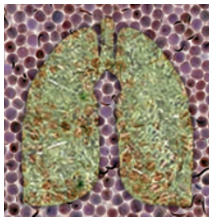

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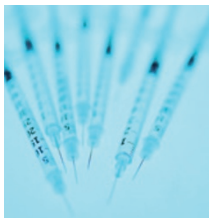

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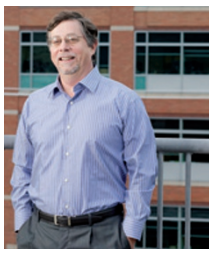

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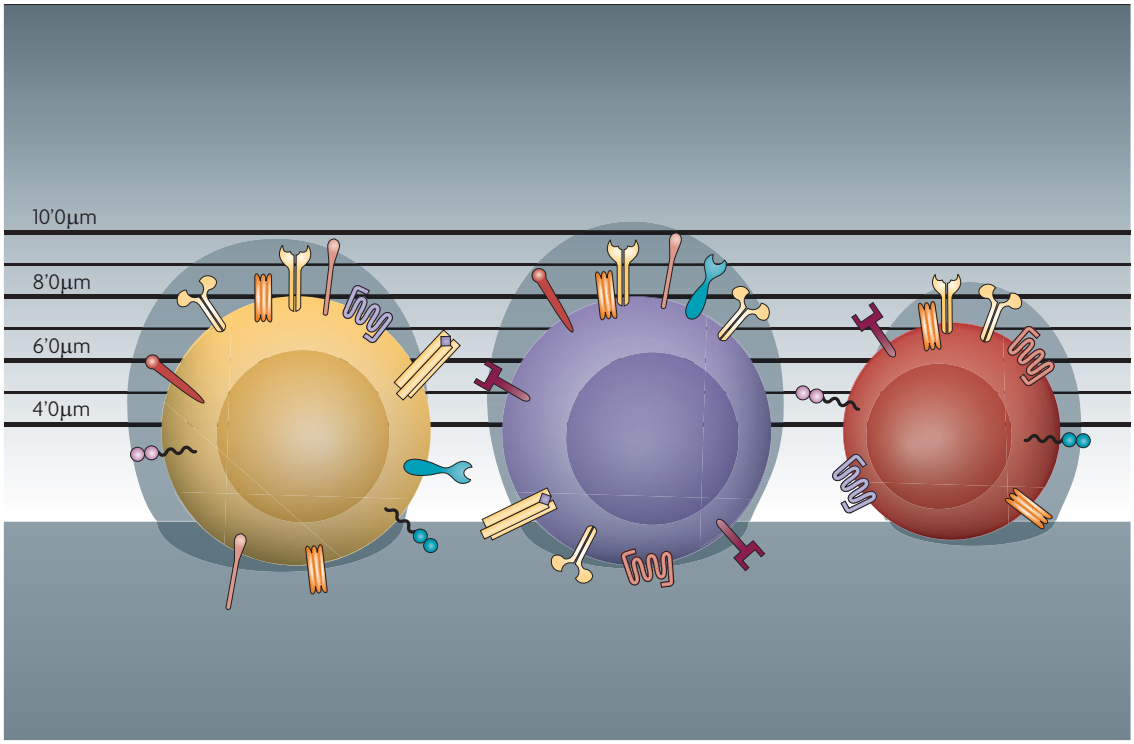


Single-cell profiling sheds new light

Advances in single-cell profiling are starting to shape drug and vaccine discovery and development efforts.

Asher Mullard

When Antoni Ribas started treating melanoma patients with an adoptive T cell therapy in 2009, he knew it would be a long shot. But armed with a new tool that measures the secretion of 20 proteins from single T cells, he was hopeful he could turn even poor efficacy into a successful experiment. As the data started to roll in, his hopes were justified; although the therapy's efficacy was not long lived, the unprecedentedly detailed single-cell analysis showed what was going wrong. "We learned things that are forcing us to tweak our approach and change how we are conducting the trial," says the oncologist, who is based at the Jonsson Comprehensive Cancer Center, University of California, Los Angeles, USA.

Ribas and his colleagues are one of a few groups who have shown in recent months how advances in single-cell profiling can shape drug and vaccine discovery and development efforts. One group has combined flow cytometry with mass spectrometry so that they can measure dozens of surface and intracellular proteins on single cells. Other researchers are capitalizing on improved sequencing technology to examine the gene expression of individual cells. Much of the early work has focused on profiling T cells and tumour cells, but other cell types are equally accessible.

As these technologies are taken through their paces, drug developers will eventually work out how much each can contribute to the evaluation of complex therapies, small molecule drugs and vaccines. But some are already sold. "It would be hard for us to continue with this adoptive T cell programme without relying on single-cell analysis," says Ribas.

Clinical insight needed

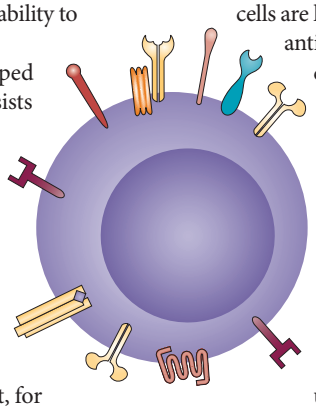
Ribas's dedication to single-cell profiling grew out of a collaboration with Jim Heath, at California Institute of Technology, USA, that started in 2005 as a result of a US National Institutes of Health (NIH) initiative aimed at uniting the fields of nanotechnology and cancer. Heath and his colleagues were making advances in surface sciences and wanted a biological outlet. Ribas, for his part, was frustrated with the available T-cell profiling approaches, which could not sufficiently tease apart the complexities of immunity.

They both agreed that the conventional tools had too many limitations for measuring secreted proteins. Cytokine flow cytometry can only measure up to five secreted proteins from a single cell and requires considerable perturbation of the protein secretion pathways. Another approach, Fluorospot, does not quantify the level of protein secretion. Consequently, when faced with a population of circulating therapeutic engineered T cells, neither approach can monitor a single cell's functional profile — that is, its ability to secrete over a dozen cytokines.

The platform Heath developed to address these concerns consists of a 1,040 microchamber chip. Each microchamber is large enough to accommodate a single cell and is plated with antibodies against 20 different secreted cytokines. It can also be coupled to standard flow sorting approaches in order to sort by phenotype prior to functional analysis (to select, for instance, only $CD8^+$ antigen-specific T cells). And because the assays are based on the standard enzyme-linked immunosorbent assay (ELISA) technology, the platform is relatively high throughput, low cost and practical, says Heath.

In the first foray into the clinic, published in May 2011, the multiparameter-measuring chips revealed profound functional heterogeneity within the adoptive T cell therapy (*Nature Med.* **17**, 738–743; 2011). The assay really showed its value, however, when it was used to follow the same cells over time. Despite initially inducing tumour shrinkage, the therapy's efficacy was short lived. The single-cell cytokine-secretion analysis showed that this was probably because the engineered T cells initially had killer cell functionality, but successively developed inflammatory and then suppressor functionality. "T cells are really complicated drugs; we developed a diagnostic that can tell us if they're doing the right thing," sums up Heath.

These findings — which suggest that the problem is not that the tumour evolves to evade the immune system — have affected Ribas's development plans. "Now we're trying to optimize manufacturing conditions so that the cells keep the killer function for longer," explains Ribas. "We're changing the way we make the cells, cryopreserve them and expand them." The team is also making plans to launch a new trial; the insight into the time course of their therapy's functionality suggests how it can be combined with Pfizer's tremelimumab, which activates the immune system by targeting the immunoregulatory protein cytotoxic T-lymphocyte antigen 4 (CTLA4).



Different ways to skin a cell

Garry Nolan, at Stanford University, California, USA, and his colleagues are taking a different approach to interrogating single cells. Similarly frustrated with the limitations of fluorescence-based measurement techniques, they developed 'mass cytometry': cells are labelled with isotope-tagged antibodies, sorted by flow cytometry and then analysed individually with a mass spectrometer. In a first showcase of the tool in May 2011, Nolan and colleagues tracked 34 parameters, including surface and intracellular proteins, on bone marrow cells (*Science* **332**, 687–696; 2011). "There should be no reason we can't measure up to 80 or 100 parameters for each cell," adds Nolan.

The mass cytometer, made by DVS Sciences, currently costs over US$600,000 and weighs half a tonne, making it an unlikely immediate addition to most laboratories or clinics. Yet, says Nolan, the data it generates provide incredible insight into how a drug affects multiple cell signalling pathways in different cell types. It can also be used to identify as-yet-unknown subsets of cells that make up heterogeneous tumours, pointing the way to the specific populations that are most important in cancer. "Just as we treat every patient as an individual now, we will eventually start treating each tumour cell as an individual."

Beyond cancer, another forefront of single-cell analysis is vaccine development. Gary Nabel at the National Institute of Allergy and Infectious Diseases, in Maryland, USA, and his colleagues have started monitoring gene expression at the single-cell level to evaluate candidates.

In one recent study, they inoculated mice with one of three different gene-based vaccines (*Proc. Natl Acad. Sci. USA* **108**, 5724–5729; 2011). Using traditional profiling approaches, all three candidates seemed to elicit the same $CD8^+$ T-cell functionality. When they used a platform made by Fluidigm that can look at the gene expression profiles of the single T cells, however, they found a more complex story: each candidate induced different sets of genes, leading to the identification of previously unrecognized subsets of cells.

> "T cells are really complicated drugs; we developed a diagnostic that can tell us if they're doing the right thing."

"This kind of analysis can be very useful to us during the evaluation of vaccines," says Nabel. In the HIV vaccine study, it showed that the three candidates were generating unique immune responses, suggesting that each was worthy of further examination. If two candidates induced similar patterns of gene expression and subpopulations of T cells, only one may be worth continuing to investigate. And once the field can leverage single-cell analysis to identify better signatures of immunogenicity or biomarkers, these same emerging techniques may be used to stop dead-end trials early or stratify patients.

Full speed ahead

Despite the potential, it is still early days for the single-cell approaches. "The technology still has to demonstrate its utility," says Nabel. "I don't think we're at a point yet where we can say that it's the be all and end all of our decision making."

As always, there are the teething pains of price, ease of use and availability of hardware. There are also questions as to the degree to which the technologies — and the different readouts they provide — will complement and compete with one another. And there is the larger looming complication of how to make sense of yet another mountain of data. Strong ties with bioinformaticians will be key. Figuring out how to identify and specifically profile the cells that matter the most will also be important, says Nabel.

But groups are nevertheless getting stuck in. Ribas and Heath have been approached by a host of laboratories who want to use the microchamber assay in immunotherapy and vaccine trials. Nolan is tweaking the mass cytometer to measure mRNAs as well as surface and intracellular proteins, and is hard at work at unravelling the heterogeneity of ovarian cancer tumours and leukaemia. And at the NIH, researchers are using gene-expression analysis to study the effects of vaccines in humans and to prioritize candidates for further preclinical work.

"I think that we've only touched the surface of what can be done with single-cell analysis," concludes Nolan.