On the surface, the link between weight loss and epilepsy is not obvious. But the ease of breeding and manipulating zebrafish means researchers don’t always need to have an exact idea of what they are looking for in their high-throughput screens. After validating that both zebrafish and human Dravet patients respond similarly to a variety of conventional drugs, the lab set out blind to find new compounds, but with speed in mind. “We decided, let’s look for paper, decided to switch up his models. Mice, with only a few offspring per litter, can be hard to scale. Two zebrafish, on the other hand, can produce up to 200 larvae at a time, an obvious advantage over rodents without deviating too far from the vertebrate lineage. He and his lab at the University of California, San Francisco set out to develop high-throughput assays, initially to measure seizure behaviors in larvae with high-speed video and later adapted to include EEG recordings to confirm seizure activity in the brain.

While Baraban was developing his assays, advances in genetics were beginning to link gene mutations to different types of pediatric epilepsy. Over 80% of patients with Dravet Syndrome have a mutation in the sodium channel gene SCN1A. When a mutation to the zebrafish equivalent of that gene turned up in a colleague’s forward genetic development screen, Baraban had an early candidate to test zebrafish’s drug discovery potential. In June 2012, the FDA approved lorcaserin as a weight loss aid for obese individuals. In early 2016, Kelly Knupp, a pediatric epilepsy specialist at Children’s Hospital Colorado, administered it to six children with Dravet Syndrome, a rare and severe form of childhood epilepsy. The initial results were impressive, says Knupp, who requested a ‘compassionate use exemption’ to try the drug in her patients who had exhausted conventional anticonvulsants. Children who had been having daily seizures for years were seizure-free for weeks at a time, she explains.

The results for five of those patients were published in Brain (140, 669–683, 2017) alongside the preclinical data connecting a weight loss drug to a treatment for epilepsy. That evidence did not come from a mouse or a nonhuman primate, nor any mammalian model for that matter. It came from zebrafish.

Aquarium to bedside
About a decade ago, epilepsy researcher Scott Baraban, senior author of the Brain paper, decided to switch up his models. Mice, with only a few offspring per litter, can be hard to scale. Two zebrafish, on the other hand, can produce up to 200 larvae at a time, an obvious advantage over rodents without deviating too far from the vertebrate lineage. He and his lab at the University of California, San Francisco set out to develop high-throughput assays, initially to measure seizure behaviors in larvae with high-speed video and later adapted to include EEG recordings to confirm seizure activity in the brain.

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moving directly from preclinical work in fish to patients in the clinic.

To Baraban, missing mice is not an issue. “At present, there’s no mouse model for Dravet Syndrome that’s been screened against 3,000 drugs like our zebrafish model and validated against known antiepileptic drugs. There’s no equivalent. So the leap has more to do with safety,” he says.

Novel treatments, however, might not have the same leeway, at least at the moment. “A new drug would be different,” Baraban says, “If we came up with a new compound or if we modify the structure of one of these drugs, I agree that would have to go through a lot more testing and the FDA would approach that finding differently.”

But that doesn’t rule out zebrafish for quickening the pace on new drug discovery. Large zebrafish screening programs are common in academic labs, but they are just starting to emerge for commercial purposes. Teleos Therapeutics, founded in 2014, is breaking zebrafish out of the academic tank. Scientific co-founder David Kokel explains, “We saw in the lab that we were finding all these great, new neuroactive compounds and we thought, well we really should be developing some of these into therapies. So that’s what motivated us to try to get some of these compounds and technologies out of the academic lab and into the company.”

Baraban’s strategy to repurpose existing drugs against a zebrafish screen is a recipe for speed—and savings. Since he started the program in 2011, he has screened over 3,000 compounds for less than three million dollars. That is less than the average annual budget of the NIH Epilepsy Screening program, which contracts similar screening research to the University of Utah for about $3 million each year, with an additional million budgeted for administrative costs. Since 1975, that program, which relies on rodents, has screened about 30,000 drugs for potential antiepileptic properties, 10 of which have been brought to market as of a May 2015 program report.

If time is money—not to mention lives—the zebrafish approach doubles-down on the savings: moving from drug screens in California to administration of lorcaserin to patients in Colorado took only 5 years. Notably, no mouse data was needed to make the case for the compassionate use exemption, an example of a discovery with safety data available from the FDA’s original approval process for lorcaserin and oversight from both the hospital’s Institutional Review Board and the FDA’s expanded access program, Knupp and her patients’ families were willing to give the drug a shot.

What’s old is new
Baraban’s 96-well plate for screening zebrafish larvae. Inset: motion tracking of a zebrafish larva modeling Dravet Syndrome (right) compared to a seizure-free control. Image adapted from *Nat. Commun.* 4, 2410, 2013.

new drugs but let’s speed up the process by purchasing libraries that contain large numbers of repurposed compounds,” he says, “with the hope that maybe we’d find something in there that was antiepileptic that no one had noticed.” They did: an antihistamine called clemizole, which they reported in 2013 (*Nat. Commun.* 4, 2410, 2013).

But clemizole had a problem. Though approved by the FDA in the 1970s, it was no longer being actively manufactured and therefore was not clinically available. A second blind screening of an active drug library identified a compound with a similar mechanism of action to clemizole that had been brought to market only a few years prior: lorcaserin. Confident in his initial validation work with the model, Baraban hoped that any compound identified through the screens could prove clinically relevant in human patients.

The aquarium and the clinic came together in early December 2015 during a Dravet Syndrome roundtable at the American Epilepsy Society meeting, where Baraban showed Knupp the zebrafish data. “I was really encouraged. The truth is, from a clinical standpoint, we have limited options for these children,” Knupp recalls. “It was reassuring to know that there was, in some animal model, some idea that this might be efficacious.”

With safety data available from the FDA’s original approval process for lorcaserin and oversight from both the hospital’s Institutional Review Board and the FDA’s expanded access program, Knupp and her patients’ families were willing to give the drug a shot.

The imaging set-up used by Teleos, nicknamed ‘Sauron.’
Teleos leverages zebrafish to screen for entire classes of compounds that may hold therapeutic benefit for a variety of central nervous system disorders. Any potential targets will be sent for medicinal chemistry and safety evaluation through more traditional mammalian means with contract research organizations before moving on to clinical trials, but they are hopeful that zebrafish will get them to that point more quickly than working with mice. “You can’t scale chemical biology in mice, or really any other system,” says Kokel. “Zebrafish really are objectively the best system.”

A future with fish

With the support of venture capital, Baraban cofounded EpyGenix Therapeutics to pursue commercial development of the antiepileptic targets. The company recently received Orphan Drug Designation for clemizole and lorcaserin and is preparing for a pre-investigational new drug (IND) meeting with the FDA.

Baraban knows his data is new territory. “It’s unprecedented to go from fish to people. And the tools to do this type of drug discovery in fish have only appeared in the last couple of years,” he says, “So it’s hard to know how the FDA is going to view this as a field.” Nevertheless, he is optimistic the agency will view the data favorably. “We’re hopeful because the drugs are safe. The purpose of the way we designed this was to find drugs that were already out there so we could quickly make these leaps.”

The FDA has no current plans to advise on zebrafish in particular as nonclinical models of efficacy for neurological disorders, though it acknowledges the potential. A spokesperson for the agency commented: “Published literature suggests that zebrafish may be useful for mechanistic investigations and for early screening. How we would interpret data from any animal model would depend on how well it has been validated and how relevant it is for a particular indication. The better the pathophysiology is understood in humans, the easier it may be to evaluate the suitability of the animal model and the more useful it may be for predicting efficacy in humans.”

For now, the FDA will be leaving it to applicants to make their case. A spokesperson explained, “Because of the lack of experience with non-mammalian efficacy models and their inherent limitations, additional data should be provided to justify the model used if nonclinical demonstration of efficacy is an important consideration.”

In addition to moving forward on results from his original drug screens, Baraban and his lab are looking to the future, which he sees in personalized medicine. Zebrafish will still be at the forefront, but the researchers will be taking a more targeted approach. “We now use CRISPR and we’re proactively making fish for all the known epilepsy genes,” he says, explaining, “CRISPR has allowed us to switch our focus away from mutagenesis and actually say, okay, this gene is in the person. Let’s make a fish to model that genetic defect.” That creates a platform for quicker testing of large numbers of compounds that may not be possible in mice, an exciting development for clinicians like Knupp who work with patients suffering from rare genetic diseases with few treatment options.

Though lorcaserin did have a “honeymoon” period, Knupp explains that it is not unusual for antiepileptics to wear off over time. Options are important. She says she’d be willing to try lorcaserin again in other Dravet patients, and would be interested in collaborating on future developments with Baraban. Ultimately, efficacy is what matters, not necessarily the model. “Children aren’t fish,” says Knupp, “but children aren’t mice either.”