January 2023 STANFORD IMMUNOLOGY SCIENTIFIC CONFERENCE

Monterey Tides
2600 San Dunes Drive
Monterey, CA 93940

Stanford MEDICINE Immunology
School of Medicine

Agenda & Abstracts

Friday, January 27 – Sunday, January 29, 2023

Stanford Immunology Quick Facts

Program launch
- 1988

Directors:
- Irving Weissman (Pathology/Developmental Biology)
- Hugh McDevitt (Microbiology and Immunology/Medicine)
- Larry Steinman (Neurology)
- Pat Jones (Biology)
- Olivia Martinez (Surgery, as of July 2018)

Director of the Institute for Immunology, Transplantation, and Infection (ITI)
- Mark Davis (Microbiology and Immunology)

Stanford MEDICINE Immunology
School of Medicine

Welcome to the January 2023 Stanford Immunology Program's Annual Conference at Monterey Tides!

We are pleased to present what promises to be an exciting retreat, loaded with presentations by faculty, staff, postdocs, and graduate students from across the Stanford Immunology community. Talks will be held in Points Ballroom. The Poster Session will be in La Grande Ballroom. Talks are divided into sessions of common theme with innovative research that crosses traditional boundaries at Stanford University School of Medicine. You won't want to miss a single talk! There are plenty of breaks to re-caffeinate, rehydrate, sugar up, and network. Faculty talks are 15 minutes, and graduate student, postdoc and staff talks are 10 minutes each. We will be enforcing the time limits to provide at least 5 minutes for questions, related insights, and to catalyze discussions throughout the weekend.

We are honored to have two **KEYNOTE SPEAKERS**

Elina Zuniga, PhD
Professor at UCSD
https://labs.biology.ucsd.edu/zuniga/

Peter Kim, PhD
Professor of Biochemistry at Stanford
https://peterkimlab.stanford.edu/

Elina Zuniga, PhD received her Ph.D. in Biochemistry from the National University of Cordoba, Argentina. She conducted postdoctoral research at The Scripps Research Institute where she held two post-doctoral fellowships from Antorchas Foundation and PEW Charitable Trust. After joining UCSD in 2007 she has received a Hellman Foundation Scholar Award, The Vilcek Finalist Prize for Creative Promise, the Leukemia and Lymphoma Society Scholar Award and the American Cancer Society Scholar Award (a lifetime honor). Prof. Zuniga is also co-founder of Global Immunotalks and has been elected for the American Association of Immunologists Vanguard Lecture and for the American Academy of Microbiology Fellowship. She is stable member of the “Virology B” Study Section at the National Institute of Health as well as member of the American Association of Immunologists Fellowship Committee.

Peter S. Kim, PhD is the Virginia and D.K. Ludwig Professor of Biochemistry at Stanford University School of Medicine, where he is also a member of Bio-X and an Institute Fellow at ChEM-H (Chemistry, Engineering & Medicine for Human Health). Prior to his arrival at Stanford served as President of Merck Research Laboratories at Merck & Co. Kim has a special interest in HIV/AIDS research, and his research has focused on design of compounds that stop membrane fusion by the AIDS virus, thereby preventing it from infecting cells. He has also pioneered efforts to develop an HIV vaccine based on similar principles. While at Merck, he oversaw the development of more than 20 new medicines and vaccines, including treatments for diabetes, cervical cancer, HIV, shingles, and hepatitis C. His current service includes the Medical Advisory Board of HHMI, the Scientific Advisory Board of the NIH Vaccine Research Center, the Scientific Advisory Board of the Harvard Medical School Program in Therapeutic Science, the Board of Scientific Advisors of the Jane Coffin Childs Memorial Fund and the Council of the National Academy of Sciences. His current research focuses on the mechanism of viral membrane fusion and its inhibition by drugs and antibodies, using the HIV envelope protein (gp120/gp41) as a model system. One branch of this research is focused on creating an HIV vaccine that elicits antibodies against a transient, but vulnerable, intermediate in the membrane-fusion process, called the pre-hairpin intermediate. Another area of Kim’s research revolves around protein surfaces that are referred to as "non-druggable,” defined empirically based on failure to identify small, drug-like molecules that bind to them with high affinity and specificity.

The Kim lab is working to characterize select non-druggable targets and developing methods to identify ligands for non-druggable protein surfaces. Kim has received several accolades for his research, including: the National Academy of Sciences Award, a DuPont Merck Young Investigator Award, an Eli Lilly Award, the Hans Neurath Award, and the Ho-Am Prize. In addition to his American Academy of Arts and Sciences membership, Kim is a member of the National Academy of Sciences, the National Academy of Medicine, and a Fellow of the Biophysical Society, the American Association for the Advancement of Science, and the American Academy of Microbiology. His publications appear in high-tier journals including Cell, PNAS, and Science.

Our ALUMNI PANELISTS include:

Justin Jarrell, PhD
Associate Director of Business Development at Teiko Bio

Rebecca (Becky) Leylek, PhD
Scientist 3 in Cancer Immunology at Genentech

Matthew H. Spitzer, PhD
Associate Professor of Otolaryngology at UCSF

Justin Jarrell, PhD is an Associate Director of Business Development at Teiko Bio. Previously, Justin served as the VP of Research at Indee Labs where he led development of non-viral based cell transfection systems to accelerate manufacturing of engineered T cell therapies. He completed his PhD in Immunology at Stanford University in 2018 where he discovered and implicated anti-cytokine autoantibodies in the pathogenesis of IgG4-Related Disease in the lab of Dr. Bill Robinson. He is the author of 17 publications, recipient of multiple grants, including 2 SBIRs, and has presented at several immunology- and translational research-focused conferences.

Rebecca (Becky) Leylek, PhD earned a B.S. in Biology from Duke University in 2014, and graduated from the Stanford Immunology PhD program in 2020. She was a student in the Idoyaga lab at Stanford, where she studied dendritic cell biology and human immunology. Upon graduation, she immediately jumped into a Research Associate position in Cancer Immunology at Genentech in South San Francisco, contributing to research and development efforts for an RNA-based personalized cancer vaccine. Becky recently transitioned into a Scientist position at Genentech taking on a more advanced role aiming to develop tolerogenic therapies for autoimmune diseases.

Matthew (Matt) Spitzer, PhD completed his training in Immunology at Stanford University in the laboratories of Dr. Garry Nolan and Dr. Ed Engleman. There, he developed experimental and analytical methods to model the state of the immune system using high dimensional single-cell data. This led Matt to develop the first reference map of the immune system, providing a framework into which new data can be integrated and compared for system-wide analysis. At Stanford, he also developed new strategies for inducing powerful immune responses against cancer. Matt moved to UCSF in the summer of 2016 as a UCSF Parker Fellow and a Sandler Faculty Fellow and is now an Associate Professor in the Departments of Otolaryngology-Head and Neck Surgery and Microbiology & Immunology and an investigator of the Parker Institute for Cancer Immunotherapy.

Game Show Friday
On Friday night, after the keynote, we will have the traditional game show featuring our faculty and Second Year Immunology students.

Poster Session Saturday
The poster session will be on Saturday night at 8:30-11:00 pm, along with a reception. A Best Poster Prize will be given to two graduate students and two postdoctoral fellows.
Poster Slam: Saturday, January 28 at 8:00-8:30 pm. Poster presenters may have the podium for several minutes to exhort our conference attendees through PG-rated means to come to their posters.
Poster Ads will run continuously throughout the retreat. The intent of these Poster Ads is to draw as many people as possible to the posters. Voting for Best Poster will be done democratically – everyone votes! Faculty are encouraged to visit each poster.

Birukova Midnight Swim
Take a plunge in the ocean on Friday night! In honor of Maria Birukova, a former School of Medicine graduate student, students, postdocs, and faculty can continue the tradition of the midnight swim on Saturday.

CDIII Updates Saturday
The CDIII (Community, Diversity and Inclusion In Immunology) committee aims to promote a culture of diversity, equity and inclusion in the Immunology Program through the identification and recommendation of long-lasting creative solutions that embrace anti-racism principles and eliminate systemic bias. They will provide updates on activities and programs happening throughout the year.

Faculty-First Years only Research Blitz
On Saturday, faculty wishing to recruit rotation students to their labs may participate in a research blitz with first year students. Each faculty member will have the opportunity to talk about their research in several minutes and converse with a First Year student. Once the allotted few minutes are up, the faculty member will meet the next first year student.

Career Panel Sunday
Graduate students and postdoc fellows have the chance to meet Stanford Immunology alumni talk about academic careers, careers in the industry, and real-life experiences.

The Annual Scientific Conference is one of the highlights of the academic year for our Stanford Immunology community – we thank you for participating in this marvelous and enriching experience.

January 2023 Immunology Conference Committee
Thank you to the conference planning committee and your dedication on making the event a success.

Conference Directors: Drs. Nima Aghaeepour and Jennifer Bando
Social Chairs: Second Year Class
Conference Committee: Lina Hansen, Dr. Olivia Martinez, and Torye Nguyen
Staff Support: Lynn Galicia, Cindy Limb, and Rita Robinson
Technical Support: Candace Liu

Stanford MEDICINE Immunology
School of Medicine

AGENDA AT A GLANCE

Friday, January 27			
12:45 PM	Conference Check-in (Captain's Table/Points Ballroom)	4:30 PM	Lodging Check in (Front Desk)
1:00 PM	Welcome and Introductions (Points Ballroom)	5:00 PM	Postdoc Happy Hour (Tides Waterfront Kitchen)
1:10 PM	Session 1	6:00 PM	Dinner with Food Trucks (Parking Lot)
2:00 PM	Session 2	7:05 PM	Keynote (Points Ballroom)
2:45 PM	Break	8:05 PM	Break
3:00 PM	Session 3	8:30 PM	Game Show & Reception (Points Ballroom)
3:45 PM	Session 4	10:00 PM	After Party (Bayview Ballroom)

Saturday, January 28			
7:00 AM	Yoga Class (Ballroom TBD)	1:30 PM	Mentor Training (Points Ballroom)
7:30 AM	Breakfast (Bayview Ballroom)	3:00 PM	Research Blitz with First Years & Faculty (Points Ballroom)
9:00 AM	CDIII Updates (Points Ballroom)	4:00 PM	Beach in a Jar Activity (Wedding Garden)
9:50 AM	Break	6:00 PM	Dinner with Food Trucks (Parking Lot)
10:05 AM	Session 5	7:05 PM	Keynote (Points Ballroom)
11:10 AM	Break	8:10 PM	Poster Slam & Reception (La Grande Ballroom)
11:25 AM	Session 6	8:30 PM	Poster Session (La Grande Ballroom)
12:15 PM	Group Pictures (Deck)	10:00 PM	Bonfire at Fire Pits
12:30 PM	Lunch (Deck)	11:00 PM	Birukova Midnight Swimming Club
12:35 PM	Faculty Meeting (Points Ballroom)	12:00 AM	Adjourn

Sunday, January 29			
7:30 AM	Breakfast (Bayview Ballroom)	11:00 AM	Break
9:00 AM	Session 7	11:15 PM	Session 8
10:00 AM	Break	12:15 PM	General Announcements
10:15 AM	Career Panel	12:20 PM	Lunch (Deck) & Checkout at Monterey Tides
		1:00 PM	Adjourn

AGENDA

STANFORD IMMUNOLOGY SCIENTIFIC CONFERENCE

Friday, January 27, 2023 at 1:00 PM – 12:00 AM (midnight)

Talks are hosted in Points Ballroom

Times, speakers, and topics may change without notice

Time	Speaker and Title
12:45–1:00 PM	**Conference Check-in** at Captain's Table/Points Ballroom
1:00–1:10 PM	**Welcome and Introductions**: Conference Directors: Nima Aghaeepour, PhD and Jennifer Bando, PhD
1:10–2:00 PM	**Session 1**
1:10–1:30 PM	**Hawa Racine Thiam, PhD,** Assistant Professor of Bioengineering, *Cellular biophysics of Neutrophils – Lessons from NETosis*
1:30–1:45 PM	**Tejas Dharmaraj,** Immunology Graduate Student, Bollyky Lab, *Engineering an Extended-Release High-Dose Bacteriophage Hydrogel*
1:45–2:00 PM	**Audrey Lee,** Immunology Graduate Student, Pulendran Lab, *Reprogramming the innate immune system to stimulate broad and durable protection against diverse pathogens*
2:00–2:45 PM	**Session 2**
2:00–2:15 PM	**Justin Arredondo-Guerrero,** Immunology Graduate Student, Mackall and Meyer Labs, *Sustained glycolysis and mitochondrial respiration via GLUT1-mediated glucose transport enhances CAR T cell effector functionality*
2:15–2:30 PM	**Oliver Wirz, PhD,** Postdoc, Boyd Lab, *Comprehensive study of SARS-CoV-2-specific B cells in infected patients and vaccinated individuals*
2:30-–2:45 PM	**David McIlwain, PhD,** Sr. Research Scientist, Nolan Lab, *Cellular correlates of protection from human influenza virus challenge*
2:45–3:00 PM	**Break**
3:00–3:45 PM	**Session 3**
3:00–3:15 PM	**Jessy Tan, PhD,** Postdoc, Andreasson Lab, *De-age the brain by targeting the macrophages: a deeper look*
3:15–3:30 PM	**Julia Adamska**, Immunology Graduate Student, Pulendran and Li Labs, *SREBP signaling is essential for effective B cell responses*
3:30–3:45 PM	**Viswanath Gunda, PhD,** Postdoc, Lewis Lab, *CRISPR regulation of respiratory tract gene expression for the prevention of influenza A viral pneumonia*
3:45–4:30 PM	**Session 4**
3:45–4:00 PM	**Xavier Rovira-Clave, PhD,** Instructor, Nolan Lab, *Highly scalable multiplex serology testing*
4:00–4:15 PM	**Gita Abhiraman**, Immunology MSTP Graduate Student, Garcia Lab, *Cytokine adaptors: soluble molecules interconvert between local immune inhibition and stimulation*
4:15–4:30 PM	**Joy Pai**, Immunology Graduate Student, Satpathy Lab, *Systematic lineage tracing reveals differentiation trajectories and long-term persistence of tumor-reactive T cells during immune checkpoint blockade*
4:30 PM	**Lodging Check in** at Front Desk
5:00–6:00 PM	**Postdoc Happy Hour** at Tides Waterfront Kitchen
6:00–7:00 PM	**Dinner** at Parking Lot
7:05–7:10 PM	**Keynote Introduction** in Points Ballroom
7:10–8:05 PM	**Keynote: Elina Zuniga, PhD**, Professor, Molecular Biology, Division of Biological Sciences at University of California, San Diego, *Type 1 interferon exhaustion*

8:05–8:30 PM	**Break**
8:30 PM	**First Year/Faculty Game Show & Reception** in Points Ballroom
10:00 PM	**After Party** in Bayview Ballroom
12:00 AM	**Adjourn**

Saturday, January 28, 2023 at 7:30 AM – 12:00 AM (midnight)
Talks are hosted in Points Ballroom

Time	Speaker and Title
7:00–7:45 AM	**Yoga Class** in Ballroom TBD
7:30–9:00 AM	**Breakfast** in Bayview Ballroom
9:00–9:50 AM	**CDIII Updates** in Points Ballroom
9:50–10:05 AM	**Break**
10:05–11:10 AM	**Session 5**
10:05–10:25 AM	**Rogelio Hernandez-Lopez, PhD,** Assistant Professor of Bioengineering, and of Genetics, *Reprogramming molecular circuits for next-generation cell therapies*
10:25–10:40 AM	**Yu Zhu, PhD,** Postdoc, Butcher Lab, *Notch signaling in endothelial cells programs cancer-associated fibroblasts to suppress anti-tumor T cell immunity*
10:40–10:55 AM	**John Hickey, PhD,** Postdoc, Nolan Lab, *Immune Cell Spatial Layering of the Human Intestine*
10:55–11:10 AM	**Preksha Bhagchandani**, Immunology MSTP Graduate Student, *Hematopoietic stem cell transplantation promotes fully MHC-mismatched islet allograft tolerance and diabetes reversal*
11:10–11:25 AM	**Break**
11:25 AM–12:15 PM	**Session 6**
11:25–11:45 AM	**Sydney Lu, PhD,** Assistant Professor of Hematology, *The accessory RNA splicing factor RBM39 regulates T cell function and the alloantigen response.*
11:45 AM–12 PM	**Josselyn Peña**, Immunology Graduate Student, Krams Lab, *Investigating the Role of Natural Killer Cells in the Control of Latent Epstein-Barr Virus Infection*
12:00–12:15 PM	**Kazuki Nagashima, MD, PhD,** Postdoc, Fischbach Lab, *Mapping the T cell repertoire to a complex gut bacterial community*
12:15–12:30 PM	**Group Pictures** on Deck
12:30–1:30 PM	**Lunch** on Deck
12:35–1:30 PM	**Faculty Meeting** in Points Ballroom: Immunology Faculty, Graduate Student, and Postdoc Representatives remain at Points Ballroom
1:30–3:00 PM	**Mentor Training** with Faculty in Points Ballroom
3:00–4:00 PM	**Research Blitz with First Years & Faculty** in Points Ballroom
4:00–5:00 PM	**Beach in a Jar Activity** in Wedding Garden
6:00–7:00 PM	**Dinner** at Parking Lot
7:05–7:10 PM	**Keynote Introduction** in Points Ballroom
7:10–8:10 PM	**Keynote: Peter Kim, PhD**, Virginia and D. K. Ludwig Professor of Biochemistry at Stanford, *Towards a universal flu vaccine and a pan-SARS-CoV vaccine*
8:10–8:30 PM	**Poster Slam** in La Grande Ballroom
8:30 PM	**Poster Session & Reception** in La Grande Ballroom
10:00 PM	**Bonfire** at Fire Pits
11:00 PM	**Birukova Midnight Swimming Club**
12:00 AM	**Adjourn**

Sunday, January 29, 2023 at 7:30 AM – 1:00 PM
Talks are hosted in Points Ballroom

Time	Speaker and Title
7:30–9:00 AM	**Breakfast** in Bayview Ballroom
9:00–10:00 AM	**Session 7**
9:00–9:15 AM	**Nathan Reticker-Flynn, PhD,** Instructor, Engleman Lab, *Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance*
9:15–9:30 AM	**Guangbo Chen, PhD,** Postdoc, Davis Lab, *Cytokines surge in the blood years prior to a cancer diagnosis in elderly individuals*
9:30–9:45 AM	**Katherine Nico**, Immunology Graduate Student, Howitt Lab, *Tuft cell-mediated type 2 immunity depletes intestinal resident T cell protection*
9:45–10:00 AM	**Sirimuvva Tadepalli, PhD,** Postdoc, Idoyaga Lab, R*apid monocyte recruitment dictates the therapeutic efficacy of focal radiotherapy*
10:00–10:15 AM	**Break**
10:15–11:00 AM	**Career Panel: Justin Jarrell, PhD,** Associate Director of Business Development at Teiko Bio, **Rebecca (Becky) Leylek, PhD,** Scientist 3 in Cancer Immunology at Genentech, and **Matthew H. Spitzer, PhD,** Associate Professor of Otolaryngology at UCSF
11:00–11:15 AM	**Break**
11:15 AM–12:15 PM	**Session 8**
11:15–11:30 AM	**Daniel Arve-Butler, PhD,** Postdoc, Monack Lab, *Eosinophils contribute to local immune responses during chronic Salmonella infection*
11:30–11:45 AM	**Madeline Lee**, Immunology Graduate Student, Blish Lab, *SARS-CoV-2 escapes direct NK cell killing through Nsp1-mediated downregulation of ligands for NKG2D*
11:45 AM–12 PM	**Frank Buquicchio**, Immunology Graduate Student, Satpathy Lab, *A unique epigenomic landscape defines CD8+ tissue-resident memory T cells*
12:00–12:15 PM	**Kameron Rodrigues,** Immunology Graduate Student, Jaiswal and Montgomery Labs, *Loss of Dnmt3a or Tet2 in stimulated macrophages alters transcription factor binding and enhances inflammatory gene expression*
12:15-12:20 PM	**General Announcements:** Announce winners of talks and posters
12:20 PM	**Lunch on Deck and Checkout at Monterey Tides**
1:00 PM	**Adjourn**

Stanford MEDICINE Immunology
School of Medicine

POSTER SESSION
STANFORD IMMUNOLOGY SCIENTIFIC CONFERENCE
Saturday, January 28, 2022
La Grande Ballroom

Presenter & Title

1. **Gita Abhiraman,** Immunology Graduate Student, Garcia Lab, *Structure- based tuning of IL-21 signaling enhances vaccination and reduces autoantibody production*
2. **Julia Adamska,** Immunology Graduate Student, Pulendran and Li Labs, *The RNA-editing enzyme ADAR1 is necessary for B cell response*
3. **Juan Aguilera, MD, PhD,** Basic Life Research Scientist, Sean N Parker Center for Allergy and Asthma, *Decreases in IL1RA, IL18, and IL8 are associated with increases in ambient air pollution during pregnancy*
4. **Meelad Amouzgar,** Immunology Graduate Student, Bendall Lab, *Unsupervised reconstruction of cell cycle progression and division in asynchronously dividing cells*
5. **Rebeca Arroyo Hornero, PhD,** Postdoc, Idoyaga Lab, *Phenotypic and functional diversification of human plasmacytoid dendritic cells: unraveling the mechanism*
6. **Šimon Borna, PhD,** Postdoc, Bacchetta Lab, *FOXP3-deficient patients have expanded autoreactive T cells originating from both regulatory and effector T cells*
7. **David Kung-Chun Chiu, PhD,** Postdoc, Engleman Lab, *Erythropoietin programs tumor-associated macrophages to suppress antitumor immunity in hepatocellular carcinoma*
8. **Blanda Di Luccia, PhD,** Basic Life Research Scientist, Monack Lab, *Gut regulatory T cells mediate immunological tolerance in Salmonella-infected super-spreader hosts*
9. **Camilo Espinosa Bernal**, Immunology Graduate Student, Aghaeepour Lab, *Multiomics modeling of preterm birth in low- and middle-income countries*
10. **Carley Fowler**, Process Development Specialist, Cancer Cell Therapy Center (CCT-LCGM), *Product characterization of CliniMACS Prodigy™ engineered T cells*
11. **Joe Gonzalez**, Immunology Graduate Student, Wang Lab, *IgG posttranslational modifications promote distinct receptor signaling pathways to elicit opposing immune activity in the lung*
12. **Lilit Grigoryan**, Immunology Graduate Student, Pulendran Lab, *Enhanced breadth of memory B cell responses following adjuvanted virus-like particle vaccination in humans*
13. **Naomi Haddock**, Immunology Graduate Student, Bollyky Lab, *The circulating phageome in lung transplantation*
14. **Maximilian Haist, MD,** Postdoc, Nolan Lab, *Spatial CD8 T-cell infiltration predicts response to primary radiochemotherapy in advanced oropharyngeal cancer*
15. **Colwyn Headley, PhD,** Postdoc, Tsao Lab, *Rescuing aging-associated CD4+ T cell dysfunction via mitochondrial transplantation*
16. **Noor Hussein, PhD,** Postdoc, Mellins Lab, *The role of CD39-expressing regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome (PANS): Ground work for a CAR-Treg therapeutic*

17. **Karan Kathuria**, Immunology Graduate Student, Davis Lab, *Modeling human immune responses to Plasmodium falciparum infection*
18. **Vimal Keerthi, MS,** Life Science Research Professional 2, Feldman and Mackall Lab, *Optimizing electroporation parameters for non-viral CAR-T cell manufacturing*
19. **Olivia Kline**, Epidemiology and Clinical Research Graduate Student, Nadeau Lab, *Chronic smoke exposure burden: Impact on the immune system of firefighters*
20. **Guo Luo, PhD,** Instructor, Mignot Lab, *Protective association of HLA-DRB1*04 subtypes in neurodegenerative diseases implicates acetylated tau PHF6 sequences*
21. **Sainiteesh Maddineni,** MD Student, Sunwoo Lab, *Identifying novel immune interactions of intraepithelial ilc1-like NK cells in head and neck cancer*
22. **Raul Maqueda-Alfaro, PhD,** Postdoc, Idoyaga Lab, *Transitional dendritic cells: identifying the role of a novel innate immune cell population*
23. **Max Miao,** Genetics Graduate Student, Satpathy, *Investigating the role of TCR signal strength in T cell differentiation and exhaustion*
24. **Ayan Mondal, PhD,** Postdoc, Mellins Lab, *Plasma from active pediatric acute-onset neuropsychiatric syndrome (PANS) induces increased BBB permeability through the disruption of junctional proteins of brain endothelial cells*
25. **Kaithlen Zen Pacheco,** Life Science Research Professional 1, Mackall Lab, *Logic-gated selection of multi-vector systems*
26. **Jimena Pavlovitch-Bedzyk,** Immunology Graduate Student, Davis Lab, *Novel skin organoid modeling of monkeypox infection and immune response*
27. **Trung Pham, MD, PhD,** Instructor, Monack Lab, *Single-cell profiling identifies ACE+ granuloma macrophages as a non-permissive niche for intracellular bacteria during persistent Salmonella infection*
28. **Kassie Press, PhD,** Postdoc, Jagannathan Lab, *Vδ2+ γδ T cell chromatin accessibility and immune function associates with prior malaria incidence*
29. **Taylor Pursell, PhD,** Postdoc, Boyd Lab, *Landscape of big brown bat (Eptesicus fuscus) splenic immune cell populations following rabies virus infection*
30. **Patrick Quinn**, Life Science Research Professional, Mackall Lab, *Cyclin dependent kinase 8 inhibition may synergize with car T-cell therapy for treatment of acute myeloid leukemia*
31. **Colin Raposo**, Immunology Graduate Student, Satpathy Lab, *Cytotoxic T lymphocyte memory after the clearance of chronic viral infection*
32. **Hayley Raquer**, Immunology Graduate Student, Idoyaga Lab, *Traveling from the epidermis to the lymph node: Langerhans cell origin determines their migration potential*
33. **Kalani Ratnasiri**, Immunology Graduate Student, Blish and Khatri Labs, *Non-human primates replicate conserved human responses to RNA viral infections*
34. **Grayson Rodriguez**, Immunology Graduate Student, Garcia Lab, *Triplekines form novel cytokine receptor complexes*
35. **Adonis Rubio**, Immunology Graduate Student, Barnes Lab, *Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains*
36. **David Seong,** Immunology MSTP Graduate Student, Idoyaga Lab, *Unraveling the mechanisms of age-associated functional disruptions in plasmacytoid dendritic cells*
37. **Fernando Sulczewski**, **PhD,** Postdoc, Idoyaga Lab, *Transitional dendritic cells are a novel source of conventional type 2 dendritic cells*

38. **Kattria van der Ploeg**, **PhD,** Postdoc Jagannathan Lab, *Malaria-exposed Ugandan women exhibit a differential SARS-CoV-2-specific T cell response*
39. **Alun Vaughan-Jackson, PhD,** Postdoc, Bassik Lab, *Developing stem cell-derived macrophages for genome wide screens of viral infectivity*
40. **Xihui Yin**, Life Science Research Professional, Utz Lab, *Anti-dopamine receptor autoantibody detection in Pediatric Acute-onset Neuropsychiatric Syndrome*
41. **Maxim Zaslavsky**, Computer Science Graduate Student, Boyd and Kundaje Labs, *Disease diagnostics using machine learning of immune receptors*

Stanford MEDICINE Immunology
School of Medicine

ORAL SESSION
STANFORD IMMUNOLOGY CONFERENCE
Friday, January 27, 2023
Points Ballroom

Session 1	Speaker and Title
1:10-1:30 PM	**Hawa Racine Thiam, PhD,** Assistant Professor of Bioengineering, *Cellular biophysics of Neutrophils – Lessons from NETosis*
1:30-1:45 PM	**Tejas Dharmaraj,** Immunology Graduate Student, Bollyky Lab, *Engineering an Extended-Release High-Dose Bacteriophage Hydrogel*
1:45-2:00 PM	**Audrey Lee,** Immunology Graduate Student, Pulendran Lab, *Reprogramming the innate immune system to stimulate broad and durable protection against diverse pathogens*

Hawa Racine Thiam, PhD, Assistant Professor
Department of Bioengineering and of Microbiology & Immunology

Cellular biophysics of Neutrophils – Lessons from NETosis

Neutrophils are innate immune cells critical for host defense again pathogens. To accomplish their tasks, neutrophils need to build cell-scale responses to rapidly move, remodel, and interact with the microenvironment. These cell-scale functions require cells to generate and transmit physical forces. Our lab aims to understand how neutrophils generate the physical forces required for the well execution of their functions in the physically challenging *in vivo* environment. Such knowledge will be critical for our long-term goal of controlling neutrophils functions and improve human health.

A specific neutrophil function my lab is currently focusing on is NETosis, a process during which neutrophils release their chromatin, antimicrobial and cytotoxic proteins to the extracellular environment to kill pathogens. However, NETosis also occurs in sterile inflammation, can damage the host, and worsen the outcome of inflammation related diseases. While the pathophysiological role of NETosis is well established, little is known about the cellular or biophysical mechanisms that drive this process. In this talk, I will present my lab's work toward closing this gap in our knowledge of the fundamental mechanisms of NETosis. I will also discuss how we will use this knowledge to reengineer neutrophils and control NETosis initiation, completion, and damaging effects on the host.

Tejas Dharmaraj, Immunology MSTP Graduate Student
Advisor: Paul Bollyky
Department of Infectious Diseases and of Microbiology & Immunology

Engineering an extended-release high-dose bacteriophage hydrogel

Bacteriophage (phage) therapy is the use of lytic viruses to treat bacterial infections. Effective phage therapy requires consistent delivery of high concentrations of active phages, but this has not always been possible, in part because existing formulations for phage therapy are rapid-burst release or impractical for clinical use. Here, we present an injectable hydrogel technology to address this problem. Our hydrogel, based on reversible dynamic covalent chemistry, utilizes hydrazine-functionalized alginate (VLVG-HYD) and aldehyde-functionalized polyethylene glycol (PEG-ALD) crosslinked via reversible hydrazone linkages to encapsulate and release bacteriophages via reversible imine coupling between phage capsid lysines and PEG-ALD. Our hydrogel encapsulates antipseudomonal bacteriophages, releases >10^10 bacteriophages/day in vitro, and eradicates Pseudomonas aeruginosa in a mouse model of Pseudomonas aeruginosa wound infections. The hydrogel is durable, self-healing, and easily integrates with standard-of-care antibiotic regimens.

Audrey Lee, Immunology Graduate Student
Advisor: Bali Pulendran
Department of Pathology

Reprogramming the innate immune system to stimulate broad and durable protection against diverse pathogens

Vaccines have conventionally been known to mediate their protective effects in an antigen-specific manner through the induction of neutralizing antibodies and specific T and B cells. However, emerging studies have found that certain vaccines, such as the Bacille Calmette-Guerin (BCG) vaccine, could induce epigenetic memory or “trained immunity” in innate immune cells, thereby potentially conferring non-specific protection against other pathogens. Using transcriptomic and epigenomic sequencing, we have also previously shown that various adjuvants could lead to long-lasting epigenetic reprogramming of myeloid cells (Wimmers et al., 2021; Lee et al., 2022; Arunachalam et al., 2021). However, the extent of epigenetic reprogramming following vaccination and mechanism of vaccine-induced heterologous protection remain poorly understood. To this end, we characterized the transcriptomics and epigenomics of myeloid and adaptive immune cell subsets following vaccination in animal models in the bone marrow and periphery at late timepoints. These findings provide a tissue level omics map of vaccine-induced responses to potentially guide rational vaccine design.

Session 2	Speaker and Title
2:00-2:15 PM	**Justin Arredondo-Guerrero,** Immunology Graduate Student, Mackall and Meyer Labs, *Sustained glycolysis and mitochondrial respiration via GLUT1-mediated glucose transport enhances CAR T cell effector functionality*
2:15-2:30 PM	**Oliver Wirz, PhD,** Postdoc, Boyd Lab, *Comprehensive study of SARS-CoV-2-specific B cells in infected patients and vaccinated individuals*
2:30-2:45 PM	**David McIlwain, PhD,** Sr. Research Scientist, Nolan Lab, *Cellular correlates of protection from human influenza virus challenge*

Justin Arredondo-Guerrero, Immunology Graduate Student

Advisors: Crystal Mackall, Department of Pediatrics and of Blood & Marrow Transplantation; and Everett Meyer, Depatment of Pediatrics

Sustained glycolysis and mitochondrial respiration via GLUT1-mediated glucose transport enhances CAR T cell effector functionality

The current limitations in efficacy of chimeric antigen receptor (CAR) T cell therapy have driven new metabolic-based approaches to circumvent immunosuppressive tumor microenvironments and promote sustained glucose consumption for T cell fitness. Lackluster patient responses that lead to diminished antitumoral activity can be attributed to activation-induced exhaustion, lack of memory population formation, or competition for available nutrients required for glycolysis and mitochondrial respiration. In this study we stably overexpressed the glucose transporter GLUT1 in primary human CAR T cells to investigate how enhanced glucose consumption affects effector functionality and metabolic flux. GLUT1 overexpression increased both glycolytic flux in stimulated CAR T cells and spare respiratory capacity resulting in increased cytokine production in response to leukemic and solid tumor cell lines. The enhanced CAR T effector functionality observed in vitro also resulted in sustained tumor control and peripheral surveillance in vivo. We found that GLUT1 promoted TCF1 expression and antitumoral proliferation against solid tumor lines with low and high antigen densities. Lastly, we observed that continuous glucose transport through GLUT1 supported mitochondrial fitness and mTORC1 activity while altering arginine metabolism at the Urea Cycle axis. Our collective findings highlight the importance of sustained glycolysis and respiration for CAR T cell effector functionality and persistence via GLUT1-mediated glucose transport.

Oliver Wirz, PhD, Postdoc

Advisor: Scott Boyd

Department of Pathology

Comprehensive study of SARS-CoV-2-specific B cells in infected patients and vaccinated individuals

B cells mount a protective immune response following SARS-CoV-2 infection or mRNA vaccination. Yet, it is still unclear to what extent infection and vaccine responses differ at a B cell clonal level. Therefore, we sorted SARS-CoV-2-specific B cells from blood of vaccine recipients and infected patients, followed by single cell sequencing to identify specific B cell receptors together with their bound antigen. We used a highly multiplexed panel of DNA-tagged antigens including full SARS-CoV-2 Spike, S1 NTD, S2, receptor binding domain (RBD), and nucleoprotein, as well as different RBDs from viral variants. Spike-specific B cells peaked one week after the second vaccine dose, whereas they increased during the first three months post-onset of symptoms in infected patients. Cross-reactive B cells binding eight different viral variant RBDs were increased among vaccinees

compared to patients, and showed diminished binding to Delta, Gamma, and Beta variant RBDs in decreasing order. Using a panel of 21 viral variant RBDs, we studied which germline encoded B cell receptor genes and mutation levels are used for the most cross-reactive B cell clones. Further, isolation of SARS-CoV-2-specific B cells from tissues of organ donors identified antigen-specific B cells with a phenotype similar to previously described atypical memory B cells. The BCR specificity of over 3500 antigen-binding B cells from 17 vaccinees, 28 infected patients and seven organ donors were characterized in these experiments. Our data demonstrate the utility of a large antigen panel to characterize timing and breadth of the B cell responses to vaccinations and infection.

David McIlwain, PhD, Sr. Research Scientist
Advisor: Garry Nolan
Department of Pathology

Cellular correlates of protection from human influenza virus challenge

Human influenza virus challenge studies are valuable mechanisms to accelerate the development and evaluation of therapeutics. Mass cytometry (CyTOF) is a powerful tool for deep profiling many circulating immune cell subsets simultaneously. Using a mass cytometry-centered approach, we first mapped the natural history of influenza virus infection during volunteer human virus challenge, this work identified a critical role for CD38 in type I interferon production in plasmacytoid dendritic cells and uncovered a previously unrecognized population of circulating pSTAT5+ plasmablasts. We then used this knowledge to define the first cellular correlates of protection for an investigational oral influenza vaccine as part of a Phase II study. Specifically, using levels of pSTAT5+ plasmablasts and mucosal homing marker positive T and B cell subsets that differed early after immunization, we created a machine-learning model to predict who would be later protected from virus shedding post-challenge. Establishing cellular correlates of protection is critically important to bring to market innovative non-traditional vaccine approaches, such as the oral vaccine platform evaluated, that may rely more heavily on mucosal and cellular immunity than antibodies.

Session 3	Speaker and Title
3:00-3:15 PM	**Jessy Tan, PhD,** Postdoc, Andreasson Lab, *De-age the brain by targeting the macrophages: a deeper look*
3:15-3:30 PM	**Julia Adamska**, Immunology Graduate Student, Pulendran and Li Labs, *SREBP signaling is essential for effective B cell responses*
3:30-3:45 PM	**Viswanath Gunda, PhD,** Postdoc, Lewis Lab, *CRISPR regulation of respiratory tract gene expression for the prevention of influenza A viral pneumonia*

Jessie Tan, PhD, Postdoc
Advisor: Katrin Andreasson
Department of Neurology & Neurological Sciences

De-age the brain by targeting the macrophages: a deeper look

Macrophages play pivotal roles in innate immunity. In aging, macrophages instigate maladaptive pro-inflammatory responses, which can be reverted by inhibiting the EP2 receptor, resulting in improved metabolic robustness and, intriguingly, restored cognitive function of the aging brain. This brings new hopes for translational application if unraveling the underlying mechanisms to identify druggable targets for humans. My ongoing studies show that the brain rejuvenation effect implemented by EP2 receptor inhibition is CNS-independent; instead, it is a periphery-to-CNS retrograde signaling transduction. The re-modified chromatin architecture is one of the significant consequences of EP2 receptor inhibition, which is co-dependent on the cellular metabolism state. Consequently, the macrophages residing in different peripheral tissues and organs are rejuvenated upon EP2 receptor inhibition leading to enhanced tissue function, less inflammatory blood condition, and more youthful blood metabolites circulating to the brain. Interestingly, targeting the tissue-resident macrophages that originated from the yolk sac leads to the improved cognitive function of the aging brain. The tissue-resident macrophages are enormously enriched in the intestine, whose permeability is substantially restored by EP2 receptor inhibition. The leaky gut is under interrogation to further address the gut-to-brain rejuvenation effects.

Julia Adamska, Immunology Graduate Student
Advisor: Bali Pulendran
Department of Pathology

SREBP signaling is essential for effective B cell responses

B cell responses are critical for generating sustained humoral immunity. Our previous human systems vaccinology study identified an association between the sterol regulatory binding protein (SREBP) pathway and humoral immune response. To further investigate the role of SREBP signaling in modulating immune responses, we generated mice with B cell or CD11c+ antigen-presenting cell (APC) specific deletion of SCAP, an essential regulator of SREBP signaling. Ablation of SCAP in CD11c+ APCs had no effect on immune responses to immunization. SREBP signaling in B cells was dispensable for their maturation and homeostatic maintenance. However, mice deficient in SREBP signaling in B cells were defective in forming germinal centers (GCs) and memory compartments upon immunization. SREBP signaling was required for metabolic reprogramming in stimulated B cells. Deletion of SCAP in GC B cells reduced lipid raft content and cell cycle progression. These studies provide mechanistic insights coupling lipid metabolism with the quality and longevity of humoral immunity.

Viswanath Gunda, PhD, Postdoc
Advisor: David Lewis
Department of Pediatrics (Immunology)

CRISPR regulation of respiratory tract gene expression for the prevention of influenza A viral pneumonia

CRISPR regulation of respiratory tract gene expression for the prevention of influenza A viral pneumonia. Viswanath Gundy,1 Jordan T. Spatz,1 Marie Faye La Russa,2 Stanley Qi,2 and David B. Lewis.1 Departments of 1Pediatrics and 2Bioengineering, Stanford University School of Medicine.

We have developed a novel CRISPR-based strategy for preventing infection with IAV or other respiratory viruses by increasing the innate immune function and resistance to viral infection of the host's respiratory tract epithelium. This host-directed therapeutic approach does not require any specific knowledge of the RNA viral strain to be targeted, and uses engineered virus-like particles (eVLPs) with a proven ability to deliver CRISPR nucleoprotein complexes to mouse tissues in vivo (e.g., liver, eye, and brain). We have modified eVLPs for effective delivery to the respiratory tract epithelium of mice, humans, and other mammals by including IAV surface proteins in the eVLP lipid bilayer. We first used these modified eVLPs to demonstrate the efficient delivery of cargo protein (luciferase) to murine or human respiratory tract epithelial cells and human lung organoids in vitro. We next used the eVLP system to deliver CRISPR/sgRNA nucleoprotein cargo to these cell types for the upregulation of gene transcription of interferon-lambda (IFN-λ), a potent anti-viral cytokine secreted by respiratory epithelial cells. This treatment resulted in the secretion of high levels (1-2 ng/ml in cell culture supernatants) of IFN-λ for at least 3 days, which provide protection against infectious challenge with mNeon labeled IAV PR8/34 virus. We next verified that modified eVLPs effectively delivered luciferase cargo to the upper and lower respiratory tracts based on in vivo IVIS imaging. Finally, we found that the intranasal delivery to B6 mice of modified eVLPs with CRISPR/sgRNA cargo for IFN-λ upregulation markedly limited upper and lower respiratory tract infection following challenge with a large but sublethal dose of IAV. These studies lay the groundwork for using modified eVLPs for the prevention/treatment of other viral infections, such as SARS-CoV-2, or for the delivery of other CRISPR or non-CRISPR-based respiratory tract therapies.

Session 4	Speaker and Title
3:45-4:00 PM	**Xavier Rovira-Clave, PhD,** Instructor, Nolan Lab*, Highly scalable multiplex serology testing*
4:00-4:15 PM	**Gita Abhiraman**, Immunology MSTP Graduate Student, Garcia Lab, *Cytokine adaptors: soluble molecules interconvert between local immune inhibition and stimulation*
4:15-4:30 PM	**Joy Pai**, Immunology Graduate Student, Satpathy Lab, *Systematic lineage tracing reveals differentiation trajectories and long-term persistence of tumor-reactive T cells during immune checkpoint blockade*

Xavier Rovira-Clave, PhD, Instructor
Advisor: Garry Nolan
Department of Pathology

Highly scalable multiplex serology testing

Population scale serology testing informs public health practice by inferring immunity to infectious agents, but most assays used are currently limited in throughput and depth of molecular correlates. Repeating an assay hundreds or thousands of times is the de facto choice for studies at a population scale, which is costly and time consuming. Here, we developed a serology assay based on mass cytometry that is scalable, multiparametric, affordable, and fast to facilitate public health surveillance at a population scale.

Gita Abhiraman, Immunology MSTP Graduate Student
Advisor: Chris Garcia
Department of Molecular & Cellular Physiology and of Structural Biology

Cytokine adaptors: soluble molecules interconvert between local immune inhibition and stimulation

Cytokines are soluble factors secreted by immune cells to coordinate complex immune processes, from the tolerance of self to the rejection of cancer. Cytokines are frequently dysregulated in disease. TGF-β and IL-10 are immunosuppressive cytokines overexpressed in cancer, and IL-23 drives inflammation in multiple autoimmune diseases. Here, we present “cytokine adaptors” – soluble molecules that convert immunosuppressive cytokines into immunostimulatory cytokines or vice versa. We develop three cytokine adaptors, which convert TGF-β to IL-2, IL-10 to IL-2, and IL-23 to IL-10. We find that these cytokine adaptors can reverse TGF-β or IL-10 mediated T cell suppression in vitro and improve tumor killing in an organotypic human cancer model. Unlike other methods of immune conversion that require cell engineering, cytokine adaptors are soluble molecules that deliver two-in-one function: blockade and agonism. We anticipate that these results will enable the accessible and modular design of other cytokine adaptors for a variety of disease contexts.

Joy Pai, Immunology Graduate Student
Advisor: Ansu Satpathy
Department of Pathology

Systematic lineage tracing reveals differentiation trajectories and long-term persistence of tumor-reactive T cells during immune checkpoint blockade

Paired single-cell RNA and T cell receptor sequencing (scRNA/TCR-seq) has allowed for enhanced resolution of clonal T cell dynamics in cancer. Here, we report scRNA/TCR-seq analysis of 162,062 T cells from 31 tissue regions, including tumor, adjacent normal tissues, and lymph nodes (LN), from patients who underwent resections for persistent or progressing lung cancers after immune checkpoint blockade (ICB). We found marked regional heterogeneity in CD8 and CD4 T cell phenotypes that was associated with heterogeneity in tumor persistence. Regions with persistent cancer cells were enriched for exhausted CD8 T cells, regulatory CD4 T cells (Treg), and follicular helper CD4 T cells (TFH). Tracking individual T cell clonotypes across tumor regions and tissues revealed that exhausted CD8 T cells and TFH CD4 T cells could be clonally linked to TCF7+ SELL+ progenitor counterparts in tumor draining LNs and progressive exhaustion trajectories of CD8 T, Treg, and TFH cells as they migrate from LNs into the tumor microenvironment. Finally, longitudinal tracking of CD8 and CD4 T cell clones with tumor-reactive features revealed persistence in the peripheral blood for years after ICB therapy; however, exhausted CD8, Treg, and TFH cells had lower persistence relative to other subsets. Strikingly, the presence of clonally linked progenitors in the LN conferred greater longitudinal persistence. Altogether, this comprehensive scRNA/TCR-seq dataset with regional, clonal, and longitudinal resolution provides fundamental insights into the tissue distribution, differentiation trajectories, and persistence of the T cells that underlie clinical responses to ICB.

Keynote	Speaker and Title
7:05-8:05 PM	**Keynote: Elina Zuniga, PhD**, Professor, Molecular Biology, Division of Biological Sciences at University of California, San Diego

Elina Zuniga, PhD, Professor

Department of Molecular Biology, Division of Biological Sciences at University of California, San Diego

Type 1 interferon exhaustion

Interferons are a family of proteins that exert central and vital roles in immunity and antiviral defense. My laboratory discovered that while interferons are initially elevated few hours after a viral infection, these antiviral mediators are subsequently and rapidly silenced despite continuous pathogen replication. We showed that there is a (host-mediated) interferon suppression or exhaustion phase, during which the infected host fails to elevate interferons in response to the presence of the original virus or an unrelated secondary pathogen, which associates with delayed viral control and enhanced susceptibility to opportunistic infections. I will present our recent work on the mechanisms underlying this Type I interferon exhaustion phase and a potential explanation why such mechanisms have evolved.

ORAL SESSION
STANFORD IMMUNOLOGY CONFERENCE
Saturday, January 28, 2023
Points Ballroom

Session 5	Speaker and Title
10:05-10:25 AM	**Rogelio Hernandez-Lopez, PhD,** Assistant Professor of Bioengineering, and of Genetics, *Reprogramming molecular circuits for next-generation cell therapies*
10:25-10:40 AM	**Yu Zhu, PhD,** Postdoc, Butcher Lab, *Notch signaling in endothelial cells programs cancer-associated fibroblasts to suppress anti-tumor T cell immunity*
10:40-10:55 AM	**John Hickey, PhD,** Postdoc, Nolan Lab, *Immune Cell Spatial Layering of the Human Intestine*
10:55-11:10 AM	**Preksha Bhagchandani**, Immunology MSTP Graduate Student, *Hematopoietic stem cell transplantation promotes fully MHC-mismatched islet allograft tolerance and diabetes reversal*

Rogelio Hernandez-Lopez, PhD, Assistant Professor
Department of Bioengineering, and of Genetics

Reprogramming molecular circuits for next-generation cell therapies

Immune engineering is an exciting field that seeks to apply engineering principles to the design of the immune system for a range of applications. In particular, T cells modified with synthetic receptors are a highly promising therapeutic strategy for cancer treatment. However, current engineered T cells still have limited efficacy, poor specificity and persistence against solid tumors.

My laboratory seeks to integrate mechanistic cell biology, synthetic and systems biology for understanding and programming fundamental cellular behaviors such as recognition and communication. I will discuss a new approach to engineer T cell specificity towards cancer cells based on antigen density sensing. We have designed a two-step recognition-activation circuit that involves two receptors. In this circuit an initial recognition event, via a SynNotch receptor, alters the potency of a subsequent response, CAR expression and activation. We have designed and expressed several SynNotch/CAR circuits and have tested their ability to achieve antigen density sensing in vitro and in xenograft models of cancer.

Yu Zhu, PhD, Postdoc
Advisor: Eugene Butcher
Department of Pathology

Notch signaling in endothelial cells programs cancer-associated fibroblasts to suppress anti-tumor T cell immunity

Scarcity of tumor-infiltrating T cells poses significant challenges to cancer treatment, but mechanisms that limit T cell recruitment into the tumor microenvironment are unclear. Here we ask if the endothelial lining of the tumor vasculature suppresses T cell infiltration. Using mouse pancreatic ductal adenocarcinoma (PDAC)

models, we found that Notch signaling in endothelial cells (ECs) suppresses the recruitment of anti-tumor T cell by inhibiting the pro-inflammatory functions of cancer-associated fibroblasts (CAFs). Abrogation of canonical Notch signaling in ECs reprograms the phenotype of CAFs from myofibroblasts into pro-inflammatory fibroblasts and stimulates T cell recruitment in a CXCR3-dependent manner to inhibit tumor growth. Moreover, the fibroblast-immune remodeling, induced upon Notch deficiency in ECs, unleashes interferon gamma (IFNg) responses in the tumor, upregulates PDL1 expression on tumor cells, and sensitizes PDAC to PD1-based immunotherapy. Collectively, these data uncover an important role of endothelial Notch signaling in shaping the tumor immune microenvironment, and suggest the potential of targeting EC-CAF crosstalk as an approach to enhance anti-tumor immunity in immunologically cold tumors.

John Kickey, PhD, Postdoc
Advisor: Garry Nolan
Department of Pathology

Immune cell spatial layering of the human intestine

Using a variety of different single cell (CODEX, snRNA-Seq, snATAC-Seq) technologies we analyzed many different regions of the intestine across spatial scales and present the first multiplexed imaging reference for healthy small intestine and colon. This analysis showed differences in immune, stromal, and epithelial cell type density, organization, and composition along the intestine. Particularly our analysis uncovered cell type organizations associated with donor metadata (BMI and hypertension) and also spatial immune microenvironments coordinated with epithelial development.

Preksha Bhagchandani, Immunology MSTP Graduate Student
Advisor: Seung Kim, Department of Developmental Biology, and of Endocrinology, Gerontology, & Metabolism; and Everett Meyer, Department of Blood & Marrow Transplantation, and of Pediatrics - Stem Cell Transplantation

Hematopoietic stem cell transplantation promotes fully MHC-mismatched islet allograft tolerance and diabetes reversal

Although islet transplantation holds promise as a curative approach in diabetes, physicians and scientists are challenged to produce safe immunosuppression-free methods to promote islet transplant tolerance. Mixed chimerism achieved by hematopoietic cell transplantation (HCT) promotes tolerance of transplanted donor-matched solid organs and tissues, but currently requires toxic bone marrow conditioning, and entails risks of graft-versus-host disease (GVHD). We developed a chemotherapy-free, non-myeloablative conditioning regimen that achieves mixed chimerism and allograft tolerance across fully-mismatched major histocompatibility complex (MHC) barriers. Durable multi-lineage mixed chimerism was achieved in immunocompetent mice using monoclonal antibody targeting of c-Kit, T-cell depleting antibodies, and low dose total body irradiation prior to transplantation of purified hematopoietic stem and progenitor cells (HSPC). Mixed chimerism allowed for long-term tolerance of donor-matched islet allografts without signs of GVHD. We applied this reduced-intensity conditioning protocol to diabetic B6 RIP-DTR mice with inducible diabetes. After diabetes induction and conditioning, fully MHC-mismatched donor-matched islets and hematopoietic cells were transplanted, resulting in 100% long-term correction of diabetes (n=9/9 mixed chimeric mice), with preservation of fertility and other measures of functional status, and without chronic immunosuppression or GVHD. Allotolerance in mixed chimeras is likely mediated by donor-derived thymic dendritic cells and host-derived peripheral regulatory T cells. Furthermore, this conditioning regimen with hematopoietic cell and donor-matched islet transplantation

applied to NOD (Non-Obese Diabetic) autoimmune diabetic mice results in complete chimerism and 100% long-term correction of autoimmune diabetes (n = 5/5). Studies of chimerism in NOD reveal that both host and donor Tregs are essential to maintain peripheral tolerance to residual autoreactive T cells. These results provide proof-of-concept for a clinically-translatable reduced-intensity conditioning regimen and cell transplantation protocol that achieve durable hematopoietic chimerism, promoting islet allograft tolerance and diabetes reversal.

Session 6	Speaker and Title
11:25-11:45 AM	**Sydney Lu, PhD,** Assistant Professor of Hematology, *The accessory RNA splicing factor RBM39 regulates T cell function and the alloantigen response.*
11:45 AM-12PM	**Josselyn Peña**, Immunology Graduate Student, Krams Lab, *Investigating the role of natural killer cells in the control of latent Epstein-Barr virus infection*
12:00-12:15 PM	**Kazuki Nagashima, MD, PhD,** Postdoc, Fischbach Lab, *Mapping the T cell repertoire to a complex gut bacterial community*

Sydney Lu, PhD, Assistant Professor
Department of Hematology

The accessory RNA splicing factor RBM39 regulates T cell function and the alloantigen response

Recent studies have identified a class of small molecules, the 'anti-cancer sulfonamides,' to target the accessory RNA splicing factor RBM39. These drugs coopt the endogenous cellular Ddb1/CUL4 E3 ubiquitin ligase and a key adapter DCAF15 to mediate ubiquitination of RBM39 as a neosubstrate, yielding its proteosomal degradation. RBM39 degraders are selective for myeloid neoplasms with RNA splicing factor mutations via a synthetic lethal mechanism, and the drug E7820 is in phase II trials for these diseases clinicaltrials.gov NCT05024994).
In addition to direct anti-cancer effects, we have recently shown that RBM39 degradation in tumor cells enhances anti-tumor responses to immune checkpoint blockade through generation of novel RNA missplicing-derived transcripts. These are then translated to immunogenic MHC I-presented peptides on tumors and elicit an endogenous anti-tumor CD8 T cell response. RBM39 degraders improve the responses to immune checkpoint blockade in preclinical models without obvious immune related toxicities, and did not appear overtly immunosuppressive.

Despite the above, rigorous evaluation of the role of RBM39 in immune cells has not been performed. Here we identify that RBM39 modulates exon recognition in T cells to regulate their function. Although essential for the survival of hematopoietic precursors and tumors, RBM39 is dispensable for peripheral T cell survival. Functional evaluation of the T cell alloantigen response in allogeneic hematopoietic stem transplantation (allo-HSCT) revealed that Rbm39 loss attenuated graft-versus-host-disease (GVHD) and improved overall survival while partially sparing graft-versus-tumor (GVT) effects.
To rigorously evaluate the role of RBM39 in immune cells, we generated an Rbm39 conditional knockout mouse by floxing Rbm39 exon 7, which encodes its first RNA recognition motif. Pan-hematopoietic deletion of Rbm39 resulted in complete failure of hematopoiesis and impaired hematopoietic stem cell (HSC) self-renewal. We next analyzed animals with T cell-specific RBM39 deletion and compared this with the effects of pharmacologic RBM39 degradation. Strikingly, in contrast to the profound effects of genetic RBM39 loss in HSCs and the inability of cancer cells to tolerate RBM39 deletion, RBM39 loss under Cd4-cre (deletion early in thymopoiesis) or distal Lck-cre (late in thymopoiesis) yielded modestly fewer viable peripheral T cells, which skewed towards a CD44+ effector/memory phenotype. This observation was also recapitulated with extended treatment of mice with the RBM39 degrader E7820.

Deep RNA-seq analysis of splenic CD3+ T cells from distal Lck-cre Rbm39fl/fl mice revealed widespread decreased efficiency of RNA splicing in Rbm39 null T cells compared to controls, indicative of altered RNA splicing in such T cells. Rbm39 KO T cells experienced global increases in exon skipping at genomic regions where upstream introns had reduced GC content. These data identify that RBM39 is required in a cell and tissue-specific manner for cell survival and further highlights the selectivity of therapeutic approaches targeting RBM39.

We next assessed the functional consequences of pharmacologic RBM39 degradation or genetic RBM39 deletion in T cells. Consistent with observations in genetic mouse models, T cells could tolerate extensive pharmacologic

RBM39 degradation without loss of proliferative capacity in response to PMA/Ionomycin or anti-CD3+CD28 stimulation. Similarly, genetic Rbm39 null T cells had an intact proliferative response to PMA/Ionomycin. Furthermore, when we evaluated the effects of RBM39 loss in the MHC-mismatched C57BL/6 => Balb/c allo-HSCT system, Rbm39 deletion in donor T cells under either CD4- or distal Lck-cre profoundly reduced GVHD severity and improved overall survival in recipients. Similar results were found with pharmacologic RBM39 degradation in the same HSCT system. Lastly, transplantation experiments with Balb/c recipients engrafted with A20 lymphoma revealed partial preservation of GVT activity using Rbm39 null T cells. Single cell RNA sequencing analyses of CD4-cre Rbm39fl/fl primary T cells revealed upregulation of the integrin beta1 subunit in both CD4 and CD8 T cells. This suggests alteration of T cell trafficking away from GVHD target organs as one potential mechanism by which RBM39 loss may alter T cell alloreactivity in allo-HSCT.

Our results identify cell-type specific roles for RBM39 in hematopoiesis and immune cells and suggest a therapeutic approach for GVHD via direct modulation of RNA splicing. Of note, as the RBM39 degrader E7820 is currently in phase II clinical trials for relapsed myeloid neoplasms, our previous studies and this data identify a treatment for myeloid neoplasms in the post-transplant setting, where RBM39 degraders mediate both direct and immune-mediated anti-tumor effects while also attenuating GVHD via modulation of RNA splicing in T cells.

Josselyn Peña, Immunology Graduate Student
Advisor: Sheri Krams
Department of Surgery - Abdominal Transplantation

Investigating the role of natural killer cells in the control of latent Epstein-Barr virus infection

Epstein–Barr virus (EBV) infection affects more than 90% of adults worldwide and is associated with several malignancies. EBV infection is typically asymptomatic in healthy individuals and remains dormant in memory B cells. Failure to control latent EBV can result in a variety of malignancies, especially in immunocompromised people. Studies in both experimental models and humans suggest that NK cells are critical in the host defense against EBV. In previous work, we demonstrated that NK cells expressing the inhibitory receptor NKG2A were able to specifically target autologous B cell lines latently infected with EBV. We also demonstrated that HLA-E-presented peptides derived from EBV latent cycle proteins can impair NKG2A recognition and its downstream inhibitory signaling, potentially leading to NK cell activation. We generated a panel of EBV-lymphoblastoid cell lines (EBV-LCL) and performed co-cultures with primary NK cells and autologous EBV-LCL, then stained with a novel mass cytometry panel of over 40 NK functional and phenotypic markers. In addition to NKG2A, EBV-LCL-responsive NK cells express increased HLA-DR and CD32 (FcγRII) and downregulate CD16 (FcγRIII). Our mass cytometry findings in combination with EBV-LCL-responsive NK cell transcriptome analysis from single-cell RNA sequencing are providing insights into the mechanism whereby NKG2A+ NK cells can recognize and kill EBV-LCL.

Kazuki Nagashima, MD, PhD, Postdoc
Advisor: Michael Fischbach
Department of Bioengineering and of Microbiology & Immunology

Mapping the T cell repertoire to a complex gut bacterial community

Certain bacterial strains from the microbiome induce a potent, antigen-specific T cell response. However previous reports have profiled strains from the microbiome under artificial conditions of mono-colonization. This approach identifies strains that has a potential to modulate immune cell function, but it is unknown how a

strain behaves in the context of the complex gut microbiome, or what all strains contribute to the net phenotype of immune modulation by the entire microbial community. Here, we colonize germ-free mice with a complex defined community (97 or 112 bacterial strains) and profile T cell responses to each strain individually. Unexpectedly, the pattern of T cell responses suggests that many T cells in the gut repertoire recognize multiple bacterial strains from the community. We constructed T cell hybridomas from 92 T cell receptor (TCR) clonotypes; by screening every strain in the community against each hybridoma, we find that nearly all of the bacteria-specific TCRs exhibit a one-to-many TCR-to-strain relationship, including 13 abundant TCR clonotypes that are polyspecific for 18 Firmicutes in the community. By screening three pooled bacterial genomic libraries against 13 pooled hybridomas, we discover that they share a single target: a conserved substrate-binding protein (SBP) from an ABC transport system. Treg and Th17 cells specific for an epitope from this protein are abundant in community-colonized and specific-pathogen-free mice. Our work reveals that T cell recognition of Firmicutes is focused on a widely conserved cell-surface antigen, opening the door to new therapeutic strategies in which colonist-specific immune responses are rationally altered or redirected.

Reference:
1) Cheng et al., Cell, 2022. Design, construction, and in vivo augmentation of a complex gut microbiome, PMCID: 36070752
2) Nagashima et al., bioRxiv, Mapping the T cell repertoire to a complex gut bacterial community

Keynote	Speaker and Title
7:05-8:10 PM	**Keynote: Peter Kim, PhD**, Virginia and D. K. Ludwig Professor of Biochemistry at Stanford, *Towards a universal flu vaccine and a pan-SARS-CoV vaccine*

Peter Kim, PhD, Professor
Department of Biochemistry

Towards a universal flu vaccine and a pan-SARS-CoV vaccine

While the rapid development of COVID-19 vaccines has been a scientific triumph, the need remains for a globally available vaccine that provides long-lasting, protective immunity against infection by present and future SARS-CoV-2 variants of concern (VOCs). We describe DCFHP, a ferritin-based, protein-nanoparticle that, when formulated with alum as the sole adjuvant (DCFHP-alum), can elicit robust and durable neutralizing antisera in non-human primates against known VOCs including Omicron BA.4/5, as well as against SARS-CoV-1. Following a booster ~one year after the initial immunization, DCFHP-alum elicits a robust anamnestic response. We show that DCFHP-alum is room-temperature stable, and we generated a cell line that can enable production of thousands of vaccine doses per liter of cell culture. DCFHP-alum has potential as a once-yearly booster vaccine, and as a primary vaccine for pediatric use including in infants, with global accessibility.

ORAL SESSION
STANFORD IMMUNOLOGY CONFERENCE
Sunday, January 29, 2023
Points Ballroom

Session 7	Speaker and Title
9:00-9:15 AM	**Nathan Reticker-Flynn, PhD,** Instructor, Engleman Lab, *Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance*
9:15-9:30 AM	**Guangbo Chen, PhD,** Postdoc, Davis Lab, *Cytokines surge in the blood years prior to a cancer diagnosis in elderly individuals*
9:30-9:45 AM	**Katherine Nico**, Immunology Graduate Student, Howitt Lab, *Tuft cell-mediated type 2 immunity depletes intestinal resident T cell protection*
9:45-10 AM	**Sirimuvva Tadepalli, PhD,** Postdoc, Idoyaga Lab, *Rapid monocyte recruitment dictates the therapeutic efficacy of focal radiotherapy*

Nathan Reticker-Flynn, PhD, Instructor
Advisor: Ed Engleman
Department of Pathology, and of Immunology & Rheumatology

Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance

The majority of cancer-associated deaths result from distant organ metastasis, yet the mechanisms that enable this process remain poorly understood. For most solid tumors, colonization of regional or distant lymph nodes (LNs) typically precedes the formation of distant organ metastases, yet it remains unclear whether LN metastasis plays a functional role in disease progression. LNs are major sites of anti-tumor lymphocyte education, including in the context of immunotherapy, yet LN metastasis frequently correlates with further disease progression. Here, we find that LN metastasis represents a critical step in tumor progression through the capacity of such metastases to induce tumor-specific immune tolerance in a manner that promotes further dissemination of tumors to distant organs. Using an in vivo passaging approach of a non-metastatic syngeneic melanoma, we generated 300 unique cell lines exhibiting varying degrees of LN metastatic capacity. We show that the presence of these LN metastases enables distant organ seeding of metastases in a manner that the parental tumor cannot, and this effect is eliminated in mice lacking an adaptive immune response. Furthermore, this promotion of distant seeding by LN metastases is tumor specific. Using flow cytometry and single-cell sequencing to perform comprehensive immune profiling, we identify multiple cellular mediators of tolerance. In particular, we find that LN metastases have the capacity to both resist NK cell cytotoxicity and induce regulatory T cells (Tregs). Furthermore, depletion of NK cells in vivo enables non-metastatic tumors to disseminate to LNs, and ablation of Tregs using FoxP3-DTR mice eliminates the occurrence of lymphatic metastases. Adoptive transfer of Tregs from the LNs of mice bearing LN metastasis to naïve mice facilitates metastasis in a manner that Tregs from mice without LN metastases cannot, and we find that these Tregs are induced in an antigen-specific manner. Whole exome sequencing revealed that neither the metastatic proclivity nor immunosuppression evolve through the acquisition of driver mutations, loss of neoantigens, loss of MHC class I presentation, or decreases in melanoma antigen expression. Rather, by RNA-seq and ATAC-seq, we show that a conserved interferon signaling axis is upregulated in LN metastases and is rendered stable through epigenetic reprogramming of chromatin accessibility resulting from chronic exposure to interferons in vivo. Furthermore, using CRISPR/Cas9, we find that these pathways are required for LN metastatic seeding, and validate their conserved significance in additional mouse models of pancreatic ductal adenocarcinoma and head and neck squamous cell carcinoma and humans with LN metastatic disease. Together, these findings demonstrate a critical role for LN metastasis in promoting tumor-specific immune tolerance.

Guangbo Chen, PhD, Postdoc
Advisor: Mark Davis
Department of Microbiology & Immunology

Cytokines surge in the blood years prior to a cancer diagnosis in elderly individuals

How the human immune system reacts prior to a cancer diagnosis is largely unknown. Here we analyze a longitudinal cohort of 133 healthy individuals (18 to over 90 years old), 28 of whom developed cancers over nine years. We find the global abundance of a broad spectrum of cytokines (26 out of 32 assayed) surges 2-3 years before cancer diagnosis but not with other conditions (such as inflammatory or cardiovascular diseases). These cytokine surges precede cancer diagnosis only in those 80 years or older, suggesting age-specific inflammatory effects. Meanwhile, in early-stage (but not late-stage) tissues across 10 cancer types, aging elevates cytokine transcriptional activity, which coincides with an age-dependent expansion of the inflammatory macrophage population. We further find that macrophage is a major producer of cytokines in cancer tissues, irrespective of cancer types. These results suggest that cancer associated macrophage is a major source of peripheral inflammatory molecules and may mobilize immunity well before a clinical diagnosis in the elderly.

Katherine Nico, Immunology Graduate Student
Advisor: Mike Howitt
Department of Pathology, and of Microbiology & Immunology

Tuft cell-mediated type 2 immunity depletes intestinal resident T cell protection

CD8+ tissue resident memory cells (TRMs) are critical players in the immune response at mucosal surfaces, as they reside at the site of initial infection and are therefore optimally primed to respond quickly to specific threats. In the gut, co-infection with an acute type 2 immune stimulus has been shown to hinder the pathogen-specific CD8 T cell response and increase infection burden, but no studies have addressed the effects of a chronic type 2 immune stimulus on long-lived CD8 T cell populations such as TRMs. We report here that induction of type 2 immunity in the ileum after tuft cell stimulation selectively disrupts local CD8 TRMs and shifts the lymphocyte landscape within the intraepithelial compartment, which impedes the host's ability to mount an immune response to oral Listeria monocytogenes infection. In the absence of tuft cell activation, L. monocytogenes is efficiently cleared from the host and generates robust CD8 T cell memory that protects thoroughly against secondary infection. However, elevated ileal succinate levels from T. mu colonization results in fewer activated CD69+CD103+ TRMs, higher bacterial burden, and decreased overall protection upon challenge with L. monocytogenes. These results support our hypothesis that non-pathogenic, chronic sources of type 2 immunity mediated through tuft cell chemosensation can alter the CD8 T cell repertoire in the gut, leading to lower protective memory responses and increased susceptibility to gastrointestinal infection.

Sirimuvva Tadepalli, PhD, Postdoc
Advisor: Juliana Idoyaga
Department of Microbiology & Immunology

Rapid monocyte recruitment dictates the therapeutic efficacy of focal radiotherapy

Tumor-associated myeloid cells modulate innate and adaptive immune responses and have an important role in cancer therapies such as radiotherapy (RT). During non-conformal RT, the recruitment of monocytes and their differentiation into monocyte derived suppressive cells is associated with a negative therapeutic outcome. However, the role of monocytes in clinically relevant conformal radiotherapy (CRT) is not explored until now. Since monocytes are known to be plastic and respond to environmental cues during cancer and inflammation induced by RT, we investigated the functional role of monocyte in the acute immune response during CRT. Contrary to non-conformal RT, we found that monocytes have a positive role in the response to CRT. CRT induces a rapid recruitment of monocytes to the tumor that minimally differentiate into tumor-associated macrophages (TAM) or dendritic cells (DC), but instead upregulate major histocompatibility complex II (MHCII) and costimulatory molecules. We establish that these large numbers of infiltrating monocytes are responsible for increasing type I interferon in the tumor microenvironment (TME), activation of CD8+ T cells and the reduction in tumor burden. Importantly, we demonstrate that rapid monocyte infiltration to the TME is hindered when RT inadvertently affects healthy tissues. Our results unravel a previously unknown positive role of monocytes during CRT.

Session 8	Speaker and Title
11:15-11:30 AM	**Daniel Arve-Butler, PhD,** Postdoc, Monack Lab, *Eosinophils contribute to local immune responses during chronic Salmonella infection*
11:30-11:45 AM	**Madeline Lee**, Immunology Graduate Student, Blish Lab, *SARS-CoV-2 escapes direct NK cell killing through Nsp1-mediated downregulation of ligands for NKG2D*
11:45 AM-12 PM	**Frank Buquicchio**, Immunology Graduate Student, Satpathy Lab, *A unique epigenomic landscape defines CD8+ tissue-resident memory T cells*
12:00-12:15 PM	**Kameron Rodrigues,** Immunology Graduate Student, Jaiswal and Montgomery Labs, *Loss of Dnmt3a or Tet2 in stimulated macrophages alters transcription factor binding and enhances inflammatory gene expression*

Daniel Arve-Butler, PhD, Postdoc
Advisor: Denise Monack
Department of Microbiology & Immunology

Eosinophils contribute to local immune responses during chronic Salmonella infection

Eosinophils are effector cells that have essential immunoregulatory roles as well as key anti-microbial effects against helminths, viruses and some bacterial species. However, the mechanisms of how eosinophils contribute to eliminating bacteria are not fully understood. Furthermore, published results are diametrically different depending on the infecting pathogen. Here we investigate the role of eosinophils during chronic Salmonella infection within mesenteric lymph nodes (MLNs) of 129x1/svJ mice. We show that eosinophil numbers increase significantly over the first 8 weeks of infection with the facultative intracellular pathogen Salmonella enterica Typhimurium (STm). The number of MLN eosinophils significantly correlated with the increase of the eosinophil chemoattractant CCL11. Furthermore, eosinophils were shown to localize to the periphery of STm-containing granuloma structures in MLNs 4 weeks post infection. Eosinophil depletion using Siglec F-specific antibodies rendered mice more susceptible to infection with increased bacterial burdens in the MLNs compared to IgG2 treated controls. In addition, eosinophil-depleted mice had a significant reduction of IFN□-producing cells and lower tissue levels of IFN□ compared to IgG2 controls, suggesting that eosinophils contribute to pathogen control by skewing the tissue microenvironment to a more bactericidal phenotype. In contrast, there was no difference in host survival, pathology or bacterial burdens during an acute, 7-day STm infection in wild type compared to eosinophil-deficient dblGATA1 mice in the BL/6 background. Collectively, our data indicate that eosinophils contribute to controlling the levels of STm within the MLN of persistently infected hosts.

Madeline Lee, Immunology Graduate Student
Advisor: Catherine Blish
Department of Infectious Diseases

SARS-CoV-2 escapes direct NK cell killing through Nsp1-mediated downregulation of ligands for NKG2D

Natural killer (NK) cells are cytotoxic effector cells that target and lyse virally-infected cells; many viruses therefore encode mechanisms to escape such NK cell killing. We interrogated the ability of SARS-CoV-2 to modulate NK cell recognition and lysis of infected cells and found that NK cells exhibit poor cytotoxic responses against SARS-CoV-2-infected targets, preferentially killing uninfected bystander cells. We demonstrate that this escape is driven by downregulation of ligands for the activating receptor NKG2D ("NKG2D-L"). Indeed, early in viral infection, prior to NKG2D-L downregulation, NK cells are able to target and kill infected cells; however, this

ability is lost as viral proteins are expressed. Finally, we found that SARS-CoV-2 non-structural protein 1 (Nsp1) mediates downregulation of NKG2D-L and that Nsp1 alone is sufficient to confer resistance to NK cell killing. Collectively, our work reveals that SARS-CoV-2 evades direct NK cell cytotoxicity and describes a mechanism by which this occurs.

Frank Buquicchio, Immunology Graduate Student
Advisor: Ansu Satpathy
Department of Pathology

A unique epigenomic landscape defines CD8+ tissue-resident memory T cells

Memory T cells provide rapid and long-term protection against infection and tumors. The memory CD8+ T cell repertoire contains phenotypically and transcriptionally heterogeneous subsets with specialized functions and recirculation patterns. While these T cell populations have been well characterized in terms of differentiation potential and function, the epigenetic changes underlying memory T cell fate determination and tissue-residency remain largely unexplored. We examined the single-cell chromatin landscape of CD8+ T cells over the course of acute viral infection, revealed an early bifurcation of memory precursors displaying distinct chromatin accessibility, and defined epigenetic trajectories that lead to a circulating (TCIRC) or tissue-resident memory T (TRM) cell fate. While TRM cells displayed a conserved epigenetic signature across organs, we demonstrate that these cells exhibit tissue-specific signatures and identify transcription factors that regulate TRM cell populations in a site-specific manner. Moreover, we demonstrate that TRM cells and exhausted T (TEX) cells are distinct epigenetic lineages that are distinguishable early in their differentiation. Together, these findings show that TRM cell development is accompanied by dynamic alterations in chromatin accessibility that direct a unique transcriptional program resulting in a tissue-adapted and functionally distinct T cell state.

Kameron Rodrigues, Immunology Graduate Student
Advisor: Sidd Jaiswal, Department of Pathology; and Stephen Montgomery, Department of Pathology, of Genetics, and of Biomedical Data Science

Loss of Dnmt3a or Tet2 in stimulated macrophages alters transcription factor binding and enhances inflammatory gene expression

Kameron B. Rodrigues, J. Gopakumar, M. Maurer, Z. Weng, T. Eulalio, S. Mitchell, D. Nachun, D. Estrada, T. Mazumder, L. Ma, Stephen Montgomery, Siddhartha Jaiswal

A source of increased age-associated inflammation is Clonal Hematopoiesis of Indeterminate Potential (CHIP). CHIP occurs when a somatic mutation arises in a bone marrow stem cell and promotes clonal expansion of that stem cell and its progeny. Furthermore, the function of macrophages derived from these bone marrow stem cells is thought to be affected by CHIP mutations, possibly explaining the increased risk of cardiovascular diseases in people with CHIP. Strangely, the mutations that cause CHIP most frequently occur in genes with opposite function: loss-of-function mutations in DNMT3A and TET2. These enzymes methylate and demethylate DNA, respectively. To evaluate how DNMT3A and TET2 contribute to inflammatory responses in the context of CHIP, we exposed bone marrow derived macrophages from Dnmt3a knockout (KO), Tet2 KO, and wild type (WT) control mice to a low dose of the inflammatory stimuli lipopolysaccharide (LPS). We then measured gene responses with RNA-seq across 12 time points from 0 to 24 hours, paired with ATAC-seq and whole genome methyl-seq for multiple time points. A key subset of inflammatory genes (including Il1b, Il6, and Il23a) were

expressed around twice that of WT in both KOs by 2 hours of stimulation, despite the opposing activity of the enzymes on DNA methylation. Further, KO of Dnmt3a or Tet2 affected DNA methylation in the cis-regulatory regions of many of these inflammatory genes. DNA footprinting analysis from ATAC-seq similarly showed that the binding of transcription factors before and during stimulation was affected by loss of either enzyme. This supports a mechanism whereby the activity of DNMT3a and TET2 alters DNA methylation, modifying binding of nearby transcription factors. Our data demonstrates how DNMT3A and TET2 could modulate DNA methylation to limit inflammatory gene expression in healthy macrophages. Targeting sites of inflammation-associated DNA methylation defects may be a therapeutic avenue for blocking the pathogenic role of CHIP for many inflammatory diseases of aging.

Stanford MEDICINE | Immunology

POSTER SESSION
STANFORD IMMUNOLOGY SCIENTIFIC CONFERENCE
Saturday, January 28, 2022
La Grande Ballroom

Presenter & Title

1. **Gita Abhiraman,** Immunology Graduate Student, Garcia Lab, *Structure- based tuning of IL-21 signaling enhances vaccination and reduces autoantibody production*
2. **Julia Adamska,** Immunology Graduate Student, Pulendran and Li Labs, *The RNA-editing enzyme ADAR1 is necessary for B cell response*
3. **Juan Aguilera, MD, PhD,** Basic Life Research Scientist, Sean N Parker Center for Allergy and Asthma, *Decreases in IL1RA, IL18, and IL8 are associated with increases in ambient air pollution during pregnancy*
4. **Meelad Amouzgar,** Immunology Graduate Student, Bendall Lab, *Unsupervised reconstruction of cell cycle progression and division in asynchronously dividing cells*
5. **Rebeca Arroyo Hornero, PhD,** Postdoc, Idoyaga Lab, *Phenotypic and functional diversification of human plasmacytoid dendritic cells: unraveling the mechanism*
6. **Šimon Borna, PhD,** Postdoc, Bacchetta Lab, *FOXP3-deficient patients have expanded autoreactive T cells originating from both regulatory and effector T cells*
7. **David Kung-Chun Chiu, PhD,** Postdoc, Engleman Lab, *Erythropoietin programs tumor-associated macrophages to suppress antitumor immunity in hepatocellular carcinoma*
8. **Blanda Di Luccia, PhD,** Basic Life Research Scientist, Monack Lab, *Gut regulatory T cells mediate immunological tolerance in Salmonella-infected super-spreader hosts*
9. **Camilo Espinosa Bernal**, Immunology Graduate Student, Aghaeepour Lab, *Multiomics modeling of preterm birth in low- and middle-income countries*
10. **Carley Fowler**, Process Development Specialist, Cancer Cell Therapy Center (CCT-LCGM), *Product characterization of CliniMACS Prodigy™ engineered T cells*
11. **Joe Gonzalez**, Immunology Graduate Student, Wang Lab, *IgG posttranslational modifications promote distinct receptor signaling pathways to elicit opposing immune activity in the lung*
12. **Lilit Grigoryan**, Immunology Graduate Student, Pulendran Lab, *Enhanced breadth of memory B cell responses following adjuvanted virus-like particle vaccination in humans*
13. **Naomi Haddock**, Immunology Graduate Student, Bollyky Lab, *The circulating phageome in lung transplantation*
14. **Maximilian Haist, MD,** Postdoc, Nolan Lab, *Spatial CD8 T-cell infiltration predicts response to primary radiochemotherapy in advanced oropharyngeal cancer*
15. **Colwyn Headley, PhD,** Postdoc, Tsao Lab, *Rescuing aging-associated CD4+ T cell dysfunction via mitochondrial transplantation*
16. **Noor Hussein, PhD,** Postdoc, Mellins Lab, *The role of CD39-expressing regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome (PANS): Ground work for a CAR-Treg therapeutic*
17. **Karan Kathuria**, Immunology Graduate Student, Davis Lab, *Modeling human immune responses to Plasmodium falciparum infection*

18. **Vimal Keerthi, MS,** Life Science Research Professional 2, Feldman and Mackall Lab, *Optimizing electroporation parameters for non-viral CAR-T cell manufacturing*
19. **Olivia Kline**, Epidemiology and Clinical Research Graduate Student, Nadeau Lab, *Chronic smoke exposure burden: Impact on the immune system of firefighters*
20. **Guo Luo, PhD,** Instructor, Mignot Lab, *Protective association of HLA-DRB1*04 subtypes in neurodegenerative diseases implicates acetylated tau PHF6 sequences*
21. **Sainiteesh Maddineni,** MD Student, Sunwoo Lab, *Identifying novel immune interactions of intraepithelial ilc1-like NK cells in head and neck cancer*
22. **Raul Maqueda-Alfaro, PhD,** Postdoc, Idoyaga Lab, *Transitional dendritic cells: identifying the role of a novel innate immune cell population*
23. **Max Miao,** Genetics Graduate Student, Satpathy, *Investigating the role of TCR signal strength in T cell differentiation and exhaustion*
24. **Ayan Mondal, PhD,** Postdoc, Mellins Lab, *Plasma from active pediatric acute-onset neuropsychiatric syndrome (PANS) induces increased BBB permeability through the disruption of junctional proteins of brain endothelial cells*
25. **Kaithlen Zen Pacheco,** Life Science Research Professional 1, Mackall Lab, *Logic-gated selection of multi-vector systems*
26. **Jimena Pavlovitch-Bedzyk,** Immunology Graduate Student, Davis Lab, *Novel skin organoid modeling of monkeypox infection and immune response*
27. **Trung Pham, MD, PhD,** Instructor, Monack Lab, *Single-cell profiling identifies ACE+ granuloma macrophages as a non-permissive niche for intracellular bacteria during persistent Salmonella infection*
28. **Kassie Press, PhD,** Postdoc, Jagannathan Lab, *V*δ2+ γδ *T cell chromatin accessibility and immune function associates with prior malaria incidence*
29. **Taylor Pursell, PhD,** Postdoc, Boyd Lab, *Landscape of big brown bat (Eptesicus fuscus) splenic immune cell populations following rabies virus infection*
30. **Patrick Quinn**, Life Science Research Professional, Mackall Lab, *Cyclin dependent kinase 8 inhibition may synergize with car T-cell therapy for treatment of acute myeloid leukemia*
31. **Colin Raposo**, Immunology Graduate Student, Satpathy Lab, *Cytotoxic T lymphocyte memory after the clearance of chronic viral infection*
32. **Hayley Raquer**, Immunology Graduate Student, Idoyaga Lab, *Traveling from the epidermis to the lymph node: Langerhans cell origin determines their migration potential*
33. **Kalani Ratnasiri**, Immunology Graduate Student, Blish and Khatri Labs, *Non-human primates replicate conserved human responses to RNA viral infections*
34. **Grayson Rodriguez**, Immunology Graduate Student, Garcia Lab, *Triplekines form novel cytokine receptor complexes*
35. **Adonis Rubio**, Immunology Graduate Student, Barnes Lab, *Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains*
36. **David Seong,** Immunology MSTP Graduate Student, Idoyaga Lab, *Unraveling the mechanisms of age-associated functional disruptions in plasmacytoid dendritic cells*
37. **Fernando Sulczewski**, **PhD,** Postdoc, Idoyaga Lab, *Transitional dendritic cells are a novel source of conventional type 2 dendritic cells*
38. **Kattria van der Ploeg**, **PhD,** Postdoc, Jagannathan Lab, *Malaria-exposed Ugandan women exhibit a differential SARS-CoV-2-specific T cell response*

39. **Alun Vaughan-Jackson, PhD,** Postdoc, Bassik Lab, *Developing stem cell-derived macrophages for genome wide screens of viral infectivity*
40. **Xihui Yin**, Life Science Research Professional, Utz Lab, *Anti-dopamine receptor autoantibody detection in Pediatric Acute-onset Neuropsychiatric Syndrome*
41. **Maxim Zaslavsky**, Computer Science Graduate Student, Boyd and Kundaje Labs, *Disease diagnostics using machine learning of immune receptors*

Gita Abhiraman, Immunology Graduate Student
Advisor: Chris Garcia Lab
Department of Molecular & Cellular Physiology and of Structural Biology

Structure-based tuning of IL-21 signaling enhances vaccination and reduces autoantibody production

Interleukin-21 (IL-21) plays a critical role in generating immunological memory by promoting the germinal center reaction, yet clinical use of IL-21 remains challenging due to its pleiotropy and association with autoimmune disease. To better understand the structural basis of IL-21 signaling, we determined the structure of the IL-21–IL-21R–γc ternary signaling complex by X-ray crystallography and a structure of a dimer of trimeric complexes using cryo-EM. Guided by the structure, we designed analogs of IL-21 by introducing substitutions to the IL-21–γc interface. These IL-21 analogs act as partial agonists that modulate downstream activation of pS6, pSTAT3, and pSTAT1. One engineered IL-21 analog enhanced the effects of influenza vaccination in human tonsil organoids, with attenuated induction of autoantibodies compared to wild-type IL-21. These results suggest a strategy for deploying engineered IL-21 variants as vaccine adjuvants to enhance B cell activation, thereby expanding the breadth of antibody responses generated upon vaccination.

Julia Adamska, Immunology Graduate Student
Advisor: Bali Pulendran, Department of Pathology; and Jin Billy Li, Department of Genetics

The RNA-editing enzyme ADAR1 is necessary for B cell response

B cell intrinsic recognition of pathogen associated molecular patterns by pattern recognition receptors (PRRs) can modulate B cell response to antigen. Under normal homeostatic conditions, self double-stranded RNA (dsRNA) is modified by adenosine deaminase acting on RNA 1 (ADAR1) to prevent sensing by PRRs. While ADAR1 is required for normal B cell development, the consequences of its loss in activated B cells is not completely understood. Using a mouse model of conditional Adar1 ablation in activated B cells and local protein-hapten immunization, we observed lower primary and recall antigen-specific antibody responses, as well as defects in memory B cell and long-lived plasma cell formation. Unexpectedly, this phenotype was present in the absence of difference in the frequency, cell cycle progression, or apoptosis of germinal center B cells at their peak. These data suggest that ADAR1 in necessary for normal terminal B cell differentiation. Current work is aimed at elucidating the precise stage and mechanism driving the phenotype.

Juan Aguilera, MD, PhD, Basic Life Research Scientist
Sean N Parker Center for Allergy and Asthma

Decreases in IL1RA, IL18, and IL8 are associated with increases in ambient air pollution during pregnancy

Rationale: During pregnancy, exposure to air pollution can negatively impact the health of both mother and fetus. However, the role of the immune system has not been fully investigated. We quantified immune cytokine levels from pregnant women living in an area of elevated air pollution. We hypothesized that there is an association between PM2.5 exposure and inflammatory cytokines of pregnant women.

Methods: We collected blood from pregnant women (n=168, age=29±6y) at 20-23 weeks of pregnancy. Plasma samples were analyzed using a human 80-plex Luminex assay for cytokine measurement. We used an age- and weight-adjusted multivariable regression model to evaluate the association between PM2.5 exposure 3 months prior to blood draw (mean=14.7µg/m3, range=8.8-26.5µg/m3) and cytokine levels.

Results: PM2.5 levels were negatively associated with the expression levels of inflammatory cytokines after FDR adjustment: IL18 ($p<0.001$), IL1RA ($p<0.001$), IL8/CXCL8 ($p<0.001$), IL16 ($p<0.001$) and MIF ($p<0.005$).

Conclusion: There was a negative association between PM2.5 exposure and inflammatory cytokine levels of pregnant women during their second trimester. Future studies should assess whether air pollution differentially impacts mother's health during other timeframes of pregnancy.

Meelad Amouzgar, Immunology Graduate Student
Advisor: Sean Bendall
Department of Pathology

Unsupervised reconstruction of cell cycle progression and division in asynchronously dividing cells

Cell cycle progression and division are tightly linked to cell fate decisions across development of the hematopoietic and immune systems. Activated T cells enter the cell cycle and rapidly proliferate into a deluge of cellular phenotypes with diverse functional capacity, which is crucial to establish protective immunity. While we understand the importance of cell cycle entry for T cell proliferation, cell cycle progression is at best coarsely studied as four phases: G1, S, G2, and M. Thus, there remains a lack of tools to study highly-resolved cell cycle progression and cell state in tandem. Using Cytometry by time of flight (CyTOF), we combine high-dimensional proteomics, CFSE-derived division id annotations, and trajectory inference to computationally reconstruct cell cycle progression across multiple divisions in millions of asynchronously dividing cultured cells. We implement a landmark-based dimensionality reduction of cell cycle targets using normal, dividing cells and a principal graph that fits a curve in a closed-loop to obtain a pseudotime of cell cycle progression. Our landmark-based dimensionality reduction approach helps generalize our cell cycle pseudotime to new data, and allows for robust estimation of the most similar cell-cycle state in perturbation conditions. We validate our model experimentally using both cell synchronization techniques and known biological differences in cell cycle progression across different cell states. We pose that highly-resolved modeling of cell cycle progression across divisions will permit granular study of the link between cell cycle timing and cell state, and the study of how extrinsic signals and cell plasticity may be controlled by an intrinsic biological system like the cell cycle.

Rebeca Arroyo Hornero, PhD, Postdoc
Advisor: Juliiana Idoyaga
Department of Microbiology & Immunology

Phenotypic and functional diversification of human plasmacytoid dendritic cells: unraveling the mechanism

Plasmacytoid dendritic cells (pDCs) are key players in antiviral immunity given their unique capacity to secrete fast and abundant type I IFN (IFN-I) upon viral encounter. Yet, it has long been known that IFN-I production is limited to a still undefined subpopulation of stimulated pDCs. Moreover, activated pDCs are also known to convert into classical DC (cDC)-like cells with the ability to present antigens and activate T cells; however, it is unknown if all pDCs have this conversion capacity. Here, using high-dimensional single-cell approaches, we demonstrate that activation of a transcriptionally homogenous pDC population results in cellular diversification, i.e., the generation of cellular states with distinct phenotypes and functions. Indeed, a fraction of activated pDCs is equipped to produce IFN-I, while another distinct portion acquire cDC-like properties. These observations reveal an intrinsic plasticity of human pDCs, and a division of labor following activation. Our analysis also revealed that pDC diversification can be modulated by targeting the IFNα and TNF signaling pathways, offering a therapeutic approach to regulate the IFN-I and T cell activation capacity of pDCs, respectively. Our current hypothesis is that harnessing pDC diversification early after infection may increase anti-viral immunity and improve clinical outcomes. This mechanistic pathway may play a critical role during COVID-19, a disease in which pDC dysregulation has been demonstrated to impact immune responses.

Šimon Borna, PhD, Postdoc
Advisor: Rosa Bacchetta
Department of Pediatrics (Stem Cell Transplantation)

FOXP3-deficient patients have expanded autoreactive T cells originating from both regulatory and effector T cells

Immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome is a life-threatening disease caused by loss of function mutations in the FOXP3 gene, a critical transcription factor for functional regulatory T cells (Treg). Yet, the presence of autoreactive T-cell and their origin has never been characterized in IPEX and the role of Treg in controlling autoreactive T-cell expansion in humans remains ill-defined. Using epigenetic analyses of Treg specific demethylated region (TSDR) as a lineage marker for Treg origin, and TCR receptor sequencing, we showed that IPEX patients have expanded autoreactive T-cells originating from two sources. The first originates from effector T cells (Teff), which expanded, likely due to reduced Treg suppressive function. The second source originates from expanded unstable Treg, where a fraction of them lose their phenotype including CD25 and FOXP3. Moreover, functional analyses of FOXP3 knockout and IPEX Treg revealed that the unstable Treg are capable of Teff function, which is consistent with the immune dysregulation occurring in IPEX patients. Collectively, we provide the first demonstration of the presence of autoreactive T cells in IPEX which could be used to monitor disease progression or treatments' efficacy.

David Kung-Chun Chiu, PhD, Postdoc
Advisor: Ed Engleman
Department of Pathology, and of Immunology & Rheumatology

Erythropoietin programs tumor-associated macrophages to suppress antitumor immunity in hepatocellular carcinoma

David Kung-Chun Chiu[1], Nathan E Reticker-Flynn[1] ,Qiang Liu[1], Bowie Yik-Ling Cheng[1], Ryan Lee[1], Sameera Kongara[1], Kazukuni Hayashi[1], Yeseo Choi[1], Lorna L Tolentino[1], Cherie Barclay[1], Joanne ND Reyes[1], Jayakumar Rajadas[1], Xiangyue Zhang[1], Edgar G Engleman[1]

[1] Stanford University, Stanford, CA.

Macrophages are typically abundant and immunosuppressive in solid tumors. They play a key role in protecting tumors from immune surveillance and compromising the efficacy of immune checkpoint blockade (ICB) therapy. However, as professional antigen-presenting cells, macrophages have the intrinsic potential to activate anti-tumor immunity if they receive appropriate environmental cues. Our experiments in mice with hepatocellular carcinoma (HCC) reveal that erythropoietin (EPO) secreted by tumor cells interacts with the erythropoietin receptor (EPOR) on tumor-associated macrophages (TAMs) to govern their immunosuppressive function. As a glycoprotein hormone secreted by the kidney, EPO is known mainly for its ability to induce erythropoiesis. However, it is also secreted by many solid tumors in response to hypoxia. In our experiments, overexpression of EPO by HCC cells results in an immunosuppressive tumor microenvironment, with decreased CD8+ effector memory T cells (TEM) and expansion of regulatory T cells (Tregs) and CD11b+ myeloid cells, thereby preventing T cell-mediated regression of the tumors. In HCC tumors, EPOR is expressed by TAMs, which mainly consist of Kupffer cells and monocyte-derived macrophages. EPO exerts its effects mainly on the TAMs, causing impaired antigen presentation, reduced pro-inflammatory cytokine production and enhanced proliferation and tolerogenic function of macrophages via EPOR. Remarkably, lineage-specific knockout of EPOR in macrophages was sufficient to induce tumor regression, prolong survival in spontaneous HCC models that are resistant to immune checkpoint blockade (ICB) and sensitize the tumors to ICB. Tumors in which TAMs lack EPOR were characterized by an increase in absolute leukocyte number, together with higher frequencies of CD86+MHCII+ macrophages and KLRG1+PD1+CD8+ TEM. These latter observations suggest a change in immune landscape from a cold ICB-resistant tumor to a hot tumor that favors ICB. Together, our results demonstrate EPO/EPOR signaling is crucial for TAMs to suppress anti-tumor immunity. This pathway represents a promising new target for immunotherapy.

Blanda Di Luccia, PhD, Basic Life Research Scientist
Advisor: Denise Monack
Department of Microbiology & Immunology

Gut regulatory T cells mediate immunological tolerance in Salmonella-infected super-spreader hosts

Salmonella Typhimurium is an intracellular enteric pathogen that causes gastroenteritis in humans and Typhoid fever-like symptoms in mice. Previous work from our lab has shown that oral infection of 129X1/SvJ mice with Salmonella results in 20-30% of the mice being high-shedder (shed >108 CFU/g feces). Since these animals can rapidly transmit infection to a naïve host, we refer to them as super-spreaders (SS). Surprisingly, these mice are disease tolerant and remain asymptomatic despite high pathogen burdens in the gut. Here we describe the super-spreader immune state and investigate the mechanisms underlying this newly established homeostasis

between host and pathogen. Super-spreader mice have a sustained Salmonella burden at 28 dpi in the gut but not in the spleen or mesenteric lymph nodes. Although mice developed robust CD4+ and CD8+ T-cell responses, they remained asymptomatic and healthy. We found that super-spreader mice had significantly more Tbet+ Tregs in the gut compared to non-superspreader mice (nonSS). The specific depletion of Tregs resulted in dramatic weight loss, increased gut inflammation, and a compromised epithelial intestinal barrier. Finally, depletion of CD4 T cells rescued the morbidity and intestinal pathology observed in Treg depleted super-spreader mice. We are currently investigating the immunological mechanism triggering this effect and ask whether this new immune- adapted state may prevent new upcoming enteric infections in super-spreader hosts.

Camilo Espinosa Bernal, Immunology Graduate Student

Advisor: Nima Aghaeepour

Department of Anesthesiology, Perioperative and Pain Medicine, and of Pediatrics - Neonatal and Developmental Medicine

Multiomics modeling of preterm birth in low- and middle-income countries

Preterm birth (PTB) is the leading cause of death in children under five years of age, yet comprehensive studies are hindered by its multiple complex etiologies. Epidemiological associations between PTB and maternal characteristics have been previously described. This work employed multiomics profiling and multivariate modeling to investigate the biological signatures of some of these maternal characteristics. Maternal covariates and plasma samples were collected during pregnancy from a cohort of 13,841 pregnant women across four low- and middle-income countries. Plasma samples from 231 participants were analyzed to generate proteomics, metabolomics, and lipidomics datasets. Machine learning-based epidemiological and multiomics models showed robust performance for the prediction of multiple relevant objectives, including PTB, time to delivery, maternal age, gravidity, and body mass index. These results simultaneously provide a novel integrated view of the epidemiological maternal factors associated with PTB and identify new biological signatures of such maternal covariates impacting this disease.

Carley Fowler, Process Development Specialist

Cancer Cell Therapy Center (CCT-LCGM)

Product characterization of CliniMACS Prodigy™ engineered T cells

Generating chimeric antigen receptor (CAR) T cell therapies involves the genetic engineering of patient-derived T cells to introduce artificial receptors targeted to a specific tumor antigen. To produce dose-relevant quantities of the cell therapy product requires isolating T cells from patient apheresis, activating the desired T cell subset, transducing with a specific CAR vector, and expanding these modified cells. One system used to automate the engineering of CAR T products is the GMP-compliant platform, CliniMACS Prodigy™. Stanford Center for Cancer Cell Therapy utilizes this process to generate sufficient dosages of CAR T cells targeting CD22 in patients with CD19- CAR-refractory large B-cell lymphoma. Despite promising clinical outcomes, not all patients treated with engineered CD22 CAR T cells enter remission. Researchers have shown that there is a correlation between CAR T cell product attributes and clinical outcomes. Here, we present a process designed to characterize patient CAR T cell products generated using the automated platform, which involves a combination of multicolor flow cytometry and functional assays to determine the phenotypic profile and cytokine secretion of the products. These data will help us develop models to connect drug product attributes with the clinical efficacy of the

therapeutic.

Carley Fowler[1], Nikolaos Gkitsas[1], Dorota Klysz[1], Vimal Keerthi[1], Sunny Patel[1], Kyle Asano[1], Matthew J. Frank[1,2], Ramya Tunuguntla[1], Shabnum Patel[1], Steven Feldman[1]

Affiliation:
[1] Center for Cancer Cell Therapy, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA.
[2] Department of Medicine, Division of Blood and Marrow Transplantation and Cellular Therapy, Stanford University School of Medicine, Stanford, CA, USA.

[1] Labanieh, L., Majzner, R.G. & Mackall, C.L. Programming CAR-T cells to kill cancer. Nat Biomed Eng 2, 377–391 (2018). https://doi.org/10.1038/s41551-018-0235-9
[2] Lock, Dominik et al. Automated Manufacturing of Potent CD20-Directed Chimeric Antigen Receptor T Cells for Clinical Use. Hum Gene Ther 28,10 914-925 (2017). https://10.1089/hum.2017.111
[3] Baird, J. H., Frank, M. J., Craig, J, et.al. CD22-directed CAR T-cell therapy induces complete remissions in CD19-directed CAR-refractory large B-cell lymphoma. Blood 137 2321–2325 (2021). https://doi.org/10.1182/blood.2020009432
[4] Brentjens, Renier J et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 5,177 (2013). https://doi.org/10.1126/scitranslmed
[5] Majzner, R.G., Mackall, C.L. Clinical lessons learned from the first leg of the CAR T cell journey. Nat Med 25, 1341–1355 (2019). https://doi.org/10.1038/s41591-019-0564-6

Joe Gonzalez, Immunology Graduate Student

Advisor: Taia Wang

Department of Medicine (Infectious Diseases) and of Microbiology and Immunology

IgG posttranslational modifications promote distinct receptor signaling pathways to elicit opposing immune activity in the lung

How antibody signaling impacts the immune environment within a relevant tissue is not well known. Here we describe how different compositions of polyclonal human IgG antibodies impact the cellular effector response in vivo. We observe that IgG compositions enriched for either afucosylated (F0) or sialylated (FS) glycoforms utilize distinct receptors to reciprocally regulate biological pathways relevant to antiviral and inflammatory immune responses in alveolar macrophage (Amϕ). Our findings suggest that differences in IgG glycosylation alone have a profound impact on the activation state of Amϕ effector cells and the broader landscape within the lung. F0 and FS IgG promote inverse transcriptional changes through respective CD16a and SIGN-R1 signaling that enhance or restrain the local immune response. The efficacy of immunotherapies and vaccination strategies could be improved through greater attention to and intention in IgG glycosylation to promote desired clinical outcomes related to immune activity.

Lilit Grigoryan, Immunology Graduate Student
Advisor: Bali Pulendran
Department of Pathology

Enhanced breadth of memory B cell responses following adjuvanted virus-like particle vaccination in humans

The emergence of SARS-CoV-2 variants has highlighted the importance of inducing broadly-reactive humoral responses with vaccination. Adjuvants are immune stimulatory agents known to enhance the magnitude, durability and breadth of the immune response to vaccination. While the ability of adjuvants to enhance antibody breadth has been observed at the level of polyclonal serum responses, it remains unknown whether adjuvants enhance the breadth of memory B cell responses at the level of individual B cell clones. In this study, we investigate the immune responses in individuals vaccinated with either an AS03-adjuvanted or non-adjuvanted virus-like particle COVID-19 vaccine. We find that the AS03 adjuvant promotes the generation of robust and durable memory B cell responses at 6 months following vaccination. Furthermore, we perform B-cell receptor sequencing of spike protein-specific memory B cells, followed by production of monoclonal antibodies (mAbs), and show an enhanced binding and neutralization breadth of B cell-derived mAbs in the adjuvanted group when compared to the non-adjuvanted vaccination. Thus, our study provides evidence for adjuvants enhancing the breadth of the memory B cell compartment.

Naomi Haddock, Immunology Graduate Student
Advisor: Paul Bollyky
Department of Infectious Diseases and of Microbiology & Immunology

The circulating phageome in lung transplantation

Cell free DNA (cfDNA) are the free DNA circulating in human blood. These sequences have been utilized in recent years for studying and diagnosis transplant rejection. However, a less utilized portion of cfDNA is the non-human portion which provide a rich reflection of the human microbiome. Bacterial viruses, phages, can be identified in these sequences and studied. We find that bacteriophage are present in the plasma cfDNA of lung transplant recipients, and that they are reflective of disease leading to transplantation as well as shifts in the circulating phageome post-transplant that differ by patient outcomes. We further find a phage signature associated with the production of donor specific antibodies and graft rejection.

Maximilian Haist, MD, Postdoc
Advisor: Garry Nolan
Department of Pathology

Spatial CD8 T-cell infiltration predicts response to primary radiochemotherapy in advanced oropharyngeal cancer

PURPOSE/OBJECTIVES: Effective anti-tumor immune responses are mediated by cytotoxic T cells (CTL) and require organized, spatially coordinated interactions within the tumor microenvironment (TME). Understanding

coordinated T-cell-behavior and deciphering mechanisms of radiotherapy resistance mediated by tumor stem cells will advance risk stratification of oropharyngeal cancer (OPSCC) patients treated with primary chemoradiotherapy (RCTx).

MATERIAL/METHODS: To determine the role of CD8 T cells and tumor stem cells for response to RCTx, we employed 7-plex immunofluorescence stains on a dedicated tissue microarray of pre-treatment biopsy specimens from 86 advanced OPSCC patients. The TMA was constructed from 1.2mm diameter cores that were punched from two representative regions of the invasive tumor front of the tumor tissue blocks. Seven-color multiplex fluorescence stains for the antigens pan-Cytokeratin, p16INK4a, CD271, PD-L1, Ki67 and CD8 was performed using the Opal Polaris 7-Color Manual Detection Kit. Single-cell based analyses of multiplex stains was carried out for all TMA cores in the open-source image analysis software QuPath and spatial interactions of the resulting cell phenotypes within the TME was explored using the R-package Spatstat. Resulting quantitative single-cell data were finally correlated with clinical parameters, such as patient demographics, smoking and alcohol consumption, treatment regimens and survival data. Primary endpoints included best response to RCTx and overall survival (OS).

RESULTS: Our observations demonstrate that a strong CTL-infiltration into the epithelial tumor compartment (HR for overall survival, OS: 0.35; $p<0.001$) and the expression of PD-L1 on CTL (HR: 0.36; $p<0.001$) were both associated with a significantly better response and survival upon RCTx that was confirmed in a multivariate Cox-regression model. By contrast, overall CTL infiltration, regardless of the affected compartment, was not associated with response or survival (see Figure 1). As expected, p16 expression was a strong predictor of improved OS (HR: 0.38; $p=0.002$) and correlated with overall CTL infiltration (r: 0.358, $p<0.001$). Tumor cell proliferative activity and the expression of the tumor stem cell marker CD271 were not associated with response or survival.

CONCLUSION: In this study, we demonstrate the clinical relevance of the spatial organization and the phenotype of CD8 T cells within the TME. In particular, we found that the infiltration of CD8 T cells specifically into the tumor cell compartment was an independent predictive marker for response to RCTx. Meanwhile, tumor cell proliferation and the expression of stem cell markers showed no independent predictive effect for response to RCTx and require further study.

Colwyn Headley, PhD, Postdoc
Advisor: Phil Tsao
Department of Cardiovascular Medicine

Rescuing aging-associated CD4+ T cell dysfunction via mitochondrial transplantation

Mitochondrial dysfunction alters cellular metabolism, increases oxidative stress, and may be principal to the dysregulated signaling and function of CD4+ T lymphocytes in the elderly. In this proof of principle study, we investigated whether the transfer of functional mitochondria into CD4+ T cells that were isolated from old mice and elderly humans, could abrogate aging-associated mitochondrial dysfunction and improve the functionality of aged CD4+ T cells. Our results show that the delivery of exogenous mitochondria to aged T cells led to cellular and mitochondrial reprogramming. This was evidenced by significant mitochondrial proteome alterations that coincided with improved aerobic metabolism and decreased mitoROS in non-activated CD4+ T cells from old mice that received healthy mitochondria. Compared to non-manipulated CD4+ T cells, CD4+ T cells that received functional mitochondria (i.e. mito-transferred CD4+ T cells from old mice) showed improvements in activation-induced TCR-signaling kinetics that correlated with increased frequencies of CD4+ T cells that displayed markers of activation (CD25), increased IL-2 production, as well as enhanced proliferation ex vivo. Immunodeficient mouse models (RAG-KO and TCR-KO) showed that the adoptive transfer of mito-transferred naive CD4+ T cells, protected recipient mice from influenza A and M. tuberculosis infections and promoted effector memory T cell

differentiation. Further, mito-transfer improved CD4+ T cell activation in human T cells by decreasing activation-induced senescence. These findings support mitochondria as targets of therapeutic intervention in aging.

Noor Hussein, PhD, Postdoc
Advisor: Betsy Mellins
Department of Pediatrics - Human Gene Therapy

The role of CD39-expressing regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome (PANS): Ground work for a CAR-Treg therapeutic

Pediatric Acute Onset Neuropsychiatric Syndrome (PANS) is a relapsing and remitting disorder, accounting for 10-20% of pediatric obsessive-compulsive disorder (OCD). To date, there is no specific therapy for PANS, though some patients respond to antibiotics, anti-inflammatory and/or anti-psychotic drugs. PANS is associated with inflammation of the basal ganglia, dysregulation of innate immune cells, and autoimmunity. Regulatory T (Treg) cells are key inhibitors of autoimmunity and play an essential anti-inflammatory role. Among the different immunological mechanisms hypothesized to be involved in OCD immunopathogenesis, alteration in Tregs frequency has been reported. A study of children with early-onset OCD showed an imbalance of circulating Tregs and T effector (Th17). However, the role of Tregs in PANS remains largely unexplored. Hence, we aimed to examine the levels of regulatory T (Treg) cells in PANS patients and healthy controls. The assessment was performed in 10 children with paired samples from PANS flare and improved status, and 6 age-sex-matched healthy controls (HCs). The percentages of circulating Treg cells were evaluated using flow cytometry. The results showed a trend towards increased frequency of CD4+CD127low/-CD25high (bona fide Tregs) in peripheral blood mononuclear cells (PBMCs) of PANS flare patients compared to HCs as well as a decrease in these cells in paired samples after PANS improvement. In bona fide Treg cells, the percentage of CD39+ cells (a marker of highly active and suppressive Tregs) was significantly higher in PANS flare compared to HCs and PANS improvement ($p = 0.04$ and $p = 0.01$, respectively). The percentage of double positive fork head box P3 (FOXP3) + (key Treg transcription factor)/CD39+ Tregs within bona fide Treg cells was significantly higher in PANS flare compared to HCs and PANS remission ($p = 0.03$ and $p = 0.04$, respectively). We hypothesize that the significant increase in circulating CD39+ Tregs in PANS flare signals the development of a strong immunosuppressive response to the severe neuroinflammation of PANS flares. Further research is in progress to determine the functional activity and stability of the CD39+ Treg subset during PANS flare and remission.

Karan Kathuria, Immunology Graduate Student
Advisor: Mark Davis
Department of Microbiology & Immunology

Modeling human immune responses to Plasmodium falciparum infection

Mechanistic studies of immune responses to Plasmodium falciparum (P. falciparum) and vaccine efficacy have been stymied by the lack of an accurate infection model; mouse models of malaria use Plasmodium species that do not infect humans. We have developed a human spleen organoid culture (HSO) system that recapitulates germinal center responses and produces antibodies in response to live attenuated influenza and SARS-CoV-2 mRNA vaccines. Given the dual role of human spleen in initiating cellular and humoral responses to blood-stage P. falciparum and in clearing parasites, we are developing a spleen-malaria infection model. Stimulation of HSO with killed P. falciparum parasites in red blood cells (RBCs) yielded CD8 T cell activation and remarkable gamma-delta T cell proliferation. In addition, we observed a five-fold increase in plasmacytoid dendritic cells

(DCs) as a percentage of all DCs. We also noted significant changes in B cell phenotype from naïve and memory cells to germinal center (GC) and pre-GC cells, accompanied by three-fold enrichment in plasmablasts compared to control HSOs. We are enthusiastically pursuing live infection cultures and are interested in studying human-parasite interactions with a full suite of genetic, cellular, and soluble factor manipulations afforded by our experimental system.

Vimal Keerthi, MS, Life Science Research Professional 2

Advisors: Steve Feldman, Department of Blood & Marrow Transplantation; and Crystal Mackall, Department of Pediatrics and of Blood & Marrow Transplantation

Optimizing electroporation parameters for non-viral CAR-T cell manufacturing

Background: Non-viral CAR-T cell manufacturing relies on electroporation to deliver nucleic acids which contain therapeutic transgenes such as Chimeric Antigen Receptors (CARs). However, implementing electroporation into a closed system process has been challenging. Methods: Here we optimized electroporation parameters using the Neon/Xenon platform, which is the only commercially available electroporator platform that can accommodate large-scale electroporation suitable for GMP application and allows for changes in the electroporation settings. We optimized electroporation settings and explored low conductivity buffers for electroporation of primary human T-cells using nucleic acid for transgene delivery.

Results: Our efforts resulted in a uniform non-viral CAR transgene expression as high as 26% CAR positive cells and up to 17-fold T-cell expansion by Day 14 of culture. Additional work to optimize cell density at electroporation and concentration of nucleic acid electroporated into primary human T-cells is ongoing and will be presented.

Conclusion: Our work will provide a blueprint for future non-viral manufacturing of T-cell therapies using a closed system electroporation platform that allows for seamless optimization of electroporation settings.

Olivia Kline, Epidemiology and Clinical Research Graduate Student

Advisor: Kari Nadeau

Department of Medicine and of Pediatrics - Allergy and Clinical Immunology

Chronic smoke exposure burden: Impact on the immune system of firefighters

Firefighters are exposed to significant levels of smoke during their careers. Long-term smoke exposure has been associated with chronic diseases and cancers in firefighters. Furthermore, smoke exposure has been associated with the long-term accumulation of heavy metals (e.g., lead, arsenic), which have toxic effects. Despite these known risk factors, the impact of this pollutant burden on the immune system of firefighters remains largely unknown. The goal of this study was to characterize the immune dysfunction caused by chronic smoke exposure and identify possible associations with heavy metals in firefighters versus controls.

Peripheral blood mononuclear cells (PBMCs) were profiled utilizing cytometry time of flight (CyTOF) and measured heavy metal levels in the cells during CyTOF by utilizing available heavy metal channels in a cohort of San Francisco firefighters (n=9; 21-52 years;) and healthy controls (n=9; 26-58 years) with a similar level of baseline pollution exposure. FlowJo was used to identify cell populations.

The firefighter group had a lower frequency of CD45- CCR7+CD27- NK cells in the live cell population

(p<0.02) and in the NK cell population (p=0.016). Higher cell counts (p<0.05) were found in the firefighter group compared to control in B cells that were positive for Cd 106, Pb 204, and W 182 and CD4+ cells that were positive for Pb 204 and Pb 208. These results suggest chronic smoke exposure may alter the frequency of CD45- CCR7+CD27- NK cells and increase accumulation of certain heavy metals compared to healthy controls.

This study expands upon current knowledge of potential mechanisms of immune dysfunction associated with smoke exposure and heavy metals in firefighters.

Guo Luo, PhD, Instructor
Advisor: Emmanuel Mignot
Department of Psychiatry and Behavioral Sciences - Sleep Medicine

Protective association of HLA-DRB1*04 subtypes in neurodegenerative diseases implicates acetylated tau PHF6 sequences

Protective association of HLA-DRB1*04 subtypes in neurodegenerative diseases implicates acetylated tau PHF6 sequences
Guo Luo, Yann Le Guen, Jean-Charles Lambert, Michael Greicius, Emmanuel Mignot (and PD/AD collaborators)

Objective
To explore genetic association between human leukocyte antigen (HLA) and neurodegenerative diseases and investigate mechanisms behind the association.

Background
Pathophysiology of Alzheimer's disease (AD) and Parkinson's disease (PD) involves accumulation of tau (neurofibrillary tangles) and amyloid-β-rich (Aβ, amyloid plaques) aggregates in AD, α-synuclein-rich aggregates (αSYN, Lewy bodies) in PD, although these aggregates may also co-occur. Likewise, consensus is growing that the adaptive immune system may play a key role in AD and PD, but the connection between human leukocyte antigens (HLAs) and tau/Aβ/αSYN is little known.

Design/Methods
We analyzed HLA associations in ~176,000 individuals with PD or AD versus controls across ancestry groups. Pursuing this, we also compared postmortem brain density of neurofibrillary tangles and amyloid plaques in brain, tau and Aβ42 levels in cerebrospinal fluid (CSF) of ~8,000 individuals (controls and AD), and examined association of HLA in ~2,500 patient with pathologically demonstrated Lewy Body Dementia. We next screened 448 overlapping tau peptides including the most frequent post translationally modifications (PTM) against multiple HLA subtypes. We further examined tau restricted T cells using tetramer HLAs and performed single-cell RNA sequencing (scRNA seq).

Results
A shared genetic association was observed across AD and PD at rs601945 (PD: odds ratio (OR)=0.84; 95% confidence interval, [0.80; 0.88]; p=2.2x10-13; AD: OR=0.91[0.89; 0.93]; p=1.8x10-22). Hierarchical protective effects of HLA-DRB1*04 subtypes best accounted for the association, strongest with DRB1*04:04 and DRB1*04:07, intermediary with DRB1*04:01 and DRB1*04:03 and absent for DRB1*04:05. The same signal was associated with decreased neurofibrillary tangle (but not neuritic plaque) density postmortem and was more associated with lower tau levels than Aβ42 level changes in the CSF. Further, protective DRB1*04 subtypes strongly bound the aggregation-prone tau PHF6 sequence, but only when acetylated at K311, a modification central to aggregation. T cells recognizing this epitope were identified and T cell receptor (TCR) clones were characterized, showing relevance of this immune response in patients with neurodegenerative disorders and suggesting a protective role of HLA DRB1*04.

Conclusion
An HLA-DRB1*04-mediated adaptive immune response, potentially against tau, decreases AD risk, offering the possibility of new therapeutic avenues.

Sainiteesh Maddineni, MD Student
Advisor: John Sunwoo
Department of Otolaryngology - Head & Neck Surgery Divisions

Identifying novel immune interactions of intraepithelial ilc1-like NK cells in head and neck cancer

Adoptive cell therapies have been growing in relevance in recent years for the treatment of solid tumors. Importantly, natural killer (NK) cell therapies have been a relatively understudied class of immunotherapy that are increasingly popular now. Compared to the significant risk of graft-versus-host disease (GVHD) associated with CAR-T therapy, allogeneic adoptive NK transfer carries minimal risk of GVHD and offers an avenue for off-the-shelf treatment of cancer. In order to optimize future NK cell therapies, an understanding of differentiated NK subsets is necessary. Recently, the Sunwoo Lab has identified intraepithelial ILC1-like (ieICL1-like) NK cells as the subset of NK cells in head and neck squamous cell carcinoma (HNSCC) with the greatest cytotoxicity. The increased cytotoxicity of these cells in HNSCC is promising for next-generation NK cell therapies that robustly curb solid tumor growth. However, a better understanding of the mechanisms by which ieILC1-like NK cells may promote tumor clearance is necessary. Specifically, investigating how ieILC1-like NK cells interact with the broader immune system can reveal novel immune interactions and provide mechanistic explanation for the potency of these cells in HNSCC.

Here, we elucidate the role ieILC1-like NK cells play in recruiting and activating dendritic cells (DCs) and T cells into the HNSCC microenvironment. First, we generated a population of ieILC1-like NK cells and validated their cytotoxicity against HNSCC cells. Next, we characterized the cytokine and chemokine secretion profiles of these cells when stimulated by contact with tumor cells and found elevated levels of GM-CSF and CXCL10. Finally, we validated these observed cytokine secretions by testing how different stimulation conditions as well as different cancer cell lines could affect production of CXCL10 and GM-CSF by ieILC1-like NK cells. CXCL10 is an important chemokine for recruitment of various immune cells, including DCs and T cells, and GM-CSF plays an important role in DC activation. Uncovering these interactions highlights the important role ieILC1-like NK cells may play in bridging innate and adaptive immune responses to improve systemic anti-tumor responses. Furthermore, these data explain how ieILC1-like NK cells may contribute to a more potent systemic immune response than other NK subsets, providing preclinical support for future clinical translation of ieILC1-like NK cells into patient trials.

Raul Maqueda-Alfaro, PhD, Postdoc
Advisor: Juliana Idoyaga
Department of Department of Microbiology & Immunology

Transitional dendritic cells: identifying the role of a novel innate immune cell population

High-dimensional approaches have revealed emerging heterogeneity within dendritic cells (DCs), including a conserved population of transitional DCs (tDCs). These cells harbor phenotypic features of both plasmacytoid DCs (pDCs) and conventional type 2 DCs (cDC2s). However, the tissue micro-localization and function of tDCs has yet to be determined. Here, we used immunofluorescence to evaluate the tDC localization in mouse spleen. We found that, different from pDCs, tDCs locate mainly in the marginal zone and bridging channels of the spleen at

steady state, which correlate with their capacity to uptake circulating particulate antigens like sheep red blood cells (SRBC). We then analyze the capacity of tDCs to respond to immune stimuli. We found that, similar to other well-known DC subsets, tDCs undergo significant transcriptional changes following Toll Like Receptor (TLR)-ligands stimulation; however, tDCs maintain their distinct transcriptional identity. We further evaluate tDC capacity to produce cytokines post-TLR stimulation, and demonstrate their capacity to secrete IL12. Finally, we demonstrate that tDC are potent antigen presenting cells that promote naïve CD4+ T cell proliferation. Our data demonstrate that tDC are functional DCs, and guide our understanding of their function during immune responses in vivo.

Max Miao, Genetics Graduate Student
Advisor: Ansu Satpathy
Department of Pathology

Investigating the role of TCR signal strength in T cell differentiation and exhaustion

T cells provide protection against a broad range of pathogens by expressing genetically arranged T cell receptors (TCRs) with diverse antigen specificity and binding avidity. The strength of TCR signaling is a key determinant of T cell differentiation and function. In chronic infection and cancer, T cell clones with higher-affinity TCRs are biased to become terminally exhausted, while those with lower-affinity TCRs are biased to become cytotoxic exhausted cells expressing killer cell lectin-like receptor (KLRs). We aim to identify genes that regulate T cell differentiation in response to varied TCR signaling strengths and improve T cell function by perturbing these genes. We developed a robust, scalable, and reproducible in vitro culture system that provides T cells with different TCR signal strengths using altered peptide ligands (APLs) of varied affinities. While chronic stimulation with either high or low-affinity APLs induced T cell exhaustion marked by co-inhibitory receptors and reduced proliferation, only the lower-affinity APL upregulated cytotoxic and effector-related genes such as granzymes and KLRs, consistent with previous observations in vivo. Transcriptional and chromatin accessibility analysis revealed that the lower-affinity APL induced transcription factors Eomes and Id2, and increased chromatin accessibility at Fos/Jun binding motifs. Finally, we performed pooled CRISPR screen to identify genes regulating cell survival and co-inhibitory receptor expression in response to APLs of varied affinities and found families of TCR signal strength-dependent regulators. These findings suggest that T cells respond to a broad range of TCR signal input by utilizing different downstream pathways, and tuning these pathways by pharmaceutical intervention or genetic engineering may improve T cells function in the context of chronic antigen exposure.

Ayan Mondal, PhD, Postdoc
Advisor: Betsy Mellins
Department of Pediatrics - Human Gene Therapy

Plasma from active pediatric acute-onset neuropsychiatric syndrome (PANS) induces increased BBB permeability through the disruption of junctional proteins of brain endothelial cells

The blood-brain barrier (BBB) is formed by a monolayer of tightly sealed blood endothelial cells (BEC) and maintained by interactions between tight junction proteins [e.g., claudin 5, occludin, and zonula occludens 1 (ZO1)] and adherens junction proteins [e.g., VE -Cadherin, β catenin]. Impaired BBB function is hypothesized to play a role in the pathogenesis of pediatric acute-onset neuropsychiatric syndrome (PANS). To begin to test this hypothesis, we used a BEC monolayer as an in vitro BBB model. We measured BEC permeability and junctional

integrity after exposure to plasma from PANS patients with active disease, the same patients after clinical improvement and age-sex-matched healthy controls. Plasma from active PANS at 1% volume significantly increased BEC permeability to 10 kDa dextran compared to healthy control plasma. Permeability consistently increased from 2 hrs to 24 hrs. In addition, plasma from active PANS patients decreased BEC expression of tight junction protein (ZO-1), adherent junction protein (VE-Cadherin), and induced actin stress fiber formation. Current investigations include analysis of transcriptional changes in BEC after exposure to PANS plasma and proteomic analysis of PANS plasma to identify factors responsible for increased BBB permeability and disruption of junctional proteins.

Kaithlen Zen Pacheco, Life Science Research Professional 1
Advisor: Crystal Mackall
Department of Pediatrics and of Blood & Marrow Transplantation

Logic-gated selection of multi-vector systems

Chimeric antigen receptor (CAR) T cell therapy is a promising area of immunotherapeutics wherein T cells are genetically engineered with synthetic receptors (CARs) to facilitate tumor antigen detection and overall destruction of cancer cells. Despite early clinical success in patients with hematologic malignancies, major challenges remain, including treatment-associated toxicities, limited efficacy against solid tumors, and high rates of relapse. Such obstacles highlight the need to develop next-generation CAR-T therapies with improved safety and ability to overcome antigen heterogeneity. However, isolating extensively engineered cell populations is costly and leads to massive cell losses when serial sorting with multiple surface markers.

Here, we introduce “STASH-select,” an AND-gate based system that uses 1 selection marker to purify multi-positive cell populations. STASH-select utilizes 3 components: an intracellular retention motif, protease with high degree of specificity, and a protease cleavage site. Between 2 separate vectors, each fused to a different protein of interest, one is engineered to encode the protease while the second encodes the protease cleavage site fused to a selection marker. Both express an intracellular motif localized to the Endoplasmic Reticulum (ER) and a ribosome skipping site (P2A) that allows for bicistronic expression of both proteins with their STASH-select counterparts. When a transduced cell receives both vectors, we expect the protease from one vector to bind to its cleavage site on the other, subsequently removing the fused intracellular retention tag and allowing the selection marker to be expressed on the cell’s surface.

For preliminary testing, we used c-Myc for our selection marker and cloned vector 1 and 2 with green fluorescent protein (GFP) and blue fluorescent protein (BFP), respectively. After cotransducing primary T cells, we ran flow cytometry and observed the highest levels of c-Myc surface expression in cells positive for both BFP and GFP, whereas those single positive for either vector expressed notably less. Currently, we are testing STASH-select with multiple CARs in hopes to further characterize T-cell functionality and enrich for multi-positive populations using GMP manufacturing platforms. While optimization is needed, STASH-select demonstrates the potential to simplify purification and understanding of the extensively engineered clinical products needed to advance cancer therapeutics.

Jimena Pavlovitch-Bedzyk, Immunology Graduate Student
Advisor: Mark Davis
Department of Microbiology & Immunology

Novel skin organoid modeling of monkeypox infection and immune response

There are currently no model systems available that recapitulate all the native elements of human skin. Currently available systems are based on using animal models or other incomplete in vitro model systems. Animal models suffer from well known differences between human and model organism biology. The in vitro model systems currently available can only model limited aspects of human skin biology often failing to incorporate critical components such as stromal cells and immune cells. Here, we create an in vitro culture system that contains all the native elements of human skin, allowing for critical studies of human skin biology. We use this system to investigate the emerging pathogen monkeypox with the goal to better understand mechanisms of infection, immune activation, and virus clearance.

Trung Pham, MD, PhD, Instructor
Advisor: Denise Monack
Department of Microbiology & Immunology

Single-cell profiling identifies ACE+ granuloma macrophages as a non-permissive niche for intracellular bacteria during persistent Salmonella infection

Macrophages mediate key antimicrobial responses against intracellular bacterial pathogens, such as Salmonella enterica. Yet, they can also act as a permissive niche for these pathogens to persist in infected tissues within granulomas, which are immunological structures comprised of macrophages and other immune cells. We apply single-cell transcriptomics to investigate macrophage functional diversity during persistent Salmonella Typhimurium (STm) infection in mice. We identify determinants of macrophage heterogeneity in infected spleens and describe populations of distinct phenotypes, functional programming, and spatial localization. Using a STm mutant with impaired ability to polarize macrophage phenotypes and maintain tissue persistence, we delineate macrophage populations that contribute to limiting infection. We find that angiotensin converting enzyme (ACE) defines a granuloma macrophage population that is non-permissive for intracellular bacteria and their abundance anticorrelates with tissue bacterial burden. Entry into ACE+ macrophages is not a limiting factor for STm to exploit these cells as a niche for persistence. ACE+ macrophages exhibit cytokine signaling and metabolic programing that are unfavorable for intracellular bacteria. Disruption of pathogen control by neutralizing TNF is linked to preferential depletion of ACE+ macrophages in infected tissues. Thus ACE+ macrophages have limited capacity to serve as a cellular niche for intracellular bacteria to establish persistent infection.

Kassie Press, PhD, Postdoc
Advisor: Pras Jagannathan
Department of Infectious Diseases and of Microbiology & Immunology

Vδ2+ γδ T cell chromatin accessibility and immune function associates with prior malaria incidence

A major component of incomplete natural immunity to Plasmodium falciparum (Pf) malaria is attenuation of cytotoxic, pro-inflammatory responses by innate immune cells following repeated malaria exposure. In order to identify mechanisms underlying Vδ2+ γδ T cell dysfunction and to characterize the longevity of this response, we obtained repeated samples from a longitudinal cohort of children living in Tororo, Uganda, before and after a district-wide insecticide campaign that dramatically reduced malaria transmission. Paired ATAC-Seq and RNA-Seq experiments utilizing sort-purified Vδ2+ cells from Ugandan children (n=20) at 3 timepoints revealed

differential chromatin accessibility and gene expression based on prior incidence of clinical malaria. We identified differential chromatin and transcription factor motif accessibility between samples from 2016 (reduced transmission) vs. 2013 (high malaria transmission) at sites associated with immune signaling (e.g. IL-19, CD8, CXCR6, STAT1) and regulation (e.g. BCL2, KLRC1). Analysis of RNA-Seq data is ongoing. In addition to defining transcriptional and epigenetic changes underlying altered cell function following repeated malaria or reduced malaria transmission, we established an in vitro system to simulate the in vivo context. Vδ2+ T cells from malaria-naïve individuals stimulated for 6 days with Pf-infected red blood cells (iRBCs) or the phosphoantigen HMBPP produced less TNFα and IFNγ and degranulated less in response to secondary stimulation compared to unstimulated cells; however, rest following stimulation partially rescued the decreased responsivity to iRBCs. Similar results were obtained using cells from malaria-uninfected Ugandan individuals, but responses from recently infected individuals were more variable. Ultimately, this work could deepen our understanding of mechanisms driving inefficient acquisition of antimalarial immunity—including potential reversibility of functional changes following repeated malaria—and could have applications for novel therapeutic approaches targeting innate immune responses in addition to adaptive responses.

Taylor Pursell, PhD, Postdoc
Advisor: Scott Boyd
Department of Pathology

Landscape of big brown bat (Eptesicus fuscus) splenic immune cell populations following rabies virus infection

Big brown bats (Eptesicus fuscus) are the North American bat species most frequently associated with rabies viral infection. E. fuscus are also one of the most widely distributed bat species and therefore play a pivotal role in the sylvatic maintenance of rabies virus (RABV) in North America. Serological and experimental evidence suggest E. fuscus have some inherent resistance to rabies infection, but the host-pathogen interactions and host factors that lead to this resistance remain unclear. Despite the evidence of novel host-pathogen dynamics and potentially novel host factors, there is a lack of basic knowledge of the E. fuscus immune system, especially the adaptive immune system. In this study, we use single cell transcriptomics to characterize splenic immune cell populations of five wild-caught, captive maintained E. fuscus individuals with variable rabies immune histories after surviving rabies challenge. Preliminary analysis shows that not only can we identify the major canonical immune cell populations, but that the adaptive immune compartment, specifically the B-cell compartment, is expanded in these bats. These data lay the foundation for more in-depth study and understanding of adaptive immunity and viral pathogen response in E. fuscus.

Patrick Quinn, Life Science Research Professional
Advisor: Crystal Mackall
Department of Pediatrics and of Blood & Marrow Transplantation

Cyclin dependent kinase 8 inhibition may synergize with car T-cell therapy for treatment of acute myeloid leukemia

Immunotherapy using chimeric antigen receptor (CAR) T-cells show promise for the treatment of acute myeloid leukemia (AML) but have limited efficacy. CDK8 is a known oncogene involved in several tumor types, including colon, breast, pancreatic, melanoma, and leukemia. CDK8 and CDK19 are the kinase subunits of Mediator kinase module which regulate transcription by phosphorylating numerous targets associated with transcription,

including RNA polymerase II. CD93 targeting CAR T-cells and Cyclin Dependent Kinase (CDK8) inhibition have both been shown to be effective against AML proliferation. Using in vitro cell cultures, our group recently reported that CDK8 inhibition augmented CAR T-cell proliferation, leading to the hypothesis that a precise dose of CDK8 inhibiting drugs could be found that might synergize the perturbation of tumor progression while enhancing CAR T-cell function. Relative to equal volumes of DMSO, the CDK8 inhibitor SEL120 was found to be less toxic to CAR T-cells at decreasing concentrations, and a 5uM dose of SEL120 was found to be tolerant to CAR T-cell expansion while remaining toxic to AML. Coculture experiments are a practical next step in assessing how well CDK8 inhibition can facilitate the clearance of AML using CD93 targeting CAR T-cells.

Colin Raposo, Immunology Graduate Student
Advisor: Ansu Satpathy
Department of Pathology

Cytotoxic T lymphocyte memory after the clearance of chronic viral infection

Chronic viral infection disrupts the normal process by which cytotoxic T lymphocytes (CD8+ T cells) form memory, instead becoming hypofunctional exhausted cells. Recently, it has been shown that clearance of infection can partially reverse this phenotype. With the clearance of chronic viral infection, exhausted CD8+ T cells take on a memory-like cell state, known as recovering exhausted T cells (TEX-REC). TEX-REC show increased functional capacity relative to exhausted T cells but are impaired relative to central memory T cells. Differential function of T cells formed from acute infection has been well characterized, so we sought to understand the phenotypic and functional diversity of TEX-REC. Using single-cell transcriptomics, we uncover that TEX-REC consist of cells in a central memory-like fate and others with a cytotoxic gene signature. Preliminary data show that these populations are clonally distinct and that central memory-like TEX-REC have increased propensity for expansion in response to viral rechallenge. Taken together, these data show that there is a specific pool of TEX-REC that has the best potential to mount a response during a secondary exposure to pathogen.

Hayley Raquer, Immunology Graduate Student
Advisor: Juliana Idoyaga
Department of Department of Microbiology & Immunology

Traveling from the epidermis to the lymph node: Langerhans cell origin determines their migration potential

Hayley Raquer-McKay[1] and Juliana Idoyaga[1,2]
[1]Immunology Program, Stanford University School of Medicine
[2]Department of Microbiology and Immunology, Stanford University School of Medicine

Myeloid cell ontogeny changes over the life of the organism, which can influence cellular function and tissue homeostasis. In particular, Langerhans cells (LC), dendritic cells of the epidermis, are known to have two distinct origins, i.e., an embryonic origin early in life and a bone marrow origin during inflammation. Yet, it is unclear if these two origins modulate LC function. To answer this question, we used a murine steady-state LC depletion model and lineage tracing. We observed that after LC depletion, embryonic LC are replaced by monocyte-derived LCs that are phenotypically indistinct from their embryonic counterparts. Interestingly, we found that without inflammatory signals, monocyte-derived LCs are not able to fully reconstitute the epidermis even after 6

months post depletion. Nevertheless, monocyte-derived LCs reach steady state levels in the skin draining lymph nodes as fast as 2 months. This implies that unlike embryonically seeded LCs, monocyte-derived LCs have a distinct preference to be localized in the lymph node than the skin. By using a competitive in vivo assay between embryonic LCs and monocyte LCs, we show that monocyte-derived LCs have an intrinsically higher migratory advantage over embryonic LCs. Further studies will aim at understanding what are the mechanisms that modulate the differential migratory capacity of monocyte derived LCs, as well as the functional consequences of this effect. Our studies aid in the understanding of functional impacts of epidermal LC origin, which is essential in the development of therapeutics targeting the skin.

Kalani Ratnasiri, Immunology Graduate Student

Advisors: Catherine Blish, Professor of Infectious Diseases; and Purvesh Khatri, Department of Biomedical Informatics, and of Biomedical Data Science

Non-human primates replicate conserved human responses to RNA viral infections

In the 21st century, a number of epidemic and pandemic viruses have emerged, with the most recent being the ongoing SARS-CoV-2-driven pandemic. The next pandemic virus is difficult to predict; however, RNA viruses make up to an estimated 44% of all emerging infectious diseases. While non-human primate (NHP) models play a critical role in understanding viral disease pathogenesis, their broad translatability across human RNA viral infections remains to be further explored. Previously, we discovered a conserved human panviral signature able to not only discern viral infections from healthy responses, but also predict disease severity. Here, we analyzed blood transcriptomic data from 22 challenge studies across five RNA viral families and over 200 macaques to show that our human-derived panviral signature is also conserved in NHPs, robustly distinguishing RNA viral infections irrespective of the biological, clinical and technical heterogeneity. Longitudinal analysis of viral challenge studies identified distinct signature dynamics of NHP infection responses by viral family: Orthomyxoviridae and Coronaviridae infections induce signatures that peak 1-3 days post-infection while Arenaviridae and Filoviridae infection responses continue to rise past7-10 days post-infection. We also confirm virus differences in human response dynamics across influenza, rhinovirus and respiratory-syncytial virus infections. Finally, we demonstrate that the strength of this panviral response correlates with known viral pathogenicity and is driven by myeloid cells. Together, our findings elucidate differences in conserved panviral response dynamics by virus in NHPs that both support NHPs as robust models for human RNA viral infections and also inform the design of future NHP viral challenge studies for studying current, emerging, and re-emerging RNA viruses.

Grayson Rodriguez, Immunology Graduate Student

Advisor: Chris Garcia

Department of Molecular & Cellular Physiology and of Structural Biology

Triplekines form novel cytokine receptor complexes

Cytokines are proteins that dimerize receptors on immune cells to initiate signaling. Natural cytokines have provided therapeutic benefits in a variety of disease contexts, but lack cell specificity and can result in undesirable toxicity. We build upon our work with surrogate agonists of cytokine receptors by inducing signaling through three receptors at once via ‘triplekine’ administration. Triplekines are composed of an engineered base cytokine linked to a binding arm for another cytokine receptor. Our work reveals the possibility of inducing non-natural signaling profiles by forming novel cytokine receptor complexes. In particular, we elucidate the effects of

simultaneous CD132, IL2Rb, and IL21R signaling in human T cells and NK cells. In CD8+ T cells, we observe modulation of pSTAT1, pSTAT3, and pSTAT5 signaling. In NK cells, we observe greater cytotoxicity and activation. These findings provide the foundation for a new catalog of engineered cytokine therapeutics and in vitro reagents for ex vivo cell expansion.

Adonis Rubio, Immunology Graduate Student
Advisor: Christopher Barnes
Department of Biology

Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains

Since the onset of the current pandemic, SARS-CoV-2-neutralizing antibodies have been comprehensively investigated and shown to be effective for the prevention and treatment of COVID-19. While first-generation antibody therapeutics against SARS-CoV-2 have been developed in record time, the emergence of variants of concern (VOCs), such as the Omicron sub-lineages, has reduced the efficacy and rendered some antibody therapeutics ineffective. Bispecific antibodies (bsAbs) can serve as a therapeutic platform with multiple advantages over existing antibody monoclonal or cocktail therapeutics including: 1) a reduction in manufacturing and regulatory obstacles associated with antibody cocktails, 2) increased resistance to viral evasion, and 3) increased avidity of binding viral antigens. Here, we determine a 3.0 Å cryo-EM structure of a broad and potent neutralizing NTD-specific antibody and identified RBD-specific antibodies whose binding geometries were compatible with an IgG-like bsAb format. We selected C1596, C1533, and C1520 NTD antibodies and C135 and C952 RBD antibodies to engineer a panel of six bsAbs that have dual specificity for the N-terminal and receptor binding domains of the SARS-CoV-2 Spike glycoprotein. We employed the CrossMabFab and XmAb bsAb platforms to facilitate expression using Expi293F mammalian cells and purification of our desired bsAb using ion-exchange chromatography and size-exclusion chromatography. Preliminary data has demonstrated the successful expression of our bsAbs and confirmed their ability to bind each of their epitopes compared to the parental antibodies. We are currently evaluating the capability of our bsAbs to potently and synergistically neutralize SARS-CoV-2 VOCs using an in vitro SARS-CoV-2-spike pseudovirus lentivirus assay, which will inform the selection of candidate bsAbs for future in vivo efficacy studies.

David Seong, Immunology MSTP Graduate Student
Advisor: Juliana Idoyaga
Department of Department of Microbiology & Immunology

Unraveling the mechanisms of age-associated functional disruptions in plasmacytoid dendritic cells

David Seong[1,2,3], Rebeca C. Arroyo Hornero[2,3], Marcela Alcántara-Hernández[2,3], Juliana Idoyaga[2,3]

[1]Stanford Medical Scientist Training Program, Stanford University School of Medicine
[2]Department of Microbiology & Immunology, Stanford University School of Medicine
[3]Immunology Program, Stanford University School of Medicine

Aging is a growing medical problem. Although aging affects all organ systems, its influence on the immune system is particularly important as evidenced by the recent COVID-19 pandemic. While much is known about lymphocyte aging, myeloid cell aging has been relatively understudied. Among myeloid cells, dendritic cells

(DCs) initiate a broad range of immune responses through a division of labor among functionally specialized subsets. Unfortunately, an understanding of how aging affects each DC subset is lacking. Here, we used a high-dimensional approach, i.e., CyTOF, to examine age-associated changes of each subset of DCs. We found that aging causes a decrease in the number of plasmacytoid dendritic cells (pDCs), a subset of DCs involved in fighting viral infections. To examine alternations in function, we evaluated pDC capacity to secrete type I interferon (IFN-I) and stimulate T cells. While IFN-I production is similar between young and elderly pDCs, we found that elderly pDCs have reduced ability to generate regulatory T cells. To begin untangling the mechanism of functional disruption, we compared the phenotype of adult and elderly pDCs. We show that two transcription factors necessary for pDC development and function, i.e., TCF4 and IRF8, are decreased in expression with age. Altogether, our data demonstrate that aging alters pDC numbers and function, and suggest a possible mechanism of these changes. This project has the potential to unravel important immunological processes critical for the development of new treatments tailored to the elderly.

Fernando Sulczewski, **PhD,** Postdoc
Advisor: Juliana Idoyaga
Department of Department of Microbiology & Immunology

Transitional dendritic cells are a novel source of conventional type 2 dendritic cells

Dendritic cells (DCs) are group of antigen-presenting cells divided into subsets based on their distinct function, i.e., plasmacytoid DCs (pDCs) are able to secrete high levels of type I interferon (IFN-I) during viral infections, whereas conventional type 1 DCs (cDC1s) and conventional type 2 DCs (cDC2s) excel at the activation of antigen-specific CD8+ and CD4+ T cells, respectively. Recently, high-dimensional single-cell technologies have revealed emerging DC subsets. One of these are transitional DCs (tDCs), which have features of both pDCs and cDCs. Yet, we lack an understanding of the developmental origin of tDCs, which pose an impediment for the development of mouse models to deplete these cells specifically, and ultimately, study their function. Here, we show that tDCs and pDCs share several developmental features, but nevertheless, tDCs do not derive from pDCs during homeostasis. Instead, tDCs originate from a bone marrow progenitor shared with pDCs and called pro-pDCs. Interestingly, we found that tDCs are able to convert into a subpopulation of cDC2s, but are different from previous described pre-cDC2s. Our findings clarify tDC ontogeny as a unique DC lineage developmentally related to pDCs and reveal the heterogeneity of cDC2s, which includes a subpopulation of cells derived from tDCs.

Kattria van der Ploeg, **PhD,** Postdoc
Advisor: Pras Jagannathan
Department of Infectious Diseases and of Microbiology & Immunology

Malaria-exposed Ugandan women exhibit a differential SARS-CoV-2-specific T cell response

While SARS-CoV-2 and its interaction with the immune response has been well-studied in resource-rich areas, there are still many unanswered questions about how it affects malaria-endemic areas in sub-Saharan Africa. Compared to other global regions, during the pandemic, hospitalization and death rates have been reported to be much lower in these areas. One possible explanation could be differential immune responses due to different infectious exposures such as malaria. Using samples collected from pregnant and non-pregnant SARS-CoV-2 exposed Ugandan women, and similarly aged adults from Stanford, we investigated the SARS-CoV-2-specific T cell response by intracellular cytokine staining (ICS) and an activation-induced marker (AIM) assay. Overall, Ugandan women had a markedly diminished SARS-CoV-2-specific T cell response compared to our Stanford

cohort. IFNg- and TNFa-producing T cells were predominant in the Stanford cohort early on and months after initial infection. However, very low or no IFNg and TNFa production was found in the Ugandan cohort. Ongoing research will investigate whether and how other pathogenic exposures, specifically malaria, may influence the T cell response. We hypothesize that previous exposure to malaria mitigates infection with SARS-CoV-2 due to a more 'tolerized' immune response. Potential explanations for our findings include epitope cross-reactivity or down-modulation of an inflammatory response that is implicated in severe COVID-19. Identifying differences in immune responses across populations will be important for future therapeutic innovations and vaccine development.

Alun Vaughan-Jackson, PhD, Postdoc
Advisor: Michael Bassik
Department of Genetics

Developing stem cell-derived macrophages for genome wide screens of viral infectivity

Macrophages are an essential surveillant and homeostatic group of immune cells essential for clearing away viral infections and for mounting immune responses to them. As such, many viruses target these cells to manipulate the immune system and aid dissemination. Good examples of this are Flaviviruses (E.g. Zika and Dengue viruses) and Human Cytomegalovirus (HCMV), each having evolved numerous active and passive methods to evade immune activation of macrophages. However, macrophage-virus interactions can have serious consequences. In severe cases of Dengue virus infection, a potentially lethal haemorrhagic fever is caused by macrophage driven plasma leakage due to breakdown in endothelial barrier integrity. Better understanding viral infection and manipulation of macrophages is needed if we are to counter macrophage-driven viral disease outcomes. Immortalised cell lines lend themselves for genome-wide screens as they are easily cultured and rapidly expanded in number. However, these cells are not ideal as they often inaccurately respond to immune stimuli and have altered metabolic states. Advances in differentiation of induced Pluripotent Stem Cells into macrophages now provide us with a close model to primary macrophages. Furthermore, genetic manipulation at the iPSC stage allows for clonal selection of edited cells for use in probing specific gene functions, a necessary feature for follow up studies after genome-wide screening. Therefore, this project seeks to leverage this new model to provide robust experimental systems to investigate direct and indirect virus interactions with macrophages, their importance in the antiviral inflammatory response, and their potential as therapeutic targets to prevent disease.

Xihui Yin, Life Science Research Professional
Advisor: PJ Utz
Department of Immunology & Rheumatology

Anti-dopamine receptor autoantibody detection in Pediatric Acute-onset Neuropsychiatric Syndrome

Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) manifests as abrupt-onset obsessive-compulsive disorder and/or eating restriction. As a post-infectious disorder, PANS is proposed to have an autoimmune etiology where anti-dopamine receptor autoantibodies dysregulate downstream Ca2+/calmodulin-dependent protein kinase II (CaMKII) activity and dopamine signaling. Here, we developed a novel bead-based immunoarray to detect direct autoantibody binding against multiple dopamine receptor isoforms. We screened for autoantibodies in the sera of 25 PANS patients selected for high OCD scores (CYBOCS patients) and 16 PANS patients selected for high CaMKII activity (CaMKII patients). We report autoantibodies in CYBOCS patients

against dopamine D1 and D2 receptors in inactive conformations that correlate with disease flare. In contrast, we do not observe a strong correlation between high CaMKII activity and autoantibody reactivity against the dopamine receptors in our assay. Together, our results suggest that a subset of PANS patients may contain autoantibodies against the dopamine receptors in the inactive conformation. More direct cell-based binding and functional assays are warranted to validate this finding.

Maxim Zaslavsky, Computer Science Graduate Student

Advisors: Scott Boyd, Department of Pathology; and Anshul Kundaje, Department of Genetics

Disease diagnostics using machine learning of immune receptors

Clinical diagnoses rely on a wide variety of laboratory tests and imaging studies, interpreted alongside physical examination and documentation of symptoms and patient history. However, the tools of diagnosis make little use of the immune system's internal record of specific disease exposures encoded by the antigen-specific receptors of memory B cells and T cells. We combined extensive receptor sequence datasets with three different machine learning representations of immune repertoires to develop an interpretive framework, "MAchine Learning for Immunological Diagnosis" (Mal-ID), that screens for multiple illnesses simultaneously. This approach can distinguish specific acute or chronic infections and autoimmune or immunodeficiency disorders. The receptor sequences that Mal-ID prioritizes for disease classification match known immune response patterns for specific viruses like SARS-CoV-2 and HIV. The model also reveals features shared across autoreactive immune responses, demonstrating how machine learning on immune repertoires can yield new immunological knowledge.

Stanford Immunology Scientific Conference
January 27-29, 2023
Thank you for attending