* than women hosting CST3. *In vitro* experiments indicated *L. crispatus* produces greater concentrations of lactic acid and inhibits *C. albicans* growth more than *L. iners* in a pH dependent fashion. Lactic acid was able to recapitulate these effects.

In a cohort of nonpregnant women, vaginal CST was associated with *Candida* colonization. Women hosting CST3 are significantly more likely to harbor *Candida* than women hosting CST1, indicating that not all *Lactobacillus* dominated CSTs have the same relationship with *Candida*. Our *in vitro* work suggests that *L. crispatus* may impede *Candida* colonization more effectively than *L. iners* through a greater production of lactic acid.

304 Mechanisms of inflammation-induced GABAergic neuronal death and hippocampal circuit disruption in epilepsy
Erin Triplet

Mechanisms of inflammation-induced GABAergic neuronal death and hippocampal circuit disruption in epilepsy

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There are over 3 million Americans currently living with epilepsy, a neurological disorder characterized by repeated unpredictable seizures, episodes of abnormal electrical activity in the brain. Despite the availability of dozens of therapeutics targeting neuronal electrophysiology, approximately ⅓ of patients do not achieve adequate seizure control. Individuals with medically refractory epilepsy suffer from serious consequences associated with ongoing seizures, including progressive decline in cognitive function and increased risk of sudden unexplained death. In recent years a growing body of evidence has demonstrated an important role of neuroinflammation in the pathogenesis of epilepsy. Aberrant electrical activity induces localized inflammation characterized by activation of resident immune cells and recruitment of peripheral innate immune effector cells, which respond by release of inflammatory cytokines including IL6, IL1β, and TNFα.

TNFα, in addition to its role in immunological signaling, also acts directly on neurons through its receptors TNFR1 and TNFR2. While considerable attention has been focused on the role of TNFα signaling in a wide variety of neurological disease states, including medically refractory epilepsy, results have been contradictory and a clear mechanism of action of TNF-mediated neuron loss has yet to be clearly elucidated. Preliminary experiments using mice infected with the Daniel's strain of Theiler's murine encephalomyelitis virus (TMEV) model of viral-induced epilepsy indicate that hippocampal parvalbumin-positive GABAergic interneurons are preferentially lost during acute TMEV infection. EEG recording from the hippocampus also reveals a reduction in GABAergic tone. These outcomes are associated with the local release of TNFα in hippocampal interstitial fluid sampled by microdialysis. Increased inflammation and loss of inhibitory neural tone is also associated with poor outcomes in behavioral tests of hippocampal function. We posit that GABAergic tone is rapidly modulated by TNFα signaling, inducing a dramatic drop-out of PV+ inhibitory neurons, which plays a fundamental role in the induction of seizures and disruption of cognition following neuroinflammation in the CNS.

305 Collaborative Wnt- and TGFβ-signaling promotes stem cell survival in wound healing and cancer
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Collaborative Wnt- and TGFβ-signaling promotes stem cell survival in wound healing and cancer

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Adult stem cells (SCs) are responsible for the regeneration of various tissues, including barrier tissue and appendages. In homeostasis, SCs are self-renewing and give rise to additional SCs, as well as generate more specialized and terminally differentiated cells. During tissue damage, however, these SCs are exposed to extremely inflammatory conditions. In order to restore tissue integrity during wound healing, SCs must turn on additional cellular programs that specifically promote survival from the collateral damage inflicted by inflammation.

Similarly, tumorigenesis arises when tumor-initiating SCs (tSCs) accumulate mutations that allow uncontrolled SC proliferation in the absence of a wound. Cancer, then, can be thought of as a wound that never heals, especially given that patients suffering from chronic wounds have increased risk of developing cancer. While studies have confirmed common gene signatures between wounded and tumorigenic SCs, a common molecular mechanism which may promote SC survival from inflammation during wound healing and which is hijacked by tSCs in tumorigenesis remains unknown.

To search for common signaling pathways that promote SC survival from inflammation during wounding and during tumorigenesis, we studied the hair follicle stem cells (HFSCs) residing in murine skin, since HFSCs are able to efficiently repair cutaneous wounds as well as give rise to skin squamous cell carcinoma (SCC) upon acquiring mutations. Using a sophisticated genetic reporter system, we have found that TGF-β and Wnt signaling is simultaneously activated in both normal HFSCs during partial-thickness removal wounding and in tSCs initiating the oncogenic Hras-driven skin SCC. Furthermore, analysis of previously published transcriptome data in both HFSCs and tSCs revealed a short list of genes (e.g. CD80) that are commonly activated in both wounded HFSCs and in SCC-tSCs. Importantly, these genes can be induced by simultaneously treating cultured SCs with TGF-β and Wnt. Preliminary chromatin immunoprecipitation analysis have shown binding of the transcription factors downstream of TGF-β and Wnt signaling to the genomic regulatory regions of this cohort of genes. Functional perturbation of these TGF-β and Wnt co-regulated genes, such as CD80, significantly impaired tSC survival.

Taken together, these preliminary data suggest that TGFβ- and Wnt-signaling collaboratively confer a survival advantage for SCs during wounding and tumorigenesis.

306 Predictors of severe postoperative pain and increased opioid consumption in Major Abdominal Surgery
Wai Lok Tsang

Predictors of severe postoperative pain and increased opioid consumption in Major Abdominal Surgery

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Background: Pain is one of the most common postoperative complaints yet is frequently inadequately addressed. 30-60% of patients are reported to have moderate to severe pain after surgery (Sommer, De Rijke et al. 2008). Postoperative pain delays discharge and resuming of daily activities. In addition to postoperative quality of life, insufficient postoperative pain therapy may have a negative effect on perioperative morbidity and mortality (Shipton and Tait 2005). Pain increases risks for pulmonary and cardiovascular complications and acute organ dysfunction (Joshi and Ogunnaike 2005).

There is a clear necessity for development of effective analgesic strategies. Ideally, analgesic strategies should be designed based on patient characteristics and surgical factors. Identification of predictive factors for postoperative pain would allow targeting of high risk patients, and facilitate early intervention. Several demographic, clinical, and psychological factors have previously been studied and found as potential predictors of postoperative pain, however, there were restrictions in the scope of potential predictors evaluated, and in the limited time points that were collected (Warfiled, Kahn et al. 1996). Therefore, we conducted a retrospective study to investigate the predictive value of a comprehensive set of demographic, preoperative, and intraoperative factors on postoperative pain and postoperative opioid consumption.

Methods: This is a retrospective study of 907 patients who underwent major abdominal surgery in the ERAS pathway from July 2015 to July 2017 at the University of Pittsburgh Medical Center (UPMC). Data were extracted from electronic medical records, through both automatic and manual extraction by chart review. Data collected include patient demographics, preoperative medical history, intraoperative medications, and postoperative medication and complications. SPSS is then used to conduct linear and logistic regression.

Results and future directions: Mean pain scores for each day were calculated, and the proportion of patients that had a pain score at or above 5 were calculated. On postoperative day 0 (POD0), 60.7% of patients had a pain score at or above 5 and on POD5, 59.3% of patients had a pain score at or above 5. Binary logistic regression revealed age, narcotic use, smoking, previous abdominal surgery, use of gabapentin and psychiatric medication, malignancy, and intraoperative spinal and toradol as predictors of developing pain score more than 5. Whereas linear regression revealed similar factors including age, narcotic use, smoking, previous abdominal surgery, use of gabapentin and psychiatric medication, intraoperative spinal and toradol, and duration of surgery are associated with increased narcotic consumption (oral morphine equivalents). With these known factors an algorithm can be produced to screen for high risk patients, and appropriate analgesic strategies can

be developed to prevent inadequately addressed postoperative pain, and conversely, to prevent excessive use of analgesics in patients at low risks for pain.

307 Pre-doctoral Physician-Scientist Training Opportunities and Perspectives in International Medicine and Global Research

Kenneth Valles

Pre-doctoral Physician-Scientist Training Opportunities and Perspectives in International Medicine and Global Research

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Introduction: While the broad interests, opportunities, and benefits of international clinical training and practice have been well-described in the literature for medical trainees, there exist no similar information for pre-doctoral physician-scientist trainees. Presently, there is little measure of both the interest and opportunities among US MD/PhD students in engaging in medical practice or research in international settings. The objective of this study was to assess current MD/PhD students' interests in and perceived opportunities to pursue formal training in international medicine and global research.

Methods: A survey to assess US MD/PhD student interest and opportunities in international medicine was developed and sent to all current students enrolled in an MD/PhD program in the US. This anonymous, voluntary survey included questions on clinical and research interests and motivations, prior knowledge and experience in international medicine or research, expected career directions, institutional and program support, and demographics. Both univariate and bivariate analysis with Fisher exact tests and two-sided p values were used to evaluate significance differences.

Results: Initial results demonstrate a strong interest in participating in international clinical practice and global engaged research. Respondents reported unsatisfactory program and institutional support for the development of formal training and career pathways in international medicine. Additionally, respondents overwhelmingly expressed that international perspectives and education was key to the future of the physician-scientist workforce, their intended medical specialty, and to their long-term careers.

308 Metabolic engineering of probiotic E. coli Nissle 1917 for therapeutic butyrate production

Max Van Belkum

Metabolic engineering of probiotic E. *coli* Nissle 1917 for therapeutic butyrate production

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Probiotic Escherichia coli Nissle 1917 (Mutaflor) is therapeutic for a form of inflammatory bowel disease. In order to apply synthetic biology approaches to potentially augment the probiotic and therapeutic potential of this bacterial strain, we decided to introduce a partially heterologous butyrate pathway into the bacterium. We decided to delete several genomic genes from Nissle involved in producing metabolites that drain carbon and reducing equivalents from theoretical butyrate production in a redox - balanced manner. We assembled the *ter* gene under control of an inducible promoter to a pre-existing constitutive butyrate producing pathway obtained from the iGEM registry of standardized biological parts, and transformed our engineered pathway into two E. Coli strains: E. coli Nissle 1917 and E. Coli BEM3. Our approach to metabolic engineering of E. Coli Nissle 1917 involves both bacterial genome editing and biobrick assembly, both of which are necessary to turn this strain into a therapeutic butyrate cell factory in the gut.

309 Diet-induced microbiota adaptation is controlled by NF-kB-dependent regulation of 4EBP in Drosophila

Crissie L. Vandehoef

Diet-induced microbiota adaptation is controlled by NF-kB-dependent regulation of 4EBP in Drosophila

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Diet and nutrition shape all aspects of physiology across taxa, including composition, adaptation, and maintenance of the intestinal microbiota. These microbiota, in turn, influence host metabolic responses. The reciprocal interactions between diet, host signaling networks, and microbiota likely define a rheostat that governs host physiology. Importantly, when these interactions are misregulated, the result is often metabolic dysfunction and disease. Thus, there is a critical need to explore the distinct cellular and molecular host signaling mechanisms that shape diet-microbe interactions and influence host physiology. In humans and lower mammals, the variables involved in shaping these interactions are innumerable and difficult to properly control. The simplicity of the intestinal microbiome and defined dietary composition of the insect model, *Drosophila melanogaster*, eliminates some of the major variables associated with mammalian models and allows for dissection of the discreet components involved in the maintaining these interactions. This work investigates diet-dependent host signaling mechanisms, driven by the evolutionarily conserved innate immune transcription factor NF-kB, that dictate intestinal microbiota composition and homeostasis. The *Drosophila* model is exploited to tissue-specifically manipulate host signaling function under various dietary conditions, and the microbiota are subsequently surveyed using culture-dependent and independent methods. Here, we provide evidence that NF-kB transcription factor function in the *Drosophila* intestine can govern microbiota adaptation/composition and metabolic signaling pathway activity in response to specific changes in dietary macronutrients, putatively influencing micro-

biota-regulated aspects of host health and dietary adaptation. More specifically, in response to high carbohydrate-and-low protein dietary macronutrient ratios, NF-kB activity can modulate transcriptional levels and function of 4EBP a conserved regulator of physiology that couples nutrition and mRNA translation. NF-kB-dependent regulation of 4EBP is required to shift microbiota composition in response to a high carbohydrate-and-low protein diet, subsequently influencing host physiology. This work has uncovered an integrated system involving transcriptional and translational regulation of host signaling, dietary macronutrients, and microbiota composition working together to impact organismal health and physiology. These findings highlight host signaling, shaped by dietary cues, as an active participant in the microbial symbiotic relationship. Furthermore, each component of the regulatory signaling mechanism uncovered here is evolutionarily conserved from *Drosophila* to humans, allowing for speculation that similar mechanisms may be active in the human intestine.

310 Soluble CX3CL1 gene therapy improves cone survival and function in mouse models of retinitis pigmentosa
Sean K. Wang

Soluble CX3CL1 gene therapy improves cone survival and function in mouse models of retinitis pigmentosa

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Retinitis pigmentosa (RP) is a genetically heterogenous disease of the retina caused by mutations in any of over 60 different genes. In RP, there is initial loss of rod photoreceptors beginning around adolescence. For unknown reasons, however, rod death is then followed by widespread degeneration of cones which are essential for high-acuity central vision. While virtually all genes implicated in RP are expressed in rods, few actually exhibit expression in cones, suggesting the existence of one or more common mechanisms by which diverse mutations in rods trigger non-autonomous cone degeneration. Elucidation of these mechanisms could help inform the development of new therapies that preserve cone vision in RP regardless of the underlying mutation.

We investigated the involvement of immune responses during non-autonomous cone degeneration in mouse models of RP and found evidence of microglia dysfunction in the retina throughout the process of cone death. We subsequently hypothesized that adeno-associated virus (AAV)-mediated delivery of microglia regulatory signals might alleviate this dysfunction, favoring cone survival. Four AAVs were generated expressing variants of either CD200 or CX3CL1, both of which have been reported to modulate microglia activity. Subretinal administration of these AAVs into RP mice at birth identified overexpression of soluble CX3CL1 (AAV-sCX3CL1) as a promising therapy to preserve cones. Compared to a GFP control virus, AAV-sCX3CL1 significantly prolonged cone survival in three separate strains of RP mice: *rd1* (*P*rd10 (*P*Rho$^{-/-}$ (*P*PP

To mechanistically understand how AAV-sCX3CL1 alleviated cone degeneration, we examined its effect on rod survival, microglia localization, and inflammatory cytokine levels in the retinas of *rd1* and *rd10* mice. Unexpectedly, none of these parameters were significantly changed by treatment with AAV-sCX3CL1. As expression of CX3CR1, the only known receptor for CX3CL1, is confined to microglia within the eye, we consequently performed RNA sequencing of sorted microglia from *rd10* retinas with and without AAV-sCX3CL1, as well as pharmacologic depletion of microglia using PLX3397, a potent inhibitor of microglia survival. Although sequencing of microglia did demonstrate significant (adjusted *P*2) up- or down-regulation of 90 genes with AAV-sCX3CL1, depletion of ~99% microglia surprisingly failed to abrogate prolongation of cone survival with AAV-sCX3CL1 (P

In summary, we have identified AAV-sCX3CL1 as a potential mutation-independent therapy to preserve cone vision in RP, a disease that currently lacks any effective treatment. While it remains unclear exactly how AAV-sCX3CL1 exerts its cone rescue effect, our findings suggest that sCX3CL1 gene therapy may be beneficial for patients affected by a broad range of RP mutations.

311 Unraveling spatially-dependent interactions of tumor-associated macrophage in the tumor microenvironment
Victor G. Wang

Unraveling spatially-dependent interactions of tumor-associated macrophage in the tumor microenvironment

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Tumor-infiltrating lymphocytes (TIL) are a strong prognostic factor in cancer patient outcomes. This includes immune checkpoint blockade (ICB) of the PD-1/PD-L1 axis, where patients with a higher abundance of pre-treatment TILs respond more frequently. However, tumors often elaborate mechanisms to exclude TILs and/or suppress their function. Identifying negative regulators of TILs is thus of great importance to improve patient outcomes and increase the patient population that may benefit from ICB. Tumor-associated macrophages (TAM) are a known modulator of TIL activity but the interaction between the two cell types is typically characterized with *in vitro* experiments and spatially-agnostic sequencing, ignoring location-specific factors contributing to the interaction's net effects. Interrogating the TAM-TIL interactions in an intact tumor microenvironment (TME) will uncover novel mechanisms responsible for TAM function and may be key to reversing TIL immunosuppression. Here we leverage bulk RNA-sequencing with histocytometry, a multiplex quantitative tissue imaging method, to spatially-resolve the TAM-TIL interactions in intact human metastatic melanoma microenvironments. Bulk sequencing distinguishes tumor samples by high and low enrichment of lymphocyte gene sets, with additional sub-stratification by macrophage enrichment. Modular repertoire analysis implicates an

interferon response in the differential lymphocyte enrichment, but does not address macrophage-dependent effects. Drawing from physical chemistry concepts, our spatial analyses of cell-cell interactions from histocytometry reveals distinct TAM populations potentially regulating TIL activity. Phagocytic TAMs in the tumor and non-phagocytic TAMs in the stroma physically contact T-cells, with the former interaction strongly correlated with lymphocyte enrichment and T-cell infiltration. Local and regional TAM phagocytosis affinities estimated by the Langmuir adsorption model further detail the nature of this interaction within the tumor, as well as the role of macrophage phagocytosis in regulating TILs. We demonstrate linear models integrating bulk sequencing and spatial metrics to quantify the relationships between genetics, cell-cell interactions, and T-cell invasiveness. Our work unravels important TAM interactions that shape the TME which have not been previously appreciated, providing novel insight into the forces driving T-cell exclusion and revealing new TAM biology to explore further.

313 The role of DDR1 in podocyte lipotoxicity and progression of Alport Syndrome
Sydney S. Wilbon

The role of DDR1 in podocyte lipotoxicity and progression of Alport Syndrome

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The glomerular basement membrane (GBM) is primarily composed of laminin and Collagen type IV. Alport Syndrome (AS) is a genetic disease of the GBM characterized by mutations in the alpha 3, alpha 4, or alpha 5 chains of collagen type IV. *De novo* production of the α1 chain of collagen type I (Col I) has been observed in mouse models of AS, in which exon 5 of the alpha 3 chain of collagen type IV is deleted (Col4a3KO). Discoidin domain receptor 1 (DDR1) is a unique tyrosine kinase receptor that is activated by collagens. Deletion of DDR1 in the Col4a3KO mice was shown to improve their survival and renal function. However, how DDR1 activation by aberrant collagen production contributes to podocyte injury and proteinuria is poorly understood.

To elucidate the mechanism, differentiated human podocytes were serum starved, followed by 18hr treatment with 50ug/mL Col I (Corning). Following collagen treatment, podocyte lipid content was determined by BODIPY 493/503 and Cell Mask Blue staining. Free fatty acid (FFA) uptake was assessed using the fluorometric free fatty acid uptake kit (abcam). Col4a3KO mice were obtained from the Jackson Laboratory for the determination of DDR1 phosphorylation.

DDR1 phosphorylation was increased in kidney cortex from Col4a3KO mice. The pDDR1 correlated with blood urine nitrogen (BUN, R^2 =0.7, pIn vitro, DDR1 was phosphorylated by collagen type I (50μg/mL, 18hr) in cultured human podocytes. Increased intracellular lipid accumulation (p

Our data suggest that col I-induced/DDR1-mediated lipotoxicity may represent a novel mechanism leading to podocyte injury in AS.

314 DDIWAS: A High-Throughput Approach to Predict Drug Interactions
Patrick Wu

DDIWAS: A High-Throughput Approach to Predict Drug Interactions

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Drug-drug interactions (DDIs), which cause approximately 30% of adverse drug reactions (ADRs), harm patients and costs the US health system billions of dollars a year. Yet, current systems to identify DDIs through clinical trials and post-market surveillance are small, passive, and reactive. Here we propose a novel, efficient, and high-throughput method, drug-drug interaction wide association study (DDIWAS), that scans the "Allergy" section of clinical notes stored in a de-identified copy of Vanderbilt's Electronic Health Record (EHR) to investigate DDIs. To demonstrate the feasibility of DDIWAS, we investigated the drug interactions with sertraline, a commonly prescribed medication for depression and anxiety disorders. We identified an initial cohort of individuals for whom providers listed sertraline as a medication in their EHR. We split this cohort into cases and controls. Cases were individuals for whom providers documented ADR(s) associated with sertraline in the "Allergy" section of their clinical notes. Whereas controls were individuals for whom providers did not document a sertraline ADR. For each individual case, we extracted all medications from the first date at which a provider listed sertraline as a medication (i.e., t=0) to (1) the first date that a provider listed an ADR associated with sertraline or (2) 2 years after t=0, whichever date occurs first. For each individual control, we extracted all medications from t=0 to (1) the first date that a provider removed sertraline as a medication or (2) 2 years after t=0, whichever date occurs first. We extracted medications by mapping brand and generic drug names to RXCUIs. We then mapped the medications to drug ingredients and removed drug ingredient RXCUIs that were contained in

315 CD137 costimulation triggers the persistence of inflammatory CD8 T cells within vascular sites of low and disturbed flow
Maria M. Xu

CD137 costimulation triggers the persistence of inflammatory CD8 T cells within vascular sites of low and disturbed flow

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Patients with systemic circulation of activated effector T cells have increased risk for developing atherosclerotic pathology and cardiovascular disease. Though T cells are not as abundant nor as well-studied as macrophages within atherosclerotic inflammation, their frequency within human plaques was recently identified as the only immune cell subset predictive of future cardiovascular events. Activated effector CD8 T cells are generated through antigen priming and costimulation, upon which they produce pro-inflammatory cytokines such as IFNγ. CD137 (4-1BB) is a costimulatory receptor induced on a range of immune cells, but also at vascular sites of low and disturbed flow (LDF). Therefore, we aimed to identify if activated effector CD8 T cells infiltrate plaque-vulnerable vasculature and if T cell expression of CD137 mediated their infiltrative or inflammatory potential. Using adoptive transfer of CD8 T cells into wild type recipient mice that were then antigen-primed and costimulated, it was discovered that CD137 costimulation robustly boosts activated effector CD8 T cell infiltration of LDF vessels under both nomo- and hyperlipidemic conditions, as evidenced by flow cytometry and immunohistofluoresence. ELISA and intracellular cytokine staining of vessel-infiltrated cells revealed that the transferred CD8 T cells possess innate-like pro-inflammatory programs that persist weeks after their initial activation and furthermore promote the infiltration of other endogenous CD8 T cells with IFNγ-producing potential into developing atherosclerotic plaque. Conversely, when wild type CD137-sufficient mice received transfer of CD137 knockout CD8 T cells, infiltration of both transferred and endogenous CD8 T cells into LDF areas was significantly decreased and stimulation of plaque-resident cells revealed diminished capacity to produce IFNγ. Overall, our studies provide novel insight into how CD137 costimulation, specifically on CD8 T cells, instigates the persistence of activated effector T cells within LDF-activated endothelium and provide mechanistic context for the clinical observation of autoimmune patients facing higher rates of cardiovascular morbidity and mortality.

316 Thyroid Transcription Factor 1 regulation of MUC5AC expression in lung adenocarcinoma
Kei-Lwun Yee

Thyroid Transcription Factor 1 regulation of MUC5AC expression in lung adenocarcinoma

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Lung Adenocarcinomas (ADs) are the United States' leading cause of cancer death with a five year survival rate of 18%. Our research focuses on the potential prognostic marker Thyroid Transcription Factor 1 (TTF-1 or known as NKX2.1), which is expressed in 60-70% of lung AD cases with its immunopositivity associated with a better patient prognosis. To further understand the functional roles of TTF-1 in lung tumorigenesis, we transfected the TTF-1 gene with a retroviral vector into the genome of A549 (TTF-1$^-$) human lung AD cells. Chemosensitivity assays, using Cisplatin, indicated that A549 cells with TTF-1 (TTF-1$^+$) have lower IC50 values compared to controls. One proposed mechanism of this apparent increase in sensitivity is that TTF-1 alters A549 cells' protein expression and consequently how the cells communicate with each other. Mass spectrometry and Western Blot analysis demonstrated that, compared to controls, A549 cells transfected with TTF-1 have markedly less expression of Mucin 5AC (MUC5AC). Moreover, this TTF-1-driven decrease of MUC5AC expression extends to the secreted exosomes of A549 (TTF-1$^+$) cells as well. MUC5AC is an important component of mucus; however, it is also associated with angiogenesis. Incubating human umbilical vein endothelial cells (HUVEC) with the harvested exosomes from the TTF-1 positive cells (TTF-1$^+$) resulted in a less angiogenic environment for the HUVECs. In view of our findings, we propose that TTF-1 may suppress the transcription of the MUC5AC gene, causing reduced levels in the exosomes and inhibition of angiogenic activity. To better understand the activity of MUC5AC and TTF-1 in lung ADs, we have transfected (TTF-1$^+$) cells with a MUC5AC transgene to resurrect the MUC5AC expression that was suppressed by the TTF-1 transgene. We speculate that altering cells to express MUC5AC, even in the presence of TTF-1, could rescue the angiogenic activity and lead to increased survival. Moving forward, we hope to use these cells to not only test our hypothesis, but also to examine more of MUC5AC's activity in order to shed light on the role TTF-1 plays in lung ADs.

317 Abcg2-Expressing cardiac side population cells contribute to cardiomyocyte renewal through fusion
Amritha Yellamilli

Abcg2-Expressing cardiac side population cells contribute to cardiomyocyte renewal through fusion

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Cardiac side population cells (cSPCs) are proposed progenitor cells that differentiate into cardiomyocytes and undergo clonal expansion in cell culture. CSPCs can only be isolated and studied *ex vivo* based on their ability to efflux fluorescent DNA dyes out of their cytoplasm. To determine the *in vivo* role of cSPCs in endogenous cardiomyocyte renewal, we generated a new mouse model to lineage-trace cSPCs utilizing their expression of *Abcg2*, a gene that encodes the transporter essential for the side population phenotype. In this mouse model, we found that cSPCs are efficiently labeled with GFP and give rise to 0.84 ± 0.24% of adult cardiomyocytes over a four-week chase period. We confirmed that labeled cSPCs specifically give rise to cardiomyocytes by ruling out the contribution of *Abcg2*-expressing bone marrow and endothelial cells with bone marrow transplantation and endothelial cell lineage-tracing experiments. Next, we evaluated how cSPCs respond to myocardial ischemic injury. We found a three-fold higher level of cardiomyocyte labeling in injured hearts compared to sham hearts. To understand the cellular mechanisms by which cSPCs give rise to cardio-

myocytes, we bred our lineage-tracing mouse model to dual-recombinase reporter mice. With these mice, we found that 85.3 ± 3.1% of lineage-traced cardiomyocytes arose from fusion of pre-existing cardiomyocytes with labeled cSPCs, while 14.6 ± 3.1% of lineage-traced cardiomyocytes arose from direct differentiation of labeled cSPCs. To critically assess whether fusion or differentiation of labeled cSPCs contributes to newly-formed cardiomyocytes, we injected *Abcg2*-lineage-tracing mice with EdU and evaluated both EdU incorporation and fusion in lineage-traced cardiomyocytes. We found a significant enrichment of GFP-labeling in newly-formed cardiomyocytes with 21% of EdU+ cardiomyocytes labeled with GFP compared to just 0.7% of EdU- cardiomyocytes. All lineage-traced, EdU+ cardiomyocytes arose from fusion of *Abcg2*-expressing cSPCs with pre-existing cardiomyocytes. Taken together, these findings show that cSPCs contribute to endogenous cardiac regeneration through fusion and that this contribution is enhanced in response to myocardial ischemic injury. Our study is the first to show that fusion between cardiomyocytes and non-cardiomyocytes triggers cell-cycle entry in 21% of newly-formed cardiomyocytes in the adult mammalian heart. Moreover, it provides preliminary evidence that cardiomyocyte fusion may be a promising mechanistic target for future regenerative therapies.

318 eNAMPT: A novel extracellular vesicle-mediated mechanism that regulates systemic NAD+ biosynthesis and aging in mammals
Mitsukuni Yoshida

eNAMPT: A novel extracellular vesicle-mediated mechanism that regulates systemic NAD$^+$ biosynthesis and aging in mammals

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eNAMPT is a circulating form of nicotinamide phosphoribosyltransferase, the rate-liming enzyme in the mammalian NAD$^+$ biosynthetic pathway. We have previously shown that eNAMPT released from adipose tissue modulates hypothalamic NAD$^+$ levels, SIRT1 activity, neural activity, and behavior in mice (Yoon *et al., Cell Metab.*, 2015). Given that SIRT1, a NAD$^+$-dependent protein deacetylase, plays a critical role in maintaining hypothalamic functions and regulating lifespan in mice, we explored the role of eNAMPT in aging and longevity control. Circulating levels of eNAMPT significantly decreased with age in both mice and humans. A prospective study in mice demonstrated that circulating eNAMPT levels in aged mice was able to predict their remaining lifespans. These findings strongly suggest that circulating eNAMPT is a part of the conserved mechanism regulating the pace of aging and lifespan in mammals. To test this hypothesis, we generated adipose-tissue specific *Nampt* knock-in (ANKI) mice and characterized their aging phenotypes. Interestingly, increased levels of eNAMPT ameliorated age-dependent decline in eNAMPT and tissue NAD$^+$ levels and tissue functions in hypothalamus, pancreas, and retina in aged ANKI mice, partly through enhancing the activity of SIRT1. Furthermore, the median lifespan in female ANKI mice was significantly extended, providing further support for the critical role of eNAMPT in the regulation of aging and healthspan in mammals. Remarkably, we found that eNAMPT was localized exclusively in the extracellular vesicles (EVs). Using primary hypothalamic neurons and isolated EVs, we also demonstrated that EV eNAMPT, which was internalized into target cells, directly enhanced intracellular eNAMPT levels and NAD$^+$ biosynthesis. Finally, the injection of EVs isolated from young mice significantly enhanced the physical activity of aged mice and extends lifespan, implicating EV eNAMPT supplementation as a viable intervention to combat aging. These new findings demonstrate that EV eNAMPT functions as a key regulatory mechanism for systemic NAD$^+$ biosynthesis and aging in mammals.

319 Developing an organoid model to study CFTR function in the gallbladder epithelium
Keyan Zarei

Developing an organoid model to study CFTR function in the gallbladder epithelium

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In many organs, infection and inflammation develop in parallel to CF disease complicating the study of CF pathogenesis. This is not the case in the CF gallbladder where a small gallbladder (microgallbladder), epithelial mucinous changes, and luminal and duct obstructions are present at birth prior to major inflammatory responses. Interestingly, the gallbladder also has one of the highest expressions of CFTR relative to other tissues. Thus, the gallbladder represents an important, but understudied area of CF research. Our goal was to develop a gallbladder epithelial organoid model with pig and human tissue to study the role of CFTR in fluid transport and mucus secretion. Pig gallbladder tissue was harvested from newborn to one-week-old non-CF and CF piglets. Human gallbladder tissue was obtained from the University of Iowa Tissue Procurement Core following the appropriate protocols. The epithelium was stripped off, and cells were suspended in Matrigel supplemented with a specialized media containing growth factors. Brightfield microscopy and immunofluorescence were used for organoid visualization and immunostaining. Organoid measurements were acquired using Fiji. Within 48 hours, organoids began to form from both porcine and human tissue. Pig gallbladder epithelial organoids expressed biliary markers with apical markers, including CFTR, being expressed on the outer surface of the organoid (inside-out orientation). Human gallbladder organoids expressed the opposite orientation (inside-in orientation). CF pig organoids were smaller and had a decreased lumen area relative to non-CF pig organoids. In response to intracellular cAMP elevation, non-CF pig organoids decreased in size and lumen area. This response was absent

in CF pig organoids. Finally, human organoids demonstrated a swelling, instead of a shrinking, response to intracellular cAMP elevation. In conclusion, pig organoids display an apical-on-the-outside orientation as opposed to human organoids, which formed with an apical-on-the-inside orientation. Loss of CFTR function is associated with morphological defects in gallbladder epithelial organoid size. Supported by NIH and Cystic Fibrosis Foundation.

320 Isoprenoid synthase domain-containing protein gene transfer improves dystroglycan glycosylation and function in models of α-dystroglycanopathy

Sanam Zarei

Isoprenoid synthase domain-containing protein gene transfer improves dystroglycan glycosylation and function in models of α-dystroglycanopathy

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The α-dystroglycanopathies are autosomal recessive muscular dystrophies characterized by skeletal muscle wasting as well as brain and eye malformations. α-dystroglycan (α-DG) which is heavily glycosylated, binds proteins in the extracellular matrix such as laminin through its sugar groups. In α-dystroglycanopathy, there is reduced glycosylation of α-DG, preventing the stabilizing connection to laminin, henceforth described as functional glycosylation. *Fukutin, Fukutin-related protein (FKRP)*, and *Isoprenoid synthase domain-containing protein (ISPD)* are amongst the most commonly mutated genes. Mutations in *FKRP* lead to Limb Girdle Muscular Dystrophy 2I (LGMD2I) and congenital muscular dystrophy (CMD), the two most prevalent α-dystroglycanopathies. Currently there is no established treatment for these diseases. The enzymes Fukutin, FKRP and ISPD are involved in the addition of ribitol-5-phosphate (Rho5P) to the sugar chain of α-DG and mutations resulting in reduced levels or activity of these enzymes lead to disease. Fukutin and FKRP transfer Rho5P onto the sugar chain while ISPD synthesizes CDP-ribitol, the precursor substrate for Fukutin and FKRP.

Interestingly, patients with mild to moderate missense mutation in *FKRP* have partial glycosylation of α-DG, suggesting decreased FKRP enzymatic activity. Therefore, this phenomenon can be harnessed as a therapeutic strategy. Increasing the substrate – CDP-ribitol – may drive the residual FKRP enzyme to normal activity, leading to an increased production of Rho5P moieties onto the sugar chain. We hypothesize that increased CDP-ribitol delivered through adenoviral *ISPD* transfer can compensate for reduced FKRP activity and increase functional glycosylation of α-DG. To study this, we have developed a primary human fibroblast model from a CMD patient with *FKRP* mutation. Fibroblasts were transfected with recombinant adenoviruses expressing wild-type *ISPD* and *DG*. Glycosylation of α-DG was evaluated by immunoblotting with anti-matriglycan antibody, a mouse monoclonal antibody to glycosylated alpha-DG. Functional glycosylation was assessed with a laminin overlay, which was further quantitated by a laminin binding assay.

Our results show that adenoviral *ISPD* gene transfer restores glycosylation of α-DG in an *ISPD* mutant human fibroblast line to levels present in control primary fibroblasts. Adenoviral ISPD gene transfer restored functional glycosylation of α-DG in a *FKRP* CMD mutant human fibroblast line up to 40 percent of levels present in control primary fibroblasts. Cells concomitantly treated with *ISPD* and *DG* showed complete recovery of functional glycosylation similar to levels present in control fibroblasts.

These results suggest the utility of adenoviral *ISPD* gene transfer in the treatment of *FKRP*-dependent α-dystroglycanopathies. We have generated a LGMD2I mouse model in which *Fkrp* is knocked down through RNA interference, which will be used to study this approach in vivo. Transgenic *Fkrp* mice will be injected systemically with adenoviral-associated virus expressing wild-type *ISPD*. Muscle histology, body weight, two-limb grip force, and respiratory function will be assessed before and after injection.

321 Understanding the Role of the Salmonella Typhi Vi Capsular Polysaccharide in Neutrophil and Macrophage Phagocytosis

Lillian F. Zhang

Understanding the Role of the *Salmonella* Typhi Vi Capsular Polysaccharide in Neutrophil and Macrophage Phagocytosis

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Salmonella Typhi is the causative agent of typhoid fever, which is a life-threatening, systemic disease, with an estimated global disease burden of 21.6 million cases annually, resulting in about 220,000 deaths. Due to the absence of convenient animals models to study *S.*Typhi and other typhoidal *Salmonella* serovars, our understanding of typhoid fever pathogenesis is still incomplete. Like other Salmonella serovars, *S.*Typhi is phagocytosed by host macrophages and survives and replicates intracellularly within these macrophages. Interestingly, one importance virulence factor of *S.*Typhi is the polysaccharide capsular antigen Vi, which, like many of the bacterial capsules expressed by extracellular bacteria, has long been thought to play a role in preventing phagocytosis and complement killing. Thus, we encounter a paradox in which a bacteria that survives and replicates within macrophages as part of its life cycle, also possesses an anti-phagocytic capsule, which is more characteristic of an extracellular pathogen. Here, we demonstrate that the *S.*Typhi Vi capsule selectively prevents phagocytosis and uptake of the bacteria depending on the host cell type. We found that interestingly, the Vi capsule prevents phagocytosis of *S.* Typhi by neutrophils, but does not prevent uptake of the bacteria by macrophages. Instead, we propose that macrophages possess cell surface receptors that specifically bind to and recognize polysaccharides present in the Vi capsule, thereby facilitating engulfment. These findings that the Vi capsule

of S.Typhi interacts differently with different host phagocytes represents a step forward in our understanding of how typhoidal *Salmonella* serovars interface with host immunity and will provide important new insights into the pathogenesis of typhoid fever.

322 Increasing antibiotic efficacy against Staphylococcus aureus aggregates in septic joints
Neil Zhao

Increasing antibiotic efficacy against *Staphylococcus aureus* aggregates in septic joints

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Septic joint infections occur at a rate of about 6 per 100,000 people per year in industrialized countries. The mortality rate exceeds 11% despite aggressive antibiotic treatment and lavage. *Staphylococcus aureus (S. aureus)*, which comprises 40% of septic arthritis cases, forms persistent fibrin(ogen) and albumin aggregates in synovial fluid that are recalcitrant to antibiotics and thus difficult to treat. *S. aureus* is able to aggregate through the expression of cell wall-anchored proteins that bind to extracellular fibrin(ogen). These microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) are themselves anchored to the cell wall by Sortase A (SrtA), a cell membrane-associated transpeptidase. We hypothesized that prevention of *S. aureus* aggregation in synovial fluid would increase the efficacy of antibiotics. In previous work, our lab had shown that mutants lacking the MSCRAMM Clumping Factor A (ClfA) no longer aggregated. In this work, we explored methods to inhibit ClfA and SrtA so as to minimize aggregation.

Supplies of human synovial fluid are limited, thus our lab has created a pseudo-synovial fluid (pSynF, composed of 5mg/ml high molecular weight hyaluronic acid, 10mg/ml bovine fibrinogen, and 12mg/ml bovine albumin all dissolved in a volume to volume ratio of 74:26 tryptic soy broth (TSB):pH 8.5 phosphate buffered saline (PBS)) which mimics the viscosity, chemical composition, and bacterial aggregation of human synovial fluid. Methicillin-susceptible *S. aureus* (MSSA) ATCC 25923 (final concentration, 10^7 bacteria/ml) were incubated in pSynF for 6 hours alone and with different combinations of 18mM $CaCl_2$ (inhibits ClfA), 30μg/ml berberine chloride (inhibits SrtA), and 60μg/ml amikacin (an aminoglycoside antibiotic).

In the presence of 60μg/ml amikacin in pSynF, a ~2 log reduction in colony forming units (CFU) was observed compared to *S. aureus* alone (p2+ (p=0.7) or 30μg/ml berberine chloride (p=0.2) to the pSynF did not significantly alter amikacin's effects on *S. aureus*. However, when the calcium cation and berberine chloride were combined with amikacin, there was about a 3-5 log reduction in CFU compared to *S. aureus* alone (p2+ (p=0.4) nor 30μg/ml berberine chloride (p=0.2) appeared to be bactericidal or bacteriostatic. In addition, *S. aureus* incubated in pSynF was not significantly different from incubation in TSB (p=1).

Therefore, calcium, which inhibits Clfa, and berberine chloride, which inhibits SrtA, appear to work synergistically to prevent *S. aureus* from aggregating in an extracellular environment that resembles synovial fluid. Unable to aggregate, *S. aureus* becomes easier to eradicate, increasing the efficacy of the antibiotic. Possible future steps include disintegrating already formed *S. aureus* aggregates with different types and combinations of Clfa and SrtA inhibitors, and testing the resulting change in antibiotic susceptibility.

323 Pre-clinical study of first-in-class NEDDylation inhibitor in pediatric acute lymphoblastic leukemia (ALL)
Shuhua Zheng

Pre-clinical study of first-in-class NEDDylation inhibitor in pediatric acute lymphoblastic leukemia (ALL)

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Pediatric acute lymphoblastic leukemia (ALL) is the leading cause of cancer-related death in children with even worse cure rate for relapsed cases. Meanwhile, 5-year event-free survival (EFS) rate for adult ALL patients remains abysmal. These facts call for the development of a new targeting strategy for ALL therapy. Data from our lab and others demonstrated that ALL cells are vulnerable towards the novel NEDD8 Activating Enzyme (NAE) inhibitor pevonedistat (pevo, MLN4924) *in vitro* and *in vivo* (Leclerc GM, Zheng S, et al, Leuk. Res. 2016). Meanwhile, we identified significant induction of Cdt1, phospho-Chk1(Ser345), phospho-p53(Ser15), γH2AX(Ser139) and PARP cleavage. Using acetylation specific antibodies, we found pevo treatment significantly induced p53 and H3 acetylation. The activity of NAD-dependent p53 and H3 deacetylase SIRT1 were inhibited in pevo-treated ALL cells with NAD level downregulation, indicating disregulation of DNA repair mechanisms in pevo-treated ALL cells. However, CRL substrates are involved in a myriad of cellular processes including cell cycle, DNA damage response and signal transductions. Thus, further clarifications on the underlying cytotoxic mechanisms of pevo-mediated NAE inhibition in ALL death are needed. We uncovered that inhibition of the MEK/ERK pathway *in vitro* and *in vivo* sensitized ALL cells to pevonedistat. The observed synergistic apoptotic effect appears to be mediated by inhibition of the MEK/ERK pro-survival cascade leading to de-repression of the pro-apoptotic BIM protein. Mechanistically, Ca^{2+} influx via the Ca^{2+}-release-activated Ca^{2+}(CRAC) channel induced protein kinase C β2 (PKC-β2) was responsible for activation of the MEK/ERK pathway in pevonedistat-treated ALL cells. Sequestration of Ca^{2+} using BAPTA-AM or blockage of store-operated Ca^{2+} entry (SOCE) using BTP-2 both attenuated the compensatory activation of MEK/ERK signaling in pevonedistat-treated ALL cells. Pevonedistat significantly altered the expression of Orai1 and stromal interaction molecule 1 (STIM1), resulting in significantly decreased STIM1 protein levels relative to Orai1. Further, we identified eIF2α as an important post-transcriptional regulator of STIM1, suggesting that pevonedistat-induced eIF2α de-phosphorylation selectively down-regulates translation of STIM1 mRNA. Consequently, our data suggest that pevonedistat potentially activates SOCE and promotes Ca^{2+} influx leading to activation of the MEK/ERK

pathway by altering the stoichiometric Orai1:STIM1 ratio and inducing ER stress in ALL cells. All these pre-clinical studies led to the entry of Phase I clinical trial of pevo in patients with relapsed/refractory ALL (NCT03349281).

324 Slug is stabilized by ATM and required for ATR activation

Wenhui Zhou

Slug is stabilized by ATM and required for ATR activation

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Replicative stress is a potent inducer of stem cell decline, and predisposes to tissue dysfunction and disease. Mice lacking the transcription factor (TF) Slug/*SNAI2* exhibit mammary tissue dysfunction and functional stem cell decline prompting us to examine whether there might be a functional link between Slug and replicative stress. In this study, we show that Slug is required for replication protein A (RPA) complex formation following DNA damage. Consequently, Slug deficient cells exhibit delayed activation of ataxia telangiectasia (ATM) and Rad3-related protein (ATR) and phosphorylation of its targets RPA32 and Chk1; this leads to a failure of RAD51 recruitment at sites of DNA damage and unresolved DNA damaged repair. In vivo, *SNAI2*-mutant mice exhibit heightened replicative stress and increased γH2AX signals. Together, these results describe the mechanistic framework of how a transcription factor that controls stem cell activity can couple replicative stress and DNA repair to maintain genomic integrity.

Highlights: *SNAI2*$^{Laz/Laz}$ mammary epithelial cells suffer stress-induced DNA damage; Slug is stabilized by ATM in response to DNA damage; Slug promotes efficient HR-mediated DSB repair; Slug is necessary for DNA-end resection and ATR/Chk1 activation.

325 METTL3 m6A Methyltransferase Activity is Regulated by Phosphorylation in Cancer and Embryonic Stem Cells

Allen C. Zhu

METTL3 m6A Methyltransferase Activity is Regulated by Phosphorylation in Cancer and Embryonic Stem Cells

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N6-methyladenosine (m6A) is the most common internal modification in mammalian messenger RNA (mRNA), and its key role in post-transcriptional gene regulation has been shown to affect biological processes such as embryonic development and cell differentiation, translation efficiency, and mRNA metabolism. METTL3 is the enzymatic component of an RNA methyltransferase complex that "writes" m6A onto mRNAs in order to modulate mRNA biogenesis, stability, and decay. Although METTL3 expression leads to increased m6A, it is not fully understood how the methyltransferase activity of METTL3 is regulated, especially in activation and post-translational modification. In this study, we used HER2+ breast cancer and melanoma cell line models to find that activation of ERK2 leads to METTL3 phosphorylation. We found that METTL3 phosphorylation may affect m6A levels due to modulation of writer activity. In mouse embryonic stem cells, we also find that lack of METTL3 phosphorylation disrupts pluripotency and differentiation by affecting levels of pluripotency factors, such as Oct4, Nanog, Rex1, and Nr5a2. Future directions of our study include elucidating downstream effects and altered pathways of phosphorylated METTL3, with transcriptome analysis and other cell lines. Our results shed light on an important mechanism of phosphorylation of METTL3 in regulating m6A RNA methyltransferase activity, and ultimately help us understand how phosphorylation of METTL3 is affected in cancer and essential for development.

326 Optical and electrical activation of central auditory pathways in a mouse model of the auditory brainstem implant

Angela Zhu

Optical and electrical activation of central auditory pathways in a mouse model of the auditory brainstem implant

Angela Zhu[1,2,4], Stephen McInturff[1], Vivek Kanumuri[1,2], Benjamin Glickman[1], Bernardo Sabatini[3,4], Anne Takesian[1], M. Christian Brown[1], Daniel J. Lee[1,2]

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Auditory brainstem implants (ABIs) are neural prostheses that provide hearing sensations in profoundly deaf patients who are not candidates for the cochlear implant. However, speech perception is poor among ABI users and the mechanisms of auditory perception during surface stimulation of the cochlear nucleus (CN) are not well understood. One explanation for modest ABI outcomes is channel crosstalk due to electrical current spread. We hypothesize that optogenetics can be used to enhance spectral resolution by increasing the number of independent auditory channels. We aim to advance optical ABIs by (1) improving delivery of light-sensitive opsin to the CN and (2) characterizing murine midbrain and cortical responses to acoustic, electrical, and optical stimulation.

To address aim 1, we tested opsin transduction efficiency using a novel composite silk fibroin/ancestral adeno-associated virus (AAV) coated implant that has been shown to enable more uniform opsin expression in neural tissue and improved anatomical alignment with a light source. Following craniotomy in CBA/CaJ mice, synthetic AAV Anc80L65 carrying the opsin Chronos was delivered to the CN via (1) Anc80L65-coated silicone discs, (2) composite silk fibroin/Anc80L65-coated silicone discs, or (3) direct microinjections (positive control). Optically-evoked auditory brainstem responses (oABRs) and multiunit activity in the inferior colliculus (IC) were recorded after 3 weeks. We observed robust multiunit IC activity and detectable oABRs in the silk fibroin/Anc80L65 cohort, which were comparable to the responses seen in positive controls. Conversely, IC activity was weaker and no detectable oABRs were observed in mice transduced with only Anc80L65-coated discs. Histology of Chronos expression in the CN corroborated these findings. These observations suggest that silk fibroin may be essential for facilitating noninvasive transduction of opsins in the CN robust enough to produce neurophysiologic responses in the IC.

To address aim 2, we compared cortical ABI vs. sound-evoked neuron activity. C57BL/6 mice were exposed to electrical ABI stimulation at a 50 hertz (Hz) pulse rate or an auditory stimulus of 70 decibel (dB) white noise for two hours. RNA in situ hybridization of neuron activity marker Npas4 identified a subset of cortical SLC32A1-expressing excitatory neurons that were activated by white noise or electrical ABI stimulation. We are performing ongoing experiments using in vivo two-photon recordings of neuron activity in the auditory cortex of awake animals with chronically implanted ABIs to better understand the differences between ABI activation and sound activation of cortical neurons.

This study is the first to demonstrate that surface transduction of opsins in the CN leads to measurable midbrain physiologic responses to light. These results further demonstrate differences in activation of higher auditory centers from auditory, electrical, or optical stimuli. This work may bring a new generation of light-based ABIs closer to clinical translation.

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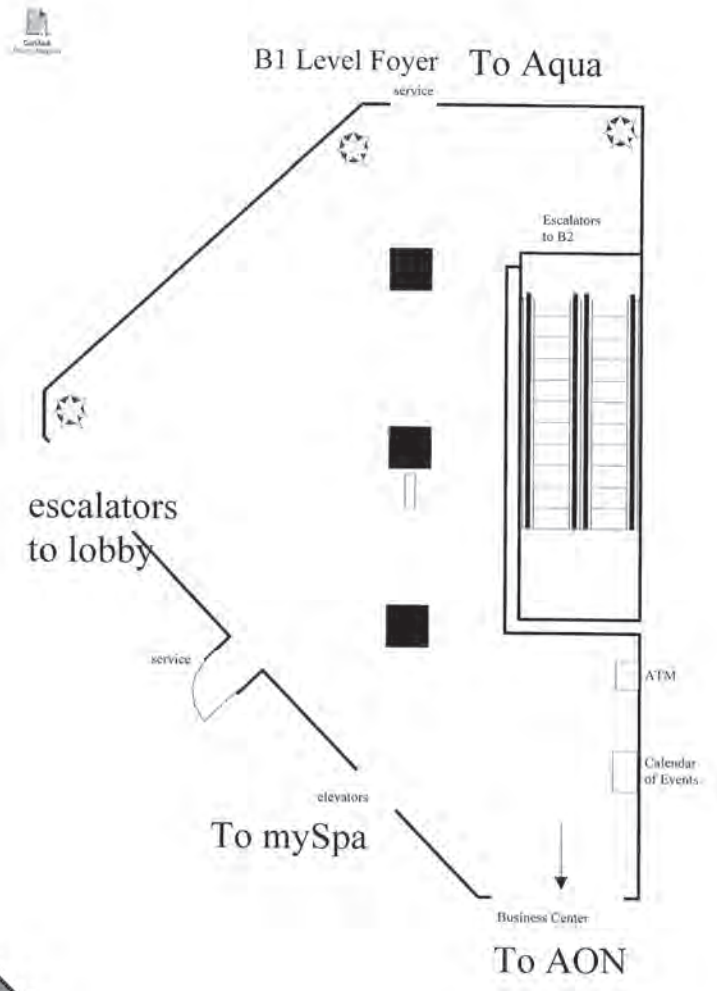

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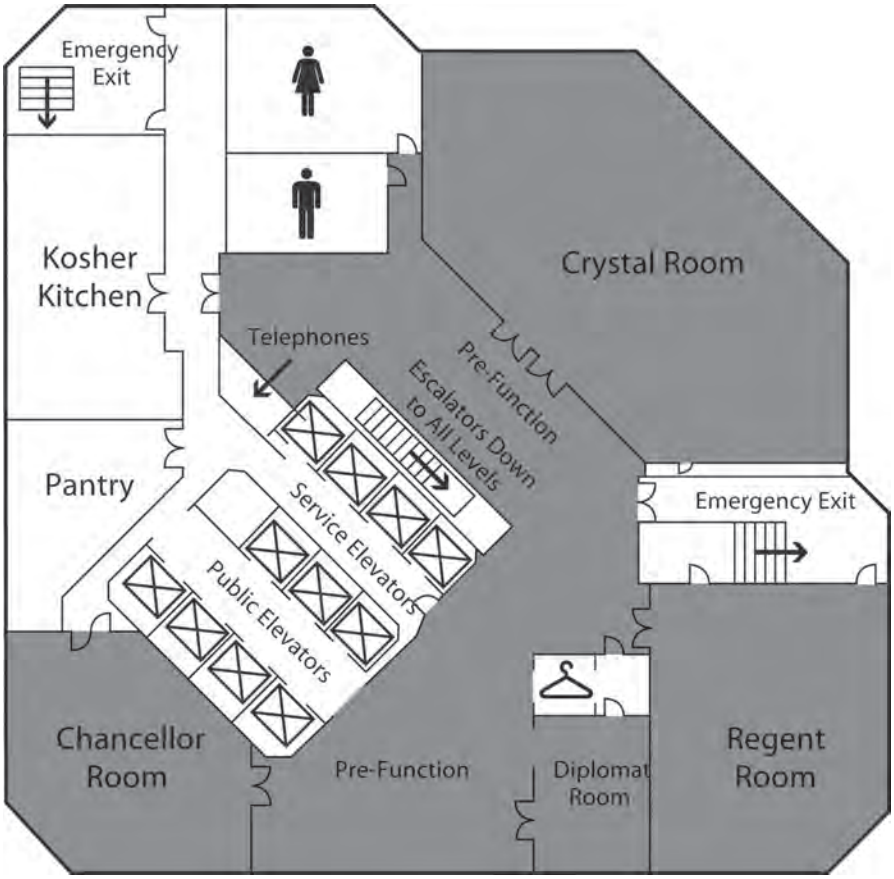

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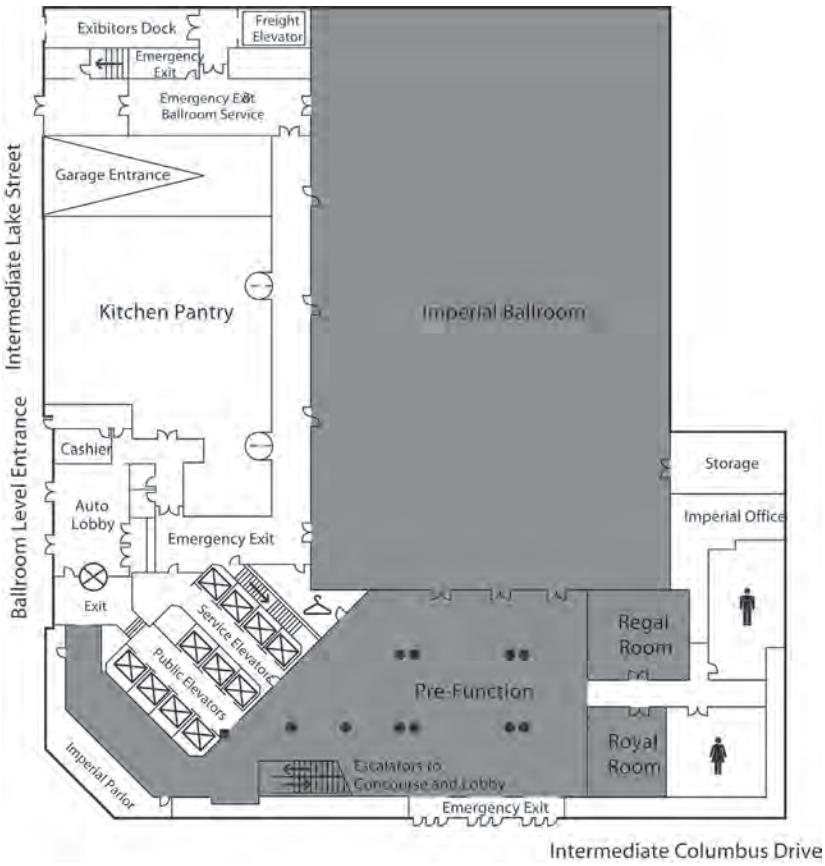

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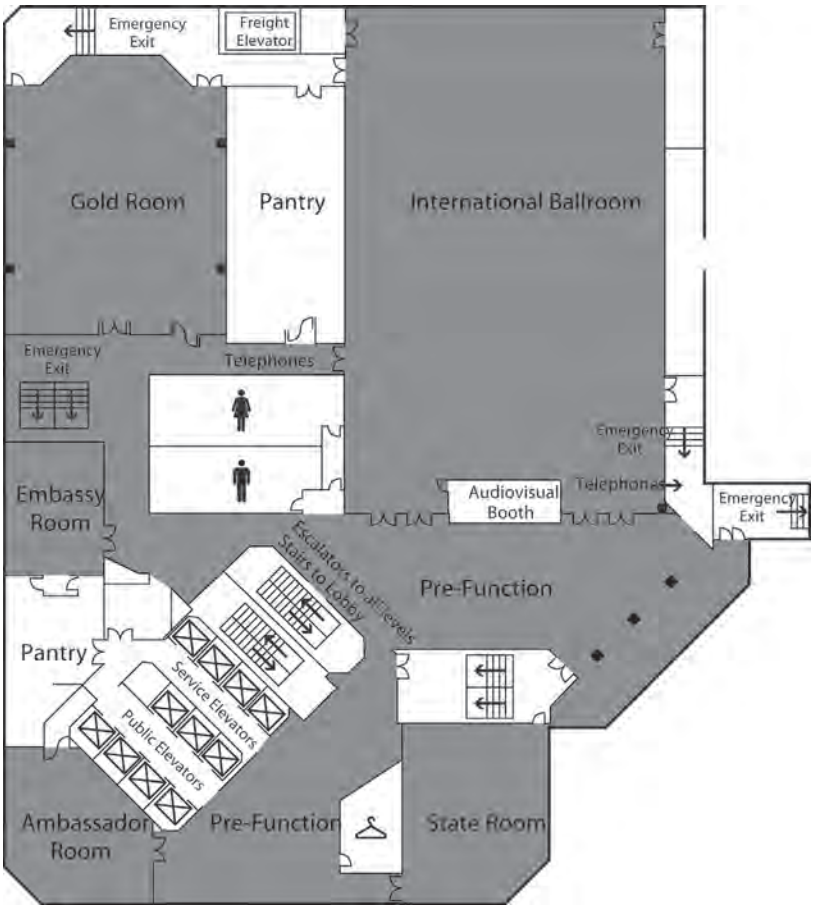

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