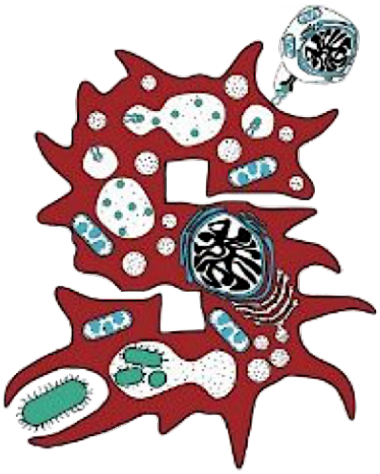

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

Stanford Immunology Annual Scientific Conference November 8–10, 2019 Agenda & Program Abstracts

Asilomar Conference Grounds
Pacific Grove, CA

Stanford MEDICINE | Immunology

Welcome to the 2018 Stanford Immunology Program's Annual Conference at the Asilomar Conference Grounds!

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 on Friday and Saturday will be held across the street from the main conference center in **Fred Farr**. The Poster Session will be in **Kiln**. We then wrap up the conference on Sunday at **Chapel**. Download the Asilomar property map here. 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 20 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:

Friday, November 8
Filip Swirski, PhD
Associate Professor of Radiology,
Harvard Medical School

Saturday, November 9
Ami Bhatt, MD, PhD
Assistant Professor of Medicine and Genetics,
Stanford University School of Medicine

Guest Speaker:

Friday, November 8
Dale Umetsu, MD, PhD
Adjunct Professor of Medicine, Stanford University
Clinical Professor of Pediatrics, UCSF

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 9:00-11:00 pm at Kiln. A Best Poster Prize will be given to two graduate students and two postdoctoral fellows.

- **Poster Slam**: Saturday, November 9 at 8:10-8:40 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 as they are the 'Super Delegates' and have decisive voting power.

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.

Birukova Midnight Swim
Take a plunge in the ocean on Saturday 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.

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.

Special Joint Session Sunday
Stanford Immunology and Stem Cell & Regenerative Medicine Institute will co-host a joint discussion session and lunch.

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.

2019 Immunology Conference Committee
Thank you to the conference planning committee and your dedication on making the event a success.

Conference Directors: Michael Howitt and Sidd Jaiswal
Social Chairs: Second Year Class
Conference Committee: Lina Hansen, Olivia Martinez, Torye Nguyen, and Rita Robinson
Technical Support: David Glass and Candace Liu

Agenda at a Glance

Times, speakers, and topics may change without notice.

Friday, November 8, 2019		Saturday, November 9, 2019		Sunday, November 10, 2019	
General Sessions in Fred Farr **Dinner in Crocker**		**General Sessions in Fred Farr** **Poster Session in Kiln** **Meals in Crocker**		**General Sessions in Chapel** **Breakfast at Crocker** **Lunch at Chapel**	
12:45 pm	Conference Check in				
1:00 pm	Welcome and Introductions	7:30 am	Breakfast	7:30 am	Breakfast
1:10 pm	Session 1	9:00 am	Session 5	9:00 am	Session 9
2:05 pm	Session 2	9:55 am	Break	10:00 am	Break
3:00 pm	Break	10:10 am	Session 6	10:10 am	Career Panel
3:15 pm	Session 3	11:05 am	Break	11:00 am	Lunch
4:10 pm	Session 4	11:15 am	Session 6 continues	11:00 am	Joint Session with Stem Cell
5:05 pm	Lodging Check in	11:55 am	Group Pictures	11:40 am	Break
6:00 pm	Dinner	12:15 pm	Lunch	11:50 am	Joint Session continues
7:00 pm	Keynote	12:15 pm	Faculty Meeting	12:55 pm	General Announcements
8:05 pm	Break	1:30 pm	Research Blitz	1:00 pm	Checkout at Asilomar
8:30 pm	Game Show	4:40 pm	Session 7		
9:30 pm	Reception	6:00 pm	Dinner		
		7:00 pm	Keynote		
		8:10 pm	Poster Slam		
		8:40 pm	Reception at Kiln		
		9:00 pm	Poster Session		
		10:00 pm	Bonfire at Fire Pit		
		11:00 pm	Birukova Midnight Swimming Club		

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

STANFORD IMMUNOLOGY ANNUAL SCIENTIFIC CONFERENCE

Friday, November 8, 2019

All talks are hosted in Fred Farr

12:45 pm	Conference Check in and Pick up name badges at Fred Farr
1:00-1:10 pm	Welcome and Introductions: Conference Directors: **Michael Howitt, PhD,** Faculty, Pathology, and **Sidd Jaiswal, MD, PhD,** Faculty, Pathology
	Session 1
1:10-1:35 pm	**Ansu Satpathy, PhD**, Faculty, Pathology, *Single-cell genomics in cancer immunotherapy*
1:35-1:50 pm	**Miles Linde**, Immunology Graduate Student, Majeti Lab, *Reprogramming Leukemia Cells into Antigen Presenting Cells as a Novel Cancer Vaccination Immunotherapy*
1:50-2:05 pm	**Wan Xing Hong, MD,** Postdoctoral Fellow, Levy Lab, *Neoadjuvant Immunotherapy for Solid Tumors*
	Session 2
2:05-2:30 pm	**Karla Kirkagaard, PhD**, Faculty, Genetics, *TBA*
2:30-2:45 pm	**Eli Gerrick, PhD**, Postdoctoral Fellow, Howitt Lab, *Interrogation of divergent immune responses induced by symbiotic protozoa*
2:45-3:00 pm	**Hannah Frank, PhD**, Postdoctoral Fellow, Boyd Lab, *They're not flying mice: Immune evolution in bats*
3:00-3:15 pm	**Break**
	Session 3
3:15-3:40 pm	**Dale Umetsu, MD, PhD**, Adjunct Professor of Medicine, Stanford University and Clinical Professor of Pediatrics, UCSF, *On the road towards developing therapies for patients with food allergy*
3:40-3:55 pm	**Hesamaldin Movassagh, PhD**, Postdoctoral Fellow, Nadeau Lab, *Dysregulation of Circulating Monocytes is Associated with Exposure to Air Pollution and Asthma Onset in Children*
3:55-4:10 pm	**Yohei Sato, MD, PhD**, Postdoctoral Fellow, Bacchetta Lab, *Engineered (LV)FOXP3 Treg-like cells protect from lymphoproliferation while preserving immune responses in humanized-mice models.*
	Session 4
4:10-4:25 pm	**Paras Minhas**, Neurosciences Graduate Student/MSTP, Andreasson Lab, *Metabolic reprogramming of myeloid cells prevents age-associated cognitive decline*
4:25-4:40 pm	**David Glass,** Immunology Graduate Student, Bendall Lab, *An integrated multi-omic single cell atlas of human B cell diversity*
4:40-6:00 pm	**Lodging Check in at Social Hall/Front Desk.** Day guest meal tickets may be purchased at the Front Desk.
6:00-7:00 pm	**Dinner** at Crocker Dining Hall
	Keynote
7:00-7:05 pm	Keynote Introduction: **Sidd Jaiswal, MD, PhD**

7:05-8:05 pm	**Keynote: Filip Swirski, PhD**, Associate Professor of Radiology, Harvard Medical School and Massachusetts General Hospital, *Lifestyle, inflammation, and cardiovascular disease*
8:05-8:30 pm	**Break**
8:30-9:30 pm	**Game Show**: Presented by Immunology Second Year Graduate Students
9:30 pm-12:00 am	**Reception** at Fred Farr

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

Saturday, November 9, 2019

All talks are hosted in Fred Farr
Poster Session is hosted in Kiln

7:30-9:00 am	**Breakfast** at Crocker Dining Hall
	Session 5
9:00-9:25 am	**John Sunwoo, MD,** Faculty, Otolaryngology - Head & Neck Surgery Divisions, *Heterogeneity of Intratumoral NK Cells and ILCs and Influences on Tumor Behavior*
9:25-9:40 am	**Berenice Mbiribindi, PhD,** Postdoctoral Fellow, Krams Lab, *EBVlatent proteins encode for peptides that prevent NK cell inhibition*
9:40-9:55 am	**Julia McKecknie,** Immunology Graduate Student, Blish Lab, *HLA upregulation during dengue virus infection suppresses the natural killer cell response*
9:55-10:10 am	**Break**
	Session 6
10:10-10:35 am	**Gilbert Chu, MD, PhD,** Faculty, Oncology, Biochemistry, *Side-by-side pairing of DNA ends couples non-homologous end joining to V(D)J recombination*
10:35-10:50 am	**Ioana Marin, PhD**, Postdoctoral Fellow, Shatz Lab, *Neuronal non-classical MHCI Qa-1 is a novel regulator of activity-dependent plasticity*
10:50-11:05 am	**Debopam Ghosh, PhD,** Postdoctoral Fellow, Mellins Lab, *The class II peptide editor, H2-M, affects the development and function of B-1 cells*
11:05-11:15 am	**Break**
11:15-11:30 am	**Adityasai Ambati, PhD,** Postdoctoral Fellow, Mignot Lab, *KIR Associations in Anti-NMDAR Encephalitis*
11:30-11:55 am	**Juliana Idoyaga, PhD,** Faculty, Microbiology and Immunology, *TBA*
	Lunch and Afternoon Activities
11:55 am-12:15 pm	**Group Pictures** in front of Fred Farr for Faculty, Graduate Students and Postdocs
12:15-1:00 pm	**Lunch** for Graduate Students and Postdocs at Crocker Dining Hall
12:15-1:15 pm	**Faculty Meeting:** Faculty, Student, and Postdoc Representatives remain at Fred Farr for Lunch
1:30-3:00 pm	**Faculty-First Year Graduate Students Research Blitz** at Fred Farr
	Session 7
4:40-5:05 pm	**Scott Boyd, PhD,** Faculty, Pathology, *TBA*

5:05-5:30 pm	**Aaron Newman, PhD,** Faculty, Biomedical Data Science, *Dissecting tumor cell states and cellular ecosystems with digital cytometry*
5:30-5:45 pm	**Amber Moore,** Immunology Graduate Student, Palmer Lab, *In-Depth Characterization of Gestational Immune Dynamics*
5:45-6:00 pm	**James Harden,** Immunology Graduate Student, Krams Lab, *Unsupervised analysis of the alloimmune response in a novel model of vascularized composite allotransplantation*
6:00-7:00 pm	**Dinner** at Crocker Dining Hall
	Keynote
7:00-7:05 pm	Keynote Introduction: **Michael Howitt, PhD**
7:05-8:05 pm	**Keynote: Ami Bhatt, MD, PhD,** Assistant Professor Medicine (Hematology) and of Genetics, Stanford University, *Unlocking the Secrets of Microbial Genomes*
	Poster Session
8:10-8:40 pm	**Poster Slam** at Fred Farr
8:40-11:30 pm	**Reception** at Kiln
9:00-11:00 pm	**Poster Session** at Kiln
10:00 pm-12:00 am	**Bonfire** at Fire Pit
11:00 pm-12:00 am	**Birukova Midnight Swimming Club**

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

Sunday, November 10, 2019

All talks are hosted at Chapel

7:30-9:00 am	**Breakfast** at Crocker Dining Hall
	Session 8
9:00-9:15 am	**Florian Wimmers, PhD,** Postdoctoral Fellow, Pulendran Lab, *The single-cell chromatin landscape of human immune responses to influenza vaccines*
9:15-9:30 am	**Prabhu Arunachalam, PhD,** Postdoctoral Fellow, Pulendran Lab, *Vaccine-induced tissue-resident memory T cells and antibodies synergize to prevent mucosal HIV infection*
9:30-9:45 am	**Abigail Powell, PhD,** Postdoctoral Fellow, Kim Lab, *Using protein nanoparticles to develop a safe and stable vaccine against Ebola*
9:45-10:00 am	**Guangbo Chen, PhD,** Postdoctoral Fellow, Khatri Lab, *Integrative proteome and clinical profiling of sJIA-PAP: an emergent lung complication associated with aTH2 response*
10:00-10:10 am	**Break**
	Session 9: Career Panel
10:10-10:50 am	**Career Panel: Marvin Gee, PhD,** Co-founder and Head of Target Discovery, 3T Biosciences, **Erika Check Hayden,** Director, Science Communication Program, University of California Santa Cruz, **Sidd Jaiswal, MD, PhD,** Assistant Professor of Pathology, Stanford University School of Medicine, **Leah Sibener, PhD,** Co-founder and Head of Therapeutic Discovery, 3T Biosciences
11:00 am-12:00 pm	**Lunch** at Chapel

Joint Session	
11:00-11:25 am	**Sidd Jaiswal, MD, PhD,** Faculty, Pathology, *TBA*
11:25-11:40 am	**Charles Chan Lab Trainee**, Surgery, *TBA*
11:40 am-11:50 am	**Break**
11:50 am-12:15 pm	**Agnieszka Czechowicz, MD, PhD,** Faculty, Pediatrics, *TBA*
12:15-12:30 pm	**Thomas Koehnke, MD,** Postdoctoral Fellow, Majeti Lab, *TBA*
12:30-12:55 pm	**Gerlinde Wernig, MD,** Faculty, Pathology, *TBA*
12:55-1:00 pm	**General Announcements**
1:00 pm	**Checkout at Asilomar.** *If you wish, you can leave your card keys with Torye or Rita at Chapel.*
	Stem Cell Conference Attendees Transition to Merrill Hall

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

Stanford Immunology Oral Presentations

STANFORD IMMUNOLOGY ANNUAL SCIENTIFIC CONFERENCE

Friday, November 8, 2019

All talks are hosted in Fred Farr

12:45 pm	Conference Check in and Pick up name badges at Fred Farr
1:00-1:10 pm	Welcome and Introductions: Conference Directors: **Michael Howitt, PhD,** Faculty, Pathology, and **Sidd Jaiswal, MD, PhD,** Faculty, Pathology
	Session 1
1:10-1:35 pm	**Ansu Satpathy, PhD**, Faculty, Pathology, *Single-cell genomics in cancer immunotherapy*
1:35-1:50 pm	**Miles Linde**, Immunology Graduate Student, Majeti Lab, *Reprogramming Leukemia Cells into Antigen Presenting Cells as a Novel Cancer Vaccination Immunotherapy*
1:50-2:05 pm	**Wan Xing Hong, MD,** Postdoctoral Fellow, Levy Lab, *Neoadjuvant Immunotherapy for Solid Tumors*

Ansu Satpathy, PhD
Assistant Professor of Pathology
Stanford University School of Medicine

Single-cell genomics in cancer immunotherapy

Immunotherapies that block inhibitory checkpoint receptors on T cells have transformed the clinical care of patients with cancer. We performed paired single-cell RNA and T cell receptor sequencing and single-cell ATAC-seq on site-matched tumors from patients with basal or squamous cell carcinoma before and after anti-PD-1 therapy. Tracking T cell receptor clones and transcriptional phenotypes revealed coupling of tumor recognition, clonal expansion and T cell dysfunction marked by clonal expansion of $CD8^{+}CD39^{+}$ T cells, which co-expressed markers of chronic T cell activation and exhaustion. However, the expansion of T cell clones did not derive from pre-existing tumor-infiltrating T lymphocytes; instead, the expanded clones consisted of novel clonotypes that had not previously been observed in the same tumor. Analysis of scATAC-seq profiles identified chromatin regulators of therapy-responsive T cell subsets and revealed an exhaustion program that governed non-response to therapy. These results demonstrate that pre-existing tumor-specific T cells may have limited reinvigoration capacity, and that the T cell response to checkpoint blockade derives from a distinct repertoire of T cell clones that may have just recently entered the tumor.

Miles Linde
Immunology Graduate Student
Advisor: Ravindra Majeti, MD, PhD
Stanford University School of Medicine, Department of Hematology

Reprogramming Leukemia Cells into Antigen Presenting Cells as a Novel Cancer Vaccination Immunotherapy

Miles H. Linde, Christopher G. Dove, Sarah F. Gurev, Paul Phan, Feifei Zhao, Eric J. Gars, Lindsay P. Miller, Ravindra Majeti

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive hematopoietic neoplasm characterized by recurrent genetic lesions resulting in B-cell maturation arrest and malignant transformation. Even with the addition of targeted therapies to conventional treatment regimens, prognosis for adults with high risk disease remains poor,

particularly for those patients with relapsed or refractory disease. Despite an arrest in B cell maturation, we previously showed that human B-ALL blasts retain the capacity for reprogramming to the myeloid lineage (McClellan et al, PNAS 2015). While the concept of forced differentiation was proposed several decades ago, no differentiation therapies have been effective in the treatment of B-ALL. Thus, we sought to investigate the therapeutic implications of myeloid lineage reprogramming of B-ALL cells.

We speculated that myeloid-reprogramming of B-ALL cells into antigen presenting cells (APCs) could induce tumor-specific T cell responses through effective presentation of aberrant tumor-associated self-peptides. To test this hypothesis, we generated murine models of B-ALL capable of reprogramming to the myeloid lineage through the inducible expression of two transcription factors, CEBPα and PU.1. Ectopic expression of these factors efficiently reprogrammed B-ALL cells into myeloid-lineage APCs, expressing myeloid markers (CD11b, CD14, CD115, and Ly6C). Reprogramming ablated the tumorigenicity of these cells as they acquired APC characteristics, including phagocytic activity and expression of antigen presentation and co-stimulation molecules: MHC-I, MHC-II, CD80, CD86, and CD40. Using chicken ovalbumin as a model antigen and DO11.10 transgenic CD4+ T cells, we demonstrated that reprogrammed B-ALL cells, but not parental blasts, can process and present both endogenous and exogenous peptides for antigen-specific T cell activation.

To explore the therapeutic potential of B-ALL reprogramming, we engrafted immunodeficient (NSG) and immunocompetent syngeneic (BALB/c) mice with our B-ALL model and induced myeloid reprogramming *in vivo*. While B-ALL reprogramming in immunodeficient mice led to a three day extension in median survival, all of the mice succumbed to their disease. Strikingly, B-ALL reprogramming in immunocompetent mice led to complete tumor regression and survival of the entire cohort 100 days post treatment, suggesting that reprogramming induced immune-mediated tumor eradication. Importantly, these animals were not susceptible to subsequent B-ALL re-challenge, demonstrating successful generation of durable, systemic, and protective immunity.
In order to investigate the mechanism underlying tumor eradication, we depleted BALB/c mice of CD4+ or CD8+ T cells. Depletion of either T cell population abrogated the therapeutic benefit of B-ALL reprogramming, indicating that reprogrammed B-ALL cells stimulate T cell activation *in vivo*. Further analysis of the CD8 T cell repertoire by TCRVβ chain usage revealed significant 10.3-fold expansion of a single TCRVβ chain family in response to B-ALL reprogramming, consistent with an oligoclonal T cell response. Following reprogramming, a 4.01-fold increase in the frequency of infiltrating T cells is observed in the bone marrow, including both activated (CD25+/CD69+) and effector memory (CD44+CD62L-) T cells. Finally, using a dual tumor model, we demonstrated that myeloid reprogramming-dependent T cell activation eradicates malignant cells systemically, as demonstrated by regression of contralateral tumors lacking reprogramming.

Together, our data suggests that (1) B-ALL cells reprogrammed to the myeloid lineage can operate as potent APCs capable of presenting both endogenous and exogenous tumor-associated antigens, (2) *in vivo* B-ALL reprogramming elicits robust immune activation, dependent on both CD4+ and CD8+ T cells, and (3) B-ALL reprogramming-induced immune activation is potent, durable, tumor-eradicating, and systemic. Thus, reprogramming of B-ALL cells into APCs represents a novel immunotherapeutic strategy with potential clinical benefit for the management of B-ALL disease progression.

Wan Xing Hong, MD
Postdoctoral Fellow
Sponsor: Ronald Levy
Stanford University School of Medicine, Department of Oncology

Neoadjuvant Immunotherapy for Solid Tumors

Hong WX[1,2], Sagiv-Barfi I[2], Czerwinski D[2], Testa S[2], Levy R[2]

1. Department of Surgery, Stanford University School of Medicine
2. Stanford Cancer Institute, Division of Oncology, Department of Medicine, Stanford University

Background
Cancer surgery remains the most effective single treatment modality for curing patients with resectable solid tumors. However, only half of all cancer patients undergo curative resection and of those a significant number will eventually relapse from occult metastases. Administration of systemic therapy prior to surgery, known as neoadjuvant therapy, has revolutionized cancer surgery. It not only decreases tumor burden, rendering previously inoperable disease operable, but also improves survival by providing systemic control in patients at high risk of having microscopic distant metastases at the time of surgery.
However, while traditional neoadjuvant therapy using conventional chemotherapy and radiation therapy has improved patient outcomes across a spectrum of solid tumors, the potential of neoadjuvant immunotherapy is currently unknown. Various immune modulating agents have achieved significant clinical success in the management of metastatic and treatment resistant cancers, yet their utility in the neoadjuvant setting remains unclear. In theory, neoadjuvant immunotherapy is particularly interesting because it utilizes the primary tumor as a rich source of antigens for T cell priming. As a result, it may be successful in eliciting an immune response where adjuvant immunotherapy treatment has failed. In our preclinical study of neoadjuvant immunotherapy, we seek to better understand how immunotherapy may be employed synergistically with surgical resection.

Methods
Utilizing an orthotopic murine model of spontaneously metastatic triple negative breast carcinoma we compared local and systemic disease control as well as survival in groups receiving neoadjuvant immunotherapy, resection alone, or immunotherapy alone. We found that neoadjuvant administration of immunotherapy offered the best survival as well as the best locoregional and metastatic disease control when compared to other groups. In fact, the neoadjuvant treatment group was the only group in which a long-term surviving cohort of mice was cured. When these long-term survivors were rechallenged with the same tumor, they were immune, confirming that the treatment successfully conferred immunological memory. When exposed to a new tumor type, however, they remained susceptible, confirming the specificity of this immunologic memory. Disease control was found to be T cell dependent as depletion of either both CD4+ and CD8+, or CD8+ only T cells resulted in loss of therapeutic benefit in mice treated with neoadjuvant immunotherapy.

Conclusion
Using an aggressive orthotopic murine model of breast cancer, we found that neoadjuvant immunotherapy successfully initiated cell mediated antitumor activity both locally and systemically at an anatomically distant disease site. This resulted in improved survival in the neoadjuvant group when compared to groups receiving resection alone or immunotherapy alone.
As increased options to incorporate immunotherapy in the clinic become available to clinicians, this study explores the timely and clinically important question of how immunotherapy may optimally be combined with surgical resection. The results of the study are readily translatable and may be utilized to inform establishment of neoadjuvant immunotherapy regimens for patients with early stage and locally advanced solid tumors.

Session 2	
2:05-2:30 pm	**Karla Kirkagaard, PhD**, Faculty, Genetics, *TBA*
2:30-2:45 pm	**Eli Gerrick, PhD**, Postdoctoral Fellow, Howitt Lab, *Interrogation of divergent immune responses induced by symbiotic protozoa*
2:45-3:00 pm	**Hannah Frank, PhD**, Postdoctoral Fellow, Boyd Lab, *They're not flying mice: Immune evolution in bats*
3:00-3:15 pm	**Break**

Karla Kirkagaard, PhD
Violetta L. Horton Research Professor and Professor of Microbiology and Immunology, Genetics
Stanford University School of Medicine

TBA

Eli Gerrick, PhD
Postdoctoral Fellow
Sponsor: Michael Howitt, PhD
Stanford University School of Medicine, Department of Pathology

Interrogation of divergent immune responses induced by symbiotic protozoa

The microbiota plays a fundamental role in immune development and modulation, as the gut is both the largest immune organ and the most microbially diverse region of the body. Accordingly, the gut microbiota can contribute to immune tolerance but is also associated with many inflammatory diseases. To determine which members of the microbiome impact the immune system, researchers have commonly utilized high-throughput sequencing of the 16S rRNA gene. Unfortunately, this technique only detects bacteria and ignores other non-bacterial members of the microbiota. Technological advances, including shotgun metagenomic sequencing, have expanded our view of the microbiome and led to the discovery of fungal and protozoan members of the human and murine microbiomes that impact intestinal immunity. For example, it was recently discovered that *Tritrichomonas muris*, a commensal protozoan of the mouse intestine, chronically activates the type 2 immune response in the small intestine. *T. muris* signals to tuft cells, taste chemosensory intestinal epithelial cells, which then orchestrate a type 2 immune response. However, we have discovered a new, closely related species of tritrichomonad, here named *Tritrichomonas casperi*, which colonizes the small intestine to high levels but does not stimulate a tuft cell-driven type 2 immune response. Instead, *T. casperi* induces a type 1 immune response upon small intestinal colonization. *T. casperi* colonization results in an accumulation of type 1 $CD4^+$ T cells (T_h1 cells), and an increase in interferon-γ production by both T_h1 cells and $CD8^+$ T cells. To dissect this highly divergent immune remodeling by closely related species, we are further characterizing the immune responses induced by *T. casperi* and *T. muris* through a combination of single cell RNA-

Sequencing, flow cytometry, and fluorescence microscopy, and are investigating the mechanisms by which these protozoa reshape intestinal immunity.

Hannah Frank, PhD
Postdoctoral Fellow
Sponsor: Boyd Lab, MD, PhD
Stanford University School of Medicine, Department of Pathology

They're not flying mice: Immune evolution in bats

Hannah K. Frank, David Enard, James Ellison, Ellie Armstrong, Cara Brook, Angelica Rodriguez, Lisandra Zepeda, Dmitri Petrov, Elizabeth A. Hadly, Scott D. Boyd

Research in model organisms has formed the foundation of current immunology knowledge and has paved the way for numerous advancements in science and medicine. However, this focus on a limited set of tractable study species means that much of the immunological diversity of life, particularly of zoonotic reservoirs, is yet to be explored. Numerous non-model organisms have interesting biology that cannot be replicated in model systems but that can yield important scientific and therapeutic insights. Bats are unique among mammals in their ability to fly and to host and shed numerous highly lethal viruses seemingly asymptomatically. For example, many bats show antibodies to rabies or can even be infected and shed the virus without showing symptoms of disease. We investigated both the innate and adaptive immune system of multiple bat species to understand the basis of their unique relationship with pathogens. We first present data from over 90 species of bats showing that pattern recognition receptors, specifically Toll-like receptors, in bats evolved early to recognize viral pathogens. We then show, using data from seven bat species, that bats express all three canonical superfamilies of Ig heavy chain V genes with similar CDR3 lengths to those observed in other mammals. This is the most comprehensive comparative dataset on bat adaptive immunity to date. Finally, we present the first data on the sequences of immunoglobulins expressed by bats in response to rabies vaccination and subsequent challenge. While researchers have noted for years that bats mount an adaptive immune response to rabies, no one has yet examined the sequences of expressed antibodies in bats. Our preliminary data suggest that innate immunity in bats has evolved differently from other mammals and may underlie their unique relationship with pathogens, while their adaptive immune system seems broadly similar to other mammals. However, comparison of bats' adaptive immune response to rabies vaccination with that of humans may further elucidate the ability of bats to resist disease from rabies and other infections.

Session 3	
3:15-3:40 pm	**Dale Umetsu, MD, PhD**, Adjunct Professor of Medicine, Stanford University and Clinical Professor of Pediatrics, UCSF, *On the road towards developing therapies for patients with food allergy*
3:40-3:55 pm	**Hesamaldin Movassagh, PhD**, Postdoctoral Fellow, Nadeau Lab, *Dysregulation of Circulating Monocytes is Associated with Exposure to Air Pollution and Asthma Onset in Children*
3:55-4:10 pm	**Yohei Sato, MD, PhD**, Postdoctoral Fellow, Bacchetta Lab, *Engineered (LV)FOXP3 Treg-like cells protect from lymphoproliferation while preserving immune responses in humanized-mice models.*

Dale Umetsu, MD, PhD
Adjunct Professor of Medicine, Stanford University
Clinical Professor of Pediatrics, UCSF

On the road towards developing therapies for patients with food allergy

Dale Umetsu was previously a tenured Professor of Pediatrics at Stanford University, and on the faculty in the Immunology, Cancer Biology and Developmental and Neonatal Biology programs. He was also Chief of the Division of Allergy and Clinical Immunology, Pediatrics before leaving in 2004 to become the Prince Turki al Saud Professor at Harvard Medical School. In 2013 he left Harvard to become Principle Medical Director and Global Development Lead for Xolair at Genentech. He retired from Genentech in 2018, although he still consults at Genentech and with several other biotech companies.

During his career, he has authored more than 250 manuscripts, was Chair of the NIH Immunological Sciences Study Section, was member of the Allergenic Products Advisory Committee of the FDA, member of the Network steering Committee of the Immune Tolerance Network (NIH, NIAID), and member of the Board of Directors of the American Board of Allergy and Immunology. He is currently an adjunct Professor of Medicine at Stanford and a Clinical Professor of Pediatrics at UCSF.

Hesamaldin Movassagh, PhD
Postdoctoral Fellow
Sponsor: Kari Nadeau, MD, PhD
Stanford University School of Medicine, Sean N. Parker Center for Allergy and Asthma Research - Lab

Dysregulation of Circulating Monocytes is Associated with Exposure to Air Pollution and Asthma Onset in Children

Hesam Movassagh[1], Mary Prunicki[1], Xiaoying Zhou[1], Jennifer A. Ataam[2], Holden Maecker[3], and Kari C. Nadeau[1]
[1] Sean N Parker Center for Allergy and Asthma Research, Stanford University
[2] Institute for Immunity, Transplantation, and Infection, Stanford University

[3] Department of Microbiology & Immunology, Stanford School of Medicine, Stanford University

Background: The impact of exposure to ambient air pollution on immune system has remained to be precisely addressed. In particular, it is not clear whether increased level of particulate matter in the environment may be associated with altered frequency and function of circulating monocytes in children. Here, we aim to investigate whether living in an area with increased level of air pollutants is associated with alteration of circulating monocytes in children. We further study whether pollution-dysregulated monocytes are associated with clinical measures of allergic asthma.

Methods: We recruited children from Fresno, California, an area with elevated air pollution and collected individual pollutant exposures (PAH_{456}, $PM_{2.5}$, PM_{10}, O_3, CO, NO_2 and NOx) using central site data and location of residence. Blood samples were collected during the visit followed by preparation of PBMC and plasma. Using cytometry time-of-flight (CyTOF), we characterized different immune cells including monocytes. CyTOF data were analyzed using FlowSOM for unsupervised clustering of immune cell types and visualized by t-SNE algorithm. Furthermore, we measured markers of inflammation such as IL-1 using Luminex 63-plex assay.

Results: Increased exposure to $PM_{2.5}$ was associated with elevation of total circulating monocytes in children. Monocytes from high $PM_{2.5}$ exposed children upregulate nuclear expression of aryl hydrocarbon receptor, AhR (n=5 per group). Interestingly, decline in spirometry results as a clinical indicator of asthma (low FEV1 to FVC ratio) was associated with increased accumulation of total circulating monocytes in children. A significant negative correlation between FEV1/FVC ratio and frequency of $CD14^+$ monocytes was observed (n=5 per group). Individual marker clustering on CyTOF data demonstrated that increase in plasma level of IL-1- is associated with elevation of both monocytic marker (CD14) and AhR in children living in Fresno. Computational analysis of CyTOF data was further validated by manual gating using FlowJo software and statistical comparison.

Conclusions: Exposure to ambient air pollution is associated with elevation of circulating monocytes in children. Upregulation of IL-1 and AhR upon air pollution exposure suggest a potential inflammasome-mediated mechanism by which monocyte functions could be dysregulated. Increased accumulation of circulating monocytes in asthmatic children may represent a novel immune signature as a prognostic biomarker in highly polluted areas.

Yohei Sato, MD, PhD
Postdoctoral Fellow
Sponsor: Rosa Bacchetta, MD
Stanford School of Medicine, Department of Pediatrics - Stem Cell Transplantation

Engineered (LV)FOXP3 Treg-like cells protect from lymphoproliferation while preserving immune responses in humanized-mice models.

Yohei Sato[1], Maria-Grazia Roncarolo[1,2], Rosa Bacchetta[1]

[1]Department of Pediatrics, Division of Stem Cell Transplantation and Regenerative Medicine, Stanford School of Medicine, Stanford, CA 94305, US
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FOXP3 is an essential transcription factor for the regulatory T cell (Treg) function and a key regulator of immune tolerance. Autologous Treg cell therapy is a promising therapeutic option for various immune mediated pathologies, including autoimmune diseases, Graft-versus-Host Disease and graft rejection.
To overcome various limitations of currently available Treg cell product, CD4+ T cells from healthy donor and IPEX patient were successfully converted into functional Treg-like cells using lentiviral (LV)-mediated *FOXP3* gene transfer ($CD4^{LVFOXP3}$), thus supporting this approach as a therapeutic for IPEX syndrome, a unique model of genetic autoimmunity due to FOXP3 mutations. Here, we have extended the in depth characterization of $CD4^{LVFOXP3}$ at the molecular level and in animal models to further assess comparability with naturally occurring Treg, and efficacy and safety prior to their *in vivo* use.

Delivery of either allogeneic or autologous $CD4^{LVFOXP3}$ to a humanized mouse model of xeno-GvHD can extend mice survival. Mice rescued from xeno-GVHD by the $CD4^{LVFOXP3}$ remain refractory to a secondary xeno-GVHD challenge. Furthermore, $CD4^{LVFOXP3}$ administration to immunodeficient mice reconstituted with human HSC knocked-out for FOXP3, can rescue the lymphoproliferative phenotype developed in this IPEX-like hu-mice model without impairing immune reconstitution.

The impact of $CD4^{LVFOXP3}$ on the host immune system was evaluated by injecting human PBMC and mature dendritic cells pulsed with several antigens. Responder cells were still able to respond to the antigens after $CD4^{LVFOXP3}$ administration. Similarly, administration of $CD4^{LVFOXP3}$ did not negatively impact on the tumor growth inhibition induced by PBMC injection, in a skin-sarcoma hu-mouse model.
In summary, $CD4^{LVFOXP3}$ exert potent suppressive activity in healthy and pathological conditions while sparing host immune system. These data support preclinical safety for the development of $CD4^{LVFOXP3}$ as a treatment for IPEX syndrome and autoimmunity of different origin.

Session 4	
4:10-4:25 pm	**Paras Minhas**, Neurosciences Graduate Student/MSTP, Andreasson Lab, *Metabolic reprogramming of myeloid cells prevents age-associated cognitive decline*
4:25-4:40 pm	**David Glass,** Immunology Graduate Student, Bendall Lab, *An integrated multi-omic single cell atlas of human B cell diversity*
4:40-6:00 pm	**Lodging Check in at Social Hall/Front Desk.** Day guest meal tickets may be purchased at the Front Desk.
6:00-7:00 pm	**Dinner** at Crocker Dining Hall
Keynote	
7:00-7:05 pm	Keynote Introduction: **Sidd Jaiswal, MD, PhD**
7:05-8:05 pm	**Keynote: Filip Swirski, PhD**, Associate Professor of Radiology, Harvard Medical School and Massachusetts General Hospital, *Lifestyle, inflammation, and cardiovascular disease*
8:05-8:30 pm	**Break**
8:30-9:30 pm	**Game Show**: Presented by Immunology Second Year Graduate Students
9:30 pm-12:00 am	**Reception** at Fred Farr

Paras Minhas
Neurosciences Graduate Student/MSTP
Advisor: Katrin Andreasson, MD
Stanford University School of Medicine, Department of Neurology

Metabolic reprogramming of myeloid cells prevents age-associated cognitive decline

Paras Minhas[1,2], Melanie McReynolds[3,4], Amira Latif-Hernandez[1], Qian Wang[1], Amanda Rubin[1], Joy He[5], Amit Joshi[6], Miles Linde[7], Esha Gauba[1], Aarooran Durairaj[1], Peter Moon[1], Ravi Majeti[7], Daria Mochly-Rosen[6], Irving Weissman[5], Frank Longo[1], Joshua D. Rabinowitz[3,4], Katrin I. Andreasson[1]

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[5]Institute for Stem Cell Biology & Regenerative Medicine, Stanford, CA, USA
[6]Department of Chemical & Systems Biology, Stanford University, Stanford, CA, USA
[7]Department of Hematology, Stanford University, Stanford, CA USA

Aging is characterized by the development of maladaptive systemic inflammation that underlies common age-associated disorders such as cardiovascular disease, cancer, arthritis, and neurodegeneration. The lipid messenger prostaglandin E_2 (PGE_2) is a potent regulator of immune responses in these disorders. Here we demonstrate that PGE_2 signaling via its EP2 receptor suppresses myeloid and microglial oxidative phosphorylation and promotes a pro-inflammatory activation state. EP2 signaling increased glycogen synthesis through pGSK3β-dependent activation of glycogen synthase and reduction of glucose flux through glycolysis. Genetic or pharmacologic inhibition of EP2 signaling increased glucose availability for cellular respiration by altering proximal glucose metabolism. In vivo, antagonizing the EP2 receptor reversed age-associated systemic and brain inflammation, loss of hippocampal long-term potentiation, and spatial memory decline. Our study reveals a novel metabolic role for EP2 signaling in aging and age-related diseases.

David Glass

Immunology Graduate Student
Advisors: Sean Bendall, PhD and Stephen Quake, PhD
Stanford University School of Medicine, Department of Pathology
Stanford University School of Medicine, Department of Bioengineering and of Applied Physics

An integrated multi-omic single cell atlas of human B cell diversity

To evaluate the impact of heterogeneous B cells in health and disease, comprehensive profiling is needed at a single cell resolution. We developed a highly-multiplexed screen to quantify the co-expression of 351 surface molecules on low numbers of primary cells. We identified dozens of differentially expressed molecules and aligned their variance with B cell isotype usage, metabolism, biosynthesis activity, and signaling response. Here, we propose a new classification scheme to segregate peripheral blood B cells into ten unique subsets, including a CD45RB+ CD27- early memory population and a CD19hi CD11c+ memory population that is a potent responder to immune activation. Furthermore, we quantify the contributions of antibody isotype and cell surface phenotype to various cell processes and find that phenotype largely drives B cell function. Taken together, these findings provide an extensive profile of human B cell diversity that can serve as a resource for further immunological investigations.

Keynote Speaker: Filip Swirski, PhD
Associate Professor of Radiology
Harvard Medical School

Lifestyle, inflammation, and cardiovascular disease

My research aims to understand how leukocytes shape and are shaped by inflammation. We use in vivo models of acute and chronic inflammation relevant to cardiovascular, infectious, and metabolic diseases, and focus on cell development, communication, and function. We think that the diversity of inflammatory conditions arises from the tension between conserved cell behaviors and specific tissue contexts. Leukocytes defend against danger, but also connect diseases and perpetuate the accrual of co-morbidities (ORCID: 0000-0002-3163-9152).

STANFORD IMMUNOLOGY ANNUAL SCIENTIFIC CONFERENCE

Saturday, November 9, 2019

All talks are hosted in Fred Farr

7:30-9:00 am	**Breakfast** at Crocker Dining Hall
	Session 5
9:00-9:25 am	**John Sunwoo, MD,** Faculty, Otolaryngology - Head & Neck Surgery Divisions, *Heterogeneity of Intratumoral NK Cells and ILCs and Influences on Tumor Behavior*
9:25-9:40 am	**Berenice Mbiribindi, PhD,** Postdoctoral Fellow, Krams Lab, *EBVlatent proteins encode for peptides that prevent NK cell inhibition*
9:40-9:55 am	**Julia McKechnie,** Immunology Graduate Student, Blish Lab, *HLA upregulation during dengue virus infection suppresses the natural killer cell response*
9:55-10:10 am	**Break**

John Sunwoo, MD
Edward C. and Amy H. Sewall Professor in the School of Medicine and Professor, by courtesy, of Dermatology
Stanford University School of Medicine

Heterogeneity of Intratumoral NK Cells and ILCs and Influences on Tumor Behavior

The presence and function of innate lymphoid cells (ILC), including natural killer (NK) cells, within human tumors has been poorly characterized. We have assessed the heterogeneity of NK and ILC populations by single-cell RNA sequencing (scRNAseq) of hundreds of individual NK cells and ILCs within primary human head and neck squamous cell carcinoma (HNSCC) tumors, matched lymph node metastases, matched peripheral blood, and blood from healthy donors. Fresh tumor specimens and blood were obtained from 8 patients undergoing surgical resection of HNSCC. The tumor samples were digested mechanically to obtain single-cell suspensions, which were stained with antibodies and sorted as single cells into 96-well plates by flow cytometry. Libraries were prepared using the Smart-Seq2 protocol. Samples were sequenced and analyzed using the Seurat R package. Unsupervised clustering revealed heterogeneous clusters of NK cells and ILC subsets in HNSCC primary tumor tissue and matching lymph node metastasis. NK cells and ILCs from blood showed a gene expression signature different than those from the tumors. Within the tumors, we observed significant heterogeneity, with distinct subsets showing profiles consistent with that of conventional NK cells, ILC1s, ILC3s, and intraepithelial ILC1s (ieILC1s). The presence of these different cell subsets within primary HNSCC tumors was confirmed by flow cytometry. Further, plasticity between the subsets is supported by in vitro and in vivo experimental data. Given the ability of ILCs to polarize the immune responses through the secretion of cytokines and the ability of certain ILCs to kill target cells, we hypothesize that the differences observed in ILC populations may result in different immune responses, influencing clinical outcomes following therapy.

Berenice Mbiribindi, PhD
Postdoctoral Fellow
Sponsor: Sheri Krams, PhD
Stanford University School of Medicine, Department of Surgery - Abdominal Transplantation

EBVlatent proteins encode for peptides that prevent NK cell inhibition

Berenice Mbiribindi[1], Josselyn K. Pena[1], Olivia Hatton[2], Claudia Romero Moreno[1], Carlos Esquivel[1], Olivia M. Martinez[1] and Sheri M. Krams[1]

[1]Department of Surgery/ Division of Abdominal Transplant, Stanford University School of Medicine, Stanford, CA.
[2]Department of Molecular Biology, Colorado College, Colorado Springs, Colorado.

Introduction: Epstein–Barr virus (EBV) infects more than 90% of adults worldwide and is associated with several malignancies, including post-transplant lymphoproliferative disorder (PTLD). Although EBV infection is typically asymptomatic in immune-competent individuals, transplant recipients are highly susceptible to EBV-associated PTLD due to the immunosuppression treatment required to prevent graft rejection. Several lines of evidence suggest that innate immune responses including Natural Killer (NK) cells are critical in host immunity to EBV. Recently, a specific NK cell subset has been demonstrated to recognize and respond to autologous B cells latently infected with EBV. This NK subset expresses NKG2A/CD94, an inhibitory receptor that recognizes HLA-E. Since EBV-infected cells induced NK cell activation despite the expression of HLA-E, we hypothesize that EBV encoded peptides play a pivotal role in the recognition and response of NKG2A+ NK cells towards latently infected EBV+ cells.

Methods and Results: Using *in silico* analysis (NetMHCpan 4.0) we generated a peptide library derived from EBV latent cycle proteins (LMPs and EBNAs) expressed during the latency III stage of EBV infection. Initial assessment resulted in a library of 61 peptides which could potentially bind to HLA-E. Using 721.174 cells as target cells, we performed a peptide stabilization assay to test the ability of individual peptides to be presented by HLA-E. We identified 37 peptides that bound and induced HLA-E expression at the surface of the target cells. The CD107a assay as a readout for NK cell activity, demonstrated that HLA-E bound EBV-derived peptides were able to inhibit NK cell activation (13 peptides), or prevent NK cell inhibition via the NKG2A receptor (8 peptides). Finally, non-inhibitory peptides prevent VAV1 dephosphorylation, a key molecule in the NKG2A inhibitory pathway. We determined that peptides encoded by LMPs tended to favor NK cell degranulation (NK cell killing) while peptides from EBNAs were inhibitory.

Conclusions: We demonstrate that EBV latent cycle proteins can encode for peptides that bind to HLA-E and promote NK cell degranulation and the elimination of EBV latently infected B cells. NK cells are generally refractory to calcineurin-based immunosuppression and thus may be an effective cellular strategy to control EBV diseases post-transplant.

My presentation has for objective to demonstrate how EBV encoded peptides can alter NK cell inhibitory pathway which lead to NK cell activation. This phenomenon will be beneficial for the elimination of EBV infected B cells.

Julia McKechnie
Immunology Graduate Student
Advisor: Catherine Blish, Phd
Stanford University School of Medicine, Department of Infectious Diseases

HLA upregulation during dengue virus infection suppresses the natural killer cell response

McKechnie, Julia L et al. “HLA Upregulation During Dengue Virus Infection Suppresses the Natural Killer Cell Response.” *Frontiers in cellular and infection microbiology* vol. 9 268. 23 Jul. 2019, doi:10.3389/fcimb.2019.00268 PMCID: PMC6663972.

Dengue virus (DENV) is the most prevalent mosquito-borne virus in the world and a major cause of morbidity in the tropics and subtropics. Upregulation of HLA class I molecules has long been considered a feature of DENV infection, yet this has not been evaluated in the setting of natural, human infection. Natural killer (NK) cells, an innate immune cell subset critical for mounting an early response to viral infection, are inhibited by self HLA class I, suggesting that upregulation of HLA class I during DENV infection could dampen the NK cell response. Here we addressed whether upregulation of HLA class I molecules occurs during *in vivo* DENV infection and, if so, whether this suppresses the NK cell response. We found that HLA class I expression was indeed upregulated during acute DENV infection across multiple cells lineages *in vivo*. To better understand the role of HLA class I upregulation, we infected primary human monocytes, a major target of DENV infection, *in vitro*. Upregulation of total HLA class I is dependent on active viral replication and is mediated largely by cytokines and other soluble factors induced by DENV infection, while upregulation of HLA-E occurs in the presence of UV-inactivated, replication-incompetent virus. Importantly, blocking DENV-infected monocytes with a pan-HLA class I Fab nearly doubles the frequency of degranulating NK cells, while blocking HLA-E does not significantly improve the NK cell response. These findings demonstrate that upregulation of HLA class I during DENV infection suppresses the NK cell response, potentially contributing to disease pathogenesis.

Session 6	
10:10-10:35 am	**Gilbert Chu, MD, PhD,** Faculty, Oncology, Biochemistry, *Side-by-side pairing of DNA ends couples non-homologous end joining to V(D)J recombination*
10:35-10:50 am	**Ioana Marin, PhD**, Postdoctoral Fellow, Shatz Lab, *Neuronal non-classical MHCI Qa-1 is a novel regulator of activity-dependent plasticity*
10:50-11:05 am	**Debopam Ghosh, PhD,** Postdoctoral Fellow, Mellins Lab, *The class II peptide editor, H2-M, affects the development and function of B-1 cells*
11:05-11:15 am	**Break**
11:15-11:30 am	**Adityasai Ambati, PhD,** Postdoctoral Fellow, Mignot Lab, *KIR Associations in Anti-NMDAR Encephalitis*
11:30-11:55 am	**Juliana Idoyaga, PhD,** Faculty, Microbiology and Immunology, *TBA*
Lunch and Afternoon Activities	
11:55 am-12:15 pm	**Group Pictures** in front of Fred Farr for Faculty, Graduate Students and Postdocs
12:15-1:00 pm	**Lunch** for Graduate Students and Postdocs at Crocker Dining Hall
12:15-1:15 pm	**Faculty Meeting:** Faculty, Student, and Postdoc Representatives remain at Fred Farr for Lunch
1:30-3:00 pm	**Faculty-First Year Graduate Students Research Blitz** at Fred Farr

Gilbert Chu, MD, PhD
Professor of Medicine (Oncology) and of Biochemistry
Stanford University School of Medicine

Side-by-side pairing of DNA ends couples non-homologous end joining to V(D)J recombination

Chun J. Tsai, Clarence Y. Cheng. Andrey V. Malkovskiy, Rhiju Das and **Gilbert Chu**
Departments of Medicine and Biochemistry, Stanford University School of Medicine

Non-homologous end joining (NHEJ) repairs the DNA double-strand breaks created by RAG1/RAG2 during V(D)J recombination. It also repair breaks induced by ionizing radiation and CRISPR.

Our previous experiments showed that the core NHEJ proteins Ku, XRCC4/Ligase IV, and XLF perform ligation even ends with the 3' overhangs created by P-nucleotide and N-nucleotide addition during V(D)J recombination. The core NHEJ proteins assemble as a megadalton protein-DNA complex containing multiple XLF and XRCC4/Ligase IV molecules.

The physical process that brings DNA ends together has remained a mystery. Here, DNAase I footprinting and exonuclease protection show assembly of core NHEJ proteins into a filament bound to DNA. Atomic force microscopy reveals both end-to-end and side-by-side pairing of DNA molecules. The unexpected side-by-side pairing of DNA couples non-homologous end joining to RAG1/RAG2 cleavage during V(D)J recombination. It also explains the spectrum of immunological defects in patients with mutations in XRCC4, Ligase IV and XLF.

Ioana Marin, PhD
Postdoctoral Fellow
Sponsor: Carla Shatz, PhD
Stanford School of Medicine, Department of Biology and of Neurobiology

Neuronal non-classical MHCI Qa-1 is a novel regulator of activity-dependent plasticity

Marin IA[1], Wei AY[1], Chew KS[1], Shatz CJ[1,2]
[1]Department of Biology, Stanford University
[2] Department of Neurobiology, Stanford University

The brain develops with an exuberant number of connections that are gradually pruned with use. This pruning is dependent on neuronal activity and is crucial during developmental critical periods. Previously we discovered a new role for major histocompatibility complex class I (MHCI) molecules in activity-mediated refinement of neural circuits. Initially thought to function solely in the immune system, recent work has revealed that MHCI proteins are expressed in the healthy brain, both in mice and humans. To date, the neuronal function of just two classical MHCI molecules (H2-K^b and H2-D), out of more than 50 family members, have been studied, demonstrating key roles in synaptic pruning and plasticity (Lee et al Nature 2014). Here, we investigate the function of a novel non-classical MHCI: Qa-1, homologous to human HLA-E. In the immune system, Qa-1 is known to present antigens to NK and T cells, but its expression and function in healthy brain have been completely unexplored until now. We find that Qa-1 is expressed by over 70% of pyramidal neurons in layer 6 (L6) of cerebral cortex, and levels are regulated by visually-driven activity. Using ocular dominance plasticity (ODP) as a paradigm for activity-dependent circuit plasticity, we found in Qa-1 KO mice that strengthening of the open eye is greater than WT, following monocular eye closure during the visual cortical critical period. This and other phenotypes suggest that Qa-1 acts as a break on synaptic plasticity. The CD94-NKG2A/C/E family are known Qa-1 receptors in the immune system. ODP is also enhanced in the visual cortex of mice carrying a mutation in Qa-1 that prevents binding to CD94-NKG2A/C/E receptors. Together, these results indicate a functional role for this ligand-receptor interaction in synaptic plasticity. Moreover, results expand understanding of MHCI function in the healthy brain, and point towards a novel and unexpected function for a non-classical MHCI expressed in CNS neurons interacting with a cognate receptor expressed in glia. Supported by NIH Grants MH071666, EY02858, the Champalimaud Foundation and the Good Ventures Foundation.

Debopam Ghosh, PhD
Postdoctoral Fellow
Sponsor: Elizabeth Mellins, MD
Stanford University School of Medicine, Department of Pediatrics

The class II peptide editor, H2-M, affects the development and function of B-1 cells

Debopam Ghosh[1], Tho D Pham[2], Lital N Adler[1], Xiao He[5], Mary E. O'Mara[6], Aaron B. Kantor[4], Deepanwita Sengupta[3], Yang Yang[4], Laurence C Eisenlohr[6], Peter E Jensen[5], Leonore A. Herzenberg[4], Scott Boyd[2], Eliver E Ghosn[7], Elizabeth D. Mellins[1]

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B-1 cells are known for their germline-like, poly-reactive B cell receptor (BCR)-repertoire, phagocytic activity and proficiency at polarizing T cells towards inflammatory cytokine-producing effector T cells, which play a critical role in host defense and autoimmunity. B-1 cells are antigen presenting cells (APCs) and express major histocompatibility complex class-II (MHCII) proteins. MHCII bind self and foreign peptides and display them to CD4+ T cells, activities essential for adaptive immune responses and also a crucial step for an autoimmune response. Non-classical MHCII protein H2-M edits peptide-MHCII complexes to ensure display of stably bound peptides by APCs. Absence of H2-M has most widely been studied for its impact on effector T cell activation, but knowledge of how this protein affects the development and function of APCs is lacking. We found that absence of H2-M down-regulated the surface expression and altered the distribution of MHCII molecules in B cells across lymphoid organs. Importantly, in H2-M knockout mice, compared to the wild-type C57BL/6 (B6) mice, the frequency and abundance of B-1 cells, but not conventional B-2 cells, was affected in the spleen and peritoneum. Interestingly, the H2-M mediated effect on B-1 cell population was only evident in B6 background (I-Ab), not in Balb/c (I-Ad/I-Ed), indicating this phenomenon is MHCII haplotype dependent. Decrease in B-1 cell number was evident in both immature and mature B-1 cells, further indicating that absence of H2-M affects the development of B-1 cells. In H2-M KO mice, B-1 cells display a significantly lower self-renewal capacity and higher rate of apoptosis compared to WT B6. Despite a lower total B-1 cell number, the frequency of B-1 cells specific for predominant self-antigens (like – phosphatidylcholine and phosphorylcholine) is increased in H2-M KO mice, indicating skewing of B-1 immunoglobulin repertoire in absence of H2-M. Collectively, these data identify a novel impact of H2-M/MHCII interaction that regulates the development of B-1 cells and influences the selection of mature B-1 cell clones in the periphery.

Adityasai Ambati, PhD
Postdoctoral Fellow
Sponsor: Emmanuel Mignot, MD, PhD
Stanford School of Medicine, Department of Psychiatry/Sleep Medicine

KIR Associations in Anti-NMDAR Encephalitis

Ambati A, Ling L, Ollila H, Finke C, Titulaer M, Honnorat J, Mignot E

Background: Novel neuroimmune disorders defined by the presence of autoantibodies in plasma and/or CSF have recently been discovered and grouped as paraneoplastic syndromes. Amongst them is the anti-NMDAR encephalitis, where serum autoantibodies against NMDAR are prevalent and this syndrome manifests with memory disturbances, psychiatric symptoms, and seizures. Anti-NMDAR cases were initially reported in association with ovarian teratoma (25%), but are now also found in many cases without evident tumors (75%). Teratomas associated with this syndrome typically contain CNS-like tissue, suggesting immunity toward the tumor being an important factor.

Methods: To unravel genetic predisposition to anti-NMDAR encephalitis, we conducted a world's first

genome wide association (GWA) screens in a multi-ethnic discovery of cohort anti-NMDAR encephalitis cases (n=306) and ethnically matched controls (n=4449) and replicated the top associations in a validation cohort of 81 cases and 636 control individuals. The first 10 genetic principle components along with demographic variables, were used in an additive linear model to find genome-wide associations with anti-NMDAR encephalitis.

Results: GWA analysis in 301 anti-NMDAR encephalitis cases revealed 4 genome-wide significant loci (pvalue < 5e-8) in the discovery cohort (ACP2, IGHG3, DMXL2, and KIR) with replication (pvalue < 5e-4) in a validation cohort of 81 cases and 636 control individuals. Intriguingly the KIR (killer immunoglobulin-like receptors) that are associated with anti-NMDAR encephalitis map to the telomeric KIR region represented by rs35189301 (effect allele T; p=6.4e-24; OR = 2.7) in the combined discovery replication cohort. KIR imputation was performed in a subset of 173 anti-NMDAR cases and matched 1556 controls, all of whom were predominantly caucasian. KIR haplotype B marked by the presence of 2DS1, 3DS1 and 2DS5 KIRs were relatively frequent in anti-NMDAR cases compared to controls. While the absence of KIR2DS4 and KIR3DL1ex4 were notably associated with anti-NMDAR as compared controls in concordance with GWAS hits.

Conclusions: Our genetic analysis reports on one of the strongest KIR associations till date and opens up new lines of investigation on the role of Natural killer cells and their cognate KIR receptors in the precipitation of paraneoplastic syndromes such as anti-NMDAR encephalitis.

Juliana Idoyaga, PhD
Assistant Professor of Microbiology and Immunology
Stanford University School of Medicine

TBA

Session 7	
4:40-5:05 pm	**Scott Boyd, PhD,** Faculty, Pathology, *TBA*
5:05-5:30 pm	**Aaron Newman, PhD,** Faculty, Biomedical Data Science, *Dissecting tumor cell states and cellular ecosystems with digital cytometry*
5:30-5:45 pm	**Amber Moore,** Immunology Graduate Student, Palmer Lab, *In-Depth Characterization of Gestational Immune Dynamics*
5:45-6:00 pm	**James Harden,** Immunology Graduate Student, Krams Lab, *Unsupervised analysis of the alloimmune response in a novel model of vascularized composite allotransplantation*
6:00-7:00 pm	**Dinner** at Crocker Dining Hall
Keynote	
7:00-7:05 pm	Keynote Introduction: **Michael Howitt, PhD**
7:05-8:05 pm	**Keynote: Ami Bhatt, MD, PhD,** Assistant Professor Medicine (Hematology) and of Genetics, Stanford University, *Unlocking the Secrets of Microbial Genomes*
Poster Session	
8:10-8:40 pm	**Poster Slam** at Fred Farr
8:40-11:30 pm	**Reception** at Kiln
9:00-11:00 pm	**Poster Session** at Kiln
10:00 pm-12:00 am	**Bonfire** at Fire Pit
11:00 pm-12:00 am	**Birukova Midnight Swimming Club**

Scott Boyd, PhD
Assistant Professor of Pathology
Stanford University School of Medicine

TBA

Aaron Newman, PhD
Assistant Professor of Biomedical Data Science
Stanford University School of Medicine

Dissecting tumor cell states and cellular ecosystems with digital cytometry

Tumors are complex ecosystems consisting of malignant, immune, and stromal elements whose dynamic interactions drive patient survival and response to therapy. A comprehensive understanding of the diversity of cellular states within the tumor microenvironment (TME), and their patterns of co-occurrence, could provide new diagnostic tools for improved disease management and novel targets for therapeutic intervention. To address these challenges, we have developed new computational tools for systematic identification and profiling of cell states and their co-associations from bulk, single-cell, and spatially-resolved transcriptomics data. Here, I will describe these approaches and their implications for dissecting TME cellular heterogeneity at high-resolution and at unprecedented scale, with implications for identifying novel diagnostics and immunotherapeutic targets.

Amber Moore
Immunology Graduate Student
Advisor: Theo Palmer
Stanford University School of Medicine, Department of Neurosurgery

In-Depth Characterization of Gestational Immune Dynamics

Amber R. Moore[1,2], Nora Vivanco Gonzalez[1,2], Katie Plummer[2], Harleen Kaur[2], Olivia Mitchel[2], Moises Rivera[2], Sean Bendall[3] and Theo D. Palmer[2]

[1]Stanford University School of Medicine Program in Immunology, [2]Department of Neurosurgery and Institute for Stem Cell Biology and Regenerative Medicine, [3]Department of Pathology, Stanford University

In both mice and humans, maternal blood comes into direct contact with fetal placental cells. This direct contact puts the pregnancy at risk because immune cells that can recognize fetal cells as foreign are present in the blood. The maternal-fetal interface is critical for the establishment and maintenance of tolerance throughout pregnancy, yet the extent to which the maternal immune system changes to accommodate the semiallogeneic fetus remains largely unknown. Furthermore, we have yet to understand how the placenta orchestrates tolerance at different stages of pregnancy or how it responds to changes in the maternal immune system. In mice, we have immuno-profiled maternal immune organs and the placenta across gestation using single-cell mass cytometry (CyTOF).

C57BL/6J CD45.2 females were crossed to C57BL/6J CD45.1 males to differentiate between maternal and fetal immune cells. Prior to dissection, pregnant females were retro-orbitally injected with anti-CD45 antibody to distinguish between endovascular- and tissue-associated immune cells. Peripheral blood, placenta and decidua were collected throughout gestation from embryonic day 10.5 (E10.5) to E18.5. Male and female placentas were run separately to examine sex differences. Over 30 phenotypic and functional markers for activation and checkpoint molecule expression were used in our CyTOF analysis.

The immune profile of the placenta changes considerably throughout gestation and is surprisingly diverse. We observe significant differences in immune cell frequency and activation between the peripheral blood and endovascular compartment of the placenta. There are also significant differences between tissue- and endovascular-associated immune cells in the placenta. For example, the majority of basophils are tissue-associated throughout gestation, whereas the majority of neutrophils are tissue-associated until E14.5 when they primarily become endovascular-associated.

Our results show extensive gestational immune dynamics and diversity in the placenta and suggest regulation of peripheral blood immune cells at the maternal-fetal interface. Our deep characterization of the placenta and blood during pregnancy will serve as a resource for studies that use mouse models to examine immune perturbations, tolerance and complications during pregnancy.

James Harden
Immunology Graduate Student
Advisor: Sheri Krams, PhD
Stanford University School of Medicine, Department of Surgery - Abdominal Transplantation

Unsupervised analysis of the alloimmune response in a novel model of vascularized composite allotransplantation

Vascularized composite allografts consists of skin, muscle, bone, and other tissues, and is more immunogenic than solid-organ allografts. Our lab developed a murine model to study mechanisms of vascularized composite allotransplantation (VCA) rejection. A limb from a donor C57BL/6 (syngeneic) or BALB/c mouse (allogeneic) is placed in the cervical region of a recipient C57BL/6 mouse, and the donor's femoral artery and vein are anastomosed to the recipient's common carotid artery and jugular vein. The skin is monitored daily for hair loss, discoloration, and dryness.

We utilized the high dimensional capacity of Mass Cytometry (Cytometry by Time of Flight; CyTOF) for unsupervised analysis of VCA immunity and designed a panel of surface and intracellular markers for the identification and characterization of leukocyte populations in the spleen. We report multiple leukocyte populations associated with day 5 alloimmunity: including Lag3+/CD8+ T cells, CD40L+/CD4+ T cells, and CD62L/Ly6C(hi) monocytes. We also report a previously unappreciated heterogeneity of alloimmune associated NK cells.

We have previous evidence of the immunosuppressive effects of donor-derived plasmacytoid dendritic cells in recipient cardiac allotransplanted mice. Here we report that pDC shows similar allograft prolongation in our VCA model, and CyTOF analysis of donor-derived pDC-treated allotransplant mice reveals an immune profile that mimics the syngeneic immune response.

Keynote Speaker: Ami Bhatt, MD, PhD
Assistant Professor of Medicine and Genetics
Stanford University School of Medicine

Unlocking the Secrets of Microbial Genomes

Ami Bhatt is a physician scientist with a strong interest in microbial genomics and metagenomics. She received her MD and PhD from the University of California, San Francisco. She then carried out her residency and fellowship training at Harvard's Brigham and Women's Hospital and Dana-Farber Cancer Institute, and served as Chief Medical Resident from 2010-2011. She joined the faculty of the Departments of Medicine (Divisions of Hematology and Blood & Marrow Transplantation) and Genetics at Stanford University in 2014 after completing a post-doctoral fellowship focused on genomics at the Broad Institute of Harvard and MIT. Prof. Bhatt has received multiple awards for her

academic scholarship including the Chen Award of Excellence from the Human Genome Organisation (HUGO).

Her team's research program seeks to illuminate the interplay between the microbial environment and host/clinical factors in human diseases. Her translational laboratory develops and applies novel molecular and computational tools to study strain level dynamics of the microbiome, to understand how microbial genomes change over time and predict the functional output of microbiomes. These innovations facilitate much improved (1) measurement of the types and functions of microbes in patients with non-communicable diseases, (2) understanding of the interactions between microbial genes, gene products, and host cells and (3) testing of the impact of microbially targeted interventions in clinical trials.

In addition to carrying out research at Stanford University, Prof. Bhatt has active collaborations world-wide including in Nigeria and South Africa. She is committed to ensuring that advances in research touch the lives of individuals in all income settings – and thus, in her spare time, enjoys volunteering for the nonprofit she co-founded, Global Oncology and serves as the Director for Global Oncology for Stanford's Center for Innovation in Global Health.

STANFORD IMMUNOLOGY ANNUAL SCIENTIFIC CONFERENCE

Sunday, November 10, 2019

All talks are hosted in Chapel

7:30-9:00 am	**Breakfast** at Crocker Dining Hall
	Session 8
9:00-9:15 am	**Florian Wimmers, PhD,** Postdoctoral Fellow, Pulendran Lab, *The single-cell chromatin landscape of human immune responses to influenza vaccines*
9:15-9:30 am	**Prabhu Arunachalam, PhD,** Postdoctoral Fellow, Pulendran Lab, *Vaccine-induced tissue-resident memory T cells and antibodies synergize to prevent mucosal HIV infection*
9:30-9:45 am	**Abigail Powell, PhD,** Postdoctoral Fellow, Kim Lab, *Using protein nanoparticles to develop a safe and stable vaccine against Ebola*
9:45-10:00 am	**Guangbo Chen, PhD,** Postdoctoral Fellow, Khatri Lab, *Integrative proteome and clinical profiling of sJIA-PAP: an emergent lung complication associated with aTH2 response*
10:00-10:10 am	**Break**

Florian Wimmers, PhD
Postdoctoral Fellow
Sponsor: Bali Pulendran, PhD
Stanford University School of Medicine, Department of Pathology

The single-cell chromatin landscape of human immune responses to influenza vaccines

Authors: Florian Wimmers[1], Alexander Kuo[1], Michele Donato[1], Tal Aschuach[2], Sanne de Jong[1], Mai Dvorak[1], Sarah E Chang[1], Mariko Hinton Foecke[1], Fabian Mueller[1], Arwa Kathiria[1], Peggie Cheung[1], Nir Yosef[2], Paul J Utz[1], Purvesh Khatri[1], Bali Pulendran[1]

Affiliations: 1) Stanford University; 2) University of California, Berkeley

Topic: vaccines, influenza, EpiTOF, ATAC, epigenome, innate immunity

Background: Epidemiological evidence has been interpreted to suggest that certain vaccines may have non-specific effects (NSEs) that convey protection not only against the specific pathogen they are designed for but also against a multitude of other pathogens. Intriguingly, molecular analyses indicate that epigenetic changes to the chromatin landscape of innate immune cells are key mediators of these effects. The significance of this so called “heterologous vaccine effect” for global health is yet to be determined. It is currently unclear which biological factors come into play in mediating these NSEs. Furthermore, whether this effect has a beneficial or detrimental, or neutral effect on protection against infections remains unknown.

Aim: Here, we determine 1) how influenza vaccines reprogram the chromatin landscape of human innate immune cells and induce lasting changes to their functionality, and 2) how the presence of a potent adjuvant impacts the nature of the induced changes.

Methods: We use single cell histone modification profiling (EpiTOF) and genome-wide chromatin accessibility analysis ([single-cell] ATAC-seq) to construct the chromatin landscape of the human immune response to the inactivated seasonal influenza vaccine TIV. Subsequently, the same analysis methods are applied to human samples from an experimental avian influenza trial with two arms: inactivated vaccine only, and inactivated vaccine plus powerful adjuvant system.

Results: Our results show that administration of TIV leads to lasting changes in histone modification profiles of innate immune cells that correlate with reduced cytokine production. Interestingly, the observed effects seem opposite to what was previously described after administration of the tuberculosis vaccine BCG which appears to convey beneficial NSEs. Isolated myeloid cells from vaccinated participants further displayed a reduction in chromatin accessibility and data from nine historic TIV trials demonstrate reduced expression of affected gene networks after vaccination. Intriguingly, when comparing the chromatin landscape of participants vaccinated with adjuvanted vs non-adjuvanted avian influenza vaccine, we observed opposing trajectories in the histone modification profiles.

ScATAC-seq and scRNA-seq analysis of the innate compartment of vaccinated participants show heterogeneity within the chromatin landscape.

Discussion: These results suggest that the nature of vaccine-induced NSEs might, indeed, depend on the type of vaccine, and hint towards a role of vaccine adjuvants as possible tools to program NSEs.

Prabhu Arunachalam, PhD
Postdoctoral Fellow
Sponsor: Bali Pulendran, PhD
Stanford University School of Medicine, Department of Pathology

Vaccine-induced tissue-resident memory T cells and antibodies synergize to prevent mucosal HIV infection

Recent efforts towards an HIV vaccine focus on inducing broadly neutralizing antibodies but combining nAbs with cellular responses may be superior. To address this, we immunized macaques with a trimeric HIV envelope protein vaccine, either alone to induce neutralizing antibodies (nAbs) or in combination with a heterologous viral vector regimen to elicit $CD8^+$ tissue-resident memory T cells (TRM) plus nAbs. After ten vaginal challenges with the autologous virus, protection was observed in both groups: 53.3% and 66.7%, respectively. A nAb titer >300 was generally protective but titers <300 were protective only in the TRM + nAb group. Protection observed in the latter group was durable as these animals resisted six additional challenges five months later. CITE-seq analysis of 15,000 single cells from vaginal tissues following ex vivo restimulation of TRMs revealed rapid induction of local antiviral immunity, which synergize with antibodies to provide enhanced and durable resistance against HIV.

Abigail Powell, PhD
Postdoctoral Fellow
Sponsor: Peter Kim, PhD
Stanford University School of Medicine, Department of Biochemistry

Using protein nanoparticles to develop a safe and stable vaccine against Ebola

Abstract: Ebolaviruses are zoonotic RNA viruses from the Filoviridae family that can cause severe human disease. No therapeutic drug or vaccine against Ebola has been widely licensed for use in humans. An experimental vaccine (rVSV-ZEBOV) has shown promising efficacy in an ongoing outbreak in the Democratic Republic of Congo. However, this vaccine contains an actively replicating virus. As a result, it requires rigorous storage conditions and can cause flu-like symptoms post vaccination. Use of the vaccine in contraindicated groups including children and pregnant women has been controversial due to concern over potential adverse events. Additionally, rVSV-ZEBOV and other Ebola vaccine candidates only provide protection against the most common pathogenic strain of Ebola, EBOV, however two other strains, BDBV and SUDV, have previously caused substantial outbreaks and are an ongoing threat.

An alternative to live-replicating viral vaccines are protein subunit vaccines. Subunit vaccine candidates composed of the trimeric Ebola surface glycoprotein (GP) generate a protective immune response in rodent disease models but have not been studied or optimized extensively. In an effort to design a protein-based vaccine against Ebola that elicits a robust immune response, I have developed a novel protein nanoparticle which displays Ebola GP trimer in 8 copies on its surface. The functionalized particles exhibit similar stability and binding properties as compared to trimer alone. Preliminary results from a mouse immunization experiment suggest that animals immunized with particles developed a significantly better antigen-specific antibody response as compared to animals immunized with GP trimer alone.

My ongoing work is focused on further characterizing the immune response to the GP functionalized nanoparticles and also modifying the particles to design a cross-reactive vaccine that is protective against all pathogenic strains of Ebola. Specifically, I will epitope map the polyclonal response to different regions of GP and characterize the neutralizing potential of the serum obtained from mice in my immunization study. I will also mask the poorly conserved regions of GP on nanoparticles by inserting N-linked glycosylation sites to skew the antibody response to the conserved, vulnerable regions of GP and determine if the resultant hyperglycosylated particles elicit a more cross-reactive antibody response.

Taken together, the results of this work will provide important insight into the development of an effective subunit vaccine against Ebola as well as strategies for creating an antigen to elicit a cross-reactive antibody response for protection against all pathogenic strains of Ebola.

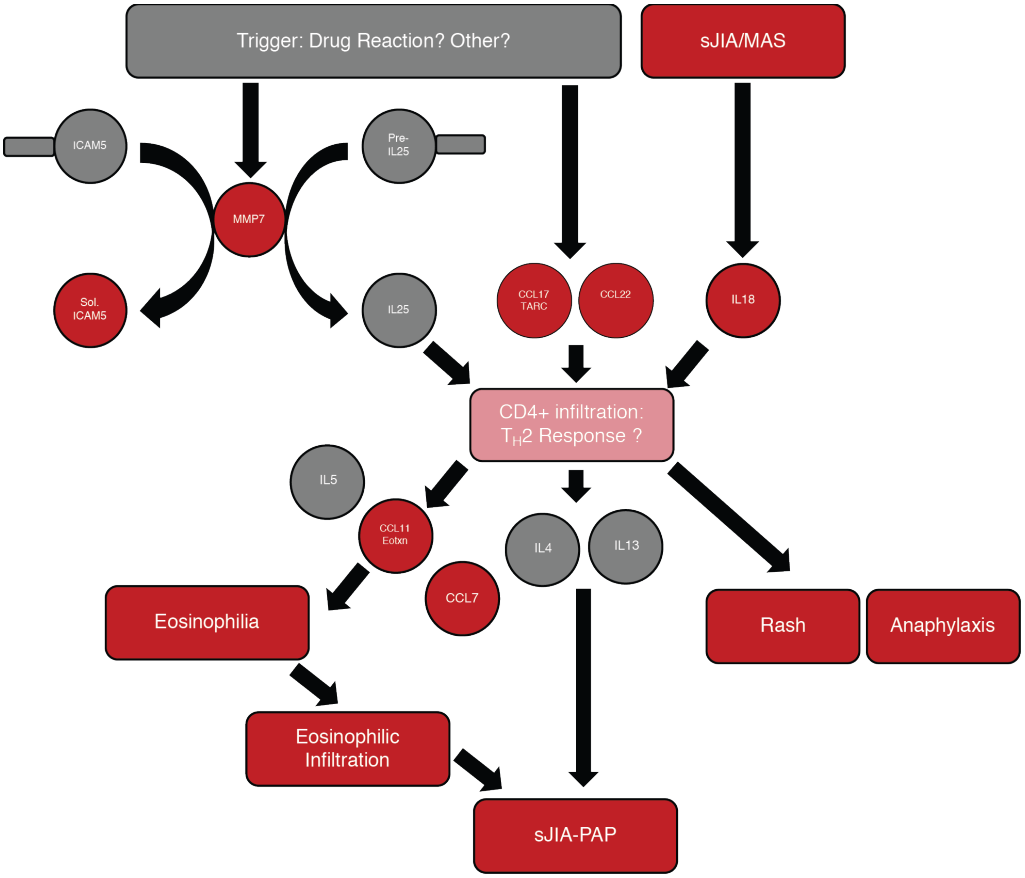
Guangbo Chen, PhD
Postdoctoral Fellow
Sponsor: Purvesh Khatri, PhD
Stanford University School of Medicine, Department of Biomedical Data Science and of BMIR-ITI Institute

Integrative proteome and clinical profiling of sJIA-PAP: an emergent lung complication associated with aTH2 response

Guangbo Chen, PhD1; Grant S. Schulert, MD, PhD2; Adriana De Jesus, MD, PhD3; Vivian Saper, MD4; Corinne Schneider5; Bruce Trapnell, MS, MD2; Alexei A. Grom, MD2; Raphaela Goldbach-Mansky, MD, MHS6; Mark Davis, PhD1; Scott Canna, MD5*; Elizabeth Mellins, MD4*; Purvesh Khatri, PhD1*

1: Institute for Immunity, Transplantation and Infection, Stanford University, Stanford, CA
2: Cincinnati Children's Hospital, Cincinnati, OH
3: NIAMS, NIH, Bethesda, MA
4: Department of Pediatrics, Stanford, Stanford, CA
5: RK Mellon Institute for Pediatric Research, University of Pittsburgh/Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA
6: NIAID, NIH, Bethesda, MA
*equal contributions

With the advent of IL-1/IL-6 inhibitor therapy in the past decade, many patients with systemic juvenile idiopathic arthritis (sJIA) can be managed successfully. However, roughly in the same period, pediatric rheumatologists noticed increased frequency of interstitial lung diseases in the sJIA population, with a variant pulmonary alveolar proteinosis (PAP) with T helper (CD4+) cell dominant lung infiltrates as the predominant pathology. Macrophage activation syndrome (MAS) often accompanied lung disease, raising the possibility that the latter may be an organ-specific manifestation of MAS. Drug reaction has also been observed in a portion of patients. We organized a multi-center serum proteome study to compare the inflammatory process of PAP cases in sJIA (sJIA-PAP) and related inflammatory conditions, such as active sJIA and MAS. In many patients, the different disease components co-occurred. We used linear modeling by LIMMA to extract the proteome alterations attributed to each component. We found that, in many patients, disease activity of sJIA-PAP, as indicated by serum proteome profiles, can sustain or progress independent of systemic MAS activity. There was little overlap in associated cytokine/chemokines between sJIA-PAP and MAS/sJIA disease component. Two chemoattractants well characterized in T_H2-mediated allergic conditions, CCL11(eotaxin) and CCL17 (TARC) were among the top elevated chemokines in the sJIA-PAP component; serum levels of CCL11 and CCL17 were significantly correlated with the eosinophil count in the sJIA-PAP cohort. The serum abundance of IL-13, a key Th2 cytokine, was not elevated in most patients, but in a subject with progressing lung disease, its abundance gradually increased to over 40 times above the healthy baseline during a period of 5 years. Most sJIA-PAP related proteins were not elevated in PAP of other causes. sJIA-PAP further augmented the serum IL-18 level, which is already elevated in both sJIA and MAS. However, in a case of compassionate use of tadekinig (IL-18 suppression), the medication failed to control the CCL11/17 elevation and eosinophil count spike, despite of lowering MAS activity. Longitudinal drug exposure analysis and investigation of T_H2 cytokines in bronchoalveolar lavage fluid are on-going. Overall, the study suggests that the etiology of sJIA-PAP differs from MAS or other forms of PAP. The possibility of a T_H2 driven eosinophilic response is consistent with our previous findings on the association between IL-1/IL-6 inhibitor exposure and unique clinical features of sJIA-PAP.


Trigger: Drug Reaction? Other?
sJIA/MAS
ICAM5
Pre-IL25
MMP7
Sol. ICAM5
IL25
CCL17 TARC
CCL22
IL18
CD4+ infiltration: T_H2 Response ?
IL5
CCL11 Eotxn
CCL7
IL4
IL13
Eosinophilia
Rash
Anaphylaxis
Eosinophilic Infiltration
sJIA-PAP

Session 9: Career Panel	
10:10-10:50 am	**Career Panel: Marvin Gee, PhD,** Co-founder and Head of Target Discovery, 3T Biosciences, **Erika Check Hayden,** Director, Science Communication Program, University of California Santa Cruz, **Sidd Jaiswal, MD, PhD,** Assistant Professor of Pathology, Stanford University School of Medicine, **Leah Sibener, PhD,** Co-founder and Head of Therapeutic Discovery, 3T Biosciences
11:00 am-12:00 pm	**Lunch** at Chapel

Marvin Gee, PhD
Co-founder and Head of Target Discovery
3T Biosciences

Marvin Gee received his B.S. degree in Biology at the California Institute of Technology in 2013, where he published and patented work engineering T cell receptors for adoptive T cell therapy in the laboratory of David Baltimore. Following, he received his Ph.D. in Immunology at Stanford University in the laboratory of K. Christopher Garcia in 2017, publishing work on technology to identify the specificities of T cell receptors for application in oncology and further work on the molecular- and systems biology-focused characterization of T cell receptor recognition of immunological targets. At Stanford, Marvin received his PhD degree with an additional core focus in Computational Immunology. Marvin has had prior work experience at the National Cancer Institute. His primary focuses are in immunology, structural biology, protein engineering, systems biology, bioinformatics, and algorithms for the application of therapeutic- and early-discovery in immuno-oncology. He is now Head of Target Discovery at 3T Biosciences since co-founding the company in 2017.

Erika Check Hayden
Director, Science Communication Program
University of California Santa Cruz

Erika became the third director of the SciCom program in January 2017. She worked from San Francisco for the news section of *Nature* from 2001 through 2016, reporting on biomedical research, emerging technologies, scientific trends, and ethics in science. Her reporting on the Ebola crisis from West Africa received multiple national honors in 2014 and 2015. Erika majored in biology at Stanford University, where she began her journalism career by writing for the *Stanford Daily* and the *Stanford Alumni Magazine*. She also has worked at *Newsweek* in New York, covering science, medicine, and breaking news.

Sidd Jaiswal, MD, PhD
Assistant Professor of Pathology
Stanford University School of Medicine

Siddhartha Jaiswal is a recent faculty recruit to Stanford in the Department of Pathology. He is no stranger to the Farm, having also obtained undergraduate, medical, and doctorate degrees at Stanford. His thesis work in Irv Weissman's lab focused on understanding the role of the innate immune signaling ligand, CD47, in macrophage tumor immunosurveillance. This work formed the rationale for the therapeutic targeting of CD47 in human cancer, which is currently in clinical trials at Stanford and elsewhere.

Dr. Jaiswal subsequently completed residency and fellowship training in pathology at the Massachusetts General Hospital and Harvard Medical School. As a post-doctoral fellow at the Broad Institute, he identified a common, pre-malignant state for blood cancers by reanalysis of large sequencing datasets. This condition, termed "clonal hematopoiesis", is characterized by the presence of stem cell clones harboring certain somatic mutations, primarily in genes involved in epigenetic regulation of hematopoiesis. Clonal hematopoiesis is prevalent in the aging population and increases the risk of not only blood cancer, but also cardiovascular disease and overall mortality. Understanding the biology of these mutations and how they contribute to the development of cancer and other age-related diseases is the current focus of work in his lab.

Leah Sibener, PhD
Co-founder and Head of Therapeutic Discovery
3T Biosciences

Leah is a co-founder and Head of Therapeutic Discovery at 3T Biosciences. At 3T she leads the R&D operations focusing on identifying new immunotherapy targets, and T cell receptors to be used in next generation cell therapies. She has co-authored work in high-impact journals such as *Cell*, *Science*, *Nature Immunology*, *Immunity*, *Nature Genetics, and PNAS*. She holds a bachelor's degree in Biophysics from Johns Hopkins University, and a PhD in Immunology from Stanford University where she helped develop 3T's target discovery platform in Chris Garcia's research group. She was named one of Forbes' 2018 30-under-30 in Healthcare and was awarded the Hugh McDevitt Award for Best Doctoral Dissertation in Stanford Immunology. Prior to Stanford and 3T, Leah was a part of research

teams at the Johns Hopkins Institute for Cellular Engineering working on engineering artificial antigen presenting cells (aAPCs) for immunotherapy, as well as at Genentech in Early Discovery Immunology.

Her list of scientific publications can be found on Google Scholar.

IMMUNOLOGY 2019 SCIENTIFIC CONFERENCE AT ASILOMAR

Stanford Immunology Poster Session

Marcela Alcantara, PhD
Postdoctoral Fellow
Sponsor: Juliana Idoyaga, PhD
Stanford University School of Medicine, Department of Microbiology and Immunology

Integrated cross-species analysis identifies a conserved transitional dendritic cell population

Marcela Alcántara-Hernández[1*], Rebecca Leylek[1*], Zachary Lanzar[1], Anja Lüdtke[1], Oriana Perez[2], Boris Reizis[2], Juliana Idoyaga[1]

[1]Stanford University Immunology IDP and Department of Microbiology & Immunology
[2]Department of Pathology, New York University School of Medicine, New York
*Equal contribution

Plasmacytoid dendritic cells (pDC) are sensor cells with diverse immune functions, from type-I interferon (IFN-I) production to antigen presentation, T cell activation, and tolerance. Regulation of these functions remains poorly understood but could be mediated by functionally specialized pDC subpopulations. Recently, we and others described a novel population of human DC contained within previous descriptions of pDC. We called these cells transitional DC (tDC) given their heterogeneous expression of pDC and classical DC (cDC) features. To identify the mouse homolog of this population, we now performed an integrated cross-species analysis using CyTOF, transcriptomics and functional assays. Our analysis shows that, similar to human, mice harbor a population of DC with a pDC-like to cDC-like phenotype, i.e. mouse tDC. Mouse and human tDC expressed transcription factors essential for both pDC (Tcf4) and cDC (Irf8, Irf4) development. However, using conditional knock-out models, we observed that tDC development was dependent on Tcf4 and not Irf8, similar to pDC. Additionally, tDC expressed lymphoid features that are characteristic of pDC and absent from cDC. *In vitro* functional assays demonstrated that tDC are specialized in antigen presentation and have limited capacity for IFN-I production in both species. Finally, during murine respiratory viral infection, pDC and tDC showed similar recruitment dynamics in the lung. Altogether, we conclude that tDC are an evolutionarily conserved DC population that is closely related to pDC. Our identification of mouse tDC provides a framework for deciphering the function of pDC and tDC during diseases, which has the potential to open new avenues for therapeutic design.

Justin Arredondo-Guerrero
Immunology Graduate Student
Advisors: Crystal Mackall, MD and Everett Meyer, MD
Stanford University School of Medicine, Department of Pediatrics - Hematology & Oncology
Stanford University School of Medicine, Department of Blood and Marrow Transplantation

Integration of orthogonal IL-2/IL-1Rb system with Chimeric Antigen Receptors

T cell based immunotherapies have been demonstrated to be effective for a wide range of immunogenic and tolerogenic applications. The potential for integrating modified growth and signaling transgenes with CAR T cell engineering has yet to be explored at depth. Orthogonal cytokines have been demonstrated to be efficacious in self-enrichment of transgenic primary mammalian T cells. Here we exploit the orthogonal signaling abilities of modified Interleukin 2 (IL-2) and IL-2Rb in the context of CAR T immunotherapy. Using an orthogonal IL-2 / IL-2Rb polycistronic vector, we have demonstrated the ability to adoptively transfer self-proliferative primary CAR+ T cells in a syngeneic host without any chemical or irradiation preconditioning of the recipient. We show that even as few as one million orthogonal CAR+ T cells introduced intravenously can engraft, proliferate, and expand to secondary lymphoid organs.

Using this model in combination with the anti-FITC mAb CAR, we have created a novel therapy that provides the specificity of monoclonal antibodies complemented by the effector functions of self-sustaining T cells.

Graham Barlow
Immunology Graduate Student
Advisors: Garry Nolan, PhD and Paul Bollyky, MD, PhD
Stanford University School of Medicine, Department of Microbiology and Immunology
Stanford University School of Medicine, Department of Microbiology and Immunology and of Infectious Diseases

Mapping the Autoimmune 'Highway System' in Human Type I Diabetic Pancreata using CODEX Highly Multiplexed Tissue Imaging

Graham Barlow[1,2], Salil Bhate[1,2], Christian M. Schürch[1,2], Darci Phillips[1,2], Nadine Nagy[2], Paul Bollyky[2], Garry Nolan[1,2]

[1]Baxter Laboratory, [2]Department of Microbiology and Immunology, Stanford University

Type 1 diabetes (T1D) is caused by autoimmune-mediated destruction of insulin-producing -cells in the pancreatic islets of Langerhans. T1D affects >1.25 million patients in the United States, resulting in debilitating complications and premature mortality. Despite large-scale research efforts, prevention or cure of T1D is still unfeasible. It has long been recognized that autoimmune infiltration in T1D is targeted towards certain islets while 'ignoring' surrounding islets. The cellular mechanisms underlying this patterning have yet to be elucidated. Cytotoxic T lymphocytes (CTLs), the primary mediators of islet destruction, localize along a network of extracellular matrix that runs through the tissue. We hypothesized that CTLs' interactions along this 'highway system' play a major role in directing islet destruction. Delineating the specific cell types regulating CTLs' migration and effector functions in the pancreas during T1D would provide targets for specifically interfering with disease progression.

Pancreata from deceased T1D patients on whom rapid autopsies were performed were obtained from the network of pancreatic organ donors (nPOD). Large areas of these tissues were imaged using CODEX (Co-Detection by indEXing) multiplexed microscopy, which enabled simultaneous visualization of 50+ antigens. This allowed for computationally integrating the localizations and activation states of CTLs, pancreatic, and immune cells in unprecedented detail. We identified specific cell types and their positions within this extracellular matrix network that direct CTL migration at the tissue scale.

The diverse cell types in the tissue surrounding islets are not simply bystanders but facilitate CTL navigation to islets. Given that analogous networks are also present in lymph nodes and the lung, insights into the regulation of lymphocyte migration in the pancreas can be extended to other immune processes such as infection and vaccination.

Ben Bell
Molecular and Cellular Physiology Graduate Student
Advisor: Peter Kim, PhD
Stanford University School of Medicine, Department of Biochemistry

Structural basis of cross-clade HIV-1 neutralization by an antibody that binds the gp41 C-heptad repeat

HIV-1 infection requires viral and cell membrane fusion, enabled by interaction of the gp41 N- and C-heptad repeats (NHR and CHR, respectively). When the CHR is folded as an alpha-helix, one face comprises highly conserved residues and is a validated target for broad-spectrum HIV-1 inhibition, suggesting this conserved helical face might be an effective vaccine target. We demonstrate that an antibody against this conserved helical face can inhibit HIV-1 infection in vitro. This antibody, A2_4.3, was isolated from selections of mutagenized yeast surface-displayed libraries of a non-neutralizing patient-derived antibody, A2_1.0, the sequence of which is close to the predicted germ-line antibody. Surprisingly, the X-ray crystal structure of A2_4.3 Fab bound to a CHR peptide shows binding via conserved framework residues typically buried at the interface between antibody variable heavy and light chain domains (VH-VL interface). A2 and its derivative antibodies validate a new, highly conserved target for HIV-1 vaccine development and suggest another potential immune mechanism for generating antibody diversity.

Kartik Bhamidipati
Immunology Graduate Student
Advisor: Bill Robinson, MD, PhD
Stanford University School of Medicine, Department of Immunology & Rheumatology

CD52 is downregulated in SLE and modulates B cell activation and differentiation

Systemic Lupus Erythematosus (SLE) is a multi-system autoimmune condition thought to be driven by pathogenic autoantibodies secreted by B cells; however, the etiology is poorly understood. To characterize B cell dysregulation in SLE we performed single cell RNA sequencing on B cells isolated from SLE patients and matched healthy controls. We identified CD52, a 12 amino acid surface glycoprotein, as downregulated on the gene expression level among SLE B cells relative to healthy. Furthermore, we confirmed lower surface expression levels and lower serum levels of CD52 in SLE patients. Next, we investigated the function of soluble CD52 (sCD52) and found that CD52 is secreted by cells upon activation, and that sCD52 dose dependently enhances cytokine secretion among TLR9 stimulated naïve B cells. Additionally, sCD52 promotes TLR9 mediated B cell proliferation, activation and intracellular signaling. In tandem, CD52 enhances the metabolic activity of TLR9 stimulated B cells as assessed by glucose uptake, mitochondrial membrane potential, mitochondrial size and cellular reactive oxygen species. Despite augmenting the short term activity of TLR9 stimulated B cells, sCD52 significantly inhibits the ability of activated B cells to differentiate into antibody secreting cells or to class switch. sCD52 additionally reduces the proportion of metabolically competent cells at later timepoints. Our results indicate that sCD52 is an immunoregulatory protein that ultimately prevents the differentiation of TLR activated B cells. Lower levels of CD52 observed in SLE patients may allow TLR stimulated B cells in these patients to more easily escape regulation and to differentiate into autoantibody secreting cells.

Volker Boehnert, PhD
Postdoctoral Fellow
Sponsor: Lingyin Li, PhD
Stanford University School of Medicine, Department of Biochemistry

Extracellular 2’3’-cGAMP is an immunotransmitter produced by cancer cells and regulated by ENPP1

Volker Böhnert, Jacqueline A. Carozza, Khanh C. Nguyen, Gemini Skariah, Kelsey E. Shaw, Jenifer A. Brown, Marjan Rafat, Rie von Eyben, Edward E. Graves, Jeffrey S. Glenn, Mark Smith and Lingyin

2’3’-cGAMP is an intracellular second messenger that leads to the activation of the innate immune STING pathway upon recognition of cytosolic dsDNA. cGAMP is degraded by ENPP1, which is an extracellular hydrolase. Using specific ENPP1 inhibitors, we could show that cGAMP is constantly being exported by cancer cells in vitro, and cGAMP export

is elevated upon ionizing radiation (IR). In tumors, depleting extracellular cGAMP by using a neutralizing protein decreases tumor-associated immune cell infiltration in a tumor cGAS and host STING dependent manner. Furthermore, depletion of extracellular cGAMP abolishes the curative effect of IR treatment. Knockout of ENPP1 in mice delays tumor growth, and systemic inhibition of ENPP1 boosts the curative effect of IR treatment. In conclusion, extracellular cGAMP is an anti-cancer immunotransmitter and ENPP1 is an innate immune checkpoint that can be harnessed to improve immunological visibility of tumors.

Kevin Brulois, PhD
Postdoctoral Fellow
Sponsor: Eugene Butcher, MD
Stanford University School of Medicine, Department of Pathology

Landscape of immune blood vascular endothelium at single cell resolution

Kevin Brulois[1*], Anusha Rajaraman[1, 2, 5*], Agata Szade[1*], Sofia Nordling[1*], Ania Bogoslowski[3, 4], Denis Dermadi[1], Milladur Rahman[1], Helena Kiefel[1], Edward O'Hara[1], Jasper J Koning[5] Hiroto Kawashima[6], Dietmar Vestweber[7], Kristy Red-Horse[8], Reina Mebius[5], Ralf H. Adams[9], Paul Kubes[3, 4], Junliang Pan[1, 2] and Eugene C Butcher[1, 2, 10].

Blood vascular endothelial cells (BECs) control the immune response by regulating immune cell recruitment, metabolite exchange and blood flow in lymphoid tissues. However, the diversity of BEC and their origins during homeostasis immune angiogenesis remains poorly understood. Here we profile transcriptomes of ~10000 BEC from mouse peripheral lymph nodes and map key phenotypes to the vasculature. In addition to known BEC subsets, our analysis identifies a novel medullary vein population which we show is unexpectedly specialized for P-selectin-dependent myeloid cell (vs lymphocyte) recruitment. We define multiple phenotypes of capillary lining BEC including a capillary resident regenerative progenitor (CRP) that displays migratory and stem cell gene signatures and that contributes to homeostatic EC turnover and to neogenesis of BEC after immunization. Trajectory analyses reveal retention of developmental programs along a progression of cellular phenotypes from CapEC to mature venous and arterial EC subsets. Overall, our single cell atlas reveals the cellular organization of lymph node blood vasculature and defines subset specialization for immune cell recruitment and vascular homeostasis.

Kassie Dantzler, PhD
Postdoctoral Fellow
Sponsor: Prasanna Jagannathan, MD
Stanford University School of Medicine, Department of Infectious Diseases and of Microbiology and Immunology

Mechanisms driving altered Vδ2+ γδ T cell function during recurrent malaria infection

Kathleen Dantzler[1], Sandy Klemm[1], Rafael Polidoro[2,3], Aditya Rao[1], Caroline Junquiera[2,3,4], Mai Dvorak[1], John Rek[5], Moses Kamya[6], Peggie Cheung[1], Alex Kuo[1], Grant Dorsey[7], Margaret Feeney[7], PJ Utz[1], Judy Lieberman[2,3], Purvesh Khatri[1], Will Greenleaf[1], Prasanna Jagannathan[1]

[1]Stanford University, Stanford, CA
[2]Harvard Medical School, Boston, MA
[3]Boston Children's Hospital, Boston, MA
[4]Fiocruz Minas, Belo Horizonte, Brazil
[5]Infectious Disease Research Collaboration, Kampala, Uganda

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Though close to 500,000 people—predominantly pregnant women and children under age 5—die from *Plasmodium falciparum (Pf)* malaria each year, much remains unknown about the short-lived nature of naturally acquired antimalarial immunity. Natural immunity provides some protection against symptomatic disease in older children and adults but is unable to eliminate parasite replication—likely due to chronic inflammation caused by innate cell activation. Repeated malaria exposure leads to attenuation of the pro-inflammatory response from the innate-like Vδ2+ γδ T cell subset, which associates with a reduced likelihood of symptoms upon subsequent *Pf* infection. Leveraging clinical samples from a longitudinal cohort in Uganda, we are utilizing several epigenetic and transcriptional approaches to identify putative mechanisms underlying this altered Vδ2+ T cell function. We used 2 parallel methods to examine epigenetic reprogramming: 1) EpiTOF, which uses CYTOF to interrogate specific histone modifications at the single cell level, and 2) ATAC-seq, which reveals sites of open chromatin across a population of cells. Results indicate significant differences in chromatin accessibility between Vδ2+ T cells from children with high *vs.* low malaria exposure, suggesting that epigenetic regulation may play a role in altering functional responses of these cells after repeated infection. We are also evaluating transcriptional regulation of Vδ2+ T cells by CITE-seq—a novel method that simultaneously measures cellular protein and transcriptome values and integrates them into an efficient, single-cell readout. Finally, we are establishing *in vitro* systems both to quantify changes in various Vδ2+ T cell functions (cytotoxicity, growth inhibition, antibody-dependent cytotoxicity) and to replicate the phenotype of altered function following repeated *in vivo* infection. By deepening our understanding of the molecular mechanisms driving inefficient acquisition of antimalarial immunity in children, this work could enable novel therapeutic approaches that enhance parasite clearance and/or reduce disease severity.

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Linking Xist ribonucleoprotein particles to increased autoimmune disease in females

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Although autoimmune diseases affect as many as one in ten Americans, females are three times more likely than males to develop diseases such as rheumatoid arthritis and multiple sclerosis and nine times more likely to develop systemic lupus erythematosus. This inflammatory disease bias towards females suggests the involvement of the XX sex complement because females have two X chromosomes and males only one. Since the long noncoding RNA (lncRNA), *Xist*, is expressed only in females for X chromosome silencing and the Xist ribonucleoprotein particle (RNP) contains many autoantigens associated with autoimmune diseases, we hypothesize that *Xist* RNPs stimulate a proinflammatory environment leading to greater autoimmunity.

We aim to determine the mechanism of *Xist* interaction with the immune system and the influence of Xist RNPs on immune responses by: 1. elucidating the mechanisms by which *Xist* interacts with the immune system through assessing the response of immune cells to *Xist* RNPs and identifying the mode of *Xist*-immune system interaction and

2. evaluating the extent to which *Xist* expression influences immune response and autoimmunity *in vivo* in a unique transgenic inducible *Xist*-expressing autoimmune disease mouse model. Our preliminary studies in mice suggest that autoantibody production is linked to Xist expression and inducing Xist expression in a male background promotes a more female-like profile in $CD4^+$ T-cells. These studies will advance the fields of RNA biology, immunology, and hematology by examining the riddle of autoimmunity from the novel angle of lncRNA-protein complexes and their role in shaping the ever-dynamic immune system.

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Epitope Selection for HLA-DQ2 Presentation: Implications for Celiac Disease and Viral Defense

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We have reported that the major histocompatibility molecule HLA-DQ2 (DQA1*05:01/DQB1*02:01) (DQ2) is relatively resistant to HLA-DM (DM), a peptide exchange catalyst for MHC class II. In this study, we analyzed the role of DQ2/DM interaction in the generation of DQ2-restricted gliadin epitopes, relevant to celiac disease, or DQ2-restricted viral epitopes, relevant to host defense. We used paired human APC, differing in DM expression (DM^{null} versus DM^{high}) or differing by expression of wild-type DQ2, versus a DM-susceptible, DQ2 point mutant DQ2α+53G. The APC pairs were compared for their ability to stimulate human $CD4^+$ T cell clones. Despite higher DQ2 levels, DM^{high} APC attenuated T cell responses compared with DM^{null} APC after intracellular generation of four tested gliadin epitopes. DM^{high} APC expressing the DQ2α+53G mutant further suppressed these gliadin-mediated responses. The gliadin epitopes were found to have moderate affinity for DQ2, and even lower affinity for the DQ2 mutant, consistent with DM suppression of their presentation. In contrast, DM^{high} APC significantly promoted the presentation of DQ2-restricted epitopes derived intracellularly from inactivated HSV type 2, influenza hemagglutinin, and human papillomavirus E7 protein. When extracellular peptide epitopes were used as Ag, the DQ2 surface levels and peptide affinity were the major regulators of T cell responses. The differential effect of DM on stimulation of the two groups of T cell clones implies differences in DQ2 presentation pathways associated with nonpathogen- and pathogen-derived Ags in vivo.

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The Heparan Sulfate Mimetic PG545 polarizes T-cell responses towards Foxp3+ Treg

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The heparan sulfate mimetic PG545 (pixatimod) is under evaluation as an inhibitor of angiogenesis and metastasis including in human clinical trials. We have examined the effects of PG545 on lymphocyte phenotypes and function. We report that PG545 treatment suppresses effector T cell phenotype and polarizes T-cells away from Th17 and Th1 cell subsets and towards Foxp3+ regulatory T cell in vitro and in vivo. Mechanistically, PG545 inhibits ERK1/2 signaling in lymphoid cells and alters T-cell polarization. Interestingly, these effects are also observed in heparanase-deficient T cells, indicating that PG545 has effects that are independent of its role in heparanase inhibition. Consistent with these findings, administration of PG545 in a mouse model of a delayed-type hypersensitivity led to reduced footpad inflammation, reduced IL-17 levels, and an increase in FoxP3+ Treg. Finally, PG545 also promoted Foxp3+ Treg induction by human T-cells. Together, these data indicate that PG545 is a potent inhibitor of Th17 effector functions and inducer of FoxP3+ Treg. These findings may inform the adaptation of PG455 for clinical applications including in inflammatory pathologies associated with delayed-type hypersensitivity.

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Engineered Type-1 Regulatory T Cells for Treatment of Graft-versus-Host Disease in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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Type 1 regulatory cells (Tr1) are a promising therapy for the prevention of graft-versus-host-disease (GvHD) in allogeneic hematopoietic stem cell transplantation (aHSCT) due to their ability to promote immunological self-tolerance in an antigen-specific manner. Tr1 cells can also exert a direct graft-versus-leukemia (GvL) effect by killing myeloid cells, demonstrating an additional therapeutic synergistic effect of Tr1 when used in concert with aHSCT to treat acute myeloid leukemia patients. Due to the scarcity of endogenous Tr1 cells, a number of different *in vitro* protocols have been developed to produce sufficient numbers of Tr1 cells for use as a cell therapy. However, subsequent characterization shows only 10-30% purity of Tr1 cells in the final product, with the remainder either not contributing to or actively hindering the suppressive potential of the product. To address this, our groups has developed a protocol to produce Tr1 cells by lentiviral transduction of the human IL-10 gene (LV-10) into human CD4+ T cells. We are able to select for IL-10 expressing LV-10 cells using the clinical grade selection marker ΔNGFR and expand them using irradiated allogeneic feeder cell mixtures. After multiple feeder expansions, LV-10 cells acquire a Tr1 phenotype. LV-10 cells constitutively express moderate levels of IL-10 that can be increased 5-10 fold upon TCR stimulation. LV-10 cells also downregulate expression of IL-4 and increase expression of IFN-γ, mimicking the classic Tr1 cytokine profile. *In vitro*, LV-10 cells are able to kill myeloid cells through a perforin/granzyme B-mediated mechanism and can also suppress the proliferation of responder CD4+ T cells through secretion of IL-10 and TGF-β1. *In vivo*, LV-10 cells can suppress xeno-GvHD in NSG mice and show the ability to synergize with PBMCs to retain a GvL effect against myeloid-derived tumors. We also performed tracking studies that demonstrate LV-10 cells do not persist within engrafted NSG mice and lack the ability to induce xeno-GvHD, suggesting they may be safe for adaptation as a third-party cell therapy. We are currently working on finding novel biomarkers that correlate with the acquired Tr1 phenotype that will help us identify cell lines suitable for use in the clinic as well as uncovering potential mechanisms for Tr1 polarization. Additionally, to determine whether LV-10 should be produced using cells from the HSC donor or as an off-the-shelf therapy, we are testing whether LV-10 cells possess any advantages in efficacy or survival in suppressing autologous responder T cells versus third party responders in a xeno-GvHD model. The

ultimate goal of this project is to determine the ideal LV-10 products and develop a production protocol that can be advanced towards clinical trials to treat GvHD in AML patients receiving aHSCT.

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Autoimmunity to hypocretin/orexin and molecular mimicry to flu in type 1 narcolepsy

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Background: Type 1 narcolepsy (T1N) is caused by a hypocretin (HCRT, also called orexin) cell loss. Association with DQB1*06:02/DQA1*01:02 (DQ0602, 98% vs. 25%), T cell receptors (TCR) and other loci indicate autoimmunity. Onset is seasonal and associated with influenza-A, notably pandemic 2009H1N1 (pH1N1) infection and the pH1N1 vaccine Pandemrix. The strong HLA association and unique effects in TCRA and TCRB suggest that autoantigen presentation by DQ0602 to CD4+ T cell is crucial. We surveyed CD4+ T cell binding to autoantigens and flu antigens presented by DQ0602 in narcolepsy versus controls, identifying the immunological basis of narcolepsy.

Methods: DQ0602 binding was tested for peptides overlapping RFX4, HCRT and flu HA, NA, PB1and NP sequences. Reactivity to ~100 tetramers was tested in 6 narcolepsy and 4 controls after expansion of cells in 10-day cultures. Higher tetramer-peptide specific $CD4^+$ T cells was found with $HCRT_{54\text{-}66\text{-}NH2}$, $HCRT_{86\text{-}97\text{-}NH2}$ ($HCRT_{NH2}$), $pHA_{273\text{-}287}$ and $NP_{17\text{-}31}$, further confirmed for $HCRT_{NH2}$ and $pHA_{273\text{-}287}$ in 77 T1N and 44 DQ0602 controls. Single cell TCR sequencing after FACS sorting was conducted and public TCR clones transfected into Jurkat76 cells to test for activation after peptide presentation by K562-DQ0602 or RM3-DQ0602 cells.

Results: Most commonly used public sequences CDR3β-TRBV4-2-CASSQETQGRNYGYTF, CDR3 -TRAV2-CAVETDSWGKLQF-TRAJ24 and TRAV26-1-CIVRSQGGSYIPTF-TRAJ6 were retrieved using both DQ0602-$HCRT_{NH2}$ and DQ0602-$pHA_{273\text{–}287}$ but not DQ0602-$NP_{17\text{-}31}$ tetramers. Sharing of clones using TRAJ24 and TRBV4-2 was notable as these exact segments (~0.8% of repertoire) are modulated by rs1154155/rs1483979 and rs1008599, polymorphisms associated with T1N. Jurkat cells transfected with some clones were activated by $HCRT_{NH2}$ and $pHA_{273\text{–}287}$, suggesting molecular mimicry. As a control, $NP_{17\text{-}31}$ involved different TCRs, notably TRAV8-6_TRAJ34 with TRBV7-9_TRBJ2-3 and was only activated by $NP_{17\text{–}31}$.

Conclusion: Our results provide strong evidence for autoimmunity and molecular mimicry with flu antigens modulated by genetic components in the pathophysiology of T1N.

Keywords: Narcolepsy, TCR, DQ0602, Tetramer, Autoimmunity, Jurkat

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Mass cytometry reveals elevated phosphorylated STAT3 (pSTAT3) in circulating CD4+ T cells and CD14+ monocytes during active Psoriatic Arthritis in association with high BMI

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Psoriatic Arthitis (PsA) is a chronic inflammatory arthritis affecting up to 40% of patients with psoriasis. Immune factors, especially T cell producers of IL-17, have been found to be involved in PsA pathophysiology. We used the high dimensionality of mass cytometry to probe for potential differences in circulating immune cells between active and inactive PsA.

Blood from adults PsA patients with active (n=15) or inactive disease (n=13) was fixed with Proteomic Stabilizer (Smart Tube) and frozen at -80°C. After thawing and red cell lysis, surface and intracellular antigens were stained using metal-labeled antibodies (Fluidigm). Cells were acquired on a Helios CyTOF instrument and data manually gated using FlowJo. Statistical analysis was performed using GraphPad Prism, SPSS and SAM analysis.
We examined baseline levels of the activated form of the signaling proteins STAT1, STAT3 and Src in 16 immune populations. Levels of phosphorylated STAT3 (pSTAT3) were elevated in the Th1, Th2, Th17, Tfh and Treg CD4+ T cell subsets of active PsA patients in comparison to inactive patients. pSTAT3 was also elevated in CD14+CD16- (classical) monocytes from active versus inactive PsA patients. The difference in pSTAT3 in Th1 cells between the two groups was still significant after Bonferroni correction. Levels of pSTAT3 in the above-mentioned cell populations were also shown to be higher in active patients using SAM analysis ($q<0.01$, FDR <0.01). Higher levels of Body Mass Index (BMI) and smoking were found in the active versus the inactive group. Correcting for BMI eliminated the difference in pSTAT3 between the groups for all cell populations, while smoking showed a minor contribution.

In summary, we found that elevated pSTAT3 is associated with active PsA in comparison to inactive PsA in CD4+ T cells and CD14+ monocytes. The effect is dependent on the higher BMI in active patients. A recent mouse study showed that overexpression of STAT3 in CD4+ T cells was sufficient to elicit all the major characteristics of PsA, reinforcing a potential role for STAT3 signaling in the promotion of an inflammatory environment in PsA. Obesity has been strongly associated with an increased probability of developing PsA; obesity in turn increases the levels of leptin and IL-6. Both of these mediators induce activation of the JAK-STAT3 signaling. Induction of STAT3 signaling by obesity-related factors may play a fundamental role in PsA pathogenesis.

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Characterization of the Composition, Structure, and Immunological Status of Human Tuberculosis Granulomas with Multiplexed Ion Beam Imaging

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Tuberculosis (TB) granulomas exhibit a high degree of variability due to their histological subtypes, variable bacterial burden, and range of outcomes. Disparate infection outcomes are thought to be driven by underlying differences in the phenotype and histologic organization of cells within the granuloma. However, high dimensional phenotypic and spatial analysis of human TB granulomas has not been conducted. Here, we utilize multiplexed ion beam imaging (MIBI) to study the cellular composition and underlying architecture of human mycobacterial granulomas. MIBI employs secondary ion mass spectrometry to image antibodies tagged with metal reporters, thus avoiding the spectral overlap of fluorophores and allowing high dimensional single-cell phenotyping that retains the spatial organization of complex tissue samples. In this study we applied MIBI to a cohort of clinical specimens from individuals infected with TB from the U.S. and South Africa. Granulomatous tissues from individuals with non-tuberculous mycobacterium (NTM) infections and sarcoidosis were also included. Our analysis revealed the complex cellular composition of granulomas, characterized by a large spectrum of myeloid phenotypes and highly ordered structure of protein expression. We also identify striking expression of immune regulatory proteins, IDO1 and PD-L1, by granuloma myeloid cells, but observe a paradoxical lack of Lag3 and PD-1 expression by lymphocytes. However, the relative abundance of CD4 and CD8 T cells in granulomas is associated with distinct granuloma 'states,' both in their composition and spatial architecture. Lastly, by comparing mycobacterial granulomas with sarcoid lesions, we identify spatial and cellular features that are generalizable across both conditions versus those that may be more specific to the mycobacterial immune response. Ultimatley, this pilot analysis of human granulomas raises the possibility of myeloid-mediated immune suppression within the granuloma and demonstrates that the immunological status of a granuloma is a function of both its cellular composition and architecture.

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Mice and humans share a similar overall immune system structure yet there are differences between the mouse immune system and human immune system that have the potential to confound or limit how well experimental results in mice translate to humans. The Utz and Khatri labs have identified a 144-gene immune Sex Expression Signature (iSEXS) that differentiates healthy male and female immune system gene expression in humans (Bongen et. al.). However, Bongen et. al. did not compare how iSEXS performed in mice. We performed an integrated multi-cohort analysis of blood transcriptome samples from five discovery cohorts with 142 healthy mice to identify the 97-gene murine immune Sex Expression Signature (miSEXS), which is differentially expressed between healthy male and female mice. However, when we compared the performed of iSEXS in mice and miSEXS in humans, the human derived signature iSEXS translated successfully to mice but miSEXS failed to accurately distinguish human females from human males. Thus, iSEXS is a robust signature of transcriptional sex differences for both humans and mice.

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Designing a safe and stable vaccine against Ebola using protein nanoparticles

Ebolaviruses are zoonotic RNA viruses from the Filoviridae family that can cause severe human disease. No therapeutic drug or vaccine against Ebola has been widely licensed for use in humans. An experimental vaccine (rVSV-ZEBOV) has shown promising efficacy in an ongoing outbreak in the Democratic Republic of Congo. However, this vaccine contains an actively replicating virus. As a result, it requires rigorous storage conditions and can cause flu-like symptoms post vaccination. Use of the vaccine in contraindicated groups including children and pregnant women has been controversial due to concern over potential adverse events. Additionally, rVSV-ZEBOV and other Ebola vaccine candidates only provide protection against the most common pathogenic strain of Ebola, EBOV, however two other strains, BDBV and SUDV, have previously caused substantial outbreaks and are an ongoing threat.

An alternative to live-replicating viral vaccines are protein subunit vaccines. Subunit vaccine candidates composed of the trimeric Ebola surface glycoprotein (GP) generate a protective immune response in rodent disease models but have not been studied or optimized extensively. In an effort to design a protein-based vaccine against Ebola that elicits a robust immune response, I have developed a novel protein nanoparticle which displays Ebola GP trimer in 8 copies on its surface. The functionalized particles exhibit similar stability and binding properties as compared to trimer alone. Preliminary results from a mouse immunization experiment suggest that animals immunized with particles developed a significantly better antigen-specific antibody response as compared to animals immunized with GP trimer alone.

My ongoing work is focused on further characterizing the immune response to the GP functionalized nanoparticles and also modifying the particles to design a cross-reactive vaccine that is protective against all pathogenic strains of Ebola. Specifically, I will epitope map the polyclonal response to different regions of GP and characterize the neutralizing potential of the serum obtained from mice in my immunization study. I will also mask the poorly conserved regions of GP on nanoparticles by inserting N-linked glycosylation sites to skew the antibody response to the conserved, vulnerable regions of GP and determine if the resultant hyperglycosylated particles elicit a more cross-reactive antibody response.

Taken together, the results of this work will provide important insight into the development of an effective subunit vaccine against Ebola as well as strategies for creating an antigen to elicit a cross-reactive antibody response for protection against all pathogenic strains of Ebola.

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The Role of Hyaluronan Glycocalyx in Cytotoxic Killing of Cancer Cells

RESEARCH ADVISORS: Ievgen Koliesnik, PhD; Catherine Blish, MD, PhD; Paul Bollyky, MD, D.Phil

The glycocalyx is a coat of glycans that surround the cell membranes of both eukaryotic and prokaryotic cells. Hyaluronan (HA) is a glycosaminoglycan and a major constituent of the glycocalyx. HA has been shown to contribute to tumor growth, cellular adhesion and metastasis. Although many studies demonstrate HA's involvement in a number of pro-cancer signaling pathways, little is known about HA's effect on immune clearance of cancer cells. To investigate the influence of the HA glycocalyx on cytotoxic killing, we conducted co-incubation experiments with NKL (natural killer) effector cells against K562 or Jurkat target cells. 4-Methylumbelliferone (4-MU), a pharmacologic

inhibitor of HA synthesis, was used in pretreatment to remove the HA glycocalyx on target cells before co-incubation. In the Calcein AM release assays, we observed that 4-MU pre-treatment increased NKL-mediated killing of K562 from 18.0% to 45.3% across five separate repeats (Wilcoxon rank sum test, p-value = 0.01587). Although not statistically significant, we observed the same trend for Jurkat T-cells; 4-MU pretreatment also increased NKL-mediated killing of Jurkats from 45% to 63% (Wilcoxon rank sum test, p-value = 0.1508). Interestingly, 4-MU pretreatment in NKLs or addition of 4-MU to the co-incubation, resulted in a reduction of NKL's ability to kill both K562 and Jurkats. Imaging studies showed HA depositing in immune synapses and forming structures that encompass both effector and target cells, suggesting that HA may help facilitate immune cell interaction with target cell. Together, these data support possible paradoxical roles of the HA glycocalyx in the immune clearance of cancer cells. On the one hand, the glycocalyx can promote clearance by stabilizing immune cell-target cell interaction. However, on the other, cancer cells can also use it as a shield for immune evasion.

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A novel dendritic cell adjuvant for boosting anti-cancer immunity during irradiation

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Harnessing the immune system against cancer through the use of checkpoint inhibitors relies on tumor-specific T cells. However, not all tumors respond to checkpoint inhibitor immunotherapy, and failure has been associated with a lack of T cells infiltrating the tumors. Consequently, there is a need to design complementary therapeutic strategies that promote the activation and accumulation of tumor-specific T cells. In particular, dendritic cells (DCs) are antigen-presenting cells unique in their capacity to initiate and modulate T cell-mediated immune responses. A subpopulation of DCs, classical type 1 DCs (cDC1) has been shown to be critical to boost anti-tumor immune responses and to promote the therapeutic success of checkpoint inhibitors in preclinical and clinical models. This evidence positions cDC1 at the center of anti-tumor immune responses and suggests that therapeutic strategies aiming at increasing their functionality would provide a unique therapeutic avenue. In this project, we use a novel immune stimulant, i.e., adjuvant, delivered to these cells. This adjuvant consists of a short double stranded DNA (dsDNA) conjugated to a monoclonal antibody (mAb) specific for DEC205 receptor, which is highly expressed on cDC1. We found that this adjuvant (referred to as αDEC-dsDNA) is able to potently activate cDCs and suppress tumor growth in a murine melanoma model. This suppression is dependent on the expression of DEC205 receptor as shown by experiments using $DEC^{-/-}$ mice. We further show that increasing the number of DCs by the administration of FMS-like tyrosine kinase 3 ligand (FLT3L) increases the effectivity of αDEC-dsDNA treatment. Finally, we demonstrate that αDEC-dsDNA can be used to improve the efficacy of radiotherapy. Altogether our results show that targeting adjuvants to tumor-localized cDCs is an efficient strategy to limit tumor growth. This non-toxic therapeutic approach for targeted adjuvant delivery has the potential improve the success of standard-of-cancer care treatments such as radiotherapy.

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Infiltrating NK cell enrichment and inhibitory killer immunoglobulin receptor expression are associated with Epstein-Barr virus (EBV) post-transplant lymphoproliferative disorder (PTLD)

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Post-transplant lymphoproliferative disorder (PTLD) is a serious complication in transplant recipients that encompasses a range of abnormal lymphoproliferations. Although many are EBV-driven, a significant minority are not. EBV employs diverse immune evasion strategies and EBV+ B lymphomas in PTLD also exhibit immunomodulatory phenotypes. To better characterize the interactions between EBV and host immunity in PTLD, we used systems biology approaches on EBV+ and EBV- PTLD tumor samples to identify immune cell types and host genes associated with EBV-driven oncogenesis.

Three gene expression datasets with EBV+ PTLD and EBV- PTLD tumor samples (n = 60) were chosen for multi-cohort analyses. We estimated various immune cell proportions in each sample via in silico deconvolution using our immunoStates basis matrix with a support vector machine-based method (CIBERSORT). To identify consistently enriched/deficient immune cell types in EBV+ PTLD tumors across datasets, we calculated the Hedges' *g* effect size of individual leukocyte proportions within each dataset. These were then incorporated to give an overall meta-effect size for each cell type across all datasets. To identify differentially expressed genes in EBV+ PTLD, we computed the Hedges' *g* effect size of each gene's expression in the individual datasets, from which we derived the gene expression meta-effect size. We then applied a random effects model, and selected genes with 1) a log2(meta-effect size) ≥ 0.59 (equivalent to a 1.5× gene expression fold change), 2) a false discovery rate ≤ 20% and 3) representation in ≥ 2 of the 3 dataset microarrays.

From these datasets, we show that NK cells are enriched in EBV+ compared to EBV- PTLD tumors. We also obtained a 189-gene signature distinguishing EBV+ from EBV- PTLD, which robustly discriminated between EBV+ and EBV- PTLD samples in each dataset. Amongst these, several inhibitory NK cell killer immunoglobulin receptors (KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2) are upregulated in EBV+ PTLD.

Our results point towards interactions between EBV, the tumor, and host immunity in EBV+ PTLD, and indicate that NK cell activity towards EBV may be suppressed in EBV+ PTLD. These may be novel clinical targets for EBV+ B cell lymphomas where host immunity is compromised.

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Immunologic profiles defining clinical states in malaria.

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Malaria due to *Plasmodium falciparum* (*Pf*) remains a compelling global health problem. A major challenge in malaria vaccine development is the knowledge gap about the mechanisms of protective immunity and pathogenesis in humans, including those driving naturally acquired clinical immunity. To better understand the development of clinical immunity, longitudinal samples from children enrolled in Tororo, Uganda were used to investigate the molecular networks underlying diverse clinical states of *Pf* infection. Peripheral blood mononuclear cells from 37 Ugandan children ages 1-11 years at three timepoints - uninfected, asymptomatic infection, or symptomatic infection- were phenotyped by CyTOF, and malaria-specific responses assessed by intracellular cytokine staining. Clustering analysis indicates that B-cells are enriched in children with symptomatic infection, and innate cells (myeloid) are abundant in children with asymptomatic infections. Through an *in vitro* stimulation induced cytokine assay, we determined that malaria-specific responses were reduced in children at symptomatic timepoints compared to asymptomatic or uninfected timepoints. These results suggest that there are marked differences among the immune profiles of children with asymptomatic and symptomatic infections. Elucidating the dynamics of these different immune responses will be crucial to understanding clinical immunity and may have implications for future vaccine development and better malarial interventions.

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Protect, modify, deprotect (PMD): A strategy for creating vaccines to elicit antibodies targeting a specific epitope

In creating vaccines against infectious agents, there is often a desire to direct an immune response toward a particular conformational epitope on an antigen. We present a method, called protect, modify, deprotect (PMD), to generate immunogenic proteins aimed to direct a vaccine-induced antibody (Ab) response toward an epitope defined by a specific monoclonal Ab (mAb). The mAb is used to protect the target epitope on the protein. Then the remaining exposed surfaces of the protein are modified to render them nonimmunogenic. Finally, the epitope is deprotected by removal of the mAb. The resultant protein is modified at surfaces other than the target epitope. We validate PMD using a well-characterized antigen, hen egg white lysozyme, then demonstrate the utility of PMD using influenza virus hemagglutinin (HA). We use an mAb to protect a highly conserved epitope on the stem domain of HA. Exposed surface amines are then modified with short polyethylene glycol chains. The resultant antigen shows markedly reduced binding to mAbs that target the head region of HA, while maintaining binding to mAbs at the epitope of interest. This antigenic preference is also observed with yeast cells displaying Ab fragments. Antisera from guinea pigs immunized with the PMD-modified HA show increased cross-reactivity with HAs from other influenza strains, compared with antisera obtained with unmodified HA trimers. PMD has the potential to direct an Ab response at high resolution and could be used in combination with other such strategies. There are many attractive targets for the application of PMD.

Aaron Wilk

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Charge-altering releasable transporters enable genetic and phenotypic manipulation of resting primary human natural killer cells

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Natural killer (NK) cells are highly recalcitrant to standard transfection techniques. To date, successful attempts at transfecting NK cells have relied on non-physiological activation methods that dramatically and permanently alter NK cell functional and cell surface phenotype. Charge-altering releasable transporters (CARTs) are cationic diblock oligomers that complex with polyanionic cargo and rearrange to neutral small molecules over time, facilitating cargo release while avoiding toxicity associated with persistent cations. Here, we report that CARTs efficiently transfect primary human NK cells with mRNA, siRNA, and Cas9 ribonucleoprotein without the need for NK cell activation. CARTs transfect primary NK cells two orders of magnitude more efficiently than published electroporation protocols, while maintaining comparable viability, cell surface phenotype, and functional phenotype. Collectively, our work provides a toolkit for genetic and phenotypic manipulation of resting primary NK cells that will enable previously unfeasible work on this understudied lymphocyte subset.

Menglan Xiang, PhD

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Single-cell trajectory analysis reflects the tissue architecture and physical relationships between lymphatic vascular niches in the mouse lymph node

Menglan Xiang, Ruben Grosso, Junliang Pan, Maria Ulvmar, Eugene C. Butcher

For decoding the mammalian vasculature functions, it will be necessary to couple single-cell data to the vascular architecture and vascular niches within the tissues. Another challenge is to identify similarities and differences across human and mouse datasets, which will allow new prospects in translational applications. Here, we address both of these points by analyzing the complex lymphatic vascular niches within the mouse and the human lymph node. We show that single-cell trajectory analysis can reveal cellular relationships directly relevant to the architecture of the lymphatic vascular network and the physical relationships between lymphatic endothelial cells and their surroundings. By using cross-species mapping of cell clusters, we provide support for a conserved pattern of lymphatic vascular niches in human and in mouse lymph nodes, but also reveals two unique populations in the human lymph node which may reflect structural differences between mouse and man. Among shared subsets we identify candidate efferent medullary lymphatic endothelial cells in the human lymph node common to mouse. Using oxazolone-induced inflammation model, we show that subcapsular floor and efferent medullary LEC, which represent major sites of cell entry and exit from the LN parenchyma, respectively, respond robustly to signaling pathways that converge on both innate and adaptive immune responses. Together, these examples demonstrate the power of single-cell trajectory analysis in elucidating endothelial cell heterogeneity and endothelial cell responses. We discuss

these findings from a perspective of lymphatic endothelial cell functions in relation to niche formations in the unique stromal and highly immunological environment of the lymph node.

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High-Parametric Evaluation of Human Invariant Natural Killer T Cells to Delineate Heterogeneity in Allo- and Autoimmunity

Human invariant natural killer T cells (iNKTs) are a rare innate-like lymphocyte population that recognize glycolipids presented on CD1d. Studies in mice have shown that these cells are heterogenous and capable of enacting diverse functions, and the composition of iNKT subsets can alter disease outcomes. In contrast, far less is known about how heterogeneity in human iNKTs relates to disease. To address this, we use a high-dimensional, data-driven approach to devise a framework to parse human iNKT heterogeneity. Our data revealed novel and previously described iNKT phenotypes with distinct functions. In particular, we found two phenotypes of interest: 1) a population with Th1 function that was increased with iNKT activation characterized by HLA-II^{+}CD161^{-} expression, and 2) a population with enhanced cytotoxic function characterized by CD4^{-}CD94^{+} expression. These populations, respectively, correlate with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation and with new onset type 1 diabetes. Our study identifies human iNKT phenotypes associated with human disease that could aid in the development of biomarkers or therapeutics targeting iNKTs.

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The mechanism and function of the maintenance of X chromosome inactivation in female immune cells

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To balance the gene dosage on the X chromosome between females and males, mammals have evolved an epigenetic mechanism by a long-non-coding RNA (lncRNA) called Xist to silence transcription of one of two X chromosomes in the early female embryos. The process of X chromosome inactivation (XCI) consists of two stages: initiation and maintenance. Xist is essential for XCI initiation by recruiting epigenetic repressors to the future inactive X chromosome (Xi). Previous research showed that once Xist deposits repressors, the Xi is stably maintained in all somatic cells throughout life via DNA methylation, which does not require Xist anymore. *Significant progress has been made to understand XCI initiation. However, the mechanism and function of XCI maintenance are largely unknown.* Increased expression of X-linked genes and aberrant Xist localization have been recently observed in immune cells from patients with autoimmune diseases, suggesting abnormal XCI maintenance may predispose women to autoimmune diseases. Given a direct contribution of Xist-mediated XCI to X-linked gene expression and the association with autoimmune disorders, it is imperative to investigate the mechanistic and functional impact of XCI maintenance in immune cells. Here, I discovered that Xist is essential for XCI maintenance of some X-linked immune genes in human B cell line, which is in contrast to the previous studies showing that XCI maintenance does not require

Xist. Importantly, the genes that are up-regulated after the loss of Xist tend to be over-expressed in patients with autoimmune disorders, suggesting that Xist-mediated XCI maintenance might be critical for proper immune B cell function. In the future, I will identify the Xist bound *trans*-factors that are essential for XCI maintenance, identify *cis* features for genes with a continuous requirement for XCI maintenance and decipher the impact of XCI maintenance in B cell activation, proliferation and antibody production.

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