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Cystic Fibrosis Patients Benefit from Mini Guts

The journey from laboratory breakthrough to help for real patients can be slow and elusive, especially in stem cell research. In one area, however—the burgeoning science around mini organs grown from living tissue, known as organoids—progress is happening remarkably fast. A team in the Netherlands headed by geneticist Hans Clevers has already begun using organoids built from gut cells to help people suffering from one of the most common hereditary conditions in the world, cystic fibrosis.

Clevers has a research group at the Hubrecht Institute, part of the Royal Netherlands Academy of Arts and Sciences, and is the research director at the Princess Maxima Center for pediatric oncology. His laboratory was instrumental to the discovery of stem cells in the gut. In 2007, he and post-doctoral fellow Toshiro Sato, now based at Keio University in Tokyo, observed that intestinal stem cells in mice do much more than divide and differentiate into different types of gut cells. With a little encouragement, these cells are able to self-assemble into blobs resembling the crypts and villi of the intestine. "They are probably the most active stem cells of our body," says Clevers.

By 2009 his team had found a way of isolating these cells from bits of tissue in culture and growing them for as long as they wanted. These 3D structures are known as intestinal organoids. "They are mini guts," Clevers explains. "Although they are tiny—they are one or two millimeters in size—when you look through the microscope, they essentially are guts." If cells are taken from a rectal biopsy, the mini gut that's grown as a result mimics the person's own. It's almost equivalent to having a living replica of their organ outside their body, with the same genotype and phenotype.

This has exciting implications for clinical research. Instead of testing drugs on patients, for example, molecules can be tested directly on the patients' intestinal organoids. There are two types of diseases that are amenable to this kind of work. One are cancers. Tumorous tissue can be removed through biopsy, before the cells are tested in vitro against an assay of cancer drugs, which can help researchers spot which drugs work best. "We've done this in collaboration with quite a few groups now. We have published on colon, pancreatic, and prostate cancer," says Clevers. They are working on breast and lung cancer, too.

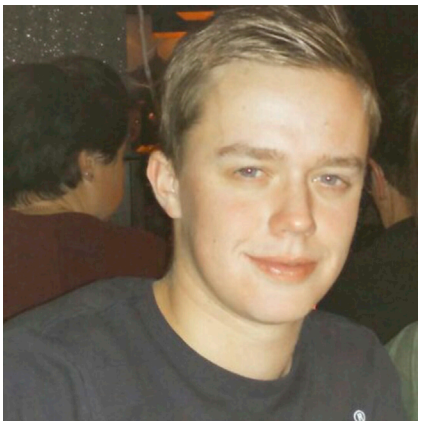
The other type are inherited diseases, such as cystic fibrosis, because they have a genetic presence in the intestinal stem cells. Cystic fibrosis is caused by a faulty CFTR protein that affects the ion channels of cells, making them struggle to move salt and water through cell walls. This leads to a buildup of thick, sticky mucus in the lungs, which subsequently becomes a cauldron for bacteria. Symptoms usually start in early childhood and include recurring chest infections, coughing, and wheezing. The same problem in the gut can cause diarrhea, bowel obstructions, and poor weight gain. This can in turn create complications such as diabetes and even infertility. Until recent decades, cystic fibrosis patients were rarely expected to survive into old age.

Around 4 years ago, Clevers was approached by Jeffrey Beekman, a former member of his department who was by then working in the Department of Pediatric Pulmonology at Utrecht University Medical School. The head of the cystic fibrosis lab there, Kors van der Ent, who is also head of pediatric pulmonology at Wilhelmina Children's Hospital, had spent many years looking at better ways to treat cystic fibrosis. Beekman wondered whether intestinal organoids might hold the key.

Cystic fibrosis is easily diagnosed after birth with a heel prick test, making it fairly straightforward to build databases of patients. In Europe, the World Health Organization estimates that one in every 2,000 to 3,000 babies is born with the condition. In the United States it's thought to be one in every 3,500. Treatments, including physiotherapy to combat the buildup of mucus and antibiotics to kill off infections, are helpful, but only recently have drugs been developed that combat the underlying symptoms. In Holland, one drug is reimbursed by health insurers under the Dutch government's medicines reimbursement system. Known commercially as Kalydeco, and developed by Vertex Pharmaceuticals, it opens up a person's ion channels to allow the easier movement of salt and water.

Although it is highly effective—indeed, it's been described as a wonder drug—Kalydeco doesn't work for every patient. There are roughly 2,000 different genetic defects that all lead to cystic fibrosis, and only a small fraction of these respond positively to the drug. If a patient's particular mutation isn't one that has been clinically tested against Kalydeco, the likelihood of success is considered too low to warrant prescription of the drug. The other problem is that Kalydeco is extremely expensive, costing hundreds of thousands of dollars per patient every year.

"Therefore it's very helpful to have, as we often call it, a sort of a brother or sister of the patient in the lab that can be used for testing," says Kors van der Ent. The team thought that perhaps intestinal organoids could play the role of these "brothers" and "sisters." With a personalized mini gut, they realized that it might be possible to test a person's response to Kalydeco quickly and cheaply, without causing them any disruption beyond a relatively painless rectal biopsy.



19- year-old Fabian, who lives in the Netherlands, has been the first Cystic Fibrosis patient to be helped using intestinal organoid technology. The photo was taken in November 2015, after his treatment with Kalydeco had begun. Image courtesy of the patient.

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By early 2015 they had their first patient, a boy called Fabian, who is now aged 19. He is unusual among people with cystic fibrosis because he has a genetic defect shared by just one other person in the world, his aunt. Since Kalydeco hadn't been clinically trialled on either of them, he wasn't eligible for the drug. His mutation was simply too rare.

At the same time, Fabian desperately needed treatment. Kors van der Ent, who has been his doctor since Fabian was young, says that his lung function at the time was down to just 30%. He was even considering a lung transplant. "He had to miss a lot of school and he couldn't do sport any more. He was in a very bad shape at that time," he explains. "So, we said, we have nothing to lose."

The team grew a mini gut using Fabian's intestinal stem cells to see whether Kalydeco might open up his ion channels. If it worked, his organoids should swell up, just the way they would in a healthy patient whose cells are processing salt and water effectively. This is exactly what happened. It meant that the drug was effective for the genetic defect that caused his cystic fibrosis. As a result, Fabian was given the drug to try in person. The results were incredible, he tells me. "The first time I used Kalydeco was astonishing because it immediately worked so good already after 4 hours… there came so much slime from my lungs." The opening of ion channels meant that the sticky mucus that had plagued his lungs and gut for so many years was finally clearing.

Van der Ent stopped treatment for a while to be sure that it was Kalydeco causing the improvement. Within a few days, his symptoms returned. Fabian was then officially prescribed Kalydeco again. He was so excited to restart it that he couldn't even wait to finish the journey home to take the pill. Told it had to be taken with food, he stopped at a McDonald's restaurant on the way and had it with some French fries. "The drug really changed my life because I don't feel I have cystic fibrosis anymore. I still have it but I don't feel it," says Fabian. Today, he is able to play hockey again and is starting university.

"I feel really proud of what we have established as a team of basic and clinical scientists, patients, and the Dutch cystic fibrosis foundation," says Jeffrey Beekman. "We moved from first observation that organoids could swell to clinical impact in only 4 years." Since Fabian, three other patients have been successfully treated the same way. The scope for testing is limited to those with rare mutations who wouldn't be prescribed the drug under ordinary conditions, but for Beekman, Clevers, and van der Ent, it nevertheless represents progress.

According to Melissa Little, a professor at the Murdoch Childrens Research Institute in Melbourne, Australia, who has done pioneering work on kidney organoids, the significance of their results is hard to overstate. "The generation of organoids from adult epithelial stem cells is now delivering personalized medicine," she says. "Its application to understanding cancer is also incredibly important and will become the standard functional genomics approach in the personalized treatment of cancers such as colon and pancreatic cancer."

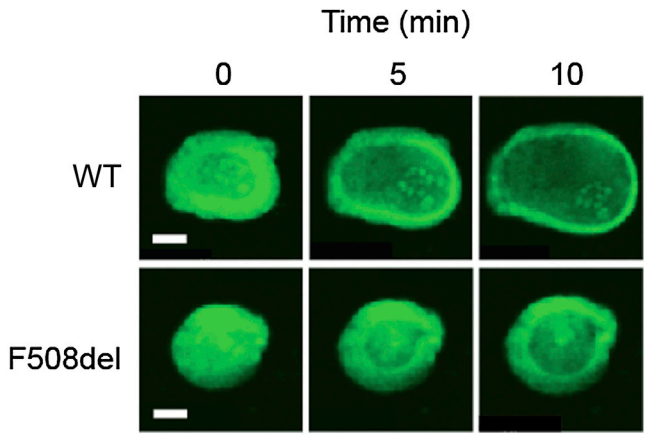
Hans Clevers' work has earned many awards and honors over the years, including the 2013 Breakthrough Prize in Life Sciences. But nothing has been more thrilling for him than to see his work on intestinal organoids being used in real patients. "It is very rewarding when you make a discovery that somebody benefits from. We've published a long string of papers in prestigious journals, but this is different. Fabian is really the first person to directly benefit from all our so-called breakthroughs," he says.

There are estimated to be 1,500 cystic fibrosis patients in Holland. Clevers would like to see every one of them deposit tissue into a biobank, to both improve research into the condition and screen them against drugs. "When my lab realized that this technology was going to be very broadly applicable, and that it would benefit enormously from large biobanks, we discussed this for a long time and in the end decided not to form a biotech but a not-for-profit foundation," he says. "Most patients would feel much more comfortable if their tissues would be in a biobank and available for research by colleagues in academia, and also by industry, if it would go through a not-for-profit organization. So that's been set up."

Biologist Rob Vries, who used to work in Clevers' lab, now runs the foundation that holds the intellectual property around his organoid research. Called Hubrecht Organoid Technology (HUB), it has already started working with Dutch health insurance companies to establish a living biobank using tissue from cystic fibrosis patients. So far, more than 300 patients have been biopsied.

The plan is to screen these patients for their suitably for Kalydeco, should they not already take it, as well as any future drugs. "That's a promise to the patients that we will keep their organoids, their mini guts, and as soon as there's a new drug, we might test again and see if there's anyone in our bank who might qualify for it," says Clevers. The obvious benefit to patients is that drugs can be tested without them even being involved. Their existing mini guts do all the work.

At the same time as all this has been happening, pharmaceutical companies have been inching closer to identifying new treatments for cystic fibrosis. "There's a lot of drugs in the pipeline,"



Organoids from a healthy individual (WT) and a cystic fibrosis patient (F508del). The WT organoids have properly functioning ion channels, which allow them to swell. With appropriate drugs, the organoid of a cytic fibrosis patient will see similar swelling. Image courtesy of Jeffrey Beekman and Kors van der Ent.

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says Clevers. ''There's currently only one drug registered in Holland. There are negotiations over a combination of two drugs. But we can already see there are five or six other drugs that work differently.''

Ultimately, Clevers would like to see the success they have witnessed so far with patients such as Fabian give way to an entirely new way of administering drugs for diseases such as cystic fibrosis. Rather than registering a drug because it happens to work on the largest statistical population, the process could instead be based on a fast, cheap personalized assay. This would prevent expensive drugs being prescribed unnecessarily, limit the disruption to patients, and allow drugs that work on only a small minority of patients to come to market. Since biopsies can be done on infants, it would be possible for a patient's particular cystic fibrosis mutation to be understood and quantified before they even develop symptoms. The burden of clinical testing shifts from in vivo to in vitro, from the organ to the organoid.

Dutch health minister Edith Schippers has been broadly supportive of these efforts. ''New drugs are ever more specific, yet ever more expensive. There is an urgent need for strategies to match individual patients with the best available drug. Fabian's organoids have provided exactly this for him,'' she says.

This clinical research need not stop with the gut, either. Theoretically, the same procedure would work for other organs, too. ''We can do this from gut, from lung, from stomach, from liver, from pancreas,'' says Clevers. ''At an individual patient level, we can isolate the disease tissue, grow it, find out what's wrong, and find drugs to correct it.''

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