Evaluating our gene drive technology

Taking a gene drive mosquito from lab to field

Target Malaria is working to develop novel genetic approaches to control the population of malaria mosquitoes in Africa. We are investigating the use of gene drive approaches as a method to achieve population control as it could allow us to develop modified mosquitoes that can pass a change in their genes to their progeny in a self-sustaining way, leading to a reduction in the malaria mosquito population over time.

Malaria is predominantly a rural disease, which has remained entrenched in African countries with populations spread over large areas and often with less well-developed transport and public health infrastructures. Despite significant progress in controlling malaria in many countries around the world in the past decade, elimination remains an elusive goal. Progress is threatened by increasing resistance to insecticides and anti-malarial drugs and the costs and complexity of repeat interventions in resource-poor settings. Gene drive approaches, because they are self-sustaining, could offer long-term, sustainable and cost-effective methods to control *Anopheles* mosquito populations in conjunction with existing tools.

We are currently investigating several options, the two most promising are:

1. A genetically modified strain with fertile males that produce predominantly male offspring, leading to a distortion in the sex ratio of 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 be expected to lead to a reduction in the mosquito populations that are the main malaria vectors over time. They could also be combined for greater impact.

While significant progress has been made in generating current versions of these strains, a substantial amount of work in the laboratories in the UK and Italy is still needed before any gene drive mosquito can be considered for further study in Africa. The long timeline for developing such a technology is due to the many steps that the mosquitoes must go through to weigh up benefits as well as potential risks to human health and the environment.

Gene Drive Development Pathway

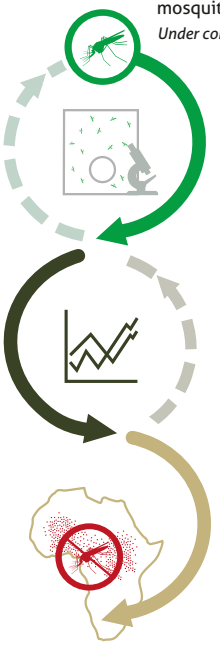
The pathway to develop our gene drive mosquitoes is informed by clear guidelines on safe and ethical research from expert organisations such as the World Health Organization (WHO), with the objective of ensuring that rigorous evaluation of genetically modified mosquito strains takes place before they are proposed for use[1]. We progress our strains gradually from research under biological containment in laboratories and insectaries, to field evaluation in countries where malaria is endemic. Moving from one step to the next is carefully considered and subject to regulatory oversight by national authorities.

Discovery

Our work begins with our discovery team in the UK. We seek to design and generate genetically modified strains that could have beneficial impacts on the size of malaria mosquito populations in the wild. This work is demanding and has been ongoing since 2005, typically involving many iterations and refinements of modifications in order to produce strains with the desired impacts on either sex ratio or fertility.

Once strains of genetically modified mosquitoes are generated in the lab, we characterise them phenotypically and molecularly, and study their population dynamics in small cages. When these studies indicate that the modification has the intended molecular properties and that the strain displays the population dynamics expected to be useful for mosquito control, then we export that strain for safety studies and further assessments in large cages at our insectaries in Italy and the USA.

Development pathway for gene drive mosquitoes



Discovery: initial laboratory studies to create new strains of gene drive mosquitoes and insectary studies to test them in small cages

Under contained use regulations

Development in containment: further contained studies in small and large cages to characterise the phenotype and molecular components

In our insectaries with the appropriate permits

Comprehensive risk assessment: modelling, literature reviews and extensive laboratory testing help us to analyse, manage and communicate about risks

Before we apply to relevant regulatory authorities

Field evaluation: release of gene drive mosquitoes that have passed the previous phases

Subject to regulatory oversight by national authorities and the agreement of local communities directly affected by project activities

Operational development: Effective control of *Anopheles gambiae* populations using our gene drive technology as part of integrated malaria elimination strategies. Reduction of malaria transmission in Africa

In line with WHO recommendations and guidance

Further development in containment

At our facilities in Italy, we use large indoor cages (up to 9 m^3) that mimic the conditions of the mosquitoes' natural environment in Africa. Over the course of several years, we use these large indoor cages to examine the ability of modified mosquitoes to compete with wild type mosquitoes for mating in large populations, to study if the modification affects different aspects of mosquito behaviour and the potential for inheritance of the genetic modification over multiple generations. This information allows us to predict the time it would take after field release for the modification to establish itself and to persist in the target population.

Key safety studies are also carried out at this stage, either in partner labs or contract research organisations. These studies are informed by analysis of potential pathways to harm arising from field release of the modified strain and by extensive discussions with external stakeholders, in particular regulators.

The project also uses external resources, such as guidance from the WHO, conversations with regulatory agencies, feedback from stakeholders, and developments from other international research to inform what studies need to be considered. The causal chain of events that would be required for each biologically plausible harm to occur is systematically mapped out to identify any studies or evidence requirements that might inform whether that harm could occur.

Key safety studies identified by analyses of such pathways to harm include comparison of the modified strain with wild mosquitoes to see if it (a) can transmit malaria or other diseases more effectively, (b) is less susceptible to insecticides, (c) could encroach beyond usual geographic ranges, potentially disrupting newly-occupied ecosystems or increasing risk of disease, by geographic expansion.

Field evaluation

If a strain of mosquitoes performs well in small and large cage assessments and passes key safety studies, only then would we consider moving to evaluations of that strain in the field. Through our partner institutions in Africa, we are working with the national regulatory authorities and all stakeholders, in particular local participating communities. The national regulatory authorities would be deciding if any future potential small-scale releases of mosquito strains could be performed and the local participating communities would decide to give their consent for the releases to take place at their sites.

It will be several years before a potential release of gene drive mosquitoes will be considered, and we are actively working with communities, experts, stakeholders, partners and authorities to understand their viewpoints, priorities and possible concerns.

Field evaluations will focus on determining whether the release of modified mosquitoes will have the desired outcome on the number of malaria mosquitoes, and ultimately on the number of malaria cases in a given area.

Prior to any regulatory approval for a field evaluation, a comprehensive risk assessment would be performed, including quantitative analysis and levels of certainty, as has been recommended in global fora.

Subject to national regulatory approval and community acceptance, we expect the field evaluations to take place through a series of iterative steps, growing from smaller scale to larger scale, starting with entomological endpoints (the impact of the modified mosquitoes on the malaria mosquito population), and subsequently incorporating epidemiological endpoints (the impact of the modified mosquitoes on the number of malaria cases) in larger scale trials where any impact on the incidence of malaria might be detected.

Using a gene drive mosquito to reduce the transmission of malaria

If field studies were successful in showing that modified mosquitoes with gene drive can effectively control wild target *Anopheles* populations and reduce malaria transmission, Target Malaria would work with WHO to evaluate the results against their criteria for a policy decision to recommend gene drive as a vector control tool against malaria in Africa. The project would also cooperate with other international authorities. Our technology would be available to any countries that wanted to use it without any commercial gain or profit.

1 See: 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.