RUTGERS

Molecular Biosciences

Graduate Student Organization

12th Annual MBGSO Research Symposium

Friday 23rd March 2018

9:30AM - 5:30PM

Atrium – Life Sciences Building

Busch Campus, Rutgers University

Table of Contents

Letter from Organizers

Welcome to the 12th Annual Graduate Student Symposium hosted by the Molecular Biosciences Graduate Student Organization (MBGSO) of Rutgers University. We are delighted to have you join us today to support the outstanding work produced by graduate students from various programs at Rutgers University.

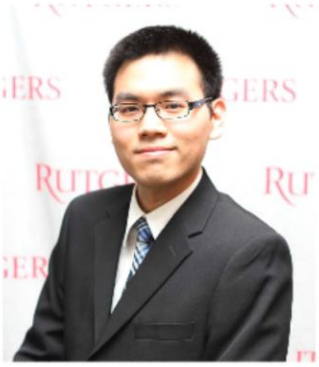
As a student organization, the goal of MBGSO is to facilitate the professional development of graduate students and promote opportunities for social interaction. By presenting their work to a critical audience, graduate students are able to hone important presentation skills and receive input on their work from faculty and peers from various departments. With these goals in mind, we organize the annual symposium and look to the university community to make it a success.

Through this symposium, we hope to not only showcase graduate student research, but to also provide a platform for professional interaction between students and faculty. We are proud to be able to provide an avenue for students from different fields of bio-science research to showcase their scientific endeavors. Through both oral and poster presentations, we seek to highlight some of the outstanding basic and translational research conducted here at Rutgers.

We want to thank Dr. Daria J. Hazuda for being our keynote speaker. We appreciate this opportunity to share her research and experience as an accomplished scientist. We also want to take this opportunity to express our gratitude to our faculty advisor, Dr. Janet Alder, for her incredible guidance and support during the planning process of this and other events throughout the year. We also want to thank the graduate student offices of the Rutgers School of Graduate Studies (Graduate Programs in Biomedical Sciences) for their help and support. We gratefully acknowledge the faculty members who have taken time out from their busy schedules to participate as judges and give students feedback on their work. this event would not be possible without the participation of our fellow graduate students and we want to we applaud their efforts and thank them. We would like to offer a very special thank you to all our generous sponsors for supporting graduate student research at Rutgers. Thank you for joining us and we hope you enjoy your time spent here today!

Sincerely,

MBGSO Executive Board 2017-2018

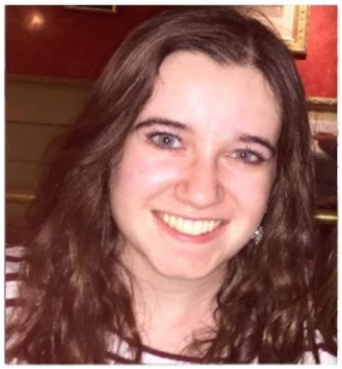


Victor
President

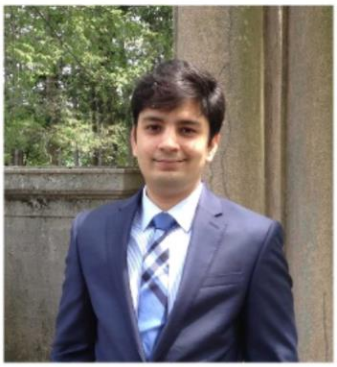
Agata
External Vice President

Vaidhy
Internal Vice President

Eve
Treasurer



Jenna
Development Officer



Vrushank
Secretary

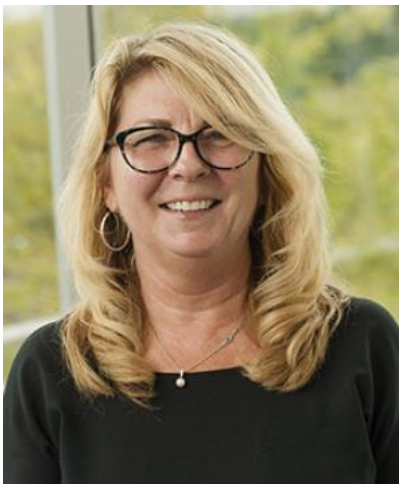
Sierra
Program Coordinator

Symposium Schedule

9:30 - 10:00AM	**Registration and Introduction**
10:00 - 11:00AM	**Oral Presentations I**
11:00 - 12:00PM	**Poster Presentations I**
12:00 - 1:00PM	**Keynote Address**
1:00 - 2:00PM	**Lunch**
2:00 - 3:00PM	**Poster Presentations II**
3:00 - 4:00PM	**Oral Presentations II**
4:00 - 4:30PM	**Award Ceremony**
4:30 - 5:30PM	**Networking event (LSB, 2nd floor Landing)**

Keynote Speaker

"HIV Drug Discovery: Past, Present and Future"



Dr. Daria J. Hazuda

Vice-President and
Therapeutic Area Head, Infectious Disease And Vaccines
CSO Merck Cambridge Exploratory Science Center
Merck Research Labs

Dr. Daria Hazuda, trained as a biochemist at the State University of New York at Stony Brook, N.Y. After completing her postdoctoral research fellowship in the department of Immunology at Smith Kline, she joined the antiviral group as a Senior Research Biochemist at Merck in 1989. Daria is currently Vice President of Infectious Disease and Vaccines at Merck Research Labs and Chief Scientific Officer of MRL Cambridge. Daria has over 20 years of experience in drug discovery and development with more than one hundred-eighty publications focused primarily on antiviral research in the fields of HIV and HCV. She led the research efforts that identified the first-in class HIV integrase inhibitor Isentress which was awarded the Prix Galien in 2008 and was responsible for pioneering work on HCV drug resistance enabling the discovery of agents with improved spectrum and efficacy including the NS5A inhibitor Elbasvir and the NS3 inhibitor Grazoprevir. Daria has been recognized with the Bernie Field Lecture Award, the David Barry DART Achievement Award for HIV Drug Development and is a Fellow of the American Society of Microbiology.

Daria is on the editorial board of the ACS Journal on Anti-infectives Research and the Journal of Viral Eradication. She is currently on the Scientific Program Advisory Council of the American Foundation for Aids Research (AMFAR) and The Forum for HCV Collaborative Research and a past member of NIH Aids Research Advisory Committee (ARAC) and the NCI Basic Sciences Board of Scientific Counselors (2010-2015).

ORAL PRESENTATIONS:

Morning session

1. Requirements for Processive Selenocysteine Incorporation

Pinkerton, MH; Sumangala, S; Copeland, P

A UGA stop codon is recoded to accommodate the incorporation of the 21st amino acid selenocysteine (Sec). For UGA to be recoded a specialized set of cis and trans factors are required and consist of: an mRNA with an in frame UGA codon, a selenocysteine insertion sequence (SECIS) in the 3' untranslated region, a SECIS binding protein 2 (SBP2), a specific translation elongation factor (eEFSec), and a selenocysteine tRNA. The N-terminus of SBP2 is believed to have no direct role in Sec incorporation because the C-terminus of SBP2 is sufficient for the incorporation of Sec into selenoproteins that have one Sec codon. Selenoprotein P (SELENOP) is an essential selenoprotein for male fertility and proper neuron function. SELENOP is also unique in that it contains 10 selenocysteines and is involved in selenium uptake and transport. Interestingly, an in vitro translation system in wheat germ, which has no endogenous selenocysteine incorporation factors, is not able to make full length SELENOP even when all the known factors are added. This contrasts with mammalian systems where full-length protein is predominantly found. This observation points to a specialized factor specific for SELENOP synthesis. We utilized in vitro translation in wheat germ lysate in order to determine the exitance of such a factor and to determine possible candidates for Selenoprotein P. We then used CRISPR knockouts in order to evaluate the importance of the factor in vivo.

2. Correction of Point Mutations by a Novel Base Editing Technology

Juan-Carlos Collantes; Huiting Xu; Katarzyna Tyc; Jinchuan Xing; Shengkan Victor Jin

Precise genome editing such as correction of point mutations requires introduction of targeted DNA double strand breaks (DSB) and activation of homology dependent repair (HDR), limiting its application to proliferating cells. To expand GE capabilities for therapeutic use in non-dividing somatic cells it is necessary to precisely modify nucleotides avoiding DSBs. Here we present a precision GE system able to edit specific nucleotides independent of DSBs. We engineered a nuclease-deficient CRISPR/Cas9 system as recruitment platform for non-nuclease DNA/RNA editing enzymes that catalyze C→T conversions by cytidine deamination. Targeted nucleotide modification was achieved with high precision in prokaryotic and eukaryotic cells. In bacteria, we tested our system targeting the rifampicin resistance determining region of the rpoB gene. Survival in rifampicin reached over 1000-fold higher than untreated cells. Sequence analysis of isolated colonies revealed targeted C→T mutations in codons known to induce rifampicin resistance. To examine whether the system can correct loss of function mutations in mammalian genome, we treated a stably integrated non-fluorescent EGFP gene containing an A→G mutation on the chromophore sequence. Fluorescence was restored in approximately 2% of cells. Sequencing confirmed G→A conversion at the target position, restoring the wild type sequence. Exome-wide sequence analysis revealed no detectable off-target effects. Taken together, the data show that our GE system represents a safe and promising technology for

correcting genetic mutations independent of DSB and HDR, with potential therapeutic value in non-dividing cells.

3. Towards a 3D Ultrasound Imaging System for Needle Guided Minimally Invasive Procedures

Mwikirize, C.; Hacihaliloglu, I.; Nosher, J.L.

Minimally invasive procedures such as percutaneous biopsies and regional anesthesia require image-guided insertion of a needle toward an anatomical target. Ultrasound is preferred due to its low-cost, radiation-free and real-time capabilities. However, needle visibility is usually impeded by signal losses between the needle and the transducer, difficulty in aligning the needle in the scan plane, and high-intensity artifacts such as bone. Inaccurate needle localization reduces efficacy of clinical procedures and could cause serious injury. Hardware advances such as mechanical needle guides, needle tracking systems and specialized needles increase cost of the imaging system and alter standard clinical workflow. To address this challenge, the focus of our work is to develop robust, accurate, automatic and real-time image enhancement techniques for needle localization in ultrasound guided interventional procedures. By modeling ultrasound signal transmission and coupling it with advanced reconstruction methods for B-mode image data from ex vivo experiments, we have achieved accurate needle localization in 2D ultrasound. Using machine learning, we have shown that a similar approach can be extended to needle localization in 3D ultrasound. By applying deep learning, we have achieved needle localization for imperceptible needles in 2D ultrasound. Work is ongoing to validate the developed methods on clinical data and achieve real-time image processing. Our work provides means for a new ultrasound imaging platform to support minimally invasive procedures. The developed algorithms will be applicable to commercially available 2D/3D cart-based and portable ultrasound systems, thus benefitting clinical practice, research and industry.

4. Determining parameters to model EGFR signaling dynamics in the developing Drosophila egg chamber

Revaitis, N.T.; Pouradier Duteil, N.; Marmion, R.A.; Niepielko, M.G.; Piccoli, B.; Yakoby, N.

Organogenesis requires the coordination among multiple cell signaling pathways to develop tissues into functional organs. While many have studied the overall impact of ligands and their associated signaling pathways, the mechanisms behind the distribution of ligands remains widely unknown. Here, we develop a mathematical model to understand the variables that shape the distribution of the TGF-α like ligand, Gurken (GRK), during Drosophila oogenesis. The GRK molecule binds to the epidermal growth factor receptor (EGFR) and subsequently activates the signaling pathway in the overlying follicle cells. By monitoring EGFR activation through diphospho-ERK (dpERK), we can determine how genetic perturbations impact signaling and the consequent changes on the Drosophila eggshell. Our model focuses on the dynamic distributions of GRK and dpERK during mid-oogenesis. The parameters used were extrapolated from literature or quantified using analyses on genetic perturbations. However, we are still left with three unknown variables: the rate of internalization of the ligand-receptor complex (Kec),

the diffusion of GRK in the perivitelline space (D), and the quantity of receptor (R0). Using CRISPR-Cas9, we developed a stable fly line that has an endogenously GFP labeled EGFR. We used this fly to follow the localization of the ligand and the dynamic interactions with the EGFR. We detect changes in the distribution of the receptor in the presence of high levels of EGFR signaling. The interplay between quantitative data and modeling aids to establish a model to study the dynamics and diversity of EGFR signaling during eggshell formation.

Afternoon session

5. Influenza infection decreases tumor burden in the lungs and promotes anti-tumor immune responses

Newman, J.H.; Aspromonte, S.M.; Bommareddy, P.K.; Aboelatta, M.; Herzog, N.L.; Zloza, A.

Recently, immunotherapies have provided improved clinical benefit to cancer patients. Unfortunately, long-term regressions have been observed only in a small proportion of patients. One reason for this may be that the focus of these immunotherapies is to boost anti-tumor immune responses. However, based on the similarity between self and tumor antigens and the efforts of the immune system to limit autoimmunity (inappropriate response to self), anti-tumor immune responses are inherently weak. Boosting such weak responses may not be sufficient to effectively treat cancer. Conversely, anti-pathogen immune responses are inherently strong, but their use as a treatment for cancer has been insufficiently studied. Therefore, we chose to study whether non-oncogenic, non-oncolytic viral infections (which constitute the majority of pathogens) could be employed to promote anti-tumor immunity. Towards this, we intravenously challenged C57BL/6 mice with 120,000 cells of the murine melanoma cell line, B16-F10, and intranasally infected with 1×10^6 pfu of influenza A/PR8/H1N1 five days later. These mice experienced both tumor development and infection in the lungs. Mice were sacrificed by 14 days after tumor challenge, and lungs were harvested for analysis. Mice concomitantly challenged with influenza and melanoma exhibited fewer tumor foci in the lungs, relative to that observed in mock-infected controls ($p<0.01$). Further, these concomitantly challenged mice demonstrated a significant increase in the proportion of tumor-specific $CD8^+$ T cells and higher concentration of IL-12 in the lungs. Our results propose that influenza infection may be harnessed as a tool to promote anti-tumor immune responses for the treatment of cancer.

6. How neurons collect and throw out their trash: The discovery of C. elegans aggresome-structure

Meghan Lee Arnold; Ryan Guasp; Ilija Melentijevic; Dr. Barth Grant; Dr. Monica Driscoll

A major health challenge to aging and diseased neurons is the maintenance of protein folding quality. Neurons can internally degrade some aggregated and misfolded proteins, but these mechanisms can become defective with age and in neurodegenerative disease. Human and mammalian studies indicate that some aggregates escape affected neurons to “infect” neighboring cells, but the transfer mechanism is unknown. We recently documented a previously undiscovered phenomenon in which toxic cytoplasmic components are selectively

extruded from C. elegans neurons in a large vesicle: the exopher (Nature, Melentijevic 2017). Exophers, produced in response to proteostatic stress, appear to help rid the neuron of potentially toxic cellular 'trash' and return to proteostatic balance. I study the cell biology of how neurons first collect their cellular trash and then how they throw it out on a scheduled 'trash day.' I found that neurons collect protein aggregates into an intermediate-filament cage structure similar to mammalian 'aggresomes', an organelle not-yet described in C. elegans . I find that these aggresome-structures can shed from the neuron and can be included in exopher vehicles. We have identified several motor and cytoskeletal proteins that participate in collection/expulsion. It is critical to understand: 1) how the neuron 'cleans house', 2) how the collected aggregate species travel (and potentially propagate) through the brain landscape, and 3) how these aggregated species are later handled by receiving tissues after shedding. The processes I study in C. elegans may be analogous to brain-wide toxic protein spread in neurodegenerative disease cases.

7. Probing mesenchymal stem cell responses based on actin-dynamics

Mishra, P; Gray, David, Moghe, P.V.

The assessment of mesenchymal stem cell (MSC) differentiation is critical prior to their applications in regenerative medicine. The traditional end-point methods rely on in-vitro induction of a small batch of cells for several days. This approach is based on the flawed assumption that the MSCs consist of homogeneous cell populations. This lack of proper MSC characterization is further aggravated by ongoing senescence and heterogeneity associated with prolonged cell culture leading to inconsistent clinical outcomes. We report a novel method for faster characterization of MSCs in culture by quantifying their real-time actin dynamics during diverse cell functions. Actin cytoskeleton is known to have functional roles in several cellular activities such as locomotion, differentiation, proliferation, aging, structural support and mechanotransduction. Due to actin ubiquity and its highly dynamic nature, we hypothesized that its turnover dynamics could be used for dynamic tracking of cellular activities. The current methods for quantifying actin turnover are limited to short-duration studies with immobile cells. The new method described here overcomes these limitations; it uses a reversible F-actin labeling probe, whose decay kinetics indicate the intracellular actin dynamics status in live cells. We used cytoskeleton perturbing drugs to validate if altered actin dynamics could be probed. Next, we measured differentiation associated altered actin dynamics within minutes. Furthermore, this method was amenable to long-term actin dynamics measurements in 3D microenvironments. Using our method, aged MSCs could be distinguished from young cells based on altered actin dynamics. This quick and robust method could be used to predict MSC fates based on very early cytoskeletal dynamics and has the potential to be extended to other in-vitro studies where temporal tracking of actin dynamics is required.

8. Re-presenting Tumor Antigens to the Immune System Using Nanoparticle Technology Improves Cancer Outcomes

Ricardo Estupinian; C. Brent Chesson; Jai S. Rudra; Andrew Zloza

Each cancer and each patient's immune responses to cancer are unique. Therefore, efforts are currently underway to combine precision medicine and immunotherapy towards identifying unique and targetable tumor antigens (including neoantigens). These efforts are expected to lead to the development of personalized vaccines for each patient based on these antigens. However, this process currently is expensive, laborious, and there is a lack of understanding which and how many antigens should ultimately be targeted. Therefore, we have developed a single-step nanoparticle antigen presentation system (SNAPS) by coupling plasma membrane and cytosolic antigens of individual tumors to nanoparticles. In vivo application (peritumoral injection) of the SNAPS coated with autologous tumor antigen results in regression of early tumors and delayed tumor growth of established melanomas. Treatment with SNAPS stimulates anti-tumor immune responses by increasing the presentation of tumor antigens within the tumor microenvironment. Our discovery provides a step toward personalized cancer immunotherapy using nanoparticle biotechnology coupled with a patient's own tumor antigens without a priori knowledge of such antigens.

POSTER PRESENTATIONS:

1. Design, synthesis, and biological evaluation of novel Keap1 Nrf2 PPI inhibitors
Dhulfiqar Abed; Xia Wen; Lauren M. Aleksunes; Longqin Hu

The Keap1–Nrf2–ARE system is an important antioxidant defense mechanism that protects cells from oxidative stress-related diseases. The Keap1–Nrf2 protein–protein interaction (PPI) has become a promising therapeutic strategy for a number of inflammatory diseases and oxidative stress conditions including pulmonary fibrosis, chronic kidney disease, chronic obstructive pulmonary disorder, and cancer chemoprevention. A number of potent inhibitors of Keap1–Nrf2 PPI has been reported, and one of these inhibitors that has been approved by the FDA is Tecfidera® to treat patients with relapsing multiple sclerosis. There are two types of inhibitors of Keap1–Nrf2 PPI which are direct and indirect inhibitors. The indirect inhibitors are electrophilic species and might cause "off-target" side effects. Recently, scientists have been focused on the development of direct inhibitors of Keap1–Nrf2 PPI. We are focusing on discovery of potent Keap1−Nrf2 PPI inhibitors by performing a comprehensive SAR (structure activity relationship) in a series of compounds based on its naphthalene scaffold. We used FP assay to test out compounds activity in vitro, and the most active compound were tested using cell based assays. Based on cell based assay, the most potent compound may have a potential therapeutic activity for treatment of inflammatory diseases by direct activation of the Keap1-Nrf2-ARE pathway.

2. Pavement System
Hiba Al-Adhami; Nenad Gucunski

One of the most important parameters to evaluate layered systems, like pavements, is the modulus of elasticity. Efforts have been made towards modulus evaluation using in situ nondestructive testing (NDT), in particular using the air-coupled Spectral-Analysis-of-Surface-Waves (SASW) method. The air-coupled SASW is an extension of the traditional SASW method, where the leaky surface waves are detected using non-contact sensors, instead of the Rayleigh waves using contact sensors. The main objective of this study is to develop an automated system for pavement modulus profiling using air-coupled acoustic testing. A numerical simulation of the air-coupled SASW test was conducted using finite elements. Several hundred hypothetical pavement configurations were used to develop an extensive database of surface wave dispersion curves. The database was further used to develop an artificial neural network (ANN) for an automated pavement modulus backcalculation. Good performance of the developed ANN in the inversion of surface wave data is demonstrated on several pavement profiles.

3. Copper Stress in Staphylococcus aureus Involves Perturbing Metal Ion Homeostasis
Hassan M Al-Tameemi; Jeffrey. M. Boyd

Staphylococcus aureus continues to be a threat to public health indicated by increasing morbidity and mortality rates as a result of infection. Antibiotic resistant strains, including

methicillin resisting strains (MRSA strains), are particularly worrisome. Microbes require metals such as iron, zinc, copper, or manganese for various biological function. During the phagolysosome killing process, vertebrates developed complex mechanisms to limit bioavailability of some transition metals to pathogens, but simultaneously flooding the phagosome vesicle with others. The macrophages limit iron, manganese, and zinc but simultaneously bombard the pathogen with copper. Therefore, homeostasis of these metals is vital for the survival of all human pathogens. Furthermore, copper and copper alloys are toxic and can kill pathogens instantaneously making it suitable intrinsic antibacterial for preventing retransmission of infection in the health facilities. Due to its significance during infection, pathogens developed sophisticated mechanisms to respond and mitigate copper effect. The persistent necessity for iron by pathogens is due to the great number of proteins that exploit the versatile redox potential of this inorganic element. The iron uptake processes is mediated by the iron dependent ferric uptake regulator (Fur). Fur represses regulated genes under iron repletion by binding to a consensus DNA sequence named Fur box sequence. To better understand copper response in S. aureus, we investigated the mechanisms by which copper toxifies cells. We built a strain that is defective in copper detoxification and we found that it exhibits sensitivity to copper. The data presented show that copper interferes with biological functions related to iron-sulfur cluster synthesis and that Fur regulon is derepressed upon Cu insult. We also found that the presence of Fur is important for the response against copper. A S. aureus strain lacking Fur had increased sensitivity to copper assault aerobically and anaerobically suggesting that a fur mutant is not killed at a greater rate because of Cu generated ROS. We also build a transposon library in the copper detoxification mutant background and were able to determine some transposons insertional knockouts that mitigated copper lethality. Of these, disruption of the manganese MntABC ABC importer allowed for increased survival against copper. Overexpressing MntABC caused increased copper sensitivity. Inactivation of mntR, the transcriptional repressor of MntABC, increased copper sensitivity. This sensitivity was mitigated in the mntR mntA double mutant. We hypothesize that iron homoeostasis is important element in copper toxicity and that MntABC has a role in copper uptake.

4. Neural progenitors derived from Tuberous Sclerosis Complex patients exhibit attenuated PI3K/AKT signaling and delayed neuronal differentiation

Zucco, A. J.; Pozzo, V. D.; Afinogenova, A.; Hart, R. P.; Devinsky, O.; D'Arcangelo, G.

Tuberous Sclerosis Complex (TSC) is a disease caused by autosomal dominant mutations in the TSC1 or TSC2 genes, and is characterized by tumor susceptibility, brain lesions, seizures and behavioral impairments. The TSC1 and TSC2 genes encode proteins forming the TSC complex, which is a major regulator of mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling, a pathway that plays an important role in promoting cell growth. It has been previously reported that TSC1/2 loss of heterozygosity (LOH) and the subsequent loss of TSC complex regulatory activity in null cells is responsible for mTORC1 dysregulation and TSC-associated brain lesions. However, it is not presently clear whether TSC1/2 heterozygous brain cells are abnormal and contribute to TSC neuropathology. To investigate this issue, we

generated induced pluripotent stem cells (iPSCs) derived from TSC patients and unaffected controls and utilized these to obtain neural progenitor cells and differentiated neuronal cells in vitro. We found that patient-derived TSC2 heterozygous neural progenitors did not display obvious defects in proliferation or viability but were delayed in their ability to differentiate into neurons. Patient-derived progenitor cells also exhibited modest activation of mTORC1 signaling downstream of TSC, and attenuation of upstream PI3K/AKT signaling. We further show that pharmacologic AKT inhibition, but not mTORC1 inhibition, causes a neuronal differentiation delay, mimicking the patient phenotype. Together these data suggest that heterozygous TSC2 mutations disrupt neuronal development, potentially contributing to the disease neuropathology, and that this defect may be due to dysregulated AKT signaling in neural progenitor cells.

5. Dispersing Listeria monocytogenes Biofilm on Food Contact Surfaces by Non-Ionic Surfactants

Albayati F; Chen D; Takhistov P.

The current cleaning methods which are used in the food processing industries are not always sufficient to prevent or disperse the Listeria monocytogenes biofilm cells. The formation of the biofilms has enabled the L. monocytogenes to survive in different types of unfavorable conditions hence making the process of eliminating them from such surfaces to be difficult. The poor management of these biofilm cells has therefore led to the development of different challenges regarding the fulfilment of the expected safety and high quality of the food products. This has therefore posed serious health-related threats to the customers. To prevent or limit these risks, it is necessary to ensure food safety in the food processing industries by creating conditions which do not favor the thriving of the L. monocytogenes on the surfaces of the equipment used in the food processing. In this experiment, the effectiveness of different types of nonionic surfactants such as Pluronic F68, Pluronic F127, Tween 20, Tween 40, Tween 80 and Brij 58 against the Listeria monocytogenes biofilm cells formed on food contact surfaces made of Low-Density Polyethylene (LDPE), Polypropylene (PP), Low-Density Polyethylene and Polypropylene (LDPE-PP), Low-Density Polyethylene and Ethylene Vinyl Acetate (LDPE-EVA), Stainless-Steel and Aluminum was determined. The quantification of the amount of the L. monocytogenes biofilm cells that were destroyed by the nonionic surfactants was done using the Acridine Orange staining (AOS) method after different time intervals of 1, 5, 15 and 30 minutes.

6. Quantitative Modeling of Essential Oil Nanoemulsion against Listeria monocytogenes in the Presence of Casein Protein as a Food Matrix

Almansoori D; No D; Takhistov P

Listeria monocytogenes is a food borne pathogen causes listeriosis,which can be acquired from processed food that have been contaminated . The reason for using EOs is to preserve food by inhibiting the growth and proliferation of microorganisms in food due to its antimicrobial effects (Perricone, Arace et al. 2015). The antibacterial effects of the essential oils derived from the

extracts of cedar wood, copaiba, fire needle, frankionce, Egyptian geranium, nutmeg, peppermint, valerian and Yiang Yiang against the Listeria monocytogenes, was investigated in the presence and absence of different, 17.59, 35.17 and 70.34 µg/ml, concentrations of the casein proteins.Various concentrations of essential oils were introduced into Brain Heart Infusion broth (BHI) to determine the minimum inhibitory concentration (MIC) for the pathogen To quantitatively evaluate the effect of each oil on L. monocytogenes from a kinetic viewpoint, the experimental data were fitted to the modified Gompertz model, and the lag phase duration and maximum growth rate were calculated and compared .The influence of the food matrix, casein proteins, was tested and determined. The effectiveness of the essential oils in absence of casein proteins and in the presence 17.59 µg/ml of casein proteins was simillar but further decreased in addition of 35.17 and 70.34 µg/ml of casein proteins. This was made possible based on the fact that in the presence of high concentration, 35.17 and 70.34 µg/ml, of casein proteins, there was the formation of the physical barrier which prevented the direct contact between the essential oils and Listeria monocytogenes.

7. MEK inhibition enhances oncolytic herpes virus immunotherapy through increased immune inflammatory gene signature and augments melanoma specific CD8 T cell mediated anti-tumor immunity

Bommareddy PK; Aspromonte S; Zloza A; Kaufman HL

Background: Herpes simplex virus, type 1 encoding Granulocyte-macrophage colony-stimulating factor (T-VEC) is the first and only FDA approved oncolytic virus for the treatment of melanoma. In this study, we sought to determine how MEK inhibition enhances tumor cell lysis and determine the therapeutic effects of combination in syngeneic melanoma model. Methods: Melanoma cells were treated with T-VEC and/or MEK kinase inhibitor (MEKi/trametinb). Cell viability was assessed by MTS assay. Viral replication was measured by plaque assay. For in vivo experiments, B6 mice bearing D4M melanoma tumors were treated with T-VEC and/or MEKi. Statistical analysis was performed using the student's t test and $P<0.05$ considered significant. Results: Combination(T-VEC/MEKi) significantly increased melanoma cell death by enhanced viral replication and increased apoptosis. In syngeneic melanoma model combination lead to decreased tumor growth, increased survival and increased melanoma antigen specific CD8 T cells. Combination effects were dependent on CD8+ T cells and resulted in immunologic memory. Gene expression analysis demonstrated that the combination lead to induction of a distinct immune inflamed signature supportive of lymphocyte recruitment to the tumors. T-VEC/MEKi seem to be dependent on Batf3 (Basic Leucine Zipper Transcriptional Factor ATF-Like 3), in Batf3-/- mice we observed significant loss in CD8+ T cells and survival benefit was lost. Combination also lead to increase in exhaustive markers, Program death ligand-1 (PD-L1) and (Program cell death protein-1) PD-1. Finally, adding PD-1 blockade to T-VEC/MEKi increased survival benefit, curing 80% of treated mice. Combining these therapies can be directly translatable to clinic.

8. Investigating the phosphoproteome of prostate cancer in response to treatment with a PI3K pathway inhibitor (LY3023414) and enzalutamide

Cheng, L. C.; Drake, J. M.

Prostate cancer is the most common and is the second leading cause of death related to cancer of men in the United States. Because prostate cancer is driven by androgen receptor signaling, androgen deprivation therapies are effective for primary disease. However, late stage prostate cancer, called metastatic castration-resistant prostate cancer (mCRPC), is lethal with an expected survival of only a couple of years. Current treatment options are limited to second-generation antiandrogens and taxane-based chemotherapy. Therefore, developing novel therapies is an important clinical need. The PI3K-Akt-mTOR pathway is frequently altered in cancer, sending signals for cell growth and survival. PTEN is a negative regulator of PI3K signaling. In prostate cancer, PTEN loss occurs in 42% of clinical cases and PI3K signaling as a whole is altered in a majority of mCRPC cases. There are currently several PI3K pathway inhibitors undergoing early phase clinical trials for prostate cancer. One dual inhibitor of PI3K and mTOR, LY3023414, is in a phase II trial with concomitant treatment with enzalutamide, a competitive AR inhibitor. We will utilize shotgun mass spectrometry (MS) to interrogate how LY3023414 transiently affects the phosphoproteome with and without enzalutamide. We will also investigate how the phosphoproteome changes with long-term treatment to identify possible mechanisms of resistance. From these findings, future work will develop a targeted phosphoproteomic assay to predict the effectiveness of combination therapy that target both AR and PI3K signaling.

9. Understanding the impact of P-glycoprotein on CNS disposition of ondansetron

Manting Chiang; Leonid Kagain

Purpose: P-glycoprotein (P-gp) is a known efflux transporter that plays a role in the tissue distribution of multiple compounds. P-gp can have an especially influential role for central nervous system (CNS) penetrating compounds that are subject to efflux at the blood brain barrier (BBB), such as ondansetron. Understanding the differences in tissue disposition, especially in the CNS, based on P-gp expression is valuable, but there is limited data available. The purpose of this work was to investigate tissue disposition of ondansetron in wild-type and Mdr1a knockout rats using plasma and cerebrospinal fluid (CSF) concentrations, and to capture and simulate the profiles for both strains using the PBPK multicompartment brain model in Simcyp Simulator. Methods: A single dose of ondansetron hydrochloride (10 mg/kg) was administered as an IV bolus to wild-type Sprague-Dawley (SD) rats and Mdr1a knockout SD rats via jugular vein cannula. Plasma samples were serially collected over a pre-specified time course; CSF samples were collected terminally. HPLC assay was developed for both plasma and CSF quantification (LOQ 10 and 5 ng/mL respectively). The data were then plotted and used for overlay onto Simcyp simulations. To utilize Simcyp, a compound file for ondansetron was built using data sourced from literature. Full-body PBPK model was used, with Method 2 (1, 2) predictions for Kp values for all tissues. An in vitro intrinsic clearance obtained from rat liver microsomes was fixed at 212 µL/min/mg protein (3), and a renal clearance of 0.22 mL/min (3)

was fixed. Plasma profiles were simulated and overlaid onto in vivo data to assess recapture of original data. After assessing the accuracy of the model, the multicompartment brain model was then used, and the Batch Processor function was utilized for the PSB (mL/min) and Pgp CLint (µL/min/fmol transporter) term to identify the best combination of values for those parameters to recapture in vivo CSF disposition between KO and WT. Results: Concentration-time profiles of ondansetron in plasma were not significantly different between wild-type and knock out strains. However, two strains showed differences in the distribution of ondansetron into the CSF. Mdr1a knockout resulted in higher concentrations of ondansetron and a similar terminal slope to that found in wild-type animals. The Simcyp simulations were able to adequately recapture the pharmacokinetics of ondansetron in plasma using parameter values obtained from literature. The Batch Processor function allowed for multiple simulations of CSF profiles based on a pre-specified range of parameter values for PSB and CLint. A PSB of 0.1 mL/min appeared to best capture profiles for both WT & KO; however, a CLint of 0.1 and a CLint of 0.3µL/min/fmol appeared to best simulate the CSF profiles for wild-type and Mdr1a knockout respectively. Conclusion: The effect of P-gp on drug penetration to the CNS has been investigated in wild-type and Mdr1a knockout rodent model. Observing raw CSF profiles between strains, KO rats observed higher ondansetron concentrations in CSF over entire time course. The kinetics of tissue disposition was described using Simcyp simulations, in particular the multicompartment brain model. These efforts will be further developed in the future to predict CNS disposition in humans.

10.Mediterranean Lifestyle And Gut Microbiota: A Holistic Approach

Sidossis, LS; Campbell, SC; Psarra, G; Georgoulis, M

Adherence to the Mediterranean diet, recommended as a healthy dietary pattern according to the 2015 Dietary Guidelines, has been shown to be beneficial to gut microbiota composition. Additional benefits may be experienced when complying not only with the Mediterranean dietary pattern, but also with the Mediterranean lifestyle (ML), including increased physical activity, socializing, conviviality, adequate rest and stress-relief. The aim of this project is to decipher how adherence to the ML impacts gut microbiota conformation. Since the ML encompasses antioxidant rich foods, physical activity and stress reduction, we hypothesize that adherence to a ML ameliorates host health by promoting an anti-inflammatory microbiota. We present here the experimental design of this project for which we have recently acquired funding and we have submitted for IRB approval. Sixty (n=60) participants will be divided into three groups: 1) elderly adhering to ML (n=20); 2) elderly not adhering to ML (n=20); and 3) young controls (n=20). Participants will come to the lab for two visits: visit 1- qualification for study entry, including assessment of medical files/history and latest routine check-up. Qualified candidates will receive instructions for completing a 5-day weighed food diary and using the fecal collection device. During visit 2, participants will provide the completed 5-day weighed food diary and the fecal sample to the study group, will give blood and will undergo an anthropometric assessment. We hypothesize that adherence to all aspects of the ML may create

a synergistic effect on promoting a healthy microbiota, which could be important for successful aging.

11. Microbial and Metabolic Responses to Exercise in C57 Wild-type and Adenylyl Cyclase 5 KO Mice

Dowden, R.A.; Wisniewski, P.J.; Guers, J.; Oydanich, M.; Vatner, S.F.; McGuinness; Kerkhof, L.; Campbell, S.C.

Healthful aging has been observed in our adenylyl cyclase type 5 knock out (AC5KO) model. Aging is known to alter the composition and diversity of the gut microbiota, however an optimal microbiota for health remains to be described. In this study, we examined microbiota in AC5KO and wild type (WT) mice in both exercise and sedentary conditions. 17 (n=6/group) 6-week old C57BL/6J male AC5KO and WT mice were randomly assigned to: (1) WT-exercise (WT-EX), (2) WT-sedentary (WT-S), (3) AC5-exercise (AC5-EX) and (4) AC5-sedentary (AC5-S). Mice underwent a treadmill test to determine maximal oxygen uptake (VO2max) and running distance. Exercise training consisted of treadmill running at 60-70% VO2max for 60-minutes 5 days/week for 5 weeks. Following training, mice received oral antibiotics to eliminate gut microbiota. DNA was extracted from fecal material using a modified CTAB extraction. Ribosomal amplicons containing the 16S rRNA subunit, 23S rRNA subunit and the internal transcribed spacer (ITS) were constructed and sequenced using the Oxford Nanopore MinION. Finally, glucose tolerance (i.p; 2 ul/kg [BW]) and insulin tolerance (i.p;1 ul/kg [BW]) testing were assessed. Measurements were taken: 1- prior to exercise, 2- post training/pre-antibiotic and 3- post antibiotic. AC5KO mice showed a unique microbiota with Helicobacter typhlonius & Bacteroides sartorii spp. being dominant only in AC5KO. Furthermore, AC5-EX mice showed altered glucose tolerance (33325 vs. 23025 AUC, $p<0.05$) and reduced exercise performance (517m vs. 258m, $p<0.05$) following antibiotic treatment. AC5KO mice demonstrate unique microbiota compared to WT mice and their enhanced phenotype may be dependent on the microbiota.

12. Tag, You're It: Using Stable Isotope Probing and DNA Sequencing to Determine Metabolically Active Microbiomes

Gadkari, P; Kurz, D; Kerkhof, L; Haggblom, M

Microbes are the most numerous and most metabolically diverse organisms on the planet, and live in almost all environments. With the rise of cost-effective DNA sequencing, culture-independent studies of microbes are amenable, though can be misleading since presence of microbes does not always indicate activity. Here, we present a novel approach to not only to determine microbiomes, but to determine metabolically active microbes by using stable isotope probing (SIP) and 16S rRNA gene sequencing. Amendments of a ubiquitous stable isotope will be metabolized by active microbes in an environment and will incorporated in biological molecules such as DNA. DNA from active microbes can separated by ultracentrifugation, and metabolically active microbes can be identified by DNA sequencing. Pharmaceutical industries can leverage this underused ecological tool for major gaps in therapeutic areas in determining

how therapeutics are impacted by patient microbiomes, and vice versa. To show these combined methodologies are viable, we have determined the metabolically active microbiome of the Arctic tundra soils at subzero temperatures. Tundra soil microcosms from Finland were amended with 13C-Cellobiose (a common tundra C-substrate) and incubated at 0, -5, and -16°C for 5-40 weeks. Certain bacterial family members were detected to be only active during specific subzero ranges, demonstrating that even when the soil is frozen, subzero temperature changes affects metabolically active bacterial composition, and potentially function. While this study has crucial findings for climate-change and global geochemical cycles, its methodologies can also be applied to numerous biomedical systems in digestive, respiratory, skin microbiomes, and disease-states.

13. Repeated mild traumatic brain injury; genetic risk factors and personalized treatment approaches

Giarratana, A.O.; Teng, S.; Zheng,C.; Thakker-Varia, S.; Alder, J.

Traumatic Brain Injury (TBI) is a serious and potentially life threatening problem. Clinicians have long noticed that certain patients recover better after TBI and we seek to identify genetic differences underlying these differences. Our lab has previously shown that after TBI, the pro-Brain Derived Neurotrophic Factor (BDNF) pathway is upregulated while the mature BDNF pathway is downregulated, which may play a role in preventing recovery. In this study, we sought to determine the effect of specific single nucleotide polymorphisms (SNPs) in the pro region of BDNF on recovery after TBI. We have investigated cellular and behavioral outcomes in mice with the BDNF Val66Met polymorphism relative to the more common Val66Val allele following repeated, mild lateral fluid percussion injury. We show that Met carriers have a larger injury volume as assessed by MRI and increased levels of neurodegeneration, apoptosis, p- tau, microglia and gliosis in the cortex compared to Val carriers at 1 and/or 21 days post injury (dpi). We have performed rotarod and balance beam testing to examine sensorimotor ability, and found no differences across genotypes. We have also done novel object recognition testing and preliminary data shows that Met carriers have worse memory. To gain insight into the mechanism of action of the cellular differences, we performed western blot analysis and found Met carriers have more proBDNF and less mature BDNF than Val carriers. We conclude that the Met allele is a risk allele after rmTBI, and are now testing a personalized therapy to rescue the genetically susceptible individuals.

14. Treating Metabolic Diseases with Safe Mitochondrial Uncoupler

Jingjing Guo, JG; Hanlin Tao, HT; Amer Alasadi, AA; Shengkan Jin, SJ

Mitochondrial uncoupling allows proton gradient bypassing ATP synthase across the inner membrane of mitochondria. The proton leakage results in lower energy efficiency and elevates lipid oxidation. By modulating cellular metabolism, mitochondrial uncoupling could be a favorable approach for treating metabolic diseases including diabetes, nonalcoholic fatty liver diseases (NAFLD) and lipid abnormalities. Niclosamide ethanolamine (NEN) is a salt form of an FDA-approved anthelmintic drug and is a safe mitochondrial uncoupler. Our previous work

demonstrated that NEN is primarily distributed in mouse liver, where it diminishes liver steatosis, increases insulin sensitivity and improves diabetic symptoms. We further examined the effect of NEN on preventing and/or treating nonalcoholic steatohepatitis (NASH) and hypercholesterolemia. NASH is an advanced stage of NAFLD progressing from benign hepatic steatosis and so far no pharmacotherapies were approved for treating NASH. We found that the long-term preventive treatment of NEN significantly reduces hepatic steatosis, hepatocytes ballooning, inflammation and fibrosis in a NASH mouse model induced by long-term western diet. Hypercholesterolemia is one of the most common lipid abnormalities, which is a major risk factor of atherosclerosis and coronary heart disease. We found NEN consistently improves hypercholesterolemia induced by either Apolipoprotein E deficiency or high-fat diet in mice. The cholesterol-lowering potency of NEN is comparable to that of statins and the mechanism of NEN might be different from that of statins. Overall, our studies demonstrate a novel approach for treating metabolic diseases by modulating cellular metabolism and identified NEN as a prototype drug.

15.The Microbiome of Vitamin A Deficiency

Honarbakhsh, M.; Ericsson, A.; Malta, K.; Chikindas, M.; Breslin, P.; Lackey, A.; Storch, J.; Isoherranen, N.; Quadro, L.

Diet is a critical driver of gut microbial community, and malnutrition, especially during childhood, affects development and maintenance of a healthy microbiota. A healthy intestine is critical to support a “healthy” microbiome which in turn helps maintaining proper intestinal functions in a feedback loop. Vitamin A-deficiency (VAD) is a major public health problem. The essential nutrient vitamin A regulates intestinal functions by modulating inflammation, cell proliferation and immunity. Thus, the VAD status has impact on intestinal health and leads to various pathological conditions of the gut. We investigated the microbiome of a mouse model of VAD. Lrat-/-Rbp-/- mice lack lecithin:retinol acyltransferase (LRAT) and retinol-binding protein (RBP), thus they cannot store or mobilize hepatic vitamin A towards the periphery of the body. Hence, they rely exclusively on dietary intake to support vitamin A-dependent functions, and when deprived of dietary vitamin A, they become severely vitamin A deficient. At 6 weeks of age, Lrat-/-Rbp-/- and WT mice were placed on either vitamin A sufficient or deficient diet for 28 days. Feces were collected throughout the experiment to perform microbiome analysis by genomic sequencing of the bacterial 16S rRNA. We confirmed that VAD impairs intestinal morphology and functions. Also, our preliminary data indicate that the vitamin A status significantly impacts the composition of the intestinal microbial communities. Specifically, the VAD status seems to be associated with an expansion of the pro-inflammatory Bacteroides sp., and a reduced percentage of the Actinobacteria, including the health-promoting probiotic Bifidobacterium sp., in the fecal microbiome.

16.Inhalable Particulate Matter Suppresses Human Immunity to Mycobacterium tuberculosis
Ibironke, O.A.; Carranza, C; Sarkar, S; Schwander, S

INTRODUCTION: Tuberculosis (TB) and air pollution both contribute significantly to global morbidity. The mechanisms by which exposure to 'real-world'-derived urban ambient particulate matter (PM) affects Mycobacterium tuberculosis (M.tb)-specific host defenses in vitro are understudied. METHODS: Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors in New Jersey. Ambient PM2.5 (aerodynamic diameter <2.5µm) were collected from Mexico City. To assess PM2.5 effects on M.tb growth control by human immune cells, PBMC were exposed to PM2.5 at final concentrations of 0, 1, and 5µg/mL and incubated at 370 C for 18 h followed by infection with M.tb. On days 0 (1h), 1, 4, and 7, cells were lysed and serial cell lysate dilutions plated in triplicate on M.tb growth media and incubated at 370 C for 21 days for colony forming unit (cfu) assays. PM2.5-induced changes to cellular viability were assessed by lactate dehydrogenase (LDH) assay. RESULTS: M.tb growth control (cfu) assays on days 1, 4 and 7 showed significantly ($p < 0.05$) higher M.tb cfu numbers in PM2.5-exposed M.tb-infected PBMC than in unexposed PBMC. Expression of intracellular IFN-γ and TNF-α (cytokines required for protective antimycobacterial host immunity) in M.tb-infected PBMC (by flow cytometry) was decreased upon PM2.5 exposures. PM2.5 exposure also downregulated the expression of the early T-cell activation marker CD69 but upregulated the expression of the immunosuppressive cytokine IL-10 in M.tb-infected PBMC. CONCLUSIONS: PM2.5 exposure leads to loss of intracellular growth control of M.tb and mitigates the expression of protective human host immune cell responses.

17.Salicylate metabolism by the dioxin and dibenzofuran degrading organism Sphingomonas wittichii RW1
Ivanovski, I.; Eleya, S. ; Zylstra, G. J.

The polycyclic aromatic hydrocarbons dibenzo-p-dioxin (DD) and dibenzofuran (DF) are among the most pervasive pollutants found in both terrestrial and aquatic environments. DD and DF are persistent in the food chain due to their lipophilic and hydrophobic properties. DD and DF both contain two benzene rings, but DD contains two bridging oxygen atoms while DF has only one bridging oxygen atom. Sphingomonas wittichii RW1 is capable of fully degrading both DD and DF by similar catabolic pathways to carbon dioxide and water. Metabolism of dibenzofuran begins with a multicomponent dioxygenase (oxygenase, ferredoxin, and reductase) forming an unstable dihydrodiol which rapidly ring-opens to form 2,2',3-dihydroxybiphenyl. Cleavage of the dihydroxylated biphenyl ring by a meta-ring cleavage dioxygenase followed by carbon chain cleavage by a hydrolase results in salicylate and a five carbon fragment. Further metabolism of salicylate in S. wittichii RW1 is currently unknown but of the two known salicylate degradation pathways it is suspected that metabolism continues through gentisate rather than through catechol. Three lines of evidence lead to this hypothesis: transcriptomic and proteomic experiments, localization of a suspected salicylate dioxygenase within the gentisate operon, and gene knockout data showing that the initial DD/DF dioxygenase and salicylate dioxygenase share a common ferredoxin and a reductase. We identified other genes and enzymes responsible for

salicylate metabolism in S. wittichii RW1 through the construction and testing of knockout mutations. Our results show that salicylate metabolism is a very complicated metabolic function in S. witiichii RW1.

18.Identifying The Role Of Hematopoietic Cell Kinase In High-Grade Serous Ovarian Cancer
Khella, C. A.; Gatza, M. L.

Ovarian cancer is the most lethal gynecological cancer and the fifth leading cause of cancer-related deaths in women. High-grade serous ovarian cancer (HGS-OvCa) accounts for 70% of ovarian cancer deaths and has a 31% five-year overall survival rate. Cytoreduction surgery and platinum/taxane-based chemotherapies are the standard-of-care therapy and while the majority of patients will initially respond, most will eventually relapse and succumb to their disease. Therefore, there is a significant need to define the underlying genetic causes of this disease in order to develop novel, rational therapeutic strategies. To identify altered signaling in poorly prognostic HGS-OvCa, we analyze orthogonal genomic and proteomic data from ~500 human tumors from the Cancer Genome Atlas (TCGA) and Clinical Proteomic Tumor Analysis Consortium (CPTAC) projects. These tumors were characterized by increased immune-related signaling, including high levels of macrophage-associated, CD68, CD44, and HCK activity. Analyses of phosphoproteomic data confirmed increased expression of pHCK and HCK-related signaling and CIBERSORT analysis indicate that poorly prognostic tumors have increased pro-tumorigenic M2 macrophage levels. These data are consistent with previous studies linking HCK activity with macrophage polarization and recruitment. While HCK is not expressed in normal ovarian or gynecological tissue, we find that HCK mRNA expression is highest in micro-dissected cancer epithelium relative to normal epithelium, tumor-associated stroma or normal stroma. These data demonstrate that HCK is highly expressed in poorly prognostic HGS-OvCa epithelial cells, is associated with increased levels of pro-tumorigenic M2 macrophage activity, and may represent an essential and potentially drug-able driver of HGS-OvCa tumorigenesis and aggressiveness.

19.ATRX, DAXX or MEN1 mutant pancreatic neuroendocrine tumors are a distinct “alpha-cell signature” subgroup
Laddha, S.V; Lewis, P; Koletsky, M; Robzyk, K; Silva,E.D.; Untch, B; Allis, C.D; Tang, L.H; Chan,C.S

Pancreatic neuroendocrine tumors (PanNETs) are a rare neuroendocrine malignancy and current classification scheme for PanNETs include grade and stage. A greater understanding of the cells of origin of PanNETs, tumor progression and pathway pathogenesis may guide the development of novel therapeutic options. The most commonly mutated genes in PanNETs are ATRX, DAXX, and MEN1. Little is known about the cells-of-origin for non-functional neuroendocrine tumors. Here, we genotyped 64 PanNETs for mutations in ATRX, DAXX, and MEN1 and found 37 tumors(58%) carry mutations in these three genes(A-D-M mutant PanNETs) and this correlates with a worse clinical outcome than tumors carrying the wild-type alleles of all three genes (A-D-M WT). We performed RNA sequencing (n=33) and Illumina 450K DNA methylation (n=32) analysis on randomly selected PanNETs to reveal two distinct subgroups

with one group consisting exclusively of A-D-M mutant PanNETs. Pair-wise correlation of gene expression,showed A-D-M mutant PanNETs are more homogeneous as a group than A-D-M WT PanNETs.Two biomarkers differentiating A-D-M mutant from A-D-M WT PanNETs were high ARX gene expression and low PDX1 gene expression with PDX1 promoter hyper-methylation in the A-D-M mutant PanNETs. Moreover, A-D-M mutant PanNETs had a gene expression signature similar to that of alpha cells (pval

20.Automated Venipuncture Device for Accurate and Reliable Blood Draws

Leipheimer, J.; Balter, M.; Chen, A.; Maguire, T.; Yarmush, M.

Venipuncture, the process of obtaining intravenous access for either intravenous therapy or blood sampling, is a standard clinical procedure with nearly 2.7 million procedures performed daily in the U.S. alone. It is also the leading cause of both patient and practitioner injury in the clinic, resulting in an upwards of $5 billion dollars in difficult venous access (DVA) related complications in the U.S. annually. This is because identifying and successfully sticking the vein relies heavily on clinician expertise and patient physiology (dark skin, obesity, age, etc.). Our group at Rutgers has developed an autonomous, robotic venipuncture device that efficiently and safely performs the venipuncture procedure using robotics, ultrasound, and near-infrared (NIR) technology. Our device combines a near-infrared imaging system, computer vision software, ultrasound technology, and a 9 DOF robotics system to autonomously perform the venipuncture procedure, without human intervention. The device works by imaging and mapping in real-time the 3D spatial coordination of subcutaneous veins via NIR cameras in order to identify and detect the most optimal vein for cannulation. Once the 3D location of the vein insertion site is selected, an attached ultrasound probe is lowered over the insertion site to provide depth information for the needle insertion phase. High accuracy robotics are used to drive the needle tip into the center of the patient's vein. Results demonstrate sub-milliliter accuracy in needle placement, with a 100% first-stick venipuncture success rate in phantom arms and veins. Future and current work is involved with miniaturizing and simplifying the device into a hand-held, portable platform that can more readily implemented into everyday clinical practice.

21.Transcriptomic and proteomic analysis reveals the metabolism of Desulfoluna spongiiphila, a marine sponge associated bacterium

Liu, J; Adrian, L; Scheer, B; Haggblom, M

Discovery of marine natural products is a very promising field because of the clinic potentials of these natural products e.g. anticancer, antiviral, antimicrobial and other bioactivities. Marine sponges are one of the richest sources for these natural products, which could produce many natural halogenated compounds. Desulfoluna spongiiphila is the bacterium isolated from sponge Aplysina aerophoba, which are able to utilize the halogenated compounds as electron acceptor. But its metabolism and interaction with sponge host is still not clear. Using the RNA sequencing and proteomic techniques, we studied the global mRNA expression and protein expression of D. spongiiphila under the condition with or without halogenated compounds as

electron acceptor. The energy generation and central metabolism pathways of D. spongiiphila are proposed to reveal how it can live on the halogenated compounds produced by marine sponge and its microbiomes. The presence of abundant multidrug efflux pumps may play an important role for the survival of D. spongiiphila in marine sponge, which is rich in diverse bioactive products.

22.TRPM7 Ion Channel Regulates Mg2+ Transport in Renal Proximal Tubule
Lou, L; Runnels, L.

Mg2+ deficiency can be identified in up to 60 % of critically ill patients and contributes to many human diseases and conditions, including hypertension and diabetes. One cause of hypomagnesemia stems from phosphate depletion and hyperparathyroidism, but the mechanisms by which hypomagnesemia develops remains undetermined. Phosphate reabsorption in this nephron segment is mediated by sodium-phosphate co-transporters, which are bound to and regulated by NHERF1 and NHERF3 scaffolding proteins. Deletion of NHERF1 from mice increases urinary excretion of Mg2+. The TRPM7 ion channel, which is associated with hypomagnesemia with secondary hypocalcemia (HSH) disease, is expressed in the proximal tubule, but its functions in this nephron segment remain unknown. To determine the impact of TRPM7 channels on Mg2+ reabsorption in the proximal tubule, we generated mice lacking the channel specifically in proximal tubule by crossing TRPM7flox/flox mice with gGT-Cre transgenic mice. TRPM7-/-(gGT-Cre) mice have lower serum Mg2+ level and showed growth retardation, a symptom of hypomagnesemia, comparing to their litter mates. This data indicates that TRPM7 is involved in controlling Mg2+ reabsorption in the proximal tubule. We conducted a yeast two-hybrid screen using a mouse kidney library and identified NHERF3 as a potential TRPM7-interacting protein. Biochemical approaches were employed to confirm the interaction of TRPM7 with NHERF3 but also showed that TRPM7 has the capacity to interact with other NHERF family members, including NHERF1. The COOH-terminal six amino acids of TRPM7 constitute a PDZ binding motif required for channel binding with NHERF1. Our experiments also indicate that NHERF1 colocalizes with TRPM7 to the apical membrane in proximal tubule epithelial cells. We conclude that TRPM7 regulates Mg2+ reabsorption in the proximal tubule and that NHERF1 plays a critical role in the presentation of TRPM7 to the brush border of epithelial cells.

23.Gene Expression Analysis of Idiopathic Autism iPSCs and NSCs
Mehta, M.; Matteson, P.G.; DiCicco-Bloom, E.; Connacher, R.; Williams, M.; Prem, S.; Millonig, J.H.

The heterogeneity of autism, encompassing genetic, epigenetic and environmental factors make it a difficult disorder to study. Advances in stem cells have made it possible to reprogram somatic cells into iPSCs, allowing for the potential to model human diseases in vitro. For the present study, blood samples were collected from sex-matched sibling pairs, one with autism and one normal sibling control. T cells were then reprogramed into iPSCs and differentiated into neural stem cells (NSCs). 3 clones from each individual were picked to serve as biological replicates. Currently, three families have been analyzed thoroughly and robust autism-specific phenotypic

differences have been reported for proliferation, neurite extension and migration. Gene expression analysis using a 23 gene multiplex Luminex assay for NSC expressed genes is now being completed for both iPSCs and NSCs for all three families, and significant changes have been observed. In addition, gene expression analyses are also being conducted on a separate set of families focusing on a Chromosome 16 CNV deletion, 16p11.2, which increases risk for autism. A separate Luminex panel has been employed to measure the expression of the 27 deleted genes, in iPSCs and NSCs. Significant differences in gene expression have been observed in one family thus far, and these results are now being replicated to include additional pairs of families. Overall, this methodology allows for a more personalized approach for studying idiopathic autism, and may provide insight into how aberrant gene expression contributes to the autism-specific proliferation and differentiation phenotypes.

24. Prokaryotic Diversity and Function at a Newly Discovered Shallow-water Gas Vent Site in the Tyrrhenian

Patwardhan S; Giovannelli D; Vetriani C

Despite their wide distribution, shallow-water geothermal environments have not been explored as extensively as their deep-sea counterparts. Tor Caldara is a newly discovered shallow-water gas vent in the Tyrrhenian Sea, Italy. The study site is a shallow submarine gas vent off the coast with a maximum depth of 3 meters. There is a significant amount of outgassing of carbon dioxide and hydrogen sulfide. A unique feature of this vent site is the absence of a thermal anomaly. In this study, we surveyed the prokaryotic diversity of the established filamentous biofilms growing in the vicinity of the venting, encrustation seen around the orifice of the gas vents as well as the sediment in the venting area. Data show that the sediment community is most diverse and includes members of Proteobacteria, Acinetobacteria Bacteroidetes, Planctomycetes, Acidobacteria, Firmicutes, Cyanobacteria etc. The crust community is slightly less diverse comprising predominantly members of Proteobacteria, Cyanobacteria and Bacteroidetes. The established filamentous biofilm community is the least diverse and most specialized and is dominated primarily by Epsilonproteobacteria, Gammaproteobacteria and Bacteroidetes. In conjunction with the 16S rRNA-based assessment of diversity, we enriched and isolated bacteria using different culture conditions. Two novel bacterial strains, TC3T and TC8T, 92.76% and 91.25 % similar to Sulfurimonas gotlandica and Magnetovibrio blackemorei respectively, were isolated. TC8T was further characterized. The integration of culture-basedand molecular analyses is providing qualitative and quantitative insight into prokaryotic diversity and function at the shallow-water vent in Tor Caldara.

25. Ungual Delivery of Ketoconazole using Solid Lipid Nanoparticles

Puri, V.; Ramzan, M.; Trehan, S.; Kaur, IP; Michniak-Kohn, B

Introduction: Nail fungal infections affect about 20% of the global population with challenging treatments. Oral antifungals are associated with serious side effects while the topical therapy is not effective. Ketoconazole (KTZ) is a broad-spectrum antifungal, practically insoluble in water. It also produces redness and irritation on skin. The nail plate comprises high water content and

for significant nail permeability, high aqueous solubility is desired. We used Solid Lipid Nanoparticles (SLNs) to enhance water solubility of KTZ and to mask its irritation. Methods: KTZ-SLNs were prepared using hot high-pressure homogenization technique. Using design of experiments, the formulation was developed and optimized for maximal loading, entrapment and minimal size. Developed KTZ-SLNs and fluorescein probe labeled SLNs were applied on the human cadaver nails using Franz diffusion cells modified with nail adapters to determine permeation into the nails. KTZ was quantified in nails and cross-sections were observed using fluorescent microscopy. Topical safety of the formulation was established using MTT cell viability assay and inflammatory mediator/cytokine assay. Results: An optimized formulation with particle size of 336.2 ± 4.6 nm and high entrapment efficiency (84.6 %) was developed. Fluorescent images of nail sections showed penetration into all layers. Permeation studies revealed significant permeability of KTZ into nails when applied as SLNs. The MTT tissue viability assay showed remarkably higher relative viability and ELISA test demonstrated the absence of IL-1β secretion. Conclusion: The developed KTZ-SLNs could overcome the well-known topical irritation of KTZ and can be a successful ungual delivery carrier for fungal infections.

26. Synergistic Combination of Kinase Inhibitors with Enzalutamide against Advanced Prostate Cancer

Tan, V.M.; Patel, H.C.; Drake, J.M.

Each year, prostate cancer ranks among the highest in number of diagnoses as well as cause of death in men. The main culprit is advancement of the disease from a treatment-naïve (responsive) state to a lethal and metastatic treatment-resistant form known as castration resistant prostate cancer (CRPC). Aberrant kinase signaling is a common feature of this advanced stage disease. However, the benefits of using kinase inhibitors in clinical trials have been inconsistent. While some clinical studies show improved patient survival, others conclude minimal marginal benefit or even lack thereof. In order to clarify the kinase signaling networks that may drive disease resistance, we utilized a multi-omics approach consisting of quantitative phosphoproteomics, genomics, and transcriptomics to characterize tumors from treatment-naïve prostate cancer patients and CRPC patients. Subsequent analysis generated a hierarchal ranking of kinases predicted to be active in the CRPC cohort, thus providing possible targets for inhibition. We then generated similar kinase hierarchies for prostate cancer cell lines LNCaP, 22RV1, and DU145. Importantly, each cell line was predicted to be driven by a unique set of targetable kinases. To confirm our predictions, we performed drug synergy studies with compounds targeting the predicted kinases, SRC and DNA-PK. Combinations with standard of care enzalutamide yielded strong synergy in concordance with our predicted kinase hierarchies for the respective prostate cancer cell lines. Collectively, our results suggest that specific targeted combinations of kinase inhibitors with enzalutamide may inform more potent therapeutic options in CRPC. We are currently working on understanding the mechanism of synergy and the possible role of these kinases in androgen receptor signaling.

27.Structural Characterization of Reelin Using Cryo-Electron Tomography

Turk, L. S.; Kuang, X.; Patel, K.; Dai, W.; Comoletti, D.

Reelin is a glycoprotein, secreted by certain neurons including Cajal-Retzius and Cajal-Retzius-like cells, that serves important functions in neuronal migration and brain development. Its altered expression has been linked to various mental health disorders, such as ASD and schizophrenia. This roughly 400 kDa protein contains a signal peptide, an F-spondin-like domain, eight Reelin repeats (RR1-8), and a stretch of positively charged amino acids at the C-terminus. Reelin has a complex degradation pattern; after secretion, it is cut into 3 discrete fragments: the N terminal fragment containing RR1-2, the central fragment of RR3-6, and the C terminal fragment containing RR7-8. Previous inquiries have identified the apolipoprotein E receptor 2 and the very-low-density lipoprotein receptor as binding partners for the central fragment of the Reelin protein. While the structures of distinct RRs have been studied, the structure of the full length Reelin protein has not been solved, most likely as a consequence of its large size and apparent flexibility. However, the solution-based technique of cryo-electron microscopy has made possible the determination of protein structures that had not previously been possible using x-ray crystallography. Reelin, owing to its large size, is a good candidate for studies using cryo-ET, and here we report preliminary structural data using techniques in cryo-ET of full length Reelin and various deletion constructs of the protein to further our understanding of its architecture and function.

28.Corticostriatal inputs from somatosensory and motor cortex have distinct effects on behavior through differential actions on striatal neurons

Lee, C. R;, Wiskerke, J.; Yonk, A. J.; Paradiso, K.; Tepper, J. M.; Margolis, D. J.

The striatum is the main input nucleus of the basal ganglia and receives excitatory afferents from neocortical and thalamic areas. It is largely unknown, however, whether 1) afferent input from sensory and motor cortical areas have different behavioral effects and 2) how distinct cortical areas influence striatal neurons. Here, we explored primary somatosensory cortical (S1) and primary motor cortical (M1) roles in controlling behavior and the cellular mechanisms underlying these functions. Specifically, we tested whether S1 corticostriatal input induces sensory-driven behavioral response inhibition, while M1 input promotes behavioral response activation. Channelrhodopsin-2 (AAV1.CamKIIa.hChR2(H134R)-eYFP.WPRE.hGH) was expressed in either S1 or M1. Mice were trained to perform a Go/No-Go task using their whiskers to discriminate between two textures for a reward. Preliminary results indicate that S1 input activation increased the number of trials without a response (correct reject and miss), while M1 input activation increased trials with a response (hit and false alarm). We investigated the possible cellular mechanisms underlying these behavioral effects using ex-vivo whole-cell recordings of identified D1 and D2 receptor-expressing MSNs and parvalbumin (PV)-expressing interneurons by optogenetic activation of corticostriatal afferents from S1 or M1. Optogenetically activated S1 inputs induced a 7-fold larger EPSP in PV interneurons when compared to either D1 or D2 receptor-expressing MSNs. In contrast, optogenetic activation of M1 inputs produced an an EPSP of similar amplitude in D1, D2, and PV neurons. Our results

suggest that input from S1 and M1 induces behavioral inhibition and activation respectively, which is likely mediated through distinct activation of striatal circuitry by these cortical regions.

29. Modeling Multisystem Biological Effects of Oxidative Stress Resulting from Human Exposures to Air Pollutants

L. Chao; T. Nguyen ; D. Mukherjee; P.G. Georgopoulos

Human exposures to photochemical air pollutant mixtures containing ozone and fine/ultrafine particles constitute a persisting and widespread problem around the globe. In addition to causing respiratory effects, photochemical pollution also impacts the cardiovascular, immune, integumentary and other physiological systems. Dermal contact can be a significant exposure route, with skin being both a barrier and target organ for pollutants. Reactive contaminants exert their detrimental effects either directly or indirectly, via the generation of reactive oxygen species (ROS). For example, ROS are produced from reactions of ozone with skin lipids as a result of dermal contact and from reactions with protein and lipid components of lung lining fluid (pulmonary surfactant) as a result of inhalation. These secondary ROS initiate series of cascading events, such as release of pro- inflammatory mediators, infiltration of immune cells, activation of aryl hydrocarbon receptor (AhR) pathways, etc. Other physiological systems are subsequently affected: the respiratory-originated pro-inflammation mediators can enter the circulatory system, initiate neuroendocrine-immune crosstalk and subsequently affect heart rate variability and blood pressure. The present work demonstrates new interconnected mechanistic modules for the exposure biology of ozone and associated photochemical pollutants in the human integumentary, respiratory and cardiovascular systems. These modules are designed as components of the MENTOR (Modeling Environment for Total Risk) multiscale computational platform for whole- body human exposure, dosimetry, toxicokinetics and toxicodynamics. MENTOR has been under continuing development at the Computational Chemodynamics Laboratory (CCL) of EOHSI and employs a spectrum of systems dynamics modeling approaches, combining differential equation and agent-based methods to quantify overlapping Adverse Outcome Pathways (AOPs) involving multiple scales (biomolecular, cellular, histological, organ) and physiological systems and endpoints, resulting from multiple exposure routes.

30. The mechanism of organ shape control

Zhenru Zhou; Herve Alegot; Kenneth D. Irvine

Understanding the mechanisms controlling tissue growth to form correct organ shape is a long-standing question in developmental biology. Cells divide, rearrange and change shape to form organs of correct shape. Altered tissue patterning due to loss of Ds-Fat signaling pathway can cause abnormal organ shape, such as shorter and rounder wings and legs in flies, and mitral valve prolapse and skeletal malfunction in humans. In the developing Drosophila imaginal disc, it's been proposed that wing shape is established by a bias in the mitotic spindle orientation, leading to growth along proximal-distal axis. However, we found that in mud mutant Drosophila, adult wing shape is still elongated, while division orientation is random. Recent improvement on long-term time-lapse live imaging of epithelial cells and computational tools to store cellular

connectivity and geometry information helps to perform multi-scale analysis of morphogenesis. In this project, we hypothesize that a combination of cell rearrangement, cell shape changes and oriented cell divisions will contribute to organ shape.

31.RNA virus host range mutations epistatically effect evolvability

Zhao, L.; Seth-Pasricha, M.; Stemate, D.; Crespo, A.; Gagnon, J.; Duffy, S.

Evolvability is often defined as the ability to generate potentially adaptive variations. Although the high mutation rates and large population sizes of RNA viruses promote evolvability, epistasis is known to constrain or potentiate different viral genotypes. Unlike previous observations, we pinpointed to the pivoting step depicting the epistatic effects of host range mutations on subsequent host range mutational neighborhoods. Through both Sanger and Illumina sequencing, we were able to inspect and confirm the epistatic interactions during host range expansion of Pseudomonas RNA bacteriophage phi6. The mutational neighborhoods of phi6-WT and its isogenic mutants phi6-E8G and phi6-G515S (harboring one non-synonymous mutation each in attachment protein P3: E8G and G515S, enabling infectivity of two other hosts in addition to the host range of the WT) were compared when host jumping to a novel host P. syringae pv. atrofaciens (PA). Sanger sequencing of 50 clones per genotype showed significantly reduced mutational neighborhoods for both phi6-E8G and phi6-G515S in the P3 coding gene, suggesting extant host range mutations epistatically affect subsequent PA host range mutational neighborhood. The mutational neighborhoods were better mapped after Illumina sequencing, which confirmed our cloning results and identified unexpected loci contributing to host range expansion in phi6-WT. Although most host range mutations arise in P3 protein, this was not the case for many phi6-WT isolates jumping to PA. Morphogenic genes on the small segment were identified to also confer expanded host range. SNP calling of deep sequencing results showed E8G potentiating, while G5151S constraining PA host range mutational neighborhoods.

32.Building a Workflow for Oxford Nanopore Sequencing Data Analysis

Anbo Zhou; Timothy Lin; Jinchuan Xing

Sequencing technologies have evolved rapidly over the last twenty years. The first-generation sequencing technology, Sanger sequencing, suffers from its low throughput and high cost per base. The second-generation technology, typically represented by Illumina, improved on the throughput but only generates short reads that lack ability to address repetitive regions. Recently, the third-generation sequencing technologies, including the nanopore sequencing technology, have emerged to tackle the previous problems. For example, the nanopore technology solves the read length problem by allowing the DNA molecules run through nanopores in a flow cell. Base pairs are called based on the electrical current created by the process. The analysis workflow in the nanopore sequencing field is in the initial stage. It is undetermined that which steps should be taken and what tools should be used. The nanopore sequencing machine creates a huge raw signal file that needs to be either processed directly or transformed to the common FASTQ file format to allow downstream analysis. The alignment

algorithm that was built around Illumina's short, accurate reads need to be adjusted to nanopore's long, error-prone reads. Finally, the variant calling algorithm need to take advantage of the long read lengths and should allow more errors. Based on above mentioned reasons, we tested a variety of tools in the field that deal with nanopore sequencing data, such as GraphMap, BLASR, and BWA for alignment, and PBHoney, Sniffiles, and Lumpy for variant call. We addressed their performance and developed a workflow that we believe work the best for the new technology at the current stage. We expect to apply this workflow in multiple future projects and make improvements to it along the way.

33. Label-free Assessment of Subcellular Dynamics

Mohammad Naser; Rene S. Schloss; Nada N. Boustany

Light scattering by subcellular organelles and interfaces such as membranes can be utilized for quantitative measurement of cellular and tissue states. Structural information of the organelles can be inferred from light-scattering by analyzing the diffraction pattern at a conjugate Fourier plane of the imaging system. Via implementation of Gabor filters on the Fourier plane, we can selectively allow only certain angles of scattering to pass. These scatter angles are directly related to the spatial frequencies of the scattering source. So in effect, the Gabor filters can probe objects of certain size/shape and orientation. Based on this property, a morphometric parameter called Orientedness was previously reported to indirectly probe the geometric aspect ratio of subcellular organelles. In this work, we propose a technique to characterize morphological changes in a more organelle-specific way by applying an unsupervised segmentation of the subcellular organelles. The segmentation acts as a mask-generator for the Orientedness which can be used to observe the morphological change of the individual organelles over time. We also propose a modification of the original segmentation algorithm for biological samples using local-energy information. To demonstrate the applicability of the proposed approach, we have conducted calcium overload experiment which renders the long mitochondria divided and round through remodeling and/or fission. We have validated our experiments with MitoTracker labeled fluorescent images. If combined with other morphological parameters such as velocity and displacement, the proposed approach can be used to provide label-free quantification of subcellular dynamics.

34. Antioxidant Nanoparticles for Inhibition of α-Synuclein Fibrillization and Intracellular Aggregation in Microglia

Nanxia Zhao, Rebecca Chmielowski, Xue Yang, Tamr Atieh, Nicola Francis, Alysha Moretti, Yue Cao, Zhiping P. Pang, Kathryn E. Uhrich, Jean Baum, Prabhas V. Moghe

Abstract: Parkinson's Disease (PD) is one of the most common age-related neurodegenerative disorders, affecting an estimated population of seven to ten million worldwide. PD is characterized by aggregated and increased levels of extracellular α-synuclein (ASYN). Microglia, the primary immune cell in central nervous system, plays an important role in α-synuclein clearance and degradation. To reduce intracellular ASYN aggregation and microglia

inflammation, we propose the use of antioxidant nanoparticles (NP) to aid the delivery of intracellular aggregation-inhibiting antioxidants. First, we investigated the use of flash nanoprecipitation to form stable nano-assembly with antioxidant compounds. We showed that antioxidant nanoparticles have more efficacy in inhibiting ASYN fibrillization *ex vivo* compared with amphiphilic macromolecule nanoparticle used in previous studies. These NPs ameliorated intracellular ASYN oligomerization *in vitro* and reduced microglia activation. Overall, antioxidant NPs fabricated via flash nanoprecipitation are a promising tool to counteract microglia activation and control neuroinflammation in the context of PD and other neurodegenerative diseases.

35.Post transcriptional regulation of CD40L and its impact on the humoral immune response
Narayanan, BN, Covey, LR

The interaction between cognate T and B cells decides the progression of an immune response to an antigen or pathogen. Ligation of CD40 on antigen-experienced B cells is associated with the initiation and development of germinal centers (GCs) resulting in the generation of high affinity antibodies and B cell memory. To understand the biological basis for activation-induced posttranscriptional regulation of CD40L, a mouse was engineered with a deletion of the mRNA stabilizing PTBP1 binding sites (CD40LΔ5). B cells rely on repeated interactions with the Tfh cells to provide direction as to how to differentiate and proliferate to optimize the immune response. We hypothesized that these interactions would be disrupted in CD40LΔ5 mice at later stages of activation and subsequently result in the Tfh being unable to provide sufficient guidance to the B cells to evolve into effective antibody factories. We saw significantly reduced levels of high affinity and class switched antibodies in the mutant mice when compared to the wild type in response to antigen challenge and this was a consequence of reduced numbers of antigen specific plasma cells. We also saw that the percentage of memory cells was significantly reduced. Unlike CD40L knock out mice, the CD40LΔ5 mice were able to produce germinal centers but they were structurally less organized than the wild type. We conclude that since the early activation stage CD40L is not affected, seeding and initiation of germinal centers occurs, but at later stages the lowered CD40L results in less effective progress of the immune response.

Participants

Dhulfiqar Abed	1
Hiba Al-Adhami	2
Hassan Al-Tameemi	3
Firas Albayati	5
Dina Almansoori	6
Meghan Arnold	Oral 6
Praveen Bommareddy	7
Longfei Chao	29
Larry Cheng	8
Manting Chiang	9
Juan-Carlos Collantes	Oral 2
Anastasia Diolintzi	10
Robert Dowden	11
Ricardo Estupinian	Oral 8
Preshita Gadkari	12
Anna Giarratana	13
Jingjing Guo	14
Maryam Honarbakhsh	15
Olufunmilola Ibironke	16
Igor Ivanovski	17
Christen Khella	18
Saurabh V Laddha	19
Josh Leipheimer	20
Jie Liu	21
Liping Lou	22
Monal Mehta	23
Prakhar Mishra	Oral 7
Cosmas Mwikirize	Oral 3
Bitha Narayanan	35

Mohammad Naser	33
Jenna Newman	Oral 5
Sushmita Patwardhan	24
Mark Pinkerton	Oral 1
Vinam Puri	25
Nicole Revaitis	Oral 4
Victor Tan	26
Liam Turk	27
Alex Yonk	28
Lele Zhao	31
Anbo Zhou	32
Nanxia Zhao	34
Zhenru Zhou	30
Avery Zucco	4

Notes:

Notes:

Sponsors

Many thanks to our sponsors for making this event possible.