Target Malaria's step-by-step development pathway

A phased pathway to develop a novel genetic approach for malaria control

Target Malaria is working to develop and share novel genetic technologies to help control malaria in Sub-Saharan Africa. We aim to do so by developing modified mosquitoes that carry a genetic trait that will result in the reduction of malaria mosquito populations and that could complement existing methods of malaria control. Reducing the number of mosquitoes that can transmit the malaria parasite would result in fewer malaria infections.

Using genetic approaches to manage insect populations is not a new concept. One of the most common approaches is the use of irradiation to make an insect sterile and then to release those sterile insects to lower the reproduction rate of a target insect population. This method has been used for the control of agricultural pests for many years, such as fruit flies[1], and it is also being used to control disease vectors such as tsetse fly in Africa[2]. Advances in genetic techniques have allowed variations of this approach to be developed. Replacing irradiation with a targeted genetic modification is currently being tested, for example to manage the population of mosquitoes responsible for dengue and chikungunya disease outbreaks[3].

Malaria is quite different compared to these diseases. In sub-Saharan Africa, malaria is mainly rural, covering large areas and regions that are often difficult to access. This geographical setting makes it difficult to rely on large scale interventions that need to be repeated over time. For dengue, for instance, much of the burden is in cities so it is possible to control the dengue mosquitoes by doing multiple releases of modified mosquitoes, enough to "swamp" the population and control it. In Africa, it would be impractical to carry out these interventions at a national or regional scale because it would mean repeatedly producing and releasing very large numbers of mosquitoes. It would not be cost effective and therefore not sustainable.

Target Malaria is seeking a long-term, sustainable and cost-effective solution to eradicate malaria. Our research is building on these precedents to develop gene drive approaches, which would allow the intended modification to become established in a target population over a relatively short period of time, thus reducing that population's ability to reproduce. This would help to decrease the population of the malaria vector, the *Anopheles* mosquitoes, and therefore result in a reduction in the number of malaria infections in sub-Saharan Africa.

While there are precedents and experience to draw on, gene drive approaches are a novel area of research that require a stepwise development pathway. Target Malaria is progressing through several phases of iterative research to enable its stakeholders and national authorities in the countries concerned to gain understanding of this new field of research and its potential.

Target Malaria's approach draws on the guidance developed by WHO, as well as expert views from national academies, regulatory bodies, other scientists and researchers who are working in this field. We have adopted a phased approach to our technology development and are constantly re-evaluating the best pathway based on the information we gather from our work, advances made by other research teams, and new guidance from experts and authorities.

Non gene drive sterile male mosquitoes

Our development pathway started with a sterile male mosquito as proof of principle in the laboratory in 2008[4]. The males were genetically modified to be sterile, so they could not have any progeny. These mosquitoes were not intended to be a viable tool for controlling malaria, because they would not provide the long-term or cost-effective benefits that malaria control requires. However, their development was an important step to gain knowledge, start a dialogue with stakeholders and provide a strain of mosquitoes that we could use to evaluate our process, procedures and preparedness.

The genetically modified sterile male mosquitoes, like all our strains, were subject to evaluation in small cages (30cm x 30cm) and then large environmental cages (up to 9.2m[5]) in laboratories in the UK and Italy as well as further safety and risk assessments, before being imported by the team at the *Institut de Recherche en Sciences de la Santé* (IRSS) in Burkina Faso, under permits granted by Burkina Faso's National Biosafety Authority, the *Agence Nationale de Biosécurité* (ANB). The team worked with the genetically modified sterile male strain for over a year in contained laboratories before conducting a small-scale controlled field release in 2019, under additional permits and with the acceptance of the local communities involved. The team at Malaria Research Training Center (MRTC) in Mali has also been granted a contained use permit by the Malian National Biosafety Committee, under the Ministry of the Environment. Work is ongoing in their insectary.

Prior to any regulatory approval for a field evaluation, a comprehensive risk assessment would be performed by the Target Malaria risk team as well as third parties, including quantitative analysis and levels of certainty, as has been recommended in global fora.

Non gene drive male bias mosquitoes

Another step in our phased development pathway has been the development of a mosquito that could successfully mate and produce offspring, but in which the genetic modification would only persist for some time before disappearing[6]. The strain(s) would hence be "self-limiting" in the sense that the modification does not persist for a very long time. This strain does not carry the gene drive element that biases inheritance. This means approximately 50% of the offspring have the genetic modification as would be expected by Mendelian inheritance. The male mosquitoes carry a modification that causes them to produce almost exclusively males offspring, biasing the ratio of the targeted mosquito population towards males (male mosquitoes do not bite and therefore do not transmit malaria).

While also an interim step that does not involve gene drives, it is useful to have strains where the modification persists for longer in order to provide additional information on how the genetically modified mosquitoes behave in a natural setting over an extended period of time. It is also an important intermediate step for stakeholders to learn more about genetic modification and for regulatory authorities to consider how they want to manage future research in this field. This strain of mosquitoes has not yet been imported by any of the teams in Africa but has undergone laboratory evaluation in the UK and Italy, as previously described.

Self-Sustaining gene drive mosquitoes

Our ultimate goal is a new vector control tool for malaria. To achieve this goal, we are developing a mosquito strain, which involves a gene drive that biases its inheritance and is self-sustaining. Malaria is predominantly a rural disease, which has remained most entrenched in African countries with populations spread over large areas and often with less well-developed transport and public health infrastructures. The complexity and cost of carrying out repeat interventions (such as spraying and bed net distributions), combined with issues of growing resistance to insecticide and anti-malarial drugs, are threatening to reverse progress towards malaria elimination.

Gene drive approaches, because of their self-sustaining nature, could - in conjunction with existing tools - offer long-term, sustainable and cost-effective methods to control *Anopheles* mosquito populations. We are currently investigating several options, the two most promising are:

1) A gene drive strain with fertile males that produce predominantly male offspring, leading to a distortion in the sex ratio and biased inheritance of the gene drive element in the targeted mosquito population;

2) A genetically modified strain with fertile males carrying a gene that will spread through the mosquito population and cause females that inherit the gene from both parents to be sterile.

Both approaches would lead to a reduction in the mosquito populations that are the main malaria vectors. We are making good progress, but these approaches are still in the discovery stages in our laboratories in the UK and Italy.

Although we are developing the approaches separately, it may also be possible to combine the two approaches for use in the fight against malaria. The project's achievements so far on development and evaluation of self-sustaining genetically modified mosquitoes have been published in peer reviewed scientific journals[7].

1 FAO-IEIA joint programme: *http://www-naweb.iaea.org/nafa/ipc/index.html*

2 IEIA: *https://www.iaea.org/newscenter/pressreleases/iaea-helps-burkina-faso-scale-up-fight-against-tsetse-flies*

3 *https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008103* and *https://www.pnas.org/content/108/12/4772*

4 Windbichler N., et al. 2008 Targeting the X Chromosome during Spermatogenesis Induces Y Chromosome Transmission Ratio Distortion and Early Dominant Embryo Lethality in Anopheles gambiae. PLoS Genet 4: e1000291. Targeting the X Chromosome during Spermatogenesis Induces Y Chromosome Transmission Ratio Distortion and Early Dominant Embryo Lethality in Anopheles gambiae. *https://doi.org/10.1371/journal.pgen.1000291*

5 Our phased development pathway builds on reports that contain clear guidelines for safe and ethical research to ensure the rigorous regulation of genetically modified mosquito strains from: 2014 World Health Organisation (WHO) Guidance Framework for Testing of Genetically Modified Mosquitoes, the 2016 National Academies of Sciences, Engineering and Medicine report Gene Drives on the Horizon, and the 2018 Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group.

6 Galizi, R. et al. A synthetic sex ratio distortion system for the control of the human malaria. mosquito. Nature Communications 5, 3977 (2014). A synthetic sex ratio distortion system for the control of the human malaria mosquito. *https://doi.org/10.1038/ncomms4977*

Pollegioni, P, North, AR, Persampieri, T, et al. Detecting the population dynamics of an autosomal sex ratio distorter transgene in malaria vector mosquitoes. J Appl Ecol. 2020; 57: 2086– 2096. https://doi.org/10.1111/1365-2664.13702

7 Kyrou, K., Hammond, A., Galizi, R. *et al.* A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat Biotechnol* 36, 1062–1066 (2018). *https://doi.org/10.1038/nbt.4245*
Simoni, A., Hammond, A.M., Beaghton, A.K. et al. A male-biased sex-distorter gene drive for the human malaria vector Anopheles gambiae. Nat Biotechnol 38, 1054–1060 (2020). *https://doi.org/10.1038/s41587-020-0508-1*