Great Lakes International Imaging and Flow Cytometry Association

GLIIFCA 18

October 2nd – October 4th, 2009
Pittsburgh, PA, USA
www.gliifca.org

2009 Program Chairs:	**Mike Sramkoski, President**
	Vera Donnenberg
	Keith Shults
Site Organizer:	**Alexander Nakeff**

Corporate Sponsors & Members:

GLIIFCA 18
Program
Table of Contents

General Information……………………..….2
General Schedule……………………………4
Speaker Schedule – At a Glance…………...5
Industrial Science Symposium..……………6
Symposium I Schedule and Abstracts…....12
Keynote Address Abstract ……………. 14
Luncheon Roundtable Topics ………….. 15
Symposium II Schedule and Abstracts….. 17
Symposium III Schedule and Abstracts….21
Poster Abstracts…………………………... 23
Steering Committee Info…………………. 35
List of Attendees………………………….. 36
Registered Vendor Representatives……... 39
Blank page for notes……..………………. 40

GLIIFCA 18 GENERAL INFORMATION

(What You Always Wanted To Know About GLIIFCA 18 But Were Afraid To Ask!)

CONFERENCE REGISTRATION DESK: Grand Ballroom, Foyer B, 2nd Floor

Conference registration fee ($80 early registration, $95 on-site) includes Friday reception, Sat/Sun breakfast, Sat. Lunch, evening reception, banquet and coffee breaks.

Friday, Oct. 2	6:00 to 11:00pm
Saturday, Oct. 3	8am to 8pm
Sunday, Oct. 4	9am to 12:30pm

POSTERS:

SET UP: Friday, October 2 after 5pm — Grand Ballroom (GB) 4/5/6

Numbers on posters correspond to poster abstract numbers 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 ***VELCRO*** only

Viewing on Friday to Sunday 10:30am

Presentation and Judging: Saturday 5:30 to 7pm

EXHIBITS:

Scheduled exhibitors will have booths in the Exhibit/Poster area (GB 4/5/6)

Booths will be open from 6pm Friday to the end of the meeting

All activities other than the plenary sessions, roundtable luncheon, Steering Committee meeting and banquet will be located in the Exhibit/Poster area (GB 4/5/6)

Please frequent the booths and show our appreciation for the generous financial support provided by the exhibitors who substantially help "pay the freight" for this meeting

BREAKFASTS:

Free continental breakfast provided for all registrants in the Exhibit/Poster area (GB 4/5/6) on:

Saturday:	7 to 8am
Sunday:	8 to 9am

Steering Committee breakfast meeting, Sunday morning (7:30 to 9am) in GB 1

COFFEE BREAKS:

Snacks and drinks available in the Exhibit/Poster area (GB 4/5/6) - no need to line up!

INDUSTRIAL SCIENCE SYMPOSIUM & FRIDAY RECEPTION:

Industrial Science Symposium presentations: 7 to10pm, Grand Ballroom 3

Reception: 5:30 to 7pm and 10 to 11pm, GB 4/5/6

Use 4 drink tickets for wine and beer

SYMPOSIA LOCATION: Grand Ballroom 3

SATURDAY LUNCHEON ROUNDTABLES (12 to 1:30 pm):

Free lunch/pop; 2/3 ham/turkey and 1/3 egg salad in Grand Ballroom 1 & 2. Sandwiches, chips and fruit at each roundtable of 10 labeled with the title of discussion topic - attendance at each table determined from sign up sheet at GLIIFCA registration desk – just pick up pop of choice and move to your roundtable of choice!

SATURDAY WINE AND CHEESE HAPPY HOUR:

5:00 to 8 pm in the Exhibit/Poster area (GB 4/5/6) with cheese and fresh fruit trays (use drink tickets).

BANQUET:

Free to registrants and paid guests
Commences at 8pm, Grand Ballroom 1/2
Numerous food stations (salad, entrée and dessert) provided to minimize waiting time
Full service bar available for drinks (use drink tickets or pay cash)
DJ with dance music until 12am; requests encouraged (get up and have fun!)

DRINKS:
Full service bar located in the Exhibit/Poster area (Grand Ballroom 4/5/6) for Friday reception and Saturday afternoon for Wine & Cheese reception and Grand Ballroom 1&2 for banquet Saturday evening. Three free drink tickets/registrant ***for beer and wine only*** – beer in bottles/cans
Mixed drinks – cash bar (your cost).
All pop in bottles/cans is free

FACILITIES/SERVICES:

Parking: use valet service or self-park in adjoining parking structure
Message boards: on easels next to the GLIIFCA Registration Desk
Xerox copying, faxing, etc: Ask at Marriott registration desks

CMLE CREDITS:

To receive CMLE credit, sign application form at the GLIIFCA registration desk

NAME TAGS AND EVALUATION FORMS: Before leaving….

Fill out evaluation form and leave at Registration/Check Out Desk with your name tag

Additional Enquiries should be directed to Dr. Alexander Nakeff or leave a message for him at the GLIIFCA registration desk.

GENERAL MEETING SCHEDULE:

All symposia to be held in the Grand Ballroom 3. Poster viewing, Vendor Exhibits, breakfasts and breaks will all be held in the Grand Ballroom 4/5/6.

Friday, October 2

10a – 3p **Resource Managers Workshop**
Sponsored by: GLIIFCA & ISAC

4p – 10p **Registration**
Grand Ballroom, Foyer B, 2nd Flr.

6:00p – 11p **Opening Reception**
Grand Ballroom 4/5/6
Sponsored by: Accuri, Beckman Coulter

7p – 10p **Industrial Science Symposium**
Grand Ballroom 3

Saturday, October 3

7a – 8a **Continental Breakfast**

8a – 11a **Symposium I**
Clinical Cytometry

9:30 – 10a **Coffee Break**
Sponsored by: Spherotech

11a –12p **The Carleton and Sigrid Stewart Keynote Lecture**

12:00-1:30p **Luncheon Roundtables**
Grand Ballroom 1/2

1:30p – 5:15p **Symposium II**
Cutting Edge Cytometry

3:30p – 3:45p **Coffee Break**
Sponsored by: Verity Software House

5:00p – 8p **Wine & Cheese Reception**
Sponsored by: iCyt

5:00p – 7p **Poster Presentations and Judging**

8p – 12a **GLIIFCA Annual Banquet**
Sponsored by: Becton Dickinson,
Grand Ballroom 4/5/6

Party Theme: GLIIFCA Graduates!
Costume Prize Sponsored by TreeStar

Sunday, October 4

8a – 9a **Continental Breakfast**

7:30a – 9a **Steering Comm. Meeting**
Grand Ballroom 1

9a – 11:45a **Symposium III**
Technical Innovations in Cytometry

10:30a – 11a **Coffee Break**
Sponsored by: iCyt and Millipore

11:45a–12p **Closing Remarks/Awards**

Chao-Huei Jeffrey Wang Memorial Achievement Award
Sponsored by: Spherotech

Alex Nakeff Young Investigator Award

Poster Awards
Sponsored by:
Jackson Immunoresearch (2), Cell Signaling Technologies (1), Tree Star (1)
Travel Stipend Awards
Sponsored by:
Tree Star (1)

BEFORE LEAVING: Please return evaluation form and name tag holder to Registration/Check out Desk. Thank you!

Speaker Schedule – At a Glance

Resource Managers' Workshop
Friday, October 2nd, 10:00a – 4:00p
Convener: Sally Quataert

Industrial Science Symposium
Friday, October 2nd, 7:00p – 10:00p
Conveners: Tom Sawyer, Karen Domenico

7:00-7:20 *Larry Duckett, Becton Dickinson*, Complex Multicolor Experimental Design Drives the Need for Specifically Configured Flow Cytometry Instrumentation.
7:20-7:40 *Matt Alexander, Beckman-Coulter*, Advancing the Science of Cytometry - New Hardware and Software.
7:40-8:00 *Clare Rogers, Accuri*, The Advantages of a Flow Cytometer with Pre-Optimized Optical Alignment and Detector Voltage/Gain Settings.
8:00-8:20 *Jerry Aultz, eBioscience*, eFluor Technologies: Robust Reagents for Multi-Parameter Flow Cytometry.
8:20-8:40 *Jonathan Rosenberg, Imgenex*, Signaling Pathways of Innate and Adaptive Immunity: Studying the Network of Toll-like Receptors, etc.
8:40-9:00 *Mark Munson, Verity Software House*, GemStone: Profiles in Cytometry.
9:00-9:20 *Jeff Clapper, iCyt,* Microfluidic Chips for the iCyt Reflection: an Economical, Disposable Solution for Cell Sorting.
9:20-9:40 *Gayle Buller, Invitrogen*, New Flow Cytometry Products.

Symposium I – Clinical Cytometry
Saturday, October 3rd, 8:00 am – 11:00 am
Convener: Vera Donnenberg

8:00-8:45 Mihai Merzianu, RPCI, A Survey of Changes and Update of Acute Leukemias of Ambiguous Lineage in the WHO Classification.
8:45-9:30 *Michael T. Lotze, Univ of Pittsburgh,* Imaging Cytometry of Autophagy in Cancer and Immunity.
9:30-10:00 Coffee Break
10:00-10:45 *Carmen Visus, Univ of Pittsburgh*, Measuring Immune Response in Cancer Patients by Flow Cytometry.

11a-12p The Carleton and Sigrid Stewart Keynote Lecture. *Maurice (Mo) R.G. O'Gorman, Professor of Pediatric Medicine Northwestern University Feinberg School of Medicine, Children's Memorial Hospital.* The Role of Flow Cytometry in the Assessment of Primary Immunodeficiency Disease: An Historical Perspective.

Luncheon Roundtables
12:00p – 1:30p
Conveners: Joanne Lannigan, Paul Champoux

Symposium II – Cutting Edge Cytometry
Saturday, October 3rd, 1:30 pm – 5:00 pm
Convener: Mike Sramkoski

1:30 – 2:15 *James Fitzpatrick, Carnegie Mellon University*, Fluorogen Activating Protein Labeling Technologies for the Super-Resolution Imaging of Living Cells
2:15 – 3:00 Marvin Nieman, CWRU, Interactions between Protease Activated Receptor 1 (PAR1) and PAR4 and the Consequences for Thrombin Activation
3:00 – 3:30 Tom Mace, RPCI, Using Imagestream Flow Cytometry to Characterize the Effects of Mild Systemic Hyperthermia on Antigen-Specific T Lymphocyte Function and Plasma Membrane Organization

3:30-3:45 Coffee Break

3:45 – 4:30 Eduardo Egea, University of Pitt., *Role of BASCs in Emphysema Repair*
4:30 – 5:00 Qing Chang, University of Toronto, Application of Multi-Parametric Flow Cytometry to Study the Effects of Tumor Hypoxia on the Cellular Heterogeneity of Human Primary Pancreatic Cancer Xenographs

5:00p – 7:30p Poster Presentation and Judging

Symposium III – Technical Innovations
Sunday, October 4th, 9:00 am – 11:45 am
Convener: Mike Sramkoski

9:00 – 9:45 Scott Tanner,U Toronto, A Mass Cytometer and Element-Tagging System for Massively Multi-Parameter Cytometry
9:45 – 10:30 Brian Grimberg, CWRU, High Throughput, Simultaneous Detection of Drug Sensitivity of P. falciparum Lifecycle Stages and Identification of Novel Antimalarials using Flow Cytometry

10:30 – 11:00 Coffee Break
11:00 – 11:45 Paul Sammak, University of Pittsburgh, Nuclear and Chromatin Dynamics in Stem Cells by Analytical Confocal Time-Lapse Imaging

Industrial Science Symposium

Friday, October 2nd
7pm – 10pm
Grand Ballroom 3

Convener: Karen Domenico, University of Toledo

7:00-7:20 Complex Multicolor Experimental Design Drives the Need for Specifically Configured Flow Cytometry Instrumentation.
Larry Duckett, Becton Dickinson.

7:20-7:40 Advancing the Science of Cytometry - New Hardware and Software.
Matt Alexander, Beckman-Coulter.

7:40-8:00 The Advantages of a Flow Cytometer with Pre-Optimized Optical Alignment and Detector Voltage/Gain Settings.
Clare Rogers, Accuri.

8:00-8:20 eFluor Technologies: Robust Reagents for Multi-Parameter Flow Cytometry.
Jerry Aultz, eBioscience.

8:20-8:40 Signaling Pathways of Innate and Adaptive Immunity: Studying the Network of Toll-like Receptors (TLRs), NF-κB Signaling Molecules, Cytokines and CD Markers Using the IMGENEX *TLRSystem*™
Jonathan Rosenberg, Imgenex.

8:40-9:00 GemStone: Profiles in Cytometry.
Mark Munson, Verity Software House.

9:00-9:20 Replaceable Precision Molded Flow Cell Nozzles for iCyt Cell Sorters.
Jeff Clapper, iCyt.

9:20-9:40 New Flow Cytometry Products from Molecular Probes - Invitrogen.
Gayle Buller, Invitrogen

Abstracts:

Complex Multicolor Experimental Design Drives the Need for Specifically Configured Flow Cytometry Instrumentation.

Larry Duckett, Lori Anderson, Becton-Dickinson

The increasing demands for more complex flow cytometry applications by researchers require not only more fluorochrome choices but sometimes, increasingly specialized instrumentation configurations better suited to their experimental needs. Researchers are increasingly looking to customized instrument and reagent solutions that provide an integrated system configured specifically to their specifications.

SORP (Special Order Research Products) are for Life Science, Research Use Only (RUO) customers who require unique, specific instrument configurations that are not offered as standard product

options. These flow cytometers and analyzers provide a method to advance research unlike any other competitive provider. These products are differentiated by functionality specifically defined by the customer, and are provided with the high quality expected of BD Biosciences products.

In this study, we'll demonstrate how an integrated customized solution using a new SORP BD LSRFortessa in the investigation of intracellular signaling pathways of immune effector cell (Th1, Th2, Th17) subsets. The instrument, experimental design and data will be discussed.

Correspondence: www.bd.com

Advancing the Science of Cytometry- New Hardware and Software from Beckman Coulter

Matt Alexander, Beckman Coulter

Introduction to the Beckman-Coulter Gallios 10 Color analyzer and an overview of the Kaluza analysis software will be presented.

Correspondence: www.coulterflow.com

Reducing Relativity: The Advantages of a Flow Cytometer with Pre-Optimized Optical Alignment and Detector Voltage/Gain Settings.

Clare Rogers, Accuri

Flow cytometric analysis relies on the interaction of a number of systems, each having inherent variability. These include the functional systems of the instrument itself (fluidics, optics, electronics and software) as well as factors such as sample preparation, reagent quality and stability, and the skill and experience of the human operator. This variability at every step in the process often frustrates efforts to standardize analysis and to compare data collected on different types of instruments at various locations. The Accuri C6 Flow Cytometer is manufactured to optimize overall instrument performance before leaving the factory. The optical alignment of lasers, flow cell, light filters and detectors is "locked down" during the manufacturing process, and the voltage and gain on photomultipliers and diode detectors is factory-set, using industry standard beads (Spherotech 8 Peak Rainbow). The result is a cytometer with highly predictable and reproducible performance. The advantages of this for the user are multiple: 1) Routine instrument characterization and quality assurance procedures are greatly simplified, 2) Spectral overlap is highly predictable, 3) Quantitative fluorescence measurements are easy to obtain, and 4) Instruments within any given manufacturing generation show low instrument-to-instrument variation in performance and fluorescence detection characteristics, making the C6 ideal for multi-center cytometry projects.

Correspondence: www.accuricytometers.com/

eFluor™ Technologies: Robust Reagents for Multi-Parameter Flow Cytometry

Jerry Aultz, eBioscience

As researchers develop more sophisticated model systems to address current questions in the life sciences, the evolution of relevant tools for this research must keep pace. Realizing the power of multi-parameter flow cytometry requires the availability of high performance fluorochromes paired with appropriate specificities to acquire consistent and reliable data. The new eFluor™ brand of fluorochromes from eBioscience includes two product lines, organic and nanocrystal-based fluorochromes, designed to provide the best performing reagents for multicolor flow cytometry.

Maximizing use of the violet laser is paramount to designing robust multicolor flow cytometry panels. The presentation will focus on the physical properties of the eBioscience nanocrystal technology and the initial offering of eFluor™ products. Data will be presented to show practical tips and advantages regarding the use of eFluor™ Nanocrystals and Organic Dyes in multicolor staining applications.

Correspondence: www.ebioscience.com

Signaling Pathways of Innate and Adaptive Immunity: Studying the Network of Toll-like Receptors (TLRs), NF-κB Signaling Molecules, Cytokines and CD Markers Using the IMGENEX *TLRSystem*™

Jonathan Rosenberg, Jonathan Rosenberg, Sujay Singh, Gita Singh, Lisa Stein, Prasanta Maiti, Imgenex

We have coalesced families of ***T***ools***, L***igands, Antibodies and other ***R***eagents into a comprehensive product line under our new ***TLRSystem*™** label to study Innate and Adaptive Immune Signaling Pathways.

***TLRSystem*™** components include antibodies, kits and reagents to study Toll-like Receptors, MyD88 and adapter molecules of the NF-κB pathway, Peptide Inhibitors of the NF-κB pathway, Pro-Inflammatory Cytokines and CD Markers an enabling portfolio for in depth understanding of the cells and pathways linking Innate and Adaptive Immune Responses.

These molecules have been combined into assays and application platforms for:

CD and TLR phenotyping via Flow Cytometry
NF-κB Activation via ELISA and Flow Cytometry
Pro-Inflammatory Cytokine Analysis via ELISA and Flow Cytometry

Flow Cytometry analysis includes use of antibody probes from IMGENEX specific for CD3, CD4, CD8, CD14, CD11b, CD25, TLR2, TLR4, TLR9, TLR3, TLR7, MD-2 conjugated with FITC, PE, APC or Alexa Fluors to analyze cell phenotype by both cell lineage and TLR expression.

Along with Flow Cytometry, cell activation states through measurement of
NF-κB pathway activation and elicited cytokine response lends insight into this network of molecules that shapes and regulates the ensuing cellular response.

Our studies will be presented as models to
Delineate profiling and presence of TLRs on Dendritic Cells, B Cells and other Innate Immune Effector Cells
Provide new information to delineate profiling and presence of TLRs on T cells and T cellsubsets
Examine activation and signaling pathways using TLR agonists and Peptide Inhibitors of the NF-κB pathway which plays a major function in immune response generation

Correspondence: www.imgenex.com

GemStone: Profiles in Cytometry

Mark Munson, Verity Software House

With an ever-increasing number of parameters becoming available to cytometrists, data display and interpretation become more cumbersome, at best - the so-called "Dimensionality Barrier." GemStone's Parametric Overlay Plot simplifies and enhances the presentation and understanding of multiparameter data analysis. A number of specific examples will be presented.

Correspondence: www.vsh.com

Replaceable Precision Molded Flow Cell Nozzles for iCyt Cell Sorters

Jeff Clapper, iCyt

Traditional flow cell nozzle assemblies are hand-built from numerous parts. The assemblies are fairly complex, difficult to clean, and expensive to replace. iCyt will discuss its newly developed precision molded flow cell nozzle. This seamless solution will be available in 70, 85, 100, 130, or 200 µm sizes. They can be used for jet-in-air sorting, sense-in-channel sorting or closed-loop analysis. They are durable enough for repeated uses, but inexpensive enough to replace when irrevocably clogged or when zero particulate or zero molecular carry-over is desired. iCyt will explain the new product in detail and share data comparing its performance to standard flow cell nozzle assemblies.

Correspondence: www.i-cyt.com

New Flow Cytometry Products from Molecular Probes - Invitrogen.

Gayle Buller, Invitrogen

From subset identification to rare event detection in stem cell analysis, multicolor flow cytometry provides answers to complex cell biology questions. Higher-plexed multicolor flow cytometry experiments reveal more information at the single cell or population level in less time, with less sample. Invitrogen recently introduced a number of novel reagents and assays that have accelerated the ability of researchers and clinicians to analyze cell function far beyond immunophenotyping. Newer reagents cover a number of areas: Apoptosis, Dead cell discrimination using SYTOX® AADvanced™ Dead Cell Stain – a dye with spectral properties similar to 7-AAD but with faster kinetics and lower CVs; Live cell cycle analysis with Vybrant DyeCycle™ Ruby Stain - detection in far red channels without cytotoxicity; Bead-based compensation with Amine reactive Compensation, or ArC™ Beads, optimized for use with the LIVE/DEAD™ Fixable Dead cell stains; Antibody Compensation Beads, or AbC™ Anti-Mouse Beads, optimized for anti-mouse antibody capture; and FxCycle™ Far Red and FxCycle™ Violet stains which allow DNA content measurements using fixed cells to be moved easily from the 488 nm laser, leaving the blue laser channels open for multiplexing. This presentation will cover a number of these assays, including cutting edge novel approaches, with practical examples and details.

Correspondence: www.invitrogen.com

Non-Presenting Vendor Abstracts

Advances in Flow Cytometry Screening: How to Design and Analyze Flow Cytometry Screening Experiments Utilizing HyperCyt Version 3 Screening Software

Linda Trinkle, IntelliCyt

Flow cytometry screening is a transformational new technology that enables high throughput analysis using powerful cell based assays. Although flow cytometry is a mature technology, it is only recently that flow cytometers could efficiently handle the challenges of sampling from 96 and 384 well microplates. The speed of data acquisition in modern digital flow cytometers and the sample throughput made possible by HyperCyt has created new challenges in managing, visualizing and reporting results of large screening experiments. HyperCyt Version 3 software provides all the tools necessary for researchers to design and annotate flow cytometry screening experiments and provides

elegant visualization features to easily analyze large-scale experiments and to identify hits and trends in screening campaigns.

Correspondence: www.intellicyt.com

Laser Imaging Cytometry – A Complementary Technology for Flow Cytometry Facilities

Scott Baldwin, CompuCyte Corporation

The nature of the samples and the types of analysis being requested to answer a wide and diverse number of questions are becoming more complex as our understanding of cells and their interaction are studied further. The tools needed to answer all these new questions are, out of necessity, being expanded to address the changing needs of laboratories and core facilities.

These can encompass sample types involving very small cell numbers derived from fluids, tissue or fine needle aspirates. Other cells may tend to clump together or, in order to derive certain accurate measurements, be much more content in their native environment within tissues. Transitional changes within compartments of cells and quantifying these changes are becoming more important with our growing understanding of cellular processes.

Flow Cytometry is able to answer many questions but the increasing complexity and importance of the new questions being asked now requires new tools to help derive the answers in a new era of expanding analytical studies. Laser Imaging Cytometry can enhance the capabilities in analyzing these new sets of criteria in cellular analysis. It is also becoming increasingly important in expanding our understanding of cell based studies and tissue studies as they become more intertwined and the knowledge from each area becomes more compelling as the information is merged together.

Laser Imaging Cytometry will show the importance of imaging capabilities along with flexibility in how samples are analyzed in order to give a more complete **image** of our understanding of these new areas of exploration.

Correspondence: www.compucyte.com

VenturiOne® Offline Data Analysis Software – Making flow faster, data exploration easier with VenturiOne®

Gillian Byrne, Applied Cytometry

VenturiOne® revolutionary off-line data analysis software from Applied Cytometry delivers exceptionally fast, simple data analysis enabling researchers to explore the true potential of their data, achieving results previously unobtainable. With the latest office 2007 look and feel, VenturiOne® has a familiar appearance coupled with highly discoverable functionality. Learn about the exceptional speed of VenturiOne® and how, in just a few simple clicks, the software enables researchers to:

Preview every combination of parameters instantaneously
View a hierarchy of plots giving an instant picture of every gating strategy
Perform DNA cell cycle analysis
Easily compensate post acquisition data with manual or automatic methods
Directly compare histogram plots using the VenturiOne® Snapshot Overlay feature
Analyse 400 data files at once in seconds
Generate simple, quick and selectable reports

Experience the simplicity of VenturiOne® yourself with a free trial version.

Correspondence: www.appliedcytometry.com

Multi-parametric Analysis of Cytokine Induced STAT Activation.

Don Weldon, Millipore Corporation

The Signal Transducers and Activators of Transcription (STAT) proteins have been implicated in a multitude of cellular functions such as embryogenesis, cell proliferation, differentiation, and cell survival (1). They transmit signals received by cell surface receptors and then translocate to the nucleus where they initiate target gene transcription. Many types of cancers both solid tumor and some blood based malignancies have been found to exhibit constitutive STAT activation (2). Interestingly, the activation within these cancer cells is generally a combination of STATs, typically STAT1, STAT3, and STAT5. This finding has led to an increase in research revolving around the activation of STAT family members.

Although there are many applications available to study STAT activation, flow cytometry has distinct advantages over other applications.
Flow cytometry can be used not only to distinguish minute changes in protein activation states by way of phospho-specific antibodies, but, also give additional information regarding the level of activation within individual cells or of an entire population. In addition, STAT activation levels can be analyzed using a dose response curve to determine the optimal concentration of cytokine for activation of a particular cell type.

Through use of a bead based detection system we then examined the expression of a panel of cytokines and chemokines prior to and following cytokine induced activation of STAT's to analyze a subset of possible target genes.

Correspondence: www.millipore.com

Symposium I

Saturday, October 3rd
8am – 11am
Clincal Cytometry
Grand Ballroom 3

Convener: Vera Donnenberg, University of Pittsburgh

8:00 – 8:45 A Survey of Changes and Update of Acute Leukemias of Ambiguous Lineage in the WHO Classification.
Mihai Merzianu, Roswell Park Cancer Institute

8:45 – 9:30 Imaging Cytometry of Autophagy in Cancer and Immunity.
Michael T. Lotze, University of Pittsburgh, School of Medicine

9:30 – 10:00 Coffee Break – Posters and Exhibits
Grand Ballroom 4/5/6

10:00 –10:45 Measuring Immune Response in Cancer Patients by Flow Cytometry.
Carmen Visus, University of Pittsburgh, School of Medicine

Abstracts:

A Survey of Changes and Update of Acute Leukemias of Ambiguous Lineage in the WHO Classification

Mihai Merzianu
Roswell Park Cancer Institute

Acute leukemias (AL) have been additionally classified into myeloid (AML) and lymphoid (ALL) lineage, neoplasms with different biology, prognosis and management. A subset of acute leukemias with either overlapping features of AML and ALL or completely lacking differentiation have posed a nosologic and therapeutic challenge for the last decades. The most recent WHO classification significantly revised this group of disease, collectively designated as acute leukemias of ambiguous lineage (ALAL), the most common subtype being mixed phenotype acute leukemia (MPAL). Molecular studies have been also incorporated into the new classification system. The implication of these changes for flow cytometry immunophenotyping of AL and the antibody panels to be utilized for AL will be reviewed. Roswell Park Cancer Institute experience with MPAL and ALAL as currently and formerly defined will be presented and the practical applicability of the new classification system will be discussed.

Correspondence: Mihai.Merzianu@RoswellPark.org

Imaging Cytometry of Autophagy in Cancer and Immunity.

Michael T. Lotze, Adam Farkas, Daolin Tang, Rui Kang, Chun-Wei Cheh, Patricia Loughran, Herbert J Zeh.
University of Pittsburgh, School of Medicine

Autophagy clears long-lived proteins and dysfunctional organelles, and generates substrates for ATP generation during periods of starvation. As such autophagy represents 'programmed cell survival' and plays a critical role counter-regulating programmed cell death or apoptosis. Measures of autophagy

have been classically done by demonstration of autophagic vesicles or so-called LC3 spots, best visualized by fluorescence microscopy or, as we have shown, by imaging cytometry. Alternative strategies which we are exploring include disappearance of the long-lived p62/SQSTM1 scaffolding protein which is degraded by autophagy. Recently, we have shown that cytosolic expression of HMGB1, a chromatin associated nuclear protein and extracellular damage associated molecular pattern molecule [DAMP], is a critical regulator of autophagy. Stimuli that enhance reactive oxygen species promote cytosolic translocation of HMGB1 and thereby enhance autophagic flux. Thus, detection of cytosolic translocation of HMGB1 as a critical pro-autophagic protein enhancing cell survival and limiting programmed apoptotic cell death, represents a novel imaging cytometric measure of autophagy which can be coupled with measures of p62 and LC3 punctae.

Correspondence: Lotzemt@upmc.edu

Measuring Immune Response in Cancer Patients by Flow Cytometry.

Carmen Visus

University of Pittsburgh, School of Medicine

Antigen-specific cytometry allows a detailed view of the role of antigen-specific lymphocytes for immune protection in cancer.

Tetramer analyses was used to measure the immune response on a randomized phase II p53 vaccine trial comparing subcutaneous direct administration with intravenous peptide pulsed dendritic cells in high risk ovarian cancer patients. In addition; sensitivity to apoptosis of virus with tumor-specific circulating CD8+ T cells was compared in head and neck and melanoma cancer patients.

We demonstrated that immune responses can be generated against a wild-type protein epitope in a significant number of ovarian cancer patients.
Tumor-specific T lymphocytes undergo spontaneous apoptosis in the peripheral circulation in head and neck cancer patients but not in melanoma patients.

Immune response can be measure by peptide-specific-MHC-tetramer staining by flow cytometry in cancer vaccines.

Correspondence: visusc@upmc.edu

The Carleton and Sigrid Stewart Keynote Lecture

Saturday, October 3rd
11:00 am – 12:00 pm
Grand Ballroom 3

The Role of Flow Cytometry in the Assessment of Primary Immunodeficiency Disease: An historical perspective.

Maurice (Mo) R.G. O'Gorman, MS., MBA, PhD, D(ABMLI).
Professor of Pediatric Medicine, Northwestern University Feinberg School of Medicine, Children's Memorial Hospital.

Over the past 50 to 60 years, approximately 165 primary immunodeficiency diseases have been described, classified and for the majority, the underlying genetic abnormality identified. Although molecular diagnosis is the penultimate goal in the continuum of clinical care for primary immunodeficiency diseases, flow cytometry has and will continue to play a major role both in the diagnosis and monitoring of these patients. Over the past 3 decades flow cytometry has emerged as an invaluable technology in the clinical laboratory and has contributed significantly to both the understanding and the evaluation of immune system abnormalities. The unparalleled ability to simultaneously identify characteristic physical cell properties, cell functions and numerous gene products at rates of thousands of cells per second has resulted in the development of a large repertoire of diagnostic, prognostic and monitoring assays. Abnormalities detected by flow cytometry can broadly be grouped as 1/ relative or absolute decrease in a specific subset or subsets, 2/ loss or abnormal expression of a specific cell associated marker or markers 3/ loss or abnormal function. During the course of this lecture, I will review the milestones in the history of primary immunodeficiency discoveries in the context of major biologic and technological advances that have converged in the development of flow cytometry based applications. I will also attempt to mirror the history of the primary immunodeficiency disease with milestones in the careers of Carlton and Sigrid Stewart.

Correspondence: mogorman@northwestern.edu

Luncheon Roundtables

Grand Ballroom 1/2

Saturday, October 3rd 12:00-1:30PM

Conveners: Joanne Lannigan, University of Virgina
Paul Champoux, University of Minnesota

Free box lunch available at each table – drinks available at side tables.

The primary aim of these discussions is to provide a forum for participants to address their interests in a variety of cytometry and image topics.

R1 - Cell cycle analysis : We will discuss advanced cell cycle analysis. This generally means more than two antibodies plus DNA content. Related issues would be cell kinetics (BrdU pulse and continuous labeling; EdU; stathmokinetics, etc.). *Moderator: Jake Jacobberger, Case Western*

R2 - Cytometry Education: A review of some approaches to successfully educate your flow cytometry users—from training to providing consultation and beyond. *Moderator: Jonni Moore and Drew Bantly, University of Pennsylvania*

R3 - Practical aspects of high speed cell sorting: Practical High Speed Sorting - All modern sorters are so-called high speed sorters. However, what does "high speed sorting" actually mean in different contexts? We will discuss instrumentation design and operation, sample preparation, data interpretation and the statistics of high speed sorting and how these topics impact on how fast cells may be sorted. *Moderator: Larry Arnold, University of North Carolina, Chapel Hill*

R4 - Fluorescent protein primer: As the fluorescent protein pallet increases, the challenges facing the cytometrist follow suit. The plight of the manic cytometrist has now included trying to understand more biology of expression vectors while juggling the basic excitation and emission characteristics of these proteins. We will discuss in general; some fluorescent protein constructs, delivery, and what that means for the cytometrist. We will also look at instrument requirements and hopefully address some of the advantages and disadvantages of a few new laser lines that have recently hit the market. If time permits and there is sufficient interest, we can touch on the world of scientific applications and their logistics; such as sorting or Fluorescence Resonance Energy Transfer (FRET). *Moderator: Joel Puchalski, University of Wisconsin*

R5 - Polychromatic Flow Cytometry: Moving from 3-4 color experiments to 6 or more colors requires not only a thorough understanding of instrument configuration but also strategic planning in the experimental design, proper controls, and data analysis considerations. In this workshop we will discuss all the necessary considerations when planning a polychromatic experiment to insure a successful outcome as determined by the generation of quality data which can be interpreted with confidence. *Moderator: Mike Solga, University of Virginia*

R6 - Tracking Dyes: This Roundtable will have a lively discussion of different methods and reagents of measuring immune cell tracking with an emphasis on monitoring cell proliferation. We will discuss (i) critical issues for measuring cell function assays using commercially available probes (ii) Staining and analysis of cells using different types of cell tracking dyes (CFSE, PKH26), and (iii) Correlation with other measure of immune function e.g. multimer staining, cytokine production, CD107a etc. The Roundtable will also consider the limitations and advantages of newer commercially available probes which expand the wavelengths usable for cell tracking. Feel free to bring your data and problems. *Moderator: Paul Wallace, Roswell Park Cancer Institute*

R7 - Grant writing and funding opportunities: NIH and the Stimulus - new formats for reviews and possible new formats for submissions. Let's discuss ARRA grants (RC1: Challenge; RC2 Grand Opportunities (GO); RC3 ....), SBIR reauthorization, and what the new man at NIH is likely to do! *Moderator: Betsy Olhsson-Wilhelm, SciGro.*

R8 - Tumor Stem Cells: *Moderator: Vera Donnenberg, University of Pittsburgh.*

R9 - Quantitative image analysis of cells in flow: The discussion will cover applications for quantitative image analysis of cells in flow including molecular translocation, cell cycle, apoptosis, autophagy, co- localization, cell conjugate and morpohology analysis. Emphasis will be given to relevant experimental design and analysis parameters as well as new features and capabilities of the ImageStream X system. *Moderator: Rich DeMarco, Amnis, Inc.*

R10 - New Clinical Diagnostics: During this session we will discuss what might be involved in developing a new assay for use in the clinical laboratory. Essentially this will be effected in a journal club format, where we will review a published report on the development of a new diagnostic test. If time permits we will discuss issues affecting the development and implementation of new flow cytometry based assays in my laboratory that are in currently in process. We will leave time for questions or issues that participants may have related to New Clinical Diagnostics. *Moderator: Maurice (Mo) O'Gorman, Northwestern.*

R11 - Clinical trial monitoring and protocol development: Sample preparation and data analysis for clinicians without research laboratories is a source of revenue for core facilities. Since clinical trial monitoring is generally not provided by diagnostic hospital labs, this is an ideal service for cores. Join us in discussions about types of assays, protocol development, standardization of data collection and data analysis. *Moderators: Kathy Schell and Dagna Sheerar, University of Wisconsin*

R12 - Signal Transduction: Analysis of cell signaling using combinations of phosphospecific antibodies is a powerful technique that can be applied to basic research and to clinical samples. However, it is technically demanding. The purpose of this roundtable is to allow exchange of information and networking between experienced individuals, and to provide contacts for those who are interested in the field". *Moderator: David Hedley, University of Toronto.*

R13 - Core laboratory financial management: It's not for someone else to worry about! Especially in these economically challenging times, it is important to become well-versed in the intricacies of your lab's budgetary concerns thereby empowering you to be proactive in its management. This Roundtable will cover financial management principles including basic budget and fee development, impacts on lab financial health, proactive measures core managers can use, and long-range planning. *Moderator: Mary Paniagua, University of Chicago*

R14 - Instrument evaluation and selection: Purchasing a new instrument is a major investment AND a major decision. Making the wrong decision for your laboratory will have a negative impact on your lab. This workshop will cover the major considerations necessary to make the best possible choice for your laboratory, from the evaluation to the selection and finally the purchase. *Moderator: Joanne Lannigan, University of Virginia*

Symposium II

Saturday, October 3rd
1:30 pm – 5:15 pm
Cutting Edge Cytometry
Grand Ballroom 3

Convener: Mike Sramkoski, Case Western Reserve University

1:30 – 2:15 Fluorogen Activating Protein Labeling Technologies for the Super-Resolution Imaging of Living Cells
James Fitzpatrick, Carnegie Mellon University

2:15 – 3:00 Interactions between Protease Activated Receptor 1 (PAR1) and PAR4 and the Consequences for Thrombin Activation
Marvin Nieman, Case Western Reserve University

3:00 – 3:30 Using Imagestream Flow Cytometry to Characterize the Effects of Mild Systemic Hyperthermia on Antigen-Specific T Lymphocyte Function and Plasma Membrane Organization
Tom Mace, Roswell Park Cancer Institute

3:30 – 3:45 Coffee Break

3:45 – 4:30 Role of BASCs in Emphysema Repair
Eduardo Egea, University of Pittsburgh

4:30 – 5:00 Application of Multi-Parametric Flow Cytometry to Study the Effects of Tumor Hypoxia on the Cellular Heterogeneity of Human Primary Pancreatic Cancer Xenographs
Qing Chang, University of Toronto

Abstracts:

Fluorogen Activating Protein Labeling Technologies for the Super-Resolution Imaging of Living Cells

James A.J. Fitzpatrick
Molecular Biosensor and Imaging Center and Department of Biological Sciences, Carnegie Mellon University

Fluorogen activating proteins (FAPs) are a new class of genetically encodable reporter proteins that generate fluorescence by binding to a range of non-fluorescent dyes known as fluorogens. Each FAP module was isolated from a library of single-chain antibodies (derived from human immune mRNA) by screening for initial affinity via magnetic bead pull-out and subsequently by the activated fluorescence of the fluorogen in question. Several FAP modules which bind and activate the fluorescence of the fluorogen Malachite Green (MG) have been evaluated for use in infrared Stimulated Emission Depletion (STED) super-resolution microscopy. Our results for imaging both extracellular and intracellular targets using these probes and the STED technique will be discussed.

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Interaction between Protease Activated Rreceptor 1 (PAR1) and PAR4 and the Consequences for Thrombin Activation

Marvin Nieman
Case Western Reserve University

A platelet rich thrombus is a hallmark of myocardial infarction, stroke and deep venous thrombosis; therefore, antiplatelet therapy is a key component of the treatment regimen for coronary artery disease. Thrombin is a major activator of platelets by binding and cleaving protease activated receptors 1 and 4 (PAR1 and 4). PAR1 and PAR4 are important receptors on platelets and are promising targets for new types of antiplatelet therapy. To define the interactions between thrombin and the PARs, purified recombinant PAR1 and PAR4 exodomains were used to determine the kinetics of cleavage by thrombin. Mutations at the cleavage site of both PAR1 and PAR4 did not influence the Km, however the kcat was decreased in each case indicating that these receptors have extended contacts with thrombin. When the full receptor was expressed on cells, an anionic region on PAR4 accounted for the interactions with thrombin away from the active site and was essential for thrombin activation of PAR4. PAR1 lowered the EC50 of PAR4 activation 10-fold when co-expressed in the same cell. In addition, PAR1 and PAR4 may form heterodimers. An assembly of PAR1-PAR4 complexes on the platelet surface may influence thrombin activation by providing unique interfaces for thrombin binding. These interfaces are potential targets for antiplatelet therapies. We have generated an antibody to the anionic region on PAR4 that blocks α-thrombin-induced platelet aggregation in human platelets. These data indicate that the anionic region on PAR4 is essential for thrombin activation of platelets. Thrombin-induced platelet aggregation is mediated by PAR1 and PAR4, therefore the antibody directed to PAR4 may target an interface between PAR1 and PAR4 to disrupt thrombin activation of PAR1-PAR4 complex.

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Using Imagestream Flow Cytometry to Characterize the Effects of Mild Systemic Hyperthermia on Antigen-Specific T Lymphocyte Function and Plasma Membrane Organization

Thomas Mace, Casey Kilpatrick, Hans Minderman, Paul Wallace, and Elizabeth Repasky
Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY

Nearly all experiments conducted to date which have contributed to our understanding of the signaling requirements for T cell activation have been conducted in vitro using a standard temperature of 37°C. However, under physiological conditions, regions of the body have temperatures which can range from 29-37°C while fever and hard exercise can bring core temperature to 39 - 40°C. We recently found that mild, in vitro heating of freshly isolated CD4+ and CD8+ T cells from BALB/c and C57BL/6 at 39.5°C resulted in the aggregation of lipid rafts, an event typically found in association with T cell signaling by antigen presenting cells. Approximately 70% of heated lymphocytes express aggregated rafts compared to only 20% of cells maintained at 37oC. CD71, a molecule which does not redistribute to lipid rafts during normal activation, serves as an effective negative control. But, does this phenomenon occur in vivo during fever-range whole body hyperthermia? And, if so, how can we identify which populations of lymphocytes respond? And, how does body temperature affect T cell function?

Using new technology which combines the ability to sort large numbers of cells based on various cell surface phenotypes with the ability to morphologically analyze these same cells in an unbiased fashion, we have been able to determine, for the first time, the effects of mild systemic heating on lipid raft organization in CD8+ lymphocytes. For these experiments we used Pmel-17 mice which are transgenic for CD8+ T cells expressing TCRs specific for the gp100 peptide. This pmel model will allow us to determine if hyperthermia has an effect on Ag-specific CD8+ T cell lipid raft aggregation and activation. We administered whole body hyperthermia (WBH) to raise the

temperature of mice to 39.5°C for 6 hours and immediately harvested and fixed cells from spleen and lymph nodes. Cells were analyzed by the Amnis Imagestream using separation by cell surface phenotype and morphological analysis of the distribution of GM1, a lipid raft marker, and CD71. We observed a 3 fold increase in the number of CD8+ T cells with lipid raft aggregates isolated from WBH mice compared to those from normothermic mice.

Considering the role that lipid rafts play in the activation and function of T cells, we investigated how various physiological temperatures could affect Ag-specific CD8+ T cell function by measuring IFN-γ production by ELISA. We found that incubating Ag-specific Pmel CD8+ T cells at higher temperatures in vitro (39.5°C) prior to activation with APCs loaded with gp100 peptide results in enhanced IFN-γ production compared to cells pre-incubated at 37° or 33°C. Further investigation is required to determine if there is a direct correlation between the effects of mild thermal stress on CD8+ T cell lipid raft aggregation and function.

Overall, these findings can help us to understand the role of physiological temperatures on lymphocyte function and assist in the rational design of thermal therapy protocols which impact immune function.

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Role of BASCs in emphysema repair

Eduardo Enrique Egea
University of Pittsburgh

Emphysema is defined as airspace enlargement that is not fully reversible. We hypothesized that bronchioalveolar stem cells (BASCs), participate in alveolar repair. BASCs possess properties of both airway and alveolar epithelial cells as observed in their expression of CC10 and SP-C respectively. BASCs are also characterized by being CD34+, SCA1+, CD45-, PECAM-. Through the utilization of fluorescence-activated cell sorting (FACS) targeting known surface markers of BASCs, and isolating these cells from whole lung digestions, we are able to analyze RNA profile changes after exposure to lung alveolar injury. Flow cytometry analysis of SP-C and CC10 also allow us to identify candidate markers to better characterize BASCs. Further attempts to understand the biology of BASC are underway in our lab and will be discussed in this talk, including culturing of BASC, BASC transplantation and in vivo methods of tracking BASC.

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Application of multi-parametric flow cytometry to study the effects of tumor hypoxia on the cellular heterogeneity of human primary pancreatic cancer xenografts

Qing Chang and David Hedley
Division of Applied Molecular Oncology, Ontario Cancer Institute/Princess Margaret Hospital, University of Toronto, Ontario, Canada.

Interactions between cancer and stroma cells, and the persistence of growth factor signaling due to mutant K-ras, appear to play a major role in the biological aggression of pancreatic cancers. Based on direct intra-operative pO2 measurements, pancreatic cancers are also strikingly hypoxic. When grown at the orthotopic site, primary xenografts show morphological features that closely resemble the surgical samples – i.e. mucin-secreting adenocarcinomas, arranged in glandular structures surrounded by a dense fibrovascular stroma.

We have used >7 early passage tumors to study the biological effects of hypoxia in these near-clinical models. Hypoxic cells were labeled using the 2-nitroimidazole probe EF5, and cell suspensions prepared for flow cytometry. We observed that some xenograft models are more hypoxic than others, with good agreement between flow cytometry and fluorescence imaging methods. These

data suggest there might be a genetic basis for hypoxia in pancreatic cancer. DNA content analysis by flow cytometry showed that the majority of cells in these tumors were of host origin. Interestingly, dual staining for DNA content and EF5 identified a significant degree of hypoxia in the stroma, as well as the tumor cells. EF5-positive labeling was only seen in stroma but not cancer cells in OCIP 16 xenograft models. In contrast, EF5-positive labeling was seen both in stroma and cancer cells in OCIP 20, 21 and 23 xenograft models. The hypoxic xenografts (OCIP20, 21 and 23) are faster growing and have a greater BrdU labeling compared to the non-hypoxic models (OCIP16, 18, 19 and 24), consistent with the idea that hypoxic cancers have a worse prognosis. To further study the relations between tumour hypoxia and cell cycle progression, we are developing flow cytometry protocols incorporating Cyclin B1, Cyclin A2, phospho-Histone H3, EF5 and DNA content. Preliminary data suggest that there are complex changes in cell cycle regulation in response to hypoxia that are potentially of therapeutic importance.

Hypoxia has also been reported to promote transition towards a mesenchymal phenotype associated with increased potential for metastasis, and cancer stem cell maintenance. To investigate this, we are developing protocols that combine differentiation markers including vimentin, cytokeratin, and E-cadherin with EF5 and the putative cancer stem cell markers CD133 and CXCR4. Preliminary data identified considerable heterogeneity in vimentin content, indicating that pancreatic cancers have the potential to adopt a more mesenchymal phenotype, and further characterization of this is ongoing. High dimensional flow cytometry appears to be a powerful technique that can be used to address major current research questions in cancer biology and treatment.

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Symposium III

Sunday, October 4th
9:00 am – 11:45am
Technical Innovations in Cytometry
Grand Ballroom 3

Convener: Mike Sramkoski, Case Western Reserve University

9:00 - 9:45 **A Mass Cytometer and Element-Tagging System for Massively Multi-parameter Cytometry**
Scott Tanner, University of Toronto, DVS Sciences, Inc

9:45 – 10:30 **High Throughput, Simultaneous Detection of Drug Sensitivity of P. falciparum Lifecycle Stages and Identification of Novel Antimalarials Using Flow Cytometry**
Brian Grimberg, Case Western Reserve University

10:30 – 11:00 **Coffee Break**

11:00 – 11:45 **Nuclear and Chromatin Dynamics in Stem Cells by Analytical Confocal Time-Lapse Imaging**
Paul Sammak, University of Pittsburgh, School of Medicine

Abstracts:

A Mass Cytometer and Element-Tagging System for Massively Multi-parameter Cytometry

Scott D. Tanner, Olga Ornatsky, Dmitry I. Bandura, Vladimir I. Baranov
DVS Sciences Inc.

Instrumentation and reagents that provide massively multi-parametric analyses of single cells at up to 1000 cells per second are described. The technology addresses the challenges of flow cytometry: as it does not involve optical interrogation and the consequent need to collimate the cells, and uses an atomic mass spectrometer for detection, we name the device a mass cytometer.

Cell biomarkers are immuno-stained using primary antibodies that are tagged with a novel metal-bearing polymer, and cellular DNA is interrogated with a metallointercalator. The metal-labeled cells are then introduced individually into a fast-reading Inductively Coupled Plasma Mass Spectrometer. Each cell is vaporized, atomized and ionized, yielding a transient of approximately 300 microseconds duration, during which time ions corresponding to the stable metal isotopes used as tags are registered. The DNA metallointercalator response is used as a trigger for cell recognition. Since there are many (more than 50) available enriched stable isotopes, and the ICP-MS provides resolution that ensures independence of detection channels over a wide dynamic range, multi-parameter assay is (truly) as simple as single channel fluorescent analysis – provided that a sufficiently non-cross-reactive antibody panel is available.

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High Throughput, Simultaneous Detection of Drug Sensitivity of *P. falciparum* Lifecycle Stages and Identification of Novel Antimalarials using Flow Cytometry

Brian T. Grimberg, Ph.D.
Center for Global Health and Diseases, Case Western Reserve University

Results of tests used to measure drug resistance levels of Plasmodium falciparum in vitro often vary greatly between laboratories. The source of this variation remains unclear. Contradictory evidence in the literature suggests that ring stage parasites are more or less resistant to antimalarial drugs, such as chloroquine (CQ), than the schizont stages. Here, we applied a recently developed flow cytometry method utilizing Hoechst 33342 and thiazole orange stains to detect the level of parasite DNA and RNA within infected erythrocytes. We also assessed parasite viability using propidium iodide and a membrane potential stain, DiIC1-5. Using this method we demonstrated that ring stage P. falciparum parasites were significantly more resistant to CQ than schizont stages across multiple strains ($P \leq 0.01$). Similar observations of reduced IC50 values in schizonts were observed when strains were exposed to mefloquine. Based upon these observations we believe the variation in IC50 determinations of other studies which used radioactive hypoxanthine uptake is the result of the differences in antimalarial drug sensitivities for various parasite life cycle stages. Additionally, when strains of P. falciparum were exposed to clinical doses of drugs for 48 hr we were able to observe several live parasites of all three stages in a single culture. This observation was confirmed using a new apoptosis-detecting membrane potential stain. The presence of these sensitive live parasites in a small culture demonstrates the potential for the selection of drug resistant parasites both in the laboratory as well as in patients with malaria. Furthermore, this method has been modified to a high throughput format to screen for new antimalarial drugs. A highly resistant strain of P. falciparum W2mef was used with this new screening techniques to identify two kinase inhibitors (Bay 43-9006 [targeting Raf/MEK/ERK], SU 11274 [targeting MET], and a non-nucleoside reverse transcriptase inhibitor (TMC 125) as compounds that exhibit potent (<1 μM) overall and ring stage in vitro antimalarial activity.

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Nuclear and Chromatin Dynamics in Stem Cells by Analytical Confocal Time-Lapse Imaging

Paul Sammak
University of Pittsburgh, School of Medicine

We have applied kinetic 4-dimensional imaging to address the problem of nuclear organization and genome wide silencing that occurs during early human development. Changes in nuclear organization also occur during tumorigeneisis and so our approach can be applied to evaluate stem cell epigenetics in drug screens, toxicological assays and cancer diagnostics. The new analytical methods in this approach can be applied to a wide variety of images to quantify amorphous morphological structures

Chromatin dynamics and organization influence gene expression patterns and developmental pathways during stem cell maturation. Chromatin plasticity can be observed by confocal time-lapse imaging of the histone GFP-H2B and measured quantitatively by novel image analysis algorithms for noising images and measuring heterogeneous nuclear texture in concentric layers. New statistical methods allow us to quantitatively measure changes in nuclear organization and dynamics and show how heterochromatin formation occurs only after pluripotent cells differentiate.

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POSTER ABSTRACTS

Abstract number corresponds to poster board number.
All posters displayed in the Grand Ballroom 4/5/6

1. Breast Cancer Dormancy and Regenerative Therapy using Adipose-Derived Stem Cells

Ludovic Zimmerlin, Vera S. Donnenberg, Melanie E. Pfeifer, E. Michael Meyer, J. Peter Rubin, Albert D. Donnenberg
University of Pittsburgh

The use of human fat as an autologous source of stem cells for breast reconstruction after cancer is confronted by the unknown effects of tissue regeneration on resting cancer cells. We investigated both in vitro and in vivo the effects of human adipose-derived stem cells (ADSC) on the proliferation of freshly isolated breast cancer cells.

Breast cancer cells were isolated from malignant pleural effusions (PE). For in vitro experiments, 5000 PE cells were plated over subconfluent CFSE-labeled ADSC or foreskin fibroblasts (FF). Tumor cell proliferation was assessed by immunohistochemistry. As an in vivo tumor xenograft model, PE were flow cytometrically sorted into: 1) low light scatter (small resting) CD90+CD31-CD45-; 2) high light scatter (active) CD90+CD31-CD45-; or 3) CD90-CD31-CD45- cells. Sorted cells (100/site) were co-injected with either ADSC, FF, heavily irradiated tumor or alone into the mammary fat pads of NOD/SCID mice (3 PE, 100 mice total, 4 sites of injection each). Animals were sacrificed up to 6 months after injection.

ADSC enhanced in vitro PE cell proliferation (number of Ki67+ cells/field: tumor alone 5.75±4 and tumor+ADSC 22±8.5). Preliminary in vivo data revealed that ADSC co-injection with sorted large CD90+ PE cells increased the number of sites in which human cytokeratin+ cells could be detected (9/20 sites compared with 0/20 sites using irradiated PE cells). ADSC co-injection did not increase the tumorigenicity of small resting CD90+ PE cells (3/20 versus 5/20 sites, respectively).

Regenerating/dividing ADSC may significantly enhance tumor growth by creating a favorable milieu for recruitment and activation of neoplastic cells. Yet no enhancing effect was detected when resting CD90+ cells were co-injected. Extended characterization of xenotransplanted animals will allow us to identify proliferating Ki67+ and non epithelial human cells at the injected sites, evaluate lymphatic spread and further characterize the differences between active and resting CD90+ tumor cells.

2. Analysis of Signalling Networks Consequent to FLT3 in Acute Myeloid Leukemia Patient Mutations

Hsiao-Wei Tina Chen, Sue Chow, Mark D. Minden, David W. Hedley
Princess Margaret Hospital, Toronto, ON, Canada

Activating mutations in the FMS-like tyrosine kinase 3 (FLT3) gene are the most common genetic alterations in acute myeloid leukemia (AML) and are associated with poor prognosis, making FLT3 an attractive target of small molecular inhibitors. Current molecular agents appear to be very promising in vitro but their efficacy as monotherapy in clinical trials is limited to a small subset of patients and the response is incomplete and/or short-lived. Our goal is to dissect the relationship between FLT3 inhibition and its consequences on

downstream signalling of different activating mutations. We are developing multicolour flow cytometric methods to monitor the responses of molecular markers including p-ERK, p-Akt, and p-STAT5. Preliminary work in cell lines reveals the signalling pattern in cells harbouring FLT3/ITD (internal tandem duplication) differ than that of the wildtype. This heterogeneity appears to impact the cell's sensitivity towards certain FLT3 inhibitors. We are now examining peripheral blood (PB) of leukemia patients. We screen PB for blast, -/+CD34+, and -/+CD117+ and examine their molecular response to SCF, FL and FLT3 inhibitors. Concurrently we are culturing short-term primary cells from patients of interest to determine the effects of inhibitors on cell cycle, proliferation, and apoptosis. Our flow cytometric methods can be applied to further investigate other mutations and signal cross-talk and will be advantageous in monitoring future clinical studies. Insights into the relationship between inhibitors, mutational status, and differential downstream pathways could be predictive of inhibitor efficacy and lead to the development of individualized combinational therapy for better clinical outcome

3. Building a User Group: Methods and Lessons from the Development of the Mid Ohio Cytometry Association (MOCA).

White AN, Burkhard MJ, McAllister C, McElwain B.

We developed the Mid Ohio Cytometry Association (MOCA) to support regional cytometry users. Developing a user group for specialized interests requires a four step process: identify needs, develop a core group, plan events, and facilitate interactions. We recognized the demand and need for a local cytometry group to bring awareness and education to users of the shared resource facilities. The primary goal of MOCA is to focus on the needs of the users themselves who are primarily in the academic research environment. MOCA has two main subgroups. Facility managers and cytometry operators form the MOCA core group and are responsible for planning events and helping to deliver educational material.Users attending MOCA meetings are primarily drawn from graduate students and laboratory technicians who desire to apply cytometry expertise to high quality reproducible research experiments. Interactions are facilitated at multiple levels including large group presentations, small group training, and one-one discussions.

4. Assessment of hES Cells by High-Content Microscopy and Flow Cytometry.

Erik Puffer, Joseph Klim, Jamie Boyd, and Kathleen Schell
University of Wisconsin–Madison Carbone Cancer Center, Madison, WI

High-content analysis of cell population data allows for accurate assessment of various cell types. These methods are crucial to our ability to assess stem cell state. The availability and quality of reagents to characterize human embryonic stem cells (hESC) by various methods has recently improved. Here we discuss methods for the evaluation of hES cells using high-content microscopy and flow cytometry. The University of Wisconsin Carbone Cancer Center Flow Cytometry Facility has developed flow cytometry-based procedures to characterize hESC. These Standard Operating Procedures (SOPs) are used by the National Stem Cell Bank to characterize various stem cell lines. We discuss the design of these SOPs, describe antigenic markers currently employed by the NSCB, and review the process by which we identify cells positive for the target antigen. We also compare Oct-4, a transcription factor important in hESC pluripotency, expression profiles in FCS data generated from microscopy images acquired on a BD Pathway BIoimager and flow

cytometry data from an LSRII. Finally, we demonstrate the ability to examine the growth and Oct-4 expression patterns of stem cells grown in a bioreactor on a 10 cm dish.

5. Clinical Research Services in a Flow Cytometry Core Facility

Dagna Sheerar, Erik Puffer, Jamie Boyd, Joel Puchalski, and Kathleen Schell
University of Wisconsin–Madison Carbone Cancer Center, Madison, WI

The University of Wisconsin – Madison Carbone Cancer Center Flow Cytometry Facility has developed a repertoire of Clinical Research Services for researchers at our university as well as for the local business and biotechnology sector. These are fee-based services and include experimental design, sample preparation, data acquisition and data analysis. Introduction of these charged services has increased facility revenue, encouraged collaboration, and probably most importantly given the flow staff important experience in taking an experiment from idea to presentable data. This experience has been invaluable when assisting facility clientele with all stages of flow cytometry experimentation, from choosing antibody–fluorochrome combinations to data analysis and sorting strategies.

6. Enhanced Wide Angle (EWA) Forward Light Scatter Improves Resolution of Red Blood Cell Microvesicles (RBCMV)

E. Michael Meyer[1], Chenell L. Donadee[2], Mark T. Gladwin[2], Albert D. Donnenberg[1,3]
University of Pittsburgh Cancer Center Flow Cytometry Facility, University of Pittsburgh, Pittsburgh, PA[1] Division of Pulmonary Allergy and Critical Care Medicine University of Pittsburgh Medical Center, Pittsburgh PA[2] University of Pittsburgh School of Medicine, Department of Medicine, Pittsburgh PA[3]

Detection of red blood cell (RBC) derived microvesicles is an area of intense interest because they hold promise as a biomarker of disease activity in sickle cell anemia, thalassaemia intermedia and cardiovascular disease. Their quantification in stored blood products may also serve as an objective quality indicator. Flow cytometry based assays have been devised to quantify RBCMV in blood on the basis of conventional forward light scatter (FSC) and annexin V binding. In such assays, RBCMV are detected as a discrete population of FSC low cells, the majority of which bind annexin V, indicating loss of phospholipid asymmetry. Intact RBC are largely annexin negative, but form one continuous population with transitional cells having slightly lower FSC and higher annexin binding. Presumably this transitional population is intermediate to the formation of RBCMV. The newly released Beckman-Coulter Gallios cytometer has the capability to detect FSC light in a mode that preferentially amplifies signals collected between 8-19º. This mode, termed Enhanced Wide Angle Forward Scatter, is particularly useful for resolving size difference among small particles (micron to submicron range).

In this study, we demonstrate that EWA FSC is able to resolve RBCMV from both the transitional population and from intact RBC in blood bank whole blood products that had been aged 3 and 21 days. Intact RBC and fragments were detected as Glycophorin-A (Gly-A, CD235a) (PE-Cy5) positive events. Annexin V binding was measured on each Gly-A+ population. With EWA FSC, intact RBCs were detected as a homogenous scatter population with a mean half-peak CV of 10.8%. This population was easily distinguished from the lower scatter transitional population and from microvesicles themselves. In contrast, using conventional FSC, RBCs formed a diffuse population (mean half-peak CV = 17.7%) that was continuous with transitional vesicles. The ability to resolve RBC populations by EWA FSC

enables further time course studies to determine whether the emergence of a transitional population predicts the subsequent degradation of RBC into RBCMV.

7. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo

Tatyana N. Milovanova,[1] Veena M. Bhopale,[1] Elena M. Sorokina,[1] Jonni S. Moore,[2] Thomas K. Hunt,[3] Martin Hauer-Jensen,[4] Omaida C. Velazquez,[5] and Stephen R. Thom[1,6]
[1]Institute for Environmental Medicine, Departments of [2]Pathology and Laboratory Medicine and [6]Emergency Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania; [3]Department of Surgery, University of California at San Francisco, San Francisco, California; [4]Department of Surgery, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and [5]Department of Surgery, University of Miami, Miami, Florida

We hypothesized that oxidative stress from hyperbaric oxygen (HBO2, 2.8 ATA for 90 min daily) exerts a trophic effect on vasculogenic stem cells. In a mouse model, circulating stem/progenitor cell (SPC) recruitment and differentiation in subcutaneous Matrigel were stimulated by HBO2 and by a physiological oxidative stressor, lactate. In combination, HBO2 and lactate had additive effects. Vascular channels lined by CD34+ SPCs were identified. HBO2 and lactate accelerated channel development, cell differentiation based on surface marker expression, and cell cycle entry. CD34+ SPCs exhibited increases in thioredoxin-1 (Trx1), Trx reductase, hypoxia-inducible factors (HIF)-1, -2, and -3, phosphorylated mitogen-activated protein kinases, vascular endothelial growth factor, and stromal cell-derived factor-1. Cell recruitment to Matrigel and protein synthesis responses were abrogated by N-acetyl cysteine, dithioerythritol, oxamate, apocynin, U-0126, neutralizing anti-vascular endothelial growth factor, or anti-stromal cell-derived factor-1 antibodies, and small inhibitory RNA to Trx reductase, lactate dehydrogenase, gp91phox, HIF-1 or -2, and in mice conditionally null for HIF-1 in myeloid cells. By causing an oxidative stress, HBO2 activates a physiological redox-active autocrine loop in SPCs that stimulates vasculogenesis. Thioredoxin system activation leads to elevations in HIF-1 and -2, followed by synthesis of HIF-dependent growth factors. HIF-3 has a negative impact on SPCs.

8. A Strong Educational Program Enhances Flow Cytometry Shared Resource Laboratory Efficiency and Effectiveness

Andrew D. Bantly, Charles H. Pletcher, Jr., Richard Schretzenmair, Pall Hallberg, Ryan Wychowanec, Gisela Brake Silla, Jonni S. Moore
Flow Cytometry and Cell Sorting Shared Resource Laboratory, Path BioResource, University of Pennsylvania School of Medicine, Philadelphia, PA

Flow cytometry and cell sorting are highly sophisticated technologies that encompass a broad range of techniques and applications. Because of the cost of the instrumentation, the complexity the technology, and the need for significant support on behalf of users, institutions recognize that shared resource laboratories are a cost effective means to provide communal access to the technology. A central goal of an academic shared resource is to balance productivity with quality, minimize the cost per research project, and keep overall costs as low as possible. Easy access to cost-effective services combined with the excellent on-site scientific and technical expertise allow investigators to maximize the benefit of the technology for their individual research projects. The Flow Cytometry and Cell Sorting Shared Resource Laboratory (FCCSRL) has implemented a comprehensive educational

program for over 10 years which, combined with enhanced sophistication and capacity of its equipment and the expertise of its staff, has led to consistently increasing utilization. This has been achieved, in part, by actively encouraging investigator-performed services, with sufficient training and guidance by the staff, so that the technical staff can focus their efforts on complex flow cytometry applications and development strategies. In this way, the Flow Cytometry and Cell Sorting Facility has been able to maximally utilize the instrumentation and increase volume while controlling total staffing costs.

9. Development of a Flow Cytometric Method for Quantification of Bcl-2 Family Members in Human Lymphoma and Leukemia Cell Lines.

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We have developed a method to measure Bcl-2 family members' protein expression levels using quantitative fluorescence cytometry (QFCM). This method is specific, sensitive and can be used to quantify Bcl-2 family members requiring fewer cells when compared to Western blot methods. It is particularly useful to identify expression levels in a specific cell population within a heterogeneous population of cells. We first characterized and validated several antibodies to various Bcl-2 family members to confirm their specificity for flow cytometry. To demonstrate that this method can be used to correlate the expression levels of Bcl-2 family members and their response to ABT-263 a panel of leukemia/lymphoma cell lines were treated with varying concentrations of ABT-263 and cell viability was determined The expression of Bcl-2 and Mcl-1 as determined by QFCM in the different cell lines correlated best with their cellular response to ABT-263 such that high expression of Bcl-2 was associated with cells sensitive to ABT-263 and Mcl-1 was high in cells resistant to ABT-263. This method can be utilized in clinical trials to facilitate patient stratification.

10. Characterization of cytokeratin positive cells in pulmonary and peripheral blood from lung resection surgery

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Introduction. We hypothesized that mechanical stress during tumor resection may result in dissemination of tumor through venous circulation. We developed a flow cytometry based method to characterize cytokeratin+ cells in pulmonary venous blood. Major vessels were ligated prior to lobectomy or pneumonectomy. After resection, an incision was made in the major venous vessel and 7-10 cc of blood was removed to a cup, and aspirated into a heparinized syringe. As a control, venous blood was also obtained from the antecubital fossa.

Methods. We used a flow cytometric adaptation of the CellSearch assay to detect nucleated (DAPI+) nonhematopoietic (CD45- and lineage (CD14, CD33, glycophorin A) negative), cytokeratin+ cells in pulmonary and peripheral blood (n=10 paired samples). Linear DAPI fluorescence allowed ploidy detection in cytokeratin+ cells.

Results. Cytokeratin+ cells were detected at low frequency in both pulmonary and peripheral blood, but were 10-fold more prevalent in pulmonary blood (0.034% and 0.004%, respectively. $P<0.001$, two-tailed paired t-test). Among cytokeratin+ cells in pulmonary blood, euploid cells outnumbered aneuploid (>2N DNA content) by 35-fold ($p<0.0005$). Whereas, in peripheral blood, euploid cytokeratin+ cells were 12-fold more prevalent than

aneuploid ($p=0.01$). When ploidy was considered in the comparison of pulmonary and venous cytokeratin+ cells, only the frequency of euploid cells was statistically significantly higher in pulmonary blood ($p<0.001$).
Conclusions. The data indicate that epithelial marker+ non-hematopoietic cells can be found at low frequency in peripheral venous blood, and at increased frequency in pulmonary blood. However, assessment of DNA content indicates that the frequency of aneuploid cytokeratin+ cells (presumptive tumor cells) is indistinguishable in these two compartments. In contrast, the frequency of euploid cytokeratin+ cells is greatly increased in pulmonary blood, suggesting that these are shed normal epithelial cells, rather than tumor.

11. Effect of inhibition of embryonic signaling pathways hedgehog and notch on in vitro hematopoietic colony formation.

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Background: Inhibition of embryonic signaling pathways active in certain cancers has been proposed as a way to specifically target cancer cells with stem cell-like properties. It has been demonstrated that cyclopamine, a hedgehog pathway inhibitor, has activity in certain cancer cell lines, while LDE225 (Novartis), a similarly acting hedghog pathway inhibitor, is currently undergoing a phase I clinical trial for the treatment of a variety of cancers. Likewise, multiple gamma secretase inhibitors (GSI), which act by notch pathway inhibition, are currently in phase I clinical trials for the treatment of multiple cancers. With the importance of therapeutic index in mind, we seek to assess the effects of these compounds on human erythroid, myeloid, and platelet differentiation.
Methods: Cryopreserved human bone marrow ($n=5$) was thawed and plated in H4434 methylcellulose medium (containing stem cell factor, GM-CSF, IL-3, and erythropoietin) and incubated with either cyclopamine (5 µM), LDE225 (1 µM), sonic hedgehog (Shh, 200 ng/mL), cyclopamine and Shh, LDE225 and Shh, GSI (Sigma, 10 nM), or no additional compounds. After 14 days of incubation, colonies were scored by microscopic observation as either erythroid or myeloid. Additionally, flow cytometry was used to assess surface expression of CD45, Glycophorin A (GlyA), CD71, CD33, CD34, and CD41. All statistical analysis was done using 2-sided paired t-tests.
Results: Cyclopamine and LDE225 inhibited erythroid differentiation by reducing the percentage of GlyA+ cells (~50%, $p \leq 0.015$ and ~20%, $p \leq 0.017$ reduction compared to control cultures respectively). In the presence of these agents, erythroid colonies in culture appeared visibly smaller, though not less in number when viewed microscopically. LDE225 also caused a small but significant increase (~10%, $p \leq 0.043$) in the percentage of myeloid cells (CD45+, CD33+, CD34-) with respect to total CD45+ cells. Within the platelet lineage (CD45+, CD41+), LDE225 caused a reduction (~50%, $p \leq 0.037$) in the percentage of high forward scatter megakaryocytes. Addition of Shh ligand to the cultures did not reverse the effects of either cyclopamine or LDE225. GSI had no detectable effects on erythroid, myeloid, or platelet differentiation.
Conclusions: Both Hedgehog pathway inhibitors resulted in partial inhibition of in vitro erythroid differentiation, but not myeloid differentiation. LDE225 also showed megakaryocytic toxicity, while GSI had no detectable effect on in vitro hematopoietic colony growth. These results predict relatively limited hematotoxicity when the study drugs are given as single agents. Future in vitro studies examining the interaction of embryonic pathway inhibitors and cytotoxic drugs may be useful in predicting hematopoietic toxicity in multi-drug regimens.

IRB approval: UPCI 99-053

12. A Novel Violet-Laser Excitable Ratiometric Probe for the Detection of Membrane Asymmetry Breakdown during Apoptosis

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Apoptosis is carefully regulated and essential part of normal tissue development and homeostasis. Regulatory changes in the apoptotic pathway has been implicated in many diseases, most notable are many types of cancers and autoimmune disorders. Normal cells exhibit a remarkable asymmetry in lipid distribution between the outer and inner cell membranes characterized by phosphatidyl-serine (PS) and phosphatidylethanolamine (PE) normally located on the inner leaflet of the cell membrane. During apoptosis PS and PE are translocated from the inner to outer leaflet of the cell membrane. Translocation of PE and PS to the external cellular environment facilitates recognition and elimination of these cells by macrophages. The most common method for detecting changes in membrane asymmetry during apoptosis utilize fluorescently labeled annexin V, which acts by binding to PS on outer membrane leaflet. The interaction of annexin V with PS requires the presence of Ca2+ ions (up to 2.5 mM). Annexin V has also been shown to associate with membrane surfaces that contain negatively charged byproducts of lipid peroxidation. Moreover, cell-harvesting techniques that utilize trypsin can produce false positives when assayed with annexin V. The Violet Ratiometric Membrane Asymmetry Probe, 4'-N,N-diethylamino- 6-(N,N,N-dodecyl-methylamino- sulfopropyl) -methyl-3-hydroxyfla vone (F2N12S), is a novel violet excitable dye for the detection of membrane asymmetry changes during apoptosis. The dye exhibits an excited-state intramolecular proton transfer (ESIPT) reaction resulting in a dual fluorescence with two emission bands corresponding to 530 nm and 585 nm, producing a two-color ratiometric response to variations in surface charge. The ratiometric probe is a self-calibrating absolute parameter of apoptotic transformation, which is independent of probe concentration, cell size, and instrument variation. The two-color ratiometric response of the F2N12S reagent allows detection of apoptosis among samples with variable cell concentrations up to 100 fold with no loss in signal resolution. The Violet Ratiometric Membrane Asymmetry Probe rapidly targets the plasma membrane and can be analyzed in as little as 5 minutes at room temperature and does not require special buffers or wash steps reducing the chance of cell death during extended sample processing. The Violet Ratiometric Membrane Asymmetry Probe has been shown to work in both suspension and adherent cell lines with good correlation to other markers for apoptosis, unlike annexin V which indicated a larger population of apoptotic cells at an earlier time in both suspension and adherent cell lines that did not correlate with other apoptosis markers. The violet laser excitation of F2N12S allows users to fully utilize their violet-laser capable cytometer and develop multi-parameter apoptosis assays while still employing commonly used blue and red excitable reagents to perform more complex and detailed apoptosis assays.

13. Reducing Variability in Flow Cytometric Analysis.

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Flow cytometric analysis relies on the interaction of a number of systems, each having inherent variability. These include the functional systems of the instrument itself (fluidics, optics, electronics and software) as well as factors such as sample preparation, reagent

quality and stability, and the skill and experience of the human operator. This variability at every step in the process often frustrates efforts to standardize analysis and to compare data collected on different types of instruments at various locations. We describe here a flow cytometer design and manufacturing process which optimizes and standardizes overall instrument performance before shipment. The result is a cytometer with highly predictable and reproducible performance: as one example, for 60 instruments manufactured over a 3 month period, the top peak position for each of 6 parameters (FSC, SSC, FL1, FL2, FL3, FL4) varied less than 1.50% at time of shipment to customers. Bead data collected both in house and from the field showing maintenance of instrument performance with time is also presented. The advantages of this pre-optimized cytometer for the user are multiple. Routine instrument characterization and quality assurance procedures are greatly simplified, requiring only that the same calibration beads used in the manufacturing process be run by the customer to validate instrument performance each day. Fluorescence spillover for common dyes (FITC, PE, APC), when used with the standard instrument filter configuration, is highly predictable and can be modeled mathematically based on empiric data. This allows definition, on dual parameter plots, of single positive and double-positive fluorescence regions for specific fluorochrome pairs. The combination of highly predictable fluorescence spillover, pre-optimized detector gain settings, and high resolution digital signal processing allows users to by-pass setting fluorescence compensation altogether, without compromising the accuracy of population identification. These features also allow users of different C6 instruments to create, and share, analysis templates which include pre-set gates specific to particular applications and sets of fluorochromes.

14. Epithelial to mesenchymal phenotypic transition in human primary tumor explants.

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Introduction. The epithelial to mesenchymal transition is thought to represent an important stage in the evolution of epithelial tumors. We have optimized the culture of primary epithelial tumor isolates using conditions developed for human embryonic stem cell (huES). In this study we used these primary culture conditions to track the expression of endothelial and mesenchymal markers on primary breast and lung tumor explants.

Methods. Freshly, isolated human normal and tumor tissues were mechanically disaggregated and directly added into culture (huES culture medium, 0.1% gelatin-coated flask, 37°C, 5%CO_2). Cells were passaged continuously. Cultures were weekly documented by photomicroscopy and characterized by 9-color flow cytometry before and after expansion (cytokeratin, CD44, CD90, CD14, CD33, Glycophorin-A, CD117, CD133, and CD45; DAPI to identify nucleated cells and characterize cell cycle).

Results. Tumor samples have currently been expanded up to passage thirteen. Primary tumor cell outgrowth was visualized microscopically as a mixture of colonies exhibiting either epithelial or mesenchymal (fibroblastic-like) morphology. Fibroblastoid cells progressively supplanted epithelial cells after serial expansion. These observations were confirmed by flow cytometry, revealing the presence of cytokeratin+ CD44+/CD90+ (tumor stem cell candidate) as a minority population at low passages and cytokeratin dim CD44+/CD90+ cells lacking CD133 or CD117 expression predominating later in culture.

Conclusions. The morphology and immunophenotypes observed in early and late passages are consistent with epithelial and mesenchymal lineages, respectively. Further experiments are required to determine whether this transition occurred at the clonal level, or was the result of outgrowth of mesenchymal-like tumor cells.

15. Macrophages respond homogeneously to apoptotic cell-mediated inflammatory suppression: cytofluorimetric analysis of macrophage pro-inflammatory cytokine expression.

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Clearance of dead cells by professional phagocytes, such as macrophages, is an essential physiological process contributing to homeostasis. Importantly, the clearance of apoptotic cells by macrophages is marked by a lowered macrophage response to inflammatory stimuli, such as bacterial lipopolysaccharide (LPS). This lowered response is evident on the level of secretion and is exerted on the level of pro-inflammatory cytokine gene transcription. Previous studies have examined apoptotic suppression in populations of macrophages. Here, we have applied single cell cytofluorimetric analysis to examine apoptotic suppression in individual macrophages. Using intracellular staining of the accumulation of a pro-inflammatory cytokine (TNF-α) as a sensitive marker of macrophage responsiveness, we find that apoptotic suppression occurs homogenously and uniformly within the entire responder population. Suppression by apoptotic cells was observed similarly when another pro-inflammatory cytokine, IL-6, was analyzed. Technically, cytokine production was monitored as the accumulation of the cytokine intracellularly in fixed and permeabilized cells following blockade of secretion (using the inhibitor of vesicular transport, Brefeldin A). The differential staining of apoptotic cells and macrophages facilitated the independent analysis of the process of phagocytosis. The evaluation of apoptotic cell phagocytosis in parallel with intracellular cytokine staining allowed us to document that apoptotic suppression, while dependent on cell-cell contact, is independent of apoptotic corpse engulfment.

16. Current Antimalarials Affect Cellular Zinc in Plasmodium falciparum

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Zinc plays a central role in many cellular processes and biochemical reactions. Prior discoveries of Plasmodium falciparum infection showed the parasite's extraordinary ability to accumulate high levels of 'free' or weakly bound zinc. This metal, which appeared to be concentrated within mitochondrial space, seems to be an essential component for parasite growth and development. Zinc deprivation showed a clear association with parasite inhibition, indicating a requirement for the metal. The studies presented here focus on the effects that current antimalarial compounds have on the essential parasitic zinc stores, with the aim of providing a wider understanding of the mechanisms of antimalarial action. The assessment of relative 'free' zinc levels was determined using the fluorescent zinc probe, Zinbo5. Multiple antimalarials including the quinoline derivatives, Chloroquine, Halofantrine, Quinacrine, and Quinine showed a decrease in essential 'free' zinc levels over the dose curve of the compound. Likewise, Atovaquone and Artemisinin which are speculated to target parasite mitochondria exhibited a similar depression in cellular zinc.

Daphnetin, an iron chelator, appears to also affect zinc at a slightly lower dose than the IC50 concentration of the drug. The effects of antimalarials on essential zinc stores may play an important role in activity, and could therefore be an important target for the development of novel antimalarial drugs.

17. MANIPULATION OF EMBRYONIC PATHWAYS TO TARGET TUMOR STEM CELLS

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Cancer dormancy is particularly important in breast cancer, where cancer recurrence may occur years after apparently successful therapy. The cancer stem cell paradigm explains, in part, tumor heterogeneity and has led to the hypothesis that therapy resistance originates in the mechanisms by which normal tissue stem cells protect themselves from toxic insults. The hypothesis underlying this work is that the Hh pathway plays a critical role in tumor self-protection, compared to normal hematopoietic and mesenchymal progenitor cells, which are less dependent on hedgehog for the activation of their protective pathways. This predicts that in breast cancer inhibition of the Hh pathway should have multiple downstream effects, resulting in increased sensitivity of breast cancer to antineoplastic agents and increased differentiation leading to increased sensitivity to hormonal therapy. The effects of inhibition of Hh signaling in breast cancer cell lines (MCF7, BT474 and MDAMB231), as compared to mesenchymal progenitor cells (specificity control) were measured using immunohistochemical detection of cytokeratin, CD44, Ki67, estrogen receptor, sonic Hh (SHh) and Gli. The addition of exogenous sonic hedgehog (C24II, 200 ng/mL, 48h) to the breast cancer cell line MCF7 resulted in increased cytoplasmic and nuclear Gli-1, higher proportion of cells in cycle (Ki67), and an unexpected increase in ER expression, without any notable increase in CD44, an adhesion molecule reported on breast cancer stem cells, or any significant decrease in cytokeratin expression. In normal bone marrow derived mesenchymal cells, positive for SHh but negative for smoothened (SMO) or Gli, addition of exogenous SHh had no effect on proliferation or differentiation of these cells. The addition of the PTCH inhibitor cyclopamine (5 μM) in MCF7 blocked SHh increased proliferation (Ki67) and ER upregulation. No effects of cyclopamine were detected in mesenchymal progenitor cells. Taken together, these preliminary findings provide early evidence for selective effects of Hh pathway inhibition of tumor cells.

18. A multiplatform method for the clonal isolation of rare cells.

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The clonal isolation of rare cells, particularly cancer and stem cells, in a population is important to cell biology. We have demonstrated that the Laser-Enabled Analysis and Processing (LEAP, Cyntellect Inc., San Diego, CA) instrument can be used to efficiently produce clones by photoablative dilution. The LEAP instrument can image cells in a multiwell plate, and then eliminate all undesired cells by laser ablation. In photoablative dilution, rare cells are deposited into a multiwell plate at 10 cells per well. Then one cell is chosen to clone, and the other cells present in the well are eliminated by laser ablation. We have successfully used LEAP to produce single cell clones in 95% of wells (originally

containing 5±2.1 cells/well). While photoablative dilution is a very effective way of producing clonal cultures, it has a fundamental limitation in the low number of cells that can be processed. This can be overcome by performing an enrichment sort using flow cytometry based cell sorting to increase the frequency of the rare cells to be cloned. Flow sorting can provide greater than 104-fold enrichment and cells can be sorted directly into a multiwell plate. With pre-enrichment, photoablative dilution can be used to clonally isolate rare cells from a sample. This is especially important in cases where the total number of potentially rare cells recovered by first stage enrichment sorting is only 10-200 cells. We have demonstrated this method by successfully producing a single rare cell (1.49% of total population) that remained viable in 40 out of 40 wells of a trial plate.

19. Mitochondrial membrane potential changes in HepG2 cells can predict drug induced toxicity using Laser Scanning Cytometry

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Thiazolidinediones (TZD) and biguanides (BD) are a group of oral hypoglycemic agents involved in the activation of the peroxisome proliferator-activated receptor gamma (PPARγ) and in the reduction of fast plasma glucose and insulin levels by reducing hepatic glucogenesis and improving glucose uptake in peripheral tissues. Despite the clear benefits of these drugs there is significant liver toxicity that resulted in the withdrawal of few of them from the market. Recent in vitro studies have demonstrated mitochondria involvement in drug-induced toxicity.

Five hypoglucemic compounds, three TZDs (Rosiglitazone, Troglitazone and Pioglitazone) and two BDs (Metformin and Phenformin) have been analyzed in hepatocarcinoma cells HepG2-based high content toxicity screening (HCTS) using Laser Scanning Cytometry (LSC) together with several strong mitochondrial toxins as controls. High-content data were obtained using a set of four fluorescent biomarkers, TMRM, Hoechst 33342, ToPro-3, and DCFDA, which enable characterization of mitochondrial membrane potential (MMP), plasma membrane permeability, DNA content, nuclear circularity, area, and reactive oxygen species (ROS) generation. In addition, cell cycle analysis was performed by flow cytometry. Cell viability was confirmed by lactate dehydrogenase assay (LDH). Moreover, the effect of glucose deprivation was analyzed by substituting glucose with galactose-containing medium. Glucose-deficient medium prevents the escape of mitochondrial-triggered cell death by glycolysis and sensitizes cells to mitochondrial toxicant-induced cell death. Our result showed that at prolonged exposure to drugs (24h of incubation) differences in the mechanisms of action of tested compounds becomes prominent. For example, Troglitazone and Phenphormin showed decreased viability accompanied by drop of MMP and, in case of Phenphormin, by the accumulation of ROS. Comparison of the pattern of behavior of different cellular parameters enabled grouping of compounds into several categories using Pearson-correlation based distances with hierarchical clustering algorithm. Obtained groups proved to be in good agreement with the degree of toxicity of tested compounds. Our results showed that disruption of MMP is the one of the earliest indicators for the mitochondrial toxicity and, moreover, drug-induced

changes in MMP in the presence of glucose as opposed to galactose/glutamine- enriched media was essential to separate mitochondrial dysfunction from general cytotoxicity. In conclusion, we have shown that HCTS enabled drug-effect characterization and grouping based on the mechanisms of mitochondria functionality and glucose dependency with future application to prediction of drug-induced toxicity.

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