



Backstory

Unifying researchers and clinicians to eliminate *ex vivo* artifacts in human immune system studies

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Standardization of techniques and measurements is notoriously difficult in large-scale multi-center studies, particularly when including single-cell technologies and proteomics from clinical samples. Individual labs and researchers often have their own preferred method, and while this works adequately for some aspects of research, it does not always easily replicate or translate to clinical applications. Basic methods may need to be re-worked to be fit for purpose for studies of common disease mechanisms or biomarker research, causing a bottleneck in translation of new laboratory findings to validation cohorts for the development of diagnostics or treatments. Blood samples are an obvious source for detecting changes in the immune system; however, a fundamental issue is background variation in samples due to processing methods, which can disguise changes related to disease states. In a recently published paper, in *iScience* by Savage et al. ([https://www.cell.com/iscience/fulltext/S2589-0042\(21\)00372-2](https://www.cell.com/iscience/fulltext/S2589-0042(21)00372-2)), researchers and collaborators from the Allen Institute for Immunology tackled the issue of blood samples changing over time after being drawn and how to future-proof large-scale immunology research to overcome sample variability. This is the first in a planned series of similar works to find true biological windows into the human immune system.

The Allen Institute for Immunology was launched in December of 2018 with a 125-million-dollar philanthropic gift from the late [Paul G. Allen](#), the co-founder of Microsoft and the founder of the Allen Institute. Mr. Allen's vision for the institute was to focus the emerging advances in single-cell, proteomic, and epigenetics technologies to focus on unprecedented deep immune profiling of the human immune system to better understand both immune health and disease. Mr. Allen's challenge to us all was to go beyond

Figure 1. Members of the Allen Institute for Immunology, Clockwise from top left: Troy Torgerson (Director, Experimental Immunology), Tom Bumol (Executive Vice President and Director), Peter Skene (Director, Molecular Biology and Biochemistry), Veronica Hernandez (Immunology), June Kim (Molecular Biology and Biochemistry), Morgan Weiss (Molecular Biology and Biochemistry), Charles Roll (Immunology).



the descriptive work and seek mechanistic insights that can directly impact translational research in humans. Those of us at the Allen Institute hold that challenge as our north star—a key metric for our research and thank Mr. Allen for his vision and leadership.

As we built a set of clinical and basic research collaborators across many geographies, our internal challenge was to unify both the sample collection and sample preparation across our collective group of investigators. Each established lab or clinic had very specific procedures and successful histories of their “process.” We reasoned that it would be important to “experimentally” unify our approach with data to ensure our overall work in studying human blood and tissues would represent “ground truth” about the human immune system in health and disease in all of its natural heterogeneity in human populations. That was the rationale behind this first paper—convince all the collaborative investigators to standardize their approach so we could compare results from eight cohorts run through the same pipeline to meet our research objectives together.

MOTIVATION

What was the motivation to launch the foundational standards program, and what are the main challenges facing this initiative?

The immune system, by its very nature, is a sentinel system constantly responding to our environment and insults to homeostasis including infectious agents. We assumed that this system does not stop its function *ex vivo* at the outset and the sensitivity of new high dimensional assays needed to be evaluated for artefacts compared to the clearest picture of physiological ground truth. As our initial set of collaborations in human cohorts aimed to study cancer, autoimmunity and in parallel healthy human subjects, it was critical that we standardized how blood and tissue samples were procured and processed at different geographic sites, as site standard operating procedures differed dramatically. We knew that we would run the core pipeline at the Allen Institute, but we needed absolute certainty on the nature of the samples as we compared normal immune variance with disease-associated variance in longitudinal studies in the future. At the core, we needed to understand what legitimate “noise” from this variable sentinel system as part of immune homeostasis versus disease and/or therapy induced “signals” to make the research advances we seek.

What do you think are the key factors that stimulate interdisciplinary research, and how do you apply those in your institute?

Our strategy, based on experiences in other organizations, was to bring together experts in both basic and clinical human immunology. We also wanted to wed the many medical disciplines that study human immunology including oncology, rheumatology, and infectious disease along with scientists willing to help define human immune health. We believed that varied perspectives would be important to discern true advances in human immunobiology.

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To date, the Allen Institute for Immunology is working with five major academic institutions covering eight cohorts of patients or volunteers. On the clinical side, we have rheumatologists and gastroenterologists working in autoimmunity, oncologists working in melanoma and multiple myeloma, and infectious disease experts in our SARS-CoV-2 research as part of the larger research network all bringing important perspectives from their disciplines. On the basic research side, we have immunologists, molecular biologists, biochemists, and computational biologists who are experts in basic research but also familiar with the many challenges of high content multi-omic data to assess data quality, data analysis and integration, and data visualization. Finally, we made the commitment to put all these data into an interactive cloud-based scientific computing environment for all of us to work together. It is termed the Human Immune System Explorer or HISE for short.

We feel that this broad commitment for the deep immune profiling pipeline, common computational analyses, data integration, and a computational cloud-based collaboration environment will bring this group together in unique synergistic ways.

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LANGUAGE

Did this project require tailoring your research methods and language to adjust to working with researchers from different backgrounds (e.g. clinical vs. basic)?

Yes, an important question. While we did standardize sample collection and we are centrally studying the core deep immune profiling pipeline the same way every day with key batch controls, all of our partners are doing research at their academic sites which we will intercalate into HISE so that all partners will get a chance to explore these findings. We believe that the integration of multiple data streams will be critical for insights. Internally, we have dubbed this as a three-step process termed DVT, for discovery-validate-translate, expanding the initial data into strong testable hypotheses in validation research while keeping a view to the healthy volunteer or patient. This should allow us to better understand the immune system in health (defining immune health) and disease where we are currently studying two cancers, two autoimmune diseases, and SARS-CoV-2 infections.

We believe these common core methodologies will allow cross-over research across varied disciplines while advancing goals of specific programs.

PUBLICATION

What are the challenges during publication of this or any such research?

The key is coordinated data sciences across data quality, data integrity, data analysis, and data visualization to really drive important research findings. While it is always a bit easier to anchor key research in what we know, we are interested in going beyond the obvious and want to take full advantage of these deep and rich data sets. We would like to build a reputation for attempting to have the highest quality and most reproducible human immune profiling possible to share immediately with our collective research alliance partners and then the broader scientific community to mine and advance the findings. Given the costs of these technologies, Mr. Allen's vision and his accompanying philanthropy allows us to set this standard.

FUTURE

What are the future outlooks of interdisciplinary research at your institution?

Interdisciplinarity is our core strategy going forward. We firmly believe that the integration of clinical test data, clinical metadata, and 21st century single-cell analyses will be a productive strategy to redefine complex biology. Importantly, we have designed our deep immune profiling pipeline to be adapted to any human physiological or disease condition where routine blood draws can be collected in longitudinal research. In 2020, we were able to strategically pivot to study SARS-CoV-2 infection without any modifications to our protocols while adding disease-specific research, in this case, COVID-19-specific immune target research. We think our approach to integrate multiple streams of data will be of great interest to understanding other important human disease and immune health questions in the future.

FINAL THOUGHTS

It has been a challenge thus far to unify the data quality, data analysis, and data visualization strategies. Established research groups are familiar with the tools they use on a regular basis, and we are attempting to unify the approach. This has required training programs in using the HISE environment and demonstrations of how this could work going forward. Asking great scientists to do their work "differently" has clearly required demonstration projects to see the future advantages.

We believe this "less glamorous" work will also help similar studies to avoid sample processing artifacts in the future, facilitating similar multidimensional research studies of the human immune system.

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