KGI KECK GRADUATE INSTITUTE

PhD Virtual Presentation Day

Friday, January 15, 2021 | 9:00 a.m.–5:00 p.m.
Via Zoom: kgi.zoom.us/j/95676482957

Presentation Agenda

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Time	Topic
9:00–9:15 a.m.	**Welcome and opening remarks** Sheldon Schuster, KGI President **PhD overview** Jim Sterling, PhD Program Director
9:15–10:30 a.m.	**Keynote Speaker Jeffrey Esko, Professor of Cellular and Molecular Medicine and Co-Director of the Glycobiology Research and Training Center at UCSD** "SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2"
10:30–11:00 a.m.	**PhD students: two minute lightning round on project research** 1. David Kent 2. Jason Lee 3. Mandar Makwana 4. Jonas Otoo 5. Noa Park 6. Dhruv Patel 7. Jonalyn Herce 8. Christine Urrea 9. Abrar Al Maghribi 10. Payam Amiri 11. Tochukwu (Dubem) Anyaduba 12. Karen Yrene Paco Mendivil 13. Joshua Yang
11:00 a.m.–5:00 p.m.	**Phd students oral presentations**
11:00–11:20 a.m.	Jonalyn Herce
11:20–11:40 a.m.	Dhruv Patel
11:40 a.m.–12:20 p.m.	Dubem Anyaduba
12:20–12:40 p.m.	Christine Urrea
12:40–1:00 p.m.	Noa Park
1:00–1:40 p.m.	Abrar Al Maghribi
1:40–2:00 p.m.	Jonas Otto
2:00–2:40 p.m.	Joshua Yang
2:40–3:00 p.m.	Mandar Makwana
3:00–3:40 p.m.	Karen Mendivil
3:40-4:00 p.m.	Jason Lee
4:00-4:40 p.m.	Payam Amiri
4:40-5:00 p.m.	David Kent

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Keynote Speaker

Jeffrey Esko, PhD
"SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2"

About the Speaker

Jeffrey Esko, PhD, is currently a Distinguished Professor of Cellular and Molecular Medicine and Co-Director of the Glycobiology Research and Training Center at UCSD. His research focuses on understanding the structure, biosynthesis, and biological roles of proteoglycans in mammalian cells and model organisms. Notable scientific contributions include his discovery of the dependence of tumor formation on heparan sulfate, the discovery and development of the first small molecule inhibitors of heparan sulfate, the action of proteoglycans as receptors for hepatic lipoprotein clearance and for delivery of therapeutic agents. Esko cofounded Zacharon Pharmaceuticals which was acquired by Biomarin in 2013, TEGA Therapeutics, and Covicept Therpaeutics. He received his PhD in Biochemistry at the University of Wisconsin and was a faculty member at the University of Alabama Birmingham before moving to the Department of Cellular and Molecular Medicine at UCSD in 1996. He has published over 300 scholarly papers, reviews, and book chapters, and was editor/author of the first textbook in the field of Glycobiology, *Essentials of Glycobiology*. This also became one of the pioneering textbooks to be distributed electronically freely online.

About the Presentation

The COVID-19 pandemic, caused by SARS-CoV-2, has swept across the world, resulting in serious clinical morbidities and mortality, as well as widespread disruption to all aspects of society. Previous studies established that the receptor for viral infection is Angiotensin-Converting Enzyme 2, which docks with the viral spike protein. Recently, we showed that SARS-CoV-2 spike protein interacts with both cellular heparan sulfate and ACE2 through its Receptor Binding Domain (RBD). Docking studies suggest a heparin/heparan sulfate-binding site adjacent to the ACE2 binding site. Both ACE2 and heparin can bind independently to spike protein in vitro and a ternary complex can be generated using heparin as a scaffold. Electron micrographs of spike protein suggests that heparin enhances the open conformation of the RBD that binds ACE2. On cells, spike protein binding depends on both heparan sulfate and ACE2. Unfractionated heparin, non-anticoagulant heparin, heparin lyases, and lung heparan sulfate potently block spike protein binding and/or infection by pseudotyped virus and authentic SARS-CoV-2 virus. We suggest a model in which viral attachment and infection involves heparan sulfate-dependent enhancement of binding to ACE2. Variation in the structure or content of heparans sulfate could explain the variable susceptibility of different cell types to SARS-Cov-2 and the stratification of infection according to sex and age. Manipulation of heparan sulfate by the microbiome or inhibition of viral adhesion by exogenous heparin presents potential opportunities to interfere with infection.

Presentation Details

Noa Park

Antimicrobial Preservatives for Vaccines Delivered as Multi-Dose Products

David Kent

Serum-free media for Adipose-derived MSC for Exosome production

Jonas Otoo

Lab-on-a-chip for Multiplexed Medical Diagnostics

Dhruv Patel

GPCRs role in TH17 pathogenicity, functions and development of Autoimmune disease

Jason Lee

Modeling fluidic shear stress found in various bioprocess systems and its effect on cells

Mandar Makwana

Characterization of upstream processes using computational fluid dynamics (CFD) as in-silico method and real time online monitoring systems

Jonalyn Herce

Understanding the role of old and young exosomes derived from erythrocytes and plasma in aging

Christine Urrea

Raman spectroscopy models for monitoring and controlling cell culture metabolites

Abrar Al Maghribi

Point of Care Infectious Disease Diagnosis via Isothermal Nucleic Acid Amplification Integrated into a Sample to Answer Device

Presentation Abstract: Sexually transmitted diseases caused by Chlamydia trachomatous (CT) and Neisseria gonorrhea (NG) remain a major global public health concerns causing over 351.7 million infections per year. Dengue fever is a mosquito born acute illness caused by Dengue Virus (DENV), is also a rising global public health concern. Proper diagnosis of these infections facilitates proper patient management which reduces long term complications and mortality, and helps prevent disease spread. Point of care sensitive and specific diagnostics are essential for low resource settings. We have developed a prototype system consisting of a cartridge and compact instrument that can execute sample preparation, isothermal Loop Mediated amplification (LAMP) and lateral flow detection of these pathogens. For CT and NG, we have established singelplex and duplex LAMP assays, in liquid and paper form, with lateral flow detection. We have performed inhibition testing, demonstrated full process execution outside the cartridge, then implement the process in the cartridge and device, demonstrating detection down to 100 EB/mL for CT and 100 CFU/mL NG in urine. For DENV, we established a pan-serotype singleplex RT-LAMP assay and a duplex assay with MS2 as internal amplification control, in liquid and paper form. We demonstrated that the master-mix in paper form is stable upon storage at RT and 40oC over 8 weeks. For sample preparation starting from whole blood we performed plasma separation coupled to amplification, with inhibitor testing. The next step is a front to back experiment and implementation in a modified cartridge for the detection of all DENV serotypes. The herein described assay processes and prototype systems can be further developed to enable point of care diagnosis for these and other pathogens in low resource settings.

Karen Yrene Paco Mendivil

Targeting Intrinsically Disordered Domains and Heparin-binding Motifs for the Development of SARS CoV-2 Vaccine Candidates

Presentation Abstract: The coronavirus disease of 2019 was caused by the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) and has posed a worldwide public health emergency. Currently, noticeable efforts have been put into controlling the pandemic that includes the design of effective vaccines and the development of accurate diagnostic tools. In this urgent need to develop a vaccine, we have performed extensive in-silico and structural analysis to predict antigenic regions of the SARS CoV-2 spike protein. The most antigenic regions were fused to Qβ virus-like particles and are being tested in mice for safety and efficacy analysis. Interestingly, antigenic regions predicted by computational algorithms show a high disordered content and are of intrinsically disordered regions (IDPRs). Previously it has been reported that glycans and other post-translational modifications might stabilize IDPRs; hence we have found computationally glycan-binding sites (GBMs) within the SARS CoV2 spike that correlate with IDPRs. Many of these domains are related to high in-silico predicted antigenicity scores. These predictions make IDPRs and GBM sites potential targets for the design of peptide-based vaccines and the development of new diagnostic tools.

Joshua Yang

The Pharmacokinetic Profile of a High Affinity Transferrin Receptor Antibody-Erythropoietin Fusion Protein Following Intraperitoneal and Subcutaneous Routes of Administration

Presentation Abstract: Objective: Erythropoietin is a potential therapeutic for Alzheimer's disease with limited blood-brain barrier permeability. The transferrin receptor monoclonal antibody fused to erythropoietin (TfRMAb-EPO), a chimeric monoclonal antibody, ferries erythropoietin into the brain. TfRMAbs have Fc-effector function-related adverse effect. To overcome this, an effectorless TfRMAb-EPO fusion protein (TfRMAb-N292G-EPO) was developed. The mutant fusion protein displays accelerated plasma clearance and reduced plasma concentrations compared to the wild-type (WT) fusion protein. The aim was to characterize the pharmacokinetic profile of TfRMAb-N292G-EPO.Methods: C57BL/6J mice were injected with a dose (3-20 mg/kg) of TfRMAb-N292G-EPO through subcutaneous (SQ) or intraperitoneal (IP) route. Plasma concentrations were determined at differential timepoints post-injection from ELISA. Mice were sacrificed 24-hours post-injection, and terminal blood was collected for a complete blood count. Brains were harvested for comparisons.Results: Plasma pharmacokinetic differences between both routes were observed. Dose escalation from 3-20mg/kg increased the plasma Cmax for both routes. SQ plasma Cmax were lower compared to IP. Brain concentrations in 3mg/kg injected mice of the mutant fusion protein were significantly lower than the WT treated mice. Reticulocyte suppression increased with an increase in dose. Conclusions: Elimination of the Fc N-linked glycosylation site reduces plasma exposure of TfRMAb-N292G-EPO. The low-plasma concentrations of the mutant fusion protein result in negligible brain uptake following 3 mg/kg dose. Reticulocyte suppression rescue by N292G mutation is a function of plasma AUC and negated at high doses. High doses are needed to achieve concentrations comparable to the WT fusion protein.

Dubem Anyaduba

Design & Development of Unit Processes for A Primer Payload System for Multiplex Loop-mediated Isothermal Amplification

Presentation Abstract: The impact of antimicrobial resistance is a re-emerging public health concern with a menacing global impact. According to the CDC, in the US alone, antibiotic-resistant infections result in more than 35,000 deaths annually. Antibiotic stewardship has been embraced as a strategy to improve prescription effectiveness; however, diagnostic turnaround time presents a limitation. With rapid point-of-care (POC) molecular diagnostics (mdx), these limitations can be assuaged, and prescriptions can be more precise for diseases with single etiologic agents such as tuberculosis, syphilis, etc. However, for polymicrobial diseases such as urinary tract infections, rapid POC mdx become more complex and requires multiplex screening platforms. Ideally, isothermal mdx methods are preferred, with loop-mediated isothermal amplification, LAMP, as our choice. However, current rapid mdx multiplexing methods are not ideal for LAMP. To exploit LAMP in the diagnosis of polymicrobial diseases in POC settings, we have designed an innovative primer-payload system that would exploit the principles of microfluidics, bead-based spectral sharing, and droplet-digital nucleic acid amplification. These principles synergistically will ensure the identification of multiple (>6) nucleic acid targets (DNA) in the same sample stream without spectral crosstalk, and non-specific target amplification.

In this talk, I will be discussing the overview of the primer payload system, our design of its unit systems, and our journey so far in developing them.

Payam Amiri

The Development of a Microfluidic Organ-on-a-Chip to Explore the Effects of Young and Old Erythrocytes on the Blood-Brain Barrier

Presentation Abstract: Blood exchanged between heterochronic partners has demonstrated that old blood has a detrimental effect on the brain health of the young animal. The primary causes are largely attributed to soluble blood factors, neglecting the role of blood cellular components, specifically erythrocytes. As we age, these cells undergo significant morphological changes, affecting how erythrocytes respond to a high shear stress environment. To explore this phenomenon, we have engineered a microfluidic brain-on-a-chip (μBBB) to monitor the effect of erythrocytes derived from an old animal on the integrity of the blood-brain barrier (BBB). The system was co-cultured with endothelial b.End3 and astrocytic C8-D1A cells. BBB integrity was monitored by the passage of FITC-dextran and trans-endothelial electrical resistance (TEER) across this synthetic BBB. The results indicate increased permeability and diminished TEER of the μBBB with the introduction of erythrocytes derived from an old animal. This effect was significantly more pronounced compared to the introduction of young animal derived erythrocytes. These results suggest a previously unknown, old erythrocytes induced detrimental effect on the integrity of the BBB which can play a role in the passage of systemic inhibitory molecules into the brain tissue resulting in brain health deterioration.

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