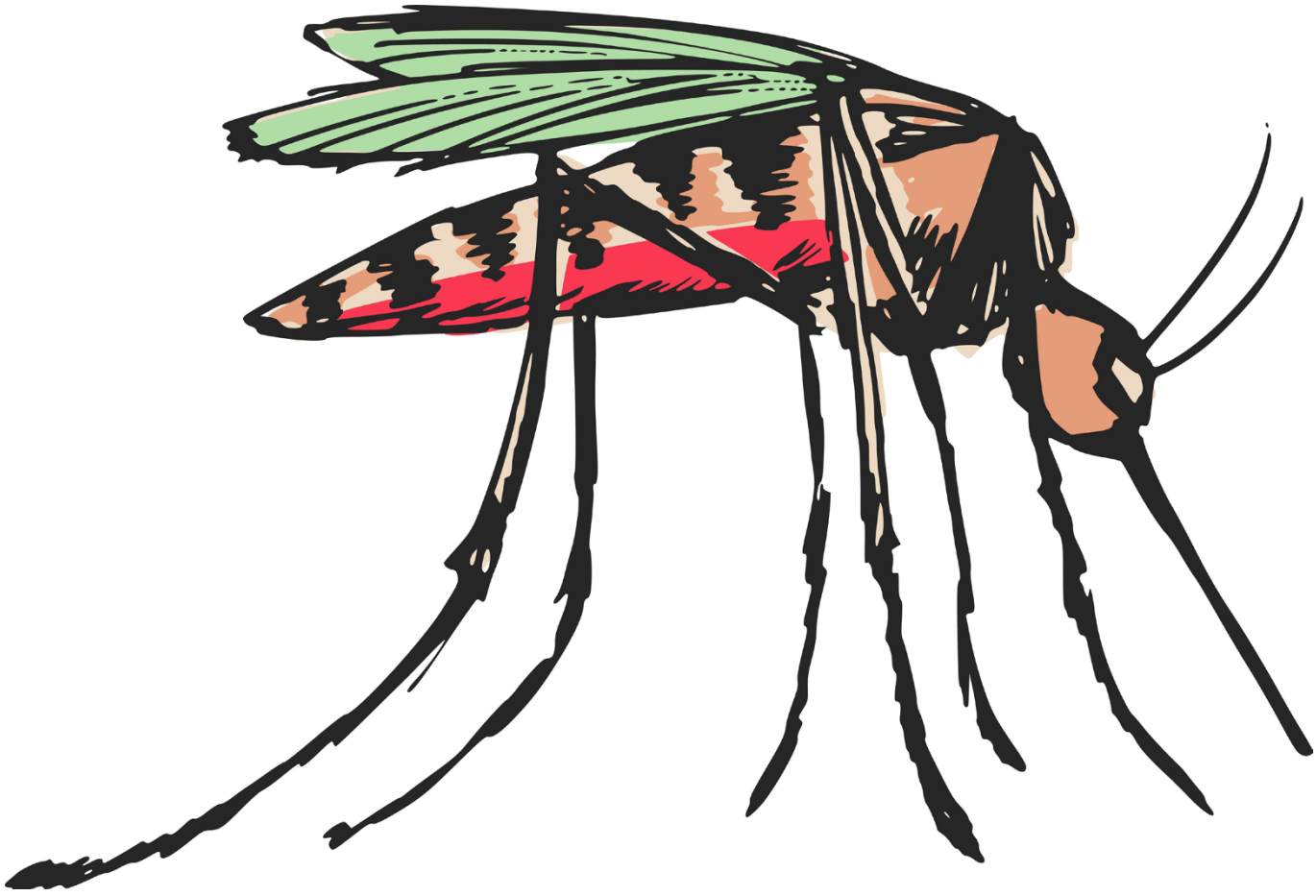

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A second look:

Efforts to repurpose old drugs against Zika cast a wide net

By Ellie Kincaid

More than two years before the current Zika virus outbreak made headlines, Johan Neyts, a virologist at the University of Leuven in Leuven, Belgium, began to search for an antiviral against the now-infamous virus. Neyts, who had spent his career until then focused on viruses such as dengue and yellow fever, had heard about the Zika outbreak in French Polynesia, which began in 2013 and affected more than 28,000 people. Neyts thought it was likely that the virus would spread further. And so, as a side project without dedicated funding, he began to research possible drug candidates against Zika in mice.

There are now many research teams around the world searching for treatments for the disease. Currently, the only options available are run-of-the-mill medicines such as Tylenol, and these just address symptoms such as fever, not the infection itself. This poses a grave problem in cases of infection during pregnancy, which can cause birth defects. According to the World Health Organization, eighteen institutions and companies, including the Butantan Institute in São Paulo, Brazil, and Sanofi Pasteur, are developing Zika vaccines—but bringing any of these candidates to the market could take years. Vaccine development for Ebola, for instance, accelerated during the 2014 outbreak in West Africa, and vaccine candidates were tested in clinical trials in 2015, yet a vaccine still has not been approved against the disease.

In search of a quickly deployable option, some researchers, including Neyts, are now studying the possibility of repurposing drugs that were originally developed for other conditions to work against Zika. These efforts are at different stages, ranging from early-stage computer modeling of how drug candidates interact with viral proteins to *in vitro* assays and animal studies that are testing the efficacy of potential drugs against viral infection.

Scientists have made attempts to repurpose drugs before, but there is special urgency to do this for Zika because of a historic lack of drug development against the virus. By contrast, researchers had already been trying to develop medicines for Ebola for some time, such that when the outbreak in West Africa was declared in 2014, there were already advanced drug candidates ready for testing. As such, the search for drugs to repurpose against Ebola was primarily "to make sure that nothing was missed," says Noel Southall, a bioinformatician at the National Center for Advancing Translational Sciences (NCATS) at the US National Institutes of Health. The NCATS maintains a comprehensive drug library of 2,500 clinically approved drugs and 1,000 investigational compounds, as well as infrastructure for rapidly screening high volumes of drugs against disease models, and the center has collaborated on research to repurpose drugs for both Ebola and Zika. "In the case of Zika," Southall says, "we're really starting from scratch."

Double duty

Shortly after Neyts's lab began to work on Zika therapeutics in early 2014, he decided

to test some previously developed antivirals that had shown activity against RNA viruses similar to Zika. The compounds that he chose were nucleoside analogues, which mimic the building blocks of RNA but disrupt replication when added to a growing chain of nucleosides. Among others, he tried 7-deaza-2′-C-methyladenosine (7DMA), a compound that Merck had begun to develop for hepatitis C but did not bring to market, owing to safety concerns that emerged during preclinical work. In mice infected with Zika, 7DMA delayed disease progression and reduced the amount of viral RNA circulating in the blood compared to infected mice that received sham treatment (*PLoS Negl. Trop. Dis.*, **10**, e0004695, 2016). The compound is "not yet potent enough to treat Zika infection," and perhaps not safe enough either, Neyts says, but even reducing the amount of viral RNA in the blood could possibly lower the likelihood of mosquitoes transmitting the virus to a healthy person after biting someone with the infection. Neyts has also tested the antiviral favipiravir, approved in Japan for treating influenza, against Zika infection in mice. Unpublished preliminary evidence suggests that it might work, he says.

> "We're going to need multiple drugs if we're really considering this."

Virologist Amilcar Tanuri of the Federal University of Rio de Janeiro is searching for a potential Zika drug among clinically approved antimalarials. Previous work by scientists has shown that the antimalarial drug chloroquine can inhibit dengue *in vitro* (*Sci. World J.*, doi:10.1155/2013/282734, 2013), so Tanuri posited that it might work against the closely related Zika virus. Tanuri exposed human blood–brain-barrier cells and human neural stem cells to Zika *in vitro*, and then treated the cells with chloroquine for five days. Flow-cytometric analysis indicated that while 80% of blood–brain-barrier cells exposed to Zika were still infected after treatment with a low dose of chloroquine, treatment with a higher dose decreased the amount of infected cells to 20%, and the higher dose protected 80% of all the cells exposed to the virus from death, according to the findings, which have not yet been peer reviewed but are posted to a preprint server (preprint at bioRxiv, http://dx.doi.org/10.1101/051268, 2016). Pei-Yong Shi, a virologist at the University of Texas Medical Branch in Galveston, says that the findings are "a very interesting first step." But a clinical trial in 2013 of chloroquine as a treatment for dengue did not show any obvious benefits (*Mem. Inst. Oswaldo Cruz* **108**, 596–599, 2013), and so Shi wants to see results from animal trials before considering whether chloroquine might be effective against Zika in humans.

Rather than focusing on one compound, Sara Cherry, a virologist at the University of Pennsylvania, has cast a wide net, screening more than 2,000 small-molecule compounds with known target proteins. The US Food and Drug Administration (FDA) has approved half of these compounds, some as antivirals or antibiotics. Cherry's lab has tested each of the 2,000 candidates in different human cell lines infected by Zika, including endothelial cells from the blood–brain barrier and placental cells. By using fluorescence microscopy, she measured viral antigen production in cells after infection. In each different cell type, more than a dozen of the drugs stopped Zika from infecting cells altogether, or otherwise decreased viral replication, but Cherry found that only a handful worked in all cell types. "It seems like there are different mechanisms being engaged in different scenarios," Cherry says; drug candidates identified in one type of cell might not work in all cases. Before publishing her research, Cherry wants to identify the host cell pathways that the drug candidates target to confirm her findings.

In as-yet-unpublished work, Raymond Schinazi, a biochemist and virologist at Emory University, used computer modeling to identify an antiviral drug that binds to a polymerase protein in Zika, and found that the drug stopped the virus from replicating *in vitro*. The drug has been used for over 40 years and is an FDA Pregnancy Category B compound, he says, meaning that in animal studies, the drug has not demonstrated risk of harm to fetuses. Ideally, women who are pregnant or want to become pregnant could take such a drug prophylactically, says Schinazi, whose lab has developed several approved drugs for HIV and the blockbuster Solvadi (sofosbuvir) for hepatitis C. He thinks that if these women were infected, the drug would prevent the Zika virus from replicating before it could cross the placenta and spread to the fetus.

Meanwhile, investigators for the OpenZika project, led by computational chemist Carolina Horta Andrade of the Federal University of Goiás in Goiânia, Brazil, are spearheading a crowdsourcing effort through IBM's World Community Grid, which is a network of volunteers' computers that runs simulations to predict how well drugs will bind to Zika proteins. Compounds already approved by the FDA or by the European Medicines Agency are prioritized. The researchers hope to begin testing compounds suggested by the computer simulation in assays and 3D cultures of human neural cells this month.

Commercial interest

Start-up companies have also ramped up their projects in drug repurposing for Zika. For the past three years, the Georgia-based pharmaceutical startup Ennaid Therapeutics has been building partnerships to explore a drug for flaviviruses such as Zika, modeled after the injectable HIV drug Fuzeon (enfuvirtide), which the FDA approved in 2003. Both drugs are fusion-peptide inhibitors, which prevent a virus from attaching to a host cell and infecting it (*PLoS ONE* **7**, e50995, 2012). "The writing was so clearly on the wall we had to respond to what was happening," says Ennaid's founder and CEO, Darnisha Grant Harrison. She hopes that the company will commence preclinical testing of their compound in animal models of Zika and begin clinical trials of the compound by early next year.

Even if scientists identify compounds that can be successfully repurposed against Zika, the necessary dose might be too high for people to take safely. Many studies were published about repurposing drugs to treat Ebola, Shi says, but the concentrations of compounds reported could not be used to treat patients. In Tanuri's research, the dose of chloroquine that was determined necessary to treat Zika in humans would be much higher than the current dose of chloroquine used as a malaria prophylaxis—and perhaps unsafe, Tanuri says.

Another challenge is that RNA viruses such as Zika often accumulate mutations through replication that might help them to quickly evolve resistance to an antiviral. "We're going to need multiple drugs if we're really considering this," Cherry says, adding that drugs with different targets will be especially important. Developing therapies for Zika is further complicated by the fact that "the real pathogenesis we're concerned about is during pregnancy, a time when we don't treat people with experimental drugs," Cherry adds.

Even if repurposing experiments identify drugs that are only weakly effective, they could still point scientists in the direction of mechanisms to target with new drugs and thus provide information that expedites the development process, says Southall. "It's going to give you a real head start in successfully developing a drug."

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