

Bugging out over Chagas:

Bioluminescent protozoans and old drugs might help unravel kissing-bug disease

By Nala Rogers

João Roberto Ripper / DNDi

Carolina Batista watched the Bolivian children playing with the bugs that carry Chagas disease. The youngsters had no idea that their imaginary cars could also bear a parasite that might one day destroy their hearts.

"The first time I saw that, I felt desperate," says Batista, who at the time in 2010 was working as a physician with Doctors Without Borders. "People were playing with their enemies, because they didn't know that this is the bug causing the disease that was killing their families, and has been for generations."

It wasn't just the children who were oblivious. Governments, doctors and pharmaceutical companies worldwide largely ignored Chagas disease after its discovery in 1909, even though the disease kills an estimated 10,000–14,000 people per year¹ and is a leading cause of heart failure in Latin America². But now, Chagas is finally getting attention. Because of global travel and relocation, the illness has appeared in places like the US, Europe and Australia, bringing the disease into the

global spotlight.

A growing network of researchers is working on ways to help the 6–7 million people who are currently infected. At long last, governments and pharmaceutical companies are funding large-scale, controlled clinical trials. So far the trials have found little support for new drugs, but they have revealed untapped potential in existing ones.

A silent killer

Chagas is caused by a protozoan parasite known as *Trypanosoma cruzi*, which travels between hosts in the guts of blood-sucking triatomine bugs, also known as kissing bugs. The acute phase of the disease can last two months, accompanied by a generic-seeming fever or totally without symptoms. But, if untreated, infected individuals spend the rest of their lives with chronic disease. About a third of these people suffer progressive damage to their hearts or digestive systems over the course of the next few decades.

In some rural villages in Latin America

more than half of adults are infected, says Batista, who is now the medical director for the Latin American wing of the Drugs for Neglected Diseases initiative (DNDi), a 12-year-old collaborative nonprofit organization that spearheads much of the current work on Chagas treatments. Cases rarely rise to the notice of doctors; fewer than 1% of infected people are thought to receive treatment³. "It's a forgotten disease among forgotten people," says Peter Hotez, dean of the National School of Tropical Medicine within the Baylor College of Medicine in Houston, who is developing a therapeutic vaccine to treat infected people.

This neglect has made for slow progress in Chagas research. The only drugs successfully developed to kill *T. cruzi* parasites, nifurtimox and benznidazole, are more than 40 years old. For most of that time, doctors rarely prescribed the parasite-killing drugs after the acute phase of the illness, mistakenly believing that organ damage in chronic Chagas disease was an autoimmune problem triggered by earlier infection. The consensus didn't

change until the early 21st century, when researchers concluded that parasites were to blame in all stages of the disease⁴. Even then, it was unclear how much benznidazole or nifurtimox helped at later disease stages. There were few controlled clinical trials, and researchers had no reliable ways to determine whether a treatment had worked. Most available data suggested that a course of treatment only cured a minority of chronic Chagas patients⁵.

Expanding options

Despite the evidence that treatment did not work for everyone infected, in 2002 the WHO cautiously recommended treating people in all phases of the disease. The new standards have been slow to catch on, in part because nifurtimox and benznidazole have severe side effects. Up to 18% of patients stop treatment early because of problems that include skin rashes and gastrointestinal upsets⁶. “I have seen someone who’s taken benznidazole and had this rash that they talk about, and it’s more than a rash. It’s ulcerating skin lesions that can be secondarily infected with bacteria. It’s really, really ugly,” says James McKerrow, a biochemist at the University of California, San Diego. “The bar has been set very low,” he adds in reference to drug toxicity. McKerrow is investigating both new and existing drugs as possible alternatives, and in July he received a grant from the US National Institutes of Health (NIH) grant to repurpose a Sanofi drug candidate that

was originally developed for chronic pain.

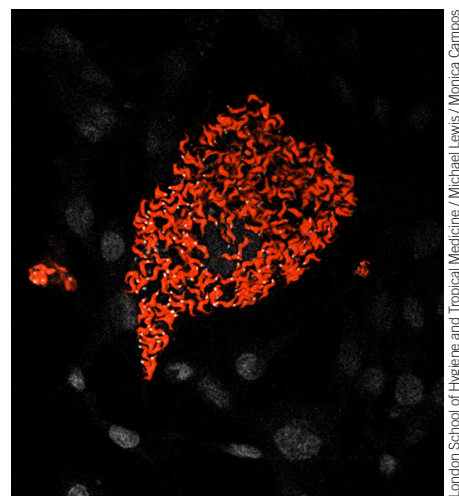
As a result of the urgent need for safer options, the search for new Chagas treatments has gathered steam over the last five years. Initially, there was tremendous excitement about a class of drugs known as triazoles, which interfere with sterol synthesis by inhibiting an enzyme called CYP51. In 2010, researchers at the Vall d’Hebron Teaching Hospital in Barcelona launched a clinical trial with a triazole called posaconazole, which is already approved as an antifungal drug. In 2011, DNDi started a trial with E1224, a prodrug of another triazole still in development. At first, the triazoles seemed perfect. They caused few side effects, and PCR tests found no parasites in patients’ bodies after treatment.

But a year later, the parasites had returned⁷. “Virtually all of the patients treated with the posaconazole or E1224 reemerged with parasitemia, so we didn’t kill all of the parasites,” says Robert Don, discovery and preclinical director at DNDi. “Both of [the drugs] were complete failures in the clinic.”

A few researchers, such as William Roush at the Scripps Research Institute in Jupiter, Florida, are still pursuing CYP51 inhibitors, but most labs have started screening out compounds that work through the same mechanism as the triazoles. Abandoning these compounds effectively cleared out the Chagas drug pipeline at DNDi, Don says. The situation is similar at the University of Dundee in Scotland, where more than half of the compounds in development for Chagas have been shelved, says Alan Fairlamb, a molecular parasitologist at Dundee’s Division of Biological Chemistry and Drug Discovery. “We don’t want to work on those, because the risk that they will go all the way to the clinic only to have the same problem as the azole drugs is too great,” Fairlamb says. Both the DNDi and University of Dundee now have promising compounds that work through different mechanisms, though these are still in early stages of development.

The mouse trap

Researchers emphasize that triazoles looked good in preclinical studies. “The mouse experiments and the *in vitro* data suggested that CYP51 inhibitors were really



Seeing the light: Bioengineered Chagas parasites are tagged with fluorescent antibodies. Opposite page: A pest control professional holds a parasitic triatomine bug, or ‘kissing bug’.

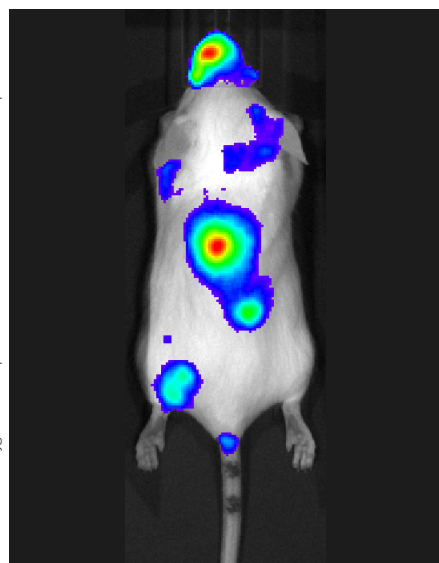
dynamite,” says Fred Buckner, a molecular parasitologist investigating Chagas treatments at the University of Washington in Seattle. “It has left everybody in the field sort of scratching their head about what’s different in the real infected humans.” The implications of the posaconazole and E1224 trials are larger than any one mechanism. If the preclinical tools couldn’t predict that

triazoles would fail in people, researchers can’t be sure they will provide useful information about the next class of drugs.

One major challenge for researchers is that *T. cruzi* parasites are notoriously hard to

detect, both in chronically infected humans and in animal models of chronic infection. The parasites are usually few in number, and they replicate inside of their hosts’ cells. These cells sometimes burst, spilling *T. cruzi* into the blood, where researchers can detect them with PCR. But, says Buckner, the blood levels of parasite are unpredictable and may even be intermittent. Researchers can examine tissue samples for replicating parasites, but it’s hard to know where the parasites are hiding. “They’re sort of scattered here and there, so you could easily do a biopsy and miss it,” says Buckner. “It’s very, very hard to prove the negative, that there are no parasites.”

John Kelly’s lab at the London School of Hygiene and Tropical Medicine has developed a new model to help solve



Shining through: Light from bioengineered parasites shines through the body of a mouse with chronic Chagas disease. Next page: A child plays on the floor in a village near Cochabamba, Bolivia.



João Roberto Ripper/DNDi

this problem, at least in mice. His team engineered bioluminescent *T. cruzi* parasites. The red light they produce travels easily through tissues, allowing researchers to see exactly where the parasites end up in a mouse's body, says Michael Lewis, a co-author with Kelly of a January 2015 paper describing the system⁸. With older animal models, researchers can't detect *T. cruzi* in mice treated with posaconazole or E1224. But with the new model, they can see that treated mice are still infected⁹. The London researchers are using their red-shining parasites to work on promising compounds identified by Fairlamb's lab, and other teams are adopting the model as well.

The recent clinical trials offered little hope for triazoles, but the findings had an unexpected upside. Benznidazole, the older drug used in the control groups, worked better than anyone expected. A year after treatment, most patients who completed treatment with benznidazole—94% in the posaconazole study, 81% in the E1224 study—had no parasites detectable by PCR⁷. This doesn't prove that the parasites would never come back, or that the patients would never develop heart failure. But it does

suggest that benznidazole is more powerful, and perhaps more flexible, than previously realized.

DNDi is now planning a clinical trial to optimize benznidazole treatment regimens. The side effects get worse as treatment progresses, Batista says. If the drug still works in lower doses or with shorter courses of treatment, it may be possible to cure patients while sparing them the worst of the side effects.

Many researchers are also hopeful about the potential of drug combinations. A team in Argentina just wrapped up a trial that included a posaconazole-plus-benznidazole arm, and they plan to release their findings this year, according to Sergio Sosa-Estani, a parasitologist at

the Instituto Nacional de Parasitología in Buenos Aires, Argentina¹⁰. The same group is also preparing results from two longitudinal studies of benznidazole efficacy¹¹. These studies may finally show whether reducing or eliminating *T. cruzi* parasites can halt the progression of organ damage—the only outcome that matters to patients.

Whether the answer lies in new drugs, old drugs, or both, the next challenge will be

getting the drugs to patients. Public health programs have helped reduce the number of new infections in some areas, though many people still don't understand the threat posed by triatomine bugs. Millions of people are living with Chagas, and hardly any have access to treatment. Among doctors and policy makers, knowledge is limited and often out of date.

But the mood is changing. "In the last few years, when I go to meetings with the governments and public health departments of countries, there is a momentum," Batista says. "People are open to talking about Chagas. They don't want to have this scar in their countries anymore."

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