FDA reviews application for field trials

Attacking Zika by modifying mosquitoes

There are over 3,000 species of mosquito, but the *Aedes aegypti* has made headlines in recent months because it is the primary vector for the Zika virus, which has reached pandemic levels since an outbreak began in Brazil just over a year ago. While some researchers are working to develop a Zika vaccine, others in the private sector and academia are using genetic engineering techniques to eliminate the source of this growing public health risk, namely the *Aedes aegypti* itself.

Most people infected with Zika only have mild symptoms, but the virus is still causing a stir around the world. Firstly, its appearance in a new geographical area is worrying because the population has no pre-existing immunity to slow it down.[1] Secondly, the virus is associated with serious birth defects such as microcephaly, as well as neurological disorders such as Guillain-Barré syndrome – links that were only picked up when the virus struck the Western hemisphere. Thirdly, the infection can be sexually transmitted between humans.

To complicate matters further, diagnosis can be tricky because existing tests cannot effectively differentiate between Zika and other flavivirus infections like dengue, for which the *Aedes aegypti* is also the main vector. Active transmission of Zika is spreading and is currently present in 48 countries and territories, according to the US Centers for Disease Control and Prevention (CDC).[2]

Therefore, the race is on to address the pandemic before it becomes worse.

Traditionally, vaccines, antiviral drugs and diagnostics have been used to address viral pandemics. Indeed, the US National Institute of Allergy and Infectious Diseases (NIAID) is carrying out research into more reliable diagnostics and it has a vaccine under development.[3] However, Phase I trials for the latter have yet to commence.

The NIAID said that an investigational Zika vaccine should be ready to enter early-stage human trials this autumn. This means that a safe and effective, fully licensed Zika vaccine will likely not be available for several years.

Fortunately, scientists are also looking at possible solutions involving genetic modification that could be implemented more quickly. This is an existing technology which is already being used in plants. It is not to be confused with gene editing technology such as the CRISPR/Cas9 research tool.

Can GM mosquitoes save the day?

UK-based biotech company Oxitec Ltd is pioneering the way, with a 'commercial release' approval from Brazil's National Technical Commission of Biosafety (CTNBio) for its genetically modified (GM) *Aedes aegypti*, OX513A, and an application to begin field trials in the Florida Keys which is under review by the US Food and Drug Administration (FDA).[4]

In an interview with *MedNous*, Oxitec CEO Hadyn Parry explained how the company's technology works. "It's actually quite straightforward. We created a strain of *Aedes aegypti* with two additional genes. The first is a self-limiting gene, which all offspring inherit. The second is a fluorescent red protein that allows us to trace the offspring and closely monitor populations."

When the company releases its transgenic males into the environment to mate with wild females, the offspring will die before they reach adulthood and the population will decline. They only release males, which do not bite or transmit disease and have a life span of about one week.

Oxitec's OX513A addresses what Mr Parry sees as the greatest hurdle of all: private property. *Aedes aegypti* live in and around homes, office buildings and schools. They can breed in minimal amounts of water, for example, discarded bottle caps, plant pots and tyres, and they spread batches of their eggs over two or more sites. Moreover, the eggs can remain dormant for up to six months.

This makes it extremely difficult to address using traditional pest control methods because "it's impractical to go into people's homes or onto private property to treat the areas with insecticides," said Mr Parry.

Using OX513A to control *Aedes aegypti* solves this problem. Populations vary in different locations, and with the red fluorescent markers as a guide, the company knows which areas need more or less treatment. "*Aedes aegypti* only fly about 200 yards in their lifetime, so based on analysis of weekly sample collections, we're able to treat areas accordingly. It's a very methodical, programmed, planned activity. This is a big contrast with insecticides because you can't control where the chemical goes," Mr Parry noted.

He said that the company had deliberately gone down a self-limiting route because it is a controlled system that has no permanent impact on the environment or ecosystem. Although continual release of OX513A mosquitoes is necessary to suppress wild populations indefinitely, it means that stopping release will result in OX513A mosquitoes disappearing from the environment within a few weeks. In addition, these GM mosquitoes do not have a harmful impact on other species of mosquito or 'beneficial' insects such as bees and butterflies, as opposed to conventional approaches that do not discriminate.

Field trials carried out by Oxitec since 2009 in the Cayman Islands, Brazil and Panama have shown an over 90% reduction in the *Aedes aegypti* population in the areas where it has been released. "This is a huge shift away from what you can achieve with insecticides because of the quick build-up of insecticidal resistance," noted Mr Parry. OX513A can repress populations of *Aedes aegypti* that carry insecticide-resistant genes, which are making conventional approaches increasingly ineffective.

The potential of OX513A to fight Zika has attracted the attention of the World Health Organization (WHO), which reviewed its ability to offer an emergency response to the Zika pandemic at its Vector Control Advisory Group (VCAG) meeting on 14-16 March 2016.[5] The VCAG recommended carefully planned pilot deployment of Oxitec's GM mosquitoes accompanied by rigorous independent monitoring.

Noting that there is an absence of data on epidemiological

impact, the VCAG also said that randomised controlled trials with epidemiological outcomes should be carried out to build evidence for routine programmatic use of OX513A against *Aedes*-borne diseases. To this end, Oxitec is setting up a steering group with representatives from the VCAG, the CDC and the NIAID, among others, to reach a consensus on the best approach for a trial design.

"Mosquitoes don't travel very far, but the problem is that people do, and you need about 300,000 people before you can demonstrate with true statistics the epidemiological outcome. It's pretty uncharted territory. Anecdotal results show that disease levels have decreased, but it does take time to prove a direct disease correlation," Mr Parry said.

Nevertheless, Oxitec is moving forward with its plans. In Brazil, the company is settling in for the long term. The first step was gaining CTNBio's approval, which allows it to do projects throughout the country. It then had to obtain a special temporary registration (which is imminent) from the National Health Surveillance Agency, Anvisa, to commercialise OX513A.

When CTNBio handed the OX513A file over to Anvisa for review, "they scratched their heads for a bit as to how to handle it because it's a first," said Mr Parry. "They decided to create a new regulatory framework for so-called 'urban pathogens'. The procedure is still being set up for similar products that arise in the future, but they are granting us the special registration based on the data we provided them," explained Mr Parry. The company is building its second mosquito production facility in Brazil so that it can scale up the release of OX513A across the country.

The process is moving more slowly in the US, where Oxitec opened a file with the FDA in 2011 – the same year it filed in Brazil. The US agency was also confused as to which regulatory route to follow, but then decided that OX513A would be evaluated as an investigational new animal drug. Thus, field trials will have to be conducted in order to generate data to support a new animal drug application.

As a first step, the FDA's Center for Veterinary Medicine (CVM), along with the CDC and the Environmental Protection Agency, reviewed Oxitec's environmental assessment (EA) on its proposal to conduct a field trial in Key Haven, Florida. After an extensive review, the CVM in March 2016 published a preliminary finding of no significant impact on the environment (FONSI).

The document was out for public comment until mid-May 2016 and an FDA spokesperson confirmed that the agency is now reviewing the comments. "Because this is a first of its kind application, the FDA understands how important the public comment period process is. The FDA is reviewing the thousands of comments received and intends to issue a final EA and FONSI, or prepare an environmental impact statement as expeditiously as possible," the FDA told *MedNous*. While there was no timeframe given, the FDA noted that the application was a top priority.

The agency has its work cut out, as the proposed trial is being met with quite a bit of controversy. Stakeholders have submitted about 20 thousand comments, some of which are from local residents who vehemently oppose the release of OX513A. The Center for Food Safety has also filed a letter, with over 55 thousand signatures, urging the FDA to reject the application. Mr Parry is well aware of the debate but remains confident that Oxitec has more supporters than opponents. Nevertheless, the company actively engages with the community to allay fears and improve trust.

As for Europe, Mr Parry said that the *Aedes aegypti* is not really a threat in European countries. However, Oxitec is developing a transgenic *Aedes albopictus*, commonly known as the Asian tiger mosquito, which is a potential disease carrier especially in southern Europe.

In academia, another potential game-changer for eradicating vector-borne diseases is gene-drive technology, otherwise known as gene editing. Research is ongoing, but it has yet to see the light of day outside the lab.

Gene drives are often described as an exception to the conventional (Mendelian) rules of inheritance, i.e. where offspring have a 50% chance of inheriting a gene. With a gene drive, the offspring will almost always receive the targeted genetic element. The result is the preferential increase of a specific genotype. In particular, the development of CRISPR/Cas9 has spurred scientists to look into applications for altering organisms that transmit infectious diseases to humans, such as mosquito vectors of Zika, dengue, chikungunya and malaria.

This technology is much more complicated than Oxitec's and will need greater scrutiny. "The objective is to put out an organism that will spread," Mr Parry pointed out. "It's a self-perpetuating system and once it's been released, you can't recall it," he added.

The US National Academies of Sciences, Engineering, and Medicine (NAS) issued a report in June 2016, which concluded that "there is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment."[6] It acknowledged the potential benefits of gene drives for basic and applied research, however.

In the UK, the House of Lords Select Committee on Science and Technology has also published a review of a report on GM insects.[7] Because modifications using gene drives would be persistent in the environment, it noted, "there must be much more rigorous assessments of the long-term environmental impacts compared to the population suppression strategies, where no such modifications persist."

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