SoCal Flow Cytometry Association SUMMIT 2014

March 24-25, 2014
Beckman Center
University of California, Irvine

SoCal Flow Cytometry Association SUMMIT 2014

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Southern California Flow Cytometry Association

presents

SoCal Flow SUMMIT 2014

March 24-25, 2014

Hosted by the Sue & Bill Gross CIRM Stem Cell Research Center
University of California, Irvine

Researchers from the Southland's regional research centers, clinical laboratories and biomedical industries join together to share their contributions to Cytometry, and network to enhance learning opportunities in the region.

Progam Highlights

(see pages 12-13 for full program)

SUMMIT 2014 Day One
March 24, 2014
8:15am - 5:20pm

Poster Session / Happy Hour
Core Managers
Dinner Meeting
Everyone is welcome
March 24, 2014
5:20pm - 8:30pm

SUMMIT 2014 Day Two
March 25, 2014
8:15am – 4:20pm

Pre-Summit Course by Expert Cytometry *(see page 3)*
Post-Summit Course by FloCyte *(see page 3)*

Invited Speakers:

Maurice R.G. O'Gorman M.Sc., Ph.D., MBA, D(ABMLI) - The Use of Flow Cytometry for the Assessment of Primary Immunodeficiency Disease

Scott Kitchen PhD - A stem cell based approach for engineering anti-HIV immunity

Keith Kelley BS - Applications of Flow Cytometry to the Characterization of Aggregates in Therapeutic Proteins

Zbigniew Darzynkiewicz M.D., PhD – Cytometric Assessment of DNA Damage - and mTOR - signaling, the Factors Contributing to Aging

Marcin Kortylewski PhD – Dissecting mechanisms of STAT3-mediated immuno suppression in mouse and human tumor models using flow Cytometry

Craig M. Walsh PhD – Using flow cytometry to investigate T cell death mechanisms and immune tolerance

Gold Sponsor Presentations

ADDITIONAL OPPORTUNITIES

Pre- and Post- meeting Training Courses offered
Separate registration required

March 23: Excyte Cytometry, "Flow Cytometry Core Principles"

March 26: FloCyte Associates, "Cytometry in Cell Necrobiology: Apoptosis, Necrosis and Beyond"

Welcome to SoCal Flow SUMMIT 2014

The Program Committee of the Southern California Flow Cytometry Association is pleased to present its third summit - SoCal Flow SUMMIT 2014, on March 24-25, 2014, held at the Beckman Center, University of California at Irvine. All flow cytometry users from Southern California's regional research centers, clinical laboratories and leading biomedical industries are invited to join together at this exciting event for networking, sharing and education with Flow Cytometry colleagues from across the Southern California counties.

We hope that you all will enjoy this exciting two-day meeting which will include scientific presentations from invited faculty, vendor presentations, a poster session, and a core manager dinner meeting open to all attendees. The scientific program includes six (6) hours of CEU made available by FloCyte Associates.

Southern California Flow Cytometry Association
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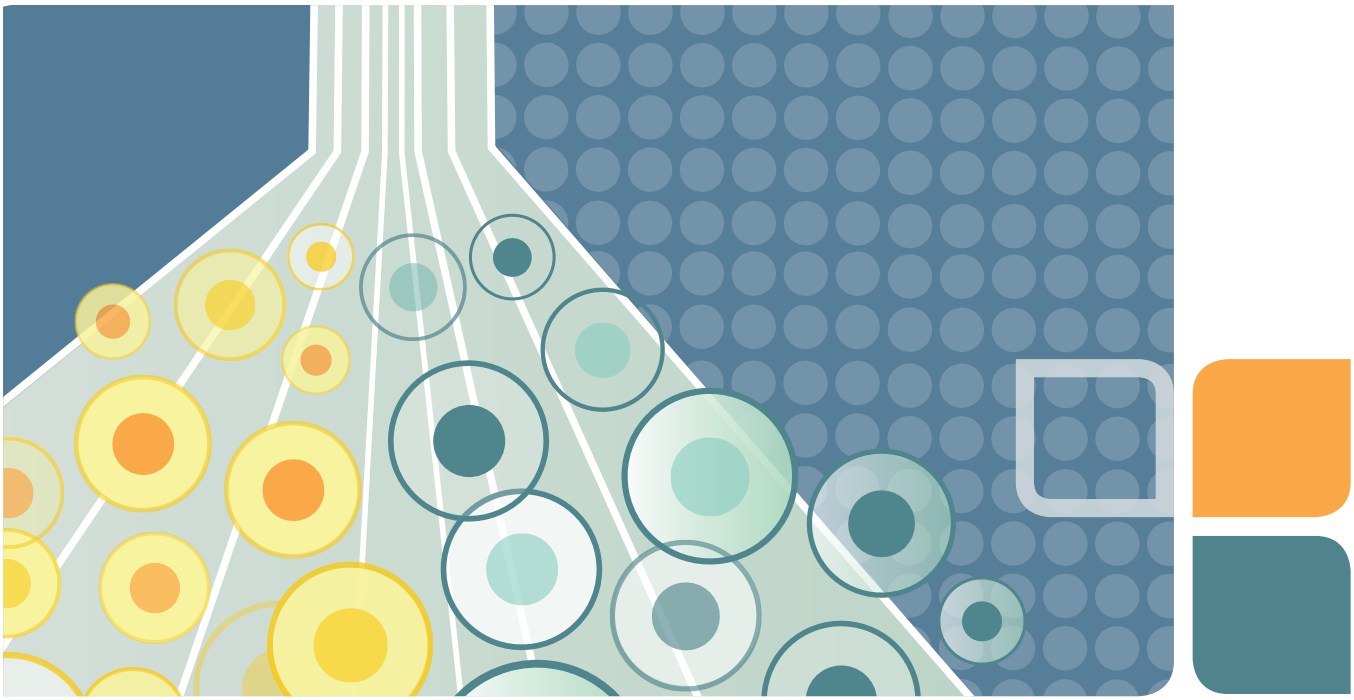

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About SoCal Flow

The Southern California Flow Cytometry Association (SoCal Flow) brings together research, clinical, and biomedical scientists from Los Angeles, Kern, San Luis Obispo, Santa Barbara, San Bernardino, Riverside, Orange County, San Diego and Imperial counties. The Association reaches out to members to enhance collaborations, for brainstorming ideas, asking questions and communicating with each other via its message board. SoCalFlow provides opportunities to further the education of its members by hosting scientific talks, workshops and training courses to keep members abreast of recent developments and advances in the field of cytometry. The inaugural November 2011 SUMMIT at USC-HSC campus was made possible by generous vendor support. The success of SUMMIT 2011 and 2013 has made possible SUMMIT 2014.

The Southern California Flow Cytometry Association is incorporated in the state of California as a tax-exempt corporation, and is non-profit 501 (c) 6 trade association. The Association has been accepted as an affiliate of the International Society for the Advancement of Cytometry (ISAC).

ISAC
International Society for Advancement of Cytometry

By attending this meeting all registered attendees will automatically become members of the Southern California Flow Cytometry Association unless they choose to opt-out. The committee encourages all SUMMIT 2014 attendees to partake of this association and become active networking members of the Southern California Flow Cytometry Community. During the SUMMIT, there will be a fifteen minute annual meeting of the Association. We hope that you will participate in this meeting and feel free to give feedback on what you would like to see the Association presenting.

SoCal Flow
Cytometry Association

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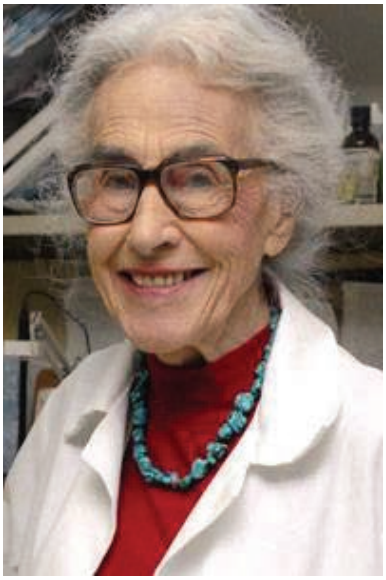
Congratulations to our Training Scholarship Winners

SoCal Flow Scholarship Recipient
Dr. Miriam Kim
Postdoctoral Scholar; Department of Hematology, University of Southern California School of Medicine

Marylou Ingram Scholarship Recipient
Linda Dukes, CLS, MBA
CLSII, GMP Core Lab Facility, Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles

This year, the SoCal Flow Cytometry Association awarded two competitive scholarships: The SoCal Flow Cytometry Association Scholarship and the Marylou Ingram Scholarship. These scholarships are awarded for flow cytometry training during SUMMIT 2014 for students currently enrolled in a Southern California university and researchers or clinical personnel of a Southern California facility. The Marylou Ingram Scholarship honors the memory of a remarkable female scientist and mentor, and is awarded to a deserving Sourthern California female scientist.



Marylou Ingram, M.D.
1920-2013

Marylou Ingram dedicated her life to science and the pursuit of knowledge. Her professional career spanned nearly 70 years and as a medical doctor her focus was in academic medicine, medical research, and teaching. Her research focused on experimental hematology, radiation biology, cellular biology, and immunology. Her innovations and pioneering work led to the discovery and development of several technologies including automated image analysis systems for the identification and enumeration of hematopoetic cells and the Histoid Bioreactor which results in the formation of 3D tumor models. Marylou has been described as an amazing woman with a zest for life and compassion for her fellow man and as an incredible mentor. As an early female scientist, she paved the way for subsequent generations of women to dream and become scientists as well.

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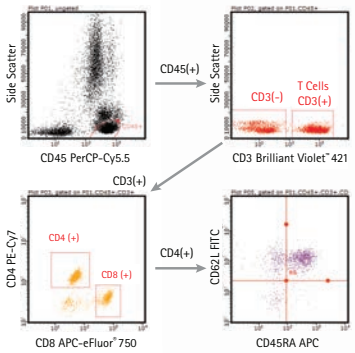

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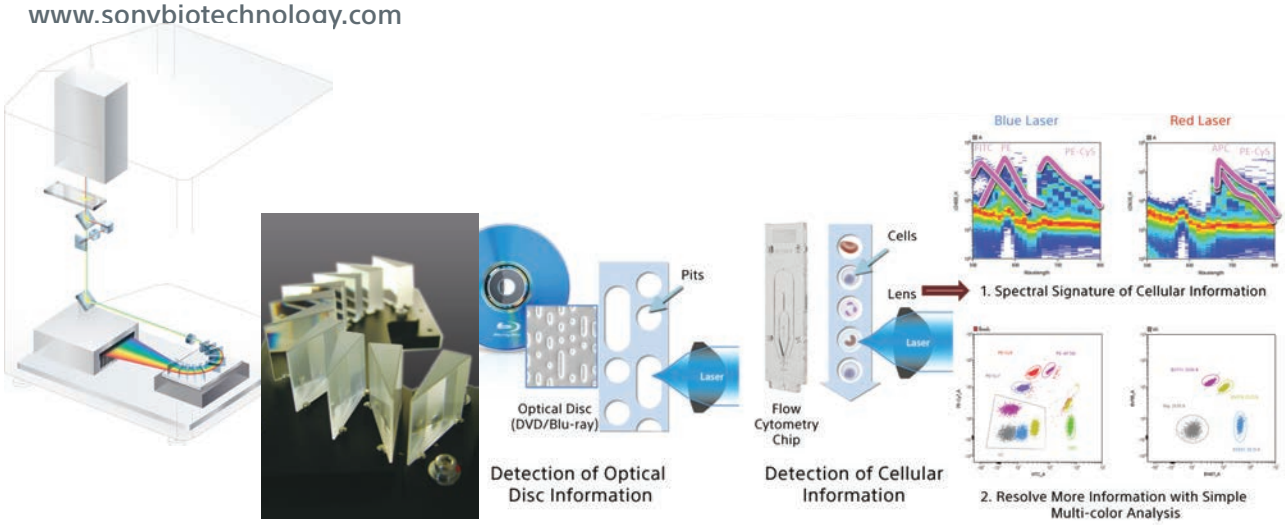


Technology for Science.

SoCal Flow SUMMIT 2014 Program

Monday, March 24, 2014

8:15am – 9:00am	Registration, Full Breakfast ***Sponsored by Life Technologies***
9:00am – 9:15am	Opening Remarks
9:15am – 10:05am	**Maurice O'Gorman, Ph.D, MSc, D(AMBLI), MBA. Children's Hospital Los Angeles.** "The Use of Flow Cytometry for the Assessment of Primary Immunodeficiency Disease"
10:10am – 10:30am	Stefan Jellbauer, Ph.D., UC Irvine: "Immunophenotyping of neutrophil subpopulations during *Salmonella Typhimurium* Colitis"
10:30am – 10:45am	Life Technologies: Jim Princi, Life Technologies. "The next generation in Acoustic Focusing - Attune NxT".
10:45am – 11:30pm	Coffee Break (Posters and Vendor Tables)
11:35am – 12:00am	DVS Sciences: Ryan L McCarthy, Ph.D. MD Anderson Cancer Center. "Pluripotency and differentiation at the single cell level: Understanding Stem Cell Heterogeneity by CyTOF mass cytometry."
12:05pm – 12:25pm	Brent Kern, B.A., Pfizer: "Receptor coccupancy and internalization of an anti-IL-7 receptor antibody"
12:30pm – 2:00pm	Buffet Lunch
2:00pm – 2:50pm	**Scott Kitchen, Ph.D. UCLA.** "A stem cell based approach for engineering anti-HIV immunity"
2:55pm – 3:10pm	Sony: Vladi Cherepakhin, Ph.D. Sony. "Spectral Flow Cytometry from Sony Biotechnology."
3:10pm – 3:55pm	Coffee break (Posters and Vendor tables)
4:00pm – 4:20pm	SoCal Flow Business Meeting
4:25pm – 5:15 pm	**Keith Kelley, Amgen.** "Applications of Flow Cytometry to the Characterization of Aggregates in Therapeutic Proteins."
5:20pm – 6:20pm	Happy Hour/Poster Session. Everyone is Welcome
6:20pm – 8:30pm	Buffet DINNER & CORE MANAGER'S MEETING Everyone is Welcome. ***Sponsored by ISAC.***

Abstracts for the oral poster presentations are available at www.socalflow.org

SoCal Flow SUMMIT 2014 Program

Tuesday, March 25, 2014

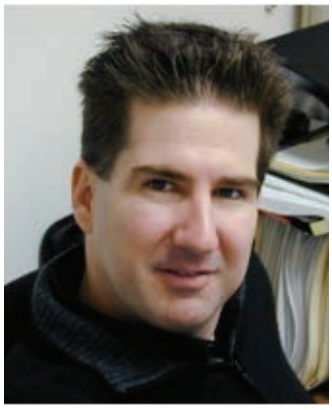
8:15am – 9:00am	Registration, Full Breakfast ***Sponsored by Life Technologies***
9:00am – 9:25am	EMD Millipore: Mark Hildebrand, Ph.D. Scripps Institution of Oceanography. "Application of Imaging Flow Cytometry for Marine Biotechnology".
9:30am - 10:20am	**Zbigniew Darzynkiewicz, MD, Ph.D. New York Medical College. Keynote Speaker.** "Cytometric Assessment of DNA Damage - and mTOR - signaling, the Factors Contributing to Aging" ***Speaker is sponsored by FloCyte Associates.***
10:25am – 10:40am	Beckman Coulter: Christopher Trindade, MD. Beckman Coulter. "Beckman Coulter Innovation: Dry Reagents and the ONE Study".
10:40am – 11:25am	Coffee break (Posters and Vendor tables)
11:25am – 11:45am	Eric D. Diebold, Ph.D, UCLA: "Digitally-synthesized beat frequency multiplexing for ultra-high throughput imaging flow cytometry".
11:50am – 12:40pm	**Marcin Kortylewski, Ph.D. City of Hope Medical Center.** "Dissecting mechanisms of STAT3-mediated immuno suppression in mouse and human tumor models using flow Cytometry"
12:40pm – 2:10pm	Buffet Lunch
2:10pm – 2:25pm	Affymetrix eBioscience: Matt Cato, M.S., Affymetrix eBioscience. "RNA by Flow Cytometry: The power of branched DNA".
2:30pm – 3:20pm	**Craig Walsh, PhD. UC Irvine.** "Using flow cytometry to investigate T cell death mechanisms and immune tolerance"
3:25pm – 3:40pm	Bio-Rad: Matt Alexander, Ph.D. Bio-Rad. "Walk away sorting with the Bio-Rad S3; Adding capability without burden".
3:45pm – 4:05pm	Christine M. Evangelista, M.S., Amgen: "Co-lyophilization of PGD2 assay control and quantum dot nanocrystals to trace experimental variables and analytes in a Whole Blood Flow Cytometric Assay"
4:05pm – 4:20pm	Adjourn; Raffle, coffee and take down posters

Abstracts for the oral poster presentations are available at www.socalflow.org

Maurice O'Gorman, M.Sc., Ph.D., MBA, D(ABMLI)
Professor, Department of Pathology,
Clinical Scholar and Professor, Department of Pediatrics,
Keck School of Medicine, University of Southern California

The Use of Flow Cytometry for the Assessment of Primary Immunodeficiency Disease

The clinical landscape of molecular cancer therapeutics is shifting. Expectations that dramatic responses to signal transduction inhibitors, similar to those seen in CML patients treated with imatinib, have not been realized. Since it is likely that most oncogenic targets have now been identified, with effective inhibitors available for clinical testing, the emphasis is shifting towards combinations of targeted agents and the challenge is patient selection for specific drug combinations (recognizing that all of these agents have side effects). Despite early optimism, a deluge of genome sequencing data has failed to identify significant numbers of previously unknown druggable targets. These genomics studies have also rediscovered the analytical complexity of tumor heterogeneity, which is what flow is all about! On the other hand, there is major interest in the areas of epigenetic and metabolic regulation of cancers, which might be more tractable in terms of novel treatments, and those fields are currently wide open for translational research. So it is timely to review the potential for flow cytometry in the field of molecular oncology. In this talk I will overview some practical issues, particularly the development and optimization of novel flow techniques that can be applied to patient samples linked to clinical trials of targeted agents. I will emphasize applications where I believe that flow has a competitive edge relative to alternative approaches.



Scott Kitchen, PhD
Associate Professor of Medicine, David Geffen School of Medicine, UCLA
Director, Humanized Mouse Core Laboratory, UCLA

A stem cell based approach for engineering anti-HIV immunity

The HIV-specific cytotoxic T lymphocyte (CTL) response is a critical component in controlling HIV replication. We are interested in the development of ways to genetically enhance the HIV-specific CTL response to allow long-term viral suppression or viral clearance. We have previously demonstrated that human hematopoietic stem cells (HSCs) can be modified with a molecularly cloned HIV-specific T cell receptor (TCR) to develop into fully functional CTL that can suppress HIV replication in a humanized mouse model. A fundamental issue with this approach is the notion that these TCRs are human leukocyte antigen (HLA)-restricted, and therefore their use therapeutically is highly restricted to individuals with specific HLA genotypes.

We are currently investigating the use of non-HLA restricted chimeric antigen receptors (CARs) that allow the recognition of HIV when expressed by a CTL. Here we report that the use of a CD4-ζ chain CAR that contains the extracellular portion of the CD4 molecule fused to the intracellular TCR-zeta signaling domain. The lentiviral vector containing the CD4-ζ CAR also expresses small hairpin (sh)RNAs specific for CCR5 and the HIV LTR to protect the developing cells from infection. We determined that CD4-ζ CAR transduced HSCs can differentiate into functional CD4 and CD8 T cells as well as NK cells in vivo in humanized mice. Importantly, we found that CD4-ζ containing cells can functionally respond to HIV over long periods of time and significantly suppress HIV replication following infection. Thus, this system allows the close examination of the engineering of antiviral immunity and non-HLA restricted HIV-specific CTL responses in vivo. Our results strongly suggest that stem cell based gene therapy may be a feasible approach in the treatment of chronic viral infections and provide a foundation towards the development of this type of strategy.

Keith Kelley, BS
Senior Scientist, Department of Medical Sciences, Amgen, Thousand Oaks,

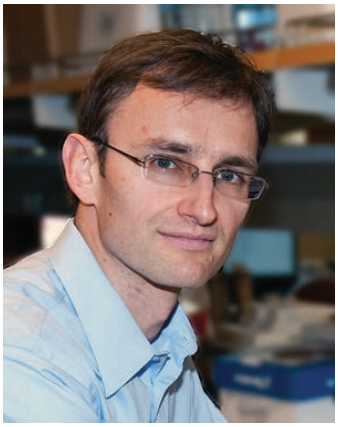
Applications of Flow Cytometry to the Characterization of Aggregates in Therapeutic Proteins

The formation of particulates and aggregates of therapeutic proteins during their production, fill into syringes or vials and storage is a major concern because the degree of aggregate formation varies as a function of (even) minor processing and storage differences. Both sub visible and visible particulates and aggregates can induce immunogenic responses directed against the therapeutic. The effects of such interactions can alter their pharmacokinetic properties, can neutralize the activity of the therapeutic, can induce hypersensitivity reactions and in some cases can react with endogenous host proteins to mediate life-threatening events. Detection and characterization of particulates and aggregates can be challenging due to their potentially large size range (which transverses into the sub visible space) and varied physical properties. No single analytical platform or method serves to meet the needs of this workstream. In this study we therefore compared flow and imaging Cytometry with currently accepted standard methods (MFI™ Micro-Flow Imaging, HIAC) to determine particle count, relative size and morphological characteristics. In addition particles were physically separated on a cell sorter based on scatter profiles and subjected to subsequent microscopic observation, imaging cytometry and component analysis. Overall, we find that flow and imaging cytometry technologies hold value as orthogonal techniques in particulate and aggregate studies, with the size range of detected particles greater than MFI or HIAC. Moreover, the cytometry platforms offer a means to characterize particles in much the same way that they are commonly leveraged to provide detailed characterization of cells.

Zbigniew Darznkiewicz, MD, PhD
Director, Brander Cancer Research Institute
Professor of Pathology, Medicine and Microbiology/Immunology
New York Medical College

Cytometric Assessment of DNA Damage – and mTOR - signaling, the Factors Contributing to Aging

Two mechanisms are being proposed as a cause of aging and senescence. Persistent DNA damage by reactive oxygen species (ROS), by-products of oxidative phosphorylation, is one of them (ROS mechanism). Activation of the mTOR/S6K1 signaling pathway by nutrients and growth factors is considered to be an alternative (mTOR) mechanism. The flow- and laser scanning- cytometric methods were developed to measure the level of the constitutive DNA damage/ROS- as well as of mTOR/S6K1- signaling. Specifically, activation of ATM and expression of γH2AX in untreated cells detected with phospho-specific Abs reports constitutive DNA damage induced by endogenous ROS. The phosphorylation of Ser235/236-of S6 ribosomal protein (RP), of Ser2448 of mTOR and of Ser65 of 4EBP1 informs on constitutive signaling along the mTOR/S6K1 pathway. The reported anti-aging agents: rapamycin, metformin, 2-deoxyglucose, berberine, resveratrol, vitamin D3 and aspirin, all decreased the level of constitutive DNA damage signaling. They also decreased intracellular level of ROS and mitochondrial trans-membrane potential $\Delta\Psi m$, the marker of mitochondrial energizing. All these agents also reduced phosphorylation of mTOR, RP-S6 and 4EBP1 in A549, TK6, WI-38 cells and in mitogenically stimulated human lymphocytes. The most effective was rapamycin. Although the primary target of each on these agents may be different the data are consistent with the downstream mechanism in which the reduction of translation rate through mTOR/S6K1 signaling is coupled with a decrease in energy production through oxidative phosphorylation and leads to a decline in the level of ROS, mitochondrial potential and oxidative DNA damage. The decreased rate of translation induced by these agents may slow down cells hypertrophy and alleviate other features of cell aging/senescence. The reduced oxidative DNA damage is expected to lower predisposition to neoplastic transformation which may result from defective DNA repair at the sites coding for oncogenes or tumor suppressor genes. The data suggest that combined assessment of constitutive γH2AX expression, mitochondrial activity (ROS, $\Delta\Psi m$) and mTOR signaling provides an adequate gamut of cell responses to evaluate effectiveness of suspected gero-suppressive agents by cytometry. **Speaker sponsored by FloCyte Associates.**



Marcin Kortylewski, PhD
Assistant Professor, Department of Cancer Immunotherapeutics & Tumor Immunology, Beckman Research Institute,
City of Hope National Medical Center, Duarte, CA

Dissecting mechanisms of STAT3-mediated immuno suppression in mouse and human tumor models using flow Cytometry

Signal Transducer and Activator of Transcription-3 (STAT3) is an oncogene and immune checkpoint commonly activated in cancer cells and in tumor-associated immune cells. We previously developed an immunostimulatory strategy based on targeted Stat3 gene silencing in Toll-like Receptor 9 (TLR9)-positive hematopoietic cells using siRNA-based strategy (CpG-siRNA conjugates). In our recent studies, we evaluated therapeutic effect of systemic STAT3 blocking/TLR9 triggering on disseminated acute myeloid leukemia (AML) in vivo utilizing multiple flow cytometry-based assays. We used a model of transplantable mouse Cbfb/MYH11/Mpl-induced leukemia, which mimics human inv(16) AML. Our results demonstrated that intravenously delivered CpG-Stat3 siRNA, but not control oligonucleotides, can eradicate established AML and impair leukemia-initiating potential in majority of mice. These antitumor effects were mediated by effector CD8+ T cells but did not require host's TLR9+ antigen-presenting cells. Instead, CpG-Stat3 siRNA had direct immunogenic effect on AML cells in vivo leading to upregulation of the surface expression of MHC class II, costimulatory molecules and proinflammatory mediators, such as IL-12, while downregulating expression of coinhibitory PD-L1 molecule. Systemic administration of CpG-Stat3 siRNA generated potent tumor antigen-specific immune responses, increased the ratio of tumor-infiltrating CD8+ T cells to Tregs in various organs and resulted in CD8+ T cell-dependent regression of leukemia. TLR9 is also commonly expressed in human AML, including leukemic progenitors (CD34+CD38+) and stem cells (CD34+CD38-). Our preliminary results indicate that STAT3 blocking/TLR9 triggering can alleviate immunosuppressive potential of primary patients' AML cells while promoting autologous T cell activation. These findings underscore the potential of using CpG-Stat3 siRNA strategy to break tumor tolerance and induce potent immunity against AML and potentially other TLR9-positive blood cancers.

Craig M. Walsh, PhD
Assistant Professor, Co-Interim Director, UC Irvine Institute for Immunology, Director, UC Irvine Multiple Sclerosis Research Center

Using flow cytometry to investigate T cell death mechanisms and immune tolerance

Our group is interested in the mechanisms that control T cell homeostasis, the process that dynamically regulates the expansion and contraction of antigen specific T cells. In particular, we are focused on the means by which cell death modulates this important balance. A major project in the lab is to understand the biology of a protein known as FADD, and how this protein contributes to T cell function. Although FADD is known to activate a form of cell death known as apoptosis, we have discovered that FADD also contributes to the activation and survival of antigenically-stimulated T cells. These intriguing results raise an important question: how can a single molecule both induce apoptosis under some circumstances and promote survival and expansion of T cells under other conditions. Understanding this paradox will contribute to our knowledge of how T cell homeostasis is maintained and will also help us understand how such a balance is disturbed in autoimmunity and lymphoproliferative disease. A second protein the lab is investigating is DRAK2, an apoptosis regulating kinase. Mice with an engineered deficiency in this death-inducing kinase are known to be hyper-sensitive to antigenic stimulation under specific conditions. We are dissecting the signaling pathways that lead to such hyper-responsiveness. We are also conducting studies to determine how the structure of the kinase regulates its function. Finally, we are determining if this kinase plays a role in the prevention of autoimmune diseases such as rheumatoid arthritis. We believe that a more complete understanding of the physiological roles of such apoptotic regulatory pathways will contribute greatly to the amelioration of diseases of the immune system. Flow cytometry is a major tool utilized by our group to investigate signal transduction and immune responsiveness in T cells.

Presentations from Event Sponsors

Life Technologies: Jim Princi, MBA; "The Next Generation in Acoustic Focusing – Attune NxT"

On behalf of EMD Millipore: Mark Hildebrand PhD; Scripps Institute, La Jolla, CA; "Application of Imaging Flow Cytometry for Marine Biotechnology"

SONY: Vladi Cherepakhin, PhD; "Spectral Flow Cytometry from SONY Biotechnology"

On behalf of DVS Sciences: Ryan L McCarthy; MD Anderson Cancer Center; "Pluripotency and differentiation at the single cell level: Understanding Stem Cell Heterogeneity by CyTOF mass Cytometry"

Beckman Coulter: Christopher Trindade, MD; "Beckman Coulter Innovation: Dry Reagent and the ONE Study"

Affymetrix eBioscience: Matt Cato, MS; "RNA by flow Cytometry: The power of branched DNA"

Bio-Rad: Matt Alexander, PhD; "Walk away sorting with the Bio-Rad S3: Adding capability without burden"

Poster Presentation Competition Winners

SoCal Flow invited undergraduate and graduate students, post-docs and non-management rank professionals (industry or academic) to submit an abstract for the Poster Session. Four poster were selected to present their work orally at the summit. The winners are:

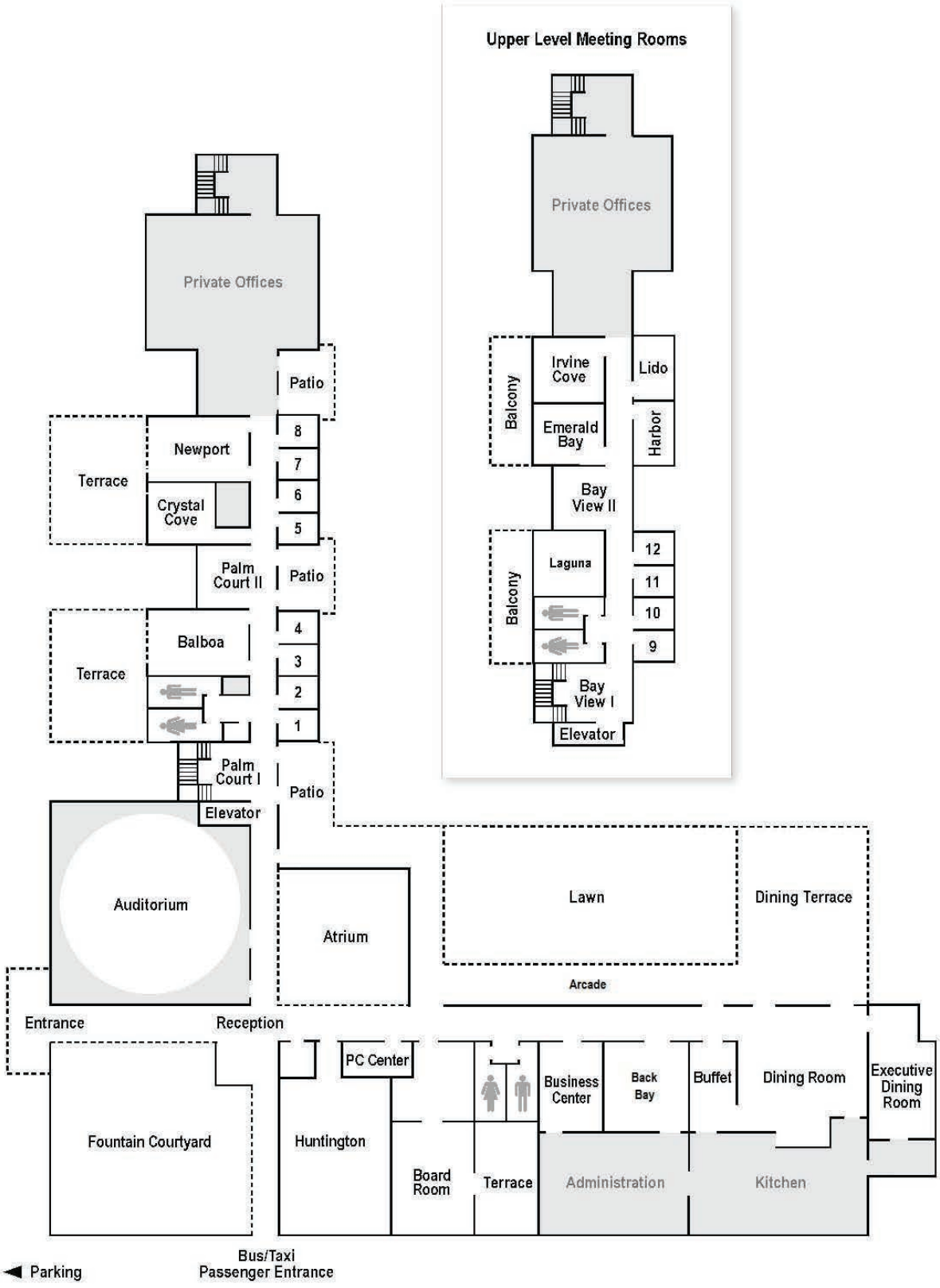
Stefan Jellbauer, PhD, Department of Microbiology and Molecular Genetics, Institute for Immunology at the University of California, Irvine: "Immunophenotyping of neutrophil subpopulations during Salmonella Typhimurium Colitis"

Brent Kern, BA, Pharmacokinetics, Dynamics, and Metabolism, Pfizer, La Jolla, California: "Receptor coccupancy and internalization of an anti-IL-7 receptor antibody"

Eric D. Diebold, PhD, Department of Electrical Engineering, California NanoSystems Institute University of California , Los Angeles: "Digitally-synthesized beat frequency multiplexing for ultra-high throughput imaging flow cytometry"

Christine M. Evangelista,MS, Department of Medical Sciences, Amgen, Thousand Oaks, CA: "Co-lyophilization of PGD2 assay control and quantum dot nanocrystals to trace experimental variables and analytes in a Whole Blood Flow Cytometric Assay"

Beckman Center Floor Plan



Please be sure to thank our sponsors.
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