Gene Drive Research Funding Recommendation Report

For the Philanthropy Advisory Fellowship

Organized by Harvard University Effective Altruism Student Group

On behalf of Thomas Mather

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Executive Summary

The purpose of this report is to provide recommendations to philanthropists interested in funding a new genetic technology called "CRISPR gene drive." This synthetic biology technology allows scientists to design genetically modified animals that can rapidly spread particular genes through wild populations. As a tactic against parasitic diseases, it could be used directly on parasites to crash their population (e.g. by forcing them to only have male offspring, who in turn only have male offspring), or against intermediate non-human hosts (such as mosquitos) which grant the host immunity to the parasite, thus interrupting the parasite's life cycle. Although the initial cost to research and develop gene drive systems are high; once developed, it offers an incredibly cost-effective means of combating infectious diseases as the gene drive is capable of spreading itself with little additional cost or human intervention. This is in stark contrast to additional anti-parasite public health programs campaigns who depend on costly massive drug manufacturing and distribution campaigns. Although many questions about this technology remain unanswered, we are optimistic about the potential of gene drives in strengthening the public health arsenal and reducing worldwide human suffering.

In this report, we present data from various sources on the top 20 transmitted diseases and parasites with the heaviest health impacts in the world. We analyzed this data to determine the top afflictions suitable for targeting via gene drives. We used the following selection criteria:

- Global disease burden
- Current funding environment
- Genetic tractability
- Targeting efficiency

This full analysis is presented in the companion spreadsheet to this report. Based on the analysis, we chose to recommend the following diseases for further research funding:

- Soil-Transmitted Helminths (STH)
    - Hookworm
    - Whipworm
    - Threadworm
- *Aedes* Mosquito borne diseases
    - Dengue
    - Chikungunya
    - Zika
- Chagas disease

Recommended labs to fund:

- STH
    - Dr. Matthew Berriman, Sanger Institute
    - Dr. James Lok, University of Pennsylvania
- Aedes aegypti

    - Dr. Omar Akbari, University of California, Riverside
- Chagas Disease
    - Dr. Najib M. El-Sayed, University of Maryland
- Basic research and safety
    - Dr. Kevin Esvelt, MIT Media Lab

Our analysis, recommendations, and rationales for these diseases are contained in this report. However, it is important to note what we have *not* included in our scope. We have not considered the political barriers to implementing gene drives, which may be greater than the scientific ones. Much research remains to be done in the area of gene drive safety and control, which we recommend funding. Even with high levels of safety, however, public perception and political feasibility remains a concern. Such a modification of the environment and biosphere is unprecedented in human history, although the case of GMO crops provides some lessons. How will states react when a gene drive, introduced by a neighboring country, spreads into their sovereign territory without their permission? Such questions still need to be addressed, but investing in public education would likely ameliorate these issues.

Although still in its infancy, gene drive research has the potential to make enormous positive impacts on global human health, and funding to answer basic questions and develop initial proofs-of-concept may lead to increased academic and donor interest for this transformative emerging biotechnology.

Introduction

What is a gene drive?

The idea of a gene drive takes inspiration from nature. Scientists had observed gene elements that can bias their own rate of inheritance before even the discovery of the structure of DNA (Serebrovskii, 1940; Vanderplank, 1944). These gene elements are able to insert extra copies of themselves into sites in the host's genome specified by a special DNA sequence, thereby overcoming limitations of traditional Mendelian inheritance. This discovery gave birth to the idea that we, too, can engineer a "selfish gene" that is able to bias its own chance to be inherited by duplicating and re-inserting copies of itself into the host genome.

In 2003, Austin Burt initiated an effort to utilize the "selfish" nature of homing nucleases to carry gender biasing gens to promote wild-type *Anopheles* mosquitos to be a single gender in order to suppress their population and mosquito-borne diseases (Burt, 2003). Unfortunately, difficulties with retargeting homing endonuclease to cut new sites represented significant roadblocks to the progression of this design. Other similar efforts have been tried as well, but all were stalled by related technological roadblocks.

It was not until the so called “CRISPR revolution” in late 2012 that scientists were able to overcome the limitations of traditional nucleases through versatility and programmability of the new CRISPR-cas9 gene editing system, paving the way for a new generation of gene drive systems coined the ‘CRISPR gene drive’.

Current State of CRISPR Gene Drive Directions

Utilizing this powerful new tool, a team of scientists led by Kevin Esvelt from George Church’s laboratory at Harvard Medical School proposed and developed a novel ‘gene drive’ system that will not only carry engineered genetic elements in the laboratory, but also utilize our understanding of CRISPR-based genome editing to bias its own rate of inheritance, thus allowing genetically altered organisms to spread their laboratory-engineered genes through wild-type populations. While there is much interest and discussion surrounding the scientific, ethical, and long-term ramifications of human genome editing using similar CRISPR-cas9 systems (Baltimore, et al., 2015), tremendous opportunities exist in utilizing CRISPR gene drive systems to edit the genomes of whole populations of other species to both relieve human suffering and improve the environment in the near future.

To this end, two independent studies were published in early December 2015 detailing successful demonstrations of CRISPR gene drive activity in two separate mosquito species: *Anopheles stephensi* and *Anopheles gambiae* (Gantz, et al., 2015; Hammond, et al., 2015), with the intent to suppress the spread of malaria. Taking inspiration from these examples, it is conceivable that we can also develop CRISPR gene drive based technologies to fight many other afflictions around the world. The fact that CRISPR gene drive activity has already been validated in yeast, fruit flies, and two species of mosquitos (DiCarlo, et al., 2015; Gantz & Bier, 2015) only further supports our confidence in this technology’s potential. In this report, we recommend Chagas disease, whipworm, hookworm, threadworm and three diseases commonly carried by the *Aedes aegypti* mosquito, as the most effective funding directions for a CRISPR gene drive project to alleviate human suffering.

Selection Criteria

Global disease burden – a holistic evaluation of the total number of affected persons in the world, the annual death-toll, as well as the severity of human suffering caused by the disease. Suffering of children is heavily weighted. Diseases with a high disease burden are valued to be better targets in this report.

Current funding environment – an evaluation of the current funding environment, both from public entities such as the government and the WHO, as well as private organizations such as the Gates Foundation and the Rockefeller Foundation for each disease. Diseases with less funding and public attention are determined to be better funding targets in this report.

Genetic tractability – an analysis of the availability of the necessary genetic information to initiate a CRISPR gene drive project. Fully annotated deep sequencing of an organism is naturally ideal, however, a partially annotated genome can be sufficient. If no genomic annotations are available, or worse - an organism has not even been sequenced, then whole genome sequencing of the target organism needs to be funded before a CRISPR gene drive project can be attempted. A suitable alternative would be to target the pathogen's host organism instead if genomic information for the host is available.

Disease versus host targeting efficiency – an evaluation of whether it is better to target a disease agent's host organism rather than the pathogen itself. For instance, some diseases are caused by multiple related parasite species that all share a common host organism. Because different species generally do not interbreed, a gene drive would need to be created for each separate pathogen species. In these cases, it is far more efficient to target the host organism. Furthermore, some parasites have long lifespans and low geographic mobility, which makes them poor targets. An important caveat to keep in mind is that this report does not support CRISPR editing of the human genome, therefore no gene drive projects targeting humans, even if they are a pathogen's primary host, are considered here.

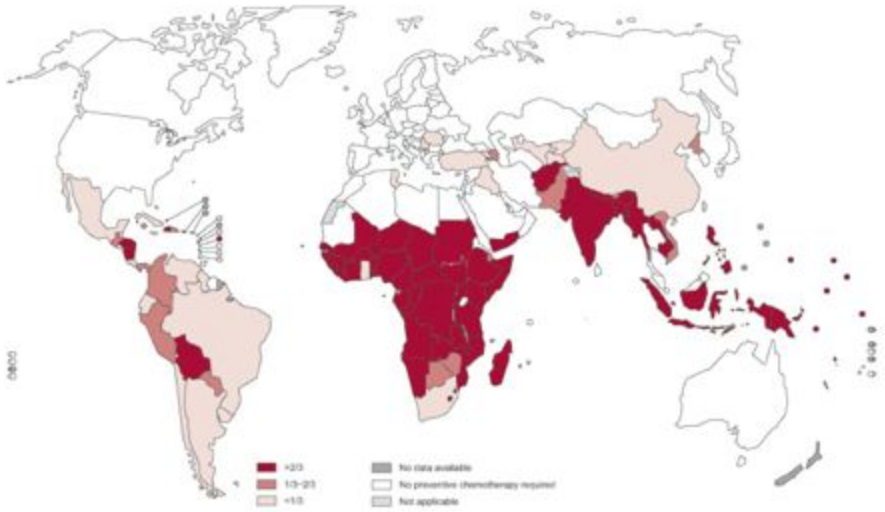
Recommended Funding Targets

Soil-transmitted Helminths (STH)

The global burden of soil-transmitted helminths

Approximately 2 billion people worldwide, including nearly 1 billion children, are infected with soil-transmitted helminth (STH) diseases. Infections are most prevalent in sub-Saharan Africa, the Americas, China and East Asia. Soil-transmitted helminths do not cause death; however, they lead to chronic nutritional deficiencies, such as anemia, which severely impair physical and mental development in children.

Proportion of children requiring preventive chemotherapy for STHs by region, and this number have now climbed to 396 million (WHO, 2015).



Prevalence and burden of different STH infections

Several different species of soil-transmitted helminths can cause disease. The ones of greatest public health importance due to the combination of their prevalence and severity of symptoms are Ascaris lumbricoides (common roundworm), Trichuris trichuria (whipworm), Necator americanus (hookworm), and Strongyloides stercoralis (threadworm).

Soil-transmitted helminth infections of human beings	**Disease**	**Estimated population infected (millions)**
Ascaris lumbricoides	Common roundworm infection	807–1221
Trichuris trichiura	Whipworm infection	604–795
Necator americanus and Ancylostoma duodenale	Hookworm infection	576–740
Strongyloides stercoralis	Threadworm infection	30–100
Enterobius vermicularis	Pinworm infection	4–28% of children
Toxocara canis and Toxocara cati	Visceral and ocular larva migrans	2–80% of children

Adapted from Bethony et al, 2006

Hookworm

Known disease-causing species - *Necator americanus; Ancylostoma duodenale*

Current disease burden and affected population distribution - between 500-750 million people are affected around the world according top estimates from the CDC. Symptoms include anemia, and protein deficiency in adults, and stunting of intellectual and physical development in children. The severity of hookworm disease is directly proportional to the number of worms in the body, as each worm ingests 0.03 ml of blood per day. While the risk of death from a hookworm infection is very low, associated symptoms such as anemia have significant effects on the lives of those affected, particularly children. Impact of hookworm is particularly severe in rural communities in developing and underdeveloped countries.

Recommended target specie(s) - *Necator americanus*, for it is the only species with available genomic information. Whole the precise impact of the two separate species of hookworms are poorly documented, *Nector americanus* is believed to be responsible approximately 85% of all hookworm infections and can cause the more severe symptom of necatoriasis (Loukas 2011); in addition, this species is responsible for ~95% of infections in the US. Therefore, targeting *N americanus* would alleviate the majority of the disease burden. Success in this species should provide sufficient interest and traction toward a gene drive project in *Ancylostoma duodenale.*

Whipworm

Known disease-causing species: *Trichuris trichuria*

Current disease burden: Approximately 800 million people worldwide are infected with whipworm. Symptoms vary with severity of infection. Dry skin and diarrhea are common with heavier infections, which can often result in anemia. In children, these heavier infections can lead to growth retardation.

Recommended target species: *Trichuris trichuria*. Since its primary host is human, the worm must be targeted directly.

Threadworm

Known disease-causing species: *Strongyloides stercoralis*

Current disease burden: Approximately 30-100 million people are infected with *Strongyloides,* and the prevalence is increasing in certain regions, including parts of Europe, the Caribbean, Southeast Asia, Latin America, and sub-Saharan Africa (Puthiyakunnon et al, 2014). The clinical presentation of infection varies widely, and can range from gastrointestinal symptoms such as abdominal pain and diarrhea to pulmonary effects such as respiratory failure.

Recommended target species: *Strongyloides stercoralis*. *Strongyloides* has the advantage of being able to mate and reproduce outside humans, in the free-living larval stage. Therefore it

would be theoretically feasible to design a gene drive that allows it to remain it the free-living stage and not infect humans.

Current treatment strategies for STH

Anti-worm medications are the current standard of care for the treatment of STH infections, but their effectiveness varies depending on the species targeted, as indicated in the table below. Mass Drug Administrations, in which anti-helminth medications are given to entire populations in endemic areas irrespective of disease status, have been the core of helminth control programs to date. However, the success of these programs has been threatened by various factors, including the apparent emergence of drug-resistant helminth populations (McCarthy et al, 2012).A novel vaccine specifically targeting hookworm infection is currently in Phase I clinical trials. (Sabin Institute, 2015)

Drug	Dose	Rate	*S. stercoralis*[a]	*A. lumbricoides*	Hookworms	*T. trichiura*	Refs
Albendazole	400 mg	CR	—	88–98.4	78.4–100	10–52.7	[22,24,33,46]
	—	ERR	—	86.5–100	64.2–100	40.3–50.8	—
Mebendazole	500 mg	CR	—	95–96.5	22.9	19–36	[24,33]
	—	ERR	—	—	—	66.7–92.8	—
Ivermectin	200 µg/kg	CR	56.6–68.1	78.4–94.2	—	35.1–44.3	[28,46,47]
	—	ERR	—	94.3–100	—	42.7–86.8	—
Pyrantel	10 mg/kg	CR	—	88	31	28.1	[24]
	—	ERR	—	87.9	56.4–75	52	—
Levamisole	2.5 mg/kg or 80 mg	CR	—	91.5	10–38.2	9.6	[24]
	—	ERR	—	—	—	41.5	—
Albendazole/ivermectin	400 mg/200 µg/kg	CR	56.6–68.1[b]	78.1–100	78.4–100[b]	38–79.6	[33,46,47]
	—	ERR	—	99.5–100	100	68–97.5	—
Mebendazole/ivermectin	500 mg/200 µg/kg	CR	56.6–68.1[b]	96.5[b]	22.9[b]	55.196.7	[33]
	—	ERR	—	—	—	—	—

[a]Studies considered are only those that used antibody responses rather than parasitologic evaluations as test of cure.
[b]In view of the lack of control studies with these combinations, values refer to the efficacy observed with the administration of the most effective drug of the combination, used as monotherapy.
CR, cure rates; ERR, egg reduction rates.
doi:10.1371/journal.pntd.0002165.t003

PLoS Neglected Tropical Diseases, 2013

Gene drive strategy to eradicate STH infections

Gene drives offer a promising alternative to current helminth control strategies, and have the potential to rapidly control the spread of STH infections. A gender biasing drive, in which worms are programmed to only produce progeny of only one sex, could rapidly deplete populations of worms over the course of a couple generations.

Major challenges of gene drive usage in STHs

Transfection: Transfection is the technique by which the CRISPR/Cas9 machinery is introduced into the worms. Currently, there is no viable transfection technique in STHs. Dr. James Lok, at

University of Pennsylvania Veterinary School of Medicine, is in the process of designing an effective transfection technique in Strongyloides.
Finding targets: There is minimal basic biology known about the STHs (conversation with Dr. Matthew Berriman). In order to design a successful gene drive, we need to find an optimal target. Characterization of candidate gene functions in the STHs is essential for the successful implementation of the drives.

Reproductive dynamics: The success of the gene drive is dependent upon its timely spread through worm populations. Therefore, with eggs remaining viable in the soil for 10 years or longer, *Ascaris lumbricoides* (roundworm) would prohibit the success of this technique, unless the gene drive targets a host species.

Transmission dynamics: The limited mobility of the STHs across geographical regions could prevent the gene drive from spreading to eradicate the disease. Understanding the mobility and transmission dynamics of these worms is crucial to the success of any gene drive.

Due to their faster generation times, gene drives may be most promising for use in Trichuris trichuria (whipworm), Necator Americanus (hookworm), and Strongyloides stercoralis (threadworm).

Potential first research steps:
- Establish transfection technique
- Transmission dynamics: sequencing genomes in different regions to gain an understanding of the population structures of the STHs

Notably, this research will be beneficial to the design of any new treatment for STHs.

Recommended funding targets

Dr. Matthew Berriman, head of parasite genomics at the Wellcome Trust Sanger Institute in the UK, in collaboration with Dr. James Lok at UPenn

Matthew Berriman is a leader in the field of STH biology, and has done extensive work on parasite genomics and deciphering mechanisms of parasitism in STH. He is enthused about the prospect of using gene drives to eradicate STH and eager to work on this project.

Dengue, Chikungunya, Zika (Targeting *Aedes* mosquitos)

Dengue and severe dengue

- **Global burden:** Approximately 400 million dengue and severe dengue infections are estimated to occur each year. About 100 million of those infected develop clinical symptoms, which include fever, joint pain, and severe headaches. About 500,000 people with severe dengue require hospitalization each year, of whom 2.5% die. Young children are disproportionately at risk of developing severe symptoms and requiring hospitalization (WHO, 2013).
- **Current treatment strategies**: There is currently no cure for dengue; medical care is aimed at managing symptoms. In addition, vaccine development has been slow due to the fact that there are four serotypes of the Dengue virus, each of which interact with antibodies in the bloodstream differently. This means that a vaccine would need to stimulate production of antibodies for all four serotypes of the virus to confer immunity. Similarly, a person exposed to one serotype of Dengue remains vulnerable to the other serotypes.

Chikungunya

Chikungunya is a virus that causes flu-like symptoms, including fever and joint pain, in approx. 1.7 million people (CDC 2015). There is no treatment currently available. Patients diagnosed with Chikungunya are generally prescribed pain medication and bed rest.

Zika Virus

- **Global Burden:** Zika virus first emerged about 50 years ago and has been traditionally perceived as relatively mild compared to the other diseases prevalent in endemic areas. However, in the recent 2015 outbreak, there has been a surge in birth defects linked to the Zika virus outbreak in Brazil. According to Brazilian officials, more than 4,000 cases of microcephaly were reported during the second half of 2015, which represents a ~20 fold increase from previous rates (CDC 2016).
- **Current treatment strategies:** Currently there are no vaccine or treatment available for the Zika virus. Furthermore, Dr. Howard Zucker pointed out at a recent public forum at the Harvard Kennedy School of Government (Feb 2016) that the Zika viral infection itself tend to be asymptomatic. This, combined with the fact that Zika can only be diagnosed with a highly specialized test, puts everyone in the Zika affected region at risk of having children with microcephaly.

Current Funding

Substantial funding for treatment and vaccine development for Dengue and Zika already exist. However, funding for Chikungunya is much more limited. It is important to stress that all of the existing funding are mainly directed toward the development of vaccine and drug development.

Recommendations

Recommended target specie(s): *Aedes* type mosquitoes are known to be the primary host species for all three viral diseases. Of these, only *Aedes aegypti* and *Aedes albopictus* have genome sequencing information available and represent the primary disease carrying species.

Theoretical gene drive strategy: Population suppression based gene drive. The basic system would involve rendering one sex infertile while spreading the gene drive through mosquitos of the opposite sex. In this instance, a male gene drive mosquito who mates with a female will only produce fertile gene drive males and infertile females.

Recommended expert to fund: Omar Akbari Ph.D., is an Assistant Professor of Entomology at the University of California, Riverside. He is very enthusiastic about the prospects of gene drive systems and is already testing prototypes in *Aedes aegypti* and actively developing a gene drive system in *Aedes albopictus*.

Chagas Disease

Known disease-causing species: *Trypanosoma cruzi*

Current disease burden: Approximately 5 – 18 million people have Chagas disease and it causes roughly 10,000 deaths annually. Transmission is limited to the Americas. In some parts of South America, prevalence rates reach 5% of the population (Stanaway et al, 2015). In 2010 Chagas disease was estimated to be responsible for 550,000 Disability-adjusted life years (DALY). 20% to 30% of those infected will develop chronic cardiovascular Chagas disease, which includes arrhythmias, thromboembolism, and heart failure. Chronic digestive symptoms will occur in another 15-20% of individuals infected. (Stanaway et al, 2015).

Theoretical gene drive strategy: *T. cruzi* is transmitted by the "kissing bugs" of the triatominae subfamily. There are 5 primary host species, of which Rhodnius prolixus is one of the most common. These insects could be targeted with a functionally similar fashion population

suppression drive currently proposed for the various mosquito borne diseases to crash their populations.

Recommended expert to fund: Najib M. El-Sayed M.D., who is an associate professor in the department of cell biology and molecular genetics at the University of Maryland.

Dr. El-Sayed is a world leading expert in *T. cruzi*, and co-authored the publication of the whole genome sequencing results of *T. cruzi* in collaboration with Dr. Peter Myler of the Center for Infectious Disease Research in Seattle. The two have been working on using genetic techniques against the spread of *T. cruzi* for over a decade.

Additional Recommendations

With CRISPR-based genome editing itself being only a three-year-old technology, many questions remain unanswered with respect to the safety, efficacy, and long term effects of releasing gene drive organisms. From a public safety standpoint, it would be reckless to consider any gene drive organism for a field trial without data to support not only the efficacy of the gene drive design, but also our ability to contain the gene drive organisms. Therefore, it is also important to support *in vivo* model organism systems to enable rapid prototyping of CRISPR gene drive designs within the confines of the laboratory. In addition, such a model system would also need to have a relatively short generational cycle so that experiments can not only be completed in a time-efficient manner, but also enable scientists to cultivate large and genetically diverse populations to simulate real world scenarios.

We recommend funding basic research on CRISPR gene drive technology in the classical model organism *C. elegans* and the highly related nematode species *C. brenneri* in the Esvelt lab. Over the long run, this project will not only have far reaching impacts for both the gene drive and developmental biology communities, but may also accelerate the CRISPR gene drive development for soil-transmitted helminths, which are related to nematodes of the *Caenorhabditis* genus.

About the Authors

Mack Darrow graduated from Princeton University in 2013 with a degree in Politics. He currently works for an investment firm in Newport Beach, California.

Eric Gastfriend graduated from the MBA Program at Harvard Business School in May 2015. He co-founded the Harvard University Effective Altruism Student Group, and currently leads the Philanthropy Advisory Fellowship, which recruits Harvard graduate students to advise philanthropists and foundations on how to maximize the impact of their charitable giving. As an active leader in the Effective Altruism community, he has volunteered for 80,000 Hours and

Future of Life Institute, and has taken the Giving What We Can Pledge to donate 10% of his lifetime income to highly cost-effective charities. Before business school, Eric was the General Manager of Happy Cloud, a venture-backed cloud gaming startup.

John Min is currently a PhD candidate at Harvard Medical School working collaboratively between the laboratories of Prof. George Church, and Prof. Kevin Esvelt at the MIT Media Labs. He has extensive experience working with DNA as a nanomaterial in the field of DNA Origami with Professor William Shih at Harvard, as well as the biochemistry of blood vessel contractility and how they contribute to heart disease with Professor Kathleen Morgan at Boston University. He holds a B.A. double majoring in Biochemistry & Molecular Biology and Economics from Boston University.

Alex Sakatos is a PhD candidate at the Harvard T.H. Chan School of Public Health, working in the laboratory of Dr. Sarah Fortune.

Conflicts of Interest

John Min is a graduate student working collaboratively between the labs of Dr. George Church and Dr. Kevin Esvelt.

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