measuring the amount of virus in the blood, HIV researchers could conduct small, rapid drug trials to discover effective drugs and drug combinations. "The reason AIDS got solved is because the approval pathway allowed a biomarker endpoint," he says.

For neurological LSDs, Kakkis would like to use similar biomarkers that measure the amount of particular molecules in the patient's urine or blood. Many rare diseases have distinct biochemical markers, directly related to disease progression, that might be used in this way. But since 1992 the FDA has agreed only two new biomarker endpoints of any type, he says. "Nobody can break through with a new endpoint because the requirements are impossible," Kakkis says. The FDA needs to formulate clear scientific criteria for assessing new biomarkers, he adds.

But there has been some progress, says Kakkis. The FDA Safety and Innovation Act of 2012 stipulated that the FDA should consider novel endpoints, "especially in instances where the low prevalence of a disease renders the existence or collection of other types of data unlikely or impractical". And in August 2015, the FDA released draft guidelines for industry on the development of orphan drugs, including an initial discussion on biomarkers. "The ball is moving," says Kakkis, although he wishes it would move a bit faster.

FINDING FUNDING

Megan Donnell knows that her foundation's work will not end with the first approved therapy for Sanfilippo syndrome. "Advocacy will be critical when we have a treatment we need funding for from the government," she says (see 'Counting the cost'). But right now, getting a therapy through clinical trials remains her focus.

With advice from Hopwood, Megan has decided to fund a gene-therapy trial at the Nationwide Children's Hospital in Columbus, Ohio. Thanks in part to this seed funding, the research has led to the creation of a spin-off company, Abeona Therapeutics, which will commercialize the therapy. Abeona has just started its first clinical trial.

Isla and Jude Donnell are now seven and five years old. "The disease is progressing in them," says Megan, "but they are doing quite well, all things considered." She hopes that they will be eligible to join Abeona's trial later in the year.

The progress in finding treatments for rare diseases continues to rely on foundations such as Megan's. "There is a community of kids that needs treatment, and we are close to having something for them," Megan says. "A little bit of focus on research in this area is going to make a big difference to a generation of patients." ■

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DRUG DEVELOPMENT

Through the barrier

Treatments that can cross the blood–brain barrier are needed if doctors are to treat the devastating neurological symptoms of many lysosomal storage disorders.

BY SARAH DEWEERDT

The first time Tim Cox used enzyme-replacement therapy to treat a patient, the effects were "like magic", he recalls. The patient was a businessman who had been forced to retire in his forties and was now confined to a wheelchair. He had a massively enlarged liver and spleen, and needed frequent transfusions of blood and platelets to stay alive.

These are the effects of Gaucher's disease. The body cannot produce the enzyme that breaks down a fatty molecule called glucocerebroside, which then accumulates to toxic levels in various body tissues. The only established treatment at the time, a quarter of a century ago, was a risky bone-marrow transplant, but none of the man's three sisters was a viable match. So Cox, a metabolic physician at the University of Cambridge, UK, obtained a cutting-edge treatment from the United States — glucocerebrosidase enzyme purified from a human placenta — and administered it to his patient by intravenous infusion.

This was the first UK use of enzyme-replacement therapy to treat Gaucher's disease, and the result was dramatic. Within months, Cox's patient was no longer dependent on either blood transfusions or the wheelchair. "He never looked back, and he's still alive today," Cox says. Without it, "he would have been dead from bleeding or some complication a long time ago."

The advent of enzyme-replacement therapy in the early 1990s revolutionized the treatment of Gaucher's disease. It also led to a new era of drug therapies for other lysosomal storage disorders (LSDs), a group of around 50 genetic conditions that compromise the body's ability to break down specific molecules, which then build up to create severe health problems (see page S146).

Today, enzyme-replacement therapies are available for more than half a dozen LSDs. Treatment options have expanded to include drugs with other modes of action too, such as partly blocking the synthesis of the troublesome molecules, and rescuing the function of mutant enzymes. But despite this broadening range of therapies, it remains difficult to get drugs into the central nervous system to treat the neurological symptoms that are the most devastating aspect of many LSDs. Researchers hope that some of the molecules being developed may finally clear this barrier. "We're right on the verge of some incredible new opportunities," says John Marshall, senior principal scientist at biotech company Sanofi Genzyme in Cambridge, Massachusetts.

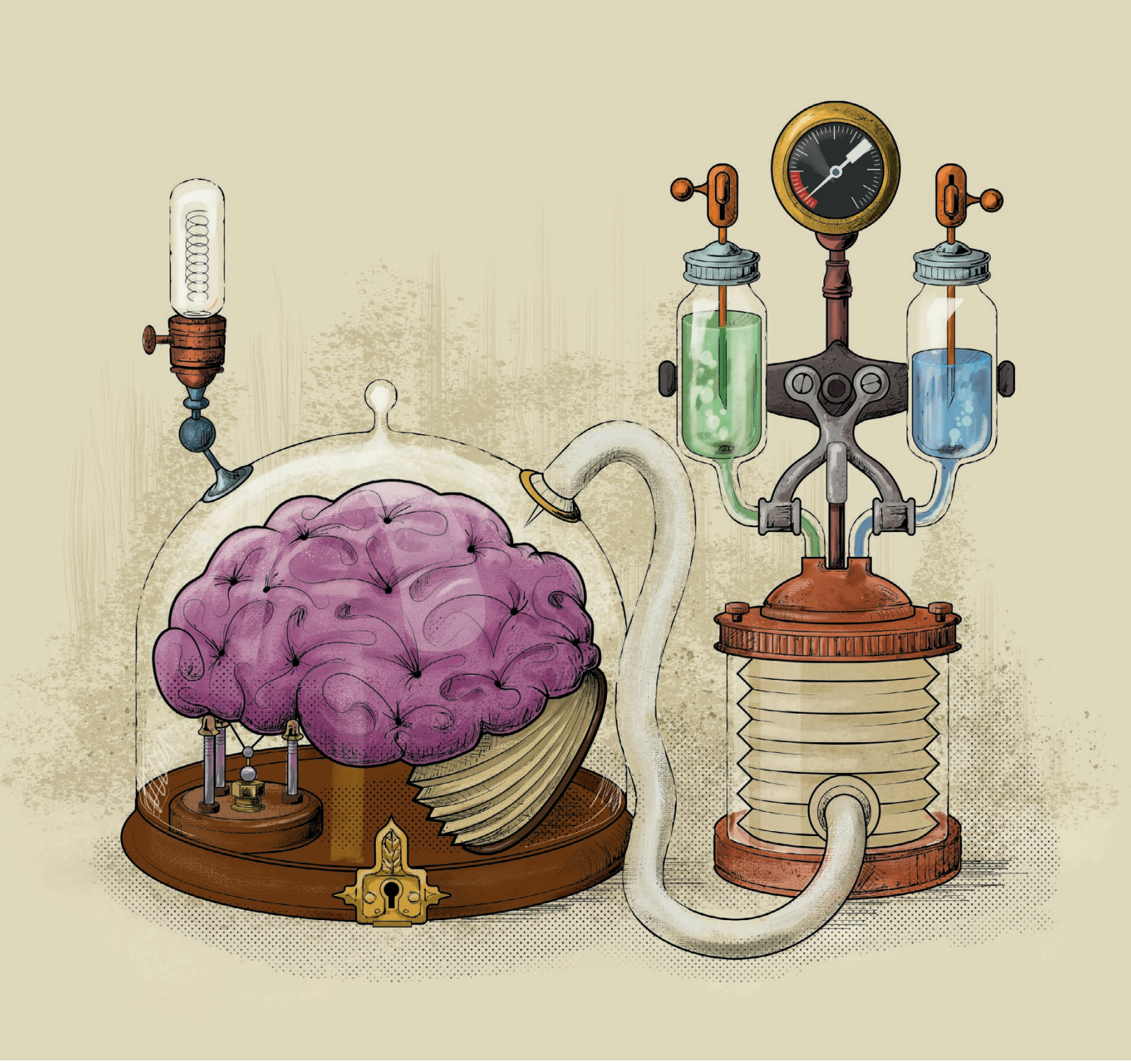
NEW FOR OLD

Enzyme-replacement therapy is exactly what it sounds like. "You're putting back the missing factor," says Paul Harmatz, a paediatric gastroenterologist at the Children's Hospital Oakland in California. He has been involved in developing enzyme-replacement therapies for a group of LSDs known as mucopolysaccharidoses.

But as so often happens, part of the development of enzyme-replacement therapy came about through a chance discovery. In the 1960s, researchers in the laboratory of Elizabeth Neufeld, a biochemist now at the University of California, Los Angeles, inadvertently mixed together cells from patients with two different mucopolysaccharidoses: Hurler and Hunter syndromes. Surprisingly, both sets of cells became physiologically normal. Neufeld and her colleagues showed that each type of cell secreted the enzyme that the other lacked, and absorbed the enzyme it needed from its neighbours — a concept known as cross-correction.

"We're right on the verge of some incredible new opportunities."

This finding was taken up by Roscoe Brady at the US National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, who had already made important discoveries regarding the diagnosis and causal mechanisms of Gaucher's disease. His team altered the glucocerebrosidase so that a sugar called mannose appeared on its surface to help the enzyme enter macrophages, the



NIK SPENCER

cell type most affected by Gaucher's. And they conducted some of the first human studies of this enzyme therapy.

This early focus on Gaucher's disease for enzyme-replacement therapy was "a very lucky shot", says Carla Hollak, a metabolic physician at the Amsterdam Lysosome Centre in the Netherlands. In many patients, Gaucher's disease progresses slowly and some of its effects are reversible. "So there is a large window of opportunity for treatment," Hollak says. Other LSDs progress much more quickly and cause irreversible damage.

Genzyme (now part of Sanofi Genzyme) collaborated with Brady and developed his enzyme replacement to create Ceredase, which was approved by the US Food and Drug Administration (FDA) to treat Gaucher's disease in 1991. The drug has since been discontinued and replaced with Cerezyme, which is produced by genetically engineered bacteria.

Today, enzyme-replacement therapies are available for Gaucher's, Fabry's and Pompe diseases, for four different forms of mucopolysaccharidosis, and for lysosomal acid lipase deficiency. More drugs are being developed, including therapies aimed at mucopolysaccharidosis VII and one form of Niemann–Pick disease.

But the treatment also has its downsides. It must be administered for life by intravenous infusion every few weeks, a regime that is burdensome for patients. It is expensive, costing about US$250,000 per patient per year, and proteins are much harder to manufacture than small-molecule drugs. Effectiveness varies: enzyme-replacement therapy is more effective in Gaucher's than in Fabry's disease, for example, because even though the two disorders involve closely related enzymes, they damage different organ systems. And some LSDs are not good candidates for enzyme-replacement therapy at all. Niemann–Pick type C and the juvenile form of Batten disease, for example, involve proteins from the membrane of the lysosome. Membrane proteins are not water soluble, so they would be difficult to purify and cannot be delivered in an intravenous solution.

But perhaps the biggest drawback of enzyme-replacement therapy is that the

NHGRI/ERNESTO DEL AGUILA

Ellen Sidransky (left) is looking for chaperones that can treat lysosomal storage disorders.

proteins cannot cross the blood–brain barrier to prevent the toxic build-up of molecules in the neurons. Some researchers are working on ways to deliver enzyme-replacement therapy directly to the central nervous system, for instance by injecting it into the brain or spinal cord. But this involves surgery to insert a port for delivery of the treatment, which carries a risk of infection — a tough call, especially when it comes to young children, who are most affected by neurological symptoms.

INTO THE BRAIN

Most proteins are too big to cross the blood–brain barrier, so many researchers are instead investigating small-molecule drugs that might have a better chance. Improved understanding of how LSDs are caused is also contributing to rapid advances. "We're becoming smarter about what's wrong with these cells and beginning to know how we can fix them," says Beverly Davidson, chief scientific strategy officer at the Children's Hospital of Philadelphia in Pennsylvania.

Instead of replacing the defective or missing enzymes, small-molecule drugs shore up various parts of the dysfunctional mechanism. One approach is substrate reduction, which involves turning down the production of the specific molecule or substrate that cannot be processed and hence builds up in a particular LSD. Substrate reduction is a well-established concept in other fields of medicine: statins, for example, lower blood cholesterol by inhibiting an enzyme involved in cholesterol synthesis.

Substrate-reduction therapies do not stop production, however — and this would not be desirable anyway, because many of the molecules that accumulate are essential to the body, even if they become toxic in large quantities. For this therapy to work, the body must have a way of breaking down the smaller amount of substrate that will still be present. "In most of the lysosomal storage disorders, the patients do have some residual enzyme activity," says Marshall. And other biochemical mechanisms can contribute to the breakdown or excretion of these molecules, he adds.

Two substrate-reduction therapies are already on the market. The first is miglustat, which was approved in 2002 to treat Gaucher's disease in people who cannot have enzyme-replacement therapy because of an allergy or an inability to have drugs administered intravenously. It has also been approved in some countries (but not the United States) for the treatment of Niemann–Pick disease type C. Miglustat is not as effective as enzyme replacement for Gaucher's disease, and has major side effects, such as tremor and gastrointestinal problems. "But the principle of substrate-reduction therapy was shown," says Hollak, which was "a step forward to developing new oral treatments".

"We're becoming smarter about what's wrong with these cells and beginning to know how we can fix them."

The second substrate-reduction therapy for Gaucher's disease, eliglustat, was approved two years ago. It has fewer side effects than miglustat and is more effective — nearly as effective as enzyme-replacement therapy. "It was beyond anybody's expectation," says Cox, who conducted several clinical trials for miglustat in the 1990s, but was not involved in the development of eliglustat. The drug might have other benefits as well. Studies in animals suggest that eliglustat may help to prevent B-cell lymphoma and myeloma — common blood cancers that often arise as complications of Gaucher's disease.

Both miglustat and eliglustat can be taken orally and so have the advantage of convenience. But they are expensive: the annual cost of eliglustat is similar to that of enzyme replacement for Gaucher's disease. And although small-molecule drugs can theoretically cross the blood–brain barrier, neither miglustat nor eliglustat does so reliably.

But other substrate-reduction candidates being developed may finally achieve this. With his colleagues at Sanofi Genzyme, Marshall is working on a molecule, currently known as Genz-682452, that has been shown in animal studies to enter the brain. The drug inhibits the first step in the synthesis of a class of molecules known as glycosphingolipids, so it could help to treat several sphingolipidosis LSDs, including Fabry's, Gaucher's and Tay–Sachs diseases, says Marshall. Sanofi Genzyme launched a phase II trial of the drug in Fabry's disease last year, and is also exploring developing the molecule to treat Gaucher's disease.

THE RIGHT FOLD

A third strategy for LSD therapy is to rescue enzymes that are present but mutated. In many patients — the exact percentage varies from one LSD to the next — mutations do not affect the active sites of enzymes but only change their shape. "They have ample activity, we just need to get them folded," says Thomas Kirkegaard, chief scientific officer of the biotech company Orphazyme, based in Copenhagen. Misfolded enzymes tend to be degraded by cells quite quickly. But if they can be folded correctly, or otherwise stabilized so they are not degraded, and then shepherded to the right site in the lysosome, they can still perform their function.

Drugs that accomplish this are known as pharmacological chaperones. Like substrate-reduction therapies, chaperones tend to be small molecules and therefore also have the potential to cross the blood–brain barrier. Chaperone therapy is new, but is already in use elsewhere in medicine: two drugs of this type have previously been approved to treat cystic fibrosis.

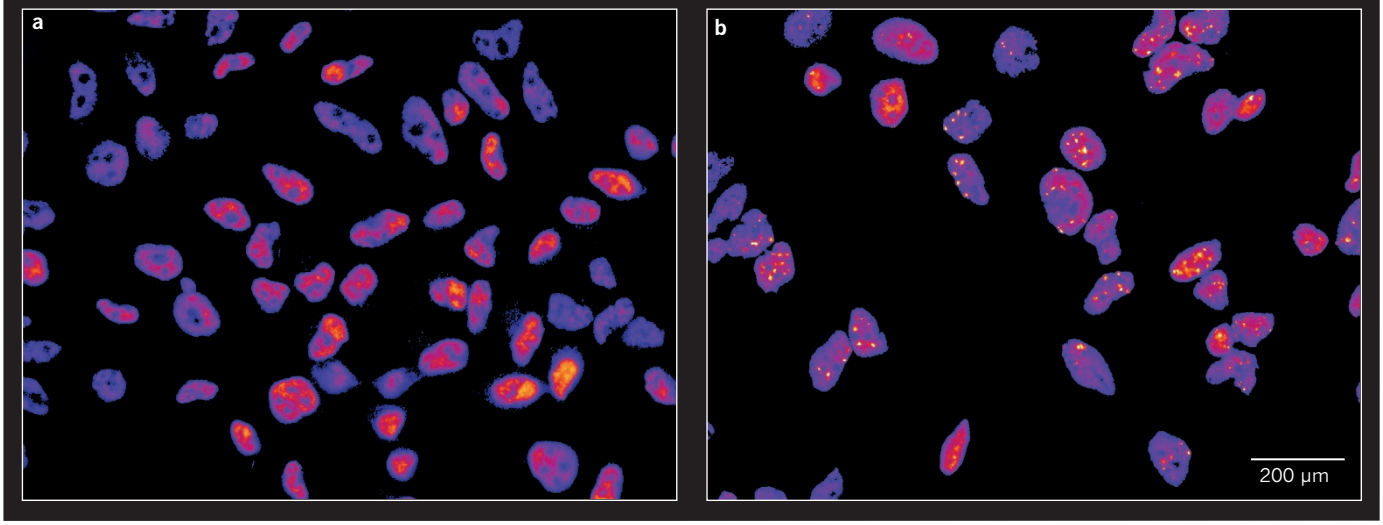
Two types of chaperone can be used in LSDs, says Ellen Sidransky, a neurogeneticist at the US National Human Genome Research Institute in Bethesda, Maryland: inhibitory and non-inhibitory. Inhibitory chaperones bind to the active site of their target enzyme and stabilize it for transport to the lysosome. Once in the lysosome, they detach so that the enzyme can do its work.

The first chaperone therapy for an LSD, which entered the European market earlier this year, is an inhibitory one. The drug is known as migalastat and has a similar chemical structure to the similarly named miglustat. It has been approved for the 35–50% of people with Fabry's disease who have any of 269 different mutations that cause misfolding of the enzyme α-galactosidase A.

CLAUS BORNAES & LUKASZ M. SOLANKO, ORPHAZYME APS

SHOCK TACTICS

Chaperones can treat lysosomal storage disorders that are the result of protein misfolding by helping to fold them correctly. Orphazyme's drug arimoclomol, which recently had a promising phase I/II trial, works by inducing HSP70, one of the body's natural chaperones known as heat-shock proteins. In unstressed cells (**a**), the transcription factor HSF1 (yellow), which activates heat-shock proteins, is distributed across the cell. When the cells are stressed (**b**), HSF1 is activated and becomes localized in nuclear stress bodies — a sign that heat-shock proteins are being produced. Arimoclomol prolongs this activation and aids the binding of HSF1 to heat-shock elements in the DNA, amplifying the production of heat-shock proteins.



The problem with inhibitory chaperones is that their development can involve difficult chemistry and dosing, Sidransky says. She is now looking for non-inhibitory chaperones, which stabilize an enzyme by binding on sites other than the active site, as these are easier to work with. However, non-inhibitory chaperones "are a little bit harder to find", she says. Her team has conducted a massive screen of a library that contains 250,000 compounds and turned up several promising candidates.

By using neurons and macrophages derived from reprogrammed skin cells from people with Gaucher's disease, Sidransky and her colleagues showed that one of these candidates, a compound dubbed NCGC607, enables mutant glucocerebrosidase to enter lysosomes and break down accumulated glucocerebroside. Crucially, the molecule does seem to cross the blood–brain barrier. "In Gaucher's disease, we don't have any therapy for the version that affects the brain," Sidransky says, but this drug could finally change that. Her results also suggest that NCGC607 might even help to treat a much more common neurological condition, Parkinson's disease (see page S160).

One limitation of chaperone therapies, however, is that most will be applicable to only one or perhaps a small number of LSDs — or even just to specific mutations in an individual LSD. Each LSD might have tens or even hundreds of different mutations that result in the same disease outcome, and it is not practical to develop chaperones to target them all.

To get round this problem, Kirkegaard is investigating drugs that ramp up the body's natural chaperones, the heat-shock proteins. In theory, this approach could help to treat dozens of disorders, including many LSDs. "It is a broadly applicable concept, particularly in protein-misfolding diseases," Kirkegaard says. Earlier this year, a small phase I/II trial of Orphazyme's drug arimoclomol showed promise for treating a non-lysosomal muscle disease called sporadic inclusion body myositis. It is also being tested against amyotrophic lateral sclerosis.

Other researchers are sceptical of these broad claims. Previous attempts to harness the heat-shock protein system to treat Huntington's disease, a common neurodegenerative disorder that involves aggregations of mutant huntingtin protein, showed promise in cell-culture studies, Davidson says, but the clinical results were underwhelming. "It's difficult for me to see how this is going to work on all lysosomal storage diseases," Davidson says. She is currently developing her own small-molecule treatments for LSDs, but declined to name their class or mechanism of action.

But Kirkegaard thinks that arimoclomol, which induces a heat-shock protein known as HSP70 (see 'Shock tactics'), could be suitable to treat the sphingolipidoses (T. Kirkegaard *et al. Sci. Transl. Med.* **8,** 355ra1118; 2016). This is because HSP70 binds to a fatty molecule inside lysosomes called BMP, which in turn is a cofactor of a variety of sphingolipid-degrading enzymes and increases their activity. So boosting the availability of HSP70 should supercharge the sphingolipid-degrading system.

Orphazyme has recently launched a phase III trial in children with Niemann–Pick type C, roughly 95% of whom have a misfolding mutation that is amenable to chaperone therapy. The study will involve at least 46 patients in Europe and the United States, and is scheduled to run for 1–2 years. If the results are favourable, the company will try the treatment in other sphingolipidoses.

Researchers are trying different approaches to find therapies that are applicable across many LSDs. A team led by geneticist Andrea Ballabio at the Telethon Institute of Genetics and Medicine in Naples, Italy, is investigating whether manipulating a master regulator of lysosome function, called TFEB, could boost the activity of lysosomes and help to clear a variety of molecules that are building up (see page S148).

Other researchers are trying to mitigate the production of incomplete enzymes, which Marshall estimates cause 10–15% of LSD cases. Read-through and exon-skipping technologies can instruct a cell's protein-making machinery to ignore the premature stop signals in genes that lead to truncated enzymes. One compound, ataluren, which has been approved in Europe for the treatment of Duchenne muscular dystrophy, is being investigated in a phase II clinical trial for Hurler syndrome.

"The beauty of it is that many of these ways of correcting the disease are complementary," says Kirkegaard. This means that the future of treatment for LSDs may lie not with one particular class of drugs, but with combinations of them, although that will require the high cost of the drugs to come down (see 'Counting the cost', page S153). But if enzyme-replacement therapy, substrate-reduction therapy, chaperone therapy and other strategies each produce small improvements, then taken together, says Kirkegaard, "you might have a very, very good treatment for some of these diseases." ■

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