Institution: University of Cambridge

Unit of Assessment: 5

Title of case study: Overcoming regulatory barriers for the implementation of Gene Drive technology to control malarial mosquitos in Africa

Period when the underpinning research was undertaken: 2011 - 2015

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Steven Russell	Professor of Genome Biology	1990 - date

Period when the claimed impact occurred: 2017 - 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact (indicative maximum 100 words)

As part of Target Malaria, a not-for-profit research consortium working across four countries in Africa, the Russell lab at the University of Cambridge showed for the first time in 2011 that gene drive technology could work in an animal. They established novel gene drive systems that were implemented effectively in *Anopheles* mosquitos, the vector for the malaria parasite. The project overcame substantial regulatory barriers to obtain the first license for importation of a genetically modified (GM) insect into Burkina Faso in 2016, followed by licenses for Mali and Uganda. Subsequently, a field release took place in Burkina Faso in 2019. By facilitating research in local environments, in collaboration with local researchers, the developed frameworks promoted capacity building, which in turn led to the development of the first regulatory approval for the use of GM invertebrates in any African country.

2. Underpinning research (indicative maximum 500 words)

The context of a global health issue

Malaria remains one of the world's major infectious diseases, responsible for approximately 405,000 deaths in 2018, with 94% of these in sub-Saharan Africa, and with an estimated annual economic impact of USD12 billion in Africa alone (WHO World Malaria Report 2019). In 2019, the World Health Organisation predicted that reliance on current methods of malaria control, such as insecticide, preventative therapies and antimalarials, would mean over 10 million cases of the disease per year would still occur in Africa in 2050. Therefore, cost-effective tools are required to control this disease. The use of genetically modified insects offers the prospect of developing new tools for malaria intervention, however, there are considerable regulatory barriers to research into transgenic insects and the deployment of modified insects in Africa, where established regulatory pathways are poorly developed (Ecuru, 2017, DOI: 10.1017/9781316585269.025).

Using expertise in a model organism for pioneering use of gene drive technology

As a world-leading centre for research on the fruit fly, *Drosophila melanogaster*, the Russell lab at the University of Cambridge participated as a collaborating partner in a consortium project, Target Malaria, to explore the use of a particular approach, gene drive, to control insect disease vectors. Gene drive is a process that promotes the inheritance of specific genes from generation to generation and while the gene drive process was well established in fungi, it had never been shown to work in an animal. Prof. Russell used expertise in *Drosophila* [R1], a tractable model organism, to lead a major project work package demonstrating that gene drive could take place in insects, transferring the technology to colleagues at Imperial who then used it to develop drive based female sterility in malaria-causing mosquitos.

The Russell lab research in the period 2005 to 2011 [R2] centred on homing endonucleases genes (HEGs): DNA-cutting enzymes found in fungi which can be used to modify genes in a precise way when engineered to recognise specific sites in the genome. HEGs reside at their target site in the genome, cutting DNA at this site in a wild-type (non-modified) chromosome when they encounter one. The cut DNA is repaired by copying information from the HEG-containing chromosome and 'pasting' it into the wild-type, generating a mutation in the gene and duplicating the HEG. When this occurs in sperm or egg cells, HEGs are inherited in a non-Mendelian way and spread throughout the population. The group proposed engineering HEGs to recognise specific sites required for female fertility in the genome of the mosquito *Anopheles gambiae,* an important malaria vector throughout sub-Saharan Africa. In this way, it would be possible to generate female sterility mutations that would be driven through a population, resulting in population suppression.

Breakthrough development

Using *Drosophila* as a model, the Russell group found that high rates of 'homing', converting a normal copy of a gene to a HEG-containing mutant copy, could be accomplished within developing male sperm using customised HEGs. This allowed the modified gene to be more often inherited and thus increase in frequency in the population. This finding (published in 2011), along with the demonstration that the homed constructs continued to exhibit HEG activity in the subsequent generation, represented the first proof-of-concept that gene drive technology could work in an animal [R1]. Prof. Russell and his group also published several papers in 2013 and 2014 with strategies to optimise HEG-based gene drive [R3-R5].

To implement the technology in *Anopheles* it was necessary to accelerate the identification of the target genes in mosquitos, as the genetics and the process of introducing modified genes were poorly established in the species. To do this, the Russell group and collaborators constructed the MozAtlas, an *Anopheles* sex and tissue-specific gene expression atlas [R6]. This tool was used in the first demonstration of a functioning gene drive in *Anopheles*, targeting three different female fertility genes [R7]. The development of this tool, and its potential as a new way to tackle malaria, provided a starting point from which to build new regulatory frameworks that would allow its use in countries with high prevalence of malaria.

3. References to the research (indicative maximum of six references)

R1. Ryder E, Blows F, Ashburner M…**Russell S** The DrosDel Collection: A Set of P-Element Insertions for Generating Custom Chromosomal Aberrations in Drosophila melanogaster. *Genetics* June 1, 2004 **167(2):** 797-813; doi: 10.1534/genetics.104.026658

R2. Chan YS, Naujoks, DA, Huen DS, **Russell S** Insect population control by homing endonuclease-based gene drive: an evaluation in Drosophila melanogaster. *Genetics* 2011 **188:** p33-44 doi: 10.1534/genetics.111.127506

R3. Chan YS, Huen, DS, Glauert R, Whitway E, **Russell S** Optimising homing endonuclease gene drive performance in a semi-refractory species: the *Drosophila melanogaster* experience. *PLoS ONE* 2013 **8:** e54130 doi: 10.1371/journal.pone.0054130

R4. Chan YS, Takeuchi R, Jarjour J, Huen DS, Stoddart BL, **Russell S**. The design and *in vivo* evaluation of engineered I-OnuI-based enzymes for HEG gene drive. *PLoS ONE* 2013 **8:** e74247 doi: 10.1371/journal.pone.0074254

R5. Simoni A, Siniscalchi C, Chan YS, Huen DS, **Russell S**, Windbichler N, Crisanti, A Development of Synthetic Selfish Elements Based on Modular Nucleases in Drosophila melanogaster. *Nucleic Acids Res.* 2014 **42:** 7461-7472 doi: 10.1093/nar/gku387

R6. Baker DA, Nolan T, Fischer B, Pinder A, Crisanti A, **Russell S**. A comprehensive atlas of tissue-specific expression in the malaria vector, *Anopheles gambiae,* reveals genes evolved for

specialized feeding and mating behaviours. *BMC Genomics* 2011 **12:** 296 doi: 10.1186/1471-2164-12-296

R7. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, **Russell S**, Burt A, Windbichler N, Crisanti A, Nolan T. A CRISPR-Cas9 Gene Drive System Targeting Female Reproduction in the Malaria Mosquito vector *Anopheles gambiae*. *Nat Biotech* 2015 **34:** 78-83 doi: 10.1038/nbt.3439

All research outputs have been published in peer-review journals.

Competitive external funding received
2005 – 2012 Homing endonuclease genes: new tools for population engineering and control, Foundation for the National Institutes of Health Grand Challenges in Global Health initiative, GBP1,237,620
2012 – 2016 Controlling the mosquito vectors of malaria with engineered endonucleases, Bill & Melinda Gates Foundation, GBP49,975
2016 – 2020 Controlling the mosquito vectors of malaria with engineered endonucleases, Bill & Melinda Gates Foundation GBP26,058,753

4. Details of the impact (indicative maximum 750 words)

Prior to the work of Target Malaria, no regulatory frameworks for the use of GM insects existed in any sub-Saharan country. The significant impacts within the assessment period are thus centred on the development of regulatory structures enabling the use of GM insects in Burkina Faso, the first example of such regulation in Africa.

From proof-of-concept to implementation
Results generated by the Russell group at the University of Cambridge from this first-in-animal use of gene drive were used by groups at Imperial College London (lead partners for Target Malaria) to develop a subsequent construct that drives female sterility in *Anopheles* mosquitos through large laboratory cage populations without the emergence of detectable resistance. This key milestone, involving results from the Russell lab, enabled further funding to be secured from the Gates Foundation to allow Target Malaria to push forward with implementation (2012-2016 USD21,000,000, 2016-2020 USD34,500,000).

The Target Malaria lead PI says, “The work of Steve Russell and his group at Cambridge Genetics was critical to the early success of the project… when we began in 2005, there were good reasons for thinking this reaction would also work in animals, but nobody had ever seen it, and the Russell group’s demonstration of homing in the *Drosophila melanogaster* model system was a key early proof of principle that we were on the right track… these various studies were invaluable to the overall efforts.” [E3]. Senior Vice-President for Science at the National Institute of Health, who served as the program officer for Target Malaria from 2005-2018, echoed these comments in another letter stating that ‘the basic research performed by Prof. Russell and team provided fundamental early insights supporting the discovery of a potentially transformational new tool’ [E1].

Development of regulatory frameworks for the first time in sub-Saharan Africa
Realising the potential of this new tool would involve being able to test it in a relevant, malaria-affected region. Burkina Faso has a significant prevalence of malaria, and is one of the countries where Target Malaria operates (the others being Ghana, Mali and Uganda), making it a suitable place to carry out first trials.

In 2016, Target Malaria achieved a licence for importation of genetically modified insects for contained use and laboratory experimentation with engineered mosquitos in Burkina Faso. The project subsequently obtained similar licences for Mali and Uganda in 2019 [E12]. These developments led to regulatory approval in Burkina Faso for small-scale field release of pilot GM mosquitos, the first such approval granted in Africa [E1]. In July 2019, the project performed the

first ever field-release of a transgenic insect in Burkina Faso, the first example of such an undertaking on the African continent [E2].

The Principal Investigator for Target Malaria in Burkina Faso wrote: "When we began this work, the regulatory framework for working with genetically engineered insects was poorly developed in Burkina Faso and across Africa… the Target Malaria consortium, working closely with the National Biosafety Agency in Burkina Faso, has greatly contributed to the development of a robust regulatory policy for controlling contained use and pilot field releases of transgenic mosquitoes'' [E3].

Influencing wider policy discussion

Using insights and expertise gleaned as a Target Malaria partner, Prof. Russell co-authored a policy-focussed paper in 2015 that developed multiple containment strategies to safeguard the use of gene drive organisms in laboratories [E4]. This work was cited in a 2018 report by regulatory agencies in the UK, Belgium, Germany and the Netherlands that laid out a framework for the risk assessment and management of gene drive technology in contained use [E5] (Microbiology and Biotechnology Unit, UK; 4 Scientific Institute of Public Health (WIV-ISP), Belgium; Federal Office of Consumer Protection and Food Safety, Germany, National Institute for Public Health and the Environment, Netherlands). The suggestions were also cited and endorsed in a report by the US National Academy of Sciences, which outlined recommendations for responsible conduct when using GM organisms [E6] and again in a report by the European Academies' Science Advisory Council regarding the policy options and public interests surrounding genome editing [E7].

The above research and Target Malaria have also been used to inform a House of Lords report in 2015 on genetically modified insects [E8] and an African Union report on gene drive for malaria control and elimination [E9] that states the technology "present[s] realistic options for effective disease control". In October 2020, the field trials in Burkina Faso were referenced in a WHO position statement on genetically modified mosquitos for the control of vector-borne diseases, which states that the "promising characteristics of self-sustaining gene drive systems have raised hopes for durable, affordable protection against disease transmission" [E10]. The inclusion of this work shows its significance and relevance as part of the global effort to tackle this major health concern.

Impact on public debate and local capacity building

The team engaged with the local community in Burkina Faso in 2017 from the early stages to co-develop their approach, which was crucial in developing these frameworks and further promoted technical, institutional and regulatory capacity building for gene drive technology within the country. From the Burkina Faso lead PI: "The capacity building work of Target Malaria…and the public engagement work of the team was instrumental in achieving this milestone, the first regulatory approval for use of a transgenic insect in Africa." [E3]. This approach was cited in April 2019 for its 'commitment to reimagining engagement' (Hartley et al. 2019, DOI: 10.1371/journal.pntd.0007233)

The Cambridge research [R6] and Prof. Russell's policy paper [E4] also attracted attention from the international press [E11], highlighting the co-creation approach taken to work with those on the ground in the countries of implementation. The stakeholder engagement manager for Target Malaria is quoted by ABC News: "It's one of the essential pillars of our work – making sure the villagers understand and are happy with what we are doing. This is why our partners…have been working with the government and the local people, talking to them, building trust…" This coverage stimulated useful debate, raising awareness and understanding of the issues surrounding the ethics, feasibility, regulation and biosafety of the technology. This engagement with stakeholders at all levels, combined with the development of the first regulatory frameworks, has made a very positive contribution to the way in which transformative new technologies can begin to be adopted.

5. Sources to corroborate the impact (indicative maximum of 10 references)

E1. Combined testimonials from PI of Target Malaria Burkina Faso, Target Malaria lead PI and Senior Vice-President for Science, National Institutes for Health
E2. Target Malaria press release on the first licence GM insects
E3. Target Malaria press release on the first release of GM insects
E4. Akbari et al., Safeguarding gene drive experiments in the laboratory. Science 349:927-929 (2015)
E5. Regulatory body paper that reiterates suggestions from Akbari paper on risk assessment and management of Gene Drive technology, page 25
E6. National Academy of Sciences - Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values (R6 cited on pages 3, 13, 16, 26, 32, 34, 39, 52-54, 58, 99, 101, 102, 117, E4 cited on pages 18, 29, 78, 88, 92, 94, 96)
E7. European Academies' Science Advisory Council Report on genome editing (2017), page 18
E8. Genetically Modified Insects - *1st Report of Session 2015-2016* by House of Lords Science and Technology Select Committee (2015) and Government Response, page 13
E9. Gene Drives for Malaria Control and Elimination in Africa by African Union and NEPAD (2018), page 2 (page 10 of document)
E10. WHO position statement on genetically modified mosquitos for the control of vector-borne diseases
E11. Combined articles on recent developments by ABC News, Gènéthique, STAT and Scientific American
E12. Nakkazi (2020), Fighting malaria with genetically modified mosquitoes, BMJ 2020;370:m2172, DOI: 10.1136/bmj.m2172