A FATHER'S FIGHT

One man's quest to give his sons weedkiller could help thousands struggling with rare genetic conditions.

BY DAVID ADAM

Red cabbage? Nick Sireau couldn't understand why the doctor was asking about red cabbage. It was late one night in October 2000, and Nick and his wife Sonya had just made an alarming discovery. Urine from their two-week-old baby son Julien had suddenly turned dark red, almost black. The physician who came to their cramped London flat assured them that it wasn't blood. Perhaps, he suggested, the pigment from some red cabbage that Sonya had eaten for lunch had made it into her breast milk and was colouring the boy's urine. He told them it was nothing to worry about.

Nick hadn't studied biology since school, but something about the doctor's explanation didn't feel right. So he and his wife sought a second opinion and were referred to a specialist at a London hospital.

The colour wasn't from the cabbage. Tests revealed that Julien had a rare genetic disease called alkaptonuria (AKU). The staining came from a rogue chemical metabolite, a by-product of not being able to fully process certain proteins. The boy's body was dumping massive amounts of the substance into his urine, which turned red when exposed to air.

Worse, the chemical was circulating in his blood and accumulating in his joints and soft tissue — in his eyes, his tendons and even his heart. By adulthood, the specialists said, Julien's body would show the wear and tear of a much older person. AKU is not usually fatal, but people with it often have to get elbows, knees and hips replaced by the time they turn 40. Nick and Sonya could limit the amount of protein in Julien's diet to slow these effects, but aside from that there was nothing to be done. No treatment was available.

"It was a real shock," Nick says. "We had never heard of it and had no idea what to do. The doctors told us not to look it up on the Internet, but of course that's the first thing we did. It was horrible."

Nick plunged himself into trying to find some relief for his son. As it turned out, researchers had identified a possible treatment. But the drug was being given to help infants survive a different condition — it wasn't approved for AKU. And the path to approval was blocked by a considerable obstacle: the need for a full-scale randomized clinical trial. That's expensive and difficult enough for medicines used to treat common diseases. It's much harder for a condition that is almost unheard of.

Now, in no small part due to Nick's efforts, this treatment could be widely available for people with AKU in Europe within the next few years. It would be the first effective way to stop the disease, which researchers identified more than a century ago. But the web of challenges that Nick faced serves as a cautionary tale for patients and researchers battling other rare disorders. The number of such conditions will no doubt increase as genetic advances identify the need for treatments targeted to smaller and smaller populations.

"We do need rigorous and robust scientific processes," says Alastair Kent, former director of Genetic Alliance UK, an umbrella body for more than 200 rare-disease patient groups. "But we also need new ways of proving the quality, safety and efficacy of new drugs."

Nick Sireau quit his job to help find an effective treatment for alkaptonuria.

BRIAN DAVID STEVENS

Nick is trying to ensure that the journey will be smoother for others than it has been for him and his family.

HISTORIC FOE

Archibald Garrod first described AKU in 1902, drawn to it by the colour-changing urine, which is caused by the build-up of homogentisic acid[1]. He noticed that it followed Mendelian inheritance patterns, and it became the first disease ascribed to an inherited cause — although the part of the genome responsible would not be discovered for another 90 years or so[2].

It occurs when a person has mutations in both copies of the gene for an enzyme known as HGD. The enzyme is found in the liver and kidneys, and helps cells to break down the amino acid tyrosine[3]. AKU became a staple of genetics textbooks, but Garrod's early description of the disease was as a harmless curiosity, and that has unfortunately stuck.

Like many rare conditions, AKU was not seen as big or lucrative enough to warrant much attention from drug developers. Physicians didn't see it as serious, and people with the disease were too few and scattered to lobby for change. So when Nick started to search the Internet for help with his son's newly diagnosed condition, he found little to go on — just some information posted by a patient in Liverpool, UK. Nick caught a train north to pay him a visit.

Bob Gregory was a straight-talking former trade-union and city-council official who had been diagnosed with AKU later in life. Like Nick and Sonya, he had been told there was no treatment. But Gregory told Nick that he was trying to change that. He had met with a consultant at Liverpool's university hospital and unleashed his frustration. Why had medicine abandoned people with this condition? The disease had solidified his joints, he said, and made it feel like he lived his life wearing a suit of chain mail. Why were scientists not trying to find a cure?

"The doctors told us not to look it up on the Internet, but of course that's the first thing we did."

The physician — Lakshminarayan Ranganath, widely known as Ranga — said he would do whatever he could do to help. Or at least, that's how Gregory remembered it — and he promptly registered Ranga (without initially telling him) as the medical director for a charity he wanted to set up. Nick came on board too, promising to raise money to support Ranga's research.

Ranga agreed to take part. But he didn't actually plan on doing much research. He was busy with his work on diabetes and obesity, and

he knew that US physicians were planning a clinical trial for a promising drug. "I really did think it was going to take up very little of my time," Ranga says. "I thought the main challenge was going to be how to make the drug available once the trial finished."

That trial was of a drug called nitisinone, which was originally developed as a weedkiller in the 1980s but was found to have toxic effects in fish and rats. Its development as a weedkiller was halted, but the company that owned it, Zeneca Agrochemicals, asked some specialists in the United Kingdom to investigate how it worked. The researchers discovered that it kills plants in an unexpected way: it chokes off their supply of chlorophyll by disabling an enzyme called HPPD. According to the scientific literature, the world's leading expert on HPPD was Sven Lindstedt, a clinician at a hospital in Gothenburg, Sweden, so in 1991, the researchers gave him a call. His response astonished them: he wanted to give nitisinone to children.

Lindstedt was searching for a way to inhibit the enzyme in order to save the lives of babies and children with a condition called type-1 hereditary tyrosinaemia (HT1), which results from a disruption to the same metabolic pathway affected in AKU. Although the weedkiller was toxic, the effects of HT1 were so devastating and deadly that it was considered worth a shot. Lindstedt described the results in a 1992 paper in *The Lancet*: the treatment worked better than anyone had hoped[4].

Under laws that popped up around the world in the 1980s, companies could reap financial incentives — such as extended patent protection — to commercialize treatments for rare diseases such as HT1. These 'orphan drugs' could command a hefty price. So, the Stockholm-based company Swedish Orphan Biovitrum acquired the license for nitisinone and marketed it as Orfadin.

Now, a compound so cheap that it was intended to be thrown on the ground is generating millions of dollars in sales — US$96 million in 2017. And all without a randomized controlled trial. HT1 was considered so serious that the drug was first given under a compassionate-use exemption, and later under an agreement that the company would gather the necessary data on safety and efficacy afterwards.

INFLEXIBLE ENDPOINTS

Physicians realized that what worked in HT1 should, in theory, also work in AKU. Disabling HPPD halts the breakdown of the amino acid tyrosine. Slowing down the metabolism of tyrosine in people with AKU should also stop them producing so much HGA.

Patients knew this, too, and many were desperate to get their hands on the drug. Some succeeded, and reported improvements, but most attempts by physicians to prescribe the drug 'off-label' were turned down by medical insurers and other gatekeepers because it was too expensive. "I couldn't get nitisinone for Bob. It cost £4,000 [US$5,000] a year," says Ranga. In the United States, the price tag was closer to $30,000. To get it covered required approval, and that meant a trial.

The US government stepped in. From 2005 to 2008, researchers at the National Institutes of Health (NIH) tested nitisinone on people with AKU. This was the trial that Ranga had hoped would report positive results. With hindsight, however, the study was doomed from the start: just 40 patients were recruited, half of whom were not given the drug. And success of the treatment — the clinical endpoint — was defined in a very narrow way. Doctors measured participants' hip flexibility. When the treatment group didn't show a significant difference over the control group in this measure, the trial was deemed a failure.

"It was very difficult news," says Wendy Introne, the NIH scientist who led the study. "I knew the scientific community was waiting and we all expected nice clinical results to come out of it."

Part of the problem was that the hip movement in the control group didn't deteriorate as much as expected. Introne speculates that this was because all trial participants received regular physical therapy as part of the study. The failure of the trial was especially frustrating, she says, because nitisinone worked exactly as expected from a biochemical perspective: HGA levels plummeted in the participants receiving treatment[5].

Still, without meeting its endpoint goals, nitisinone was not going to win approval in the United States. And insurers and other health officials refusing to pay for off-label use elsewhere now had another reason: the evidence suggested that it didn't work. Suddenly, the Liverpool charity was the disease's next great hope. "We knew it could work," Ranga says. "And we knew we could show that."

A LARGE TRIAL

For a clinical trial to succeed, and to convince regulators, the Liverpool team would have to find a more-reliable endpoint. That meant tracking how the disease progressed, both in animals and in people. This would require more research, and that meant money.

Nick's first fund-raising effort was probably not what he initially had in mind. In 2005, he ran a sponsored half marathon to raise the money needed to transport the dead body of a 74-year-old with AKU from Nottingham to Liverpool. ("It cost £450," Ranga says. "I've still got the receipt.")

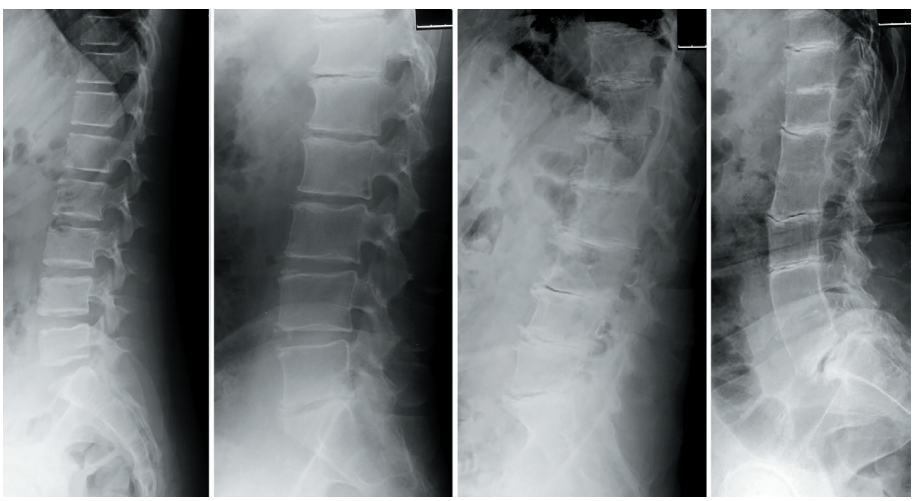
The results of the post-mortem were intriguing: although the woman's soft tissues were completely rigid and black with HGA build-up, the patterning in tissue such as bone was patchy. This suggested a complicated mechanism by which the damaging deposits build up. "That was the starting point," Ranga says. "Our ideas for researching AKU began from the findings of the post-mortem."

Nick — who gave up his job and started working on AKU fund-raising full time in 2010 — secured £500,000 (US$633,00) from Britain's Big Lottery Fund. This went to James Gallagher, a musculoskeletal researcher at the University of Liverpool, to develop a mouse model of the condition. With the right gene knocked out, mice showed the expected build-up of HGA — and the expected decline in that chemical when they were given nitisinone[6].

By writing to Britain's 60,000 general practitioners, the Liverpool group then managed to identify and analyse the symptoms of 81 people with AKU in the United Kingdom. (An astonishingly high number given that only 70–280 such people exist in the region.) The researchers used these observations to generate a severity index and assess the combined impact of symptoms.

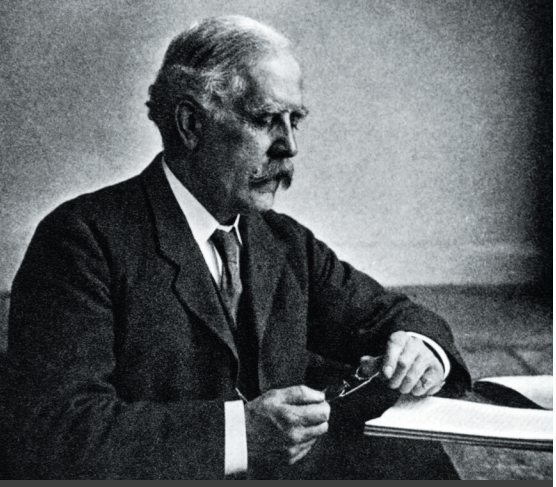
Next was the big one: a full-scale clinical trial. "We knew more about AKU than the NIH by then," says Ranga. "We thought we could do it right." In 2012, the European Commission agreed and said it would hand over £5 million to fund a full-scale trial.

A complication emerged, however. Thanks



LAKSHMINARAYAN RANGANATH

Homogentisic acid can obliterate cartilage in the spine as alkaptonuria progresses (from left to right).



SPL

History of an orphan

Alkaptonuria (AKU) has been known about for more than a century and has been a prominent disease in the course of modern medical genetics.

1500 BC

Signs of the disease are seen in the Egyptian mummy Harwa.

1902

Archibald Garrod (pictured) proposes that AKU follows Mendelian inheritance patterns[1], making it the first genetic disease ever identified.

1958

Bert La Du and colleagues demonstrate biochemically that a single missing enzyme is responsible for the condition[3].

1993

Martin Pollak and colleagues pinpoint chromosome 3 as the location of the gene encoding that enzyme[2].

2003

Bob Gregory starts the AKU society with physician Lakshminarayan Ranganath.

2005–08

A US clinical trial exploring the use of nitisinone finds some clinical benefit for AKU, but does not meet its primary endpoints[5].

2012

The National Alkaptonuria Centre launches in Liverpool, UK. Nitisinone is made available to residents as part of an observational study.

2015

A large-scale, randomized controlled trial begins to test nitisinone in Europe. It includes surrogate endpoints and no placebo, but should satisfy European regulators.

2019

European trial is expected to end.

in part to Nick's lobbying, the UK National Health Service (NHS) agreed to make nitisinone freely available to all patients over the age of 16 in England and Scotland, as long as they travelled to a centre in Liverpool (that is now named after Gregory, who died in 2014).

In a sense, Nick had achieved his original goal: his son Julien, and younger son, Daniel, who is also affected, would get access to nitisinone. But now, patients in the United Kingdom couldn't join the trial — too many had access to the drug through the NHS. So the team looked abroad and identified another 400 people with the disease across countries in Europe and beyond. The new crop of recruits includes a group of 19 patients from Jordan, where AKU is unusually prevalent in some rural villages. Mohammed Alsbou, who is coordinating efforts there, says that he has uncovered large pockets of the disease. "Many of them are relatives from my village in the south of the country," he says.

> *"I think we've made a real difference and it's lovely to be able to do that."*

The European nitisinone trial began in 2015 with 138 patients, and the researchers were able to stretch some of the usual confines of randomized trials. Unusually, there is no placebo — the drug stops urine from turning black, so it's obvious to patients whether they are on it. Instead, the control group is left untreated, and are very much aware of that. That was a "heartbreaking" decision, says Nick. Still, only a dozen or so of those patients dropped out — which Ranga says demonstrates their dedication to finding a treatment. "These are motivated patients since they were previously completely isolated," he says.

The European trial is bigger than the NIH effort, but there is a another, more-important difference. Instead of having to demonstrate a significant clinical benefit (in hip rotation or any other anatomical measure) regulators suggested that the trial be judged mainly on a surrogate endpoint — reduced levels of HGA — accompanied by a vague 'positive trend' in alleviation of symptoms. If these can be met, nitisinone will be approved for AKU in Europe.

That's a good example of trial flexibility, says Kent. He suggests that regulators also consider variations such as *N*-of-1 trials, in which a treatment is introduced one patient at a time to build up a trend. He also argues for efforts that move more quickly towards conditional approval, which requires more data to be gathered after patients get access. Such steps will become more important for common diseases, too, he says, as genetic analyses split patient populations into smaller distinct subgroups. "At that point, the traditional approach starts to fall apart," he says.

Around the world, regulators are under pressure to speed up the approval of therapies without sacrificing safety and efficacy assessments. Some of these efforts are controversial — a scheme in Japan to approve stem-cell treatments before they are known to work, for example and 'right to try' laws in the United States that allow people who are terminally ill to take unlicensed medicines. Nick co-founded another charity in 2012 to help people with rare diseases and their carers advocate for orphan-drug development.

In the AKU trial, everybody involved expects people receiving the treatment to show the necessary improvements — not least because of some positive results reported earlier this year by the NHS, which officially labelled its access programme as an 'observational study'. The results showed not only the anticipated drop in circulating HGA, but also a reduction in the speed of disease progression, as measured by the symptom-severity index developed by the Liverpool team[7]. The paper concludes simply: "Nitisinone is a beneficial therapy for alkaptonuria."

Ranga says: "I can tell you the difference is immense. I think we've made a real difference, and it's lovely to be able to do that."

In large part thanks to his father, Julien Sireau — now 18 — received his first dose of nitisinone in August last year. His brother Daniel should get it soon, too. If all goes well in the European study, approval of the drug will put what is now a temporary UK supply on a more secure footing, and patients across Europe will gain access.

But that's not the case elsewhere. Nitisinone will still need to be approved by the US Food and Drug Administration to reach patients there. And unlike the Europeans, US officials have signalled that a surrogate endpoint won't be acceptable. "I haven't given up. I'm still optimistic about the drug," says Introne. Yet for many more couples who find red–black staining in their infant's urine, the long journey through — and against — the system is just beginning. ■

David Adam *is* Nature*'s Editorials editor and is writing a book on AKU with Nick Sireau.*

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CORRECTION

The timeline in the News Feature 'A father's fight' (*Nature* **565**, 148–151; 2018) erroneously located the National Alkaptonuria Centre in Cambridge, UK. In fact, it is in Liverpool, UK.