Child Health Research Centers
Annual Retreat

PROGRAM

September 5-7, 2014
Boston, Massachusetts

Co-Hosted by:

Boston Children's Hospital
Harvard Medical School Teaching Hospital
Yale-New Haven Children’s Hospital
## 2014 CHRC Annual Retreat
### September 5-6, 2014
#### Seaport Hotel
##### Boston, Massachusetts

<table>
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<th>TIME</th>
<th>ACTIVITY AND LOCATION</th>
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<td><strong>Friday, September 5, 2014</strong></td>
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<tr>
<td>12:00 pm – 2:00 pm</td>
<td>Registration and light lunch for early arrivals</td>
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<td>Lighthouse 2</td>
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<tr>
<td>1:15 pm – 2:00 pm</td>
<td>Scholars Career Development Session</td>
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<td></td>
<td>30 Years in Translation: Basic Research in Hematopoiesis and Stem Cell Genetic Therapies</td>
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<td><em>David Williams, MD</em></td>
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<td><em>Chief, Division of Hematology and Oncology</em></td>
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<td>2:00 pm – 3:00 pm</td>
<td>Scholars Break-out Session with Mentors</td>
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<td>2:00 pm – 3:00 pm</td>
<td>Program Director’s Meeting</td>
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<td>Lighthouse 2</td>
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<tr>
<td>3:00 pm – 3:30 pm</td>
<td><strong>Break</strong></td>
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<td>Seaport/Mezzanine Lobby</td>
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<tr>
<td>3:30 pm – 3:45 pm</td>
<td><strong>Welcome</strong></td>
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<td><em>Kevin Churchwell, MD</em></td>
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<td><em>Chief Operating Officer</em></td>
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<td>Seaport Ballroom</td>
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<td>3:45 pm – 4:36 pm</td>
<td>Scholar Introduction of Each Poster (Poster Presenters)</td>
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<tr>
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<td>Moderator: Jordan Kreidberg, MD, PhD</td>
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<td>Seaport Ballroom</td>
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<tr>
<td>4:40 pm – 5:00 pm</td>
<td>Poster Presenters Set-up Posters</td>
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<td>5:00 pm – 6:30 pm</td>
<td><strong>Poster Session</strong></td>
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<td>Plaza Lobby</td>
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<td>7:00 pm – 9:30 pm</td>
<td><strong>Dinner</strong></td>
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<td><strong>Keynote Speaker</strong></td>
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<td><em>George M. Church, PhD</em></td>
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<td><em>Director of NHGRI Center for Excellence in Genomic Science</em></td>
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<td><em>Harvard Medical School</em></td>
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<td><em>Plaza C</em></td>
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<td><strong>Saturday, September 6, 2014</strong></td>
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<td>7:00 am – 8:00 am</td>
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<tr>
<td>8:00 am – 8:20 am</td>
<td><strong>Introduction/Welcome</strong></td>
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<td></td>
<td><em>Gary Fleisher, MD</em></td>
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<td><em>Physician-in-Chief</em></td>
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<td><em>Egan Family Professor of Pediatrics</em></td>
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</table>
| 8:20 am – 10:00 am | **Platform Presentation Session 1**  
The Immune System and Disease  
Moderator: George Lister, MD  
Plaza AB                     |
| 10:00 am – 10:30 am | **Break**                                      
Plaza Lobby                   |
| 10:30 am – 12:10 pm | **Platform Presentation Session 2**  
Development and Genetics  
Moderator: Gary Fleisher, MD  
Plaza AB                     |
| 12:10 pm – 1:30 pm | **Lunch**                                       
Plaza C                       |
| 1:30 pm – 3:10 pm  | **Platform Presentation Session 3**  
Infectious Disease  
Moderator: Jordan Kreidberg, MD, PhD  
Plaza AB                     |
| 3:10 pm – 3:30 pm  | **Break**                                       
Plaza Lobby                   |
| 3:30 pm – 4:50 pm  | **Platform Presentation Session 4**  
Oncology and Vascular Biology  
Moderator: Clifford Bogue, MD  
Plaza AB                     |
| 4:50 – 6:00 pm    | **Dismantle Posters**                           
Plaza Lobby                   |
| 6:30 pm          | **Dinner**                                      
Plaza Ballroom                |

**Sunday, September 7, 2014**

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| 6:00 am – 8:00 am | **Breakfast in Hotel**  
Plaza C  
Depart for Airport |
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<td>Yee-Ming Chan</td>
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<td>Luke Judge</td>
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<td>Michael Keller</td>
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<td>Shane McAllister</td>
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<td>Marlin Touma</td>
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<td>David Van Mater</td>
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<td>Michael Watson</td>
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<td>Pamela Winterberg</td>
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<td>Yuhua Zheng</td>
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Friday, September 5
12:00 pm – 2:00 pm  Registration and light lunch for early arrivals

1:15 pm – 2:00 pm  Scholars Career Development Session – Lighthouse 1
   30 Years in Translation: Basic Research in Hematopoiesis and Stem Cell Genetic Therapies
   David Williams, MD
   Chief, Division of Hematology and Oncology
   Boston Children’s Hospital

2:00 pm – 3:00 pm  Scholars Break-out Session with Mentors
       Lighthouse 1

2:00 pm – 3:00 pm  Program Director’s Meeting
       Moderator: Karen Winer, NIH/NICHD
       Lighthouse 2

3:00 pm – 3:30 pm  Break - Seaport / Mezzanine Lobby

3:30 pm  Welcome
       Kevin Churchwell, MD
       Chief Operating Officer
       Boston Children’s Hospital

3:45 pm  Scholar introduction to each poster: 3 min/3 slides:

Moderator: Jordan Kreidberg

3:45  THE IMPACT OF MODULATING MXI1 AND MXI0 EXPRESSION ON N-MYC-MEDIATED NEUROBLASTOMA TUMOR PATHOGENESIS AND CHEMOSENSITIVITY.
       MB Armstrong, C Ellis, S Widemon, and DS Wechsler, Durham, NC.  Duke University
       Poster 1
       Page 10

3:48  MECHANISMS OF METABOLIC DERANGEMENT IN STEM CELLS FROM OFFSPRING OF OBESE MOTHERS.
       PR Baker, K Boyle, A Buti, D Debelea, L Barbour, and JE Friedman, Aurora, CO.  University of Colorado
       Poster 2
       Page 11

3:51  THE KISSPEPTIN-STIMULATION TEST AS A NOVEL DIAGNOSTIC TOOL FOR THE EVALUATION OF DELAYED PUBERTY.
       Y Chan, MF Lippincott, VE Christopoulos, CX Li, and SB Seminara, Boston, MA.  Boston Children’s Hospital
       Poster 3
       Page 12
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<tr>
<td>3:54</td>
<td>EXOME SEQUENCING IN THE DIAGNOSIS OF PEDIATRIC CANCER PREDISPOSITION.</td>
<td>VY Chang, H Lee, T Davidson, N Dorrani, E O’Leary, PA Ganz, and SF Nelson, Los Angeles, CA.</td>
<td>University of California Los Angeles</td>
<td>Poster 4</td>
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<td>3:57</td>
<td>ALTERED TOLL-LIKE RECEPTOR GENE EXPRESSION IN A MURINE MODEL OF BRONCHOPULMONARY DYSPLASIA.</td>
<td>JR Dalton, AP Popova, NW Lukacs, and MB Hershenson, Ann Arbor, MI.</td>
<td>University of Michigan</td>
<td>Poster 5</td>
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<td>4:00</td>
<td>CLUSTER ANALYSIS OF THE OBESE ASTHMATIC PHENOTYPE IN PUERTO RICAN CHILDREN.</td>
<td>E Forno, W Chen, J Brehm, Y Han, E Acosta-Perez, M Alvarez, A Colon-Semidey, G Canino, and QC Celedon, Pittsburgh, PA and San Juan, Puerto Rico.</td>
<td>University of Pittsburgh</td>
<td>Poster 6</td>
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<td>4:03</td>
<td>CELECOXIB ANALOGS AS ANTIFungal SMALL MOLECULES: IN VITRO ACTIVITY, IN VIVO EFFICACY AND MECHANISM OF ACTION.</td>
<td>JV Green, L Didone, K Koselny, D Savage, R Boeckman, Jr., and M Wellington, D Krysan, Rochester, NY.</td>
<td>University of Rochester</td>
<td>Poster 7</td>
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<td>4:06</td>
<td>MODELING BAG3 CARDIOMYOPATHY IN HUMAN PLURIPOTENT STEM CELLS.</td>
<td>LM Judge, J Yoo, A Truong, J Perez-Bermejo, Y Miyaoka, P Lizarraga, MJ Spindler, T Nguyen, PSO, and BR Conklin, San Francisco, CA.</td>
<td>University of California, San Francisco</td>
<td>Poster 8</td>
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<td>4:09</td>
<td>DONOR CMV STATUS IMPACTS THE PHENOTYPE OF ANTIVIRAL CYTOTOXIC T-LYMPHOCYTE PRODUCTS.</td>
<td>MD Keller, P Hanley, and C Bollard, Washington, DC.</td>
<td>Children’s National Medical Center</td>
<td>Poster 9</td>
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<td>4:12</td>
<td>THE AIRWAY MICROBIOME OF THE NEWBORN AND MOTHER.</td>
<td>KM Kloepfer, D Nelson, R Tepper, and SD Davis, Indianapolis, IN.</td>
<td>Indiana University School of Medicine</td>
<td>Poster 10</td>
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<td>4:15</td>
<td>AUTOCRINE β-ADRENERGIC SIGNALING DRIVES POST-CONFLUENT GROWTH OF ENDOTHELIAL CELLS INFECTED WITH KAPOSI SARCOMA HERPESVIRUS.</td>
<td>SC McAllister and RS Hanson, Minneapolis, MN.</td>
<td>University of Minnesota</td>
<td>Poster 11</td>
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<td>4:18</td>
<td>THERAPEUTIC TARGETING OF NEUTROPHIL CD64 AND REATIVE OXYGEN SPECIES IN PEDIATRIC CROHN'S DISEASE.</td>
<td>P Minar, Y Haberman, Y Tsai, I Jurickova, and LA Denson, Cincinnati, OH.</td>
<td>Cincinnati Children's Hospital Medical Center</td>
<td>Poster 12</td>
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4:21  A SYNDROME OF ENTEROCOLITIS AND AUTOINFLAMMATION (SCAN4) CAUSED BY MUTATION OF NLRC4.

_N Romberg, KA Maoussawi, M Choi, NA Podoltsev, B Kazmierczak, and RP Lifton, New Haven, CT. Yale University School of Medicine_  
_Poster 13  
Page 22

4:24  A LIN28B/RAN/AURKA SIGNALING AXIS PROMOTES Neuroblastoma TUMORIGENESIS.

_RW Schnepp and JM Maris, Philadelphia, PA. University of Pennsylvania_  
_Poster 14  
Page 23

4:27  PIGGYBAC-MEDIATED PHENOTYPIC CORRECTION OF FACTOR VIII DEFICIENCY.

_JM Staber, MJ Pollpeter, A Arensdorf, P Sinn, DT Rutkowski, and PB McCray, Iowa City, IA. University of Iowa_  
_Poster 15  
Page 24

4:30  ALTERED CALCIUM HANDLING AND DIASTOLIC DYSFUNCTION DURING CHRONIC KIDNEY DISEASE (CKD).

_PD Winterberg, R Jiang, B Wang, S Harbaran, and MB Wagner, Atlanta, GA. Emory University_  
_Poster 16  
Page 25

4:33  S-ADENOSYL METHIONINE INHIBITS β-CATENIN ACTIVATION IN LIVER AND COLON CANCER.

_Y Zheng, T Li, and S Lu, Los Angeles, CA. Children’s Hospital Los Angeles, University of Southern California_  
_Poster 17  
Page 26

4:40 pm – 5:00 pm  Poster Presenters set up posters – Plaza Lobby

5:00 pm – 6:30 pm  Poster presentations including snacks – Plaza Lobby

7:00 pm  Dinner – Plaza C

_Keynote Speaker_  
New technologies for diagnosis and prevention of childhood diseases: NGS, FISSEQ, CRISPR  
George M. Church, PhD  
Director of NHGRI Center for Excellence in Genomic Science  
Harvard Medical School

_Saturday, September 6, 2014_  
7:00 am – 8:00 am  Breakfast in hotel - Plaza C

8:00 am – 8:20 am  Opening Comments - Plaza AB

_Gary Fleisher, MD  
Physician-in-Chief  
Egan Family Professor of Pediatrics  
_Boston Children’s Hospital_  
George Lister, MD  
Chair, Department of Pediatrics  
_Yale School of Medicine_
Moderator: George Lister

8:20  UTILIZING T PROGENITORS TO INCREASE TEC REGENERATION AND THYMOCYTE CROSS-TALK IN A MURINE TRANSPLANT MODEL.
HE Stefanski, MJ Smith, LM Jonart, and BR Blazar, Minneapolis, MN. University of Minnesota Page 27

8:40  ROLE OF BCL11B IN HUMAN THYMOPOIESIS AND T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA.
CA Parekh, V Ha, A Luong, P Chaudhary, R Bhatia, and G Crooks, Los Angeles, CA and Duarte, CA. Children's Hospital Los Angeles, University of Southern California Page 28

9:00  SUPPRESSION OF ADOPTIVE CD8 T-CELL KILLING BY INFLAMMASOME ACTIVATION IN THE BONE MARROW MACROPHAGES OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA.
JC Shand, JT Mariano, MW Winter, and LM Toia, Rochester, NY. University of Rochester Page 29

9:20  FOLLISTATIN LIKE PROTEIN 1 REGULATES IL-17 SIGNALING BY INFLUENCING IL-17 RECEPTOR EXPRESSION.
BT Campfield, Y Chaly, R Hirsch, and J Kolls, Pittsburgh, PA and Iowa City, IA. University of Pittsburgh School of Medicine Page 30

9:40  MICROBIOTA, MHC AND AUTOIMMUNITY: CAN GUT BUGS PROTECT FROM TYPE 1 DIABETES?
MA Silverman, C Benoist, and D Mathis, Boston, MA. Harvard University Page 31

10:00–10:30 am Break
Plaza Lobby

Moderator: Gary Fleisher

10:30  NEONATAL MOUSE HEART MATURATION: CHAMBER SPECIFIC EXON ENRICHMENT OF CELL CYCLE GENES.
MTouma, K Xuedong, L Jae-Hyung, X Xinshu, and Y Wang, Los Angeles, CA. University of California Los Angeles Page 32
10:50  THE ROLE OF DCHS2 IN VERTEBRATE PATTERNING AND CONGENITAL HEART DISEASE.
E Deniz, A Robson, MA Choma, M Brueckner, and MK Khokha, New Haven, CT. Yale University
Page 33

11:10  POST-TRANSCRIPTIONAL CONTROLS IN GASTROINTESTINAL GENE EXPRESSION.
L Ghanem and SA Liebhaber, Philadelphia, PA. University of Pennsylvania  Page 34

11:30  OF MICE AND MEN: MODIFIERS OF VASCULAR DISEASE IN WILLIAMS SYNDROME.
B Kozel, St. Louis, MO. Washington University School of Medicine  Page 35

11:50  EXPLORING THE ROLE OF IMPAIRED ARGinine SYNTHESIS IN ASL DEFICIENCY USING ZEBRAFISH.
OA Shchelochkov, J Jung, L Yu, J Downward, LM Teesch, CM Brenner, and RA Cornell, Iowa City, IA. University of Iowa  Page 36

12:10 pm – 1:30 pm  Lunch - Plaza C

Platform Presentation Session 3
Infectious Disease
1:30 pm – 3:15 pm
Plaza AB

Moderator: Jordan Kreidberg

1:30  AIRWAY EXOSOMAL MICRORNA SECRETION AND IMMUNE PROFILES DURING RHINOVIRUS INFECTION IN EARLY CHILDHOOD.
GR Nino, G Perez, K Pancham, S Huseni, D Preciado, R Freishtat, A Colberg-Poley, E Hoffman, and MC Rose, Hummelstown, PA and Washington, DC. Children’s National Medical Center  Page 37

1:50  T REGULATORY CELLS MODULATE THE CYTOTOXIC T CELL RESPONSE FOLLOWING RIFT VALLEY FEVER VIRUS VACCINATION.
AK McElroy, J Cruz, C Munoz-Fontela, ST Nichol, and CF Spiropoulou, Atlanta, GA and Hamburg, Germany Emory University  Page 38

2:10  INTERLEUKIN-17A AND HOST RESPONSES IN ASYMPTOMATIC AND INFLAMMATORY MODELS OF STREPTOCOCCUS PYOGENES INFECTION.
ME Watson, S Ahmed, I Laczkovich, JB Weinberg, and SR Dawid, Ann Arbor, MI. University of Michigan  Page 39

2:30  PULMONARY CERAMIDE DEPLETION AMPLIFIES AIRWAY HYPER-RESPONSIVENESS & ENHANCES GRANULOCYTE CHEMOTAXIS IN A MODEL OF ALLERGIC ASTHMA.
AW Lindsley, K Rehn, and R Edukulla, Cincinnati, OH. Cincinnati Children’s Hospital Medical Center  Page 40

2:50  INACTIVATION OF A GENE INVOLVED IN OXIDATIVE STRESS INFLUENCES FITNESS OF MORAXELLA CATARRHALIS.
AS Evans, C Pybus, JS Kahn, and EJ Hansen, Dallas, TX. UT Southwestern Medical Center  Page 41

3:10 pm–3:30 pm Break
Plaza Lobby
Platform Presentation Session 4
Oncology and Vascular Biology
3:30 pm – 4:50 pm
Plaza AB

**Moderator: Clifford Bogue**

3:30  **THE FANCONI ANEMIA (FA) SIGNALING NETWORK REGULATES SPINDLE ASSEMBLY CHECKPOINT TO PREVENT ANEUPLOIDY AND CANCER.**

  *G Nalepa, Indianapolis, IN. Indiana University*

3:50  **A NOVEL SMALL MOLECULE INHIBITOR OF THE GAS6/TAM PATHWAY, IN COMBINATION WITH ADP/P2Y ANTAGONISTS, MEDIATES SYNERGISTIC INHIBITION OF PLATELET AGGREGATION AND DECREASE ARTERIAL & VENOUS THROMBOSIS.**

  *BR Branchford, L Law, G Acevedo, C Brzezinski, S Sather, G Brodsky, D DeRyckere, W Zhang, HS Earp, S Frye, DK Graham, and JA Di Paola, Denver, CO, Aurora, CO, and Chapel Hill, NC. University of Colorado*

4:10  **DISSECTING THE ONCOGENIC ACTIVITY OF LRH-1 IN THE COLON.**

  *JR Bayrer and RJ Fletterick, San Francisco, CA. University of California San Francisco*

4:30  **A ROLE FOR INJURY IN SARCOMAGENESIS.**

  *D Van Mater, L Ano, JM Blum, and DG Kirsch, Durham, NC. Duke University*

4:50 pm  **Dismantle Posters**

6:30 pm  **Dinner – Plaza Ballroom**

**Sunday, September 7, 2014**

6:00 am – 8:00 am  **Breakfast in Hotel and Depart for Airport**

Plaza C
Neuroblastoma is the third most common malignancy of childhood. Despite intensive treatment, outcomes for children with advanced disease remain poor. The MYCN gene is frequently amplified, portending a poor prognosis, however, the etiology is unclear. My laboratory is investigating the role of Mxi1 and Mxi0, two Myc family members with the ability to modulate the function of N-Myc. As a Myc antagonist, Mxi1 represses transcription of Myc-regulated genes resulting in decreased growth and proliferation of neuroblastoma cells in vitro. Furthermore, we identified Mxi0, a novel, alternatively transcribed Mxi1 isoform that lacks transcriptional inhibitory activity of Mxi1 suggesting that the Myc-inhibitory activity may be modulated by Mxi0. Our hypothesis is that the balance of Mxi1 and its analogue, Mxi0, are critical to normal neuroblast growth and also to the pathogenesis of neuroblastoma tumors. We are exploring the interactions of Mxi1 and Mxi0 with each other in respect to their roles in modulating N-Myc function in neuroblastoma. I feel that my background makes me uniquely suited to approach this project. I gained extensive experience in molecular biology during my Ph.D. training. I expanded these skills during my post-doctoral fellowship during which time I began studying the molecular pathophysiology of neuroblastoma. I am now looking to build on this scientific foundation and knowledge of neuroblastoma pathophysiology and begin the transition to an independent investigator. Also, my clinical background in Pediatric Hematology-Oncology grants me a practical perspective in which to battle this devastating disease and pushes me to facilitate the translation of basic science discoveries into the clinical arena. As Director of the therapeutic 131I-MIBG program at Duke and institutional PI for early phase clinical trials in neuroblastoma, I have demonstrated my commitment to finding better ways to fight neuroblastoma. I look forward to utilizing my molecular biology skills and clinical expertise to provide a brighter future for the children impacted by this terrible disease.

The Impact of Modulating Mxi1 and Mxi0 Expression on N-Myc-Mediated Neuroblastoma Tumor Pathogenesis and Chemosensitivity
D. Christian Ellis, R. Scott Widemon, Daniel Wechsler, and Michael Armstrong
Duke University Medical Center, Durham, NC

**Background:** Neuroblastoma is the most common extracranial malignancy of childhood. The Myc family regulates cell growth and proliferation is implicated in the etiology of many cancers. MYCN amplified neuroblastoma carries a poor overall survival. Investigating specific tumor pathways will further our understanding of neuroblastoma pathogenesis and lead to future therapeutic options. Mxi1 is a member of the MAD family which inhibits N-Myc function. Mxi0 is an alternatively-spliced variant of Mxi1 whose function has not been determined. We hypothesize that Mxi1 and Mxi0 impact N-Myc-dependent neuroblastoma cell growth. **Design:** We expressed Mxi1 and Mxi0 in SHEP neuroblastoma cells and SHEP cells stably transfected to express high levels of MYCN (SHEP/MYCN). We also utilized native neuroblastoma cell lines with inducible expression of Mxi1 and Mxi0. Cell proliferation and survival were quantified using BrdU and MTT assays. Apoptosis was measured by propidium iodide staining and caspase-3 immuno-histochemistry. Cellular localization of Mxi1 and Mxi0 proteins was detected by immunofluorescence. **Results:** Overexpression of Mxi1 inhibits N-Myc mediated cell proliferation. In the absence of N-Myc, Mxi1 overexpression independently inhibits cell proliferation and induces cell apoptosis. Conversely, overexpression of Mxi0 in MYCN amplified neuroblastoma cell lines leads to enhanced proliferation, suggesting that Mxi0 has a counter-regulatory role to that of Mxi1. Expression of Mxi0 made the cells more chemoresistant. Finally, examination of Mxi1 and Mxi0 cellular location reveals that Mxi1 resides in the nucleus while Mxi0 is found primarily in the cytoplasm. **Conclusions:** Overexpression of Mxi1 in neuroblastoma cell lines leads to inhibition of N-Myc-mediated cell proliferation while Mxi0 appears to promote cell growth. Mxi1 expression enhanced chemosensitivity of neuroblastoma cells, while Mxi0 had the converse effect. A better understanding of the interaction between Mxi1 and Mxi0 and how the balance of these proteins affect neuroblastoma physiology may aid in developing more effective targeted therapies to improve outcomes in children with neuroblastoma.
Peter R. Baker II, MD
Assistant Professor
University of Colorado

Dr. Baker received his undergraduate degree at Arizona State University and did his medical training at the University of Arizona. After medical school, Dr. Baker completed a residency in pediatrics at the University of Oregon Health & Science University and fellowships in clinical genetics and biochemical genetics at the University of Colorado. He also completed a post-doctoral research fellowship in the Pediatric Scientist Development Program, under the late George Eisenbarth at the Barbara Davis Center for Childhood Diabetes. In early 2013, Dr. Baker joined the laboratory of Dr. Jed Friedman at the University of Colorado in the Nutrition and Obesity Research Center, to pursue further post-doctoral research training. Dr. Baker is currently an assistant professor in the Department of Pediatrics, Section of Clinical Genetics and Metabolism at the University of Colorado, School of Medicine, where he is interested in metabolism and bioenergetics in obesity. Utilizing a unique umbilical cord derived mesenchymal stem cell (MSC) model, he is studying the effects of maternal obesity in the setting of a high fat Western diet on maternal/fetal metabolism and fetal programming. Dr. Baker is particularly interested in understanding the effects of maternal over-nutrition on fetal mitochondrial metabolism, specifically catabolism of branched chain amino acids and fatty acids, in MSC differentiated myocytes and adipocytes. These metabolic derangements are potentially the result of programmed epigenetic changes in the infant that lead to early onset childhood obesity and metabolic syndrome observed in offspring of obese women.

Mechanisms of Metabolic Derangement in Stem Cells from Offspring of Obese Mothers.

Peter Baker, K Boyle, A Buti, D Dabelea, L Barbour, and J Friedman, University of Colorado, Aurora, CO

Background: Childhood obesity is a world-wide epidemic. “Developmental programming” is a major contributor to obesity-associated pathology recognized as early as age 6 years. Although there is increasing evidence for the roles of excess lipid exposure, epigenetic modifications, and mitochondrial dysregulation the mechanisms by which these effects are transmitted from mother to offspring, particularly in humans, are not well understood. Adipocytes and myocytes from obese subjects have alterations in key mitochondrial energy pathways (including branched chain amino acid (BCAA) and fatty acid oxidation (FAO) metabolism), and they have a common cellular precursor: the mesenchymal stem cells (MSC). MSC have not yet been used to investigate obesity and fetal programming. Our hypothesis is that obesity risk in offspring of obese mothers is manifest in offspring MSC mitochondrial metabolism and epigenome. Objectives: Test the hypothesis that umbilical derived MSC, from infants of obese versus normal weight (NW) mothers, manifest impaired mitochondrial metabolism. Design/Methods: The Healthy Start Study deeply phenotypes 1,400 pregnant women enrolled prospectively before 24 weeks of gestation, and followed for maternal-infant macronutrient utilization, inflammatory markers, and body composition through the first year of life. Umbilical derived MSC from enrollees have been collected and stored. Undifferentiated MSC from infants of normal weight (n=5) and obese (n=5) mothers were grown and differentiated into myocytes. On day 21 of differentiation all cells were tested for total mitochondrial FAO flux and amino acid concentrations in media. Results: MSC from infants of NW mothers had 2-fold higher flux through the FAO pathway versus cells from infants of obese mothers (p=0.08). BCAA (leucine, isoleucine, and valine) were higher in media of MSC from infants of obese versus NW mothers (p=0.01, 0.04, 0.07 resp.). These studies are ongoing. Conclusions: Early studies in MSC indicate programmed mitochondrial inflexibility and increased intermediary metabolism in offspring of obese mothers. This MSC model will be valuable in the targeted study of energy metabolomics, transcriptomics, and epigenomics in fetal programming and obesity.
Dr. Chan received his undergraduate degree from Yale University and received his M.D. and Ph.D. from the Medical Scientist Training Program at the University of California, San Francisco (UCSF). He stayed at UCSF for his residency in general pediatrics, and he arrived at Boston Children’s Hospital for his fellowship in Pediatric Endocrinology. During his fellowship training, he joined the research group of Dr. Stephanie Seminara in the Reproductive Endocrine Unit at Massachusetts General Hospital. Dr. Chan is currently in the Division of Endocrinology, Department of Medicine at Boston Children’s Hospital and is studying puberty and the physiology of the reproductive endocrine system. One focus of his work is using the recently discovered hormone kisspeptin as a diagnostic test to help determine whether an adolescent with delayed puberty has a self-limited condition (constitutional delay of puberty) or a more permanent problem (hypogonadotrophic hypogonadism). He is also starting to use human genetic approaches to elucidate the genetic basis for the delay in pubertal timing in constitutional delay.

The Kisspeptin-Stimulation Test as a Probe of Reproductive Endocrine Function

Yee-Ming Chan, Margaret F. Lippincott, Voula E. Christopoulos, Cindy X. Li, Stephanie B. Seminara

1Division of Endocrinology, Boston Children’s Hospital, Boston, MA, USA, 2Reproductive Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA

Background: The neuropeptide hormone kisspeptin directly and specifically stimulates the secretion of GnRH (gonadotropin-releasing hormone). Administration of kisspeptin may therefore be used as a probe of GnRH neuronal function to chart normal physiology and to identify pathophysiologic conditions. In healthy adults, responses to kisspeptin have been found to differ between men and women as well as between women across the changing sex-steroid milieu of the menstrual cycle, though the precise reason for these differences is unclear. In adolescents with delayed puberty, kisspeptin may help to distinguish between two conditions that present with delayed puberty: constitutional delay of puberty (CDP), a self-limited delay in pubertal timing in which underlying GnRH neuronal function is normal, and idiopathic hypogonadotropic hypogonadism (IHH), a pathological condition due to impaired GnRH secretion or action. Methods: In rodent studies, LH responses to kisspeptin 5 nmol SC vs. saline were measured in 4-6 week old male and female mice that were intact, gonadectomized, or gonadectomized with sex-steroid replacement. In human studies, adolescents with delayed puberty were given kisspeptin 0.24 nmol/kg IV x1 and blood was drawn every 10 minutes to chart both baseline and kisspeptin-stimulated LH secretion. Results: Intact female mice responded robustly to kisspeptin (baseline 0.02 ± 0 mIU/mL, stimulated 3.2 ± 0.2 mIU/mL, P = 0.001). This response was abolished after gonadectomy (1.8 ± 0.5 mIU/mL to 1.9 ± 0.4 mIU/mL, P = 0.9) and restored by treatment with sex steroids (0.02 ± 0 mIU/mL to 1.41 ± 0.3 mIU/mL, P = 0.01). In contrast, gonadectomized male mice retained their response to kisspeptin (0.8 ± 0.3 mIU/mL to 4.4 mIU/mL, P = 0.03). 2) To date, two adolescents with delayed puberty have received kisspeptin; neither responded. Both subjects have impaired senses of smell, suggesting that they have Kallmann syndrome, in which IHH is seen in combination with anosmia. Conclusions: There are intrinsic differences in responsiveness to kisspeptin between the sexes in rodents as there are in humans, as loss of sex steroids abolishes responses to kisspeptin in female but not male mice. Adolescents who are likely to have IHH fail to respond to kisspeptin. Ongoing recruitment will determine whether adolescents with CDP will respond to kisspeptin and whether the “kisspeptin-stimulation test” will be the long-sought method to definitively and prospectively distinguish CDP from IHH.
Vivian Y. Chang, MD, MS  
Clinical Instructor  
University of California, Los Angeles

Dr. Chang received her undergraduate degree in Biological Chemistry with a minor in English from Wellesley College. She then attended medical school at the University of Chicago, Pritzker School of Medicine, where she stayed on to complete her general pediatrics residency at Comer Children’s Hospital. In July 2009, she started her pediatric hematology-oncology fellowship at University of California, Los Angeles Mattel Children’s Hospital. During her fellowship, she completed a Master’s degree in Bioinformatics and began working with next-generation sequencing data, in the laboratory of Dr. Stan Nelson. After fellowship, she joined the faculty at UCLA and has since been developing a unique multidisciplinary genomics clinic, the Pediatric Cancer Predisposition Clinic. Through this clinic, she provides a clinical service for patients at high-risk of cancer, and collects samples and data for a prospective biorepository that serves as a resource for research. Currently, she is using whole exome sequencing in these patients to identify novel germline cancer predisposition genes. She is also exploring the use of cutting-edge genome-engineering techniques such as transcription activator-like effector nucleases and clustered regularly interspaced short palindromic repeats to introduce site-specific mutations of interest and to design functional assays.

Exome Sequencing in the Diagnosis of Pediatric Cancer Predisposition

Vivian Y. Chang, Hane Lee, Tom Davidson, Naghmeh Dorrani, Erin O’Leary, Patricia A. Ganz, Stanley F. Nelson, Julian A. Martinez-Agosto  
University of California, Los Angeles, Los Angeles, CA.

Background: Currently, there are few guidelines on genetic testing of pediatric patients for cancer predisposition and on appropriate cancer surveillance recommendations once a genetic cancer predisposition is diagnosed. Previous research studies estimated that only about 5-10% of pediatric cancers are due to a heritable cause. However, this may be an underestimation of the true incidence in the pediatric population. Objectives: The aims are (1) To identify true incidence of genetic predisposition in pediatric cancer patients and (2) To identify novel cancer predisposition genes and their function, and to expand phenotype and understanding of known cancer predisposition genes.

Design/Methods: Patients referred to a multi-disciplinary Pediatric Cancer Predisposition Clinic with an increased risk of cancer due to a genetic condition were evaluated and when indicated, clinical exome sequencing (CES) was used as the first-line diagnostic test. Results: There were 49 patients evaluated in the first 18 months. CES resulted in four reportable, likely pathogenic mutations in PTEN, NF-1, SUFU, and PRF1. Six patients had no clinically significant genetic variants identified, and 13 patients had variants of uncertain clinical significance, which represent potentially new cancer predisposition genes. Conclusion: More thorough and broad scale germline analyses are needed to accurately inform patients of inherited risk to cancer, as it is not always obvious based on clinical presentation or family history. The routine use of CES in testing the germline genomes of children with cancer holds the promise to augment diagnosis, treatment, and screening, and to permit the exploration of early initiation of cancer prevention strategies of patients with high risk of cancer.
Jennifer Dalton, MD  
Clinical Lecturer  
University of Michigan

Dr. Dalton did her undergraduate training at Purdue University and attended medical school at The Ohio State University College of Medicine and Public Health. She completed her pediatric residency and neonatal-perinatal medicine fellowship at the University of Michigan. During her fellowship, Dr. Dalton joined the laboratory of Dr. Cory Hogaboam in the department of pathology, where she focused on the epigenetic regulation of cytokine production in bronchopulmonary dysplasia. She has since transitioned to the laboratory of Dr. Nicholas Lukacs, also in the department of pathology, where her study of epigenetics in bronchopulmonary dysplasia and the preterm neonatal immune system continues. She is particularly interested in the mechanisms behind the aberrant cytokine production seen in extremely preterm neonates.

**Altered Toll-like Receptor Gene Expression in a Murine Model of Bronchopulmonary Dysplasia**

*Jennifer Dalton*; Antonia Popova*; Nicholas Lukacs*; Marc Hershenson*

*University of Michigan Health System, Ann Arbor, MI.

**Background:** Bronchopulmonary dysplasia (BPD) is a prematurity-related disease that results in significant morbidity and mortality and disordered inflammation is known to play a role in its development. Sequence variation in toll-like receptor (TLR) genes, which allow cells to distinguish between pathogens and initiate the inflammatory cascade, have been linked to the development of BPD. **Objective:** To determine the pattern of TLR3 and TLR4 gene expression in response to TLR3 and TLR4 agonists in infantile mice with an experimental model of hyperoxia-induced BPD as compared to control infantile and adult mice. **Methods:** Beginning on day of life 2, C57BL/6 mice underwent hyperoxia treatment for 14 days in 75% oxygen. Both hyperoxia-exposed and control mice were harvested on day of life 16. Five month-old C57BL/6 mice were used as adult controls. CD14+ monocytes were isolated from the mouse spleens and were cultured in the presence of a TLR3 agonist (Poly:IC) or a TLR4 agonist (LPS). After 24 hours of stimulation, the mRNA from these cell cultures was processed and quantified by real-time quantitative reverse transcription PCR. **Results:** There was no difference in TLR3 or TLR4 gene expression between adult, infantile and infantile hyperoxia-exposed CD14+ monocytes at baseline. Upon stimulation with a TLR3 agonist (Poly:IC), the adult and infantile control CD14+ monocytes had no change in TLR3 or TLR4 gene expression, while the hyperoxia-exposed infantile mice had a significant increase in both TLR3 and TLR4 gene expression as compared to baseline (both p<0.05). Upon stimulation with a TLR4 agonist (LPS), none of the CD14+ monocytes had a change in TLR3 gene expression over baseline. When TLR4 gene expression was evaluated in the presence of LPS, the adult control CD14+ monocytes had significantly lower expression (p<0.05), the infantile control CD14+ monocytes had no change in gene expression, and the hyperoxia-exposed infantile CD14+ monocytes had significantly higher gene expression when compared to baseline (p<0.05). **Conclusions:** Infantile mice with a hyperoxia-induced model of BPD had a different pattern of TLR3 and TLR4 gene expression upon stimulation with TLR3 and TLR4 agonists than adult and infantile control mice. These findings suggest that differences in the TLR pathway of pathogen recognition may contribute to the lung pathology seen in BPD.
Cluster analysis of the “Obese Asthmatic” phenotype in Puerto Rican children

E Forno1, W Chen1, J Brehm1, YY Han1, E Acosta2, M Alvarez2, A Colón2, G Canino2, JC Celedón1

1Division of Pediatric Pulmonary Medicine, Allergy, and Immunology, University of Pittsburgh, Pittsburgh, PA; 2University of Puerto Rico, San Juan, PR.

Rationale: There is growing evidence of an “obese asthmatic” phenotype in children. Since “obese asthma” is likely complex, we sought to characterize sub-phenotypes in a cohort of children with asthma. Methods: In a study of 678 Puerto Rican children with (n=351) and without (n=327) asthma aged 6-14yrs, protocol included questionnaire data, anthropometry, pulmonary function testing, allergy skin testing and measurement of total/ specific IgE. We used factor analysis to select covariates representing the spectrum of asthma severity, then unsupervised hierarchical cluster analysis to identify “obese asthmatic” sub-phenotypes. We included 94 overweight/obese (BMI≥85th percentile) children with complete data. The resulting clusters were then compared to 182 cases of normal weight using generalized linear models. Results: Cluster analysis yielded 4 distinct “obese asthma” clusters (O1-O4). Compared to lean asthmatics, all 4 clusters had increased BMI (by definition) but O3 had percent body fat, waist circumference, and waist-hip ratios that more closely resembled those of lean asthmatics (p<.05 vs O1, O2, or O4; p>.05 vs lean). Clusters O1 and O4 had the highest percent body fat. Clusters O1 and O2 had more girls, and clusters O3 and O4 more boys. Cluster O1 had lower FEV1 and FEV1/FVC (p<.05 in all instances), with more school absences and higher odds of severe exacerbations. Compared to other clusters, cluster O2 had lower odds of atopy (defined by skin test reactivity to at least one allergen), severe asthma exacerbations, or use of inhaled corticosteroids (ICS). Compared to other clusters, cluster O3 had later onset of asthma, more environmental tobacco smoke (ETS) exposure in early life, and higher airway hyper-responsiveness. Compared to the lean cluster, children in cluster O4 had lower FEV1/FVC and were more likely to be on ICS, and tended to have less current ETS exposure. Conclusions: Unsupervised cluster analysis identified four sub-groups of obese asthmatic children with distinct phenotypic characteristics. Further studies are needed to better characterize and validate these clusters.
Julianne Green, MD, PhD  
Senior Instructor  
University of Rochester Medical Center

Dr. Green received her undergraduate degree in chemistry from Purdue University and her PhD in Organic Chemistry from the University of Cincinnati, specializing in the synthesis, stereochemical characterization, and structure-activity relationships of small organic molecules. Her postdoctoral work in Baltimore, Maryland included collaborative studies at Johns Hopkins School of Medicine, evaluating the anti-cancer potential of numerous synthetic compounds. Dr. Green then spent ten years developing novel isolation/purification schemes for pharmaceuticals at Abbott Laboratories in North Chicago, IL. She then transitioned to a career in medicine, receiving her MD degree from the University of Louisville. At Cincinnati Children’s Medical Center she completed pediatrics residency and fellowship in Pediatric Infectious Diseases, where she studied the interaction of Candida albicans with heparin in relation to biofilm formation. She is currently a Senior Instructor at the University of Rochester Medical Center Division of Pediatric Infectious Diseases, working under the mentorship of Dr. Damian Krysan. Her research interests include the identification of new antifungals via repurposing of FDA-approved molecules with known biological activity. Her recent efforts include elucidating the mechanism of action of OSU-03012, a celecoxib analog with potent antifungal activity.

CELECOXIB ANALOGS AS ANTIFUNGAL SMALL MOLECULES: IN VITRO ACTIVITY, IN VIVO EFFICACY AND MECHANISM OF ACTION

J Green1; L DiDone1; K Koselny1; D Savage2; R Boeckman Jr.2; M Wellington1; D Krysan1,2  
1Department of Pediatrics, 2Department of Chemistry, University of Rochester, Rochester, NY, USA

Background: Invasive fungal infections are an important cause of morbidity and mortality for people living with compromised immune function. Although medical advances have increased the number of people at risk for invasive fungal infections, the pace of new antifungal drug development has been very slow. The echinocandins, the newest class of antifungal drugs, were discovered over 30 years ago and have been in clinical practice for over ten years. Objective: Discovery of novel fungicidal small molecules via repurposing of compounds with known biological activity. Method: A novel high throughput screening strategy that relies on the release of the intracellular enzyme adenylate kinase (AK) as a marker of fungicidal activity led to the identification of OSU-03012 as a potent antifungal compound. Results: OSU-03012 is a pyrazole-based molecule related to the COX2 inhibitor celecoxib. OSU-03012 is a multi-kinase inhibitor with reported activity against human PDK1 and PAK kinases; it has entered phase I clinical trials as a targeted anti-cancer therapy. In vitro, OSU-03012 has good antifungal activity against C. albicans and C. neoformans (MIC 4 µg/mL), and synergistic activity with fluconazole (FIC and time-kill) toward C. neoformans. In a mouse model of disseminated candidiasis, OSU-03012 reduced kidney fungal burden ~1 log10 cfu/g relative to untreated controls. In a mouse model of cryptococcosis, OSU-03012 was inactive alone but synergized with fluconazole to reduce brain burdens by 1 log10 cfu/g relative to fluconazole alone. We have begun studies to identify the fungal target of OSU-03012; initial results indicate it disrupts calcium homeostasis in fungal cells. Conclusions: OSU-03012 is a potent anti-fungal compound with in vitro and in vivo activity. Ongoing efforts to determine its mechanism of action point to modulation of calcium homeostasis. Further efforts to elucidate OSU-03012’s target will allow for structure refinement and potency optimization.
Luke Judge, MD, PhD
Clinical Fellow
University of California, San Francisco

Dr. Judge received his undergraduate degree at Pomona College, followed by medical and graduate training at the University of Washington. His dissertation research was performed with Dr. Jeffrey Chamberlain, focusing on mouse models of Duchenne’s Muscular Dystrophy. After medical school, Dr. Judge completed a residency in pediatrics and fellowship in neonatal-perinatal medicine at the University of California, San Francisco. During his fellowship he joined the laboratory of Dr. Bruce Conklin as a post-doctoral scholar at the Gladstone Institute of Cardiovascular Disease. His fellowship research has focused on modeling inherited cardiomyopathy using human pluripotent stem cells. Dr. Judge is particularly interested in the BAG3 gene product, a co-chaperone protein that is critical for the function of both skeletal and cardiac muscle. He is using genome and tissue engineering methods to study the function of BAG3 in human cardiomyocytes.

Modeling Bag3 Cardiomyopathy in Human Pluripotent Stem Cells.
LM Judge1,2, J Yoo2, A Truong2, J Perez-Bermejo2, Y Miyaoka2, P Lizarraga2, MJ Spindler2, T Nguyen2, PL So2, BR Conklin.2
1University of California, San Francisco, CA 2Gladstone Institute of Cardiovascular Disease, San Francisco, CA

Background: Large-scale genomic studies are generating a wealth of information about genetic variants associated with cardiovascular disease, but unraveling the functional consequences of these variants provides new challenges. Recent genetic studies implicate protein-coding variants in the BAG3 gene in both inherited and sporadic forms of dilated cardiomyopathy (DCM), the most common indication for cardiac transplantation in the U.S. Objectives: To generate an allelic series of isogenic human induced-pluripotent stem (iPS) cell lines with various BAG3 mutations, in order to model the functional consequences of protein coding variants in engineered human tissue. We hypothesized that cardiomyocytes derived from a BAG3 null iPS line would have a cardiomyopathy phenotype, which could be compared with various point mutations. Design/Methods: We designed Transcription Activator-Like Effector Nucleases (TALENs) to induce targeted homologous recombination in a healthy human iPS cell line, in order to produce an isogenic series of BAG3 mutant iPS lines. Targeted cells were isolated either by antibiotic resistance, or a novel method recently published by our laboratory (Miyaoka et al, 2014). All cell lines were characterized and differentiated into cardiomyocytes using previously described methods. Results: We generated an allelic series of iPS lines with the following targeted BAG3 mutations: heterozygous null, homozygous null, P209L, and C151R. All mutant cell lines had a normal karyotype, expressed pluripotency markers, and efficiently differentiated into functional cardiomyocytes. Homozygous BAG3 null cardiomyocytes demonstrated sarcomeric disorganization, as determined by quantitative image analysis. Conclusions: Human iPS cells with disease-associated BAG3 mutations retain pluripotency and efficient cardiac differentiation, however cardiomyocytes with loss of BAG3 expression show sarcomeric disorganization that consistent with human and mouse pathology. Studies are ongoing to determine the functional consequences of individual point mutations in BAG3.
Donor CMV Status Impacts The Phenotype of Antiviral Cytotoxic T-Lymphocyte Products

**Michael Keller,** MD  
**Assistant Professor**  
**Children's National Medical Center**

Dr. Keller received his undergraduate degree from the University of Virginia, and received his medical degree from Duke University. While there, he was an HHMI research scholar in the laboratory of Dr. Rebecca Buckley. He completed his pediatrics residency at Johns Hopkins, and went on to fellowship in Allergy & Immunology at Children's Hospital of Philadelphia. During fellowship, he joined the lab of Dr. Jordan Orange, where he pursued new mendelian causes of primary immunodeficiency disorders, with a particular focus on natural killer cell deficiency. In July 2013, he joined the laboratory of Dr. Catherine Bollard at Children's National Medical center, where he is investigating the host and viral factors influencing T-cell repertoire in cytotoxic T-lymphocyte (CTL) products through the use of ex vivo T-cell culture as well as an open clinical protocol of CTL therapy using either CMV seropositive or CMV naïve donors for CTL generation. He is also very interested in the use of adoptive therapy in primary immunodeficiency disorders, and has an open protocol for use of CTL therapy in primary immunodeficiency patients prior to hematopoietic stem cell transplantation.

**Donor CMV Status Impacts The Phenotype of Antiviral Cytotoxic T-Lymphocyte Products**

**MD Keller,** P Hanley, and C Bollard  
Children's National Medical Center, Washington, DC

**Background:** Adoptive immunotherapy using virus-specific cytotoxic T-lymphocyte (CTL) products has been highly successful in treatment or preventing viral infections after hematopoietic stem cell transplantation, and new protocols allow CTL generation from virus-naïve donors by use of naïve T-cell selection and serial stimulation with antigen presenting cells. However, the cellular phenotype of T-cells and NK cells differ in products derived from these two donor sources, which we hypothesize could impact their clinical efficacy and persistence. **Objectives:** To examine the differences in cellular phenotype and anti-viral effector function between CTL derived from CMV seropositive versus naïve donors. **Design/Methods:** CTL were generated from healthy donors using established protocols. Briefly, a rapid protocol using pepmix for CMV proteins pp65 and IE1 was used for CMV seropositive donors, and CD45RA selection and serial stimulation with donor-derived antigen presenting cells were used for CMV-naïve donors. Cultures were analyzed by flow cytometry, chromium release assay, and Interferon-γ ELISpot to evaluate differences between groups. **Results:** Flow cytometry showed similar T-cell populations in each product, with a predominance of central memory T-cells (36% versus 58% in CMV-naïve versus seropositive CTL, respectively). Interferon-γ production in response to CMV pp65 was comparable between the CMV naïve versus CMV seropositive-derived CTL (224±24 versus 417±155 spots/well respectively). Curiously, CMV naïve donor-derived CTL showed little to no killing of pp65-pulsed PHA blasts in vitro compared with the seropositive group (2.9% lysis versus 36.6% at an effector:target ratio of 40:1). Of note, significantly more Natural Killer (NK) cells were seen in CMV naïve donor-derived CTL (5-15% versus <1%), though they lack CD16 or NKG2C expression. **Conclusions:** CTL derived from the two donor groups have similar antiviral specificity, but the CMV-naïve donor-derived CTL have low cytotoxic activity and mostly immature NK cells, suggesting that both T-cells and NK cells derived from these donors are not fully matured during ex vivo culture. The clinical importance of these differences will be answered in an ongoing clinical trial.
Kirsten Kloepfer, MD, MS  
Assistant Professor  
Indiana University School of Medicine

Dr. Kloepfer received her undergraduate degree at Texas A&M University and her medical degree at St. George’s University. After medical school, Dr. Kloepfer completed a residency in pediatrics at the University of Arkansas for Medical Sciences and a fellowship in allergy and immunology at the University of Wisconsin. During fellowship she obtained her Master of Science degree in Clinical Investigation at the University of Wisconsin. Dr. Kloepfer is currently in the Department of Pediatrics, Division of Pulmonary, Allergy and Sleep Medicine at Indiana University School of Medicine, where she is interested in the role of the airway microbiome on airway function and inflammation. Obtaining nasal samples and lung function measurements in newborns, she is studying the upper airway microbiome to determine if shifts in airway bacteria (dysbiosis) correlate with airway inflammation and the development of airway obstruction. Dr. Kloepfer is particularly interested in understanding how environmental exposures at birth and in early life influence the development of recurrent wheezing. She anticipates translating her findings into intervention methods to prevent recurrent wheezing and the subsequent development of asthma.

The Airway Microbiome of the Newborn and Mother  
Kirsten M. Kloepfer, David Nelson, Robert Tepper, Stephanie D. Davis  
Indiana University School of Medicine, Indianapolis, IN, USA

Background: Changes in the upper airway microbiome have been associated with asthma in both children and adults. However, our understanding of the airway prior to the first wheezing episode is limited. To address this gap in knowledge, we designed a study to prospectively follow children from birth to 12 months to determine if an increase in pathogenic bacteria with an associated decrease in commensal bacteria, known as dysbiosis, is associated with future episodes of recurrent wheezing. Because multiple exposures can influence the airway microbiome, sampling begins prior to discharge from the hospital to determine if the newborn airway was similar to the airway of the mother. We hypothesized that the airway microbiome between mother and infant would be similar. Methods: 10 newborns born to mothers with asthma were recruited within 72 hours of birth. Nasopharyngeal samples were obtained from both the mother and newborn. Methods used by the Human Microbiome Project were followed. Bacterial DNA was extracted from samples and 16S-rRNA gene sequences targeting the V3-V5 region were analyzed utilizing Metastats. Results: Starting a prospective study at a new academic institution presented unforeseen challenges. Obtaining IRB consent and authorization from various hospital networks was a lengthy process and delayed our anticipated recruitment start date. We recently began recruiting for this study and results will be presented at the meeting. Conclusion: Utilizing new bacterial detection techniques, we plan to examine the infant microbiome prior to development of wheeze and subsequent asthma. We plan to recruit newborns and prospectively follow them to determine if dysbiosis of the upper airway microbiome at birth correlates with the development of recurrent wheeze. With the prevalence of asthma increasing each decade, our focus is on understanding the pathogenesis of asthma so we may develop methods of prevention.
Dr. McAllister graduated from the Medical Scientist Training Program at Oregon Health & Science University in 2007. He subsequently completed a Pediatrics residency and Infectious Diseases Fellowship at Stony Brook University in New York. His research in graduate school and during fellowship was centered on γ-Herpesvirus pathogenesis. Since joining the faculty at the University of Minnesota in 2012 his laboratory has been focused on oncogenic mechanisms of human Herpesvirus 8/Kaposi sarcoma Herpesvirus and the development of novel therapeutic strategies for Kaposi sarcoma amenable for use in limited resource settings.

**Autocrine β-adrenergic Signaling Drives Post-Confluent Growth of Endothelial Cells Infected with Kaposi Sarcoma Herpesvirus**

SC McAllister and RS Hanson
University of Minnesota Medical School, Division of Pediatric Infectious Diseases
Kaposi sarcoma (KS) is a common tumor in sub-Saharan Africa, but economic constraints hinder successful treatment in many patients. Another vascular lesion, infantile hemangioma, is successfully treated with the generic β-adrenergic antagonist propranolol. Based on pathogenic similarities between these two vascular lesions, we tested the hypothesis that propranolol decreases proliferation of endothelial cells infected with KS Herpesvirus (KSHV). We have found that cells infected with KSHV secrete the catecholamines norepinephrine and epinephrine, which drive post-confluent growth of infected cells through β2-adrenergic receptor (β2AR) signaling. Chemical inhibition of the β2AR with propranolol resulted in a dose-dependent decrease in proliferation of post-confluent KSHV-infected cells. Furthermore, we have demonstrated that cyclin A2, cyclin E, and cyclin-dependent kinase 2 (cdk2), proteins required to by cells to pass the G1-S restriction point and complete S phase of the cell cycle, were significantly upregulated in KSHV-infected cells. Treatment of infected cells with propranolol lead to a dose-dependent decrease in expression of cyclin A2 and cyclin E proteins, resulting in loss of Rb phosphorylation. Proliferation of post-confluent infected cells was similarly decreased by roscovitine, an inhibitor of cdk2 holoenzymes, confirming a role for cyclin A2, cyclin E, and cdk2 in driving KSHV-associated proliferation. In addition, we have found that propranolol increases the susceptibility of KSHV-infected cells to the cytotoxic effects of bleomycin, a chemotherapy agent available for treatment of KS in some African centers. Together, these data suggest that propranolol may be useful in the treatment of KS, particularly in limited resource settings.
Phillip Minar, MD  
Assistant Professor  
Cincinnati Children's Hospital Medical Center

Dr. Minar received his undergraduate degree at Duquesne University in Pittsburgh and did his medical training at the Medical College of Wisconsin. After medical school, Dr. Minar completed a residency in pediatrics at the Medical College of Wisconsin/Children's Hospital of Wisconsin and a fellowship in pediatric Gastroenterology, Hepatology and Nutrition at Cincinnati Children's Hospital Medical Center. In July of 2013, Dr. Minar joined the Department of Pediatrics, Division of Gastroenterology at Cincinnati Children's Hospital Medical Center as an Assistant Professor with Dr. Ted Denson serving as his primary mentor. Dr. Minar is primarily interested in the development of biomarkers in inflammatory bowel disease to objectively monitor treatment response, predict sustained remission and estimate disease severity. Dr. Minar's lab is currently exploring the utility of a neutrophil surface receptor in predicting treatment response as well as its effect in the pathogenesis of intestinal injury. Dr. Minar is also conducting preclinical studies of a novel neutrophil inhibitor that may have efficacy in patients with severe, treatment refractory IBD.

**Therapeutic Targeting of Neutrophil CD64 and Reactive Oxygen Species in Pediatric Crohn's Disease.**

**P. Minar**, Y. Haberman, Y. Tsai, I. Jurickova, LA. Denson  
Cincinnati Children's Hospital Medical Center, Cincinnati, OH

**Background:** Crohn's disease (CD) results from a dysregulated immune response to the gut microflora in a genetically susceptible individual. There is growing evidence that local infiltration of activated polymorphonuclear neutrophils (PMN's) contribute to the intestinal epithelial damage in CD by producing excessive reactive oxygen species (ROS). Ileal transcriptome analysis of newly diagnosed CD patients revealed a significant up-regulation of Fcy receptor I (CD64). **Objectives:** We hypothesize that elevated ileal CD64 expression is an early indicator of severe, treatment refractory CD. We also investigated the relationship of intestinal and peripheral blood (PB) PMN CD64 and its role in the pathogenesis of ROS-induced intestinal damage. We hypothesized that high PMN CD64 (intestinal and PB) results in increased ROS production that will be reduced with a novel small molecule pharmacologic agent (Phox-I) in-vitro. **Design/Methods:** RNA sequencing was performed on ileal biopsies from 217 treatment-naive CD patients and 43 non-IBD controls. Expression of CD64 and the NOX2 genes (*NCF1, NCF2, NCF4, CYBA, and CYBB*) was explored and compared to clinical outcomes. 28/217 CD patients received early (<90 days form diagnosis) anti-TNF therapy. In a separate cohort of CD patients, we evaluated the PB PMN CD64 expression, fMLP-induced PMN oxidative burst by flow cytometry and tested the effect of in-vitro inhibition of PMN oxidative burst with a novel NOX2 ROS inhibitor (Phox-I). **Results:** We found ileal *CD64* expression at diagnosis was significantly higher (p=0.02) in the 7/28 patients who failed to achieve clinical remission at 6 months. We also found that ileal *CD64* expression significantly correlated with expression of *NCF2* (r=0.84), *CYBB* (r=0.76), *NCF1* (r=0.66), and *NCF4* (r=0.61) in CD. Next, we found IFNy-stimulated PMN's caused a 4-fold (mean) increase in PMN CD64 surface expression and a 1.5-fold (mean) increase in ROS compared to unstimulated PMN's. In-vitro Phox-I caused a dose-dependent inhibition of PMN ROS. **Conclusions:** Ileal *CD64* at diagnosis predicts a more severe disease course and a poor response to anti-TNF therapy in pediatric CD while high PB PMN CD64 is likely contributing to ROS-induced intestinal injury. Planned murine colitis models will explore the effect of Phox-I on intestinal injury in-vivo.
Neil Romberg, MD
Assistant Professor
Yale University School of Medicine

Neil Romberg received his undergraduate degree from the University of Michigan, Ann Arbor and did his medical training at the Pennsylvania State College of Medicine. After medical school, Neil completed a residency and chief residency at New York University Medical Center followed by a fellowship in allergy and clinical immunology at Yale University. In 2009, Neil joined the laboratory of Eric Meffre PhD in Yale’s Division of Immunobiology to pursue post-doctoral training in the field of B-cell development. Currently, Neil is an assistant professor in the Department of Pediatrics, Division of Pediatric Allergy and Clinical Immunology at Yale University. He is interested in the identification of new primary immune diseases and investigating their molecular mechanisms.

A syndrome of enterocolitis and autoinflammation (SCAN4) caused by mutation of NLRC4

N Romberg, KA Moussawi, M Choi, NA Podoltsev, BI Kazmierczak and RP Lifton
Yale University School of Medicine

Upon detection of pathogen-associated molecular patterns, receptors of the innate immune system initiate inflammatory responses. These receptors include cytoplasmic NOD-like receptors (NLRs), whose stimulation recruits and proteolytically activates caspase-1 within the inflammasome, a multi-protein complex. Caspase-1 mediates the production of interleukin-1 family cytokines, leading to fever and inflammatory cell death (pyroptosis). Mutations that constitutively activate these pathways underlie several autoinflammatory diseases with diverse clinical features. We describe a family with a previously unreported syndrome featuring neonatal-onset enterocolitis, periodic fever, and fatal/near-fatal episodes of autoinflammation caused by a de novo gain of function mutation in NLRC4 that co-segregates with disease. Mutant NLRC4 causes constitutive Interleukin-1 family cytokine production and macrophage cell death. Stimulation of mutant NLRC4 leads to formation of abnormal inflammasomes polarized towards pyroptosis. These findings describe and reveal the cause of a life-threatening but treatable autoinflammatory disease that underscores the divergent roles of the NLRC4 inflammasome.
Robert Schnepp, MD, PhD
Instructor
The Children's Hospital of Philadelphia

Dr. Schnepp is a pediatric hematologist-oncologist who investigates how genetic variation and consequent disrupted signaling promotes the development and maintenance of the pediatric cancer neuroblastoma. He received his M.D. and Ph.D. at the Perelman School of Medicine at the University of Pennsylvania. His Ph.D. thesis demonstrated that the tumor suppressor protein menin, the mutation of which results in the development of Multiple Endocrine Neoplasia type I, promotes apoptosis and inhibits cell cycle progression. He completed his residency in General Pediatrics and his fellowship in Pediatric Hematology-Oncology at The Children’s Hospital of Philadelphia. Dr. Schnepp joined the laboratory of Dr. John Maris during fellowship to study how genetic variation drives aberrant molecular signaling to promote the development and maintenance of neuroblastoma. In July 2013, he became an Instructor and Attending Physician at The Children’s Hospital of Philadelphia. Dr. Schnepp is continuing his research in the laboratory of Dr. John Maris, where he is defining how the RNA binding protein LIN28B serves as a master regulator to promote the malignant phenotype in neuroblastoma. He is ultimately interesting in determining whether the LIN28B pathway, which regulates diverse biological processes, including cell growth, metabolism, and stemness, can be targeted for therapeutic benefit in neuroblastoma and other pediatric solid malignancies.

A LIN28B/RAN/AURKA Signaling Axis Promotes Neuroblastoma Tumorigenesis
RW Schnepp, JM Maris
The Children's Hospital of Philadelphia, Philadelphia, PA.

Background: Neuroblastoma is a childhood cancer of the sympathetic nervous system that accounts for approximately 15% of pediatric oncology deaths. The genetic basis of neuroblastoma has begun to be elucidated, with genome-wide association studies performed by our laboratory uncovering BARD1, LMO1, and, more recently, LIN28B, as susceptibility genes and oncogenic drivers in a large subset of established neuroblastomas. LIN28B, which binds mRNAs directly and is a master regulator of the let-7 family of tumor suppressor microRNAs, is of particular interest, as high expression in primary tumors is associated with poor overall survival. Objectives: To define the key downstream signaling cascades influenced by LIN28B and to determine how LIN28B promotes the oncogenic phenotype. Methods: To determine LIN28B-influenced signaling networks, we analyzed mRNA expression data sets from 648 primary neuroblastomas. To study the consequences of LIN28B signaling, we employed siRNA and shRNA-based approaches to perturb the levels of LIN28B and its key downstream effectors in multiple human neuroblastoma cell lines. Results: We observed a strong positive correlation between expression of LIN28B and the oncogene RAN in multiple datasets. LIN28B and RAN RNA and protein levels correlated with each other in neuroblastoma cell lines, and shRNA-mediated depletion of LIN28B reduced RAN RNA and protein levels. LIN28B directly bound RAN mRNA, likely promoting its transcription and/or translation. RAN promotes the phosphorylation and activation of Aurora kinase A (AURKA). We demonstrated that LIN28B promotes AURKA activation by influencing RAN. Moreover, we revealed AURKA to be a let-7 target, demonstrating a mechanism by which LIN28B/let-7 directly influences AURKA expression. Finally, we showed that RAN depletion results in decreased neuroblastoma proliferation, phenocopying the effects of LIN28B depletion. Conclusions: Collectively, these results demonstrate that one of the mechanisms by which LIN28B promotes the malignant phenotype is by influencing the expression of the oncogenes RAN and AURKA. As AURKA in turn stabilizes the oncogene MYCN, our studies reveal LIN28B to be a master regulator of multiple oncogenes implicated in neuroblastoma pathogenesis, nominating it as a potential candidate for therapeutic targeting.
Janice Staber, MD  
Assistant Professor  
University of Iowa

I obtained my medical degree at the University of Iowa, Carver College of Medicine. I completed my residency and fellowship training at the University of Iowa Children's Hospital. I am an Assistant Professor in Pediatric Hematology/Oncology and the Director of the Hemophilia and Thrombosis Center at the University of Iowa Hospitals and Clinics. The goal of my research is to develop a novel nonviral integrating vector for application for hemophilia A. My research project utilizes the development and application of a novel non-viral vector developed from an insect derived DNA transposon termed "piggyBac" (PB). This vector may offer advantages over existing tools in this field. Our overall goal is to transduce hepatocytes with this vector in vitro and in a mouse model of hemophilia A and restore protein production and effect correction of the disorder. I started as a CHRC junior investigator on July 15th, 2010.

PIGGYBAC-Mediated Phenotypic Correction of Factor VIII Deficiency

J Staber, M Pollpeter, A Arensdorf, P Sinn, D Rutkowski, and P McCray  
Carver College of Medicine, University of Iowa, Iowa City, IA

Background: Hemophilia A, caused by a deficiency in factor VIII (FVIII), is the most severe inherited bleeding disorder. Hemophilia A is an attractive gene therapy candidate because even small increases in FVIII levels will positively alter the phenotype. While several vectors are under investigation, gene addition from an integrated transgene offers the possibility of long term expression. Nonviral DNA transposons are genetic elements consisting of inverted terminal DNA repeats which in their naturally occurring configuration flank a transposase coding sequence. The insect derived transposon, piggyBac (PB), can be engineered to carry a therapeutic transgene. Objectives: We hypothesize that a PB transposon vector carrying a codon-optimized human FVIII cDNA long with a hyperactive transposase (iPB7) will confer persistent gene expression and correction of the hemophilia A bleeding phenotype. Design/Methods: We engineered PB transposon to carry a codon-optimized human FVIII B-domain deleted cDNA (coFVIII-BDD). We evaluated the in vivo gene transfer efficiency in hemophilia A mice by hydrodynamic tail-vein injection using PB coFVIII-BDD driven by the murine albumin enhancer/human alpha anti-trypsin promoter. Factor VIII null mice received 25 micrograms each of the PB coFVIII-BDD transposon and iPB7 to determine long term expression and phenotypic correction. Results: Evaluation of gene transfer efficiency in FVIII null mice demonstrated that piggyBac containing the FVIII cDNA, delivered via hydrodynamic injection to immunocompetent hemophilia mice, conferred persistent gene expression, attaining mean FVIII activity of 65% wild type mouse levels with few mice developing inhibitors. In addition to efficacious expression, a goal of gene transfer-based therapies is to develop vectors with low toxicity. To assess endoplasmic reticulum stress in hepatocytes stably expressing the transgene, we evaluated levels of ER stress markers via qRT-PCR and found no evidence of cell stress. To evaluate phenotypic correction, a tail clip assay performed at the end of the study revealed reduced blood loss. Conclusions: These data demonstrate that the piggyBac vector can be used to achieve sustained FVIII expression and long-term therapeutic benefit in a mouse model.
Pamela D. Winterberg, MD  
Assistant Professor  
Emory University School of Medicine

Dr. Winterberg received her undergraduate degree in Biology from San Jose State University in San Jose, CA and earned her M.D. degree from the University of Maryland School of Medicine in Baltimore, MD. She completed her residency in Pediatrics at the Oregon Heath and Sciences University in Portland, OR and fellowship in Pediatric Nephrology at UT Southwestern in Dallas, TX. She is currently an Assistant Professor of Pediatrics at Emory University in the division of Pediatric Nephrology, and a member of the Center for Cardiovascular Biology at the Emory and Children’s Pediatric Research Center. Her research is focused on the mechanisms of cardiovascular disease during childhood chronic renal failure. She is particularly interested in the molecular and biochemical mechanisms of cardiac and vascular stiffness during chronic kidney disease.

Altered Calcium Handling And Diastolic Dysfunction During Chronic Kidney Disease (CKD).

**Pamela D. Winterberg**, MD, Rong Jiang, MD, PhD, Bo Wang, Sonal Harbaran, Mary B. Wagner, PhD.  
Emory University Department of Pediatrics and Children’s Healthcare of Atlanta, Atlanta, GA, USA

**Background:** Cardiovascular disease is the leading cause of mortality for children with chronic kidney disease (CKD) and can manifest as hypertension, left ventricular hypertrophy (LVH), arrhythmias, and diastolic dysfunction. The underlying mechanisms contributing to diastolic dysfunction during CKD are poorly understood, limiting treatment options. We aimed to determine if altered calcium (Ca²⁺) handling in cardiomyocytes contributes to diastolic dysfunction in a mouse model of CKD.

**Methods:** CKD was induced in male 129X1/SvJ mice through five-sixths nephrectomy (5/6Nx) in a two-stage surgery. Age‐matched mice that underwent no surgeries (NC) served as controls. Transthoracic echocardiography was performed at 8 weeks post-CKD to assess heart structure and function. In addition to traditional measures of function (e.g. ejection fraction), we employed speckle‐tracking based strain analysis to detect early cardiac dysfunction. In a separate experiment, cardiomyocytes (CM) isolated from mice with or without CKD were loaded with Fura 2-AM, paced by field stimulation at 0.5, 1, and 2 Hz, and imaged with a dual‐excitation fluorescence photomultiplier system (IonOptix Myocam System, Ionoptix Inc, Milton, MA) to measure sarcomere length and Ca²⁺ transients. Sarcoplasmic reticulum (SR) Ca²⁺ content was determined by measuring Fura-2 intensity following rapid application of caffeine.  

**Results:** CKD mice displayed concentric LVH and decreased longitudinal left ventricular strain (19.0%, s.d. 4.08) compared to NC (30.0%, s.d. 2.28) mice (unpaired, two‐tailed t‐test, p<0.0001) consistent with diastolic dysfunction. Diastolic sarcomere length was significantly shorter in CM isolated from mice with CKD compared to normal mice (1.86 nm vs 1.89 nm at 1Hz pacing; p = 0.016), suggestive of impaired CM relaxation during CKD. Unexpectedly, the baseline cytosolic Ca²⁺ content was lower in CKD CM (1.22 vs 1.46 AU at 1Hz pacing, p=0.003). However, during caffeine stimulus, the SR Ca²⁺ content (absolute amplitude 0.60 vs 0.59 AU, p=0.59) was comparable between CKD and normal cardiomyocytes.  

**Conclusions:** Mice with CKD have signs of LVH and diastolic dysfunction on echocardiography. CM from mice with CKD have shorter diastolic sarcomere length implying impaired relaxation. However, CKD CM unexpectedly displayed decreased cytosolic Ca²⁺ with preserved SR Ca²⁺ content. Areas of further investigation include assessing altered calcium channel/transporter expression leading to decreased cytosolic Ca²⁺ and changes in sarcomere structural proteins to explain impaired relaxation during CKD.
Yuhua Zheng, MD, MS
Assistant Professor
Children's Hospital Los Angeles, University of Southern California

Dr. Zheng received her Master of Science degree at Peking University and did her Medical training at Peking University Health Science Center, China. After medical school, Dr. Zheng completed her first residency in pediatrics at Peking University First Hospital in China, second residency in pediatrics at White Memorial Medical Center in US and a fellowship in pediatric GI at Children's Hospital Los Angeles. Prior to her residency in US, Dr. Zheng perused post-doctoral research training at UCSF. In July 2012, Dr. Zheng joined the laboratory of Dr. Shelly Lu at Keck School of Medicine, University of Southern California for gastroenterology research. Dr. Zheng is currently an assistant professor in the Department of Pediatrics, Division of Gastroenterology and Nutrition at the Children's Hospital Los Angeles, where she is interested in investigating molecular mechanisms of digestive tract diseases. Utilizing mouse models of colitis/cancers and cell lines, she is studying the signal transduction/pathways of S-Adenosymethionine and its role in colon and liver diseases. She is particularly interested in understanding the pathogenesis of inflammatory bowel disease (IBD). Currently Dr. Zheng is focusing on applying these studies to investigate the molecular mechanisms of the protective effect of Prohibitin1 in IBD.

S-Adenosylmethionine Inhibits β-catenin Activation in Liver and Colon Cancer
Tony Li¹, Yuhua Zheng², and Shelly C. Lu¹

¹ University of Southern California, Los Angeles, CA; ² Children’s Hospital Los Angeles; CA

Background: S-Adenosylmethionine (SAMe) is a nutritional supplement that serves as the primary methyl donor for biological methylation reactions. SAMe and its byproduct-Methylthioadenosine (MTA), have been shown to induce apoptosis and inhibit cell growth in colon and liver cancer cells, but it is hepatoprotective and has no toxic effects on normal colonic epithelial cells. WNT/β-catenin pathway is well known to play an important role in many cancers. Our previous work has shown that SAMe/MTA reduced β-catenin level in colon tumor tissue of azoxymethane/dextran sodium sulphate (AOM/DSS) induced murine colitis colon cancer model. Here we investigated the mechanism of how SAMe/MTA affect β-catenin.

Methods: Human colon and liver cancer cell lines with either aberrant (SW480 and HepG2) or normal (RKO and Huh7 cell lines) WNT/β-catenin signaling pathway were used. Western blot, confocal microscopy, TOPFLASH reporter assay and real time PCR were performed. Results: β-catenin protein levels were reduced in AOM/DSS colon tumors in SAMe/MTA treated mice, and in liver cancers derived from injected Hep3B liver cancer cells overexpressing methionine adenosyltransferase 1A (which elevates intracellular SAMe levels) as compared to vector control in an orthotopic liver cancer mouse model. In cancer cell lines with constitutively active β-catenin, SAMe/MTA treatment had no effect on total mRNA or protein level of β-catenin. However it reduced the nuclear content of β-catenin on confocal microscopy and Western blot. In cancer cells with normal WNT/β-catenin pathway, SAMe/MTA lowered WNT3A-mediated activation of β-catenin. The inhibitory effect of SAMe/MTA on β-catenin required the multifunctional kinase GSK-3β, which phosphorylates β-catenin, making it a target for proteasomal degradation. SAMe/MTA increased the level of β-catenin phosphorylation by GSK-3β. In addition, the GSK-3β inhibitor TDZD-8 abolished SAMe/MTA’s effect on β-catenin. These results suggest that SAMe/MTA affect β-catenin stability through GSK-3β kinase. Conclusions: In human liver and colon cancer cell lines with aberrant WNT/β-catenin, SAMe/MTA lowered nuclear β-catenin content, whereas in cancer cell lines with normal WNT/β-catenin signaling pathway, SAMe/MTA reduced total β-catenin levels by promoting its degradation via increasing phosphorylation of β-catenin by GSK-3β. The ability to inhibit β-catenin activation regardless of mutation in WNT/β-catenin pathway makes SAMe/MTA attractive as chemopreventive and/or chemotherapeutic agents.
Heather Stefanski, MD, PhD
Assistant Professor
University of Minnesota

Dr. Stefanski received her B.A. from Smith College in 1993. She then went on to attain her Ph.D. at the University of Minnesota and immunology in 1999. She completed her medical degree at the University of Minnesota 2004. She completed her pediatric training at the University of Minnesota Children's Hospital in 2007. She finished her Pediatric Hematology/Oncology and BMT fellowship in 2010 at the University of Minnesota Children's Hospital. She is board certified in Pediatrics and Pediatric Hematology/Oncology. Dr. Stefanski is an assistant professor of Pediatrics in the division of Blood and Marrow Transplantation. Her clinical interests include ALL, AML, solid tumors and immune deficiencies. Dr. Stefanski's research is focused on expediting immune reconstitution after transplant. T lymphocytes are critical for effective responses to viruses and fungal infections and to prevent relapse; there are essentially no functional T cells until one year after transplant. In her laboratory, she is currently working on T-cell reconstitution in order to decrease morbidity and mortality.

UTILIZING T PROGENITORS TO INCREASE TEC REGENERATION AND THYMOCYTE CROSS-TALK IN A MURINE TRANSPLANT MODEL.
Heather E. Stefanski, Michelle J. Smith, Leslie M. Jonart and Bruce R. Blazar
University of Minnesota Children's Hospital, Minneapolis, MN

Background: Our goal is to improve immune recovery after hematopoietic stem cell transplant (HSCT) by overcoming the adverse effects of conditioning regimen injury to thymic epithelial cells (TECs) necessary for providing survival and differentiation signals to T progenitor cells (Tprogs) and developing thymocytes. Objectives: We wanted to determine whether TEC regeneration and thymopoietic recovery is limited by inadequate cross-talk between thymocyte precursors and TECs post-HCT. Recently a Notch1- based culture system (OP9-DL1) has been utilized to generate committed Tprogs in vitro. Ex vivo expansion of Tprogs utilizing the co-culture system and adoptive transfer of murine Tprogs enhances immune reconstitution after BMT. Design/Methods: Murine hematopoietic stem cells were cultured on OP9DL1 stromal cells in the presence of cytokines for 15 days. 8e6 Tprogs with 5e6 TCD BM were injected IV into lethally irradiated hosts. Three weeks later the number of donor, host and Tprogs were determined in thymi. To determine timing of homing to the thymus, we found both 1 and 8e6 Tprogs were visualized in the thymus immediately after injection. Moreover we could see Tprogs at a dose of 1e5 at day 4 and 7. This data suggested that Tprogs immediately home to the thymus. We wanted to determine if there are differences in Tprog migration due to radiation conditioning. Tprogs were labeled with dye and added to explanted thymi from Ubiquitin-GFP transgenic B6 mice that had been given radiation. Thymi were embedded, cells were overlayed and incubated overnight on thymic slices prior to 2 photon imaging. Results: We found that by week 3, DN2 cells have preferentially increased the hematopoietic portion of the thymus, while DN3 cells have preferentially stimulated mTEC recovery. For timing of homing to the thymus, we found both 1 and 8e6 Tprogs were visualized in the thymus immediately after injection. Moreover we could see Tprogs at a dose of 1e5 at day 4 and 7. This data suggested that Tprogs immediately home to the thymus. We wanted to determine if there are differences in Tprog migration due to radiation conditioning. Tprogs were labeled with dye and added to explanted thymi from Ubiquitin-GFP transgenic B6 mice that had been given radiation. Thymi were embedded, cells were overlayed and incubated overnight on thymic slices prior to 2 photon imaging. We found that radiation disrupted thymic architecture and distinction between cortex and medulla. There was also a significant decrease in the displacement and velocity of Tprogs in irradiated thymi. Conclusions: We have found that different subsets of Tprogs have different effects on thymic development. We have also found that thymic architecture is impaired due to radiation and has effects on Tprog migration through the thymus. It is our hope that addition of Tprogs post transplant will ameliorate some of these affects by increasing TEC number and thymic cross-talk.
Chintan Parekh, MBBS
Assistant Professor
Children’s Hospital Los Angeles
University of Southern California Keck School of Medicine

Dr. Parekh did his medical training at the Seth GS Medical College, Mumbai, India. After medical school, Dr. Parekh completed a residency in pediatrics at The Women and Children’s Hospital of Buffalo and a fellowship in pediatric hematology oncology at Children’s Hospital Los Angeles. He did a research fellowship and a clinical instructorship in the field of human hematopoiesis in the laboratory of Dr Gay Crooks at The University of California Los Angeles (2008-2012). Dr. Parekh is currently an Assistant Professor in the Department of Pediatrics, Division of Pediatric Hematology Oncology at Children’s Hospital Los Angeles and the University of Southern California Keck School of Medicine. His research focuses on molecular mechanisms underlying human hematopoiesis and leukemogenesis. Utilizing in vitro and in vivo human thymopoiesis and leukemogenesis models he is studying the role of the transcription factor BCL11B in normal thymopoiesis and T cell leukemogenesis. Dr. Parekh is particularly interested in understanding molecular regulatory mechanisms during the early stages of human thymopoiesis, and how aberrations in these mechanisms lead to T cell acute lymphoblastic leukemia.

Role of BCL11B in human thymopoiesis and T-cell acute lymphoblastic leukemia

C Parekh1,2, VL Ha1, A Luong1, P Chaudhary2, R Bhatia3, GM Crooks4. 1Children’s Hospital Los Angeles, Los Angeles, CA, 2University of Southern California, Los Angeles, CA, 3City of Hope Medical Center, Duarte, CA, 4University of California Los Angeles, Los Angeles, CA, USA.

Background: T‐cell acute lymphoblastic leukemia (T‐ALL) represents a malignant expansion of developmentally arrested immature thymic progenitors. Normal thymopoiesis (T cell differentiation) involves the migration of CD34+ progenitors from the bone marrow to the thymus followed by maturation through defined progenitor stages. Deciphering the mechanisms underlying normal human thymopoiesis is critical for the elucidation of T cell leukemogenesis. The transcription factor bcl11b is required for differentiation, and repression of oncogenes during murine thymopoiesis. 16% of pediatric T‐ALL cases harbor BCL11B mutations. Our goal is to define the function of BCL11B in human thymopoiesis and T cell leukemogenesis. Objectives: Hypothesis: BCL11B represses oncogenes, leading to normal T cell differentiation during human thymopoiesis. BCL11B insufficiency in T‐ALL blocks differentiation and leads to increased leukemic cell proliferation; and restoration of BCL11B function down regulates oncogenes and inhibits proliferation of T‐ALL cells. Methods: Human cord blood CD34+progenitors were transduced with a BCL11B shRNA (BCL11B knockdown cells) or non‐targeting scrambled shRNA lentiviral vector (control cells). The cells were then cultured in an in vitro thymopoiesis model (OP9DLL1 stromal co‐culture). T cell differentiation was assessed by flow cytometry. T‐ALL cells were transduced with a BCL11B cDNA (BCL11B overexpressing cells) or empty lentiviral vector (control cells). Viable cell counts and flow cytometry were used to assess proliferation and apoptosis respectively. Results: BCL11B knockdown induced a differentiation arrest at an early stage of thymopoiesis. BCL11B overexpression inhibited proliferation and induced apoptosis of T‐ALL cells. Conclusions: Our in vitro studies suggest that 1) BCL11B is a tumor suppressor gene that is important for normal T‐cell differentiation during the early stages of human thymopoiesis; and 2) BCL11B insufficiency promotes survival of T‐ALL cells. These results provide new insights into T‐cell leukemogenesis, forming the impetus for ongoing further studies to define: (1) effects of BCL11B insufficiency on (a) oncogene expression ; and (b) human thymopoiesis and T‐cell leukemogenesis in in vivo (immune deficient mouse xenotransplantation) models; and (2) tumor suppressor pathways activated by BCL11B.
Dr. Shand received her undergraduate degree from Johns Hopkins University, as well as her Master's Degree in Microbiology and Immunology from the Johns Hopkins School of Public Health. She worked as an immunologist in the biodefense industry prior to entering medical school at the State University of New York at Buffalo. During medical school, Dr. Shand participated in the Howard Hughes Medical Institute Cloister Scholars Program (2003-4) in the laboratory of Dr. Crystal L. Mackall (Pediatric Oncology Branch, National Cancer Institute) developing strategies to enhance immunogenicity of dendritic cell cancer vaccines. She went on to complete residency training in Pediatrics at the University of Rochester, graduating with a distinction in research for her work in the laboratory of Dr. Craig Mullen. She then completed fellowship training in Pediatric Hematology-Oncology at the Johns Hopkins-National Cancer Institute Combined Program, where she performed research in the laboratory of Dr. Terry Fry (Pediatric Oncology Branch, National Cancer Institute), studying tissue-inflammatory determinants of graft-versus-leukemia effects. She returned to the University of Rochester in 2012 to begin her current faculty position in the Department of Pediatrics, Division of Pediatric Hematology-Oncology, where she is developing an independent laboratory program dedicated to understanding how the innate immune cell populations in the bone marrow of acute lymphoblastic leukemia patients may interfere with the success of adoptive T-cell therapy. Dr. Shand intends to translate these studies to develop a multi-modal immunotherapy approach that accounts for patient-specific immune responsiveness factors in the bone marrow to increase the curative potential of adoptive therapy.

**Suppression of adoptive CD8 T-cell killing by inflammasome activation in the bone marrow macrophages of patients with acute lymphoblastic leukemia.**

Jessica C. Shand, John T. Mariano, Michael W. Winter and Liana M. Toia

University of Rochester Department of Pediatrics, Division of Hematology-Oncology

Relapse of B-lymphoblastic leukemia (B-ALL) is the leading cause of pediatric cancer death. T-cell suppressive signals generated in the bone marrow microenvironment of some ALL patients may explain the failure both of native T-cell surveillance and non-curable responses to adoptive CD8 therapy. We hypothesized that canonical inflammasome signaling, characterized by caspase-1-driven IL1-beta secretion, is generated by bone marrow macrophages in response to apoptotic ALL cells, contributing to CD8 T cell (CD8) exhaustion in this setting. To determine whether the cytolytic function of adoptive T-cells could be suppressed by the interaction of ALL cells and bone marrow macrophages (BM-MM), healthy human CD8 isolated from peripheral blood were treated with supernatants from cultures of ALL alone, ALL with BM-MM and ALL with marrow stromal cells. CD8 treated with supernatants from cultures of ALL alone and BM-MM lost their ability to proliferate to anti CD3/CD28 T-cell receptor stimulation (0% proliferation by CFSE dilution, p<0.0001) and had significantly reduced cytolytic capacity (2-fold reduction in lysosomal degranulation by CD107a externalization assay, p=0.04), compared to CD8 exposed to cultures of ALL alone or ALL with stromal cells (both with 100% proliferation). The active, cleaved form of caspase-1 was induced, both by qPCR (3.7-fold expression over endogenous control) and Western blot, in BM-MM cultured with ALL, with corresponding levels of IL-1-beta secretion that exceeded macrophages treated with flagellin, a bacterial protein known to induce the canonical inflammasome pathway. Taken together, these data suggest that induction of a caspase-1 dependent suppressive inflammatory in bone marrow macrophages is sufficient to suppress T-cell antitumor responses. Current studies are aimed at selecting and reversing targets in this pathway as a platform for the development of adjunct therapies that could be used to optimize the bone marrow niche prior to adoptive T-cell therapy to promote durable cure.
Brian Campfield, MD
Assistant Professor
University of Pittsburgh School of Medicine

Dr. Campfield received his undergraduate degree from the University of Virginia and completed medical school at the University of Pittsburgh School of Medicine. Following medical school, Dr. Campfield completed a pediatrics residency at the Children’s Hospital of Pittsburgh and remained for fellowship in Pediatric Infectious Diseases. In 2010, Dr. Campfield began fellowship research in the laboratory of Dr. Raphael Hirsch, and subsequently joined the faculty of the University of Pittsburgh School of Medicine to continue host-defense studies under the mentorship of Dr. Jay Kolls. His primary focus is examining the role of Follistatin-like protein 1 (FSTL-1) in IL-17 mediated inflammation and, specifically, the impacts of FSTL-1 on host defense against bacterial infection in the lung using murine models. This line of investigation reflects Dr. Campfield’s larger interest in protective immune responses and vaccines against multi-drug resistant bacterial infections.

FOLLISTATIN LIKE PROTEIN 1 REGULATES IL-17 SIGNALING BY INFLUENCING IL-17 RECEPTOR EXPRESSION.

B Campfield, Y Chaly, R Hirsch, J Kolls
The Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, PA and The University of Iowa Hospitals and Clinics, Iowa City, IA

Background: IL-17, the canonical Th17 cytokine, is critical for the development of Collagen Induced Arthritis (CIA) and host defense. Follistatin-like protein-1 (FSTL-1), a poorly characterized glycoprotein, mediates the development of CIA in a T-cell dependent manner. Objectives: We aimed to assess the role of FSTL-1 on IL-17 mediated inflammatory signaling in vitro. Methods: ST2 cells with shRNA for FSTL-1 (shFSTL-1) and control (shCtrl) sequences, primary murine C57Bl/6 wild-type (WT) or FSTL-1 knock-out (FSTL-1 KO) littermate bone marrow stromal cells (BMSC), or FSTL-1 transfected FSTL-1 KO BMSCs were stimulated with 8ng/mL rIL-17 and 2ng/mL rTNFα. Transcript abundance was assessed by qRT-PCR. Protein secretion was assessed by Luminex. IL-17 receptor surface expression was assessed by FACS analysis. Results: Compared to ST2 shControl cells, shFSTL-1 cells had 1) decreased transcripts of IL-6 (275 vs. 43), G-CSF (1802 vs. 1325) following stimulation, but no difference in IL-17RA (1.246 vs. 1.139) and IL-17RC (1.51vs. 2.15) at baseline, 2) decreased protein secretion of IL-6 (25142 vs. 2542pg/ml), G-CSF (388 vs. 27.8 pg/ml), and 3) decreased IL-17RA surface expression (52.3% vs. 37.2%) despite similar IL-17RC surface expression (93.6% vs. 91.4%). Compared to WT primary BMSCs, FSTL-1 KO had 1) decreased transcripts of IL-6 (664.5 vs.159.9), G-CSF (76.2 vs. 6.1) following stimulation, but similar IL-17RA (0.676 vs. 0.891) and IL-17RC (0.513 vs. 1067) at baseline, 2) decreased protein secretion of IL-6 (2006 vs. 1407 pg/ml), G-CSF (8497 vs. 436 pg/ml) and 3) decreased IL-17RC surface expression (90.9% vs. 50.3%). Complementation of FSTL-1KO BMSCs with a CMV-promoted FSTL-1 or control vector resulted in 1) increased transcripts of FSTL-1 (30.83 vs. 1.80), IL-6 (81.4 vs. 44.4), G-CSF (3.53 vs. 1.01) following stimulation and 2) increased protein secretion of IL-6 (1801 vs. 1407 pg/ml). Conclusions: FSTL-1 mediates IL-17 stimulated IL-6 and G-CSF production in ST2 cells and primary bone marrow stromal cells, and FSTL-1 deficit can be rescued in complementation studies. FSTL-1 influences IL-17 receptor surface expression, suggesting that the effects of FSTL-1 on IL-17-mediated cytokine production occur at the level of the IL-17 receptor complex.
Michael Silverman, MD, PhD
Instructor
Boston Children’s Dr. Silverman was born in Richmond, Virginia in 1975. He graduated summa cum laude from Cornell University in 1998. Michael then completed the MD/PhD program at the University of Pennsylvania School of Medicine in 2007. For his dissertation research, he investigated the mechanism of FcεRI signaling in mast cells in the laboratory of Dr. Gary Koretzky. Michael then completed a residency in pediatrics at Boston Children’s Hospital (BCH) in 2010 and fellowship in pediatric infectious diseases at BCH in 2013. Michael then joined the faculty of the division of infectious disease at BCH in 2013 and attends on the immunocomprised infectious disease consultation service. He is currently investigating how gut microbes regulate the development and function of the immune system in the laboratory of Drs. Diane Mathis and Christophe Benoist at Harvard Medical School.

Microbiota, MHC and autoimmunity: Can gut bugs protect from type 1 diabetes?

MA Silverman,* C Benoist** and D Mathis**
*Boston Children’s Hospital, Boston, MA, **Harvard Medical School, Boston, MA.
The major histocompatibility complex (MHC) and human leukocyte antigen (HLA) loci possess the strongest genetic association with type 1 diabetes (T1D), in mice and humans respectively. Some loci such as the MHC class II E molecule offer dominant protection from T1D, but the mechanisms for this protection remains poorly understood. Commensal microbiota also influences the development of the immune system and affects the risk for developing T1D. The goal of this project is to explore interactions between gut microbiota, the MHC/HLA loci and the development of T1D in the non-obese diabetic (NOD) strain of mice and its closely related but non-diabetic transgenic line, expressing the protective MHCII E molecule (Eα16.NOD). Our laboratory and others have demonstrated that expression of the MHCII E molecule leads to protection from autoimmune inflammation of the pancreatic islets (insulitis) and offers complete protection from T1D. Our preliminary data indicate that antibiotic disruption of the gut microbiota induces insulitis in Eα16.NOD mice. We have also demonstrated maternal transfer of protection from insulitis and T1D from Eα16.NOD mothers to NOD pups, suggesting transfer of protective microbiota from mother to pup. Therefore, we hypothesize that the diabetes-protective effect of the E molecule reflects an impact on the gut microbiota, which has a secondary influence on the immune system. To further test this hypothesis, we are defining the gut microbiome of Eα16.NOD mice using 16s rDNA sequencing, and comparing the gut immune system between NOD and Eα16.NOD to identify potential immune mechanisms of protection in Eα16.NOD mice. Ultimately, these experiments will shed light on the relationship between MHC and HLA loci, commensal microbiota and autoimmune diabetes. Moreover, identification of immunomodulatory bacteria and their associated immune system targets offers the potential for novel therapies for T1D, and perhaps other autoimmune diseases.
Dr. Touma joined the Division of Neonatology and Developmental Biology at UCLA in July 2011 after completing her second pediatric residency at the University of Texas and her Neonatology Fellowship at Children’s Hospital Boston/Harvard Medical School. Dr. Touma’s medical education was at the Damascus University Medical School, followed by a pediatrics residency, board certification, and a Master’s level thesis at the same institution. Dr. Touma was then appointed as a Senior Pediatrician in Saudi Arabia with both academic and clinical responsibilities. In 2002 Dr. Touma immigrated to the United States with family, and served for over two years as a research assistant in the pediatric endocrinology at Loma Linda University. During fellowship Dr. Touma received research training in Drs. Christou and Kourembanas laboratories where she demonstrated interest in cardiovascular research exploring the pathobiology of pulmonary hypertension and validating Echocardiographic indices in the murine hypoxic model of the disease. In addition, Dr. Touma completed training in complete genomic analysis and statistical genetics. Upon joining the pediatric faculty at UCLA Dr. Touma enrolled in UCLA-STAR program for advanced research training and PhD pursuit in Molecular, Cell and Integrative Physiology, under the mentorship of Dr. Yibin Wang at University of California Cardiovascular Research Laboratory, where she is interested in elucidating the molecular mechanisms underlying heart transcriptome maturation and remodeling during perinatal circulatory transition in neonatal mice utilizing next generation RNA-seq technology and functional biology studies. Supported by K12-Child Health Research Center award Dr. Touma research is particularly focused on exploring the role of alternative RNA splicing in dictating chamber specificity in neonatal heart ventricles during physiologic maturation, and their alterations in pathological conditions including hypoxia and hemodynamic stress.

Neonatal Mouse Heart Maturation: Chamber-Specific Exon Enrichment of Cell Cycle Genes.

Marlin Touma, M.D.1, Xuedong Kang, Ph.D.1, Jae-Hyung Lee, Ph.D.1, Xinshu Xiao, Ph.D.1 and Yibin Wang, Ph.D.1 (1) University of California-Los Angeles, Los Angeles, California, United States

**Background:** Postnatal maturation of heart during perinatal circulatory transition involves dramatic changes of neonatal cardiomyocytes that include proliferation arrest and terminal exit from the cell cycle (CC). However, transcriptome-wide analysis of CC programs has not been performed in perinatal stages among different cardiac chambers. In particular, the contribution of alternative RNA splicing to programming the chamber-specific CC activities is unexplored. **Objective:** To achieve transcriptome-wide analysis of differential expression (DE) and alternative splicing (AS) of CC-related genes in left ventricle (LV) versus right ventricle (RV) during maturation. **Design/Methods:** Deep RNA-seq was performed on male newborn mouse (C57BL) LV and RV at 3 time points of perinatal circulatory transition: P0, P3 and P7. Reads were mapped to mouse Transcriptome, and to mouse Genome. Transcriptome-Wide difference in inclusion of individual exons was performed using MATS. DE genes and AS variants were defined as those with fold change ≥2, at a false discovery rate ≤5% for genes expressed at ≥3 RPKM in at least one sample. Significantly enriched gene ontology (GO) terms were determined at P ≤0.05. Transcripts expression levels were validated using qRT-PCR. **Results:** Altogether, 2116 DE genes and 1162 AS events were observed. Among them, 109 CC-related genes were further analyzed. Distinct temporal patterns of DE and GO enrichment of CC genes in LV vs. RV during maturation were observed and negatively correlated with Wnt signals. Transcriptome-wide analysis of CC genes revealed 77 AS events. Skipping exon accounted for 41% of splicing events. Among 30 spliced exon variants, significant chamber-and temporal-specific exon inclusion levels were identified. Interestingly, the majority of AS variants exhibited opposing patterns of exon usage in RV vs. LV at p7. **Conclusions:** Our findings suggest novel molecular basis for chamber-specific programming of cellular proliferation in neonatal heart, including Wnt signaling, dynamic splicing regulation, and exon enrichment of CC genes. Further functional and mechanistic studies to decipher the chamber-specific molecular programming of CC genes during maturation will likely lead to novel chamber-targeted therapies.
Engin Deniz, MD
Instructor
Yale School of Medicine

Dr. Deniz completed medical school at the University of Istanbul, Turkey. After medical school he completed a residency in Pediatrics at the Cohen Children's Medical Center of New York and a fellowship in Pediatric Critical Care Medicine at Yale University. Currently he is an Instructor in the Department of Pediatrics, Section of Critical Care Medicine at Yale School of Medicine. Dr. Deniz is interested in the genetics of congenital heart disease. He joined the laboratory of Dr. Michael A Choma (Biomedical Optics) during his fellowship to investigate the impact of candidate congenital heart disease genes on the biomechanical function and structure of the developing heart using micro-scale imaging modalities. He successfully developed and applied micro-scale imaging techniques to assess cardiac function and structure of the embryonic frog heart. Following his fellowship, he moved to the laboratory of Dr. Mustafa Khokha (Pediatrics and Genetics) where he is currently focusing his studies on a novel gene regulating cardiac development that was identified in human genetic studies. Dachsous2 (DCHS2) is a cadherin protein that is important in cell-to-cell adhesion and planar cell polarity signaling. Dr. Deniz utilizes the frog *Xenopus tropicalis* as the animal model to characterize the mechanisms by which DCHS2 regulates vertebrate heart development.

THE ROLE OF DCHS2 IN VERTEBRATE PATTERNING AND CONGENITAL HEART DISEASE

E Deniz, A Robson, MA Choma, M Brueckner, M Khokha, Yale University, New Haven, CT.

**Background:** Congenital heart disease (CHD) is present in approximately 8 out of 1000 live births and is a significant cause of morbidity and mortality in infants. Currently, human genomic studies are identifying candidate genes for CHDs but assigning disease causality and determining the underlying developmental mechanisms remains challenging. We developed and applied a high-throughput *in vivo* comprehensive cardiac phenotyping assay in the frog, *Xenopus* to test these novel CHD candidate genes for effects on cardiac development which identified dachsous2 (DCHS2). When knockdown by morpholino oligonucleotides in the frog, *Xenopus tropicalis*, we found a loss of cardiac trabeculations, craniofacial malformations, and loss of epidermal cilia. **Objectives:** Previous work in fruitflies and mice show that DCHS2 family members are transmembrane cadherin proteins that play an important role in establishing planar cell polarity (PCP) although no role for DCHS2 has currently been identified. The PCP pathway is critical to polarize cells within a tissue and govern cell growth, proliferation and migration as well as ciliogenesis. We hypothesized that DCHS2 plays a role in planar cell polarity signaling, ciliogenesis, and cardiac/craniofacial morphogenesis. **Methods/ Results:** First to determine the mechanisms by which DCHS2 affects ciliogenesis, we analyzed the four steps of ciliogenesis beginning from centriole generation, centriole migration, basal body formation and cilia elongation. We found that DCHS2 protein localized to the base of the cilium, which is lost when knockdown with morpholino. Then to determine the mechanisms by which DCHS2 affects cardiogenesis we applied microscale high speed imaging to morphants and demonstrated loss of cardiac trabeculation, a phenotype reminiscent of the cardiac non-compaction phenotype seen in our DCHS2 patient. Finally our initial results indicate that DCHS2 knockdowns lack the proper formation of branchial arches in tadpole, suggesting disruption of neural crest induction or migration. **Conclusions:** We successfully employed our “reverse translation” approach, taking gene discovery from the bedside to the bench in order to understand the underlying CHD disease process. We use human congenital malformations (ie disease subjects) for gene discovery and then a high-throughput animal model for disease mechanism discovery: a new paradigm for developmental biology. To our knowledge DCHS2 is the first gene within the PCP pathway related to cardiovascular defects in humans and a novel gene regulating ciliogenesis.
POST-TRANSCRIPTIONAL CONTROLS IN GASTROINTESTINAL GENE EXPRESSION


**Background:** Inflammatory bowel disease (IBD) is a prevalent, heterogeneous disorder typified by chronic intestinal inflammation that often leads to severe morbidity and growth failure in children. Defects in post-transcriptional controls mediated by RNA-binding proteins (RBPs) underlie several well-described models of IBD. While highlighting the importance of post-transcriptional controls in IBD, these models reflect neither the vast genetic heterogeneity in IBD, nor the full diversity of RNA-binding proteins and their corresponding role(s) in inflammation. The poly-C binding proteins, PCBPs, comprise a ubiquitous family of RBPs that regulate mRNA processing and stability. PCBPs can serve as mediators of negative feedback in the inflammatory process; however, their function in the GI tract is ill-defined. We hypothesize that PCBPs regulate critical steps in post-transcriptional regulation of gene expression within the gastrointestinal tract and contribute to pathologic states of intestinal inflammation.

**Objectives:** 1) Characterize PCB expression and PCB bound mRNAs during normal gastrointestinal development and in inflammatory states; 2) Evaluate PCB1 and PCB2 tissue-specific knockout on development and function of the intestinal mucosa and in models of intestinal inflammation.

**Methods:** Normal and inflamed murine gastrointestinal tissue will be assessed for PCB expression by immunohistochemistry and qPCR. Differential gene expression in these tissues will be determined by PCB:RNA immunoprecipitation coupled to microarray. Constitutive and conditional PCB1 and PCB2 knockout mice will be derived. Qualitative and quantitative measures of development and response to inflammatory stress will be made.

**Results:** PCB1/2 exhibit broad-based GI tract mucosal expression, are enriched in progenitor and mature serous exocrine cells of the gastric epithelium, and have a distribution that is dependent upon axial positioning within intestinal villi. Mice haploinsufficient for PCB1 are 30% smaller than littermate controls and otherwise are phenotypically normal. PCB1 null mice have an embryonic lethal phenotype. Phenotyping of PCB2 haploinsufficient mice and studies of inflammatory stress in PCB haploinsufficient mice are underway.

**Conclusions:** Our observations suggest there exists a developmental or functional role for PCB’s in gastric glands and along the intestinal crypt-to-villus axis. Embryonic lethality associated with PCB1 knock-out indicates the closely related isoforms PCB1 and PCB2 have non-redundant functions and that PCB1 is required for mouse viability. Tissue-specific inactivation will be required to fully assess the effects of PCB isoform deletion upon gastrointestinal development and inflammatory stress.
Beth Kozel, MD, PhD  
Assistant Professor  
Washington University School of Medicine

Dr. Kozel received her undergraduate degree at Washington University in St. Louis and did her MD-PhD training through the MSTP program at Washington University School of Medicine. After medical school, Dr. Kozel completed residencies in pediatrics and clinical genetics at St. Louis Children's Hospital. In January of 2009, Dr. Kozel initiated a post-doctoral project under the guidance of Dr. Robert Mecham and Dr. Jim Cheverud. Currently, Dr. Kozel is an Assistant Professor of Medicine in the Department of Pediatrics, Division of Genetics and Genomic Medicine at Washington University School of Medicine and is the director of the Williams Syndrome Center at St. Louis Children's Hospital. Her research combines studies in mouse models and humans to identify genetic and environmental factors that modify the severity of elastin mediated vascular disease. She has identified several pathways that exacerbate or reduce the severity of vascular disease in this population, offering the potential to impact risk assessment and treatment.

OF MICE AND MEN: MODIFIERS OF VASCULAR DISEASE IN WILLIAMS SYNDROME

B Kozel, Washington University School of Medicine, St. Louis, MO.

Background: Williams syndrome (WS) is a multisystem disorder caused by the deletion of 26-28 genes on the 7th human chromosome. Individuals with WS have a characteristic vascular phenotype, typified by focal arterial stenosis, narrow conducting vessels, and hypertension. Cardiovascular symptoms in this population are caused by the loss of the elastin (ELN) gene as part of the microdeletion. Objectives: Although all patients with WS have ELN haploinsufficiency, vascular disease severity varies from life-threatening to undetectable. The goal of this project is to identify genetic modifiers that affect vascular disease risk and outcome in this population. Design/Methods: Using the Eln+/− mouse, we performed two separate quantitative trait locus (QTL) analyses to identify genomic loci that modify the severity of hypertension and vascular narrowing. Using bioinformatics, expression analysis, congenic mice and double mutants, we narrowed these loci to specific modifiers. Once modifiers had been vetted in the animal model, we evaluated a well-phenotyped human cohort of individuals with WS for variation in those same gene sets. Results: Our QTL studies revealed multiple peaks, with LOD scores up to 17. Subsequent work with congenic and double mutant mice identified Ren1 and Ncf1, among others, as strong modifiers of hypertension and vascular diameter in the Eln+/− mice, while expression studies implicated LTBP2 and a second family of oxidases. Additional confirmatory studies are underway. Additional work in a large WS cohort confirmed the effect of NCF1 gene dosage on the risk of hypertension and vascular stiffness. Conclusions: The Eln+/− mouse offers a powerful way to screen for genes that modify the severity of vascular disease in WS. The modifiers identified by this study appear to be readily translatable to humans with the disease as parallel dosage effects were seen in the initial candidates studied. Because elastin is secreted and deposited during a very restricted developmental window, the identification of modifiers offers additional therapeutic targets to treat vascular and end organ disease in this population.
Dr. Oleg Shchelochkov received his MD degree at Tashkent Pediatric Medical Institute (USSR). After his medical school, he completed residency training in Pediatric Intensive Care and Anesthesia (Tashkent, Uzbekistan, former USSR). After obtaining the ECMFG certificate in 2002, Dr. Shchelochkov completed pediatric residency at the University of Iowa Hospitals and Clinics in 2006. He completed fellowship in Clinical Genetics in 2008 and Medical Biochemical Genetics in 2009, both at Baylor College of Medicine (Houston, TX). During his training at Baylor College of Medicine, Dr. Shchelochkov joined Brendan Lee’s laboratory. In 2009, Dr. Shchelochkov joined the faculty at the University of Iowa, Department of Pediatrics, Division of Genetics. Dr. Shchelochkov studies nitrogen metabolism using model organisms (yeast, zebrafish, mouse) to gain insight into the mechanisms of ammonia toxicity and urea cycle with the ultimate goal of developing new treatment strategies for patients with ureagenesis disorders.

EXPLORING THE ROLE OF IMPAIRED ARGININE SYNTHESIS IN ASL DEFICIENCY USING ZEBRAFISH

Oleg A. Shchelochkov, J. Jung, L. Yu, J. Downward, L. Teesch, C. Brenner, R. Cornell

1. Stead Family Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA, 2. Department of Biochemistry, University of Iowa, Iowa City, IA, 3. Department of Chemistry, University of Iowa, Iowa City, IA, 4. Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA

Intellectual disability is a significant complication of the argininosuccinate lyase (ASL) deficiency. Hyperammonemic injury is an important factor, but it does not explain intellectual disability occurring in patients without episodes of hyperammonemia. Murine null and conditional hypomorphic ASL knockout models are limited in their ability to delineate specific mechanisms of intracellular arginine deficiency underlying damage of the developing brain. The key impediment to the investigation of the arginine’s role in brain development in vivo is accumulation of ammonia, leading to encephalopathy and death, thus precluding evaluation of nitrogen flux in the urea cycle. In principle, studies of intracellular metabolism in vivo are possible if three conditions are met: the organism (1) does not depend on the urea cycle for effective excretion of ammonia, (2) contains evolutionary conserved components, and (3) has tractable genome. Zebrafish is a vertebrate model that meets all three outlined criteria as hatched larvae and adult zebrafish primarily excrete ammonia through gills and do not depend on the urea cycle to dispose of the nitrogenous waste. We reasoned that phenotypes in zebrafish harboring mutations in the urea cycle genes would result from changes in nitrogen metabolism, rather than from hyperammonemia. Our preliminary data reveal that ASL, a urea cycle enzyme responsible for the synthesis of arginine, is expressed in zebrafish after 24-48 dpf. ASL knockdown in unhatched embryos and in larvae results in abnormal eye development and brain damage. Co-injection with human ASL mRNA attenuates the neuronal phenotype induced by ASL knockdown. Importantly, consistent with the hypothesis of arginine deficiency, co-injection of ASL morpholino with L-arginine also results in the attenuation of the ASL knockdown effects. These results prompted us to generate an ASL knockout zebrafish model using TALEN-mediated genome editing. We demonstrated transfer of loss-of-function alleles in F1 embryos and are currently raising asl heterozygous mutants. To determine specific biochemical events associated with brain damage in ASL deficient zebrafish, we developed NMR and mass spectrometry methods measuring the transfer of ammoniac 15N to arginine and its downstream metabolites. NMR demonstrated that injections of 15N-labeled ammonia into developing zebrafish embryos lead to incorporation of 15N into glutamine and guanidino compounds. These data were further corroborated by mass spectrometry of zebrafish embryonic tissues. Soaking of zebrafish embryos in 15NH4Cl resulted in the incorporation of 15N into glutamine, but there were no measurable changes in the isotope pattern in arginine. These results suggest that similarly to mammal organisms, glutamine serves as a temporary storage of ammoniac nitrogen. Arginine, on the other hand, is not an efficient nitrogen sink, but serves to triage the guanidino nitrogen to its downstream metabolites, possibly nitric oxide, creatine, polyamines and agmatine. In summary our experiments have illuminated the details of nitrogen metabolism in an ASL-deficient vertebrate organism in which the complicating factor of ammonia toxicity has been eliminated.
Dr. Gustavo Nino, MD, is double board-certified in Pediatric Pulmonology and Sleep Medicine. He is currently an Assistant Professor in the Division of Pulmonary and Sleep Medicine at Children’s National Medical Center and is part of the multi-disciplinary airway biology research group at Children’s Research Institute and Research Center for Genetic Medicine. He earned his medical degree from the National University of Colombia, South America and finished pediatric residency at Maimonides Medical Center in Brooklyn, NY. After his residency, he completed Pediatric Pulmonary fellowship at Children’s Hospital of Philadelphia and post-graduate training in Sleep Medicine at Pennsylvania State University (PSU). He has received young investigator distinctions from the American College of Chest Physicians (ACCP), American Academy of Sleep Medicine (AASM), and the International Congress of Pediatric Pulmonology (CIPP). In 2012 Dr. Nino was awarded a K12 Lung Genomics Award from NHLBI and in 2013 began his K12 Child Health Research Career Development Award from the CHRC. His current research aims to investigate novel mechanisms of airway genetic reprogramming mediated by viruses via exosomal miRNAs.

AIRWAY EXOSOMAL MICRONRNA SECRETION AND IMMUNE PROFILES DURING RHINOVIRUS INFECTION IN EARLY CHILDHOOD


Background: Innate immune responses are fine-tuned by small non-coding RNA molecules termed microRNAs (miRs) that modify gene expression in response to the environment. During acute infections miRs can be secreted in extracellular vesicles (exosomes) to facilitate cell-to-cell genetic communication. Interestingly, the potential role of exosomal miRs in mediating Th2 immune reprogramming of the airways during rhinovirus (RV) infection, the most common cause of asthma exacerbations in children, has not been investigated.

Hypothesis: RV infection in young children elicits airway exosomal miR responses that modulate antiviral and Th2 immune responses during early life.

Design/Methods: Nasal airway secretions were obtained from 125 infants and toddlers (<3 years of age) during PCR-confirmed RV infections. Airway Th1/Th2 cytokine levels were measured with a magnetic-bead multiplex array (15 analytes). Nasal exosomes were isolated with polymer-based precipitation (exoquick method) and miRs profiled in 20 subjects using NanoString microarrays (800 probes). Exosomal miRNA results were contrasted with in vitro data from air-liquid interface (ALI)-differentiated human bronchial epithelium (HBE). Data was analyzed with nCounter software, Ingenuity Pathway Analysis and Partek Genomics Suite T-test and ANOVA (p<0.05).

Results: RV infection was associated with increased nasal levels of the Th2 pro-asthmatic molecules thymic stromal lymphopoietin (TSLP; mean 16.7 pg/ml in RV vs. 5.5 pg/ml in controls; p=<0.01) and eotaxin1 (CCL-11; mean 21 pg/ml in RV vs. 11.9 pg/ml in controls; p=<0.01). Nasal exosomal miR profiling overlapped significantly with exosomal miRs isolated from in vitro HBE secretions indicating an epithelial secretory origin of the isolated nasal exosomes. Nasal exosomal miR profiling identified miR-155 as the top miR present in children with RV (n=10) but not in control subjects (n=10). Through the use of bioinformatics tools, we identified that miR-155 predicted target genes regulate antiviral immunity, Th2 responses and toll-like receptor (TLR)-mediated signaling. Conclusions: Our data indicate that acute RV infection in young children is associated with airway secretion of the Th2 pro-asthmatic molecules TSLP and eotaxin-1 as well as exosomal miR 155, which is predicted in silico to regulate antiviral immunity and Th2 responses. Further characterization of airway TSLP/eotaxin-1 biology and the potential immune regulatory role of virally-induced exosomal miR secretion will enhance our knowledge about the origins of virally-induced asthma and may identify new strategies to treat and monitor this condition in children.
Anita McElroy, MD, PhD
Instructor
Emory University School of Medicine

Dr. McElroy received her undergraduate degree in Microbiology at the University of Maryland, College Park and then went on to obtain her PhD in Biology from the University of California, San Diego. Her graduate work focused on the dysregulation of the cell cycle by human cytomegalovirus. After graduate school, she completed a postdoctoral fellowship at the US Army Medical Research Institute for Infectious Diseases where she studied the persistence and pathogenesis of Hantaviruses and participated in vaccine development for several emerging viruses. Anita attended medical school at George Washington University School of Medicine and in 2007, moved to Atlanta for her pediatric residency at Emory University, where she also completed her fellowship in Pediatric Infectious Diseases and is now an Instructor in the Division of Pediatric ID. She is also a guest researcher in the Viral Special Pathogens Branch at the US Centers for Disease Control. Anita's research focuses on the host immune response to hemorrhagic fever virus infections such as Ebola virus and Rift Valley fever virus.

T REGULATORY CELLS MODULATE THE CYTOTOXIC T CELL RESPONSE FOLLOWING RIFT VALLEY FEVER VIRUS VACCINATION

AK McElroy1,2, ST Nichol 1, J Cruz, C Munoz-Fontela and CF Spiropoulou 1
1US Centers for Disease Control and Prevention, Atlanta, GA and 2Emory University, Division of Pediatric Infectious Diseases, Atlanta, GA. 3Heinrich-Pette-Institute, Hamburg, Germany

Background: Rift Valley fever virus (RVFV) can cause hepatitis, hemorrhagic fever or encephalitis in both adults and children in Africa and areas of the Middle East. The components of the adaptive immune response necessary for protection are not fully understood. Using total CD4 T cell depletion, we have recently demonstrated that CD4 T cell responses are critical for the generation of an effective immune response to a live attenuated vaccine (Del NSs RVFV) in the mouse model. Evidence of excessive immune activation in the lymph nodes and brains of CD4 depleted mice accompanied by clinical encephalitis in one third of the mice suggested that a missing regulatory component might be responsible for the observed phenotype. Objectives: Evaluate the role of T regulatory cells in the generation of an immune response against Del NSs RVFV. Methods: We took advantage of the DEREG mouse model, which is a model in which T regulatory cells can be specifically depleted upon diphtheria toxin administration. Mice were treated with DT or PBS and infected with Del NSs RVFV. At three different times post infection, a characterization of T cell subtypes and their activation status was performed in multiple tissues using flow cytometry. Results: The T regulatory cell depleted animals exhibited T cell infiltration into their brains and one third of these mice had a distinct CD4+ CD25+ CD107a+ population, suggestive of a CD4 T cell with cytotoxic activity. However, animals did not exhibit signs of clinical encephalitis. Conclusions: During the immune response to Del NSs RVFV vaccination, T regulatory cells modulate the cytotoxic CD4 T cell response in the brain, but their absence does not result in acute clinical consequences.
Michael E. Watson, Jr., MD, PhD  
Clinical Lecturer  
University of Michigan Medical School

Dr. Watson studied biology as an undergraduate at Truman State University in Kirksville, MO. He subsequently attended medical school at the University of Missouri in Columbia, earning his M.D. and a Ph.D. in Microbiology studying the pathogenesis of nontypeable *Haemophilus influenzae* with Arnold L. Smith, M.D. He then completed a residency in Pediatrics at St. Louis Children’s Hospital and stayed at Washington University School of Medicine for fellowship in Pediatric Infectious Diseases. During his fellowship, Dr. Watson began investigating *Streptococcus pyogenes* mucosal colonization and carriage in the lab of Michael Caparon, Ph.D., in the Department of Molecular Microbiology. Dr. Watson is currently a Clinical Lecturer in the Department of Pediatrics and Communicable Diseases, Division of Pediatric Infectious Diseases, at the University of Michigan Medical School. He is continuing his research on *S. pyogenes*, currently within the lab of Suzanne Dawid, M.D., Ph.D., where he remains interested in the host-pathogen interactions contributing to streptococcal infections. His current research is examining the role of host innate and adaptive immune responses to streptococcal infection and mucosal colonization, specifically to identify mechanisms by which *S. pyogenes* evades or disables the host immune response. He is a previous recipient of a Pediatric Infectious Diseases Society Fellowship Award, a T32 Fellowship Training Grant from Washington University Department of Pediatrics, and a NIH Pediatric Research Loan Repayment Award. Dr. Watson joined the Child Health Research Center K12 program in July 2013. He is a member of the Pediatric Infectious Disease Society (PIDS), the Infectious Disease Society of America (IDSA), the American Academy of Pediatrics (AAP), and the American Society for Microbiology (ASM).

**INTERLEUKIN-17A AND HOST RESPONSES IN ASYMPOTOMATIC AND INFLAMMATORY MODELS OF STREPTOCOCCUS PYOGENES INFECTION**

**M. E. Watson,** S. Ahmed, I. Laczkovich, J. B. Weinberg, and S. R. Dawid  
University of Michigan, Ann Arbor, MI

**Background:** The bacterial pathogen *Streptococcus pyogenes* is capable of causing significant disease at multiple sites, including the skin and mucosal tissues (e.g., cellulitis and vulvovaginitis). In response to many bacterial infections the adaptive immune response via Th17 helper T lymphocytes promotes recruitment of innate immunity cells (i.e., neutrophils) to sites of infection, largely via interleukin-17A (IL-17A) signaling. However, the role of IL-17A and its effector functions with regards to clearance of *S. pyogenes* remains poorly characterized. **Objectives:** Our goal was to assess the role of IL-17A in promoting clearance of *S. pyogenes* from two different conditions, including an asymptomatic mucosal colonization and an inflammatory subcutaneous infection. **Design/Methods:** Host cytokine induction was assessed via RT-PCR for transcript detection and ELISA for protein content. Murine infection assays with *S. pyogenes* included a vaginal model of asymptomatic mucosal carriage and an inflammatory subcutaneous infection model. Mouse strains utilized included C57BL/6 wild-type (WT) mice and IL-17A−/− mice of C57BL/6 background. Histologic examination of skin biopsy, vaginal epithelium, and vaginal wash fluid was conducted to compare neutrophil recruitment to infected sites between WT and IL-17A−/− mice.

**Results:** Following inoculation with *S. pyogenes* into vaginal or subcutaneous tissue in WT mice there was significant elevation of IL-17A, in addition to IL-1 and IL-23, compared with non-inoculated tissues. IL-17A−/− mice exhibited significantly prolonged vaginal carriage compared to WT mice, with greater colony counts of *S. pyogenes* recovered from vaginal washes. Examination of neutrophil recruitment into vaginal fluid demonstrated an attenuated influx of neutrophils in colonized IL-17A−/− mice compared to WT. In the inflammatory subcutaneous infection model, IL-17A−/− exhibited a 48-72 h delay in the development of maximal skin ulcer size compared to WT mice, with a lag in neutrophil recruitment in IL-17A−/− mice to the site of infection. However, the maximal skin ulcer size was ultimately significantly larger in IL-17A−/− mice compared to WT mice. **Conclusions:** IL-17A plays a critical role in coordinating host responses against *S. pyogenes* in these two distinct models of infection. The delay of neutrophil recruitment exhibited in IL-17A−/− mice suggests that neutrophils are an important component of IL-17A-mediated effector activity promoting clearance of *S. pyogenes.*
Andrew W. Lindsley, MD, PhD
Instructor
Cincinnati Children’s Hospital Medical Center

After obtaining his undergraduate degree from Princeton University, Dr. Lindsley began his postgraduate scientific training in the cardiovascular development lab of Simon Conway Ph.D. at Indiana University School of Medicine, where he earned a Ph.D. dissecting the molecular mechanisms driving persistent truncus arteriosus in the Splotch2H mouse model. After obtaining his medical degree as part of a combined MD-PhD program, he continued his clinical training in the Categorical Pediatric Residency program at Cincinnati Children’s Hospital Medical Center (CCHMC). During residency, he enrolled in the American Academy of Pediatrics-sponsored Integrated Research Pathway (IRP), which is a modified residency training program allowing for up to 11 months of basic research during clinical residency training. Throughout residency, Dr Lindsley worked in the lab of Marsha Wills-Karp Ph.D. and undertook an asthma genetics association study involving two pediatric asthmatic cohorts with >1000 subjects. After completing residency, he continued his medical training as an Allergy & Immunology Clinical Fellow at CCHMC and extended his work on the pathogenesis of asthma by investigating the molecular functions of ORMDL3, showing that ORMDL3 regulates ceramide synthesis. Dr Lindsley is also interested in humoral immune deficiency, with a special focus on terminal B cell differentiation. He joined the faculty of the Division of Allergy & Immunology in July 2013 and his research lab focuses on pediatric asthma pathogenesis, with a special focus on sphingolipid signaling.

**PULMONARY CERAMIDE DEPLETION AMPLIFIES AIRWAY HYPER-RESPONSIVENESS & ENHANCES GRANULOCYTE CHEMOTAXIS IN A MODEL OF ALLERGIC ASTHMA**

A. Lindsley, K. Rehn, R. Edukulla
Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

**Background:** Sphingolipids are critical signaling molecules which regulate cell proliferation, apoptosis, differentiation and chemotaxis. Ceramide is the prototypical sphingolipid and is the central metabolic substrate for many sphingolipid signaling cascades. Altered ceramide levels have been implicated in the pathogenesis of pulmonary diseases such as emphysema and cystic fibrosis, but little is known about the specific roles of ceramide signaling in asthma pathogenesis. Herein, we depleted pulmonary sphingolipid levels by intratracheal (I.T.) administration of myriocin, a potent inhibitor of de novo ceramide synthesis. **Objective:** To test whether pulmonary ceramides regulate immune responses in a model of allergic asthma.

**Design/Methods:** Seven week-old Balb/c male mice were pre-treated with myriocin or vehicle & then sensitized to house dust mite extract (HDM) by I.T. exposure with/without co-administration of myriocin. Airway resistance was evaluated by invasive plethysmography, followed by bronchial lavage (BAL) cytology & cytokine quantification. Lung histology, lipid analysis & cell re-stimulation was performed. **Results:** Pulmonary myriocin treatment alone induced a mild neutrophil infiltrate, without airway hyper-responsiveness (AHR). Cxcl1 (KC) was elevated in BAL fluid of myriocin-treated mice while the neutrophilic chemotactic factors anaphylatoxin C5a, leukotriene B4 (LB4) and IL-17 were unaffected. HDM treatment combined with myriocin led to a dramatic enhancement of AHR and increased mixed granulocyte pulmonary infiltrates vs HDM or myriocin alone. Airway eotaxin-1 (Ccl11) and Cxcl1 levels were also greater in HDM & myriocin co-treated mice than in mice treated with either agent alone. Isolated neutrophils were cultured in the presence or absence of myriocin and apoptosis assessed by flow cytometry. Neutrophil survival was enhanced in a dose-dependent manner by myriocin treatment.

**Conclusions:** These findings reveal that pulmonary sphingolipids modulate airway granulocyte inflammation and allergen-induced AHR. Sphingolipid pathways represent novel targets for possible future anti-inflammatory asthma medications.
Amanda Evans, MD
Assistant Professor
UT Southwestern Medical Center

Dr. Evans received her undergraduate degree at The University of Texas at Austin and did her medical training at the University of Texas Medical Branch in Galveston. After medical school, Dr. Evans completed a residency in pediatrics at Georgetown University Hospital in Washington, DC and a fellowship in pediatric infectious diseases at UT Southwestern in Dallas, Texas. In 2012, Dr. Evans joined the faculty at UT Southwestern as an assistant professor in the Department of Pediatrics, Division of Infectious Diseases. Her interests include the pathogenesis of respiratory diseases in children. She is exploring viral and bacterial co-infection interactions in a human cell tissue model, and the subsequent dynamic changes that occur within the bacterial pathogens which influence virulence and attachment. Her current research project, involving a *Moraxella catarrhalis*/*RSV in vitro co-infection* model, aims to identify *M. catarrhalis* adherence or virulence factors involved in the pathogenesis of acute otitis media, with the goal of identifying new therapeutic or prophylactic targets for this bacterium.

**INACTIVATION OF A GENE INVOLVED IN OXIDATIVE STRESS INFLUENCES FITNESS OF *MORAXELLA CATARRHALIS***

**AS Evans, C Pybus, JS Kahn, EJ Hansen**
UT Southwestern Medical Center, Dallas, TX

**Background:** The pathogenesis of acute otitis media in children is associated with nasopharyngeal bacterial colonization and the acquisition of common respiratory viruses, like Respiratory Syncytial Virus (RSV). The interactions of *Moraxella catarrhalis*, a frequent bacterial pathogen in cases of acute otitis media, with RSV-infected human cells have not been elucidated. RSV infection has been shown to induce reactive oxygen species in human lung cells in vitro and in a murine model. For *M. catarrhalis* to establish infection, it must be able to deal with oxidative stress. **Objectives:** Our hypothesis is that host infection by RSV changes gene expression in *M. catarrhalis*, and that some of these changes involve mechanisms to combat oxidative stress. The objectives of this study are to determine the effect of mutations in genes important in oxidative stress on both the attachment ability and biofilm formation ability of *M. catarrhalis*. **Methods:** Human bronchial epithelial (HBE) cells were grown with and without RSV infection. In previous experiments, *M. catarrhalis* RNA was isolated after incubation with RSV-infected versus mock-infected HBEs, and bacterial DNA microarray analysis was performed. *M. catarrhalis* attachment assays were performed 48-hrs after viral infection to compare the attachment ability of wild-type *M. catarrhalis* and mutants with deletions in genes important in resisting oxidative stress. Crystal violet-based biofilm assays were also performed with these strains. **Results:** Bacterial DNA microarray analysis demonstrated an increase in expression of genes encoding products associated with combating oxidative stress, such as glutaredoxin and glutathione reductase. A glutaredoxin deletion mutant demonstrated a decrease in attachment to HBE cells when compared to wild-type. In addition, this mutant demonstrated a 50% reduction in biofilm production when compared to wild-type, (O.D. of 0.995 vs 0.492, p>0.001). **Conclusion:** RSV infection of HBE cells increases the expression of *M. catarrhalis* genes associated with oxidative stress. Inactivation of the bacterial gene encoding glutaredoxin had an adverse effect on both bacterial attachment to RSV-infected human cells and biofilm formation. Combating host-generated oxidative stress is an important mechanism that bacteria use to help establish infection.
Grzegorz Nalepa, MD, PhD
Assistant Professor
Indiana University School of Medicine

Dr. Nalepa is interested in understanding the mechanisms of genetic instability in Fanconi anemia and other bone marrow failure/cancer predisposition syndromes. Upon completion of graduate and postdoctoral training at Baylor College of Medicine and Harvard Medical School, Dr. Nalepa employed functional genomics to identify candidate tumor suppressors that prevent genomic instability by monitoring chromosome segregation during cell division (JCB 2013). His work revealed that the FA signaling network is essential for the mitotic spindle assembly checkpoint (JCI 2013), providing novel mechanistic insights into the origins of aneuploidy that spontaneously develops in FA patient cells. Dr. Nalepa’s laboratory employs super-resolution microscopy and functional genomics to dissect the mitotic signaling networks affected by loss of FA signaling. A new mouse model of FA developed in Dr. Nalepa’s laboratory recapitulates high predisposition to cancer and other key features of human Fanconi anemia, highlighting the translational importance of abnormal mitosis in FA and providing a preclinical model to study novel anti-cancer therapeutic strategies in the context of aneuploidy resulting from loss of FA signaling. As a clinician, Dr. Nalepa is interested in providing multidisciplinary diagnostics and therapeutics for pediatric patients with Fanconi anemia and other bone marrow failure syndromes.

The Fanconi anemia (FA) signaling network regulates spindle assembly checkpoint to prevent aneuploidy and cancer
Grzegorz Nalepa*

*Indiana University School of Medicine Department of Pediatrics, Indianapolis, IN, USA
Fanconi anemia (FA) is a heterogenous genetic disease with a high risk of cancer. The FA proteins are essential for interphase DNA damage repair; however, it is incompletely understood why FA-deficient cells also develop gross aneuploidy, leading to genomic instability and malignant transformation. The spindle assembly checkpoint (SAC) is the tumor suppressor signaling network that orchestrates high-fidelity chromosome segregation in mitosis. We found that FA signaling is essential for the SAC and thus prevents aneuploidy and hypersensitivity to the microtubule poisons. We demonstrated that FA proteins differentially localize to the mitotic apparatus in a cell cycle-depending manner. Consistent with these findings, our time-lapse imaging revealed abnormal mitotic progression in unperturbed and taxol-challenged FA patient cells. Importantly, our novel murine model of Fanconi anemia recapitulated the key clinical features of human FA and provided in vivo evidence for the essential role of FA-dependent chromosome segregation in tumor suppression. Collectively, our findings provide insight into the origins of genomic instability in FA and cancers resulting from somatic inactivation of the FA/BRCA pathway. Our work may provide rationale for targeted therapeutics of selected signaling pathways in cancer.
A Novel Small Molecule Inhibitor of the Gas6/TAM Pathway, in Combination with ADP/P2Y Antagonists, Mediates Synergistic Inhibition of Platelet Aggregation and Decrease Arterial & Venous Thrombosis

Brian R. Branchford1, Luke Law1, Gilbert Acevedo1, Christine Brzezinski1, Susan Sather1, Gary Brodsky1, Deborah DeRyckere1, Weihe Zhang3*, H. Shelton Earp III2,3, Stephen Frye2,3*, Douglas K. Graham1, Jorge A Di Paola4

1Department of Pediatrics, University of Colorado School of Medicine, Aurora; 2Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3Division of Medicinal Chemistry and Natural Products and Department of Medicine and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 4Pediatrics and Human Genetics and Genomics, University of Colorado School of Medicine, Aurora, CO

Background: ADP/P2Y inhibitors used for arterial thrombosis prophylaxis exhibit inter-individual response variability and bleeding complications. Inhibition of the Growth Arrest Specific gene 6 (Gas6)/Tyro3/Axl/Mer (TAM) signal axis decreases platelet activation and thrombus stabilization, and protects mice from thrombosis, without increasing tail bleeding times. Hypothesis: Gas6/TAM pathway blockade with a novel Mer-selective small molecule inhibitor (SMI) would decrease platelet aggregation and thrombosis, comparable to that seen with known ADP/P2Y inhibitors. Methods: We compared concurrent administration of P2Y1 and P2Y12 antagonists to a novel Mer-selective SMI (“UNC Mer TKI”), using light-transmission aggregometry and two murine thrombosis models. Results: ADP/P2Y1&12 antagonists and UNC Mer TKI both mediated decreased aggregation of washed human platelets. Mean maximum aggregation in 1 µM UNC Mer TKI-treated samples (n=7, 68.9 +/- 2.2%, p<0.05), differed significantly from vehicle-treated controls (n=7, 79.7 +/- 1.6%) and from null TKI-treated controls (n=7, 76.7 +/- 1.8%, p<0.01). 100 µM ADP/P2Y1&12 inhibitor-treated samples (n=7) exhibited a mean maximum aggregation of 62 +/- 5.2% (p<0.01), and samples treated with a combination of 100 µM each of ADP/P2Y1&12 inhibitors and 1 µM UNC Mer TKI had a maximum aggregation of 31.3 +/- 7.7% (n=7, p=0.001). The Chou-Talalay Combination Index was 0.78, indicating synergy. Similarly, the Bliss additivity equation predicted 38% aggregation inhibition for an additive interaction, but a 64% inhibition was actually observed, (p<0.05), also consistent with a synergistic effect for the combination. Survival times following venous injection of collagen and epinephrine significantly differed between mice treated with 3 mg/kg ADP/P2Y1&12 antagonists (n=5, 19.4 +/- 4.4 minutes, p=0.001) or 3 mg/kg UNC Mer TKI (n=9, 19.9 +/- 4.9 minutes, p=0.05), compared to vehicle control (n=21, 3.21 +/- 2.4 minutes). Mice treated with 1.5 mg/kg ADP/P2Y1&12 antagonists, however, exhibited shorter survival times (n=6, 11.9 +/- 5.0 minutes). Interestingly, combination of 3 mg/kg UNC Mer TKI and the lower dose of ADP/P2Y1&12 antagonists recapitulated the longer survival times seen with the higher dose ADP/P2Y1&12 antagonists (n=7, 25.9 +/- 3.8 minutes). FeCl3 application induced shorter carotid artery occlusion times in mice treated with 3 mg/kg ADP/P2Y1&12 antagonists (n=3, 11.4 +/- 2.2 min, p<0.001) or 3 mg/kg UNC Mer TKI (n=12, 42.2 +/- 4.7 minutes, p=0.05), compared to vehicle control (n=15, 53.2 +/- 0.3 minutes). Mice treated with 1.5 mg/kg ADP/P2Y1&12 antagonists, however, exhibited longer artery occlusion times (n=5, 52.7 +/- 0.2 minutes). Again, combination of 3 mg/kg UNC Mer TKI and the lower dose of ADP/P2Y1&12 antagonists recapitulated the shorter occlusion times seen with the higher dose ADP/P2Y1&12 antagonists (n=6, 31.6 +/- 9.2 minutes). Conclusion: A novel Mer-selective SMI mediated inhibition of platelet aggregation and protection from arterial/venous thrombosis in a manner comparable to known ADP/P2Y inhibitors. Additionally, a combination of the two inhibitor types mediated synergistic inhibition of platelet aggregation and allowed for ADP inhibitor dose reduction with similar thrombosis protection. Combination therapies consisting of a Mer inhibitor and an ADP/P2Y inhibitor may, therefore, allow for dose reduction of one or both agents, thereby decreasing off-target effects and/or bleeding complications.
Dr. Bayrer received his undergraduate degree at Claremont McKenna College and did his MD and PhD at Case Western Reserve University, training the laboratory of Dr. Michael Weiss. After medical school he completed his pediatrics residency at the University of California San Francisco. He stayed at UCSF for fellowship in pediatric gastroenterology, hepatology, and nutrition. In July 2011, Dr. Bayrer joined the laboratory of Dr. Robert Fletterick in the department of Biochemistry and Biophysics working to understand the role of nuclear receptor function in colon cancer. He is currently an Assistant Adjunct Professor in Pediatrics at UCSF, where he is interested in nuclear receptor function in normal and disease states in the bowel. Dr. Bayrer is particularly interested in applying structural biology towards fundamental pathways in mucosal healing and tumorigenesis with the goal of developing small molecule modulators of transcription factor activity. He recently has begun a collaboration to bring in an ex-vivo intestinal culture system as a test bed for epithelial growth and drug screening for novel inflammatory bowel disease therapies.

Dissecting the Oncogenic Activity of LRH-1 in the Colon

JR Bayrer,* RJ Fletterick, PhD.#
Departments of Pediatrics* and Biochemistry#, University of California San Francisco

Background: Colorectal cancers (CRC) account for nearly 10% of all cancer deaths in industrialized countries. Recent evidence points to a central role for the nuclear receptor Liver Receptor Homolog-1 (LRH-1) in intestinal tumorigenesis. Interaction of LRH-1 with the Wnt/b-catenin pathway, highly active in a critical subpopulation of CRC cancer cells, underscores the importance of elucidating LRH-1’s role in this disease. Reduction of LRH-1 diminishes tumor burden in murine models of CRC; however, it is not known whether LRH-1 is required for tumorigenesis, for proliferation, or for both.

Methods: To evaluate the contributions of LRH-1 to CRC growth, we chose two well-characterized CRC cell lines with high and average LRH-1 expression levels. We stably transduced the cells with Tet-inducible shRNA directed against LRH-1. Cell growth of the LRH-1 knockdown lines were compared to those expressing a non-silencing control RNA. Cell cycle analysis was performed to assess for cycling arrest and for induction of apoptosis. To explore alterations in gene expression due to LRH-1 suppression, we performed a microarray analysis of our knockdown cell lines. Results: LRH-1 mRNA knockdown results in significantly impaired proliferation in cells that highly express the receptor whereas cells with moderate expression have mild proliferative impairment. LRH-1-sensitive cells undergo S-phase arrest, which is consistent with LRH-1’s regulation of Cyclin E1. Cluster analysis of microarray gene expression demonstrates significant alterations in signal transduction, bile acid and cholesterol metabolism, and control of apoptosis. Conclusions: Silencing of LRH-1 expression in CRC cell lines leads to impaired cell growth. LRH-1 may exert its effects via multiple signaling networks, with inhibition leading to cell cycle arrest. Our results suggest carefully selected patients could benefit from selective LRH-1 inhibitors.

Support: K12 HD07222, F32 CA163092, T32 DK007762
Dr. Van Mater received his undergraduate degree at the University of Rochester and did his graduate and medical training at the University of Michigan. Dr. Van Mater remained at the University of Michigan to complete a residency in pediatrics. He then went to Duke University to complete a fellowship in pediatric hematology/oncology. In July 2010, Dr. Van Mater joined the laboratory of Dr. David Kirsch in the Department of Radiation Oncology, and he is now continuing his post-doctoral training there. Dr. Van Mater is currently in the Department of Pediatrics, Division of Pediatric Hematology/Oncology at Duke University School of Medicine, where he is interested in mouse models of soft tissue sarcoma. Dr. Van Mater is utilizing genetically engineered mouse models to explore the relationship between injury and sarcoma initiation and to better understand the impact of tumor heterogeneity on tumor propagating potential and metastasis. He has recently discovered a molecular pathway that regulates the process by which injury promotes rapid sarcoma formation, and he is using dual recombinase technology to label distinct cell populations within sarcoma and determine their functional role.

A ROLE FOR INJURY IN SARCOMAGENESIS.

D Van Mater, L Añó, JM Blum, DG Kirsch
Duke University School of Medicine, Durham, NC.

Background: Many sarcoma patients report a history of an injury at the location of their tumor. We developed a primary mouse model of soft tissue sarcoma and discovered that tissue injury dramatically accelerates sarcoma formation at the site of injury in our model system. Objectives: We utilized a genetically engineered mouse model of soft tissue sarcoma to explore the mechanism of injury-mediated sarcoma formation. Design/Methods: Pax7-CreERT2 (P7) mice were kindly provided by Dr. Chen-Ming Fan of the Carnegie Institute. P7 mice express a tamoxifen-inducible Cre downstream of the endogenous promoter for the satellite cell (muscle stem cell) transcription factor Pax7. P7 mice were crossed to genetically engineered mice containing a lox-STOP-lox cassette upstream of oncogenic K-rasG12D (K) in addition to 2 floxed p53 alleles (P) to generate P7KP mice. P7KP mice were injected with systemic, intraperitoneal (IP) tamoxifen to mediate Cre-dependent deletion of p53 and activation of Kras in Pax7+ cells throughout the animal. Cardiotoxin was injected into the gastrocnemius muscle at time points either before, concurrent, or after IP tamoxifen to determine the effect of injury on sarcoma formation. Results and Conclusions: When P7KP mice were injected with systemic, IP tamoxifen, they developed sarcomas throughout the body with 100% penetrance with a median onset of 45 days. In contrast, P7KP mice treated with IP tamoxifen and concurrent intramuscular (IM) cardiotoxin developed sarcomas at the site of injury with a median onset of only 15 days. Cardiotoxin was next injected into the gastrocnemius muscle at time points either before, concurrent, or after IP tamoxifen to better define the effect of injury on sarcoma formation. Cardiotoxin promoted highly efficient transformation when administered up to 3 days before and up to 21 days after IP tamoxifen administration. Lineage tracing studies showed no appreciable proliferation of recombined Pax7+ cells in the absence of cardiotoxin, suggesting that sarcoma formation requires factors in addition to loss of p53 and activation of K-ras to cause sarcoma. In contrast, IM injection of HGF stimulated proliferation in recombined muscle progenitor cells and promoted an increased rate of sarcoma formation. Our experiments suggest that injury acts as a classic promoter in the initiator/promoter model of tumorigenesis. The majority of muscle progenitor cells remain inactive following mutation of genes with high transformation potential, however tissue injury alters their fate and unlocks their full transformation potential by “flipping a switch”, converting resting satellite cells into the proliferative phase.
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<tr>
<th>Registrant Name</th>
<th>E-mail Address</th>
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<tbody>
<tr>
<td>Michael Armstrong</td>
<td><a href="mailto:michael.armstrong@duke.edu">michael.armstrong@duke.edu</a></td>
</tr>
<tr>
<td>Peter Baker</td>
<td><a href="mailto:peter.bakerii@ucdenver.edu">peter.bakerii@ucdenver.edu</a></td>
</tr>
<tr>
<td>Mark Batshaw</td>
<td><a href="mailto:mbatshaw@childrensnational.org">mbatshaw@childrensnational.org</a></td>
</tr>
<tr>
<td>James Bayerer</td>
<td><a href="mailto:bayrerj@peds.ucsf.edu">bayrerj@peds.ucsf.edu</a></td>
</tr>
<tr>
<td>Clifford Bogue</td>
<td><a href="mailto:clifford.bogue@yale.edu">clifford.bogue@yale.edu</a></td>
</tr>
<tr>
<td>Brian Branchford</td>
<td><a href="mailto:brian.branchford@ucdenver.edu">brian.branchford@ucdenver.edu</a></td>
</tr>
<tr>
<td>Garrett Brodeur</td>
<td><a href="mailto:brodeur@email.chop.edu">brodeur@email.chop.edu</a></td>
</tr>
<tr>
<td>Lou Ann Brown</td>
<td><a href="mailto:lbrow03@emory.edu">lbrow03@emory.edu</a></td>
</tr>
<tr>
<td>Brian Campfield</td>
<td><a href="mailto:brian.campfield@chp.edu">brian.campfield@chp.edu</a></td>
</tr>
<tr>
<td>Valerie Castle</td>
<td><a href="mailto:vcastle@umich.edu">vcastle@umich.edu</a></td>
</tr>
<tr>
<td>Juan Carlos Celedon</td>
<td><a href="mailto:juan.celedon@chp.edu">juan.celedon@chp.edu</a></td>
</tr>
<tr>
<td>Yee-Ming Chan</td>
<td><a href="mailto:Yee-Ming.Chan@childrens.harvard.edu">Yee-Ming.Chan@childrens.harvard.edu</a></td>
</tr>
<tr>
<td>Vivian Chang</td>
<td><a href="mailto:ntadros@mednet.ucla.edu">ntadros@mednet.ucla.edu</a></td>
</tr>
<tr>
<td>Rehana Chowdhury</td>
<td><a href="mailto:rchowdhu@mail.nih.gov">rchowdhu@mail.nih.gov</a></td>
</tr>
<tr>
<td>Wade Clapp</td>
<td><a href="mailto:kmatteso@iu.edu">kmatteso@iu.edu</a></td>
</tr>
<tr>
<td>Jennifer Dalton</td>
<td><a href="mailto:jendalto@umich.edu">jendalto@umich.edu</a></td>
</tr>
<tr>
<td>Stephen Daniels</td>
<td><a href="mailto:stephen.daniels@childrenscolorado.org">stephen.daniels@childrenscolorado.org</a></td>
</tr>
<tr>
<td>Stephanie Davis</td>
<td><a href="mailto:jillbarr@iu.edu">jillbarr@iu.edu</a></td>
</tr>
<tr>
<td>Engin Deniz</td>
<td><a href="mailto:engin.deniz@yale.edu">engin.deniz@yale.edu</a></td>
</tr>
<tr>
<td>Sherin Devaskar</td>
<td><a href="mailto:ntadros@mednet.ucla.edu">ntadros@mednet.ucla.edu</a></td>
</tr>
<tr>
<td>Amanda Evans</td>
<td><a href="mailto:amanda.evans@utsouthwestern.edu">amanda.evans@utsouthwestern.edu</a></td>
</tr>
<tr>
<td>Polly Ferguson</td>
<td><a href="mailto:polly.ferguson@uiowa.edu">polly.ferguson@uiowa.edu</a></td>
</tr>
<tr>
<td>Donna Ferriero</td>
<td><a href="mailto:donna.ferriero@ucsf.edu">donna.ferriero@ucsf.edu</a></td>
</tr>
<tr>
<td>Jeffrey Fineman</td>
<td><a href="mailto:jeff.fineman@ucsf.edu">jeff.fineman@ucsf.edu</a></td>
</tr>
<tr>
<td>Gary Fleisher</td>
<td><a href="mailto:gary.fleisher@childrens.harvard.edu">gary.fleisher@childrens.harvard.edu</a></td>
</tr>
<tr>
<td>Erick Forno</td>
<td><a href="mailto:erick.forno@chp.edu">erick.forno@chp.edu</a></td>
</tr>
<tr>
<td>Michael Freemark</td>
<td><a href="mailto:michael.freemark@duke.edu">michael.freemark@duke.edu</a></td>
</tr>
<tr>
<td>Jed Friedman</td>
<td><a href="mailto:jed.friedman@ucdenver.edu">jed.friedman@ucdenver.edu</a></td>
</tr>
<tr>
<td>Raif Geha</td>
<td><a href="mailto:raf.geha@childrens.harvard.edu">raf.geha@childrens.harvard.edu</a></td>
</tr>
<tr>
<td>Louis Ghanem</td>
<td><a href="mailto:ghaneml@email.chop.edu">ghaneml@email.chop.edu</a></td>
</tr>
<tr>
<td>Frank Gigliotti</td>
<td><a href="mailto:francis_gigliotti@urmc.rochester.edu">francis_gigliotti@urmc.rochester.edu</a></td>
</tr>
<tr>
<td>Charles Gomer</td>
<td><a href="mailto:cgomer@chla.usc.edu">cgomer@chla.usc.edu</a></td>
</tr>
<tr>
<td>Doug Graham</td>
<td><a href="mailto:doug.graham@ucdenver.edu">doug.graham@ucdenver.edu</a></td>
</tr>
<tr>
<td>Eva Grayck</td>
<td><a href="mailto:eva.grayck@ucdenver.edu">eva.grayck@ucdenver.edu</a></td>
</tr>
<tr>
<td>Julianne Green</td>
<td><a href="mailto:Juliane_Green@URMC.rochester.edu">Juliane_Green@URMC.rochester.edu</a></td>
</tr>
<tr>
<td>Laura Haneline</td>
<td><a href="mailto:tahicraw@iu.edu">tahicraw@iu.edu</a></td>
</tr>
<tr>
<td>William Hay</td>
<td><a href="mailto:bill.hay@ucdenver.edu">bill.hay@ucdenver.edu</a></td>
</tr>
<tr>
<td>Stacy Heilman</td>
<td><a href="mailto:sheilma@emory.edu">sheilma@emory.edu</a></td>
</tr>
<tr>
<td>Marc Hershenson</td>
<td><a href="mailto:mhershen@umich.edu">mhershen@umich.edu</a></td>
</tr>
<tr>
<td>Raphael Hirsch</td>
<td><a href="mailto:raphael-hirsch@uiowa.edu">raphael-hirsch@uiowa.edu</a></td>
</tr>
<tr>
<td>Margaret Hostetter</td>
<td><a href="mailto:margaret.hostetter@cchmc.org">margaret.hostetter@cchmc.org</a></td>
</tr>
<tr>
<td>David Hunstad</td>
<td><a href="mailto:hunstad_d@kids.wustl.edu">hunstad_d@kids.wustl.edu</a></td>
</tr>
<tr>
<td>Erin Janssen</td>
<td><a href="mailto:erin.janssen@childrens.harvard.edu">erin.janssen@childrens.harvard.edu</a></td>
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