THE ORGANOID ARCHITECT

Hans Clevers pioneered lab-built mini-organs that can serve as models of disease

By **Gunjan Sinha**, *in Utrecht, the Netherlands*

By her 50th birthday, Els van der Heijden felt sicker than ever. Born with the hereditary disorder cystic fibrosis (CF), she had managed to work around her illness, finishing college and landing a challenging job in consulting. But Van der Heijden, who lives in a small Dutch town, says she always felt "a dark cloud hanging over my head." When she began feeling exhausted and easily out of breath in 2015, she thought it was the beginning of the end.

Then she read a newspaper article about a child with CF named Fabian whose life had been saved after scientists grew a "mini-organ" from a tissue sample snipped from his colon, one organ that CF affects. Doctors had used the mini-organ to test ivacaftor (Kalydeco), a drug so expensive that Dutch insurers refuse to cover it without evidence that it will help an individual CF patient. No such data existed for Fabian, whose CF was caused by an extremely rare mutation. But his minigut responded to ivacaftor, and he improved within hours of taking it. His insurance eventually agreed to pay for the drug.

A basic biologist at heart, Clevers says he never expected his findings to benefit patients.

Van der Heijden's doctor arranged to have a minigut made for her as well; it responded to a drug marketed as Orkambi that combines ivacaftor and another compound, lumacaftor. Within weeks after she began taking that combination, "I had an enormous amount of energy," she says. "For the first time ever, I felt like my body was functioning like it should."

PHOTO: SANDER HEEZEN

The life-altering test was developed in the lab of Hans Clevers, director of the Hubrecht Institute here. More than a decade ago, Clevers identified a type of mother cell in the gut that can give birth to all other intestinal cells. With the right nutrition, his team coaxed such stem cells to grow into a 3D, pencil tip–sized version of the gut from which it came. The minigut was functionally similar to the intestine and replete with all its major cell types—an organoid.

That was the start of a revolution. Clevers and others have since grown organoids from many other organs, including the stomach, pancreas, brain, and liver. Easy to manipulate, organoids are clarifying how tissues develop and repair injury. But perhaps most exciting, many researchers say, is their ability to model diseases in new ways. Researchers are creating organoids from tumor cells to mimic cancers and introducing specific mutations into organoids made from healthy tissue to study how cancer arises. And as Clevers's lab has shown, organoids can help predict how an individual will respond to a drug—making personalized medicine a reality. "It is highly likely that organoids will revolutionize therapy of many severe diseases," says Rudolf Jaenisch, a stem cell scientist at the Massachusetts Institute of Technology in Cambridge.

For Clevers, the bonanza has come as a surprise. A basic biologist at heart, he says he never had real-world applications in mind. "I was always driven by curiosity," he says. "For 25 years we published papers with no practical relevance for anyone on this planet."

ON A BRIGHT JULY MORNING at the Hubrecht Institute, Clevers listens patiently to presentations during a weekly lab meeting. One postdoc presents data on her efforts to develop an organoid model for small-cell lung cancer; another reports progress on culturing hormone-secreting organoids from human gut tissue. Whenever their research questions strike him as uninspired, Clevers urges them to be more ambitious: "Why don't you pursue something you don't know?" he asks.

"Hans is capable of raising questions that are not contaminated by the anticipated answer," says Edward Nieuwenhuis, chairman of pediatrics at University Medical Center Utrecht (UMCU) and a good friend. "He has a better nose than most for sniffing around and finding interesting stuff," says Ronald Plasterk, who co-directed the Hubrecht lab with Clevers from 2002 to 2007 and is now the Dutch Minister of the Interior and Kingdom Relations. That approach has earned Clevers many awards. In June, for example, he was inducted into the Orden Pour le Mérite, an elite German order with just 80 members worldwide.

Miniature body parts

Organoids, lab-grown miniature versions of organs, are transforming science and medicine. Researchers have grown them from many different organs; they have also created organoids from tumor cells to mimic cancers.

How to grow a gut organoid

Hans Clevers's lab grows intestinal organoids (shown in a 3D rendering, above) from individual patients. They can be used to test drugs.

1. Take a tissue sample
A very small biopsy is taken from the epithelium, the tissue lining the gut.

2. Incubate
The tissue is bathed in a mix of growth factors designed to let gut stem cells replicate.

3. Harvest
Organoids, a millimeter or less in diameter, emerge in up to 3 weeks and can be frozen for later use.

What to do with organoids

Scientists can use organoids in many different ways. Here are examples of current and potential applications in basic research and medicine.

Regenerative medicine
Organoids grown from healthy tissue could be placed back into a patient to help repair damaged tissue.

Drug testing
Candidate drugs can be tested on organoids to help predict their effects in patients.

Personalized medicine
Organoids grown from individual patients can help predict their response to new or existing drugs.

Toxicity testing
Toxicologists can use organoids to test the effects of chemicals on the liver and other human organs.

Microbiome studies
Scientists can study how normal human intestinal bacteria interact with gut organoids.

Modeling infections
Organoids can be infected with viruses or bacteria to study how pathogens affect cells.

Cancer studies
Scientists can study how cancer develops by introducing mutations in organoids grown from healthy tissues.

Clevers began his career studying immune cells as a postdoc at the Dana-Farber Cancer Institute in Boston. He landed his first job at UMCU's clinical immunology department in 1989, where he quickly became department head. Most of the work was clinical, such as leukemia diagnostics and blood work for transplants. "But my research interests were always much more basic than the environment that I was in," he says.

In early work, he identified a key molecule, T cell–specific transcription factor 1 (TCF-1), that signals the immune cells known as T lymphocytes to proliferate. Later he found that TCF-1 is part of the larger Wnt family of signaling molecules that's important not only for immune responses, but also for embryonic development and tissue repair. In 1997, his lab team discovered that mice lacking the gene for one of those signals, TCF-4, failed to develop pockets in their intestinal lining called crypts. Soon after, a study with Bert Vogelstein at Johns Hopkins University in Baltimore, Maryland, showed that TCF-4 also helps initiate human colon cancer. Fascinated, Clevers switched his focus from the immune system to the gut.

Inspired by a flurry of research on stem cells at the time, Clevers began hunting for intestinal stem cells. More than 50 years ago, researchers deduced that rodent crypts produce many cells that survive only a few days, suggesting some unidentified, longer-lived source for the cells.

After almost a decade of tedious experiments, Clevers's postdoc Nick Barker struck gold in 2007: He discovered that cells carrying a receptor named LGR5 give rise to all cells in mouse intestines and that molecules in the Wnt pathway signal those cells to divide. Barker later found LGR5-positive cells in other organs as well. In some, the cells were always active; in others, such as the liver, they multiplied only when tissues sensed injury.

At the time, culturing stem cells was notoriously hard, but after combing through previous lab experiments, another postdoc in Clevers's lab, Toshiro Sato, concocted a mix of growth factors that coaxed the gut stem cells to replicate in a dish. He hoped to see a flat layer of cells. But what emerged in 2009 from a single LGR5-positive cell was "a beautiful structure that surprised and intrigued me," says Sato, now at Keio University in Tokyo: a 3D replica of a gut epithelium. The structure self-organized into crypts and finger-shaped protrusions called villi, and it began making its own biochemicals. A paper about the feat was rejected several times before being published. Clevers recalls: "No one wanted to believe it."

Soon, the lab began culturing LGR5-positive cells and growing organoids from the stomach, liver, and other organs. "It was

an exciting time, and I really felt like we were on the frontiers of discovery," says another postdoc at the time, Meritxell Huch, now at the Gurdon Institute in Cambridge, U.K. "But we certainly didn't think we were opening a new field."

CAPTIVATED BY STEM CELLS and their potential to regenerate tissues, other labs were starting to make organoids. A few months before Sato's 2009 paper, Akifumi Ootani, a postdoc in Calvin Kuo's group at Stanford University in Palo Alto, California, reported using a different strategy to grow gut organoids. Kuo's method starts with tissue fragments rather than individual stem cells and grows them in a gel partly exposed to air instead of submerged in nutrient medium. Around the same time, Yoshiki Sasai of the RIKEN Center for Developmental Biology in Kobe, Japan, cultured the first brain organoids, starting not with adult stem cells but with embryonic stem cells. Other researchers grew organoids from induced pluripotent stem cells, which resemble embryonic stem cells but are grown from adult cells.

The various methods create different kinds of organoids, each with advantages and drawbacks. Kuo's organoids contain a mix of cell types, which enables "observation of higher-order behaviors such as muscle contraction," he says. Because those organoids include stroma, a scaffold of connective tissue essential for tumor growth, they may prove better for studying therapies that target the stroma, such as cancer immunotherapy. Clevers's mix of growth factors grows organoids consisting primarily of epithelial cells, so his technique doesn't work for the brain and other organs with few or no epithelial cells. Nor can his organoids be used to test drugs targeting blood vessels or immune cells because organoids have neither.

Both methods can generate organoids from individual patients, producing a personalized minigut in just 1 to 3 weeks. (Although Clevers's organoids originate from adult stem cells, isolating those cells isn't necessary; culturing a tissue fragment with the right nutrients is enough.) The methods are reproducible, and the organoids remain genetically stable in culture; they can also be stored in freezers for years.

In 2013, Clevers and others founded a nonprofit, Hubrecht Organoid Technology (HUB), to market applications. Clevers first proposed using organoids for tissue transplants, says HUB Managing Director Rob Vries. Studies showed that healthy organoids implanted in mice with diseased colons could repair injury. "But we bagged the idea because there were too many regulatory hurdles and the chance of success was low," Vries says.

The idea of enlisting organoids to treat CF came from Jeffrey Beekman, a researcher at UMCU who studies that disease. All Dutch newborns are screened for CF, and colon biopsy samples are taken from babies who test positive. The tissue is tested to gauge how dysfunctional the defective gene is and then stored. Growing organoids from those samples would be relatively simple, argued Beekman, who has since spearheaded the project.

CF can arise from more than 2000 mutations in one gene, which cripple the ion channels that move salt and water through cell membranes. The disease affects all tissues, but the primary symptom is excess mucus in the lungs and gut, causing chest infections, coughing, difficulty breathing, and digestive problems.

Ivacaftor and the combination drug lumacaftor and ivacaftor, both marketed by Vertex Pharmaceuticals in Boston, restore the ion channels' function. But the drugs don't work equally well for everyone, and they have been tested and approved only for people with the most common mutations, together accounting for roughly half of all CF patients. Vertex, which declined to answer questions for this story, has been reluctant to spend millions on trials in patients with rare mutations because the potential payoff is small. And with the price tag—both drugs cost between €100,000 and €200,000 per year in Europe—health services and insurance companies have been unwilling to pay for the medicines for people with those untested mutations.

Van der Heijden falls into that category because only two other people in the Netherlands share her mutation. But when organoids grown from her gut were exposed to lumacaftor and ivacaftor, the organoids swelled like normal gut tissue, a sign that the defective protein was working and that salt and water were flowing through. The result helped persuade Vertex to give her the drug through a compassionate-use program, without payment. (Regulatory agencies require her to be monitored in a clinical trial.) Her side effects included fatigue, nausea, and diarrhea, but after a few months, "it was as if someone opened the curtain and said, 'Look, the sun is there, come out and play,'" she says. "And I did."

In collaboration with Vertex, HUB has tested ivacaftor on organoids grown from CF patients who had taken part in a clinical trial of that drug. The study confirmed that organoids can predict who will respond to the drug.

HUB has also tested ivacaftor on organoids from 50 patients with nine rare muta-

Cystic fibrosis patient Els van der Heijden received a new drug combination based on organoid tests. Within weeks, "I had an enormous amount of energy," she says.

PHOTO: TESSA NEDEREND

tions. On the basis of the results, insurers agreed to pay for the drug in six more Dutch patients, and Vertex is following up with the first clinical trial of ivacaftor in CF patients with rare mutations. Meanwhile, HUB is building a biobank, financed by Dutch health insurers, containing organoids from all 1500 Dutch CF patients for testing both existing drugs and new candidates.

"This is the next big thing in CF research," says Eitan Kerem, head of pediatrics at Hadassah Medical Center in Jerusalem, who is building a similar biobank and has launched a trial in patients with rare mutations. Organoids are especially useful because no great animal models for CF exist, Kerem says; ferrets and pigs are sometimes used, but "they are expensive and not available to most researchers."

Drug and biotech companies are now striking deals with HUB to explore organoids in other diseases. The success with CF suggests that they can model other single-gene disorders, such as α-1 antitrypsin deficiency, which causes symptoms primarily in the lungs and liver. Some companies are also testing failed drugs on organoids and comparing the results with animal and clinical data, hoping to find ways to predict and avoid such failures.

CANCER IS ALSO a major target. By growing organoids from tumor samples, researchers can create minitumors and use them to study how cancer develops or to test drugs. Soon after the minigut paper came out in 2009, David Tuveson, who heads the cancer center at Cold Spring Harbor Laboratory in New York, began prodding Clevers to develop organoids for pancreatic cancer, which is notoriously hard to treat. Existing cell culture models were not very realistic, Tuveson says, and creating genetically engineered mice took up to a year, compared with up to 3 weeks for pancreatic cancer organoids.

The organoids have already helped clarify new pathways that lead to pancreatic cancer, Tuveson says, and unpublished data suggest that they will help researchers predict which treatments will be most effective. He and Clevers are trying to make the organoids resemble real cancer more closely by adding stroma and immune cells. The Hubrecht lab is also involved in two trials to assess whether colon cancer organoids grown from individual patients can predict drug response.

Charles Sawyers of Memorial Sloan Kettering Cancer Center in New York City is trying to make prostate cancer organoids, but he says they are finicky. Organoids from primary tumors generally don't grow; those from metastatic tissue sometimes do, but normal cells often outgrow cancer cells. "They seem to need a lot of tender love and care, and there is no method to the madness," says Sawyers, who has succeeded with only 20 patients so far.

But Sawyers discovered that he could easily grow organoids from normal prostate tissue—"it just works beautifully," he says—and then use gene-editing techniques such as CRISPR to study any cancer mutation he wants. "Is this a tumor suppressor gene? Is this an oncogene? Does it collaborate with gene *XY*? You can play the kind of games on the scale that you always wanted to," he says. As Kuo puts it, "We can build cancer from the ground up."

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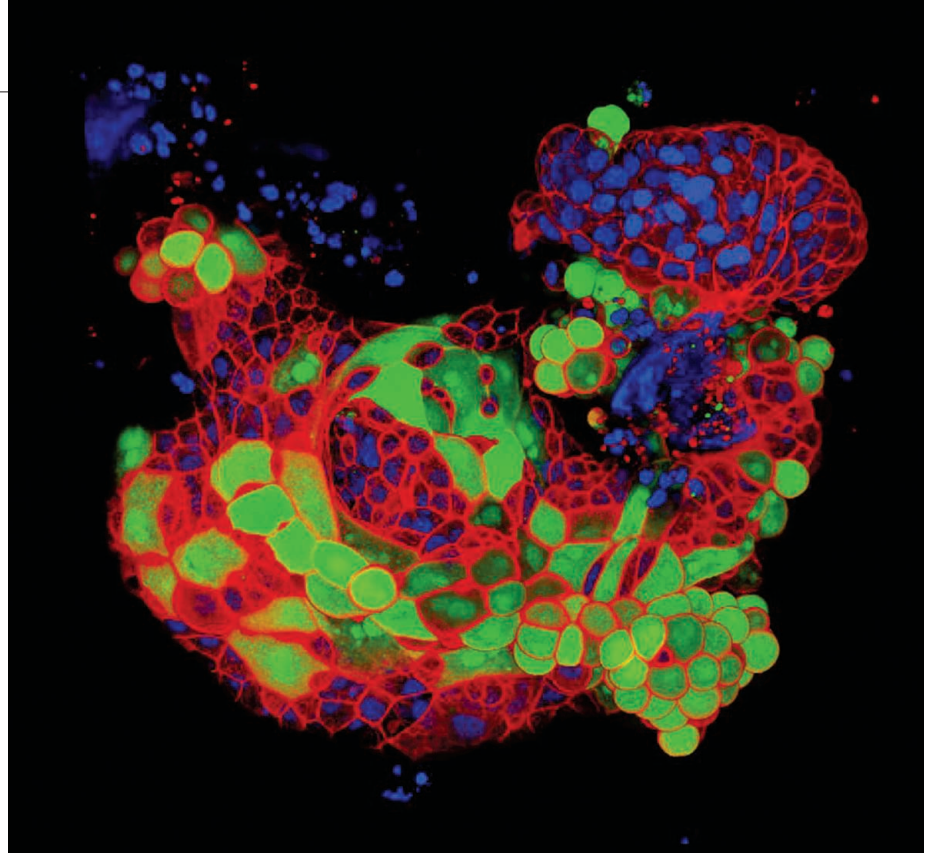
> **Rudolf Jaenisch,** Massachusetts Institute of Technology

Other cancer researchers want in, too. Tuveson received so many requests for organoid training that he began hosting regular workshops at his laboratory. In 2016, the U.S. National Cancer Institute launched a scheme to develop more than 1000 cell culture models, including organoids, for researchers around the world to use, together with Cancer Research UK in London, the Wellcome Trust Sanger Institute in Hinxton, U.K., and HUB.

Using personalized organoids to treat cancer still faces hurdles. Organoid culture time, which varies by cancer, must be shortened, and the cost, a few thousand dollars per patient, needs to come down. Also, cancers accumulate genetic mutations as they progress, which could mean that an organoid grown from a patient's cancer early on might not reflect its later state. Nevertheless, "from my perspective it's the most transformative advance in cancer research that I know of," Tuveson says.

If all of that excites Clevers, he rarely shows it. He avoids emotional language while discussing his research, preferring instead to describe and explain. Even close friends sometimes find his pragmatism puzzling. "He talks about his research like someone talking about screwing in a screw," Nieuwenhuis says.

Clevers says he gets his high from "the satisfaction of finding something novel," regardless of practical applications. Recent experiments, for instance, suggest that when an organ lacks LGR-5-positive cells, differentiated cells may be able to "dedifferentiate" and repair tissues—a radical change from the one-way street toward specific identities that stem cells were thought to travel. "Some organs may not have a professional stem cell at all," Clevers says, with a hint of wonder. But when asked how he felt when he saw his findings have profound benefits for patients such as Fabian and Els van der Heijden, he simply says, "I did not expect that." ■



Organoids can be used to study how pathogens interact with human tissues. In this lung organoid grown in Hans Clevers's lab, cells colored green are infected with respiratory syncytial virus.

PHOTO: NORMAN SACHS

The organoid architect

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Science **357** (6353), 746-749.
DOI: 10.1126/science.357.6353.746