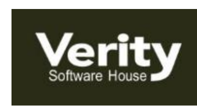
Great Lakes International Imaging and Flow Cytometry Association

CINCINNATI MARRIOTT AT RIVER CENTER

FRIDAY, SEPTEMBER 28 – SUNDAY, SEPTEMBER 30, 2018

Cincinnati, Ohio

GLIIFCA 27



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ABOUT GLIIFCA

GREAT LAKES INTERNATIONAL IMAGING AND FLOW CYTOMETRY ASSOCIATION, INC.

EIN# 16-1545169

The Great Lakes International Imaging and Flow Cytometry Association (GLIIFCA) started in 1992 by Carleton and Sigrid Stewart and Alex Nakeff fosters the interaction of professionals from Great Lakes region (U.S. states of Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin as well as the Canadian province of Ontario) with interests in flow and image cytometry. GLIIFCA organizes affordable annual meetings allowing the Great Lakes region cytometrists to learn about latest developments in cytometry and related fields, network with colleagues, and share the excitement about their research.

THE HISTORY OF GLIIFCA - WRITTEN BY CARLETON C. STEWART

In 1992 GLIIFCA began as... GLIFCA (the Great Lakes International Flow Cytometry Association - the word Imaging was added in 1993) and was first part of an outreach program for a Clinical Cancer Resource Grant that I was writing at Roswell Park Cancer Institute in Buffalo, NY. I had been thinking and discussing forming an organization that would primarily focus on young investigators and technologists doing research in cancer using imaging and flow cytometry. The established leaders in each of these fields were invited to form the first Steering Committee. Carleton C. Stewart was elected first President, Alexander Nakeff first Vice President, Sigrid Stewart, first Secretary/Treasurer and James Jacobberger first Educational Officer. Alan Landay and Maurice O'Gorman were to be the Scientific Chairs for the next meeting which was held October 1 - 3, 1993 at the Hotel St. Regis in Detroit, MI. Other members of the Steering Committee were Paul Robinson, Waclaw Jaszcz, David Hedley, Betsy Ohlsson-Wilhelm and James Leary. This Steering Committee was established to help determine the policies of the organization, maintain a budget, and create the program for the annual meeting. In addition to funding for this organization by the grant, I envisioned participation by the vendors as equal members of GLIIFCA. The reps from each company were instrumental in passing the word to their customers throughout the capture area. And you know the rest of the story...

Our first meeting of the Great Lakes International Flow Cytometry Association was held September 25 - 27, 1992 at the Hotel St. Regis in Detroit, MI: The meeting was an overwhelming success. The scientific agenda included sessions on Immunophenotyping, tumor biology and instrumentation. Over 130 charter members, some of which had never attended a flow meeting before, were able to attend because the total cost per person amounted to under $150 including registration and accommodations. Because of the central location of Detroit to the Great Lakes Region most people were able to carpool. The meeting started with a well-attended reception on Friday evening with food and an open bar sponsored by Becton Dickinson Immunocytometry Systems. On Saturday the Flowdown, sponsored by Coulter Cytometry, was thoroughly enjoyed by all. Most participants stayed until the very end. The greatest single state/province represented was Ontario, Canada. US charter members were from Michigan, Indiana, Minnesota, Illinois, Ohio, New York, Pennsylvania, Wisconsin and New Jersey. We even had two guests from Russia, which truly made this an international event. While our main region of emphasis is the Great Lakes, we welcome our colleagues from across the land to participate.

GLIIFCA 27 Sponsors

The GLIIFCA 27 meeting is kindly supported by:

- ACEA Biosciences, Inc. support for Coffee Break
- Agilent Reagent Solutions, DAKO
- Bangs Laboratories, Inc., support for Coffee Break
- Beckman Coulter- support for Beer Steins
- Becton Dickinson – support for the Poster Awards, Travel Awards, and Speaker
- BioLegend – support for Speaker
- Bio-Rad – support for Coffee Break
- Bio-Techne – support for Poster Awards
- Cell Microsystems
- Cytek – support for the Wine and Cheese Reception
- DeNovo Software
- Enzo
- FlowJo, LLC
- Fluidigm – support for the Wine and Cheese Reception and Beer Steins
- Immudex – support for the Immudex Translational and Clinical Cytometry Lecture
- Intellicyt
- International Society for Advancement of Cytometry – support for the ISAC Lecture Program
- Kitaka Used Instruments
- Leinco Technologies, Inc. – support for Beer Steins
- MilliporeSigma
- Miltenyi Biotec – support for Poster Awards
- Nanocellect
- nanoString
- Nexcelom Biosciences
- Ohio River Valley Cytometry Association – support for Speaker and Poster Awards
- Propel Labs, LLC
- Roswell Park Comprehensive Cancer Center – support for printing Conference Programs
- Sony Biotechnology – support for the Friday Evening Opening Reception
- Spherotech – support for Coffee Break
- Streck
- Sysmex – support for Beer Steins
- ThermoFisher Scientific – support for Coffee Break and Beer Steins
- Union Biometrica
- Verity Software House

FRIDAY

GLIIFCA 27 CORE FACILITY MANAGERS' WORKSHOP (MADISON I AND II)

12:00 PM – 5:00 PM

This workshop offers Core Managers and staff the opportunity to hear the latest on management practices and technical topics while meeting colleagues facing similar challenges running flow cytometry core facilities. Ample opportunity will be provided to network with attendees discussing and meeting the challenges of planning for the expanding complexity in flow cytometry technology. Core Managers and other attendees are encouraged to come prepared to participate actively in all sessions. We look forward to learning from everyone's experiences.

12:00 PM - 12:30 PM	*Opening Networking Luncheon: Welcome and Introductions*, **Matt Cochran and Monica DeLay**, GLIIFCA Organizing Committee
12:30 PM – 1:00 PM	*Anatomy of a Leader*, **Joanne Lannigan**, Director, Flow Cytometry Core Facility, University of Virginia
1:00 PM – 1:30 PM	*Panel Discussion - Leadership in a Dynamic Shared Resource Laboratory*. Each panelist will be the moderator of a specific topic
1:30 PM – 2:30 PM	*Thinking Outside the "Mouse and Human Sample" Box: Analyzing and Sorting Unexpected Samples*, **Kathryn Fox**, University of Wisconsin Carbone Cancer Center Flow Lab; **Christiane Hassel**, Indiana University Bloomington Flow Cytometry Core Facility
2:30 PM – 3:00 PM	*Bridging Administration and SRLs*, **Paula Turpen**, Director of Research Resources, University of Nebraska Medical Center
3:00 PM – 3:15 PM	*Coffee and Refreshments*
3:15 PM – 3:45 PM	*Aquatic Organisms and Instrument Modifications*, *Nicole Poulton*, Director, Facility for Aquatic Cytometry, Bigelow Laboratory for Ocean Sciences
3:45 PM – 4:45 PM	*Panel Discussion - Innovation and Novel Applications*
4:30 PM - 5:00 PM	*Parking Lot Discussions*
	Thank you to GLIIFCA for generous sponsorship of this workshop Organizing Committee: **Matthew Cochran**, **Monica DeLay**, **Vicki Smith,** and **Sally Quataert**

OPENING RECEPTION (COVINGTON BALLROOM)

The Opening Reception will take place in the **Covington Ballroom** from **6:00 PM** to **9:30 PM**. Come and interact with the Vendors and fellow GLIIFCA conference participants!

GLIIFCA CONFERENCE OPENING RECEPTION SPONSORED BY

SONY

Industrial Science Symposium (Riverview Ballroom)

Session Chairs: Christiane Hassel (Indiana University), Karen Domenico (Shriners Hospitals for Children), and Louis King (Michigan State University)

6:30 PM - 6:35 PM	**Industrial Science Symposium Introduction**
6:35 PM - 6:50PM	*Validation of Antibody Panels for High-plex Immunohistochemistry Applications*, **Indira Medina**, Nanostring Technologies Inc.
6:50PM - 7:05PM	*Streamlined Human Immune Monitoring with Mass Cytometry: 29 Markers in a Single Tube and Automated Data Analysis*, **Michelle M Poulin**, Fluidigm
7:05PM - 7:20PM	*COPAS VISION Flow Cytometer Captures Images and Sorts*, **Yongwoon Kim**, Union Biometrica, Inc.
7:20 PM – 7:35 PM	*Using FCS Express to Accelerate your Flow Cytometry Reporting and Results* ,**Sarah Schuett**, DeNovo Software
7:35 PM – 7:50 PM	*New CellStream™ Flow Cytometer*, **Todd Salomon**, MilliporeSigma
7:50PM - 8:05PM	*Expanding Application Capabilities Using Full Spectrum Cytometry*, **Dave Kennedy**, Cytek Biosciences
8:05PM - 8:20 PM	*Novel Aspects of Sorting in a Closed System: The MACSQuant Tyto*. **Dr. Patrick Sean Murphy**, Miltenyi Biotec
8:20PM - 8:35PM	*Multi-application Sorting with the Sony MA900*, **Steve Whitaker**, Sony Biotechnology Inc.
8:35PM - 8:50PM	*Sorting Simplified: Introducing the BD FACSMelody*, **David Morris**, BD Biosciences

Saturday

Breakfast will be available in the Covington Ballroom from 7:00 AM to 8:00 AM

All of the Oral Presentations will be Delivered in **Riverview Ballroom**

8:00 AM - 8:15 AM	*Welcome Address*: **Sherry Thornton** (GLIIFCA President)

Session 1: Mechanisms of Infectious Pathogens (Riverview Ballroom)

Session Chairs: Sherry Thornton (Cincinnati Children's Hospital and University of Cincinnati)
Karen Domenico (Shriners Hospitals for Children)

8:15 AM - 9:00 AM	*Biolegend Lecture: Maternal Schistosomiasis: Understanding the Implications of Immune-modulation*, **Keke Fairfax**, Purdue University
9:00 AM - 9:45 AM	*Imaging Non-conical New Roles for Natural Killers in Health and Disease*, **Stephen Waggoner**, Cincinnati Children's Hospital and University of Cincinnati
9:45 AM - 10:30 AM	*Herzenberg Lecture*: *Taming and Training Natural Killers*, **Eric Long**, NIH National Institute of Allergy and Infectious Diseases, Molecular and Cellular Immunology Section
10:30 AM - 11:00 AM	*Coffee Break* sponsored by ACEA Biosciences and Bangs Laboratories

The 2018 Carleton and Sigrid Stewart Keynote Lecture (Riverview Ballroom)

Introduction: James Jacobberger (Case Western Reserve University)

11:00 AM - 11:45 AM	*Flow Cytometry and Small Molecule Drug Development in Pharma*, **Vincent Shankey**, Shankey Biotechnology Consulting

Lunchtime Roundtable Workshops (Madison I, Madison II, Gazebo, and Terrace IV)

Workshop Organizers: Dagna Sheerer (University of Wisconsin), and Catherine Behrmann (University of Cincinnati)

11:45 AM - 1:30 PM	*Multiple parallel roundtable workshops.*

Session 2: Imaging (Riverview Ballroom)

Session Chair: Matthew Cochran (University of Rochester Medical Center)

1:30 PM - 2:15 PM	*Monitoring Immune Response by Imaging Flow Cytometry*, **Orla Maguire**, (Marylou Ingram ISAC Scholar), Roswell Park Comprehensive Cancer Center
2:15 PM - 3:00 PM	*A Myeloid Niche Revealed by Imaging Flow Cytometry*, **Katie Seu**, Cincinnati Children's Hospital
3:00 PM - 3:30 PM	*Coffee Break* sponsored by Bio-Rad and Spherotech

Session 3: Organisms and Organoids Cytometric Analysis (Riverview Ballroom)

Session Chair: Rachel Sheridan (Van Andel Institute)

3:30 PM - 4:00 PM	*ISAC Emerging Leader Lecture*: *Exploring Aquatic Microbial Biodiversity using Cytometry and Single Cell Genomics*, **Nicole Poulton**, Bigelow Laboratory for Ocean Sciences
4:00 PM - 5:15 PM	*Isolation and High-throughput RNA Sequencing of, C. elegans Neural Cells Reveals an mRNA Important for Proper Chemotaxis*, **Heather Hundley**, Indiana University
5:15 PM - 6:00 PM	*Enteroendocrine Regulation of Nutrient Absorption*, **Heather McCauley,** Cincinnati Children's Hospital

Poster Session (Covington Ballroom)

6:30 PM - 8:00 PM	*Poster Session*
6:30 PM - 7:30 PM	*Wine and Cheese Happy Hour* sponsored by Fluidigm and Cytek

The Wine and Cheese Happy Hour is Sponsored by

Social Activities (Gazebo and Riverview Ballroom)

8:00 PM - 11:30 PM	GLIIFCA 27 Banquet
11:30 PM - 2:00 AM	Post-banquet Scientific Networking

SUNDAY

Breakfast will be available in the Covington Ballroom from 8:00 AM to 9:00 AM

STEERING COMMITTEE MEETING (MADISON I AND II)

8:00 AM – 8:50 AM	GLIIFCA Steering Committee Breakfast

SESSION 4: TRANSLATIONAL AND CLINICAL CYTOMETRY (RIVERVIEW BALLROOM)

Session Chairs: Jessica Back (Wayne State University)
Kathy Schell (WiCell Research Institute)

9:00 AM - 9:45 AM	*Immudex Translational and Clinical Cytometry Keynote*: *Immune Monitoring from a Core's Perspective – the Past, the Present, and the Future*, **Janet Staats**, Duke University
9:45 AM - 10:30 AM	*Not Just Little Adults: Considerations and Caveats of Flow Cytometric Evaluation of Pediatric Bone Marrow*, **Robert Lorsbach**, Cincinnati Children's Hospital and University of Cincinnati
10:30 AM - 11:00 AM	*Coffee Break* sponsored by ThermoFisher Scientific

SESSION 5: SINGLE CELL BIOLOGY

Session Chair: Joseph D. Tario, Jr. (Roswell Park Comprehensive Cancer Center)

11:00 AM - 11:45 AM	*Single Cell Surface Proteomics in Cell Lines, Primary Explants and Heterogeneous Tissues,* **Albert Donnenberg**, University of Pittsburgh
11:45 AM - 12:30 PM	*Defining Novel Multilineage Progenitor Populations using Single-cell RNA-Seq*, **Nathan Salomonis**, Cincinnati Children's Hospital and University of Cincinnati
12:30 PM - 12:35 PM	*Closing Remarks*

GLIIFCA 27 Roundtable Lunch Workshops

***For those not attending a Roundtable Luncheon Workshop, boxed lunches will be available in a designated area in the Gazebo.

Section: Single Cell Biology

Topic 1. Single Cell Omics: Opportunities and Challenges, *Albert Donnenberg, University of Pittsburgh*
Location: **Madison I**

Analytical flow cytometry and preparative flow-based cell sorting are key technologies to facilitate single-cell Omics of heterogeneous samples. Although examples abound of single cell proteomics, transcriptomics and genomics, there are limitations to all of these applications. This round-table discussion will examine common pitfalls, including purity of sorted cells and lower limits of transcript detection, that currently limit single-cell Omics, and attempt to identify potential solutions.

Topic 2. Single Cell Sorting for Culture and Genomics, *Rachael Sheridan, Van Andel Institute, Grand Rapids, Michigan.*
Location: **Madison I**

With the increase in single cell genomic analyses and utility of CRISPR-derived clones, researchers are requiring more single cell sorting. We'll discuss validation of sorter performance for single drop sorts into plates compatible with downstream genomic analysis or culture to maximize success and minimize sample handling. There will be ample opportunity to share experiences with these sorts and the collaboration required with another core that may or may not be within the institution.

Topic 3. Cell Sorting: Setting Yourself Up for Success, *Lauren Nettenstrom, University of Wisconsin Comprehensive Cancer Center Flow Cytometry Laboratory*
Location: **Madison I**

We will discuss the steps you need to take, whether you are a sort operator or a customer, well before you get to the instrument to set yourself up for a successful sort. Topics will include education, biosafety, panel design.

Section: Imaging

Topic 4. Tissue Clearing and Deep Tissue Imaging, *Evan Meyer, ORVCA Board, Cincinnati Children's Hospital Medical Center*
Location: **Madison II**

Advances in tissue clearing techniques and imaging methodologies have enabled cytometry deeper into a variety of intact tissues including whole animal models. Selecting the optimal methodology for tissue preparation, imaging, and data analysis is essential for effective outcomes. This round-table discussion will focus on the factors that go into deciding what clearing technique is right for the scenario and considerations for how the data will be acquired and analyzed.

Topic 5. Maximizing the Potential of your Imaging Flow Cytometer, *Orla Maguire, Roswell Park Comprehensive Cancer Center*
Location: **Madison II**

Discussion points to include (we may not get to all!):

- Start at the beginning: How do I attract researchers to use IFC?
- Quality control: Appropriate QC for the instrument and your experiments
- How to publish IFC data

SECTION: EXPERIMENTAL DEVELOPMENT

Topic 6. High Parameter Flow Cytometry and Panel Design, *Victoria Smith, UNMC*
Location: **Madison II**

Fluorochromes, antigen density and co-expression, instrument characteristics, and knowledge of the biology being studied are vital in panel design. How can we minimize spectral overlap and use resolution impact to our benefit when planning our experiments? What are some other factors we need to consider when planning our experiments? Good quality high parameter data depends on great panel design.

Topic 7, Flow Cytometry and Small Molecule Drug Development in Pharma: If Flow is so Good, How is Pharma using it? *T. Vincent Shankey, Shankey Biotechnology Consulting*
Location: **Gazebo**

Discussion of assessment of drug development in Pharma, including new methodologies that incorporate machine learning and other novel concepts.

SECTION: IMMUNOTHERAPY AND CANCER

Topic 8. CD4 Voltage Titration: A Method to Optimize Cytometer Performance, *Derek Jones, Flow Cytometry and Cell Sorting Resource laboratory, University of Pennsylvania*
Location: **Gazebo**

How can we ensure that our cytometers are performing optimally? The discussion will address the purpose and procedure of CD4 voltage titrations, the practicality within a shared resource laboratory setting, the benefits provided to users, and alternative methods used to achieve optimization. Detailed SOPs and lists of required reagents will be made available.

Topic 9. Clinical Test Validation: Transforming Research Flow to Clinical Testing, *Mary Reynaud, Lisa Neumeier, Cincinnati Children's Hospital Medical Center*
Location: **Gazebo**

We will review the criteria for validating a flow cytometry test for use in a clinical diagnostic laboratory. We will discuss the requirements of accrediting agencies to demonstrate a test is robust, accurate and precise for use in making diagnoses. We hope to provide researchers with some idea of what goes into making a good diagnostic assay and emphasize the differences from testing performed for research.

Section: Non-Mammalian Cytometry

Topic 10. Beyond Cells: "We Sort all of the Above," *Kathryn Fox, UWCCC Flow Cytometry Laboratory & Christiane Hassel, IUB Flow Cytometry Core Facility*
Location: **Terrace IV**

Sometimes preparing samples for analysis and sorting requires thinking outside the box. We'll discuss what to consider when working with samples that either aren't human or mouse in origin, or are tricky to analyze or sort in general.

Section: Core Management

Topic 11. SRL Best Practices: The Role in Rigor and Reproducibility, *Joanne Lannigan, University of Virginia*
Location: **Terrace IV**

In November 2016 an ISAC Shared Resource Laboratory (SRL) Task Force published a document outlining a set of "best practices" for SRLs or Core Facilities to use as a general guide for achieving and maintaining standards of excellence in the services they provide. Recently there has been a great deal of focus on rigor and reproducibility in science. How will the implementation of best practices support rigor and reproducibility? What role do SRLs have in promoting rigor and reproducibility? How will these efforts ultimately benefit the SRL? Come join the discussion to find out.

Topic 12. SCYM Exam, *Jessica Back, Karmanos Cancer Institute, Wayne State University*
Location: **Terrace IV**

The new Specialist in Cytometry Certification (SCYM) was created through a joint effort by ISAC, the International Clinical Cytometry Society (ICCS), and the American Society for Clinical Pathology Board of Certification (ASCP BOC) and was launched in fall of 2017. The SCYM replaces the International Cytometry Certification Exam (ICCE) and the ASCP BOC's Qualification in Cytometry (QCYM) recognition program. The eligibility requirements for new applicants, credential maintenance, and frequently asked questions about the SCYM will be addressed.

GLIIFCA Conference Coffee Breaks are Supported by

GLIIFCA 27 Presentation Abstracts

Single Cell surface Proteomics in Cell Lines, Primary Explants and Heterogeneous Tissues

Albert D. Donnenberg and Vera S. Donnenberg (University of Pittsburgh School of Medicine)

The characterization of cell surface protein expression on individual cell types has historically been a gradual process requiring the synthesis of multiple investigations over time. For the best studied cell types such as T lymphocytes, a picture of the cell-surface proteome has gradually emerged, and with it an in depth understanding of within-type phenotypic and functional heterogeneity. Often, markers proposed to identify the cell type on which they were first observed (e.g. Thy-1/CD90 on thymocytes) have later been demonstrated on disparate cell populations, exposing a misleading nomenclature which often persists in the literature. Recent advances in high throughput flow cytometry (i.e. plate-based sample loaders), analytical software, and the commercial availability of a products with a large array of antibodies directed against human cell surface markers (Lyoplate and FACSCAP Lyoplate, Becton Dickinson), now facilitate a comprehensive analysis of the cell-surface proteome of heterogeneous tissue samples as well as cell lines and culture expanded primary cells. We have used these systems to compare breast cancer cell lines with primary explant cultures, provide a definitive phenotype for native and cultured adipose derived stromal cells, and to trace phenotypic changes of bone marrow mesenchymal stromal cells from their native state through senescence. Ongoing studies examining the cell surface proteome of tumor cells, tumor infiltrating lymphocytes and tumor associated macrophages in malignant effusions, provide leads for therapeutic targets and inform repolarization and immune checkpoint blockade strategies.

BioLegend Lecture

Maternal Schistosomiasis: Understanding the Implications of Immuno-modulation

Keke Fairfax (Purdue University)

Approximately 40 million women of childbearing age are at risk for schistosomiasis and at least 10 million women in Africa have schistosomiasis during pregnancy, yet little is known about the impact of prenatal exposure on the immune responsiveness of offspring. Findings from previous studies indicate that a fetus can be exposed to helminth antigens *in utero*, so the high frequency of schistosomiasis in this population represents a significant potential public health problem. The underlying mechanisms through which maternal infection with helminths, such as *Schistosoma mansoni*, function to negatively impact neonatal vaccine responses, however, remain to be discovered. We sought to determine the immunological mechanisms through which responses to heterologous immunization are altered following prenatal *S. mansoni* infection in an experimental mouse model. Interestingly, offspring from infected mothers exhibited decreased follicular dendritic cell (FDC's) and CD21/35 area at steady state (35 days of age) compared to offspring from uninfected control mothers. Corresponding with reduced FDCs. Offspring from infected mother have reduced transcription of *baff*. These differences persisted following immunization with tetanus/diphtheria. Offspring born to *S. mansoni* infected mothers exhibited smaller germinal centers, reduced frequencies of IL-4 secreting

TFH cells, and memory B-cells in comparison to age-matched offspring of uninfected mothers at days 8 and 14 post immunization with tetanus/diphtheria. These profound alterations suggest a possible mechanism for the previously reported reductions in long-term vaccine specific titers in people in helminth endemic areas.

Isolation and High-throughput RNA Sequencing of *C. elegans* Neural Cells Reveals an mRNA Important for Proper Chemotaxis

Heather A. Hundley (Indiana University)

Adenosine deaminases that act on RNA (ADARs) are proteins that alter gene expression both by catalyzing adenosine(A)-to-inosine(I) RNA editing and binding to regulatory elements in target RNAs. It is well established that A-to-I editing within coding regions of specific human transcripts alters the physiological properties of the encoded proteins, a requisite for proper neuronal function. Consistent with this important role of ADARs, alterations in ADAR protein levels and editing activity have been observed in several human neuropathological diseases. In addition, loss of ADARs affects neuronal function in all animals studied to date. In particular, *Caenorhabditis elegans* lacking ADARs exhibit reduced chemotaxis to a number of volatile chemicals. Loss of *C. elegans* ADARs likely affects the ability of the neurons to sense these chemicals as the chemotaxis defect is less severe with increasing doses of these volatiles; however, the targets responsible for the chemosensory defects of *C. elegans* ADARs were unknown. Though multiple studies have used high-throughput sequencing to examine RNA editing and the role of ADR-2, the only A-to-I editing enzyme in *C. elegans*, these studies were limited to analyzing RNA isolated from whole worms. However, recently a method using chemomechanical disruption of worms followed by fluorescent activated cell sorting (FACS) has been used to obtain cells of interest from whole worms. By expressing a fluorescent marker in neural cells, we used this method to isolate and sequence the transcriptome of neural cells from wild-type and *adr-2* deficient worms. High-throughput sequencing revealed over 7,300 editing sites in the neural editome. Additionally, differential expression analysis identified 169 genes with altered expression in neural cells lacking *adr-2*. Together this analysis revealed that *clec-41,* a gene previously found to be important for worm locomotion, was edited in the 3' untranslated region of the transcript and *clec-41* transcripts were differentially expressed in *adr-2(-)* neural cells. Strikingly, transgenic expression of *clec-41* in neural cells of *adr-2* deficient worms was sufficient to rescue the aberrant chemotaxis of these animals. This is the first study to link noncoding A-to-I editing and altered expression of a specific transcript with a neurological consequence resulting from loss of ADARs.

2018 ORVCA Herzenberg Lecture

Taming and Training Natural Killers

Eric Long (NIAID, National Institutes of Health)

The immune system includes cytotoxic cells, known as Natural Killers, that eliminate virus-infected cells and tumor cells. Unlike cytotoxic T cells, NK cells do not have antigen-specific receptors. Instead, multiple levels of regulation provide tight control of NK cell cytotoxicity. First, there is separation of duties among activation receptors. Adhesion receptors are required for tight contact with target cells and polarization of the cytotoxic machinery toward the target. Other activation receptors must be engaged to release inducers of programmed cell death from polarized cytolytic granules in NK cells. This degranulation is activated through synergistic combinations of pairs of co-activation receptors. Second, NK cells are kept in check by dominant inhibitory receptors, some of which bind MHC class I molecules. Most healthy cells express MHC-I and resist attack by

NK cells. Third, a revocable license must be issued to NK cells for permission to kill. Licensing (taming) of NK cells is a task assigned to MHC-I specific inhibitory receptors. All of these properties are conferred to NK cells by innate, germ line-encoded receptors. In addition, NK cells have an Fc receptor for IgG called FcγRIIIa or CD16, which is a potent activator of antibody-dependent cellular cytotoxicity (ADCC). NK cell activation via CD16 bypasses the requirement for synergy with co-activation receptors. We have new data showing that expansion of a subset of 'adaptive' or 'trained' NK cells with enhanced ADCC correlates with antibody-dependent protection against malaria in people living in a region with intense transmission of *Plasmodium falciparum* parasites. *P. falciparum* growth in vitro is inhibited through lysis of infected red blood cells by NK cells in presence of IgG from the serum of resistant adults living in the malaria-endemic region.

Not Just Little Adults: Considerations and Caveats of Flow Cytometric Evaluation of Pediatric Bone Marrow

Robert Lorsbach (Cincinnati Children's Hospital Medical Center and University of Cincinnati)

Flow cytometry is an important tool for the clinical evaluation and diagnosis of an array of neoplasms and reactive disorders that may involve the bone marrow. There is significant overlap in the immunophenotypic findings between adult and pediatric bone marrow. However, several important differences exist that may pose diagnostic challenges during flow cytometric analysis of pediatric bone marrow, due to differences in the frequency of normal cell populations in pediatric versus adult marrow as well as an array of reactive/genetic disorders and neoplasia that are encountered predominantly or exclusively in the pediatric setting. Our discussion will provide an overview of a diagnostic approach to the flow cytometric evaluation of pediatric bone marrow. We will then turn our attention to discussion of cell populations, e.g., hematogones (B-cell progenitors), and disease states which have immunophenotypic findings that may lead to the misinterpretation of normal marrow constituents as malignant cells or vice versa. We will conclude with discussion of the flow cytometric analysis of malignancies that preferentially occur in the pediatric age group, such as acute megakaryoblastic leukemia, with an emphasis on key immunophenotypic properties and diagnostic caveats. Participants should gain a better appreciation of the distinctive immunophenotypic findings of pediatric bone marrow that may be encountered during flow cytometric analysis and have greater understanding of potential diagnostic pitfalls.

Monitoring immune response by imaging flow cytometry

Orla Maguire (Roswell Park Comprehensive Cancer Center)

The immune system plays a critical role not only in defense from pathogen attacks, but also in cancer surveillance and more recently as a tool in immunotherapy-based treatments. Immune cell functions are tightly regulated, including by important transcription factors such as NF-κB and NFAT, both of which play essential roles. Monitoring the activity of immune cells—including phenotyping immune cell subsets, tracking cell proliferation, and measuring cytokine production—can provide insights into the overall status of immune function in patients, particularly those undergoing immunosuppression after transplants, enduring cancer treatment, or suffering from autoimmune disease or other pathologies that effect the immune system. In this seminar the current studies in our lab into the activity of immune cells focusing in on the NFAT transcription factor pathway will be presented. These will include the role of multimers in determining an individual's

immune status towards a particular antigen; the role of factors in the tumor microenvironment, such as exosomes, in immune response; and the impact of chemotherapies on immune function.

Enteroendocrine Regulation of Nutrient Absorption

Heather McCauley (Cincinnati Children's Hospital Medical Center)

Enteroendocrine cells (EECs), which are nutrient-sensing cells found throughout the gastrointestinal tract, secrete more than 20 hormones which individually and collectively regulate many physiologic processes in response to nutrient ingestion. While EECs only comprise 2-3% of the intestinal epithelium, mice and humans without EECs suffer from severe malabsorptive diarrhea, demonstrating that EECs are essential regulators of nutrient absorption. Surprisingly, it is not known how EECs control nutrient absorption. As EEC differentiation depends on the bHLH-containing transcription factor *Neurogenin 3 (NEUROG3),* we used CRISPR/Cas9 to induce a null mutation in *NEUROG3* in pluripotent stem cells (PSCs) and directed their differentiation into human intestinal organoids (HIOs) which lack EECs. We found no obvious defects in the expression or localization of glucose transporters, peptide transporters or lipid transporters in HIOs lacking EECs. However, *in vivo* transcellular transport of nutrients from the luminal brush border to the basal lamina propria depends on electrochemical gradients of sodium, potassium, chloride, and other electrolytes. These electrochemical gradients are sensitive to inputs from the enteric nervous system, such as vasoactive intestinal peptide (VIP) as well as to EEC hormones, such as peptide YY (PYY). We hypothesized that the absence of EECs would disrupt normal enterocyte electrochemical gradients and thus impair electrogenic nutrient absorption. In support of this, we detected enhanced epithelial anion secretion in response to VIP in *NEUROG3*-null intestinal epithelium that could be tempered with addition of exogenous PYY. Moreover, we observed an enhanced electrogenic response to luminal glucose in *Neurog3*-null mouse intestine that could be partially rescued by exogenous addition of PYY. Taken together, these results support a central role for EECs in controlling nutrient absorption by coupling an epithelial-neurohormonal signal with nutrient and ion transport.

ISAC Lecture

Exploring Aquatic Microbial Biodiversity using Cytometry and Single Cell Genomics

Nicole J. Poulton (MacIsaac Facility for Aquatic Cytometry Bigelow Laboratory for Ocean Sciences)

Aquatic unicellular microorganisms - including bacteria, archaea, and protists - comprise a vast majority of biological diversity on our planet and perform a significant portion of the global biogeochemical processing, such as carbon and nitrogen fixation, organic material degradation, and recycling of the life-sustaining elements phosphorus, sulfur, and iron. Recent estimates indicate the existence of over a trillion microbial species, only $\sim 10^4$ of which have been successfully brought into laboratory cultures using classical microbiology approaches. Due to this large biological diversity, molecular research tools that circumvent cultivation, collectively called "meta-omics," are becoming increasingly important sources of information about the composition and function of diverse microbiomes, the impact and mitigation of global environmental changes and anthropogenic contaminants, and exploration for new, natural products for bioenergy and biotech applications. Single cell genomics recovers the hereditary information from the most basic units of biology and has become a complement to cultivation-based and microbial community research

approaches. Single cell genomics (SCG) is relevant in aquatic microbiology, where most organisms are composed of individual cells and are difficult to cultivate, and cannot be analyzed using traditional molecular approaches. Microbial SCG has enabled the recovery of genomic blueprints of many uncultured aquatic microbial groups, revealed unrecognized biogeochemical processes, and identified novel pathogenic and symbiotic interactions *in situ.* Methods for SCG consist of a series of integrated processes (SCG pipeline), beginning with the collection and preservation of aquatic environmental samples, followed by single cell sorting, lysis and whole genome amplification of individual cells, and culminating in genomic sequencing and the interpretation of encoded biological features.

The combination of single cell sorting and genomics has been instrumental in identifying metabolic features and inter-organismal interactions of the uncultured microbial groups that dominate many aquatic environments.

Defining Novel Multilineage Progenitor Populations using Single-cell RNA-Seq

Nathan Salomonis (Cincinnati Children's Hospital Medical Center and University of Cincinnati)

Advances in single-cell transcriptome profiling have contributed to new insights into the cellular states and underlying regulatory networks that govern lineage commitment. Such cell states include multipotent progenitors that can manifest as mixed-lineage patterns of gene expression at a single-cell level. Multipotent and other self-renewing progenitors are often difficult to isolate and characterized by subtle transcriptional differences that are challenging to define. Using scRNA-Seq and innovative new computational methods, we have defined novel subsets of multilineage progenitors that challenge existing dogma and provide new insights into trajectories for lineage specification.

A myeloid Niche Revealed by Imaging Flow Cytometry

Katie Seu (Cincinnati Children's Hospital Medical Center)

Mammalian erythropoiesis occurs within erythroblastic islands (EBIs), niches where erythroblasts differentiate in close contact with a central macrophage. While it is generally accepted that the macrophages play an important role in erythropoiesis, our understanding of how EBI macrophages function has been limited due to difficulty identifying and isolating them for further study. Thus, little is known about the regulation that occurs within these niches and how EBIs fit into the broader context of hematopoietic cell interaction in the bone marrow.

We developed a novel method for phenotypic analysis and quantitative comparison of isolated erythroblastic islands and their central macrophages using multispectral imaging flow cytometry (IFC). We first used this method to evaluate markers of murine EBI macrophages which had been previously suggested by *in situ* immunofluorescence or flow cytometry studies. EBI macrophages from mouse bone marrow consistently expressed F4/80 and VCAM1 as expected, but displayed heterogeneous expression of CD169. Importantly, we observed that CD11b was not expressed on the central macrophages, but was abundantly expressed by non-erythroid cells associated with the EBIs. Using flow cytometry and RNA-seq, we identified these CD11b+ cells to be immature granulocytic precursors which appear to be specifically enriched in the EBIs relative to the total bone marrow.

Given the prevalence of myeloid precursors in the EBIs, we hypothesized that myelopoiesis and erythropoiesis may occur together within this niche. To test this, we applied IFC to examine the proportion of CD11b to CD71+ cells within the EBIs in multiple models of altered granulopoiesis. In models of anemia of inflammation, erythropoiesis is dramatically suppressed in favor of increased numbers of CD11b+ myeloid cells associated with the F4/80+ central macrophages. Alternatively, in the *gfi1-/-* mouse, a model of congenital neutropenia, myelopoiesis is blocked from a very early stage and the ratio shifts to more CD71+ cells within the EBIs. These results support a model in which granulopoiesis and erythropoiesis are regulated and balanced through signaling within the erythroblastic islands.

In summary, these data demonstrate the utility of IFC for the analysis and characterization of EBI structure and function. Our finding that granulocytes and granulocyte precursors are specifically associated with EBIs suggests they are a site for granulopoiesis as well as erythropoiesis, and that production of these lineages is dynamically regulated within this erythro-myeloblastic niche.

2018 Carleton and Sigrid Stewart Lecture

Flow Cytometry and Small Molecule Drug Development in Pharma: if Flow is so Good, How is Pharma using it?

T. Vincent Shankey (Shankey Biotechnology Consulting)

Pharma is facing increasing challenges: the cost to develop and market new small molecule drugs is steadily growing, increasing numbers of currently marketed pharmaceuticals are coming off-patent, and there is a rising societal pressure to lower the price of prescription drugs. A recent paper (DOI: 10.1056/NEJMp1500848) indicates that the current cost to develop a single new drug is approaching $2.6 billion, up from $800 million estimated in 2003. In this same time period, most large Pharma companies have significantly reduced R&D funding and decreased their research staff. Anecdotal evidence suggests that the majority of cell-based assays performed during drug development employ image analysis and that many of these image-based assays use whole field/population signal averaging rather than single-cell analyses. Published data also show that the majority of image-based tests used by Pharma operate measuring 2-3 independent features. The status of flow cytometry in Pharma is less clear, as the industrial R&D groups may not publish their most valuable data. That said, published studies show the use of up to 4-6 dimensions in flow cytometry based drug-development assays. With limited exception, computational analysis of the results is generally limited to uncomplicated univariate or bivariate techniques.

During the past five years, I worked with a start-up CRO (AsedaSciences) to develop multi-parametric flow-based assays for early assessment of compound risk. The screen employs state-of-the-art hardware such as auto-samplers, robotic liquid handling systems, and multiparametric flow cytometry. Simultaneously measuring multiple cellular responses across a range of compound concentrations, the measured biological features are organized in tensors of numerical values, jointly describing dissimilarities between controls and measured samples in biological feature space. The resultant tensors characterize the tested compounds and can be compressed, compared, and used as inputs for predictive machine learning algorithms. This approach departs from the tradition of representing compound “toxicity” as EC50 (logarithm of half maximal effective concentration) for each individual phenotypic marker. Instead, the method integrates all measurements to produce single values representing the likelihood of specific cellular stress. Abandoning the univariate cell-stress/compound toxicity metric via EC50 and adopting a genuinely multivariate representation of cell responses facilitates assignment of probabilistic scores to compound-cell interaction fingerprints. This provides a framework for quantitative evaluation and validation of assay performance via sensitivity and

specificity measures typically used for diagnostic tests. Using this approach, the assay recognizes ~50% of compounds present in the test database which "failed" (included compounds that failed for multiple reasons in more advanced stages of development , e.g. organ-specific toxicities, DILI, toxic metabolites, efficacy, etc.). The assay also offers remarkable precision (positive predictive value) of 98%. The described work demonstrates an example of a broader philosophy of cellular-stress testing, emphasizing simplicity and reproducibility paired with sophisticated computational analysis and machine learning. I will argue that the future of drug development will depend on the broader use of massively parallel and machine-learning-aided screening systems, coupled to well validated and reproducible assays. This philosophy emphasizing single cell analysis implicitly relies on cell population heterogeneity to characterize compounds in early phases of drug development and differs dramatically from costly and complex tissue and animal models.

IMMUDEX TRANSLATIONAL AND CLINICAL CYTOMETRY KEYNOTE

IMMUNE MONITORING FROM A CORE'S PERSPECTIVE – THE PAST, THE PRESENT, AND THE FUTURE

JANET STAATS (DUKE UNIVERSITY)

Flow cytometry has contributed significantly to understanding immunological changes associated with diseases/conditions (predictive biomarkers), as well as responses to novel treatments (pharmacodynamic or PD biomarkers). In the late 1980's, we began providing "cutting-edge", 2-color, flow-based immune monitoring in support of HIV clinical trials. Later, as the paradigm shifted to HIV vaccine trials, the assay endpoints approached the limits of quantitation, necessitating higher quality standards for assays, reagents, instruments, and operators. These same high quality, HIV-centric, assays stood the test of time, traveled well, and provided a stepping-stone for expanding to other areas of medicine. As a result, the Duke Immune Profiling Core is currently engaged in comprehensive, high dimensional, immune profiling activities that encompass infectious diseases, transplantation, cardiovascular disease, autoimmunity, maternal-fetal medicine, pulmonary diseases, surgical outcomes, rare diseases, chemical warfare agents, pain management, and neoplastic diseases. I will review key milestones of flow cytometry, and describe how these have contributed to immune correlative studies conducted within our Core.

IMAGING NON-CONICAL NEW ROLES FOR NATURAL KILLERS IN HEALTH AND DISEASE

STEPHEN N. WAGGONER (CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER AND UNIVERSITY OF CINCINNATI)

Natural killer (NK) cells are conventionally valued for their ability to make interferon-gamma and to kill virus-infected or cancerous cells. Our work reveals new functional roles for NK cells in regulating the duration, robustness, and character of immune responses. On one hand, we show that NK cells respond to inflammation and immunopathology during chronic virus infection by up-regulating expression of BAFF (B-cell activating factor belonging to the tumor necrosis factor family) and concomitantly providing support to maintain marginal zone macrophages and B cells. Chronic infection in mice devoid of NK cells results in complete and sustained loss of the marginal zone, which is associated with increased susceptibility to secondary bacterial infection. These data reveal a crucial and previously unappreciated role for NK cells in

sustaining immune function during chronic inflammation. In contrast, we find that NK cells can also suppress adaptive immune function via perforin-dependent cytolytic targeting of activated CD4 T cells. This activity constrains development of follicular helper T cell and germinal center B cell responses following immunization. The reduced magnitude of germinal center responses is linked to decreased vaccine-elicited antibody titers and limited affinity maturation of antibodies. NK cell suppression of the germinal center is associated with transient localization of NK cells in the splenic white pulp. This discovery reveals that targeting of NK cell immunoregulatory function may enhance efforts to promote vaccine-elicited production of high affinity antibodies against pathogens like HIV, for which we do not have effective vaccine regimens.

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1. IL-4 Mediated Stromal Cell Expansion Is Critical For Development of a Type 2, But Not a Type 1 Immune Response

Diana Cortes-Selva[1], Andrew Ready[1], Bartek Rajwa[2], Keke C. Fairfax [1]

[1] Department of Comparative Pathobiology, [2]Department of Basic Medical Sciences and Bindley Biosciences, Purdue University, West Lafayette IN

IL-4 is critical for differentiation of Th2 cells and antibody isotype switching, but our work demonstrated that it is produced in the peripheral lymph node (LN) under both Th2 and Th1 conditions, raising the possibility of other functions. We found that IL-4 is vital for proper positioning of hematopoietic and stromal cells in steady state, and the lack of IL-4 or IL-4Rα correlates with disarrangement of both follicular dendritic cells, and $CD31^{+}$ endothelial cells. We observed a marked disorganization of B cells in these mice, suggesting that the lymphocyte-stromal cell axis is maintained by the IL-4 signaling pathway. This study showed that absence of IL-4 correlates with significant down-regulation of Lymphotoxin alpha (LTα) and Lymphotoxin beta (LTβ), critical lymphokines for the development and maintenance of lymphoid organs. Moreover, immunization of IL-4 deficient mice with Th2 antigens failed to induce lymphotoxin production, lymph node re-organization, or germinal center formation, while this process is IL-4 independent following Th1 immunization. Additionally, we found that Th1 antigen mediated lymph node re-organization is dependent on IFN-γ in the absence of IL-4. Our findings reveal a role of IL-4 in the maintenance of peripheral lymphoid organ microenvironments during homeostasis and antigenic challenge.

2. Testing Conventional Wisdom in Post-sort Viability: Head-to-Head Comparisons

Kathryn C. Fox, University of Wisconsin Cancer Center, Madison, WI

In an effort to sort as many cells as possible in short appointments, researchers and instrument operators are drawn to small nozzles at high pressures. Customers sometimes question the necessity of viability dyes or suggest the reagents themselves are detrimental to viability. We deposited single cells in 96-well plates and used colony formation as a measure of long-term post-sort viability. We found an increase in colony formation when a viability dye was used to gate out dead cells, even with restrictive FSC/SSC gating. We also found more colonies when sorting with large nozzles at low pressures compared to smaller nozzles at higher pressures. These preliminary experiments provide data to support the notion that there truly is a trade-off between speed and viability, and set the stage for additional questions about optimization of sort conditions.

3. Streamlined Human Immune Monitoring with Mass Cytometry: 29 Markers in a Single tube and Automated Data Analysis

Loh, C.[A], Selvanantham, T.[A], Fung, L.[A], **Poulin, M.M.**[A], Bagwell, C.B.[B], Inokuma, M.[B], Lott, S.T.[A]
[A] Fluidigm Corporation, South San Francisco, CA; [B] Verity Software House, Topsham, ME

Introduction. Immune monitoring is an essential method for quantifying changes in immune cell populations in health and disease. The extreme heterogeneity of immune cells demands a high-parameter approach to more fully and efficiently quantify these changes. Mass cytometry is an ideal solution, enabling simultaneous detection of over 40 phenotypic and functional markers in a single tube of sample.

Methods. We developed a single-tube, 29-marker panel for mass cytometry based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel [Maecker et al. *Nature Reviews Immunology* (2012)], expanded to allow identification of additional leukocyte subsets, particularly T cells. Automated data analysis with Verity Software House GemStone™ has been developed specifically for data collected with the panel providing users with results in minutes, reducing time-to-answer and variability inherent in manual gating.

Results. Repeatability was tested with a single PBMC sample stained by a single technician in two technical replicates and acquired in triplicate on two Helios™ mass cytometers. SDs for percent of parent were 1% or less for 16 identified populations. Reproducibility was tested by determining the variability in measurements of five PBMC lots stained by five technicians and collected on two Helios instruments. CVs on mean percent of all measured populations were under 20%. R^2 values for agreement of percent parent populations using the full 29-marker panel compared to a 10-marker panel for T cell populations were 0.94 or higher.

Conclusions. We conclude that this panel kit can provide consistent immune population identification and enumeration for any given lot of PBMC.

4. Tools for ImageStream Training: Using a Single Cell Line to Demonstrate 5 Unique Morphological Characteristics

Shifu Tian*, Richard D. Schretzenmair*, Derek D. Jones*,Jonni S. Moore*
*Department of Pathology and Laboratory Medicine, Abramson Cancer Center Flow cytometry and Cell Sorting Shared Resource Laboratory, Perelman School of Medicine, University of Pennsylvania,Philadelphia, PA

Goal: As a shared resource laboratory, our goal is not to just provide staff performed ac- quisition and analysis, but to train users to be able to perform these functions indepen- dently thereby enabling technology access. ImageStream training is especially challeng- ing, not just because users are not familiar with the operation of the instrument and soft- ware packages, but also the entire concept of image processing is alien to most flow cy- tometrists. To this end, we sought to develop a quick, easy and inexpensive method to teach as many morphological determinations as possible.
Method: THP-1, is a human monocytic cell line, easy to maintain and inexpensive to grow. It can be differentiated into M1 like macrophages by incubating with 150nM Phorbol 12-myristate 13-acetate(PMA) for 2-3 days then resting for 3-5 days. The rested cells are fully differentiated Macrophages (rPMA). THP1 and

rPMA are both treated at several time points with 1µm fluorescent orange latex beads. Cells were stained with Alexa Fluor-647 conjugated WGA (Wheat Germ Agglutinin) to identify the surface membrane. Cells were then fixed, permeabilized, and stained with LysoTracker Red (staining for Lysozome), NFκβ and DRAQ5 (staining for nuclei). Data is acquired on the Image-StreamX, 3 laser, 2 camera, 12 channel system.
Results: Five morphological characteristics can be demonstrated using the ImageStream that cannot be determined using standard flow Cytometry. The change in shape between THP-1 and rPMA can be demonstrated with the Circularity feature. Internalized beads can be distinguished from adherent beads using the Internalization Wizard. The Spot Count Wizard is then used to count the number of internalized beads. Colocalization of the beads to lysosomes and phagosomes stained with LysoTracker Red can be demonstrated. Finally translocation of NFκβ to the nucleus is then demonstrated. Other features,
such as cell cycle or death (Apoptosis, Autophagy), can be added as needed. Thus, with minimal resources, users can be trained to do multiple simultaneous measurements on the ImageStream that demonstrate the unique capabilities of this instrument.

5. S6K1 Level as an Indicator of Treatment Outcome in Breast Cancer

Catherine Behrman, University of Cincinnati Medical Center, Cincinnati, Ohio

S6K1, amplified in 12% of TCGA breast cancers, is a signaling mediator downstream of mTOR Complex 1. Because it can function both upstream and downstream of the estrogen receptor α (ERα), S6K1 amplification can contribute to oncogenic estrogen signaling in breast cancer. Data show that inhibition of S6K1 reduces substrate phosphorylation in cells. This inactivation also reduces the progression of cells into S phase. For breast cancer patients who have failed on Estrogen ablation therapy, an approved second-line treatment is the mTORC1 inhibitor, Everolimus, in combination with an ERα antagonist. A survey of ERα+ cell lines revealed a wide range of S6K1 protein expression levels. We propose that patients with lower levels of S6K1, and therefore lower substrate phosphorylation, who complete a course of combination treatment will have better outcomes.

6. Single-cell RNA-seq Reveals Functional Heterogeneity Between Short- and Long-lived Plasma Cells

Derek D. Jones*, Joel R. Wilmore*, Brian T. Gaudette*, Jonni S. Moore, and David Allman
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*Equal contribution

Plasma cells are terminally differentiated effectors of the B cell lineage, and are responsible for mediating long-lasting protective antibody responses to pathogens. Within the phenotypically-defined plasma cell pool, there exist at least two main subsets: short-lived cells which provide rapid and transient antibody responses, and long-lived plasma cells that produce antibodies for decades or more. However, whether additional heterogeneity exists between and within these subsets, and which cellular processes determine short- versus long-lived fate decisions, remains unclear. Using existing bulk RNA sequencing data, we curated a custom

panel of 350 genes of interest with a focus on apoptosis, ER stress pathways, and autophagy. Autophagy is a process by which degradation and recycling of cellular components promotes survival and energy maintenance, especially under conditions of stress; thus, we sought to better understand the role of autophagy proteins in short- and long-lived plasma cells.

Using the BD Rhapsody platform, we performed targeted single-cell RNA-seq on 20,000 sort-purified spleen and bone marrow plasma cells. Computational analytic approaches, including tSNE, resolved discrete clusters of short- and long-lived plasma cells based on the expression of the CD45 isoform B220, and these clusters further segregated based on antibody isotype. Additional analyses identified the expression of several genes involved in cell cycle and autophagy within the $B220^+$ subset, consistent with studies designating them as newly generated cells. Interestingly, although the role of the autophagy program has been implicated in the context of plasma cell function, the higher resolution provided by our single-cell data revealed numerous autophagy genes from the Atg4 family not previously shown to be expressed in plasma cells. Atg4 family members are preferentially induced by reactive oxygen species (ROS); therefore, we tested whether high-level ROS are present in plasma cells. Indeed, we detected considerably higher ROS in plasma cells than in resting follicular B cells. Since malignant plasma cells in multiple myeloma are known to be highly proliferative and secrete immense amounts of immunoglobulin, we reasoned that autophagy might play an even greater role in this context than in normal plasma cells. To address this, we employed a mouse model of myeloma in which the transfer of malignant cells into congenic recipients allowed for the comparison of normal and malignant plasma cells in the same mouse. Preliminary studies using this model revealed an even greater magnitude of autophagy in malignant versus normal plasma cells. In total, our data support a model in which highly metabolically active plasma cells produce large amounts of protein and organelles during antibody secretion, resulting in the production of ROS and induction of autophagy, and ultimately promoting survival. These studies also highlight the importance of autophagy in malignant plasma cells, and suggest that therapeutic targeting of autophagy proteins may be a viable treatment for multiple myeloma.

7. Validation of Antibody Panels for High-plex Immunohistochemistry Applications

Douglas Hinerfeld, Kristi Barker, Heather Metz, Chris Merritt, Lucas Dennis, Philippa Webster, Joseph Beechem

Introduction: Characterization of the spatial distribution and abundance of key proteins within tissues enables a deep understanding of biological systems. However, it has proven difficult to perform such studies in a highly-multiplexed manner on FFPE tissue sections. There has been significant progress in developing technologies with expanded capabilities to analyze higher numbers of proteins, however, the validation of these technologies and their associated affinity reagents remains a significant barrier to adoption. We have developed a validation pipeline that ensures optimal sensitivity and specificity for high-plex antibody panels for the analysis of FFPE sections using the NanoString Digital Spatial Profiling (DSP) platform. The DSP is designed to simultaneously analyze up to 96 proteins by detecting oligos conjugated to antibodies that can be released via a UV-cleavable linker.

Methods: Antibodies targeting immuno-oncology proteins were tested for specificity and sensitivity by immunohistochemistry on FFPE human tissues, as well as human cell line pellets to evaluate binding specificity of both unconjugated and oligo-conjugated antibodies. The sensitivity and dynamic range were tested using FFPE cell pellets with target-specific positive and negative cells at different ratios. An interaction screen was performed to evaluate potential deleterious effects of multiplexing antibodies, and a human tissue

microarray (TMA) containing normal and cancer tissues was employed to assess assay robustness. The reproducibility of the panel on DSP was tested on serial FFPE tumor specimens by correlating the expression of all markers across 24 spatially-registered regions of interest (ROI) as well as the ability to reveal biological heterogeneity within lymphoid tissue by characterizing the expression of 40+ proteins in a spatial grid of 100um x 100um ROIs.

Results: Immunohistochemical analysis of unconjugated and oligo-conjugated antibodies displayed indistinguishable staining patterns on control tissues and cell lines. Mixed cell pellet assays revealed strong correlations between observed counts and positive cell numbers. Antibody interaction studies showed similar count values for antibodies alone or in combination, and TMA hierarchical clustering analysis demonstrated expected patterns of expression across tissue types. Analysis of all markers across 24 registered regions of interest across serial FFPE sections were highly correlated. Spatial analysis of lymphoid tissue revealed high levels of biological heterogeneity across multiple germinal centers.

Conclusion: These results demonstrate the validation and application of high-plex protein panels to accurately interrogate the immune biology within FFPE tissue using the NanoString DSP platform.

8. Failure to Upregulate Calmodulin Underlies the Suppressed KCa3.1 Function and Enhanced Sensitivity to Adenosine in CD8+ T Cells of Head and Neck Cancer Patients

Ameet A. Chimote[1], Vaibhavkumar S. Gawali[1], Trisha Wise-Draper[2] and Laura Conforti[1]

Department of Internal Medicine, [1]Division of Nephrology, [2]Division of Hematology-Oncology, University of Cincinnati, Cincinnati OH

The limited ability of the cytotoxic CD8+ T cells to infiltrate solid tumors presents a major roadblock to developing effective immunotherapy. Adenosine (Ado) accumulates in high concentrations in solid tumors where it contributes to the suppression of T cell function. We have previously shown that Ado reduces the chemotactic ability of peripheral blood CD8+ T cells (PBTs) from head and neck squamous cell carcinoma (HNSCC) patients by suppressing KCa3.1 channel function. Herein, we conducted experiments to elucidate the mechanism of KCa3.1 dysregulation in HNSCC PBTs. KCa3.1 channels are calcium-activated and require binding of calmodulin (CAM). PBTs were isolated from HNSCC patients and healthy donors (HD), and activated with CD3/CD28 antibodies. Using flow cytometry, we showed that CAM levels decreased post-activation in HNSCC PBTs (by ~24%, n=7) while they increased in HD PBTs (by ~37%, n=6, p=0.001). To study whether CAM downregulation contributes to KCa3.1 dysfunction, we transfected HD PBTs with siRNA against CAM (siCam). siCam transfection decreased CAM expression and KCa3.1 currents, but not KCa3.1 expression. To restore defective KCa3.1 channel function, we replaced intracellular Ca^{2+} in HNC PBTs, which did not improve the KCa3.1 function. However, addition of 50μM intracellular CAM significantly improved the KCa3.1 function. (p=0.008, n=4). We also observed that CAM downregulation affects the T cell chemotactic response to Ado in HD PBTs. Control HD PBTs migrated towards CXCL12 in the presence of Ado, but downregulation of CAM abrogated their chemotactic ability in the presence of Ado (~71% inhibition, n=8, p<0.012), which was reversed by KCa3.1 activator 1-EBIO. Our data suggest that downregulation of CAM decreases KCa3.1 activity and suppresses chemotaxis in HNSCC PBTs which may contribute to their limited ability to effectively penetrate Ado-rich tumors.

(Funding: RO1CA095286 to LC)

9. Towards Understanding & Uncovering New Key Players in T Cell Development upon Aging

Hanane Boukarabila[1] *and Hartmut Geiger* [1,2]
[1] Cincinnati Children's Hospital Medical Center, Division of Experimental Hematology and Cancer Biology, Cincinnati, USA. [2] Institute of Molecular Medicine, University of Ulm, Ulm, Germany.

Background: The decline of the immune system, which is referred to as aging-associated immune remodeling (AAIR), that occurs upon aging is due to many factors acting in concert. AAIR leads to an impaired ability to respond to vaccinations, fight infections and therefore impacts negatively on the quality of life of elderly individuals. In contrast to other blood lineages, which develop exclusively in the bone marrow (BM), the T-cell lineage also develops in the thymus by continuous replenishment of thymic seeding progenitors (TSPs) from the BM. Several studies have linked AAIR to thymic involution. However, there is novel and mounting evidence that also aging of hematopoietic stem cells (HSCs) and lymphoid-primed multipotent progenitors (LMPPs) are immune system intrinsic players in AAIR. Very little, though, is known on the mechanisms by which aging of HSCs and LMPPs contribute to AAIR.

Aim: This work aims at exploring the few possibilities that could individually or in combination explain such a defect in an intrinsic fashion: aged HSCs could give rise to a defective LMPP population associated with either: 1- size reduction, and/or, 2- lineage potential bias, and/or, 3- homing defect, and/or 4- thymic developmental defect that would fail to engage into normal T-cell differentiation.

Results: Our findings demonstrate that both aged HSCs and aged LMPPs retain the T-lineage potential *in ex-vivo* at the single cell level and *in vivo* upon transplantation assays. However, the detailed analysis of transplanted young recipients by aged HSCs shows significant decrease in the pool size of LMPPs in the BM and early thymic progenitors (ETPs) in the thymus, confirming that the old BM and thymic microenvironment could not be the sole reason behind this defect. Importantly, we were able to show that aged LMPPs are associated with a dramatic disadvantage in T-cell production *in vivo* when transplanted along young LMPPs in competitive settings, pointing towards a potential homing defect and/or a developmental defect. Surprisingly, our preliminary homing assays using the CFSE labeling system did not detect a significant delay when unfractionated BMs from young and aged mice were compared as to homing to the thymus. Lastly, in order to rule out any homing disadvantage and shed light on a potential thymic developmental defect that could occur with age, we are currently investigating T-cell developmental stages within the thymus upon intra-thymic transplantations of aged LMPPs along young LMPPs in competitive settings into young recipients.

Conclusion: AAIR is a consequence of multiple immune parameters at play and we present here exciting new data demonstrating role of HSCs and LMPPs. Understanding how aged HSCs and LMPPs could possibly drive this AAIR phenomenon at the cellular and the molecular level is of crucial importance for developing new mouse models that are needed to improve drug therapies and achieve healthy aging.

10. TEACHING PRINCIPLES OF FLOW CYTOMETRY WITH FUNCTIONAL INSTRUMENT MODELS

EDWARD PODNIESINSKI, ALEXIS CONWAY PHD, JOSEPH D. TARIO, JR. PHD, ORLA MAGUIRE PHD, HANS MINDERMAN PHD, PAUL WALLACE PHD, ROSWELL PARK COMPREHENSIVE CANCER CENTER, BUFFALO, NEW YORK 14263

For the past 3 years our department has offered a university level course “Principles in Flow and Image Cytometry”, (RPG564). This comprehensive curriculum is intended to serve as a spring board for young scientists to learn Flow Cytometry and apply it to their experimental planning. The course covers topics ranging from mechanical concepts in flow and image cytometry, basic/ advanced labeling techniques, amongst other relevant applications. The course is comprised of weekly didactic lectures and laboratories. One of the labs is the demonstrating principles of Cytometry through hardware models which were constructed to review principles, theoretical background and applications of current flow cytometry techniques.

There are 4 cytometric functional hardware models each teaching flow cytometery basics. The objectives of the cytometer models are to demonstrate basic particle interrogation, converting detected photon information into the dynamics of a representative electronic pulse signal. Multiple work stations progress from simple flow cell conceptual physics to flow cell fluid hydrodynamic focusing, fluorescence/ scatter detection, multi-laser spacial separated interrogations, Spectral Flow cytometry basics and lastly Micro Vesicle detection using multi-laser interrogations.

These 4 models will be presented within this poster.

2018 ISS ABSTRACTS

The Industrial Science Symposium will take place Friday evening, September 28th. This session is intended to provide our exhibitors, a forum to present new technology, instrumentation, etc. Each presentation lasts approximately 15 minutes.

NANOSTRING TECHNOLOGIES INC.

VALIDATION OF ANTIBODY PANELS FOR HIGH-PLEX IMMUNOHISTOCHEMISTRY APPLICATIONS

Indira Medina (imedina@nanostring.com)

Douglas Hinerfeld, Kristi Barker, Heather Metz, Chris Merritt, Lucas Dennis, Philippa Webster, Indira Medina, Joseph Beechem

Introduction: Characterization of the spatial distribution and abundance of key proteins and cells within tissues enables a deep understanding of biological systems. However, it has proven difficult to perform such studies in a highly-multiplexed manner on FFPE tissue sections. There has been significant progress in developing technologies with expanded capabilities to analyze higher numbers of proteins, however, the validation of these technologies and their associated affinity reagents remains a significant barrier to adoption. We have developed a validation pipeline that ensures optimal sensitivity and specificity for high-plex antibody panels for the analysis of FFPE sections using the NanoString Digital Spatial Profiling (DSP) platform. The DSP is designed to allow visualization and detection of cells in FFPE sections, and simultaneously analyze up to 96 proteins using antibodies conjugated to oligonucleotides that can be released via a UV-cleavable linker for quantification. **Methods**: Antibodies targeting immuno-oncology proteins were tested for specificity and sensitivity by immunohistochemistry on FFPE human tissues, as well as human cell line pellets to evaluate binding specificity of both unconjugated and oligo-conjugated antibodies. The sensitivity and dynamic range were tested using FFPE cell pellets with target-specific positive and negative cells at different ratios. An interaction screen was performed to evaluate potential deleterious effects of multiplexing antibodies, and a human tissue microarray (TMA) containing normal and cancer tissues was employed to assess assay robustness. The reproducibility of the panel on DSP was tested on serial FFPE tumor specimens by correlating the expression of all markers across 24 spatially-registered regions of interest (ROI) as well as the ability to reveal biological heterogeneity within lymphoid tissue by characterizing the expression of 40+ proteins in a spatial grid of 100um x 100um ROIs. **Results**: Immunohistochemical analysis of unconjugated and oligo-conjugated antibodies displayed indistinguishable staining patterns on control tissues and cell lines. Mixed cell pellet assays revealed strong correlations between observed counts and positive cell numbers. Antibody interaction studies showed similar count values for antibodies alone or in combination, and TMA hierarchical clustering analysis demonstrated expected patterns of expression across tissue types. Analysis of all markers across 24 registered regions of interest across serial FFPE sections were highly correlated. Spatial analysis of lymphoid tissue revealed high levels of biological heterogeneity across multiple germinal centers. **Conclusion**: These results demonstrate the validation and application of high-plex protein panels to accurately interrogate the immune biology within FFPE tissue using the NanoString DSP platform.

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STREAMLINED HUMAN IMMUNE MONITORING WITH MASS CYTOMETRY: 29 MARKERS IN A SINGLE TUBE AND AUTOMATED DATA ANALYSIS

Michelle M. Poulin (MICHELLE.POULIN@FLUIDIGM.COM)

Immune monitoring is an essential method for quantifying changes in immune cell populations in health and disease. The extreme heterogeneity of immune cells demands a high-parameter approach to more fully and efficiently quantify these changes. Mass cytometry is an ideal solution, enabling simultaneous detection of over 40 phenotypic and functional markers in a single tube of sample.

We developed a single-tube, 29-marker panel for mass cytometry based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel (Maecker et al. *Nature Reviews Immunology*, 2012), expanded to allow identification of additional leukocyte subsets, particularly T cells. Automated data analysis with Verity Software House GemStone™ has been developed specifically for data collected with the panel providing users with results in minutes, reducing time-to-answer and variability inherent in manual gating.

We will describe the kit and available data analysis options, as well as presenting data from extensive internal testing of the kit for repeatability, reproducibility and panel performance comparing population frequency data using the full 29-marker panel to that obtained with several 10-12 marker panels.

UNION BIOMETRICA, INC.

COPAS VISION FLOW CYTOMETER CAPTURES IMAGES AND SORTS

Yongwoon Kim (YKIM@UNIONBIO.COM)

We have developed instrumentation for large particle flow cytometry that can capture brightfield images in flow. Adding imaging capability to flow cytometry greatly enhances the phenotyping of samples by providing morphological and spatial information of the sample constituents not collected by conventional flow cytometers. Traditional measurements of size, optical density, and fluorescence, as well as Profiler data, are also collected, and these measurements are used for making sorting/dispensing decisions. The collected images and flow cytometry measurements are synchronized so that objects dispensed to wells of multiwell plates can be traced back to their corresponding image. Our COPASTMtechnology platform is designed for large particles making it ideally suitable for large single cells, cell clusters, and small model organisms. The COPASTMVISIONinstrument is based on this platform and is ideally suited for samples made up of particles of varying sizes and shapes. Our data from the COPAS VISIONshows proof-of-principle support for increased level of phenotyping of these types of samples.

DENOVO SOFTWARE

USING FCS EXPRESS TO ACCELERATE YOUR FLOW CYTOMETRY REPORTING AND RESULTS

Sarah Schuett (SARAH.SCHUETT@DENOVOSOFTWARE.COM)

Most flow cytometry data analysis software focuses on generating plots, gates and stats. But those are rarely your final results. Most researchers spend a significant amount of time transferring data from their flow analysis software into other packages (like Excel, Prism, Jump, Powerpoint and many others) for downstream processing. This dramatically increases the time it takes for you to get your results. FCS Express 6 has a wide variety of tools to bring your final results to you directly in your flow analysis software. Integrated bar and

pie charts update instantly as you change your gates. A fully functional spreadsheet lets you apply sophisticated calculations and regressions that change automatically with your analysis. Direct R integration as well as SPADE and vSNE plots provide state of the art high dimensional analysis and visualization. New index sorting compatibility allows for quick single cell and well review at a click. Come see how FCS Express can help you produce your results in record time.

MilliporeSigma

New CellStream™ Flow Cytometer

Todd Salomon (TODD.SALOMON@EMDMILLIPORE.COM)

Discover unparalleled fluorescence sensitivity and flexibility in a compact and affordable flow cytometry system. With patented Amnis® optics technology inside, the new CellStream™ flow cytometer uses a camera for detection to rapidly capture low-resolution cell images and transform these into high-throughput intensity data. Whether you are looking for small particles (200nm) or feint signals, the CellStream™ flow cytometers can be fully configured with 1 to 7 lasers and are easy to upgrade directly in the laboratory and with your applications in mind.

Cytek Biosciences

Expanding Application Capabilities Using Full Spectrum Cytometry

Dave Kennedy (DKENNEDY@CYTEKBIO.COM)

The Aurora continues its development path with expanded capabilities. Data will presented with the addition of 561 laser. Panel data with 4 lasers, 20+ colors. Examples of autofluorescence extraction, now enhanced with 2.0 software.

Miltenyi Biotec

Novel Aspects of Sorting in a Closed System:The MACSQuant® Tyto®

Patrick Sean Murphy (PATRICKM@MILTENYIBIOTEC.COM)

The MACSQuant® Tyto® is a revolutionary microfluidics based cell sorter allowing for gentle, high purity cell isolation in a closed, sterile environment. Designed around the world's fastest mechanical valve, the Tyto® enables sorting of fragile and hard-to-isolate cell types without high-pressure or charge, resulting in unparalleled post-sort viability and functionality. The unique sort mechanism and disposable cartridge completely eliminates aerosols and the risk of sample cross-contamination, making Tyto® an ideal choice in highly regulated environments. Applications regularly demonstrate >90% purity while maintaining >95% viability, even with delicate and rare populations such as iPS cells and mesenchymal stromal cells. The MACSQuant® Tyto® represents the state-of-the-art in closed system sorting, providing full sterility, safety and simplicity for cell isolation.

Sony Biotechnology Inc.

Multi-application Sorting with the Sony MA900

Steve Whitaker (STEPHEN.WHITAKER@SONY.COM)

The MA900 is a precision benchtop cell sorter that is easy to use, supports 12-fluorescence parameters, 4-way sorting and single cell deposition into multiple plate types. Use of modern technologies and integrated automation dramatically simplify operation to make sorting less subjective and improve reliability.

System startup, aseptic cleaning, QC, and sort setup operate with a touch of a button to ensure optimal daily alignment of the sorting chip to lasers, precise targeting, and rapid recovery from clogging. Guided workflows for cleaning cycles simplify maintenance and can be customized for aseptic sorting. Versatile system design provide capability to sort a wide range of applications ranging from single cell sorting to multicolor immune cell panels.

BD Biosciences

Sorting Simplified: Introducing the BD FACSMelody

David Morris (DAVID.MORRIS@BD.COM)

In this presentation, we will introduce the audience to the BD FACSMelody system. We will show how the simplified operation of the system brings truly easy cell sorting to shared research core facilities and individual laboratories. The FACSMelody combines proven and exclusive BD technology found in our high-end sorters with new automation, simplified software interfaces, and guided workflows. This means researchers are empowered to set up and optimize their own sorts in a fraction of the time it takes to set up other sorters. The standard 100 mm nozzle in the FACSMelody gives researchers flexibility to efficiently sort bacteria, yeast, lymphocytes, or mammalian cell lines. Equipped with optical filters optimized for the brightest commercially available dyes, the FACSMelody facilitates the separation of rare populations using up to nine fluorochromes.

ALBERT DONNENBERG, PHD

UNIVERSITY OF PITTSBURGH MEDICAL CENTER

Albert Donnenberg studied Philosophy as an undergraduate at the University of Colorado, Boulder. He received his Ph.D. in Infectious Disease Epidemiology at the Johns Hopkins University in 1980, studying cellular immunity to Herpes Simplex Virus. Upon graduation he was elected to Delta Omega, the honorary Public Health Society. After a postdoctoral fellowship under the direction of Dr. George Santos at the Johns Hopkins Oncology Center, Dr. Donnenberg was appointed Instructor of Oncology in 1982, Assistant Professor in 1983, and Associate Professor in 1989. He worked on adoptive transfer of donor immunity during allogeneic bone marrow transplantation, and on the development and clinical implementation of T-cell depletion of bone marrow to prevent graft versus host disease. He also performed early studies on cellular immunity in HIV infection, and co-developed the concept of T-cell homeostasis. In 1991, Dr. Donnenberg was recruited to the University of Pittsburgh to serve as the Director of Laboratory Research in the Bone Marrow Transplant Program. He has also served as program Co-director, and as Interim Director. He has directed the UPMC Adult Hematopoietic Stem Cell Laboratory and since 1998 and the UPMC Pediatric Hematopoietic Stem Cell Laboratory and since 2000. He was promoted to Professor of Medicine in 2001. From 1998 to 2018 he also directed the University of Pittsburgh Cancer Center's Cytometry Facility. His current research interests are in cellular therapy and graft engineering, the role of stem cells in neoplasia, and immunotherapy for metastatic cancer, projects he pursues with his scientific and life partner Dr. Vera Donnenberg. He is an internationally recognized expert in therapeutic cell processing and flow cytometry. Dr. Donnenberg has co-edited two editions of the CRC Handbook of Human Immunology and has authored more than 200 scholarly publications. He is the proud father of 4 daughters and one son and the grandfather of a boy and a girl. He and Vera live on Pittsburgh's Southside where their hobbies are winemaking and collecting art.

KEKE FAIRFAX , PHD

PURDUE UNIVERSITY

Dr. Fairfax received her PhD from Yale University School of Medicine which was followed by a postdoctoral fellowship at the Trudeau Institute. She continued her research at Washington University in St. Louis and is currently an Assistant Professor at the Purdue University College of Veterinary Medicine in the Department of Comparative Pathobiology. Her current research interests focus on infection and immunology. Infection by pathogens that range from parasites to bacteria elicits vigorous antibody responses critical for host protection. It is clear that optimal humoral immune responses depend on T follicular helper (TFH) cells and follicular dendritic cells (FDCs), which are subsets of immune cells that form direct, or cognate, interactions with B cells in secondary lymphoid organs to promote germinal center formation, isotype class-switching, and affinity maturation of the B cell receptor. While these two cell types have recently been well characterized, there remains a dearth of knowledge about the factors produced by TFH and FDCs that imprint organ specific trafficking on plasma cells, as well as memory and effector B cells. The overall focus of my research program uses the models I developed during my postdoctoral studies in B cell and TFH cell biology in response to the parasitic helminth Schistosoma mansoni, which chronically infects ~200 million people worldwide, to define the mechanisms of organ/lymphoid specific B cell trafficking in response to multiple infectious and protein based stimuli.

Heather Hundley, PhD

Indiana University

Dr. Hundley has a broad background in molecular biology and biochemistry and is an expert in RNA biology. Her training in biochemistry and molecular biology began with her graduate work with Dr. Elizabeth Craig at the University of Wisconsin, where she used both budding yeast and human cell lines to study the function of ribosome-associated molecular chaperones, publishing a first author paper in *Science* and two co-first author papers in *PNAS* and *Molecular Microbiology*. As a postdoctoral fellow in Brenda Bass's lab, she learned the intricacies of undertaking RNA editing experiments. Using *C. elegans* and human cell lines, she was the first to demonstrate that mRNAs with edited 3' UTRs were efficiently translated *in vivo*, thus challenging the paradigm that inosine-containing RNAs were nuclear retained. Dr. Hundley is currently an Assistant Professor in the Medical Sciences Program at Indiana University, Bloomington (primary appointment in Biochemistry and Molecular Biology, Indiana University School of Medicine). She has established her research group in the field of RNA editing as evidenced by publishing several research articles in top tier journals (*eLife*, *Cell Reports, NAR, JBC*), invitations to present our work at international conferences, and election as co-Chair of the 2017 RNA Editing Gordon Research Conference. She has assembled a group of talented scientists, currently consisting of three graduate students, two research technicians and a number of undergraduate students. To directly support my research program, I have obtained a number of internal grants as well as external funding that includes a Research Scholar Grant from the American Cancer Society and a collaborative grant from the US-Israel Binational Science Foundation.

Eric Long, PhD

National Institute of Allergy and Infectious Diseases, National Institutes of Health

Dr. Long has a biochemistry degree from the ETH Zürich, Switzerland, and a Ph.D. in molecular biology from the University of Geneva, Switzerland. After postdoctoral research at the Carnegie Institution for Science and the National Cancer Institute, he returned to Geneva as a faculty member in the Department of Microbiology. There, he applied molecular approaches to isolate the first cDNA clones for MHC class II molecules. In 1983, he joined the Laboratory of Immunogenetics at the National Institute of Allergy and Infectious Diseases, NIH. While studying processing pathways for antigen presentation to CD4 T cells, he discovered that MHC-II can present endogenous antigens through a pathway that is independent of the transporter for antigen presentation. He became Senior Investigator and Head of the Molecular and Cellular Immunology Section in 1988. In the mid-90's, his main interest turned to the regulation and function of natural killer (NK) cells, after his team identified molecular clones for KIR, a family of NK cell inhibitory receptors that prevent killing of healthy cells. In 2001, this work was selected as one of the Basic Discoveries in Biology by the NIH Intramural Research Program. The discovery of the signaling basis for inhibition by these receptors was named one of the "classic papers that present seminal insights into natural killer cell function" by *Nature Immunology* in 2008, and one of the "Pillars of Immunology" by the *Journal of Immunology* in 2013. More recently, his research reported on imaging of the dynamics of receptor–ligand interactions at inhibitory immunological synapses, demonstrated activation of NK cells through synergistic combinations of coactivation receptors, and a reprogramming of NK cells to promote vascular remodeling through a specialized receptor for soluble HLA-G. His current work includes studies on the role of NK cells in providing protection against malaria in individuals living in an area of high *Plasmodium falciparum* transmission.

Robert Lorsbach, MD, PhD

Cincinnati Children's Hospital Medical Center and University of Cincinnati

Dr. Robert Lorsbach is currently director of hematopathology at Cincinnati Children's Hospital Medical Center in Cincinnati, Ohio. He graduated with a combined MD/PhD from the University of Kansas and subsequently completed residency and fellowship training in anatomic pathology and hematopathology at Brigham and Women's Hospital in Boston. He has been a hematopathologist and researcher at St. Jude Children's Research Hospital, was director of hematopathology at the University of Arkansas for Medical Sciences, and since October 2014 has served as director of hematopathology at Cincinnati Children's Hospital and professor of Pathology at the University of Cincinnati College of Medicine. He has interests in all facets of hematopathology; however, pediatric hematopathology has been a major focus of his clinical and investigational activities, with particular interest in the pathology of acute leukemia, bone marrow failure and lymphoma in the pediatric setting. Significant research focuses have included the first comprehensive characterization of pediatric follicular lymphoma and the cloning of a novel t(10;11) translocation breakpoint in an AML, identifying *TET1* as an *MLL*fusion partner and the initial characterization of the TET family of proteins, which have emerged as important mediators of epigenetic modification in development and cancer.

Heather McCauley, PhD

Cincinnati Children's Hospital Medical Center

Heather McCauley obtained her PhD in the lab of Geraldine Guasch, where she identified two novel roles for TGFβ signaling in epithelial differentiation and in cancer stem cells. She then joined the lab of Jim Wells at Cincinnati Children's Hospital Medical Center, where she is using pluripotent stem-cell derived human intestinal organoids to understand how enteroendocrine cells regulate nutrient absorption. Heather is the recipient of a postdoctoral fellowship from the American Diabetes Association.

Orla Maguire, PhD, Marylou Ingram ISAC Scholar

Roswell Park Comprehensive Cancer Center

Orla Maguire, Ph.D., is a senior cytometry specialist in the department of Flow and Image Cytometry at Roswell Park Comprehensive Cancer Center in Buffalo, New York and Marylou Ingram ISAC Scholar for 2018-2022 term. She obtained her Ph.D. from Ulster University in Northern Ireland, United Kingdom, studying the activity of nuclear-receptor transcription factor pathways in cancer. Her post-doctoral research included developing applications of imaging flow cytometry (IFC), with special attention to cell signaling. Her current research interests focus on elucidating the mechanisms involved in dampening of the immune response by the tumor microenvironment and chemotherapy, especially with regard to exosomes.

Nicole Poulton, PhD, ISAC Emerging Leader

Bigleow Laboratory for Ocean Sciences, J.J. MacIsaac Facility for Aquatic Cytometry

Nicole is Director of the J.J. MacIsaac Facility for Aquatic Cytometry, and a Research Scientist at Bigelow Laboratory. Nicole's research is focused primarily on phytoplankton ecology, including harmful algal blooms and the role of phytoplankton in the global carbon cycle. Her research uses aquatic cytometry, single cell sorting/genomics and unique imaging cytometry tools to examine phytoplankton and bacterioplankton in the natural aquatic environment. She has 25 years of flow cytometric experience in the aquatic sciences. Nicole

received a BS and BA in 1993 in Biology and Chemistry from Virginia Tech, and a PhD in Biological Oceanography in 2001 from the Massachusetts Institute of Technology and Woods Hole Oceanographic Institution Joint Program. She joined Bigelow Laboratory for Ocean Sciences in 2001 as a Postdoctoral Research Scientist and became a Research Scientist in 2008 and Director of the Facility for Aquatic Cytometry in 2013. She is very active in education and outreach at Bigelow Laboratory and is also a Research Professor at Colby College and teaches a field course in the Fall Semester-in-residence program.

NATHAN SALOMONIS, PHD

CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER AND UNIVERSITY OF CINCINNATI

Dr. Salomonis and his group are on the cutting edge of developing new software and algorithms to identify complex functional relationships from whole transcriptome data. They have developed several open source analysis tools including AltAnalyze, LineageProfiler, GO-Elite, and NetPerspective. The advent of single-cell genomic profiles has created many new opportunities for understanding stochastic decisions mediating stem cell differentiation to distinct cell fates and the regulation of distinct gene expression and splicing programs. They are capitalizing on this new technology to explore these decision-making processes at a resolution never previously possible. Last year, they worked collaboratively with a dozen investigative research teams within Cincinnati Children's to develop new methods for evaluating whole genome transcriptome datasets. These methods include: 1) the detection of distinct gene and splicing populations from bulk and single cell genome profiles, 2) predicting implicated cell types present in complex fetal maternal biological samples and 3) identifying new disease regulatory networks related to pediatric and adult cancers, cardiovascular disease and spinal cord injury.

KATIE SEU, PHD

CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER

My long-term research objective is to understand the mechanisms which regulate erythropoiesis in the bone marrow and how erythroblastic island macrophages contribute to and support erythropoiesis. My previous research experiences have provided me with a strong background in biochemistry, biophysics, membrane biology, and fluorescence microscopy. I began my predoctoral work with Dr. Jennifer Hovis studying the contribution of proteins and phospholipids to membrane curvature. This work was conducted primarily using fluorescence microscopy and I gained significant knowledge of fluorescent properties and imaging techniques and measurements (i.e. TIRF, FRET, FRAP). Dr. Hovis unexpectedly left Purdue at the end of my second year; this was an unfortunate set back at the time, but it opened up a new opportunity to apply my biophysics background to the study of the red cell membrane. I joined the laboratory of Dr. Phil Low where I completed my doctoral dissertation on dynamic regulation of membrane-cytoskeletal interactions and the mechanical properties of the red cell membrane using single molecule diffusion measurements. During this time I published several papers on this and related research projects thanks to numerous collaborations with other groups at Purdue as well as hematologists and researchers throughout the country and around the world. While working with Dr. Low, I was supported for two consecutive years by an NIH Biophysics Training Grant which is awarded to only 5 graduate researchers at Purdue each year.

Through my graduate training, it became clear to me that the dynamic properties of mature red cells were just the end result, that ultimately their function both in health and disease was determined much earlier in their time as immature erythroblasts undergoing erythropoiesis and the final step, enucleation. However, trained as a biophysicist and having studied primarily mature red cells which are highly specialized and lack a nucleus, I knew that I lacked the cell biology knowledge and experience to navigate and contribute

substantially to the field of erythropoiesis. With this in mind, I sought a post-doctoral position with Dr. Theodosia Kalfa at Cincinnati Children's Hospital, an expert on signal transduction and erythropoiesis. Working with Dr. Kalfa, I have used models of murine erythropoiesis to study the erythropoietic niche within the bone marrow, known as erythroblastic islands, and also investigated defects in the peripheral blood and iPSC-derived erythroblasts from patients with congenital dyserythropietic anemia and hereditary hemolytic anemias.

VINCENT SHANKEY, PHD

SHANKEY BIOTECHNOLOGY CONSULTING

I have spent the past ~20 years working on analyzing cell populations from complex mixtures, including our published research on signal transduction pathways utilized by different human bone marrow cell populations as they differentiate from CD34+ down the myeloid differentiation pathway to mature granulocytes, monocytes or erythrocyte precursors. This project included the development of fixation and permeabilization techniques for whole bone marrow (or peripheral blood), and modifications of this technique to allow staining of cell surface plus intracellular (signaling) epitopes. In addition, we needed to carefully design and validate all of the reagents used in these studies (frequently measuring 4 independent signaling pathways plus 6-10 cell surface CD's to monitor myeloid differentiation pathways). Finally, the daunting task of data analysis for these studies demonstrated to me that descriptive, "gating" dependent data analysis approaches to convert complex (flow) data into information with statistical significance is limiting the impact of cytometry to basic science. While much of this research is aimed at helping to develop a fundamental understanding of the biological complexity of heterogeneous cell populations, some work has focused on specific clinical problems (e.g. measuring ZAP-70 protein expression in CLL; Cytometry 70B:259-269, 2006). Much of my research from 2001-2013 was performed as part of a unique industrial-academic research program that I convinced Beckman Coulter (my employer) to invest in as a mechanism to advance "Signaling Cytometry". This program produced 7 patent applications and over 12 joint-publications. Throughout much of my scientific career, I have placed a significant emphasis on education. In addition to my teaching responsibilities at Loyola and later teaching at the University of Miami (which included one of my favorite talks to the Bioengineering students on "Is the cell a digital or an analog machine?"), in the past 20 years, I have lectured and participated in workshops nationally and internationally, including multiple US-Indian and ASEAN Flow Cytometry Workshops I have had the privilege in participating (lectures and wet workshops) at most of the National Flow Cytometry courses held at either Bowdoin College, or Los Alamos Laboratory (now at UNM) for the past 15 years. My chair at Loyola repeatedly cautioned me that these activities would not contribute to grants, publications or promotion and tenure. But it does contribute to developing the critical resource of the next generation of scientists.

JANET STAATS

DUKE UNIVERSITY

Over the past 27 years, Janet's work has focused extensively on flow cytometry technology. She is highly committed to implementing and sharing best practices in flow cytometry. She chaired the Research Triangle Cytometry Association, was founding Director of the Center for Quantitative Cytometry, participated in the development and implementation of 10 independent flow-based standardization and proficiency programs, coordinated numerous flow-based training workshops (locally, nationally and internationally), developed the Flow Cytometry training program for the Duke Center for AIDS Research that is currently used by operators and investigators, designed methods for creating novel polychromatic flow cytometry (PFC) panels, and

developed 64 unique PFC panels. She has performed immune monitoring assays in support of 65 Phase I/II clinical trials and served on protocol development teams for 14 of those. These flow-based immune monitoring studies included assessments for basic lymphocyte subsets, markers for cellular activation, maturation, regulation, exhaustion, proliferation, cell death, function, and multimer binding. Functional assays included antigen-specific intracellular cytokine assays (ICS) and CFSE proliferation assays. Flow-based units reported include %, cells/uL, and quantitative measures for antigen density, Molecules of Equivalent Soluble Fluorescence (MESF) and Antibody Binding Capacity (ABC). She is currently overseeing the development and implementation of high dimensional (50-colors) flow cytometry, including highly standardized and formerly validated assays. She routinely provides flow cytometry support for clinical trials and basic research endeavors to Duke investigators and outside pharmaceutical companies working with Duke investigators.

STEPHEN N. WAGGONER, PHD

CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER AND UNIVERSITY OF CINCINNATI

Born and raised in Frederick, Maryland, Dr. Waggoner began his research career with a high school internship in the laboratory of Joost Oppenheim and Zack Howard at the National Cancer Institute. After obtaining a BA in Biology and Chemistry at St. Mary's College of Maryland in 2000, he pursued his interests in viral immunology by performing PhD studies in Microbiology at the University of Virginia. In the laboratory of Young Hahn, Dr. Waggoner revealed immunosuppressive interactions between the nucleocapsid protein of hepatitis C virus and human complement receptors that likely contribute to viral persistence and chronic infection. In 2007, Dr. Waggoner joined the laboratory of Raymond Welsh at the University of Massachusetts Medical School, where he studied innate immune responses during virus infection in mice. This work led to the discovery of crucial immunoregulatory functions of natural killer cells that determine the incidence and severity of infection-associated disease. Following promotion to Instructor in 2011, Dr. Waggoner was named a New Scholar of the Lawrence Ellison Foundation in 2012 and was further promoted to Assistant Professor of Pathology. In 2013, Dr. Waggoner joined the faculty at Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine Department of Pediatrics. As an Assistant Professor in the Center for Autoimmune Genomics and Etiology (CAGE), he heads a team of exceptional researchers focused on how the immunoregulatory functions of natural killer cells limit vaccine efficacy, prevent severe disease by enforcing tolerogenic mechanisms during infection, preserve immune structures that are vital for host defense during chronic inflammation, and curtail autoimmunity. The laboratory is supported by an NIH Director's Pioneer and Avant-Garde Award that concerns innovative manipulation of the immunoregulatory functions of natural killer cells to enable development of an efficacious preventative vaccine for HIV, with specific consideration for how to address the impact of HIV/AIDS in the context of drug abuse. Additional funding support from the Ralph and Marion Falk Medical Research Trust fosters collaborative efforts between Dr. Waggoner's laboratory and clinicians in the Division of Rheumatology to harness the immunoregulatory activity of natural killers as a transformative new therapy for systemic autoimmune diseases, including systemic lupus erythematosus. Dr. Waggoner also serves as Chair of the Cincinnati Children's Medical Research Center Institutional Biosafety Committee.

GLIIFCA 27 GENERAL INFORMATION

CONFERENCE REGISTRATION DESK

- The registration desk is located in the lobby of the Marriott Cincinnati RiverCenter:
  - Friday, Sep. 28 – 12:00 pm to 10:00 pm
  - Saturday, Sep. 29 – 8:00 am to 11:00 pm
  - Sunday, Sep. 30 – 9:00 am to 12:30 pm
- The conference registration fee includes all coffee breaks, the Friday Opening Reception, Saturday breakfast, lunch, Wine & Cheese reception, banquet, and also Sunday breakfast.

POSTERS

- Posters set up: Friday, September 28 after 12:00 pm in **Covington Ballroom**
- Numbers on posters correspond to poster abstract order in the program
- Poster board size = 3 ft. wide and 4 ft. high
- Please mount one poster on **each** side of a poster board using the provided **VELCRO** only
- Poster viewing: from Friday 6:30 pm to Sunday 10:30 am
- Poster presentation and judging: Saturday 6:30 pm to 8:00 pm

EXHIBITS

- Scheduled vendors will have booths in the Exhibit/Poster area **(Covington Ballroom)**
- **Please Note: Booth set-up for Covington Ballroom starting at 10 am on Sept. 28 with formal opening of Covington Ballroom at 6:00 pm. Break down starting at 11:30 am on Sunday, Sept. 30**
- All activities other than the Core Managers' Workshops **(Madison I and II)**, Plenary Sessions **(Riverview Ballroom)**, Roundtable Luncheon **(Madison I, Madison II, Gazebo and Terrace IV)**, Steering Committee Meeting **(Madison I and II)** and Banquet **(Gazebo,** and **Riverview Ballroom)** will be located in the Exhibit/Poster area **(Covington Ballroom)**
- **Please frequent the vendor booths, and show your appreciation for the generous financial support provided by the vendors who substantially help "pay the freight" for this meeting**

LOCATION OF ALL ORAL PRESENTATIONS (SAT/SUN): RIVERVIEW BALLROOM

BREAKFASTS

Free continental breakfast will be provided for all the registrants in the Exhibit/Poster area (**Covington Ballroom**) on Saturday from 7:00 am to 8:00 am, and on Sunday from 8:00 am to 9:00 am.

COFFEE BREAKS

- The refreshments will be served in **Covington Ballroom**:
    - Friday – 3:00 pm to 3:15 pm (Core Managers' Meeting; **Madison I and II**)
    - Saturday – 10:30 am to 11:00 am, and 3:00 pm to 3:30 pm
    - Sunday – 10:30 am to 11:00 am

INDUSTRIAL SCIENCE SYMPOSIUM

- Industrial Science Symposium presentations: 6:30 pm to 8:50 pm, **Riverview Ballroom**.

SOCIAL ACTIVITIES

OPENING RECEPTION

- 6:00 pm to 9:30 pm, **Covington Ballroom**
- Use 3 drink tickets for wine and beer (drink tickets can be used at either the Friday Reception, Saturday Happy Hour, or the Saturday Banquet)
- Any unused drink tickets can be donated to the Common GLIIFCA Drink Pool at the Opening Reception

SATURDAY LUNCHEON ROUNDTABLES (11:45 AM TO 1:30 PM)

- Free box lunch; available in **MADISON I** (Tables 1-3), **MADISON II** (Tables 4-6), **GAZEBO** (Tables 7-9); **TERRACE IV** (Tables 10-12); **GAZEBO** (for non-participants).
- Select a lunch (roast beef, turkey, or veggie wrap; plus fruit, chips) and beverage **-** and then move to selected roundtable labeled with the title of discussion topic. The attendance at each table is determined from the **sign-up sheet** at GLIIFCA registration desk and is limited to 10 registrants per table.

SATURDAY WINE & CHEESE HAPPY HOUR

- 6:30 pm to 7:30 pm in the Exhibit/Poster area **(Covington Ballroom)** with cheese trays (you can use your drink tickets for beer and wine).

GLIIFCA BANQUET (THEME: OCTOBERFEST)

- Free to registrants, available to paid guests.
- Commences at 8:00 pm **(Gazebo)**
- Buffet style (salads, entrées, side dishes, and dessert).
- Full service bar available. Use drink tickets for beer and wine, cash for mixed drinks.
- DJ with dance music 9:00 pm – 11:45 pm; **(Riverview Ballroom)** requests encouraged (get up and have fun!)
- Any unused drink tickets can be donated to the Common GLIIFCA Drink Pool at the GLIIFCA Banquet

DRINKS

- Full service bar will be located in the Exhibit/Poster area **(Covington Ballroom)** for the Friday opening reception, the Saturday Wine & Cheese reception, and also in the **Gazebo and Riverview Ballroom** rooms for the banquet on Saturday evening
- Three free drink tickets per registrant ***for beer and wine only*** – beer in bottles/cans
- Mixed drinks – cash bar (your cost)
- All pop is free

FACILITIES/SERVICES

- Message Board: on easel next to the GLIIFCA Registration Desk
- Xerox copying, faxing, etc.: ask at the Registration Desk

OTHER INFORMATION

GLIIFCA WEBSITE

- `http://www.gliifca.org`

NAME TAGS AND EVALUATION FORMS:

- Remember! Before leaving, please fill out evaluation form and leave at Registration/Check Out Desk with your name tag

ADDITIONAL ENQUIRIES, COMMENTS, SUGGESTIONS

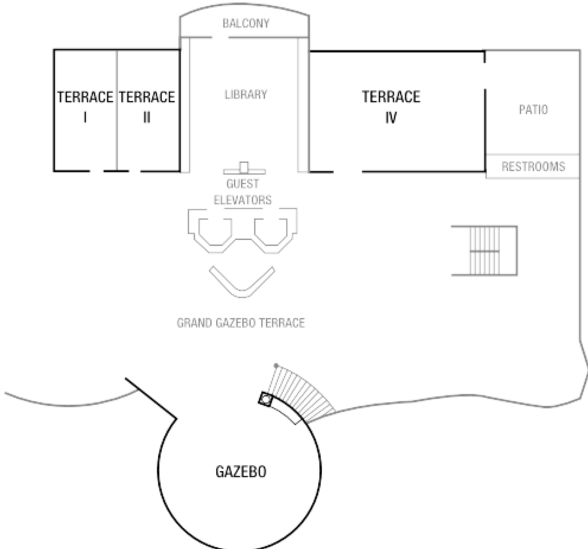
- Contact Dr. Alexander Nakeff (e-mail: caralex3@comcast.net, cell: (313) 820-6227) or leave message at the GLIIFCA registration desk
- Comments and suggestions – e-mail Dr. Sherry Thornton at **SHERRY.THORNTON@CCHMC.ORG** or Dr. Joseph Tario at president@gliifca.org

STEERING COMMITTEE BREAKFAST MEETING

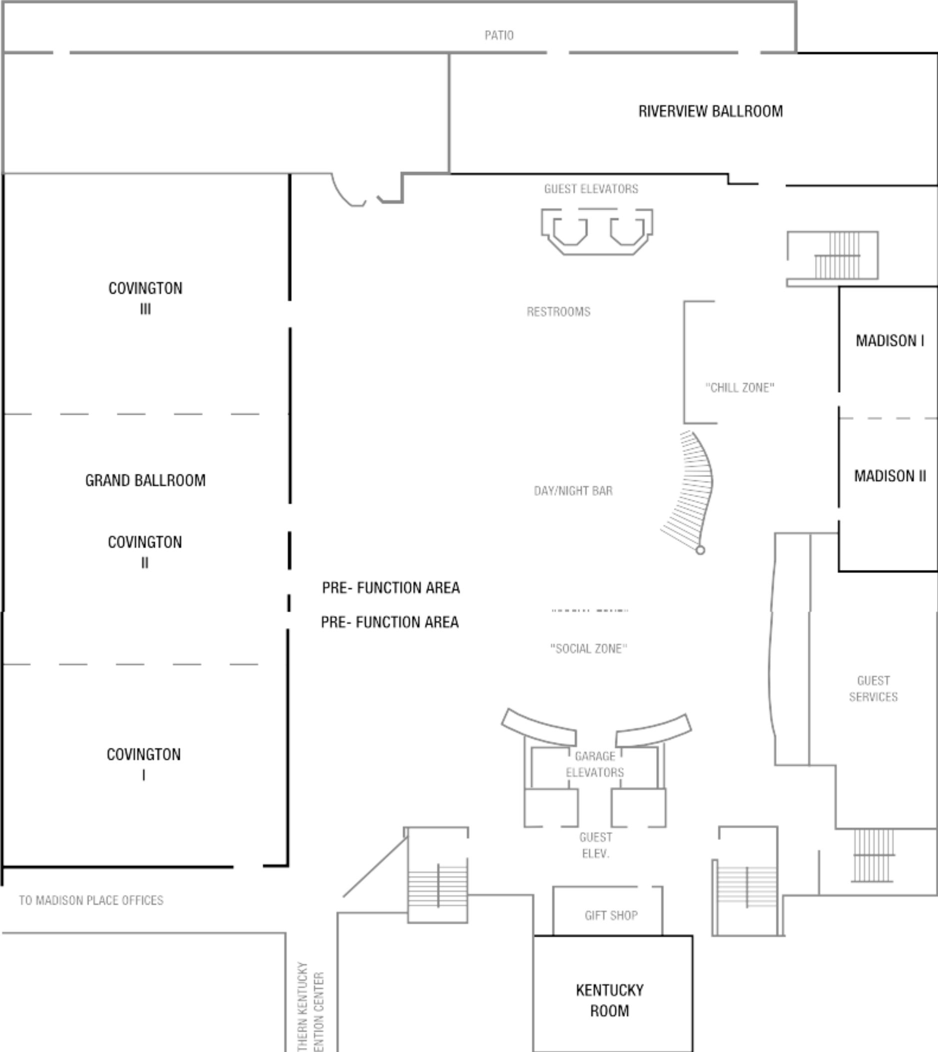
- Sunday morning (8:00 am to 9:00 am) in **Madison I and II.**

MARRIOTT CINCINNATI RIVERCENTER MAP

SECOND FLOOR



FIRST FLOOR



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