RAPID-FIRE
Q&A

Let's talk CyTOF with

MARCELO SZTEIN, MD

STANDARD BIOTOOLS™

Marcelo Sztein, MD, Associate Director for Basic and Translational Research at the University of Maryland Center for Vaccine Development and Global Health and Professor in the Departments of Pediatrics, Medicine and Microbiology and Immunology, provides his insightful perspective on the use of CyTOF® flow cytometry in translational research.

Why CyTOF

I have been in the flow cytometry field since the early 80s, when we started with two-color flow cytometry, moving up in colors until we reached 14 or 15 using conventional flow cytometry a few years ago.

I work in translational research in the immunology, infectious disease and vaccine fields, and I had a great need to increase the number of parameters that I could study per cell. CyTOF provided a golden opportunity to be able to work with very few cells, typically all that can be obtained from human specimens, and extract a lot of information from that limited number of cells to contribute to advance the vaccine and infectious disease fields.

Over time, our research has expanded to the use of new sample types like human gut biopsies in addition to peripheral blood mononuclear cells, which yields even more limited cell numbers. Since we use these samples regularly in our research, CyTOF has played a critical role in many projects over the past decade.

Getting more from limited samples

We usually work with very limited amounts of specimens. Thus, we try to get as much information as possible from these limited numbers of cells to evaluate not only phenotypic characteristics but also those associated with function and homing, among others. This is particularly important in our studies because of the high impact of the research derived from studying human specimens. If we increase the number of parameters that we can measure in every cell, that will help us dissect the complex immunological phenomena associated with, in my case, development of vaccines and host immunity following exposure to wild type infectious organisms. We are currently able to measure 50-plus parameters per cell.

Before CyTOF

Before I was able to use CyTOF, I was very limited in the amount of information I could get on an individual cell basis. CyTOF really allows me to go way beyond what I could do before adopting this technology.

CyTOF in unique applications

In recent years, one of the main applications that CyTOF has been instrumental in is to study epigenetics using EpiTOF. This has a definitive advantage over other technologies like studying the methylome or doing ATAC-seq, single-cell ATAC-seq or other technologies to study

epigenetics. Those are molecular biology and sequencing approaches, and they are very, very expensive. Thus, these measurements are typically performed in only a very limited number of cells.

The remarkable advantage of EpiTOF is that it makes it possible to look at multiple epigenetic marks at the same time that information is collected regarding cell phenotypes and activation status in millions of individual cells, including methylation, acetylation and other epigenetic modifications.

For example, we have demonstrated that exposure to *Salmonella Typhi*, an important bacterial pathogen, induces very well-defined sets of epigenetic modifications in various cell types. Our publication was the first of its kind and was made possible by using CyTOF.

Immune complexity and CyTOF

When deciding which tool to use for a project, investigators should look at the expected use of CyTOF in the context of what are the biological questions that they want to answer. It might be something simple that can be done with fluorescence-based flow cytometry. But if there is a need for a very detailed analysis of the complex immune responses, then eight or 10 markers per cell are no longer sufficient in most cases.

Immunology has become extraordinarily complex with multiple activation and regulatory pathways acting in concert. Thus, there is great need for a technology that allows you to go into a lot of detail about which populations are stimulated, what phosphorylation pathways are activated, what activation and homing markers are expressed, etc., after exposure to antigenic stimulation. To address these questions, there is a great need for a high number of parameters to be measured simultaneously, and CyTOF can do that.

Learn how CyTOF is transforming clinical and translational research:

standardbio.com/cytof-summit-2023-videos

Unleashing tools to accelerate breakthroughs in human health™