Research

Caring for the rare cases

by Hubert Filser

Source: Verena Müller

"I want to be able to give my patients answers," says Christoph Klein, LMU pediatrician and clinical researcher. He studies rare diseases, seeking to determine their causes and find experimental therapies. This takes time, but many of his patients have little to spare.

It is a strangely uplifting moment. Äyä is lying in an isolation ward. The little girl has been ill since birth, has undergone many operations, has lost much of her intestinal tract, and cannot digest most normal foods. But now, as she gazes up at Christoph Klein, she has a blissful look in her eyes. Klein, pediatrician and Director of the Dr. von Hauner Children's Hospital, has just explained to the 9-year- old that her illness is a very exceptional one. Indeed she is the first person in the world known to have it. "We have discovered the cause of this very rare disease you have," he says. "A single one of the 3.2 billion genetic letters in your cells is out of order, and we now know which one it is. We have found the needle in the haystack." Äyä leaves off eating her soup, and her eyes light up for a moment. "And no one else has it?" she asks. "Then I'm famous." In this instant the child feels very special. "There is one other girl from Beirut, but we owe the discovery of this disease to you," Klein says.

Äyä's condition still doesn't have a name. The findings made by Klein and his team won't be published until next year. But, at her bedside, Äyä's mother asks: "What happens next? Can Äyä now be helped?" Unfortunately, here, Klein has to dampen hopes of rapid improvement. "That will take time," he replies.

This is another of those research breakthroughs that spark great expectations, but provide little immediate benefit for patients. It often takes years or even decades to translate research findings into effective treatments. And for sick children, these may come too late, as Christoph Klein is all too aware. "Nevertheless, these children and their fate are my motivation; they are the point of departure for my work," he says. Klein sees himself as a clinician and as a scientist. As a pediatrician and oncologist, Klein supervises the treatment of all his patients at the hospital. As a researcher he focuses on the rarest conditions. These are often called "orphan diseases", as no pharmaceutical firm wants to spend money studying them because the small numbers of patients mean that the economic returns would be paltry.

Replacing the defective gene

Klein has won many awards for his work, including the Leibniz Prize, the most important German accolade for research, in 2010, and a highly endowed Advanced Investigator Grant from the European Research Council (ERC) in the following year. But in his chosen field, "light and shade lie very close together," as he puts it. A new treatment, such as a genetic therapy for the rare Wiskott-Aldrich syndrome (WAS), is a bright patch, but then there are the shadowed areas, such as the story of Sulin. Her case put him on the right track as a researcher, but the right treatment came too late to save her.

Hardly anybody has ever heard of Pompe's disease, the Chiari malformation or the Wiskott-Aldrich syndrome just mentioned. These terms refer to uncommon diseases that are named after the doctors who first described their characteristic symptoms. About four-fifths of all such diseases are due to single gene defects. As in Äyä's case, a single error in the genetic code can result in a devastating, often fatal, disease. And Äyä is only one of approximately 100,000 individuals in Germany who suffer from some very rare, often poorly defined, inborn immune defect. As that figure reveals, in absolute terms, such disorders are not so rare at all. Germany has cases of between 5,000 and 7,000 rare diseases, and the total number of patients affected is on the order of 4 million. "In Europe a disease is referred to as rare, if it affects fewer than 5 out of every 10,000 people," Klein explains.

Scientists like Christoph Klein try to correct such deleterious genetic errors by replacing the defective gene with an intact copy. Seven years ago, in one of the first attempts to apply gene replacement therapy undertaken anywhere, he and his

colleagues began to treat WAS patients using this approach. One in a million newborns suffers from WAS. These children are immunodeficient, develop eczemas and are very susceptible to infection. Blood coagulation is also perturbed, as these patients don't produce enough of the platelets that initiate the process, and those they have are small. While normal individuals have several hundred thousand platelets per milliliter of blood, in WAS patients the number can be less than 10,000.

Klein designed an experimental stem-cell-based therapy to treat patients with this disorder. Hematopoietic stem cells are isolated from affected children, and viruses are used as vehicles to insert intact copies of the WAS gene into the genomes of these cells in place of the defective version. To avoid side-effects and ensure its long-term expression, the new gene must be integrated correctly and stably. "This was terra incognita for everyone," Klein points out. "With genomic manipulations, there will always be side-effects, and applying such experimental therapies to children poses ethical questions, and confronts parents with a difficult decision." So, only children who had little chance of survival otherwise were considered at all. "We also committed ourselves to long-term monitoring and care of these patients, irrespective of the cost," he adds.

So far, ten patients with WAS have been treated with the modified stem cells in the context of a clinical trial. In none of these cases could a suitable donor be found to enable a classical allogeneic bone-marrow transplant to be carried out. In 2006, 3-year-old Sergey from Russia became the first patient to be enrolled in the trial. Without treatment, most of these children die between the ages of 10 and 15. And they and their families live on a knife-edge. Take Felix from Koblenz, who also took part in the study. Every little fall could have led to fatal internal bleeding, and only now has his anxiety begun to diminish. He is now rated as healthy. "But there is no telling what may happen in 20 years," Klein says.

His caution is well-founded, as the therapy has not been an unmitigated success. Unfortunate after-effects continue to appear, so the treated children come to Munich for a thorough clinical evaluation every three months. Sergey, who is now 10, developed leukemia about a year ago, and five others also have this blood-cell cancer. "On the one hand, we were pleasantly surprised how successful the treatment turned out to be," Klein says. "On the other, the long-term side-effects are entirely unacceptable. We must now construct new gene-delivery vehicles and make appropriate changes to the study design before we can recruit any further patients." Happily, the doctors have been able to arrest the progression of leukemia in all of the six children affected. "Without the help of donations, the treatment would have ruined us financially," says Klein.

The patients treated by his French colleague Alain Fischer are also exposed to the risks associated with genetic manipulations. Of the 20 children in his study, five have developed leukemia. "A new therapy is always a journey into the unknown," Klein says. The ideal gene therapy requires a kind of high-precision surgery. The damaged gene must be removed, and the intact version inserted, without altering the DNA on either side in the slightest. "There have been hopeful technical developments in this area," says Klein, "but clinical applications are a long way off."

In Sulin's case too, the margin between success and failure was very narrow. Klein takes up the story again: "Sulin was 9 and suffering from jaundice when I first saw her. Her rate of growth had slowed, and her liver was scarred." She was listed to receive a liver transplant, but the ultimate cause of the inflammation of her liver remained unknown, and Klein and his team were determined to identify it. "As doctors, we have to pin down causes, especially where the symptoms do not conform to textbook patterns," he says. His best guess was that single-celled microorganisms of the genus Cryptosporidia might be involved. In a healthy individual, these protozoan parasites induce transient diarrhea, but Klein suspected that Sulin's immune system was too weak to eliminate them, provoking chronic inflammation of the bile ducts and liver. After three years of intensive research his intuition was vindicated. He found a genetic error in the gene for the so-called interleukin-21 receptor in Sulin's genome, the first patient

A single one of the 3.2 billion genetic letters in Äyä's cells is out of order. *Source: Verena Müller*

worldwide with this defect. Interleukin-21 is a signal molecule that promotes the differentiation of immune memory cells. Lack of functional memory cells would explain why a parasite infection could become chronic. "And in Sulin's case, these cells did not function correctly," says Klein, "because she did not have the receptor for interleukin-21."

A glance at the statistics reveals how important it is to have a definite diagnosis. No less than 21% of children who suffer from a rare disease have been seen by at least five doctors without receiving an accurate diagnosis. "False diagnoses are very frequent in this field," Klein confirms. "Many of these illnesses, like Sulin's, are literally unknown." Of course, the researchers set out to correct the functional defect in Sulin's memory cells by replacing her entire immune system. Transplantation of hematopoietic stem cells from healthy tissue-matched donors allows all types of immune cells to be replaced, and would effectively cure her immune deficiency. Sadly however, Sulin's liver was already so extensively damaged that a stem-cell transplant could not save her. "Had we identified the defect sooner, she would very probably still be alive," says Klein. "We dedicated the discovery posthumously to her, and we hope that other children will profit from it."

Thanks to his international contacts, Klein has found two siblings in the US, who have the same genetic defect as Sulin and can now be treated. The Dr. von Hauner Hospital is a "Care for the Rare Center", one of the coordinating institutions in a worldwide network which is devoted to doing everything possible for children with rare diseases. The Care for the Rare Foundation was founded by Klein himself, with the intention of gathering and collating research findings and case reports from physicians in Europe, Africa and Asia. The idea is to compile sufficient data to enable reliable conclusions to be drawn. In Germany, there are currently 12 networks for rare diseases. In the Munich Center patients and their parents can seek help and receive a diagnosis directly, without having to embark on a long and often vain search for the right specialist. Klein takes this issue personally. "When a child asks: 'What is wrong with me, doctor?', I want to be able to answer the question."

His daily rounds keep reminding Klein of the need for early identification of genetic defects. He and his team plan to set up a model screening procedure for the detection of cases in which immune cells are missing or non-functional. "For me, pediatrics has always been a branch of preventive medicine," he says. "If one correctly identifies disorders in children early enough, treatments can begin early." Children with immune deficiencies can be helped by infusions of immunoglobulins or by bone-marrow transplantations, and success rates are higher, the sooner the intervention is performed.

"So far, health insurers have refused to support the screening project, arguing that it is purely a research venture," Klein says. It is an argument he cannot comprehend. "Nowadays, medicine is viewed above all in terms of economics. Of course we must make responsible use of our limited resources, but one should not forget that, in university hospitals particularly, we also have a responsibility to the academic tradition, to continuously extend the boundaries of our knowledge. Practical medicine owes its progress to advances based on rational, scientific thinking. And new discoveries have often come from studies on single patients." Insights gained from rare cases sometimes do put researchers on the trail of critical mechanisms that are of general significance in cell biology and gene regulation. And when they do – as in the case of Äyä – Klein and his colleagues can set about developing effective therapies. And with luck, in a few years, perhaps Äyä herself will reap the benefits of their tireless efforts.

LMU pediatrician and clinical researcher Christoph Klein: children with rare diseases and their fate are his motivation. *Source: Manfred Lehner*

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