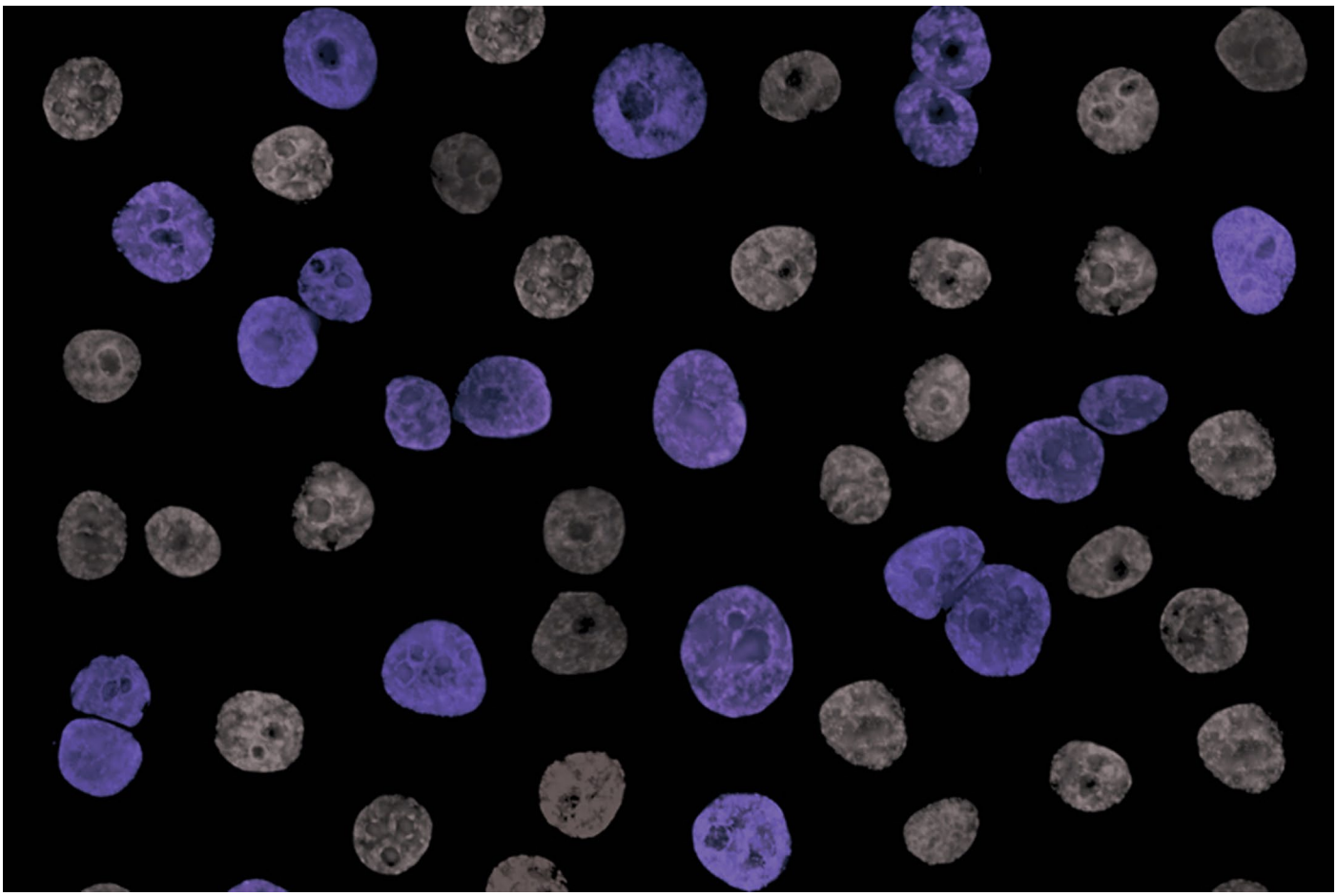


Credit: Photo courtesy of Nicholas Navin

Single-cell sequencing edges into clinical trials

Parsing the DNA of individual cells gives a higher-resolution view of health and disease.

Amanda B. Keener

In late June, the Chan Zuckerberg Initiative announced the recipients of $68 million in funding to create the Human Cell Atlas (HCA), a genetic map of the cells that make up a healthy human. Each of the 38 international research groups will tackle an organ or system, such as the heart, immune system and gastrointestinal tract. Some will catalog the same tissues, but from different populations of people. Using samples from donated blood, deceased organ donors, plastic surgery and more, the group plans to build a reference map of what 'healthy' looks like from the perspective of our cells.

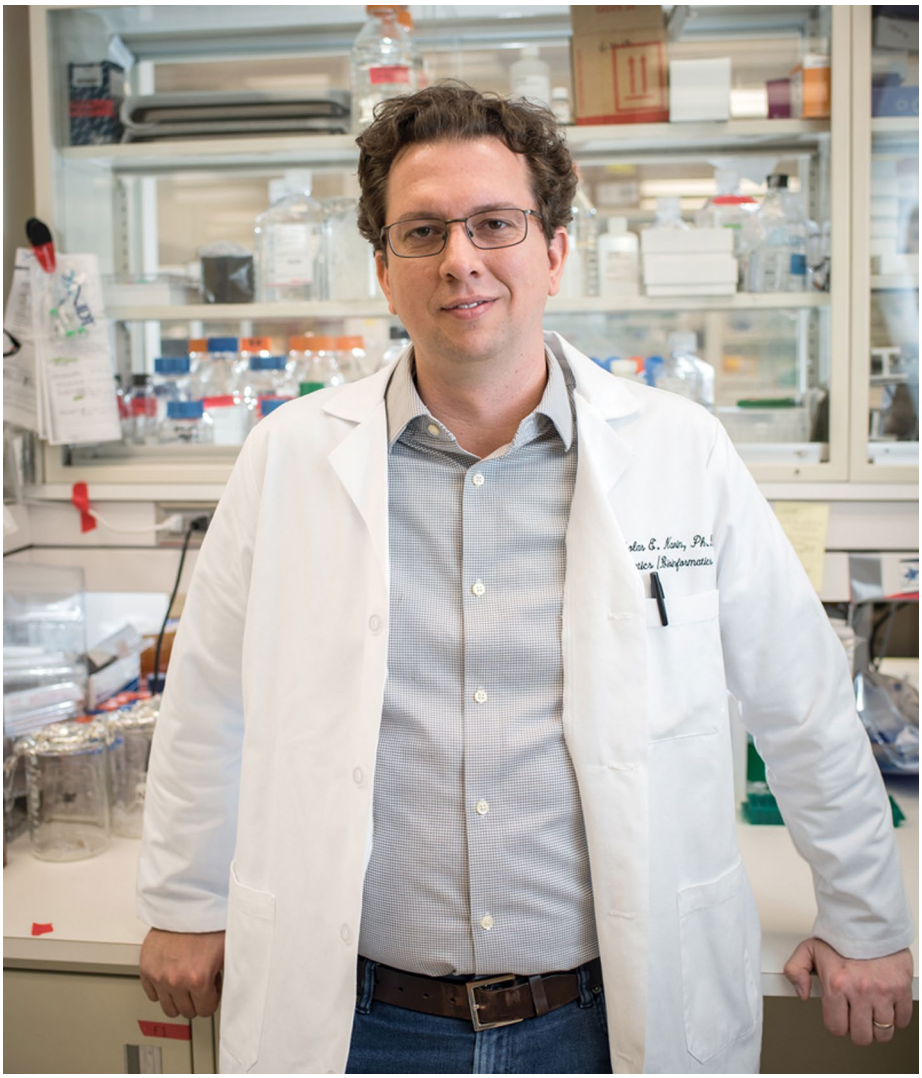
In the early days of his career, Nicholas Navin, a geneticist at MD Anderson Cancer Center who is leading one of the HCA's teams on breast tissue, could not have envisioned a world where single-cell sequencing would be the thrust of a multimillion-dollar grant supported by the CEO of Facebook. In fact, Navin had a lot of trouble convincing colleagues of the value in the technique. As a graduate student, he developed the first method to sequence individual mammalian cell genomes, but when he first tried to publish a paper on it, the initial feedback he got was, "why would you ever want to do this?" Now, he says, single-cell sequencing is such a widespread research tool, it is rare for him to hear of a clinical study that does not include it.

As little as a decade ago, the only way to study which genes were mutated in a kidney tumor or which genes were turned on and made into RNA transcripts during an infection in the liver was to analyze the genetic information from thousands or millions of cells together at a time. Although those bulk data are useful, says Nikolaus Rajewsky, a biologist at Max Delbrück Center for Molecular Medicine in Berlin, it is only an average—a muddled view of the true biology happening in an organ. "The function of a tissue or of an organ is defined by the different cell types, the way they're arranged in space and how they communicate with each other," he says,

adding that if you grind everything up and look at it as a homogenous slurry, "you cannot say which contributions come from which cells."

This surge in interest has closely trailed improvements in the technologies that have made it possible to decipher DNA or RNA sequences from tens of thousands of individual cells in the time and at the expense it once took to sequence just 100 cells. In some cases, weeks of work have been cut down to a couple of days. Over the last decade, for example, researchers have developed methods to encapsulate individual cells into tiny droplets and attach each cell's RNA onto a unique nanoparticle before sequencing so that they can keep track of which transcripts came from which cells. Such developments mean researchers can track mutations and changes in gene activity in specific cell types rather than having to average everything going on in a hunk of tissue or tube or blood. "It's exciting days for a huge swath of biology," says Aviv Regev, MIT and Broad Institute biologist and co-organizer of the HCA, whose research group came up with one of the most popular methods of single-cell RNA sequencing, called DropSeq. "[Biologists] basically have this bigger microscope."

Single-cell sequencing is allowing researchers to define new cell types and map the contributions of cell types in disease. "We're gonna find out about diseases at single-cell resolution in a way that we've never seen before," says Scott Budinger, a pulmonologist who studies lung biology at Northwestern University. Many are also using the technique to monitor how cancer responds to treatment, and highlight potential drug targets and therapy combinations. The next challenge researchers are taking on is turning that information into diagnostic tests, treatments and even disease prevention.



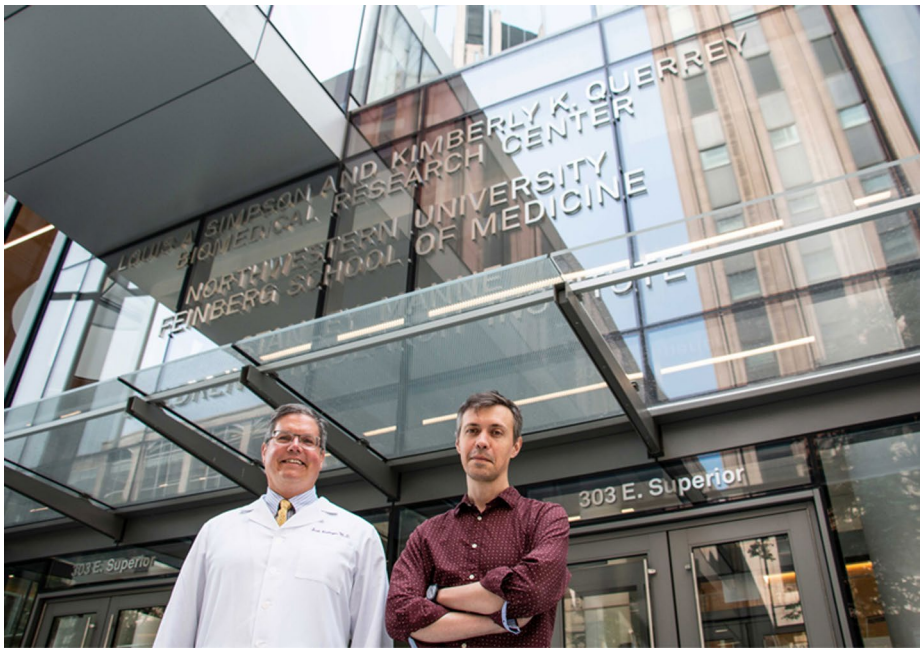
Cell profiler: Nicholas Navin tracks chemotherapy-resistant cells. Credit: Nicholas Navin

A bigger microscope

Pulmonologists like Budinger have two drugs approved by the US Food and Drug Administration (FDA) to pick from when treating their patients with lung fibrosis, a disease in which scar tissue progressively replaces normal lung. Both drugs have bad side effects, and neither works very well according to Budinger. "Even beyond that, nobody really knows how these drugs work," he says. But to design new drugs for this fatal disease, researchers first need to know which cells are responsible for driving it. In 2017, Budinger and his Northwestern University colleague, Alexander Misharin, also a pulmonologist, found that in mice, a subset of immune cells called monocyte-derived alveolar macrophages were present in fibrotic but not healthy lungs, and played a big role in causing disease. But their experiments relied on genetically modified mice whose macrophages glowed when the cells expressed certain genes[1]. They could essentially watch the cells turn certain genes on and off, and diverge into two distinct lineages, something they had never be able to do in samples from patients.

To test whether their findings held up in humans, the team used single-cell sequencing to map all of the RNA transcripts in lung biopsies from patients with disease and in healthy organ donor samples. They found that, like the sick mice, patients with lung fibrosis carried a distinct population of macrophages expressing genes that are highly active in mouse models of fibrosis[2]. Budinger says those cells could be good targets for news drugs or serve as indicators of disease progression. He and Misharin have now begun a study profiling gene expression in patients' lung fluid cells before treatment and six months later in search of 'signatures' of those patients who respond well and those who do not.

This approach of using single-cell sequencing to monitor disease progression and response to therapy is already widely used in cancer research. Researchers use these methods track which 'clones'—or cells with a particular genetic profile—are eliminated by chemotherapy and which ones persist or become resistant over time. Last year, for example, Navin and his colleagues published a study, in which they described tracking of chemotherapy-resistant cells from 20 women with aggressive breast tumors before, during and after treatment[3]. They found that the cells with the genetic



Parsing biopsies: Scott Budinger (left) and Alexander Misharin use single-cell sequencing. Credit: Alexander Misharin, Scott Budinger, Northwestern University

abnormalities responsible for drug resistance in half of patients are present in the tumor before the treatment begins. Navin's lab is performing similar experiments on tumor samples from MD Anderson's ongoing ARTEMIS trial, which is looking for biological signs of chemotherapy sensitivity in several hundred breast cancer patients.

Single-cell sequencing is not being used as a tool to make treatment decisions just yet, but it could soon have a big impact in matching patients to the right cancer drugs, says Peter Sims, a systems biologist at Columbia University. Tumors are complex environments composed of different cell types at different stages of growth, death and evolution, says Sims. "A given targeted therapy is not going to be able to take out all the different cell types that co-occur in an individual patient. The key missing piece of information is what cell type in the tumor is targeted by a given drug?" Studying tumor gene expression at an individual cell level can easily furnish that information.

How well a drug works also depends on what is happening in the surrounding tissue or microenvironment. Immunotherapies, for example, only work if there are enough immune cells in a tumor and those cells are already programed to attack cancer cells. Regev recently used single-cell RNA sequencing to compare how the 'programming' differs between T cells within melanoma tumors from patients that did or did not respond to checkpoint inhibitor therapy. She and her colleagues found a subset of cancer cells had a program—or pattern of gene activity—that predicted which tumor T cells would be resistant to activation by the checkpoint inhibitor anti-PD-1. Then, in lab experiments, they screened drugs for their ability to repress that program and re-sensitize T cells to anti-PD-1. Regev's colleagues at the Dana-Farber Cancer Institute are now setting up a clinical trial to test one of those drugs, a CDK4/6 inhibitor, in combination with anti-PD-1 therapy in patients with melanoma[4]. Regev says the project went quickly because both drugs are already FDA-approved, but it is a prime example how just taking a higher-resolution view of a tumor can open up new treatment options that might otherwise be overlooked.

Last year, Regev co-founded Celsius Therapeutics, a biotech company based in Cambridge, Massachusetts that applies single-cell RNA sequencing to the search for biomarkers of treatment response in cancer and some autoimmune diseases. In July 2019, the company announced its first pharmaceutical industry collaboration with Janssen Biotech. Celsius researchers will use single-cell sequencing to look for cell programs that predict a positive treatment response among patients with ulcerative colitis who are enrolled in a phase 2 trial testing a combination of two immunosuppressive antibody drugs, guselkumab and golimumab.

Life from a cell's perspective

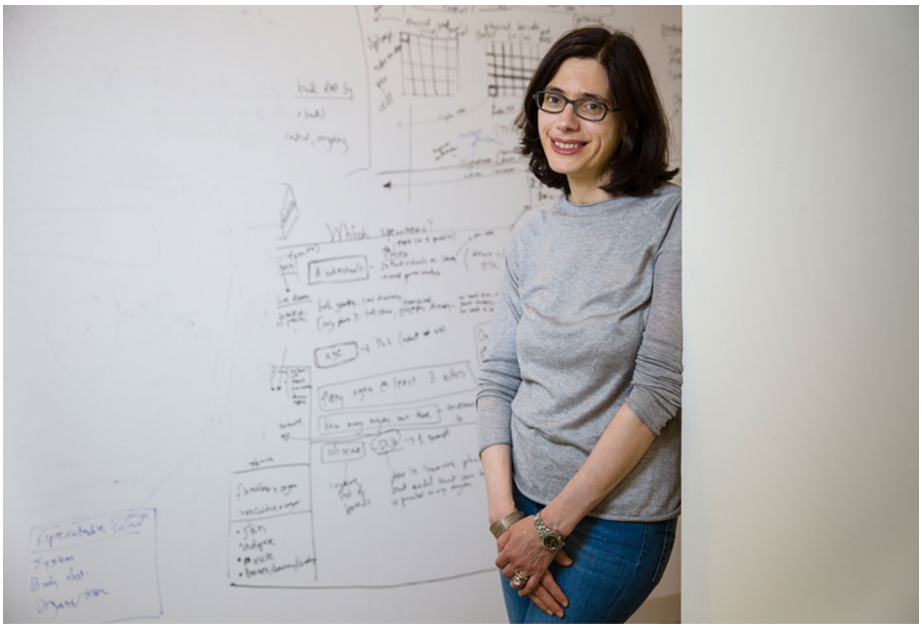
Single-cell sequencing's clinical applications may be furthest along in the realm of cancer, but the technique is causing a shift in every area of biomedical research. Single-cell RNA sequencing in particular has potential to reveal new cell types and cell states because it allows researchers to group cells based on a comprehensive view of the active genes in each cell rather than on one or two protein markers. Two cells may technically be the same type based on such markers, but one might secrete signals that promote an inflammatory response whereas the other might make molecules encouraging tissue growth. Before, the two would be lumped together despite having different roles in a tissue. Single-cell RNA sequencing also removes a lot of bias because researchers do not have to know before they begin an experiment that those two subtypes of cells exist.

The combination of this potential to get an unbiased picture of all the cell types in a tissue with the ability to sequence tens of thousands of cells at once easily convinced Regev and others that the time was ripe a human cell atlas. By 2014, Regev was mentioning the idea of mapping out whole organ systems at the end of every talk she gave. "After a fair bit of evangelizing, I guess I got a reputation," she says.

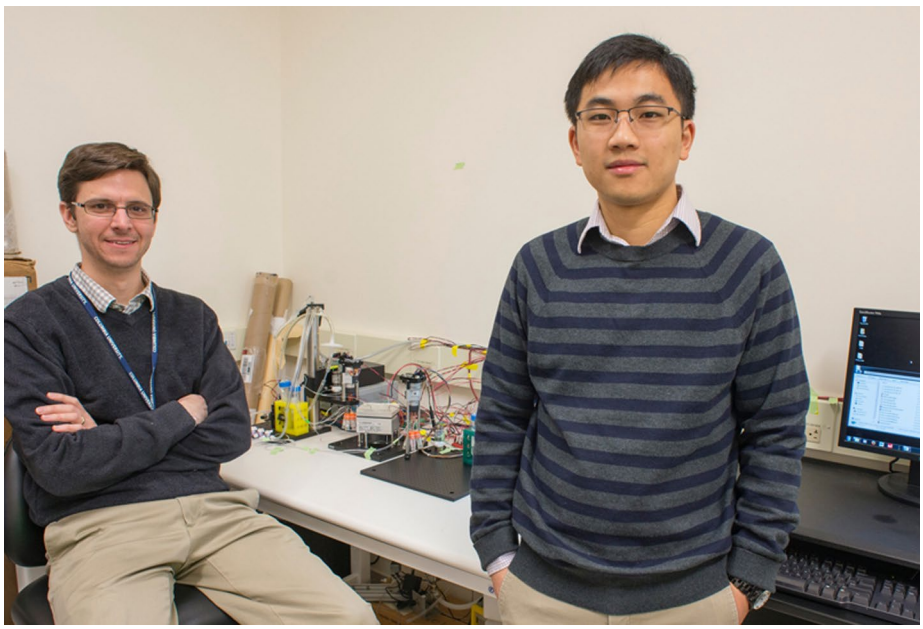
Regev found herself at the center of a community of researchers with the same passion, and in 2016, she and several colleagues organized a meeting hosted by the Wellcome Sanger Institute in London to hash out just what these maps would look like. Three years later, the consortium is well into its first 'draft' of a human cell atlas, focusing on several major organs, such as the heart, breast and lung, but also on systems including immune and female reproductive systems.

The atlas also includes studies of how these organs and systems develop over time and how they differ among various populations. Navin's contribution, for example, is a breast atlas that will compare tissue from women at various reproductive stages including menopause, pregnancy and post-partum. Another group at the University of Indiana School of Medicine, meanwhile, will contribute a breast atlas including samples from ethnically diverse populations of women.

The HCA is focused on healthy tissue, which requires some creativity on the part of the researchers since healthy people do not usually get biopsies. Navin says his team will source some breast tissue from plastic surgeries, for example. Sims is part of an HCA team that will survey immune cells present in human organs from different age



Precise to a T: Aviv Regev has studied T cells with single-cell technology. Credit: Casey Atkins Photography



Express yourself: Peter Sims (left) and postdoc Jinzhou Yuan study what makes cells different, including gene expression. Credit: Peter Sims

groups, and some of their tissue samples will come from organ donors. For RNA sequencing, tissues samples must by fresh. That means there must always be someone on call to acquire next-of-kin consent and collect donor samples, and someone ready to run to the lab and process the tissue. It is not trivial work, but having a healthy reference map of immunity would be huge, Sims says. "There are papers coming out every day where people have isolated immune cells from tumors—my lab as well—but the problem is we don't have the corresponding normal healthy reference."

In addition to being a reference guide, these atlas studies could bring clarity to our understanding of genetic diseases. For example, sequencing individual cells of the mouse lung led Regev and her colleagues to a cell type that had never been seen in the lungs before and could have a role in cystic fibrosis[5]. The cell type, called ionocytes, are found in the skin of some fish and frogs where they exchange ions with surrounding water to keep molecules like sodium and calcium in balance. Although they made up less than half a percent of the total cells in the mouse lung, these cells were responsible for making more than 50 percent of the RNA transcripts from the cystic fibrosis transmembrane conductance regulator (CFTR) gene—the gene that, when mutated, causes cystic fibrosis. The team analyzed cells from the lungs of organ donors and reported that in ionocytes also carried the bulk of the CFTR transcripts in human lung tissue samples. "The majority of the expression of that gene was in the cell type that wasn't known, which is crazy," Sims says. Knowing which cell populations are most involved in expressing disease-related genes could help scientists know where to target therapy approaches, like CRISPR-Cas, he says.

Trajectories of disease

Cataloging disease at a single-cell level has a unique potential to let researchers spot illnesses at much earlier stages because individual cells change before entire organs do. "Eventually, what we want is to prevent the disease rather than treating the disease," says Kai Tan, a molecular biologist at the University of Pennsylvania who is involved in the National Cancer Institute's Human Tumor Atlas Network (HTAN). To that end, half of HTAN's supported projects are focused on conditions that are likely to become cancer, such as colon polyps or lesions of abnormal cell growth in the lungs or cervix. Research groups in the HTAN will study samples from people at risk for colon, lung, breast, skin and blood cancers in search of single-cell-level changes that precede disease.

Navin is doing similar work on ductal carcinoma in situ (DCIS), a condition in which cancer-forming cells are found contained in the milk ducts of the breast. In DCIS, the cancer cells may never exit the ducts, but if they do, they can cause fatal disease. The diagnosis leaves many patients with a difficult decision about whether to undergo surgery and chemotherapy. Navin's team is comparing RNA transcript profiles between individual DCIS cells and invasive breast tumor cells to look for characteristics of DCIS that is likely to become malignant.

Rajewsky says there can be hundreds or thousands of molecular steps that turn

a healthy cell into a sick cell—and that allow sick cells to cause disease. Mapping out those small changes is the idea behind the newly minted LifeTime Initiative, of which Rajewsky is co-coordinator. The pan-European consortium aims to identify 'molecular symptoms' that appear years, sometimes decades, before any clinical symptoms do. The goal is to find ways to screen for those changes the way we screen for prostate or colon cancer, and then actually intervene before the next step happens and disease develops. Maybe one day, Rajewsky says the standard of care could be to treat cells rather than organs.

LifeTime Inititative officially launched in early May when the researchers involved gathered at the Max Delbrück Center for Molecular Medicine and laid out the general approach they will take to achieve this goal. Along with single-cell sequencing and personalized organoid research, they will focus heavily on developing machine learning methods that can interpret sequencing data and turn it into information about a patient's risk of developing a disease and about which drugs might lower that risk. "What we will do is deliver, for the first time, the massive amounts of data that machine learning needs to start to be helpful for explaining how cells change on a molecular level," Rajewsky says. Some big data companies, including Google and Amazon, have signed on to offer support to help make this happen.

One thing the LifeTime consortium did not do at the meeting in May was decide which diseases to start with. This, Rajewsky says, will be a group decision based on information currently being compiled by clinical experts. A lot of factors will go into that decision, including which diseases are most amenable to sampling over time, how many people a disease impacts, and how treatable it is. There will doubtless be many more meetings over the next year as the consortium puts together its road map, which is due to the European Commission in 2020. "There's a huge amount of work in front of us," Rajewsky says. ❐

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