Celebrating 10 Years of
Computational & Systems Immunology

CSI SYMPOSIUM 2023

MONDAY
OCTOBER 30

MUNZER
AUDITORIUM

Presentation talks and social hour.

Stanford MEDICINE School of Medicine
Computation & Systems Immunology

Stanford MEDICINE School of Medicine
Institute for Immunity, Transplantation and Infection

CSI SYMPOSIUM 2023

We are celebrating the 10th year anniversary of the Computation & Systems Immunology (CSI) program on October 30-31, 2023. Through generous support from the Stanford Institute for Immunity, Transplantation and Infection (ITI), we are inviting a significant number of external speakers, bringing back our alumni, and having our current community come together to celebrate this important milestone.

Symposium and Program Co-Directors: Drs. Nima Aghaeepour and Purvesh Khatri

AGENDA AT A GLANCE

Monday, October 30, 2023		
8:00 – 8:30 AM	Coffee/tea & light breakfast will be served	Beckman Bistro Patio
8:30 – 9:00 AM	**Mark Davis, PhD**, Professor and Director of Stanford Institute for Immunity, Transplantation and Infection, *Welcome Address & History of CSI*	Munzer Auditorium
9:00 – 9:15 AM	**Purvesh Khatri, PhD**, Associate Professor and Co-Chair of CSI, *Overview of CSI*	Munzer Auditorium
9:15 – 9:30 AM	**Nima Aghaeepour, PhD**, Associate Professor and Co-Chair of CSI, *Recent changes in CSI and Overview of the Day*	Munzer Auditorium
9:30 – 10:05 AM	**Arup Chakraborty, PhD,** John M. Deutch Institute Professor, *The antibody response to repeated vaccination*	Munzer Auditorium
10:05 – 10:40 AM	**Matt Spitzer, PhD**, Associate Professor at UCSF, *Understanding and improving immune responses to cancer*	Munzer Auditorium
10:40 – 11:00 AM	Break	Beckman Bistro Patio
11:00 – 11:35 AM	**David Furman, PhD**, Associate Professor at The Buck Institute, *Lever-aging space biology to model immune decline*	Munzer Auditorium
11:35 – 12:10 AM	**Bjoern Peters, PhD**, Professor at La Jolla Institute of Immunology	Munzer Auditorium
12:10 – 1:30 PM	Catered lunch will be served	Alumni Lawn in front of LKSC
1:30 – 2:30 PM	Career Panel: **Leah Sibener, PhD**, Co-Founder, Vice President, Head of Therapeutic Discovery at 3T Biosciences; **Jacob Glanville, PhD**, Chief Executive Officer & President at Centivax	Munzer Auditorium
2:30 – 3:05 PM	**Ferhat Ay, PhD,** Associate Professor at La Jolla Institute of Immunology, *Computational approaches for studying the 3D chromatin structure of the human immune cells*	Munzer Auditorium
3:05 – 3:20 PM	**Qin Li, PhD**, Postdoc, Genetics, Li Lab, *Insufficient RNA editing is a key genetic driver for inflammatory diseases*	Munzer Auditorium

3:20 – 3:35 PM	**Meng Sun, PhD**, Postdoc, ITI, Davis Lab, *Toddlers have limited mucosal protection against Live Attenuated Influenza Virus in human tonsil organoids*	Munzer Auditorium
3:35 – 3:50 PM	**Simone Thair, PhD**, Senior Data Scientist, ITI, *Prospective multi-site validation of 11-gene host response signature for influenza diagnosis*	Munzer Auditorium
3:50 – 4:05 PM	Coffee/tea will be served	Beckman Bistro Patio
4:05 – 4:20 PM	**Zinaida Good, PhD**, Instructor, Stanford Cancer Institute, *Lineage tracing of CAR T cells in patients with B cell malignancies*	Munzer Auditorium
4:20 – 4:35 PM	**Alma-Martina Cepika, MD, PhD**, Instructor, Pediatrics, *Epigenetic landscape and key transcriptional regulators of antigen-inducible, FOXP3-independent Tregs*	Munzer Auditorium
4:35 – 4:50 PM	**Camilo Espinosa Bernal**, Graduate Student, Immunology, Aghaeepour Lab, *CorALS: an open-source framework for unraveling the coordination of complex biological systems*	Munzer Auditorium
4:50 – 5:05 PM	**Candace Liu**, Graduate Student, Immunology, Angelo Lab, *Pixie: A pipeline for robust phenotyping of highly multiplexed tissue imaging data using pixel-level clustering*	Munzer Auditorium
5:05 – 5:20 PM	**Noah Greenwald**, Graduate Student, Pathology, Angelo and Curtis Labs, *The temporal influence of the tumor microenvironment in response to checkpoint blockade*	Munzer Auditorium
5:30 PM	Dinner + Networking	Alumni Lawn in front of LKSC

Mark Davis, PhD

The Burt and Marion Avery Professor of Microbiology and Immunology and Director of Stanford Institute for Immunity, Transplantation and Infection

Purvesh Khatri, PhD

Associate Professor of Medicine, and of Biomedical Data Science and Co-Chair of Computation and Systems Immunology

Nima Aghaeepour, PhD

Associate Professor of Anesthesiology, Perioperative and Pain Medicine, and of Pediatrics - Neonatal and Developmental Medicine and Co-Chair of Computation and Systems Immunology

SPEAKERS

Arup Chakraborty, PhD, John M. Deutch Institute Professor at MIT

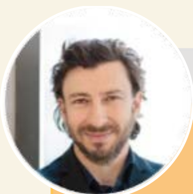
The antibody response to repeated vaccination

Arup K. Chakraborty is one of the 12 Institute Professors at MIT, the highest rank awarded to a MIT faculty member, and holds the John M. Deutch Institute Professorship. He is also a Professor of Chemical Engineering, Physics, and Chemistry at MIT. He served as the founding Director of MIT's Institute for Medical Engineering and Science, and he is a founding member of the Ragon Institute of MIT, MGH, and Harvard. For over two decades now, Chakraborty's work has largely focused on bringing together approaches from statistical physics, immunology, and virology. His interests span T cell signaling, development of the T cell repertoire, and a mechanistic understanding of virus evolution, antibody responses, and vaccine design. Since 2016, Chakraborty has also been interested in the role of phase separation in gene regulation. Chakraborty is one of only 26 individuals who are members of all three branches of the US National Academies – National Academy of Sciences, National Academy of Medicine, and National Academy of Engineering. He is also a Fellow of the American Academy of Arts & Sciences, and has received many other honors including the NIH Director's Pioneer Award, the E. O. Lawrence Medal (DOE), a Guggenheim Fellowship, the Max Delbruck Prize in Biological Physics from the American Physical Society, the Colburn, Professional Progress, and Prausnitz Institute Lectureship from the AIChE, and three honorary doctorates. Chakraborty has received 7 awards for his classroom teaching and he is a co-author of the book "Viruses, Pandemics, & Immunity". Chakraborty is a member of the Board of Governors of the Wellcome Trust, and serves as a consultant for biotechnology companies.

Matt Spitzer, PhD, Associate Professor at UCSF

Understanding and improving immune responses to cancer

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



David Furman, PhD, Associate Professor at The Buck Institute

Lever-aging space biology to model immune decline

Dr. David Furman is Director of the Stanford 1,000 Immunomes Project at Stanford School of Medicine, Associate Professor at the Buck Institute for Research on Aging and Chief of the AI Platform at the same institute. He obtained his Doctoral degree in immunology (summa cum laude) from the School of Medicine, University of Buenos Aires, Argentina, for his work on cancer immune-surveillance. During his Postdoctoral training at the laboratory of Professor Mark M Davis (Stanford), he conducted cutting-edge research in Data Science and Systems Immunology to predict clinical outcomes using multi-omics technologies in large human cohorts. The aim was to answer scientific questions with strong potential for translational medicine, including the effect of immunity in age-related disease and longevity. Dr. Furman moved to University of Bordeaux, France, where he was appointed as Visiting Scientist and investigated the involvement of the endocrine and immune systems in human aging and in kidney transplantation. After France, Dr. Furman helped create the Systems Biology Department at the Sidra Medical Research Center in Doha, Qatar. Dr. Furman was then re-appointed at the Stanford School of Medicine to assume the role of Consulting Professor at the Institute for Immunity, Transplantation and Infection (ITI), and his work involved the use of high-bandwidth/high throughput technologies to measure immune function in humans and Machine Learning tools to better define the role of the immune system in disease and longevity.

Dr. Furman is inventor of over 20 patents and has published dozens of scientific articles with over 6,000 citations in top peer reviewed journals such as The Lancet, Nature Medicine, Cell, etc. Current i10 index of Dr. Furman is 32.

Bjoern Peters, PhD, Professor at La Jolla Institute of Immunology

Research in the **Peters** lab is focused in three areas, all relating to the development of computational tools to address fundamental questions in immunology.

Starting as a PhD student in 2000, Dr. Peters has worked on the development and validation of tools to analyze and predict which parts of a pathogen, allergen or cancer cell are targeted by immune responses. Identifying these specific molecular targets of immune responses, called epitopes, recognized by diseased individuals opens a path towards the development of diagnostics, vaccines and therapeutics for that particular disease. The tools the Peters lab develops aim to reduce the experimental effort required to identify these targets; computer-based predictions allow researchers to focus on the components most likely to be recognized rather than screening thousands of molecules.

The second research area of the lab is the identification of differences between immune cells in individuals with opposite disease outcomes. Powerful experimental tools have been developed to detect differences in how cells utilize the diverse parts of the genome. The Peters lab is using these tools to characterize how immune cells from diseased individuals differ from healthy individuals. These cells are isolated using disease-specific epitopes (or reagents based on them), so our epitope-identifying algorithms directly aid our disease-focused work. This research helps us understand how the disease develops and identifies potential targets in the genome for interventions to treat or prevent the disease.

Finally, the Peters lab is deeply involved in the development of community standards for knowledge representation to promote interoperability and re-use of data. The Peters and Sette lab maintain the Immune Epitope Database (www.iedb.org), which catalogs all published experiments on immune epitope recognition. This requires transforming free text information from journal publications into a structured format, and to make the information

optimally useful, connecting it with information stored elsewhere. Doing this efficiently requires a community consensus on knowledge representation. Dr. Peters' team is contributing to such consensus building and standardization efforts through active work on scientific community initiatives such as the Ontology of Biomedical Investigations (OBI, http://obi-ontology.org/).

Ferhat Ay, PhD, Associate Professor at La Jolla Institute of Immunology

Computational approaches for studying the 3D chromatin structure of the human immune cells

Ferhat Ay is the Institute Leadership Associate Professor of Computational Biology at the La Jolla Institute for Immunology (LJI) with adjunct appointments at the UC San Diego School of Medicine, Moores Cancer Center, Institute for Genomic Medicine and Bioinformatics and Systems Biology Ph.D. program. Before LJI, he was a Research Assistant Professor at Northwestern University (2015) and a CRA Computing Innovation Fellow in William Noble's lab in the Department of Genome Sciences at the University of Washington (2011-2014). Ferhat completed his Ph.D. in Computer Science at the University of Florida (2007-2011) and his B.S. degrees in Computer Engineering and Mathematics both from Middle East Technical University (METU), Turkey, in 2007. His lab is particularly interested in analyzing and modeling the 3D genome organization from high-throughput chromatin conformation capture data to understand how changes in this 3D structure affect outcomes such as development, differentiation, and disease progression.

CAREER PANELISTS

Jacob Glanville, PhD

Chief Executive Officer & President at Centivax

Jacob Glanville is a serial entrepreneur, and computational immuno-engineer. He built and sold his first company Distributed Bio from founding in March of 2012 to a 104M dollar sale to Charles River Laboratories in December of 2020. During that period, he developed the core business model, the research teams, and the technologies that enabled Distributed Bio to become profitable without investment. As part of the acquisition agreement, he founded Centivax Inc and spun-out his assets in COVID-19 therapeutics, broad-spectrum vaccines, antivenom antibodies, anti-wound pathogen antibodies, anti-CXCR5 autoimmunity therapeutics, and blood-brain barrier translation technologies into Centivax, where he is now CEO.

He has developed multiple seminal methods in the fields of high-throughput antibody repertoire sequencing (PNAS 2009), repertoire decoding algorithms (Nature 2017), single-cell TCR receptor & phenotype sequencing (Nature Biotech, 2014), deconstructing genetic variation in the adaptive immune system (Nature Communications 2015, Nature Reports 2016, PNAS 2011, TI 2017), and computationally guided antibody library engineering (JMB 2011, JMB 2013, COSB 2015). He is the inventor of the Centivax Universal Vaccine technology, the SuperHuman discovery library technology, and the Tumbler technology. He is a Stanford University Scientific Advisory Committee member for the Sean Parker Center for Allergy and Asthma Research, a Scientific Advisory Board

member for the University of San Francisco's Biotechnology program, a repeat Gates Foundation/Stanford University Computational and Systems Immunology Grant Recipient while a PhD Candidate with Mark Davis at Stanford, a Recipient of Pfizer Achievement award 2010 while Principal Scientist at Pfizer, and has been a course-founding instructor and guest lecturer for multiple graduate-level applied computational and systems immunology courses at Stanford and USF. Growing up watching his parents grow a successful hotel and restaurant business (La Posada de Santiago) in Guatemala in a Tzutujil village during a civil war, he was happy to find running a biotechnology startup to be similar in many respects, with many of the lessons learned in team management, product refinement, client recruitment and haggling to be surprisingly translatable. From 2012-2019, he nurtured the vision of universal vaccines through the creation of a profitable business to support the work, the building of an animal facility in Guatemala to prove the technology, a collaboration with the University of San Carlos, supported an international research team over 4 years to prove the technology in-vivo, and managed to gather a team of remarkable scientists from USF, Pfizer, Genentech and other places who agreed to join and participate in manifesting the vision.

Leah Sibener, PhD

Co-Founder, Vice President, Head of Therapeutic Discovery at 3T Biosciences

Dr. Sibener is a molecular immunologist and protein engineer responsible for leading discovery efforts at 3T. She holds a PhD in Immunology from Stanford and Bachelors in Biophysics from Johns Hopkins University. While at Stanford, under the supervision of K. Christopher Garcia, she discovered novel mechanisms of tuning T cell receptor signaling and developed technology to decipher T cell receptor specificity and cross-reactivity. She as previously worked at the Institute of Cellular Engineering at Johns Hopkins, and within gRED at Genentech. Leah has been featured in *Forbes* 30-under-30 in Healthcare, and in the *San Francisco Business Times* as a rising leader in biotech.

GRADUATE STUDENTS, POSTDOCS, AND STAFF

Qin Li, PhD, Postdoc, Stanford Genetics

Insufficient RNA editing is a key genetic driver for inflammatory diseases

Innate immunity is the first line of defense against infection, yet unwanted immune response caused by self sensing can lead to severe inflammation and autoimmune disease. Here we show that ADAR-mediated adenosine-to-inosine RNA editing, a post-transcriptional event vital for suppressing sensing of self double-stranded RNA (dsRNA), is an important potential mechanism underlying genetics of common autoimmune diseases. We identified and characterized 30,319 cis-RNA editing QTLs (edQTLs) across 49 human tissues. These edQTLs were significantly enriched in GWAS signals for autoimmune and immune-mediated diseases. Colocalization analysis of edQTLs with disease risk loci further pinpointed key, putatively immunogenic dsRNAs formed by expected inverted repeat Alu elements as well as unexpected, highly over-represented cis-natural antisense transcripts. Furthermore, autoimmune disease risk variants, in aggregate, were associated with reduced editing of nearby dsRNAs and induced interferon

responses in autoimmune diseases. This unique directional effect agrees with the established mechanism that lack of RNA editing by ADAR1 leads to the specific activation of the dsRNA sensor MDA5 and subsequent interferon responses and inflammation. Our findings implicate cellular dsRNA editing and sensing as a previously under-appreciated mechanism of autoimmune diseases.

Meng Sun, PhD, Postdoc, Stanford ITI

Toddlers have limited mucosal protection against Live Attenuated Influenza Virus in human tonsil organoids

Globally, infectious diseases remain the leading cause of death among children under five. Given their unique response patterns to vaccination and other treatments, children require separate clinical trials. This is partly because we don't have much information about how their immune systems differ from adults. Notably, we have recently developed a high-resolution in vitro model of human immunity to vaccination using tonsil organoids. These organoids have demonstrated a remarkable ability to generate specific antibody and T-cell responses to vaccines across a broad age spectrum, spanning from 2 to 70 years old. This presents us a unique opportunity to investigate the variations in immune responses between children and adults following vaccination. To comprehensively explore the systematic cellular response to live attenuated influenza virus in human tonsil organoids, we employed single-cell RNA-sequencing via the BD Rhapsody platform, complemented by flow cytometry. Our data showed marked differences between toddlers (2-4 years) and adults (19-39 years). In comparison to adults, toddlers exhibited lower levels of flu-specific IgA and IgG antibodies, as well as cytotoxic T cells, including gdT and CD8 cells, which significantly contribute to immune protection. Additionally, toddlers displayed a less comprehensive enrichment of cytokines, including FLT3L, IL2, and IL15, which play pivotal roles in promoting class-switched antibody production. Notably, FLT3L was known to enhance the growth and maturation of dendritic cells, crucial to antigen presentation and cytokine secretion. Furthermore, interactions between T and B cells were less extensive in toddlers compared to adults. Our data also indicated a metabolic transition within germinal centers as individuals progress from early childhood to adulthood, shifting from oxidative phosphorylation to glycolysis. This transition is notable for its enhancement of immune function. Our study can offer valuable insights to aid vaccine design and administration practices. It may also identify vulnerabilities in the immune systems of children with implications for improving therapeutic treatments for infectious diseases.

Simone Thair, PhD, Senior Data Scientist, Stanford ITI

Prospective multi-site validation of 11-gene host response signature for influenza diagnosis

The development of a biomarker to predict risk of severe outcomes in influenza infections is urgently needed. Here we sought to validate our previously described blood-based 11-gene signature, the Influenza Meta Signature (IMS), for diagnosis of influenza infections in two prospective cohorts (1 adult (n= 699), Australia; 1 pediatric (n = 182), United States PICFLU study) across 38 clinics, hospitals, and ICUs. We further hypothesized that detection of influenza viral RNA in blood integrated with the host response to influenza would be prognostic. Using NanoString we assayed 11 IMS genes as well as 15 influenza genes covering 3 influenza strains from which we derived the Blood Flu Score (BFS), quantifying influenza virus RNA detection in the blood. In adults, using clinically diagnosed infections, including nasopharyngeal swab RT-PCR (NP+) to diagnosis influenza, the IMS score accurately distinguished NP+ influenza

patients from healthy controls, bacterial infections, other viral infections, or those with no pathogen detected (NPD) with AUROCs= 0.95, 0.88, 0.77 and 0.83 respectively. While the IMS and BFS scores positively correlated (r=0.47, p<2.2e-16), many patients with high BFS were not severely ill. We discovered the ratio of BFS-to-IMS was significantly higher in patients admitted to ICU versus not (p=7.1e-05). In PICFLU patients, the BFS-to-IMS ratio was significantly higher in those who progressed to acute respiratory distress syndrome (ARDS; p=6.3e-03), multi-organ dysfunction (MODS; p=4.5e-03), extracorporeal membrane oxygenation (ECMO; p=1.1e-04), and mortality (p=2.2e-04). These results thus validate IMS for diagnosing influenza and demonstrate a robust prognostic signature when combined as a ratio with the novel discovery of BFS.

Zinaida Good, PhD, Instructor, Stanford Cancer Institute

Lineage tracing of CAR T cells in patients with B cell malignancies

Autologous T cells genetically engineered to express a chimeric antigen receptor (CAR) targeting CD19 and/or CD22 have achieved high complete response rates in patients with hematologic malignancies, but >50% of patients progress following therapy. Here, we sought to understand key T cell intrinsic factors impacting efficacy: CAR T cell expansion, persistence, and homing to the tumor. Using an endogenous T cell receptor (TCR) sequence as a ‘barcode’, we followed individual T cell clonotypes at the single-cell level from pre-manufacture apheresis and infusion products to tumor-involved lymph node and blood at peak and late expansion in 22 adult patients with relapsed or refractory large B cell lymphoma (LBCL) or acute lymphoblastic leukemia (ALL) treated with axicabtagene ciloleucel, an FDA-approved CD19-CAR T cell immunotherapy, or bispecific CD19/CD22 CAR T cells on an investigator-initiated trial (NCT03233854). The resulting CAR T cell atlas comprises matched transcriptome (scRNA-seq) and surface protein expression (CITE-seq) for 846,344 cells from 97 samples, with 251,175 unique TCR clonotypes identified, including 20,093 unique clonotypes that could be traced across 2+ timepoints in CAR+ cells. This atlas enabled us to ask: “What were the phenotypes of ‘successful’ CAR T cell clonotypes with optimal homing, expansion, and persistence properties at the time of infusion or pre-manufacture apheresis?” We found that successful T cell clonotypes at apheresis had juvenile features, including IL7R expression. Conversely, successful clonotypes in the infusion product had elevated interferon pathway activity and effector signatures, including GZMB expression. Further, we mapped blood CAR T regulatory (Treg) cells that are linked to disease progression back to pre-existing natural Treg cells. Finally, we defined dynamics of TCR clonotypes with predicted specificities for viral and self-antigens. These analyses pinpoint the identities of source T cells and infusion CAR T cells with properties impacting efficacy, and also identify cell of origin for CAR Treg cells, with implications for future modulating strategies to enhance CAR T cell efficacy.

Alma-Martina Cepika, PhD, Instructor, Stanford Pediatrics

Epigenetic landscape and key transcriptional regulators of antigen-inducible, FOXP3-independent Tregs

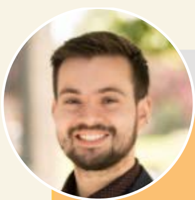
Introduction: Type 1 regulatory T (Tr1) cells are a subset of regulatory T cells that arises from mature peripheral CD4+ T cells exposed to chronic antigen stimulation in tolerogenic conditions, such as in the presence of interleukin (IL)-10. Tr1 cells are abundant in patients that become tolerant to allogeneic transplants, and Tr1 cell adoptive transfer can induce tolerance to allogeneic grafts in murine models. Human alloantigen (alloAg)-specific Tr1 cells can be rapidly induced in vitro, which we leverage therapeutically in two ongoing clinical trials, in patients with hematological

malignancies treated with allogeneic hematopoietic stem cell transplantation (ClinicalTrial.gov IDs NCT03198234, NCT04640987). Tr1 cells have a similar phenotype and comparable suppressive function to thymic-derived regulatory T cells (Tregs) that express transcription factor (TF) FOXP3. While FOXP3 orchestrates FOXP3+ Treg development and regulates their identity and suppressive function, TF(s) that play the equivalent "master regulator" roles in human Tr1 cells are unknown.

Methods: To identify master regulator TFs of human Tr1 cells, we developed a functional epigenomics approach. We generated alloAg-specific Tr1 cells (n = 5) using the same protocol as in cGMP Tr1 production, then compared their epigenome (ATAC-seq) and transcriptome (RNA-seq) to 3 control T cell subsets. Differential TF binding site analysis, TF footprinting, and computational integration of Tr1 cell-specific epigenome and transcriptome to identify the domains of regulatory chromatin (known to be enriched for master regulator TFs) revealed Tr1-specific TFs. The functional role of candidate TFs in Tr1 cell differentiation was established by their CRISPR-activation (CRISPRa) or CRISPR/Cas9-mediated knock-out (KO) in parental CD4+ T cells, which were then differentiated into alloAg-specific Tr1 cell products. Read-outs were Tr1 cell abundance, phenotype, and function of the TF-modified Tr1 products in comparison to the wild-type controls.

Results: Our functional epigenomics approach revealed new mechanistic insights into transcriptional regulation of human Tr1 cell differentiation and function. Using our clinically-relevant Tr1 differentiation model, we first established that EOMES, which is a proposed master regulator TF of alloAg-specific murine Tr1 cells, plays no role in human alloAg-inducible Tr1 cell differentiation. Second, our CRISPRa experiments revealed that early activation of MAF, known to regulate IL-10 (a key growth factor for human Tr1 differentiation), increases Tr1 cell abundance in alloAg-specific Tr1 products. Interestingly, CRISPR KO of MAF impaired the ability of Tr1 cells to produce IL-10, but did not affect their differentiation or suppressive function. Finally, CRISPR KO of IRF4 significantly reduced Tr1 cell abundance, expression of co-inhibitory proteins LAG3 and CTLA-4, and suppressive function of alloAg-specific Tr1 products.

Conclusions: Our functional epigenomics platform revealed master regulator TFs of antigen-inducible, FOXP3 independent human regulatory T cells also known as Tr1 cells. Specifically, we show that MAF is sufficient, but not necessary to boost Tr1 differentiation, whereas IRF4 is necessary for both Tr1 differentiation and their suppressive phenotype and function.



Camilo Espinosa Bernal, Graduate Student, Stanford Immunology

CorALS: an open-source framework for unraveling the coordination of complex biological systems

Advanced measurement and data storage technologies have enabled high-dimensional profiling of complex biological systems. For this, modern multiomics studies regularly produce datasets with hundreds of thousands of measurements per sample, enabling a new era of precision medicine. Correlation analysis is an important first step to gain deeper insights into the coordination and underlying processes of such complex systems. However, the construction of large correlation networks in modern high-dimensional datasets remains a major computational challenge owing to rapidly growing runtime and memory requirements. Here we address this challenge by introducing CorALS (Correlation Analysis of Large-scale (biological) Systems), an open-source framework for the construction and analysis of large-scale parametric as well as non-parametric correlation networks for high-dimensional biological data. CorALS features off-the-shelf algorithms suitable for both personal and high-performance computers, enabling workflows and downstream analysis approaches. We illustrate the broad scope

and potential of CorALS by exploring perspectives on complex biological processes in large-scale multiomics and single-cell studies.

Candace Liu, Graduate Student, Stanford Immunology

Pixie: A pipeline for robust phenotyping of highly multiplexed tissue imaging data using pixel-level clustering

While technologies for multiplexed imaging have provided an unprecedented understanding of tissue composition in health and disease, interpreting this data remains a significant computational challenge. To understand the spatial organization of tissue, imaging studies typically focus on cell-level phenotypes. However, images can capture important objects that are outside of cells, such as the extracellular matrix. We developed a pipeline, Pixie, that achieves robust annotation of pixel-level features and show its application across a variety of biological contexts and imaging platforms. Furthermore, current cell phenotyping strategies that rely on clustering can be labor intensive and require large amounts of manual adjustments. Cell phenotyping of intact tissue poses challenges that are not encountered when analyzing data from assays that use dissociated single cells. We demonstrate how pixel clusters that lie within cells can be used to improve cell annotations and decrease the amount of manual adjustments needed. To demonstrate the ability of Pixie to discover biological insights, we used Pixie to characterize the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC). Normal breast myoepithelium is a thick cellular layer between the stroma and ductal cells. In DCIS, the myoepithelium becomes stretched out in a thin layer with few cell bodies. Therefore, classical cell phenotyping strategies fail to capture the myoepithelial phenotype. Pixie allowed us to assign phenotypes to the acellular features of the myoepithelium. Importantly, a high abundance of ECAD+ myoepithelium pixels was the number one predictor of IBC recurrence in this study, highlighting the utility of Pixie.

Noah Greenwald, Graduate Student, Pathology

The temporal influence of the tumor microenvironment in response to checkpoint blockade

Immunotherapies have produced a paradigm shift in cancer treatment across a continually growing list of disease subtypes. However, our understanding of why certain patients respond and others do not, as well as our ability to predict this response, is still quite limited. This is due in large part to the complexity of the tumor microenvironment (TME), which contains cancer cells, immune cells, and stromal cells of diverse lineages. These differing cell types can have context-dependent roles in either promoting on inhibiting anti-tumor immune responses, making it challenging to identify features which reliably correlate with patient outcome. In addition, the roles of these diverse cell types are often influenced by their spatial location within the TME, meaning that assays which do not preserve spatial information may be missing key information that would help to resolve this complexity. To address these gaps, we performed multiplexed imaging to capture 37 distinct proteins across 109 metastatic triple negative breast cancer patients enrolled in a clinical trial testing anti-PD1 immunotherapy. We collected metastatic samples prior to treatment and during treatment to understand how checkpoint blockade reshaped the TME. We also collected archival material from the original primary tumor for each patient. We generated multiplexed imaging data across all of these samples using MIBI-TOF. We found a striking temporal effect on features associated with response, with features that came up as prognostic prior to treatment showing no effect once treatment had begun. Intriguingly, we were able to observe features in the original primary tumors which predicted subsequent (metastatic) response to

immunotherapy. We then compared our ability to predict response by integrating these features into a multivariate model. We observed substantial differences in predictive accuracy based on timepoint, with on-treatment samples exhibiting the best performance and primary tumor samples exhibiting the worst. These findings shed new light on the determinants of immunotherapy outcome, and may shape the design of subsequent trials to better understand the temporal aspects of checkpoint blockade.