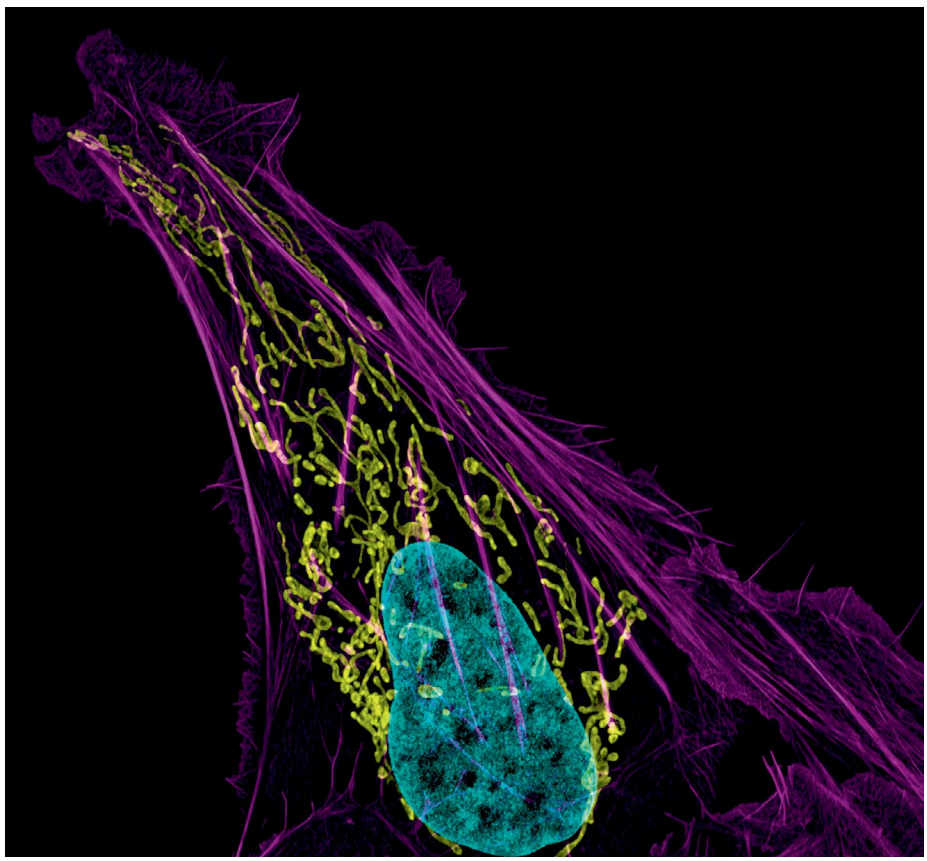
Can single-cell biology realize the promise of precision medicine?

Biology's quiet revolution is underway, as single-cell tools fuel the next-wave of drug discoveries and promise to match therapies to the individual.



A bone cancer cell with single-cell 'omics can reveal therapies matched to a person's tumor type.

CREDIT: SCIENCE PHOTO LIBRARY / ALAMY STOCK PHOTO

By Cormac Sheridan

Cellarity's single-cell-based approach to drug discovery is at the heart of a recent agreement with Novo Nordisk to develop new drugs for a form of chronic fatty liver disease associated with metabolic dysfunction. The hope is that this shift away from molecular targets and toward identifying the underlying cellular dysfunction will offer deep insights into the biology of the condition, which will, in turn, lead to improved therapies. Just as the microscope enabled the development of microbiology in the seventeenth century, high-resolution, single-cell methods are opening up whole new biological vistas (Table 1).

Single-cell methods are already embedded in basic research, where they have powered agenda-setting research initiatives, such as the recently published map of the human brain and the Human Cell Atlas, a global initiative to map every cell in the human body. But single-cell 'omics and imaging techniques are not confined to basic research. Because of the exquisite sensitivity of single-cell analysis in detecting biological signals, its potential is likely to spill over into myriad areas of medicine too. At this point, single-cell analysis is nowhere near routine clinical practice, but a handful of pioneering studies points to its potential in realizing the largely unattained goal of precision medicine – that is, accurately matching an individual patient to the therapies from which they are most likely to benefit. "Clinically, I would say, we're at the very early stages," says Joseph Powell, of the Garvan Institute of Medical Research in Darlinghurst, Australia.

The cell has been a central focus of biological research for almost 200 years, following its recognition in the 1830s as the fundamental structural and functional unit of life. But until recently, genomic, epigenomic, proteomic and other 'omic analyses could only be conducted on cells isolated in bulk. The resulting data provided an average view of a given biological sample, but glossed over rare cells, infrequently expressed transcripts, and metabolites present in low concentrations.

The widespread adoption of next-generation sequencing over the last two decades has ushered in the present era of single-cell biology. A whole-transcriptome analysis of a single mouse blastomere, published in 2009 by a team of scientists from the University of Cambridge and Applied Biosystems (now part of Thermo Fisher Scientific), is generally held to represent the first example of single-cell transcriptomics in action. The term 'single cell' applied literally in this case: the group used micropipetting techniques to handle the individual cells, which are present in very low numbers during early embryonic development.

But the whole value of single-cell biology lies in the ability to conduct parallel analyses of individual cells at massive scale. This relies on automated cell-handling platforms based on microfluidic and cytometric technologies, coupled with powerful computational platforms for managing and interpreting the data,

The migration of single-cell techniques from research labs to the clinic still requires test developers to build an evidence base to support their diagnostic or prognostic claims. That effort remains early stage. Mission Bio, for example, recently launched a single-cell assay for detecting measurable residual disease in patients with acute myeloid leukemia. It evaluates a panel of 40 genes and 19 protein markers, as defined in European LeukemiaNet guidelines, and one recent academic study suggests it may be ten

Table 1 | Selected clinical studies involving single-cell analyses

Trial	Sponsor	Indications	Description	Outcome
Carfilzomib in combination with daratumumab, lenalidomide and dexamethasone in transplant-ineligible multiple myeloma patients	Tel Aviv Sourasky Medical Center, Israel	Multiple myeloma	Single-arm clinical trial combined with longitudinal scRNA-seq to study drug resistance mechanisms in patients whose disease failed to respond to or underwent early relapse on bortezomib-based induction therapy	The study attained an ORR of 88% and identified a gene signature that predicted drug resistance and disease progression
EXALT-1	Medical University of Vienna	Relapsed hematological malignancies	Interventional study investigating the feasibility of single-cell functional precision medicine based on AI-driven ex vivo drug screening to guide treatment decision-making	ORR of 55%; 21% of patients had exceptional responses, defined as triple the expected duration of progression-free survival
Phase 1 trial of NP137	Centre Léon Bérard (Lyon, France)	Endometrial carcinoma	First-in-human trial of a humanized monoclonal antibody directed against netrin-1	According to an interim readout, 8 of 14 patients (57%) had stable disease; bulk and single-cell RNA-seq and spatial transcriptomics on pre-treatment and on-treatment biopsies established that NP137 induced tumor cell death and inhibited tumor epithelial-to-mesenchymal transition
Dabrafenib + trematinib + PDR001 in colorectal cancer	Massachusetts General Hospital (Boston)	BRAF V600E-mutated metastatic CRC	Single-arm proof-of-concept phase 2 trial combining a B-RAF inhibitor, a MEK1/2 inhibitor and a programmed cell death 1 inhibitor	The study attained its primary endpoint, with a 24.3% overall response rate; single-cell analysis of pre-treatment and on-treatment tumor biopsies revealed a correlation between increases in tumor-specific immune effector cells and duration of response
EXALT-2	Medical University of Vienna	Relapsed hematological malignancies	Study comparing the effectiveness of single-cell functional precision medicine, genomic profiling and physicians' choice in guiding treatment decision-making	Ongoing
Excyte-1	Exscientia	Ovarian cancer	Prospective observational study investigating relationship between ex vivo drug response in tumor cells, as evaluated with AI-based image analysis, and patients' clinical responses	Ongoing
Osteomics	Relation Therapeutics	Bone disorders	Observational study of bone homeostasis to identify new drug targets; includes single-cell analyses to understand the diversity of cell types within bone	Ongoing
Single cell RNAseq breast cancer	Regina Elena National Cancer Institute (Rome)	Triple-negative breast cancer	Characterization of persistent tumor cells remaining after chemotherapy and of tumor-associated macrophages to guide targeted treatment selection	Ongoing
Genomics in infection and sepsis to predict organ dysfunction and outcomes in sepsis	Chinese University of Hong Kong	Community acquired pneumonia	Prospective cohort study using single-cell leukocyte transcriptomic profiling and plasma DNA tissue mapping to identify patients with pneumonia experiencing sepsis, predict the level of organ dysfunction involved, and identify the molecular phenotypes of sepsis	Ongoing

AI, artificial intelligence; CRC, colorectal cancer; ORR, objective response rate; scRNA-seq, single-cell RNA sequencing. Sources: ClincalTrials.gov, PubMed.

times more sensitive than current methods (based on multicolor flow cytometry) in distinguishing residual leukemic cells from preleukemic cells or myeloid precursor cells. But it is still a translational research tool, as it has yet to be validated and approved as a clinical diagnostic.

The company has explicitly chosen to measure DNA instead of RNA, with that goal in mind. "Doing next-generation sequencing on RNA has never been approved by any regulator anywhere as an IVD [in vitro diagnostic]," says the Mission Bio's chief medical officer, Todd Druley. Although RNA is easier to isolate and work with than DNA, he says, its instability and variability in expression — both from person to person and from cell to cell — make it less reliable for clinical diagnosis. "It's really hard to get the same answer in two places," he says.

Single-cell spatial transcriptomics adds a further layer of analytical resolution. It allows researchers to pinpoint the locations of different cell types within a tissue sample while also identifying their gene expression profiles. The two main approaches involve either sequencing methods, in which tissue sections are analyzed on pixelated surfaces containing barcoded DNA primers, or imaging-based methods, involving fluorescence in situ hybridization or in situ sequencing.

Single-cell spatial analysis has resulted in strong growth for instrument maker 10x Genomics, which sold 100 of its Xenium systems within eight months of the product's launch. These analyzers provide users with richly detailed visualizations of tissue sections. "You're essentially looking at an image," says Ben Hindson, the company's CSO and co-founder. But it's an image with a wealth of associated biological data, which can be called up with a mouse click — it's akin to a virtual reconstruction of the original tissue. 10x Genomics scientists recently reported they used the system to perform high-resolution mapping of the breast cancer tumor microenvironment, which has suggested biological insights into the progression of cancers from ductal carcinoma in situ to invasive carcinoma.

Single-cell analysis is not confined to 'omic analytes, either. Exscientia, of Oxford, UK, is throwing its weight behind another foundational technology: high-throughput single-cell imaging. When combined with machine learning, it can yield important insights into the status of a cell exposed ex vivo to a drug. This approach to drug discovery is inherently unbiased — it does not require prior knowledge of the genomic profile of a cancer to recommend a particular course of action but relies instead on artificial intelligence-driven image analysis of cells following their exposure. The company gained the technology through its acquisition of Vienna-based Allcyte in 2021.

A prototype of the platform has already demonstrated its potential in guiding therapeutic selection in a prospective academic study, called EXALT-1, in patients with late-stage hematological cancers. Seventy-six of 143 patients had cells isolated from their tumors and collectively exposed ex vivo to a panel of 139 different drugs. Fifty-six of these patients received therapies suggested by this 'single-cell functional precision medicine' approach; the other 20 patients opted to receive physicians' choice. At median follow-up of 23.9 months, 30 of the 56 patients (54%) in the single-cell–drug-matched arm attained progression-free survival that was at least 1.3-fold longer than what they had experienced on their previous therapies. This is unusual in cancer care — typically, therapeutic success diminishes with succeeding lines of therapy. What's more, 12 patients were deemed to have experienced an exceptional response, seeing progression free-survival times that were three times longer than expected. "Eleven of our 56 patients on our EXALT-1 trial are still in remission," says Philipp Staber of the Medical University of Vienna, in Austria. "These are patients who were without any treatment options." No such survival benefit accrued to those on physicians' choice, although the two arms are not directly comparable given the variety of cancers included in the trial.

Staber and his team are now recruiting patients into a second prospective study in patients with hematological cancer, EXALT-2, which is comparing the utility of genomic screening, single-cell functional precision medicine and physicians' choice in guiding therapy selection. "Here, we're investigating three decision-making strategies. We're not investigating the drugs," he says. Roche, of Basel, Switzerland, is collaborating on the study, which is employing its FoundationOne Heme test to provide genomic profiles of patients' cancers. The study will use laser-based flow cytometry, instead of Exscientia's high-content microscopy technology, to evaluate the effect of its panel of drugs on patients' cancer cells. "We learned that actually the flow cytometer needed even less material than we used before," Staber said.

These functional single-cell approaches to precision medicine could benefit more patients than is currently the case with genomic tumor profiling. "It's a tiny minority of cancer patients who benefit from these purely genomic approaches," says Anthony Letai of the Dana-Farber Cancer Institute. The extraordinary success of imatinib, which was approved back

News in brief

First desmoid tumor drug

The US Food and Drug Administration (FDA) has greenlighted Ogsiveo (nirogacestat) as a treatment for desmoid tumors. The oral γ-secretase inhibitor developed by SpringWorks Therapeutics is the first agent approved for these rare, non-cancerous but locally invasive soft-tissue tumors, which cause pain, movement difficulties and decreased quality of life. Previous treatments relied on off-label use of chemotherapy and tyrosine kinase inhibitors.

Ogsiveo is also the first γ-secretase inhibitor to receive the FDA's nod. This drug class was originally investigated for its ability to block amyloid-β build-up in Alzheimer's disease, but unwanted effects on Notch signaling halted development. Yet, given the role of Notch in cancer, Ogsiveo's originator company Pfizer and then the National Cancer Institute ran early-stage trials with the small-molecule agent in patients with desmoid tumors, which overexpress Notch1. Clinical trials were then taken over by SpringWorks, spun out of Pfizer in 2017 to focus on rare disease, with the support of the patient advocacy group the Desmoid Tumor Research Foundation. By inhibiting γ-secretase–mediated cleavage of Notch receptors and preventing Notch signaling, Ogsiveo blocks tumor proliferation.

The approval is based on clinical trial data showing that Ogsiveo reduced disease progression by 71% and induced a response in 41% of treated patients, as compared with 8% of placebo-treated patients. The drug also improved pain, other tumor-specific symptoms, physical functioning and health-related quality of life. Because several side effects were observed, the drug carries warnings for diarrhea, ovarian toxicity, hepatotoxicity, non-melanoma skin cancers, electrolyte abnormalities, and embryo and fetal toxicity.

Another γ-secretase inhibitor, Ayala Pharmaceuticals' AL102, is in phase 2/3 trials for desmoid tumors. Ogsiveo is also being tested in other tumors associated with excessive Notch signaling, including ovarian granulosa cell tumors and multiple myeloma.

in 2001 for treating patients with Philadelphia chromosome-positive chronic myeloid leukemia (CML), raised unrealistic expectations about creating highly potent therapies simply by identifying the genetic drivers of different cancers. "We were just going to march along from cancer to cancer, and that's what we were going to do," says Letai. CML proved to be an exceptional case, however. For example, the overall response rate on the recently concluded NCI-MATCH trial, sponsored by the National Cancer Institute, was just 10.3%.

In most cases, the relationship between the genotype of a cancer cell and its phenotype is typically more complex than is it is for CML. As noted by Nikolaus Krall, who is executive vice president of precision medicine at Exscientia, other genetic, epigenetic, transcriptomic and immunological factors also influence cancer development. "Really understanding this interplay at a molecular level to such an extent that you can make predictions is very hard," he says. "In some settings, an unbiased phenotypic approach is much easier conceptually."

It's not a question of one approach replacing another but of what works best in a given setting. "The way that we assay information on cells is going to continue to evolve," says Powell. "We shouldn't be precious about what technology we are using." Krall concurs: "I see no point in competing with mutational analysis in CML, for example."

The clinical application of single-cell biology is not confined to cancer, even if that area consumes most of the current attention. For example, London-based Relation Therapeutics is running an ambitious observational study, Osteomics, which is designed to generate insights into bone biology that, it hopes, will form the basis of novel disease-modifying therapies. Co-founder and chief innovation officer Jake Taylor-King says Osteomics is set up as a single-cell expression quantitative trait locus study in order to explore disease-associated genes at a high level of resolution. It is applying single-cell analyses to bone tissue obtained from patients undergoing orthopedic surgery, including those with bone disorders, such as osteoporosis, and those without underlying disease who have experienced a bone injury. It will integrate these data with molecular biology, genetic and environmental data and employ machine learning to enable a 'lab-in-the-loop' interchange between wet lab experimentation and predictive modeling. "We're already starting to get some hits," he says.

The emergence of clinical applications will be necessarily slower. "There are regulatory hurdles as well as application uses," says Powell. Data, as always, will drive adoption. The potential rewards, both in terms of better medicines and better use of existing drugs, are high.

Cormac Sheridan
Dublin, Ireland

History

Row over Curie building ends on a high note

A building in Paris where Marie Curie worked will be dismantled, decontaminated and rebuilt nearby to make space for a new cancer research center. Rima Abdul Malak, France's minister of culture, intervened to halt the planned demolition of the Pavillon des Sources, established in 1909 as the Institut du Radium and now known as the Institute Curie, after discussions with the institute's head. Curie, born Maria Skłodowska in Warsaw, Poland, moved to France to study in 1981 and married Pierre Curie in 1895. She won the Nobel Prize in Physics 1903 and the Nobel Prize in Chemistry in 1911. The Pavillon des Sources was due to be torn down to make way for new construction. But a campaign to save the building and have it listed as a national monument found an ally in the minister of culture. Work was suspended on the construction project to allow time for alternative solutions to be considered. On 31 January, a resolution was announced: the old building, which was not Curie's lab but a place where radioactive materials for research were once housed, would not be destroyed, but instead dismantled and rebuilt nearby. In its place a $14 million cancer research center will rise.

CREDIT: EQROY / ALAMY STOCK PHOTO

Michael Francisco