

*A conversation with Andrea Crisanti,  
Nikolai Windbichler and Tony Nolan,  
Imperial College London*

## “It’s a Question of Risk and Benefit”

Photo: James Gathany/Center for Disease Control and Prevention

In 2010, the WHO estimated that about 216 million people worldwide contracted malaria, 655,000 died. Responsible for this harrowing body count is a tiny, unicellular parasite that has made itself at home in mosquitoes of the genus *Anopheles*. Measures to control transmission of the disease are desperately needed. *Lab Times* talked to Andrea Crisanti, Nikolai Windbichler and Tony Nolan from Imperial College London, who pin their hopes on genetically modifying disease-transmitting mosquitoes.

**L**ab Times: When it comes to battling malaria or other vector-borne diseases, there exist several strategies like developing vaccines or drugs, using insecticides. But what are the advantages of using genetically modified (GM) insects?

**Andrea Crisanti:** Historically, it has been shown that the most effective malaria control measures are those that target the vector. You will never be able to control malaria just by treating the case; you really need to decrease the transmission. This has been best achieved by the use of insecticides, in the past. However, the use of insecticides has several problems: they require quite complex logistics, they are relatively expensive and mosquitoes develop some kind of resistance against them. And there is another component in the control of malaria, which is the sustainability. What happens very frequently is that malaria-endemic countries imple-

ment vector control measures with the support of the local agency and international help but as soon as these fade away also the malaria control system loses impetus and efficacy. So, the sustainability is the major problem. But the sustainability is quite important and this is the reason why the genetic modifying of mosquitoes becomes attractive because, ideally, we

want to manipulate the mosquito in such a way that they do the job for us.

**Nikolai Windbichler:** But you shouldn’t see it as mutually exclusive, if you work on genetically modified mosquitoes then you don’t need vaccines or you don’t need other things... they can work together.

**Crisanti:** Actually, if you have a vaccine, which has limited or low efficacy, when you decrease the transmission it becomes much more effective. There is various experimental evidence that indicates that efficacy of a vaccine depends on the transmission. For high transmission you need a very effective vaccine, for low transmission you can get along with a much less effective vaccine. So in this case, by decreasing the number of mosquitoes able to transmit malaria, you release pressure from the vaccine efficacy. As Niki said, they complement very nicely, the two.

**Tony Nolan:** Like combination therapy in drugs.

*When targeting the insects, there are also a few approaches like the Sterile Insect Technique (SIT) or an advanced GM version of it known as RIDL (Release of Insects carrying a Dominant Lethal), developed by British com-*

*pany Oxitec (portrayed in LT 6-2011). How do they compare to your own approach?*

**Crisanti:** The SIT is currently used for the medfly [the Mediterranean fruitfly, *Ceratitis capitata*], are you familiar with the medfly story? It’s an agricultural pest that can ruin the harvest of many fruits;

it’s a threat in several countries like Turkey, California and Central America. And the release of sterile insects has proven very successful in controlling it. One very good thing

that the sterile insect technique has is that, compared to insecticides, it is very species specific. Insecticides are not species specific; they kill many, many insects. SIT won’t interfere with any other ecosystem. It is very attractive. It has a lot of potential and good effects.

**Windbichler:** It does require sterilisation of insects, which is classically done with radiation. But that’s not very good for mosquitoes; they cannot tolerate radiation very well. They lose competitiveness and viability. It also requires production of insects on an industrial scale. You have to release millions.

*And the RIDL?*

**Windbichler:** The RIDL is very comparable to the Sterile Insect Technique (SIT).

*“By decreasing the number of mosquitoes able to transmit malaria, you release pressure from vaccine efficacy.”*

*“Sustainability is the major problem of malaria control strategies and this is the reason why the genetic modifying of mosquitoes becomes attractive.”*

The basic thing is that you also have to release a lot of mosquitoes to achieve control.

**Crisanti:** Both the SIT and the RIDL do not address the issue of sustainability because you rely on several releases in different places; you rely on a big logistic.

*This sounds very ineffective. Would you say that maybe the RIDL technique should not be developed any further?*

**Crisanti:** No, we don't say it shouldn't be developed further. I think that developing this system paves the way for much more effective measures. Because, in principle, we are on a learning curve to understand what kind of process we have to follow in order to obtain authorisation, how genetically modified mosquitoes behave in the field, what is the best way to release them. So, I think we will gain a huge amount of very valuable information and I think the experiments should be done. Also because the SIT, like RIDL, have much fewer ethical issues...

**Windbichler:** It's a very risk-conservative system because the only thing they transmit to their children is sterility. So there is not so much you can potentially think of in terms of risks. And it can be use-

ful to eradicate geographically or ecologically isolated populations.

**Crisanti:** I think it's very good that these RIDL experiments have been carried out in this learning phase of using genetically modified insects.

**Windbichler:** Absolutely.

*Oxitec already released millions of GM insects, also into populated areas. Is that also part of that learning experience?*

**Crisanti:** They have carried out small scale pilot experiments to show these mosquitoes don't pose any threat to the human environment and animals. They have convinced themselves, convinced local authorities and policy making people. I feel quite comforted.

*"Ideally, we want to manipulate the mosquito in such a way that they do the job for us."*

*And if they also released their mosquitoes here in London?*

**Nolan:** If they were releasing appropriate insects, yes, but there is no malaria problem here... we ourselves work with transgenic insects every day...

**Crisanti:** It's a question of risk and benefit. I'm sure if you cross the street, you take

a risk because you are German and you're not used to looking right first at the side of the road. So, you have a small but real risk. There's nothing without risk. There's no such thing in the world. One has to balance benefit and risk.

**Windbichler:** Right now, it seems a bit abstract, to say releasing them here in Lon-

don. There's nothing to be gained from it but if there were a huge number of Dengue cases, where there's

no vaccine and no treatment, in London, suddenly the perception would shift.

**Crisanti:** From a different point of view, wouldn't we be happy if we had something that would eradicate the spring-summer encephalitis? Wouldn't we be relieved if it could be eradicated?

*Probably.*

**Crisanti:** Probably, yes, because I heard in Germany it's a big problem. Germans have implemented a very complex surveillance and treatment system. Everybody who is bitten by a tick has to go to the doctor, several times, do the vaccine check and so on. So this is quite expensive. Not only quite expensive but the tick-borne encephalitis is a very serious disease and the vaccine doesn't work all the time.

*Are you then aware of any GM approach to the tick issue?*

**Crisanti:** No, there's none that I'm aware of. But this is a very interesting case as there is no way to control it. – Coming back to what was said earlier. You have to see the problem in perspective, to us who are sitting here comfortably in London. Well, we don't want to see genetically modified mosquitoes because we don't have any problem. But there, where they have a problem and have had this problem for centuries, and then suddenly there's a solution at hand? I think you have to weight the benefit and the risk.

*People on the Cayman Islands, where Oxitec released millions of their RIDL mosquitoes, were not so happy...*

**Crisanti:** Oh, well! Listen, all technological novelty, when exposed to the population, always raises a lot of concern. Have you seen some of the caricatures that were printed here in England, when Jenner introduced the vaccination against smallpox? Everyone was scared that he meant to transform people into cows. Everybody was very

## Malaria Busters



**Andrea Crisanti** (centre) studied medicine at the University of Rome, specialising in medicine and surgery. His scientific career path then led him to the Basel Institute of Immunology and the Zentrum für Molekulare Biologie at the University of Heidelberg (ZMBH), before returning to Rome as Principal Investigator in the Italian Ministry for Research and Technology and director of the laboratory of Immunology and Molecular Parasitology at the Institute of Parasitology of "La Sapienza" from 1991-94. Since 1994 he has been affiliated to the Imperial College for Science Technology and Medicine, London, where he's currently working on the development of gene transfer technology for anopheline mosquitoes.

**Nikolai Windbichler** (left) has been a post-doctoral research associate in the lab of Andrea Crisanti since 2006. He's mostly interested in the action and evolution of selfish genetic elements. **Tony Nolan** (right) also post-doctoral research associate in the lab of Andrea Crisanti focuses on the molecular biology aspect of vector control.

sceptical; on top of that the vaccine caused some problem because it was a very dangerous vaccine. But on the other hand, everybody used it. And nobody was transformed into a cow!

*Let's talk a bit more about your own research. How did you come up with the idea to use a genetic drive system to control the malaria mosquito?*

**Crisanti:** I think the idea came from the observation that you could genetically modify the fruit fly, *Drosophila melanogaster*. Most molecular technology has been first developed in the fruit fly because it's a very versatile experimental model. There is a lot of genetic knowledge and when the first experiments were done in *Drosophila*, showing that it's possible to introduce genetic material, which can be inherited by the progeny this, of course, generated a possibility of modifying the phenotype of insects that are an agricultural pest or transmit disease. I think it wasn't an idea originated by somebody; it was the obvious concept of a technological breakthrough made in *Drosophila*. Then, of course, the next step was to transfer this technology from *Drosophila* to mosquito and this was not trivial. Right now we are working on yet another step and have been able to show some progress. This is to transfer the genetic modification from a few laboratory mosquitoes to entire populations. So, in contrast to SIT and RIDL, you release only a few mosquitoes and then these few will spread the genetic modification to all the resident mosquitoes.

*How does this work?*

**Windbichler:** There are two basic strategies. One is to actually reduce the number of mosquitoes that are out there and the other strategy is to replace these wild mosquitoes with mosquitoes that cannot transmit malaria, which is called population suppression, population replacement. If you think about population replacement, you need two components. You need a gene that will make the mosquito refractory to transmit the parasite and then you need a second component that can spread this other gene throughout the population. And this second component is what we call a genetic drive system, that's what we are working on. For this, we are utilising selfish genetic elements like homing endonuclease genes (HEGs). These are genes that can in-

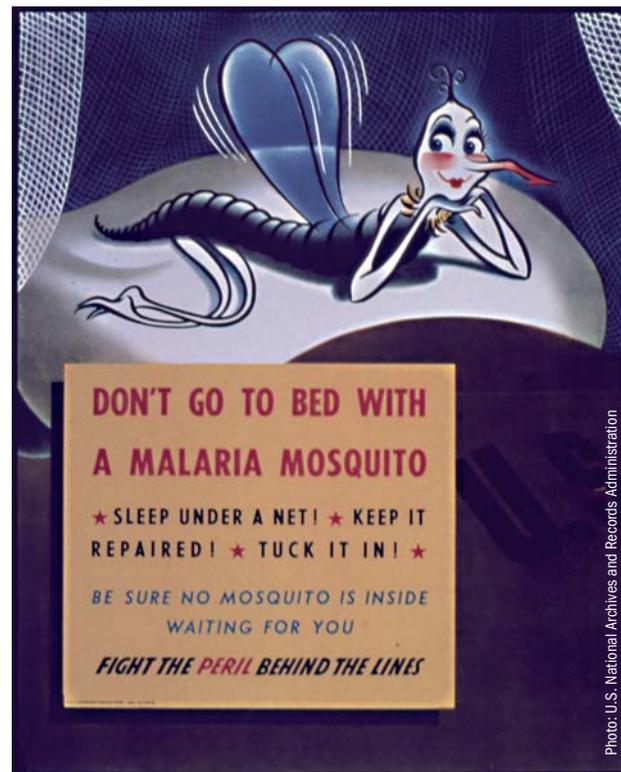
crease their frequency in a gene pool, despite having a negative effect on the individuals; that's why they are called selfish. They can spread through a population just because they are able to do it. You can compare it to a virus...

**Crisanti:** I would not, haha.

**Windbichler:** You can compare it to a virus. But those selfish genetic elements are internal, in the genome, they never leave the cell. You probably don't want to compare it to a virus.

**Crisanti:** Well, let's say, comparable to a virus in terms of their ability to replicate but they are not really like a virus. They also don't replicate from one cell to another, they duplicate themselves only during gametogenesis, during the formation of gametes. So, in principle, if you have an insect that carries this modification, it cannot transmit this modification horizontally. But what it can do is, it

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More than 60 years ago, there wasn't much one could do about malaria, other than give some good advice.

can change the way the progeny inherits the trait. Let's say, if your mother was blonde and your father has brown hair, you probably have brown hair but now to your progeny... some will have inherited the brown gene, some will have inherited the blonde

gene. Now, what our genetic modification does is it acts in such a way that *all* will inherit the brown gene or *all* will inherit the blonde gene.

**Windbichler** (smiling): Blondes are recessive.

*How's this happening at the genetic level?*

**Nolan:** It's a little bit complicated but what happens is, the way this gene copies itself over in the germ line, so in the gonads, where you produce all your gametes, it copies itself over to the opposite chromosome. And therefore, instead of being transmitted to 50% of the progeny, it's transmitted to 100%, potentially. For the population reduction strategy, you take advantage of the actual process of this selfish element moving and when it does so it disrupts a mosquito gene. As it disrupts that mosquito gene, it will have an effect on the mosquito reproductive success and, therefore, it will eventually lead to reduction in the mosquito population. But in order to do that you need this selfish genetic element in a specific location, you need to

recognise a specific location within the target gene you want to disrupt. Does this make sense?

**Windbichler:** It's probably a bit difficult to understand just by describing it in words. But imagine you have two chromosomes and here you have a gene that is required for mosquito fertility, so females need this gene to be fertile. What you could do is, you could insert into this gene your HEG construct. The way this selfish gene element works is, after being inserted into one of the chromosomes, it also makes a cut in the other. Because broken chromosomes need to be repaired, the one containing the HEG construct is used as a repair template. So, what you end up with are two identical chromosomes, right? And where-

as this one, this female that only has one copy of the HEG and one functional copy of the fertility gene is still fertile, the female, which has now two broken copies of the fertility gene, is no longer fertile. That's the basic mechanism.

*What about the population replacement strategy, what are possible “effector genes”?*

**Nolan:** It could be something that interferes with the parasite during its development. There are several potential kinds of receptors, for example, that have been identified or potential immune molecules in the mosquito that act against the parasite. All of them could potentially be used in this approach but we’re not at this stage yet. We’re concentrating more on the first approach that Niki mentioned, the population reduction.

*There’s also another “genetic spreading system” developed by Australian scientists. It involves the parasitic bacterium Wolbachia that infects mosquitoes...*

**Crisanti:** This is quite interesting; it works very nicely, yes. I mean, they show it works. They were able to get approval for a field release and it is very promising. The mosquito, when infected with these para-

site bacteria and for reasons that we do not fully understand, loses its ability to transmit the virus; in these cases, the dengue virus. This is quite useful because for dengue there is no vaccine, no therapy and with such a simple approach, you can solve this problem. It is great. Okay, one should say that it’s 95% suppression of transmission;

*“The most powerful and the final kind of technology we want to develop is the type that would spread through the whole population.”*

*Coming back to your own “milestone”, what’s your estimation, how long will it take before your GM insect approach is “ready for the field”?*

**Crisanti:** We have a kind of commitment (laughs) with our sponsors. We will have mosquitoes ready to be released in three years and will probably do the first pilot experiment then.

**Windbichler:** But there are different variants of these HEG mosquitoes. Some

it’s not 100% but still, it’s very good. Unachievable with insecticides. It’s great, I think it’s a milestone.

of them are like the RIDLs, they are sterile. So, they are very risk-conservative, they can work in a small area to have an effect. Others are self-limiting; the gene can spread to a certain extent but not actually spread through the whole population. And then eventually, the most powerful and the final kind of technology we want to develop is the type that would spread through the whole population.

**Crisanti:** We are doing them in parallel. Of course, in the field we will test the most conservative approach first.

*Do you have some more exact plans?*

**Crisanti:** Yes, but we cannot go into details. We have a line of efforts and resources to be able to test these mosquitoes in the field, in Africa. We have identified three locations and we are now working closely with the people there. It’s too early, we don’t want to compromise their efforts, their commitment. So, we can’t say more at this point.

INTERVIEW: KATHLEEN GRANSALKE