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Stanford Team Uses DVS Sciences' CyTOF for Massive Hematopoietic Cell Protein Signaling Project

May 13, 2011

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By [Adam Bonislawski](#)

Using DVS Sciences' CyTOF mass cytometry platform, Stanford University scientists have completed an investigation of human hematopoietic cells that measured 34 parameters — including expression levels of 31 proteins — simultaneously in single cells to develop a system-wide view of normal hematopoietic signaling as it relates to cell phenotype.

The study, published this month in the journal *Science*, confirms expected immune system behavior and offers new insights into the transition of hematopoietic cells through various established stages of development, said Stanford professor Garry Nolan, leader of the study. It also, he told *ProteoMonitor*, provides a profile of signaling in healthy human hematopoiesis against which profiles of diseased cells such as cancer cells can be compared.

Based in Markham, Ontario, DVS began sales of the CyTOF in 2009 and has thus far placed six of the instruments, which list for around \$600,000. Combining capabilities of flow cytometry and atomic mass spectrometry, the device is able to simultaneously quantify as many as 100 protein biomarkers in individual cells at a rate of roughly 1,000 cells per second.

This is a significant increase over traditional flow cytometry, which can typically measure only 10 to 20 parameters at once due to overlaps in the emission spectra of the fluorophores used for such assays. Mass cytometry uses antibodies linked to stable isotopes of elements, which can then be read with high resolution via time-of-flight mass spectrometry.

"With mass cytometry you're just looking at individual masses [of the attached elements]," said Nolan, whose lab owns two CyTOF machines. "So that gives us easily 80 to 100 parameters to read per cell."

For their first study with the machine, Nolan's lab limited themselves to 34 parameters comprising 31 proteins, plus measurements of viability, DNA content, and relative cell size. The 31 proteins included 13 core surface markers tied to various human hematologic cell types as well as 18 intracellular epitopes like kinase substrates that reflect intracellular signaling states.

The idea, Nolan said, was that the scientists could follow protein signaling patterns in response to various stimuli like cytokines across the stages of hematopoietic development. The work, he said, while largely confirming current understandings of immune processes, demonstrated that the lines between hematopoietic cell stages are blurrier than often envisioned.

"A hematopoietic stem cell doesn't have certain [protein] surface markers, but it has other markers like CA34," Nolan said. "And as those cells mature, they pick up additional markers along the way or lose some of those markers. Most of those events are really functional events — they're picking up receptors, moving receptors — so those markers are occurring for the purposes of the immune system and immunologists have taken advantage of them as markers to stage specificity."

"People have become accustomed to thinking of these cells as sitting in fairly discrete stages," he said, but by comparing the cells and their signaling systems along 34 different parameters, the researchers demonstrated that "in fact there's a near infinite number of stages that go between one defined state and another. The stages are for our convenience, but for the immune system there are the functional stages, but also the transitions in between them."

Within the immunology community, where to draw these distinctions has long been an issue, Nolan said, noting that "people get into nearly religious arguments" on the subject.

"We decided to be fairly agnostic and under-interpret the map by making more subdivisions than perhaps actually existed," he said, noting that by adding to the data the observed signaling functions of the cells, they were able to identify relationships and divisions between and within cell groupings that conventional definitions might not have predicted.

"We started going in and tickling the cells with different cytokines and looking at different downstream signaling elements and all kinds of new things started to show up," he said. "Borders would appear in cell groupings where you wouldn't have know there should be some subdivision. That was really the objective of the experiment in the first place — not to just recapitulate everything that was already known, but also to describe new subdivisions within the boundaries we didn't know existed before."

Building the Blueprints

This mapping effort, Nolan noted, is still in the early stages. To handle the bioinformatic demands of analyzing the grouping of thousands of cells by 34 different parameters, his

team developed a density normalization, agglomerative clustering, and minimum-spanning tree algorithm called SPADE. Since submitting the *Science* paper, they've built new forms of the algorithm that Nolan said he thought would help better classify the cells into functional substates.

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Envisioning the computing power such massively multiparameter experiments would require, his lab started several years ago "developing a very large informatics structure," Nolan said, adding that he is now working to build an infrastructure that will let other researchers access the data.

"It would take other laboratories five to six years if they needed to do it from the ground up to get where we are," he said. "So we've basically built the blueprints for other people so they can replicate it in fairly short order."

One drawback to the CyTOF machine, Nolan noted, is its relatively slow speed. While a flow cytometer can read around 20,000 cells per second, mass cytometry tops out around 1,000. However, he said, speed isn't a significant issue "because the data depth we get is easily 100-fold or more than what we get with a standard flow cytometer."

Moving forward, the researchers plan to continue the mapping work by doubling the number of parameters they measure. They're also currently investigating several types of cancer cells, including ovarian cancer, follicular lymphoma, and myelogenous and acute lymphoblastic leukemia cells.

Initial data from five ovarian cancer patients has suggested that cancer cells, like healthy cells, can be ordered and divided based on their stages of differentiation, a finding that could have important implications for treatment.

"Every patient's cancer within an individual class is sufficiently different from each other that once you see the structures you would know to treat those patients differently," Nolan said. "For instance, in some early studies we're seeing that [patients] have a kernel of the cancer that has a large number of stem cell markers, but branching off from this kernel are three or four different [developmental] arms."

"In some of the patients, at least half of the cells continue to express these stem cell markers," he said. "The other half of the patients may have only very few of these and [instead] the bulk of the cells seem to have driven into later differentiation. So you would say one patient should get a stem cell therapy and the other patient should get a therapy targeted toward more mature cells."

According to DVS CEO Scott Tanner, the company is working on several improvements to the CyTOF device for launch in the next several months including an autosampler that will come out this summer and a new cell introduction system for fall that will improve the efficiency with which the instrument uses sample.

Currently, the machine "only analyzes one out of every three cells that are introduced," he told *ProteoMonitor*, noting that the company hopes to bring that down to a one-to-one ratio.

The company, which has 25 employees, is in the middle of a big hiring push, Tanner added, with three new workers hired this week on the production side, and another scheduled to come aboard next week.

Also this week DVS held the grand opening of a new 14,000-square foot manufacturing and R&D center in Markham. It plans to open another facility this summer in Sunnyvale, Calif., which will focus on sales and marketing and conjugating metal-tagged antibodies for use with the CyTOF system.

This fall, Tanner said, the company plans to put out a catalogue of 20 to 50 pre-conjugated antibodies that it will sell as reagents for use with the platform and is in discussion with "a number of antibody manufacturers" to provide antibodies for metal tagging, but it has yet to sign a deal.

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