Stanford MEDICINE | Immunology

# 2020 IMMUNOLOGY SCIENTIFIC CONFERENCE

**November 6, 2020**
**11:00 am – 9:30 pm**
**Schedule & Abstracts**

We are pleased to present an exciting retreat, taking place virtually this year. It will be filled with presentations and posters by postdocs and graduate students from across the Stanford Immunology community. We are honored to have Keynote Speaker, Christopher Barnes, PhD, from Caltech speaking about "Structural classification of neutralizing antibodies against the SARS-CoV-2 spike receptor-binding domain suggests vaccine and therapeutic strategies." He will also be available for 30 minutes prior to the talk to informally meet with trainees and answer questions they may have regarding career path, work/non-work balance, and future research. Dr. Barnes will be joining the Stanford faculty in the Department of Biology next year. We then wrap up the retreat with a fun Social Hour.

**Keynote Speaker: Christopher Barnes, PhD**
HHMI Hanna Gray Postdoctoral Fellow
Division of Biology and Biological Engineering at Caltech

Christopher Barnes is a postdoctoral scholar in the laboratory of Pamela Bjorkman and a 2017 Hanna Gray Fellow. He and his colleagues are currently working in one of the few labs still open on campus (using social distancing and taking other necessary precautions) to develop detailed three-dimensional structures of antibodies bound to the novel coronavirus spikes, which could ultimately lead to antibody-based therapies.

**Poster Session**
The poster session will be via the Gather Town link. Please see the instructions on the conference webpage. Presenters and abstracts are listed on page 16 of the program.

**Social Hours**
Join students, postdocs, and faculty for trivia and games via the Gather Town link. Second Year Immunology Graduates have prepared some fun virtual activities.

**2020 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, and Torye Nguyen

**AGENDA**

**STANFORD IMMUNOLOGY VIRTUAL CONFERENCE**

**Friday, November 6, 2020 at 11:00 AM – 9:30 PM**

Talks via ZOOM | Meeting ID: 949 5035 7519 Passcode: 343434 | Register here

Poster Session and Social Hours via Gather Town

| Time (PDT) | Speaker & Title |
|---|---|
| 11:00 - 11:05 am | Conference Directors: Michael Howitt, PhD, and Sidd Jaiswal, MD, PhD, *Welcome and Introductions* |
| ***Session 1*** | Graduate/Postdoc Talks |
| 11:05 am - 11:20 am | **Graham Barlow**, Bollyky and Nolan Labs, *Tissue Schematics: assemblies of cellular neighborhoods as maps of tissue architecture* |
| 11:20 - 11:35 am | **Sizun Jiang**, Nolan Lab, M*ultiplexed Ion Beam Imaging of Nucleic Acids and Proteins Resolves the Immune-Pathogen Landscape of Viral Diseases* |
| 11:35 - 11:50 am | **Erin McCaffrey**, Angelo Lab, *Multiplexed imaging of human tuberculosis granulomas uncovers immunoregulatory features conserved across tissue and blood* |
| 11:50 am - 12:05 pm | **Felix Hartmann**, Bendall Lab, *Single-cell metabolic profiling of human immune cells* |
| 12:05 - 12:15 pm | ***Break*** |
| ***Session 2*** | Graduate/Postdoc Talks |
| 12:15 - 12:30 pm | **Aaron Wilk**, Blish Lab, *Multimodal single-cell profiling of COVID-19 immunity reveals neutrophil activation and monocyte silence are associated with disease severity and outcome* |
| 12:30 - 12:45 pm | **Jiaying Toh**, Khatri and Martinez Labs, *Pan-virus multi-cohort analysis of host immune response identifies immune correlates associated with the severity of viral infection* |
| 12:45 - 1:00 pm | **Aditya Rao**, Khatri Lab, *Multi-cohort analysis of host immune response identifies conserved protective and detrimental modules that predict infection severity irrespective of viral pathogen* |
| 1:00 - 1:15 pm | ***Break*** |
| 1:15 - 2:00 pm | **Lunch/Poster Session via Gather Town.** See page 16 for presenters and abstracts. |
| ***Session 3*** | Graduate/Postdoc Talks |
| 2:00 - 2:15 pm | **Amy Fan**, Majeti Lab, *Cytokine Rescue of Inflammation-Sensitive RUNX1 Deficient Human CD34+ Hematopoietic Stem and Progenitor Cells* |
| 2:15 - 2:30 pm | **Ariel Calderon**, Bendall Lab, *Characterization of Natural Killer Cell Hematopoietic Precursors in Human Hematopoiesis* |

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| 2:30 - 2:45 pm | **Caleb Glassman**, Garcia Lab, *Structural basis for IL-12 receptor assembly enables functional dissection of actions on T and NK cells* |
| 2:45 - 3:00 pm | **Nora Vivanco**, Bendall and Palmer Labs, *Gestational Immune Profiling Reveals Specialized PD-L1 expressing Mononuclear Phagocytes at the Feto-Maternal Interface* |
| 3:00 - 3:10 pm | ***Break*** |
| ***Session 4*** | Graduate/Postdoc Talks |
| 3:10 - 3:25 pm | **Lawrence Bai**, Habtezion and Khatri Labs, *Molecular and Clinical Data Integration Identifies an Association Between Decreased Colectomy Rates and Atorvastatin Exposure in Ulcerative Colitis Patients: A Retrospective Cohort Study* |
| 3:25 - 3:40 pm | **Camille Brewer**, Robinson Lab, *Rheumatoid Arthritis Autoantibodies Bind Oral Bacteria Detected in Blood Prior to Flare* |
| 3:40 - 3:55 pm | **Kartik Bhamidipati**, Robinson Lab, *CD52 is Elevated on B cells of SLE Patients and Regulates B Cell Function* |
| 3:55 - 4:10 pm | **Yoni Rubin**, Habtezion Lab, *Effects of processing conditions on stability of immune analytes in human blood* |
| 4:10 - 4:20 pm | ***Break*** |
| 4:20 - 4:50 pm | **Meet Christopher Barnes, PhD** |
| 4:50 - 5:20 pm | **CDIII: Community, Diversity and Inclusion in Immunology** |
| 5:20 - 6:00 pm | ***Dinner*** |
| 6:00 - 7:00 pm | **Keynote: Christopher Barnes, PhD**, *Structural classification of neutralizing antibodies against the SARS-CoV-2 spike receptor-binding domain suggests vaccine and therapeutic strategies* |
| 7:00 - 7:30 pm | ***Break*** |
| 7:30 - 9:30 pm | **Social Hours via Gather Town** |

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## ORAL SESSION
## STANFORD IMMUNOLOGY VIRTUAL CONFERENCE
**Friday, November 6, 2020**

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| 11:35 - 11:50 am | **Erin McCaffrey**, Angelo Lab, *Multiplexed imaging of human tuberculosis granulomas uncovers immunoregulatory features conserved across tissue and blood* |
| 11:50 am - 12:05 pm | **Felix Hartmann**, Bendall Lab, *Single-cell metabolic profiling of human immune cells* |

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

**Tissue Schematics: assemblies of cellular neighborhoods as maps of tissue architecture**

Understanding how a biological system such as an organ operates requires understanding how it is built and the programs that construct it. A schematic of such a system is a representation of its pieces and how they combine, incorporating the system's components into a single reference map through which alterations, as would be the case in malignancy, development, or evolution, can be aligned. High-parameter imaging technologies provide detailed views of tissues and computational techniques have been developed to automatically identify tissue structures such as cells, i-niches, cellular neighborhoods (CNs), and communities. However, a representation of tissue that incorporates tissue components and how they fit together to generate complex tissue behavior is lacking. We present an approach for creating 'spatial schematics' of tissues: computational maps of how these tissues are built. A spatial schematic of a tissue consists of i) identified CNs (each defined by a characteristic local composition of cell types); ii) a map of spatial contexts (distinct microenvironments defined in terms of local spatial contacts between CNs); and iii) a map of the tissue's CN assembly rules (describing how complex structures involving multiple spatially related CNs are assembled). Our results illuminate two distinct ways in which complex functions result from the spatial organization of CNs: through the construction of spatial contexts and modular assembly of tissue components.

**Sizun Jiang, PhD**
Postdoctoral Fellow
Advisor: Garry Nolan, PhD
Department of Microbiology and Immunology

**Multiplexed Ion Beam Imaging of Nucleic Acids and Proteins Resolves the Immune-Pathogen Landscape of Viral Diseases**

Orchestrated immune events are fundamental to our understanding of disease progression, such as spatial features of host-disease interactions in situ. Here, we developed Multiplexed nucleic Acid and Protein Tissue Imaging (MAP-TI), a sensitive method for simultaneously quantifying the expression of DNA, RNA, and protein targets to interrogate the cellular composition, variability, and microenvironments in fixed tissues. We applied MAP-TI to measure >30 parameters simultaneously across large sections of archival lymphoid tissues in Simian Immunodeficiency Virus non-human primate (NHP) challenge studies, as well as Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in NHP models and human autopsies. These data provide a multi-modal framework to reveal insights into the higher-level spatial coordination during viral infections and can be readily applied towards diseases involving tumor viruses, copy amplifications, and non-coding RNAs.

**Erin McCaffrey**
Immunology Graduate Student
Advisor: Robert Angelo
Department of Pathology

**Multiplexed imaging of human tuberculosis granulomas uncovers immunoregulatory features conserved across tissue and blood**

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis that is distinctly characterized by granuloma formation within infected tissues. Granulomas are dynamic and organized immune cell aggregates that limit dissemination, but can also hinder bacterial clearance. Consequently, outcome in TB is influenced by how granuloma structure and composition shift the balance between these two functions. To date, our understanding of what factors drive granuloma function in humans is limited. With this in mind, we used Multiplexed Ion Beam Imaging by Time-of-Flight (MIBI-TOF) to profile 37 proteins in tissues from thirteen patients with active TB disease from the U.S. and South Africa. With this dataset, we constructed a comprehensive tissue atlas where the lineage, functional state, and spatial distribution of 19 unique cell subsets were mapped onto eight phenotypically-distinct granuloma microenvironments. This work revealed an immunosuppressed microenvironment specific to TB granulomas with spatially coordinated co-expression of IDO1 and PD-L1 by myeloid cells and proliferating regulatory T cells. Interestingly, this microenvironment lacked markers consistent with T-cell activation, supporting a myeloid-mediated mechanism of immune suppression. We observed similar trends in gene expression of immunoregulatory proteins in a confirmatory transcriptomic analysis of peripheral blood collected from over 1500 individuals with latent or active TB infection and healthy controls across 29 cohorts spanning 14 countries. Notably, PD-L1 gene expression was found to correlate with TB progression and treatment response, supporting its potential use as a blood-based biomarker. Taken together, this study serves as a

framework for leveraging independent cohorts and complementary methodologies to understand how local and systemic immune responses are linked in human health and disease.

**Felix Hartmann, PhD**
Postdoctoral Fellow
Advisor: Sean Bendall, PhD
Department of Pathology

**Single-cell metabolic profiling of human immune cells**

Hartmann, Felix J et al. “Single-cell metabolic profiling of human cytotoxic T cells.” *Nature biotechnology*, 10.1038/s41587-020-0651-8. 31 Aug. 2020, doi:10.1038/s41587-020-0651-8

Cellular metabolism regulates immune cell activation, differentiation and effector functions, but current metabolic approaches lack single-cell resolution and simultaneous characterization of cellular phenotype. In this study, we developed an approach to characterize the metabolic regulome of single cells together with their phenotypic identity. The method, termed single-cell metabolic regulome profiling (scMEP), quantifies proteins that regulate metabolic pathway activity using high-dimensional antibody-based technologies. We employed mass cytometry (cytometry by time of flight, CyTOF) to benchmark scMEP against bulk metabolic assays by reconstructing the metabolic remodeling of in vitro-activated naive and memory CD8+ T cells. We applied the approach to clinical samples and identified tissue-restricted, metabolically repressed cytotoxic T cells in human colorectal carcinoma. Combining our method with multiplexed ion beam imaging by time of flight (MIBI-TOF), we uncovered the spatial organization of metabolic programs in human tissues, which indicated exclusion of metabolically repressed immune cells from the tumor–immune boundary. Overall, our approach enables robust approximation of metabolic and functional states in individual cells.

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| 12:30 - 12:45 pm | **Jiaying Toh**, Khatri and Martinez Labs, *Pan-virus multi-cohort analysis of host immune response identifies immune correlates associated with the severity of viral infection* |
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**Aaron Wilk**
Immunology Graduate Student
Advisor: Catherine Blish, MD, PhD
Department of Medicine – Infectious Diseases

**Multimodal single-cell profiling of COVID-19 immunity reveals neutrophil activation and monocyte silence are associated with disease severity and outcome**

Many studies indicate that immune responses in COVID-19 are key determinants of disease severity and outcome, yet our ability to predict and prevent severe disease remains limited. To elucidate immunological pathways that lead to immunopathology or protective immunity in COVID-19, we analyzed peripheral immune cells of 72 COVID-19 patients across a broad spectrum of disease severity using three single-cell modalities: single-cell RNA sequencing, single-cell ATAC sequencing, and mass cytometry. We found that peripheral neutrophils in COVID-19 patients displayed a severity-specific increase in type I interferon signature, enhanced expression of pro-inflammatory cytokine-encoding genes, and chromatin regulator expression associated with activation and neutrophil extracellular trap (NET) forming capacity. Conversely, peripheral monocytes showed downregulation of HLA class II-encoding genes and decreased chromatin accessibility at pro-inflammatory cytokine-encoding gene loci. Additionally, while natural killer (NK) cells had disease severity-specific activation signatures, they failed to proliferate and upregulate cytotoxic effector molecules in fatal COVID-19 cases, suggesting that NK cell activity may be associated with disease outcome. Finally, we identified a population of immature neutrophils that appeared uniformly in patients who succumbed to infection, implying that emergency granulopoiesis is a feature of fatal COVID-19. Our results provide a multimodal single-cell atlas of peripheral immunity in COVID-19 and identify several immunological targets for therapeutic intervention.

**Jiaying Toh**
Immunology Graduate Student
Advisors: Olivia Martinez, PhD, Department of Surgery - Abdominal Transplantation
Purvesh Khatri, PhD, Department of Medicine (Biomedical Informatics) and of Biomedical Data Science

**Pan-virus multi-cohort analysis of host immune response identifies immune correlates associated with the severity of viral infection**

The current SARS-CoV-2 pandemic, which is the fourth pandemic of the decade, has highlighted existing gaps in global pandemic preparedness and the need for generalizable clinical diagnostics to avert overwhelming healthcare systems worldwide. These diagnostic tools should be applicable despite differences in patient age, sex, ethnicity, and viral pathogen, whether novel or re-emerging. We have previously described the Meta-Virus Signature (MVS), a gene signature representing a conserved host response against acute respiratory viral infections. Here, we integrated 4,780 blood transcriptome profiles from patients (<12 months to 73 years) with one of 16 viral infections across 34 independent cohorts from 18 countries, and scRNA-seq profiles of 264,000 immune cells from 71 samples across 3 independent cohorts to identify host response modules associated with severity of viral infection, irrespective of the infecting virus. Despite the biological, clinical, and technical heterogeneity across these cohorts, we found that the conserved host response to viral infection, represented by the MVS, is correlated with the severity of infection. Secondly, we identify monocytes as the primary source of this conserved host response. In addition, cases of mild and severe viral infections follow distinct trajectories, which are defined by genes indicating that immune responses from NK cells, suppression from myeloid cells, and hematopoiesis are associated with the severity of viral infection. Together, our findings provide insights into immune response dynamics during viral infection, and identify biological factors that may influence infection outcomes.

**Aditya Rao**
Immunology Graduate Student
Advisor: Purvesh Khatri, PhD
Department of Medicine (Biomedical Informatics) and of Biomedical Data Science

**Multi-cohort analysis of host immune response identifies conserved protective and detrimental modules that predict infection severity irrespective of viral pathogen**

The SARS-CoV-2 pandemic, which is the fourth pandemic of the decade, has underscored gaps in global pandemic preparedness and the need for generalizable tests to avoid overwhelming healthcare systems worldwide. These tests should be irrespective of age, sex, location, and the infecting virus. We integrated 4,780 blood transcriptome profiles from patients (<12 months to 73 years) with one of 16 viral infections across 34 independent cohorts from 18 countries, and scRNA-seq profiles of 264,000 immune cells from 71 samples across 3 independent cohorts. Despite the biological, clinical, and technical heterogeneity across these cohorts, we identified distinct clusters of patients with non-severe and severe viral infections. Furthermore, we were able to find four gene modules that represented distinct trajectories associated with non-severe and severe outcomes. Using these modules, we defined a diagnostic Severe or Mild (SoM) score that was able to distinguish non-severe from severe viral infection with clinically useful accuracy. Together, our findings offer crucial insights into the underlying immune dynamics of viral infection severity, and show promising results for a clinical tool which clinicians could use to more accurately triage patients.

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**Amy Fan**
Immunology Graduate Student
Advisor: Ravindra Majeti MD, PhD
Department of Hematology

**Cytokine Rescue of Inflammation-Sensitive RUNX1 Deficient Human CD34+ Hematopoietic Stem and Progenitor Cells**

Loss-of-function mutations in RUNX1 are commonly found in hematopoietic malignancies and confer particularly poor prognosis in acute myeloid leukemia (AML). However, it remains unclear how RUNX1 functions during hematopoietic and leukemic development. To evaluate the role of RUNX1 in hematopoiesis and characterize intrinsic and extrinsic factors involved in RUNX1 deficient clonal expansion and leukemic transformation, we disrupted the RUNX1 locus using CRISPR/Cas9 and AAV6-mediated homology directed repair in human CD34+ hematopoietic stem and progenitor cells (HSPCs). RUNX1 deficiency caused monocytic skew and proliferative defects in vitro, and defective competitive engraftment in immunodeficient NSG mice in vivo. RNA-seq and ATAC-seq revealed the broad upregulation of NFkB-mediated inflammatory programs and downregulation of E2F-dependent cell cycle programs. We next sought to determine which cytokines are sufficient to drive RUNX1 KO cell expansion. RUNX1 KO cells not only expanded preferentially in NSG mice expressing human SCF, GM-CSF, and IL-3 (NSGS mice), but also were no longer defective in competitive transplants in NSGS mice. Further, only treatment with IL-3 was sufficient to significantly expand RUNX1 KO cells in vitro. Flow cytometry revealed that CD123 is upregulated in RUNX1 KO cells compared to control. Ongoing efforts are aimed at determining whether targeting CD123 and IL-3 signaling may be a viable therapeutic in the prevention or treatment of RUNX1-mutant malignancies using primary AML samples. In summary, our findings reveal how RUNX1 mutations may initially behave in a deleterious manner but can ultimately confer an advantage to HSPCs under certain environmental conditions.

**Ariel Calderon**
Immunology Graduate Student
Advisor: Sean Bendall, PhD
Department of Pathology

**Characterization of Natural Killer Cell Hematopoietic Precursors in Human Hematopoiesis**

Natural Killer (NK) cells have been characterized as cytotoxic lymphocytes that are key in regulating viral infections and combatting cancer. In contrast to B and T cells, there has not been a detailed examination of the developmental trajectory of human NK cells. Conflicting reports in literature state that NK cells can develop from both lymphoid and myeloid cell progenitors. Here, we utilized mass cytometry (CyTOF) to perform a multiplexed analysis of NK cell development on healthy human bone marrow. To address the current gap in knowledge, we built a comprehensive CyTOF panel to include lymphoid markers, myeloid markers, and various regulatory factors shown to be important both in mouse and human NK cell development across 4 donor samples. We analyzed this data with multiple supervised and unsupervised single cell trajectory algorithms in order to model NK developmental processes. This approach allows for unbiased observations across developmental lineages and the reconciliation of the conflicting literature. The ability to simultaneously measure multiple cellular features, including phenotypic proteins, transcription factors, and regulatory enzymes allowed us to identify a novel potential NK lineage-restricted progenitor in adult human bone marrow. We have found potential CD34+ and CD34- NK progenitors with different combinations of surface markers previously used to identify NK progenitors and regulatory markers such as IRF8, NFIL3, ID2 and TdT. Our findings here show that NK cells may be generated through two distinct fate pathways. Our next step is to analyze the capacity of this novel progenitor subpopulation to generate mature and functional CD56+ NK cells in vitro, through co culture with the OP9 and OP9-DL4 cell lines. Ultimately, our studies will shed light on the knowledge of early stages of NK lineage commitment.

**Caleb Glassman**
Immunology Graduate Student
Advisor: Chris Garcia, PhD
Department of Molecular & Cellular Physiology and of Structural Biology

**Structural basis for IL-12 receptor assembly enables functional dissection of actions on T and NK cells**

Interleukin-12 (IL-12) is a heterodimeric cytokine produced by antigen-presenting cells to regulate cytotoxicity and IFNg production of T cells and NK cells. IL-12 signals through a receptor complex composed of IL-12Rb1, a receptor which is shared with IL-23, and IL-12Rb2, an IL-12 specific receptor. Using cryo-electron microscopy and crystallography, we identified the unique mechanism of IL-12 receptor assembly in which IL-12Rb1 directly engages the p40 subunit of IL-12 and IL-23. IL-12 plays a central role in immune responses to intracellular pathogens and cancer leading to interest in IL-12 as an immunotherapy, however, systemic administration of IL-12 results in toxicity due to robust induction of IFNg by NK cells. Using our structural insight into IL-12 receptor assembly, we designed a panel of IL-12 partial agonists that preserved CD8+ T cell IFNg induction and tumor cell killing but impaired cytokine production from NK cells due to differences in IL-12Rb1 expression on T and NK cell.

**Nora Vivanco Gonzalez**
Immunology Graduate Student
Advisors: Sean Bendall, PhD, Department of Pathology
Theo Palmer, PhD, Department of Neurosurgery

**Gestational Immune Profiling Reveals Specialized PD-L1 expressing Mononuclear Phagocytes at the Feto-Maternal Interface**

The immune system of a pregnant woman must be carefully modulated to induce and maintain tolerance of the semiallogeneic fetus while retaining the ability to fight off pathogens. Protecting the fetus from maternal immunological recognition and attack depends on the coordination of maternal, fetal and placental mechanisms. The extensive immunological adaptations during pregnancy have remained unknown due to the ethical and technical challenges of collecting tissues during human pregnancy. Using a mouse model, we uncovered basic tenants of the maternal immune system during pregnancy. Single-cell mass cytometry allowed us to evaluate the maternal innate and adaptive immune compartments at homeostasis. Maternal peripheral blood, decidua and placenta were collected starting at E10.5 and ending at E18.5. In addition, we used over 30 phenotypic and functional markers to assess the maternal immune status throughout gestation. We hypothesized that the maternal immune profile would change throughout gestation at the feto-maternal interface to accommodate tissue remodeling and sustain tolerogenic adaptations.

Our analyses demonstrate distinct distribution and phenotypes of the main immune cell subsets across organs and within endovascular structures in the placenta and decidua. Furthermore, in depth analysis of the mononuclear phagocyte (MP) compartment reveals specialized MP PD-L1+ subsets in the placenta and decidua. Phenotypic distinctions between MP PD-L1+ subsets are attributed to distinguishing CD64, Ly-6C and MHC-II levels. We identified highly abundant tissue remodeling MPs in mid gestation (E10.5), with immune suppressive MPs enriched in later gestation (E14.5). Our observations point to the establishment of vascular remodeling and fetal tolerance as the main driving forces behind MP specialization.

Together this resource gives us insight that can be used for mechanistic studies and targeted interventions that improve pregnancy outcome and probe immune tolerance in general. Moreover, this study is not only a resource for pregnancy research, but also a framework for comprehensive tissue-immune profiling by mass cytometry at the organism level.

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**Lawrence Bai**
Immunology Graduate Student
Advisors: Aida Habtezion, MD, MSc, Department of Gastroenterology & Hepatology
Purvesh Khatri, PhD, Department of Medicine (Biomedical Informatics) and of Biomedical Data Science

**Molecular and Clinical Data Integration Identifies an Association Between Decreased Colectomy Rates and Atorvastatin Exposure in Ulcerative Colitis Patients: A Retrospective Cohort Study**

Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract with limited effective therapeutic options for long-term treatment and disease maintenance. We hypothesized that multi-cohort analysis of independent cohorts, which represent real-world heterogeneity of UC patients, would identify a robust transcriptomic signature to improve repurposing of FDA-approved. We performed a multi-cohort analysis of transcriptome profiles of 272 colon biopsies across 11 publicly-available datasets to identify a robust UC disease gene signature. We compared the gene signature with in-vitro transcriptomic profiles induced by 781 FDA-approved drugs. We used a retrospective cohort study design modeled after a target trial to evaluate the protective effect of predicted drugs on colectomy risk in patients with UC from two independent cohorts, the Stanford Research Repository (STARR) database and Optum Clinformatics DataMart. The in vitro transcriptome profile of atorvastatin treatment had the highest inverse-correlation with the UC disease gene signature among any non-oncolytic FDA-approved therapy. In both the STARR cohort (n=827) and Optum cohort (n=7821), we found that atorvastatin use was significantly associated with a decreased risk of colectomy compared to patients who were prescribed a comparator drug (STARR: HR=0.47, p=0.03; Optum: HR=0.66, p=0.03). These findings suggest that atorvastatin might serve as a novel therapeutic option for ameliorating disease in patients with UC. Importantly, we provide a systematic framework for integrating publicly available heterogeneous molecular data with similarly heterogeneous clinical data at a large scale to repurpose existing FDA-approved drugs for a wide range of human diseases.

**Camille Brewer**
Immunology Graduate Student
Advisor: Bill Robinson
Department of Immunology and Rheumatology

**Rheumatoid Arthritis Autoantibodies Bind Oral Bacteria Detected in Blood Prior to Flare**

Periodontitis is an established risk factor for rheumatoid arthritis (RA), and ongoing periodontal inflammation is associated with increased RA disease activity. Nevertheless, the mechanistic link between periodontitis and RA pathogenesis remains unclear. In an analysis of finger stick RNA sequencing (RNAseq) that tracked RA flares over time, we discovered increased expression of oral bacterial genes in the blood prior to flare. Additionally, we observed increased antibody binding to supragingival bacteria during flare when compared to baseline. Thus, we explored the immune responses in RA to these oral bacteria. We found that recombinant monoclonal antibodies derived from RA blood plasmablasts (rmAbs) cross-bind citrullinated human and bacterial antigens. Using mass spectrometry, we identified in situ citrullinated oral bacterial antigens in periodontitis saliva. We demonstrate that multiple RA plasmablast rmAbs cross-bind an abundant oral in situ citrullinated bacterial antigen and citrullinated human antigens present in RA synovium. We also identified enrichment of mucosal immune response signatures in RA synovium suggesting a microbial-driven immune response may directly contribute to ongoing synovial inflammation. These observations suggest a model wherein periodontitis leads to citrullination of oral flora and repeated breaches in the periodontal mucosal barrier, which together result in repeated subclinical episodes of bacteremia that activate ACPA B cells. In turn, the activated ACPA B cells contribute to bouts of joint inflammation that manifest as clinical flares in RA.

**Kartik Bhamidipati**
Immunology Graduate Student
Advisor: Bill Robinson
Department of Immunology & Rheumatology

**CD52 is Elevated on B cells of SLE Patients and Regulates B Cell Function**

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease characterized by B cell dysregulation and breaks in tolerance that lead to the production of pathogenic autoantibodies. We performed single-cell RNA sequencing of B cells from SLE patients and healthy control patients and identified CD52, a small glycoprotein, as upregulated in SLE patients. SLE patients had significantly higher B cell surface expression and soluble plasma levels of CD52, which positively correlated with measures of lupus disease activity. Using JeKo-1 CD52 knockout cells, we showed that cells lacking surface CD52 expression are hyper-responsive to B cell receptor (BCR) signaling, suggesting an inhibitory role for the surface-bound protein. In human B cells, antigen-specific BCR-activation initiated CD52 cleavage in a phospholipase C dependent manner, significantly reducing cell surface levels. Experiments with recombinant CD52-Fc showed that soluble CD52 inhibits BCR signaling via Siglec-10. Moreover, incubation of unstimulated B cells with CD52-Fc resulted in the reduction of surface immunoglobulin and CXCR5. Prolonged incubation of B cells with CD52 resulted in the expansion of IgD+IgMlo anergic B cells. In summary, our findings suggest that CD52 is a homeostatic protein that inhibits responses to BCR signaling, can be cleaved upon antigen engagement, and suppresses B cell function in an autocrine and paracrine manner. Moreover, we propose that the elevation of CD52 in SLE patients is a homeostatic mechanism to suppress B cell hyperactivity.

**Yoni Rubin, PhD**
Postdoctoral Fellow
Advisor: Aida Habtezion, MD, MSc
Department of Gastroenterology & Hepatology

**Effects of processing conditions on stability of immune analytes in human blood**

Gottfried-Blackmore, Andres et al. “Effects of processing conditions on stability of immune analytes in human blood.” *Scientific reports* vol. 10,1 17328. 15 Oct. 2020, doi:10.1038/s41598-020-74274-8

Minimizing variability in collection and processing of human blood samples for research remains a challenge. Delaying plasma or serum isolation after phlebotomy (processing delay) can cause perturbations of numerous analytes. Thus, a comprehensive understanding of how processing delay affects major endpoints used in human immunology research is necessary. Therefore, we studied how processing delay affects commonly measured cytokines and immune cell populations. We hypothesized that short-term time delays inherent to human research in serum and plasma processing impact commonly studied immunological analytes. Blood from healthy donors was subjected to processing delays commonly encountered in sample collection, and then assayed by 62-plex Luminex panel, 40-parameter mass cytometry panel, and 540,000 transcript expression microarray. Variance for immunological analytes was estimated using each individual baseline as a control. In general, short-term processing delay led to small changes in plasma and serum cytokines (range -10.8 to 43.5%), markers and frequencies of peripheral blood mononuclear cell phenotypes (range 0.19 to 3.54 fold), and whole blood gene expression (stable for >20K genes) – with several exceptions described herein. Importantly, we built an open-access web application allowing investigators to estimate the degree of variance expected from processing delay for measurements of interest based on the data reported here.

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## POSTER SESSION
## STANFORD IMMUNOLOGY VIRTUAL CONFERENCE
**Friday, November 6, 2020 at 1:15 – 2:00 PM**

Via Gather Town: https://gather.town/app/6HLfbd2zNR5dAbjW/StanfordImmunology

1. **Pui Yan Ho**, Czechowicz Lab, *Development of Lentiviral Gene Therapy in Combination with Anti-c-Kit Antibody Conditioning for Treatment of X-linked Severe Combined Immunodeficiency*
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**Pui Yan Ho, PhD**
Postdoctoral Fellow
Advisor: Agnieszka Czechowicz, MD
Department of Pediatrics - Stem Cell Transplantation

**Development of Lentiviral Gene Therapy in Combination with Anti-c-Kit Antibody Conditioning for Treatment of X-linked Severe Combined Immunodeficiency**

Hematopoietic stem cells (HSCs) have the proven ability to cure blood and immune diseases. However, their use within allogeneic hematopoietic cell transplantation (allo-HCT) can have severe side effects including immune-mediated graft attack, infections caused by medications used to limit immunologic graft rejection, and toxicities from the conditioning methods used to enable successful HSC engraftment. This prevents its expansion to all patients that could benefit from such treatment. Genetic correction of autologous HSCs is a potent alternative that overcomes many allo-HCT toxicities, although current strategies are inefficient with significant potential off-target effects. Additionally, autologous HCT still requires classic chemotherapy/irradiation conditioning to allow effective HSC engraftment. Lentiviral gene editors that permit robust and efficient editing and monoclonal antibody-based conditioning strategies that enable toxicity-free HCT may overcome these hurdles. Here we propose to fully develop such agents for autologous HSC-modification and test them both individually and in combination. As proof-of-concept we will study correction of single nucleotide mutations identified in the IL2RG gene of SCID-X1 patients and subsequently assess immune reconstitution in both in vitro and in vivo models. Ultimately these findings may be applicable for the safe and effective treatment of all genetic blood and immune diseases.

**YeEun Kim**
Immunology Graduate Student
Advisors: Sean Bendall, PhD, Department of Pathology
William Greenleaf, PhD, Department of Genetics

**A Highly Multiplexed Single Cell Proteomic Screen Reveals the Phenotypic and Functional Landscape of the Human Lympho-Myeloid Differentiation Axis**

While single-cell sequencing techniques have elucidated transcriptomic and epigenetic heterogeneities among human hematopoietic stem and progenitor cell (HSPC) populations, the corresponding proteomic level information, where the action of these regulatory networks manifests, is still missing. As cell sorting relies on surface markers, the functional capabilities and lineage specificities of HSPCs can only be evaluated after interrogation of the proteome at a single cell resolution. To that end, we quantified the simultaneous expression of 353 surface molecules and 79 functional intracellular molecules - TFs, chromatin regulators, metabolic enzymes - with mass cytometry, or CyTOF. We analyzed 556,226 CD34+ bone marrow HSPCs across 3 individuals and identified 81 molecules expressed by HSPCs, with heterogeneous expression among conventionally-defined HSPC cell types. We further analyzed human bone marrow mononuclear cells with markers identified from the screen and discovered a subpopulation of GMP (Granulocyte-Monocyte Progenitor) with high lymphoid marker expressions, as well as lymphoid primed epigenetic landscape. Overall, we supply a quantified summary of the proteomes of human HSPCs and create a framework to identify and characterize progenitor populations with unique functional states along the lympho-myeloid developmental process.

**Claudia Macaubas**
Research Scientist
Advisor: Elizabeth Mellins, MD
Department of Pediatrics - Human Gene Therapy

**Extracellular Vesicles in Systemic Juvenile Idiopathic Arthritis**

Claudia Macaubas, Justine Maller, Terry Morgan, Mayu Morita, Yunshin Jung, Katrin J. Svensson, Elizabeth Mellins

Background: Systemic juvenile idiopathic arthritis (sJIA) is a chronic pediatric inflammatory disease, characterized by fever, rash, hepatosplenomegaly, serositis (i.e., pericarditis, pleuritis) together with arthritis, which can be erosive. The mechanisms driving sJIA are incompletely characterized. We hypothesized that intracellular communication, mediated by extracellular vesicles (EVs), contributes to sJIA pathogenesis and predicted that the number and cellular sources of EVs would differ between inactive and active states of sJIA and relative to healthy controls.

Methods: We evaluated plasma from healthy pediatric controls and sJIA patients with active systemic flare or inactive disease. We isolated EVs by size-exclusion chromatography and determined total EV abundance and size distribution using microfluidic resistive pulse sensing. Cell-specific EV subpopulations were measured by nanoscale flow cytometry.

Results: Total EV concentration did not significantly differ between controls and sJIA patients. EVs with diameters <200 nm were the most abundant, and the majority of cell-specific EV subpopulations were within this size range. Relative to controls, sJIA patients had significantly higher levels of EVs from platelets, monocytes, myeloid and endothelial cells. Differences were most pronounced in EV populations from activated platelets, intermediate monocytes, and chronically activated endothelial cells, with the latter two significantly more elevated in sJIA flare relative to inactive disease and controls.

Conclusions: The observation of elevated EVs from specific cellular sources and activation states suggests that EVs play a role in modulating immune activity in sJIA. Our findings indicate that multiple cell types contribute to altered circulating EV profiles in sJIA. The EV differences between sJIA disease states and healthy controls implicate EV-mediated cellular crosstalk as a potential driver of sJIA disease activity.

**Hunter Martinez**
Immunology Graduate Student
Advisor: Paul Bollyky, MD, PhD
Department of Infectious Diseases and of Microbiology and Immunology

**CD44 standard isoform inhibits IL-2 mediated proliferation**

T lymphocyte activation involves antigen recognition through a T cell receptor, the engagement of co-stimulatory receptors and production of cytokines such as IL-2. IL-2 is a pleotropic cytokine which signals through the trimeric IL-2 receptor complex leading to proliferation and differentiation. During T cell proliferation, the receptor CD44 is upregulated. CD44 is primarily envisioned as a receptor

involved in T lymphocyte trafficking through interactions with hyaluronan, a ubiquitous component of the extracellular matrix. However, CD44 has other roles in addition to trafficking. Herein, we observe the standard isoform of CD44 negatively influences proliferation of the IL-2 dependent T cell line, CTLL-2. CD44 exerts the influence on proliferation through an IL-2-pSTAT5 independent pathway. Furthermore, using an inducible system to control CD44 expression, strong induction of CD44 results in CTLL-2 death through an unknown mechanism. These observations suggest CD44 may limit a T cell response to IL-2 either through inhibition of an IL-2 mediated growth pathway or a cell death mechanism.

**Katherine Murphy**
Immunology Graduate Student
Advisor: Crystal Mackall, MD
Department of Pediatrics - Hematology/Oncology

**Genome-wide CRISPR screen reveals negative regulators of CAR-T cell anti-tumor activity**

CAR-T cell therapies have demonstrated remarkable efficacy in the treatment of hematological cancers, but still many patients do not respond to treatment. Recent advances in CRISPR gene editing technology have made it possible to genetically modify T cell products for use in humans. It is likely that some genes expressed in T cells diminish anti-tumor activity and genetic disruption of such genes could result in superior therapeutic effects. To determine which genes limit CAR-T cell anti-tumor activity, we conducted a genome-wide CRISPR knock-out screen in human primary CAR-T cells. We identified several candidate genes which negatively influence proliferation and cytokine production. Targeted deletion of these genes resulted in CAR-T cells with increased expansion, cytokine production, and in vivo tumor clearance. These findings may provide insights into which pathways influence CAR-T cell activity in humans.

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

**Multi-regional analysis of T cell responses in acquired resistance to anti-PD-1 therapy in lung cancer**

While the treatment of non-small cell lung cancer (NSCLC) with immune checkpoint inhibitors such as anti-PD-1 antibodies has demonstrated remarkable efficacy in a subset of patients, response to these therapies not only varies between patients but also in each individual, as differential responses even within the same tumor have been observed. This regional heterogeneity in responses is likely driven by a number of immune and tumor-intrinsic factors, chief among them perhaps being antigen-specific T cell dynamics. Here, we examine the spatial distribution of the T cell repertoire by performing paired single-cell RNA- and T cell receptor (TCR)- sequencing on 8 regions within the same tumor for 3 NSCLC patients treated with anti-PD-1 therapy. This multi-regional sampling approach revealed that while the majority of TCR clones are specific to one region, highly expanded clones are ubiquitous throughout all tumor regions. Moreover, these ubiquitous clones are enriched in effector T cell phenotypes and are more likely to be found circulating in blood than regional clones. Additionally, correlation of T cell phenotypes with pathological features of each tumor site revealed that regions

containing viable tumor are enriched for exhausted CD8+ and follicular helper T cell clones in comparison to nontumor lung regions. These results suggest that the spatial localization of the intratumoral T cell repertoire may shape regional responses to immunotherapy.

**Medeea Popescu**
Immunology Graduate Student
Advisor: Paul Bollyky, MD, PhD
Department of Infectious Diseases and of Microbiology and Immunology

**A bacteriophage modulates neutrophil migration and function in response to bacterial infection**

Pseudomonas aeruginosa (Pa) is a major human pathogen, particularly effective at colonizing the airways of cystic fibrosis (CF) patients. The immune response of these patients to Pa infection is characterized by excessive neutrophil, monocyte, and macrophage influx, altered cytokine secretion, and impaired myeloid cell effector function. We have previously shown that Pf4, a temperate filamentous bacteriophage produced by Pa, contributes to the local immune dysregulation associated with Pa infection. We report now that human macrophages incubated with Pf exhibit significant downregulation of the potent neutrophil chemoattractant CXCL5 in response to LPS. Furthermore, conditioned media from Pf and LPS-treated cells are significantly less effective at inducing neutrophil migration in vitro than media from LPS-treated cells. In a Pseudomonas acute pneumonia model, mice infected with Pf-overproducing Pa show significantly less neutrophil infiltration in BAL fluid than mice infected with low-Pf-producing Pa. Correspondingly, CF patients colonized with Pf-infected Pa have significantly lower levels of CXCL5 in sputum, indicating that Pf may play a role in the innate immune response to Pa in these patients.

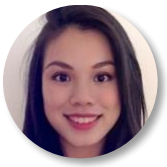
**Aditya Rao**
Immunology Graduate Student
Advisor: Purvesh Khatri, PhD
Department of Medicine (Biomedical Informatics) and of Biomedical Data Science

**A novel host transcript signature for distinguishing bacterial and viral infections**

Anti-microbial resistance is one of the biggest issues facing humanity today. A major contributing factor to anti-microbial resistance is inappropriate prescription of antibiotics due to inaccurate diagnosis of the type of infection. Existing pathogen-based diagnostics are insufficient to prevent the over-prescription of antibiotics due to several limitations. A growing body of work from us and others has shown that host-based gene expression is a sensitive and specific biomarker for diagnosis of the presence and type of infection.

Using 3,731 samples across 63 datasets, we show that host response-based diagnostic signatures identified using only samples from the US or Western Europe do not distinguish intracellular bacterial infections from viral infections with clinically useful accuracy, and are not generalizable to regions where intracellular bacterial infections are more prevalent. We hypothesized that including intracellular bacterial infections would identify a generalizable host signature for distinguishing bacterial and viral infections. Using a novel statistical framework, we integrated 4,223 samples from 20 countries across 69 independent datasets that represented biological, clinical, and technical

heterogeneity observed in the real-world patient population. We identified an 8-gene Bacterial-or-Viral Infection (BoVI) signature that accurately distinguished extracellular and intracellular bacterial infections from viral infections. We validated the accuracy of this signature in two prospective cohorts from two countries using RT-PCR. Importantly, the BoVI signature had 90% sensitivity and 89% specificity in prospective cohorts, which met the target product profile described by the WHO and FIND for a diagnostic test to reduce antibiotic over-prescription, supporting further validation of BoVI in larger cohorts.



**Erin Soon**
Immunology Graduate Student
Advisor: Robert Angelo
Department of Pathology

**Decidual Immune Microenvironment Dynamics Across Healthy Gestation Revealed By Multiplexed Ion Beam Imaging**

Dynamic and tightly regulated interactions between the fetally derived placenta and maternal decidua underpin the establishment of healthy pregnancy. Invasion of specialized fetal cells, or trophoblasts, from the placenta into the decidua and maternal blood vessels, is crucial for healthy placentation. This involves controlled downregulation of the maternal immune system in a manner that does not cause rejection of the fetus. This delicate balance of finely orchestrated immune processes within the complex spatial architecture of the decidua and placenta lends itself to a unique biological problem. Here, we use Multiplexed Ion Beam Imaging (MIBI), which captures high dimensional single cell proteomic data while retaining the positional information of the tissue structure and individual cells, to examine samples from electively terminated pregnancies from 80 patients across weeks 6-20 of gestation. We use an immune-centric panel to comprehensively profile the composition of maternal and fetal cells, and find that these cells cluster into cellular “neighborhoods” or microenvironments, each characterized by unique cell lineage combinations. We observe that certain cell types express differing levels of functional markers, depending on the immune composition of the microenvironment they are present in.

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