**Inflammation, Infection, Immunity, Immunotherapy (I-4ward) Task Force-2023 Updates**

**Introduction**: The I-4ward research portfolio includes programs studying the microbes that promote health and cause disease, the immune system that both protects us from infection and causes damaging inflammatory processes in tissues and immune-based medicines and therapies. The long-term goal of the I-4ward priority research pillar is to integrate the expertise and resources at UAB to build unified, cutting-edge interdisciplinary basic and clinical programs that are focused on understanding the fundamental rules that govern inflammatory and infectious processes and translating that basic knowledge into new therapeutics that can be used by clinicians to prevent and treat patients suffering from immune, inflammatory and infection mediated-diseases. The I-4ward Theme membership includes >300 faculty. The progress report was prepared by the I-4ward strategic Task Force members: Andre Ballesteros-Tato, Khurram Bashir, Mark Banaszak-Holl, David Kimberlin, Frances Lund, Carlos Orihuela, and Rakesh Patel.

**Immediate Goals for the I-4ward Task Force**: Following the establishment of I-4ward as one of four high-priority research focus areas in the HSOM, the I-4ward Task Force identified our constituents and developed and deployed a survey to engage with our constituents. We evaluated the data to define the immediate and long-term needs of the 300+ I-4ward researchers – who are members of 39 departments and more than 20 centers/institutes that are spread across 9 schools within UAB. From the survey results, we identified immediate needs that were articulated by many of the survey respondents. These included: access to current cutting-edge technology in cores with highly trained staff; expertise in basic bioinformatic "analyses" so that first-pass "omics" data can be evaluated for quality and outcomes; informatics expertise that is tailored to I-4ward research; and an I-4ward "service center" to connect researchers to collaborators, expertise, samples, models systems and patient cohorts. From these needs, the Task Force defined 3 immediate Priority Areas for investment. Progress in Priority Areas 1 and 2 are highlighted below and future plans to advance Priority Area 3 are described.

**Priority 1: Technology for I-4ward Researchers**. Our immediate priority is to provide our researchers with access to cutting-edge technologies that have the potential to drive transformational advancements in our understanding of the mediators of immune, inflammatory, and infectious diseases. The I-4ward Task Force recommended investments in technologies focused on integrated single-cell imaging. With that in mind, the Task Force suggested increasing our capabilities to perform multi-omic analyses in *tissues* with **single-cell spatial resolution**. With matching investments by the HSOM Immunology Institute (II) and the O'Neal Comprehensive Cancer Center (O'CCC), I-4ward purchased the **Lunaphore COMET multiplex imaging system, high-performance computer workstations, and access to Visiopharm software**. The COMET can be used to stain fixed or fresh tissue sections with up to 40 antibodies in a single day. The Viopharm software, which uses deep learning-based algorithms to define tissue and cellular structures, can then be used to analyze the images. The COMET multiplex immunofluorescence staining platform was purchased in the Spring of 2023 and arrived in early summer. It is located and managed by the UAB Flow Cytometry and Single-Cell Core (FCSCC), which is a member of the UAB Institutional Research Core Program (IRCP). Working together, Drs. Julie Carstens (Asst. Professor in Hematology and Oncology), Harish Pal (FCSCC Scientist), Aaron Silva-Sanchez (Instructor in Clinical Immunology and Rheumatology), and Jeremy Foote (Assoc. Professor in Microbiology), have overseen the installation of the COMET and the optimization/validation of an initial set of antibody panels for staining tissue arrays. At this time 56 antibodies that are specific for either human (28 antibodies) or mouse (28 antibodies) proteins that cover the main immune cell types and tissue defining markers have been validated for specificity and reproducibility on tissue arrays from healthy and diseased mouse and human tissues. The Visiopharm software seats are now at UAB and training for early adopters is complete. Training sessions were recorded and posted on a UAB Kalutra channel. In collaboration with Research Computing, Azure virtual computing systems have been built and are running the analysis suite. Work is ongoing to support user access to Long Term Storage solutions for the very large datasets. Panel design has been discussed with 16 investigators and at least 10 grants have been submitted or will be submitted this cycle listing this service. The first 2 investigators submitted samples and are generating data. Ongoing work includes fine-tuning antibody stains, expanding the antibody bank, and finalizing pricing, billing, and sample workflow. Within the next three months, the COMET technology platform and associated software will be advertised in the UAB Research Matters and HSOM weekly newsletters as well as through flier distribution to

Center and Institute list-serves. Potential users of the technology and software will be able to sign up on the FCSCC website for a consultation session to design the experiment and then will be able to book time on the instrument through the FCSCC web-based software calendar.

To complement the single-cell resolution spatial proteomics capabilities of the COMET, I-4ward championed investment in the **10X-Genomics Xenium single-cell resolution spatial transcriptomics platform**. This platform allows for true single cell resolution of gene transcripts in cells found in fresh or fixed tissue sections. Excitingly, the COMET and Xenium platforms can be linked to use the *same* tissue sections for both proteomic and transcriptomic spatial profiling. In conjunction with the Disruptive Technology to Empower Precision Health (D-TECH) and Brain Health and Disease Across the Lifespan Task Forces, I-4ward submitted an HSF-GEF grant application for the Xenium platform and associated informatics specialists (see Priority Area 2). We raised >$600,000 in matching funds from 11 Departments and Divisions and 14 Institutes and Centers. Awards for the HSF-GEF application will be announced at the end of October.

*Next Year Goals for Priority Area 1* include(1) Full rollout of COMET technology to interested researchers; (2) installation, optimization and rollout of Xenium technology in the FCSCC; (3) development of a methodology to integrate COMET and Xenium omics on the same tissue sample; and (4) investment in other omics platforms that will support cell spatial multi-omics https://www.nature.com/articles/s41576-023-00580-2 and single cell metabolomics.

**Priority 2: Strategic Hires to Bolster, Expand, and Build I-4ward Relevant Research**. Although the I-4ward investments in single cell omics platforms are critical for advancing our understanding of inflammatory, infectious, and immune-mediated diseases, we are still limited by our ability to analyze and integrate the datasets in a true multi-omic fashion. This requires hiring individuals with specialized training in building pipelines for transforming all types of single cell omics datasets into formats that can be easily interrogated by researchers who may not possess the computational skills needed for these analyses. In addition, informatics experts who can develop the platforms that will allow integration of the spatial proteomic and transcriptomic data sets are needed. To address this need, I-4ward has partnered with D-TECH and the Brain Health and Disease groups to **invest in an informatics group that will *specialize* in single cell datasets**. This group will be located within the UAB Biologic Data Science Core (BDSC), which is a member of the UAB IRCP. We expect to hire at least two full-time informaticists – one for an early 2024 start date and one in the second half of 2024.

*Next Year's Goals for Priority Area 2* include: (1) hire two informatics staff members and embed them in BDSC; (2) staff members will build pipelines and shiny apps to allow investigators to interrogate their single cell data sets; (3) staff members will design a multi-omic platform to integrate different types of single cell data sets; and (4) a single cell consulting service will be established. The service, which will include a team of single cell BDSC-associated informatics specialists and the FCSCC scientists, will meet with researchers to assist with experimental design and proposed analysis of these multi-omics datasets.

**Priority 3: Service Centers to Develop Resources for Bench, Translational and Clinical Researchers**. *Next Year's Goals for Priority Area 3* include the following. We plan to complement the single cell and associated informatics platforms with a service center that will help bench, translational, and clinical researchers link their biologic data sets to the clinical data associated with the tissue samples. As one part of this, I-4ward will partner with the UAB Health Equity Task Force to provide resources to deploy the Learning Health System Platform that is being developed by faculty and staff from COERE, RISC, CCTS, and the Informatics Institute. This platform will allow researchers to define data bundles of clinical information that can be queried for cohorts of interest on demand. In the coming year, I-4ward will provide resources and expertise to build and validate I-4ward-centric clinical data bundles. A second goal is for I-4ward to invest in infrastructure that will allow researchers to more easily navigate and mine existing data repositories (particularly published data). A third goal is to develop a platform to link I-4ward scientists with UAB clinicians who can identify patient cohorts and patient repository samples for studies.

**Summary:** I-4ward has partnered with multiple groups across UAB, including the other HSOM strategic research Task Forces, to build infrastructure that will support the basic, translational, and clinical research programs of our constituents. We will evaluate the impact of the investments that we've already made and are actively examining

financial models to ensure that these new initiatives can be sustained over time. Finally, we will ask our constituents to help identify and prioritize future infrastructure investments.