Using CRISPR to Combat Human Disease Vectors.

Omar S. Akbari

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The World’s Deadliest Animals
Number of people killed by animals, 2015

~1 Million
Mosquito

580,000
Human

60,000
Snake

24,200
Sandfly

17,400
Dog

8,000
Kissing bug

4,400
Freshwater snail

3,500
Scorpion

3,500
Tsetse fly

1,600
Tapeworm

500
Hippopotamus

100
Elephant

60
Bee

40
Jellyfish

6
Shark

MOSQUITO BORNE DISEASES

1 MILLION
DEATHS EACH YEAR FROM MOSQUITO BORNE DISEASES

- MALARIA:
  207 MILLION CASES PER YEAR
  1000/day
  Mostly children under age of 5
  ~1 child every 2-min

- DENGUE FEVER:
  390 MILLION CASES PER YEAR

- WEST NILE VIRUS:
  30 THOUSAND CASES PER YEAR

- YELLOW FEVER:
  207 THOUSAND CASES PER YEAR

HOT SPOT FOR ALL THE DISEASES

3.2 BILLION PEOPLE
ALMOST HALF OF THE WORLD'S POPULATION ARE AT RISK OF MALARIA

Why are they so deadly?

Aedes aegypti
Anopheles Gambaiae
Aedes albopictus
Commonly Used Practices for Mosquito control

**First Line of Defense: Reduce Contact**
- Bed Nets
- Repellents
- Lures/Traps

**Modify Environment**
- Wet Humid
- Drastic ecological implications
- Not Practical

**Cost money/Continuous Application/Distributed**

**Insecticides/Larvicides**

**Pathogen Removal-Drugs**

**Genetic Technologies**
- Not sustainable
- Huge Effort

- Expensive/Short term/Evolve Resistance/Bounce Back.
- **ALL** these approaches are critical for any sort of sustained success.

- Oxitec
The mosquito is an obligatory vector for Pathogen transmission

How do we block this chain of transmission?

1. Holy Grail - Develop effective vaccines
   Tons of research - many clinical trials nothing so far with sufficient immunogenicity for most vectored pathogens.

2. Engineer Mosquitoes to be Immune to disease
   Both approaches will block disease transmission, the technologies I will discuss focus on utilizing the second approach.
Mosquitoes can be engineered to resist infection by plasmodium-Marcelo Jacobs Lorena and Anthony James

Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite

...and they can be engineered to resist infection by dengue virus. Ken Olsen and AA James

Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified Aedes aegypti
We have engineered *Ae. aegypti* mosquitoes to be resistant to ZIKA Virus.

Buchman et al, 2019, PNAS

**Zika Virus Experts Available for Interviews**

Entomologists and medical professionals can comment on everything from novel genetic technologies to prevention methods aimed at Zika virus.

**By Sean Nealon On JANUARY 27, 2016**

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**CSIRO**

**RIVERSIDE, Calif.** (www.ucr.edu) — A relatively new mosquito-born virus, ZIKA virus, has recently caused concern because of its connection to neurological birth disorder and due to its rapid spread throughout the world. The University of California, Riverside has several experts who can comment on the virus:

**Omar Akbari**, an assistant professor of entomology, is focused on developing novel genetic based gene-drive technologies for mosquitoes that can be used to rapidly replace entire wild populations with genes that confer resistance to vectored diseases. His lab is currently focused on engineering technologies for *Aedes aegypti* mosquito, which transmits Zika virus. [Read more](http://www.ucr.edu/34497/aedes-aegypti-cdc-gathany)
We have engineered *Ae. aegypti* mosquitoes resistant to DENV Virus

Together these observations reveal that the Mosquito immune system can be harnessed to resist infection by Malaria, Dengue and ZIKA.
To prevent insect-borne disease......

1. Engineer insects to resist infection

2. Replace the wild insect population with engineered counterparts that cannot transmit disease

Less transmission by Mosquitoes = less Human disease.
Engineer Disease Resistant Mosquitoes

Population Replacement - Replace the wild population with individuals refractory to disease transmission

Release Into Wild Disease Transmitting Mosquitoes

Disease Transmitting Mosquitoes
The Grand Challenge
How do we cause a trait to rapidly spread throughout a population?
Problem: Genes that confer pathogen resistance are likely to result in a fitness cost to carriers.
Solution: **Link genes for pathogen resistance with a gene drive system.**

Gene drive systems have the ability to spread in a population, even if they decrease the fitness of the animals in which they reside.
CRISPR-CAS9: GENE DRIVE
Now Cell encodes the editing machinery
Population Replacement Kinds of Gene Drives

Threshold independent Replacement Gene Drives

In principal a single organism release into a wild population could spread that trait throughout the wild population.

- Driving an engineered anti-pathogen gene into a neutral site to convert the entire population into a disease resistant population.
- Ecologically For population modification the engineered population would persist in the environment but they would no longer be able to transmit pathogens. disease vectors
Threshold independent **Suppression Gene Drives**

- Drive a population to extinction
  - e.g. Drive into a recessive gene required for female fertility/viability
  - **Ecologically** For population suppression the engineered population would persist in the environment until fixation then the entire population would crash and be eradicated.

In principal a single organism release into a wild population could spread that trait throughout the wild population.
Categories of Gene Drives

Population Replacement
- Frequency of transgenes:
  - Transgenes spread to fixation
  - Unstable equilibrium

Population Suppression
- Frequency of transgenes:
  - Transgenes spread to fixation
  - Population Declines
  - Unstable equilibrium

Self-Limiting
- Frequency of transgenes:

**Controversies**
- Invasive - Spread beyond borders
- Uncontrollable
- Non-Reversible
- Difficult to measure Risks
- Regulatable?
- Public acceptance?

**Desired Features**
- Controllable
- Removeable
- Safe
- Effective
Categories of Gene Drive

Population Replacement
- Transgenes spread to fixation
- Unstable equilibrium
- Frequency of transgenes vs. # of Generations

Population Suppression
- Transgenes spread to fixation
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- Unstable equilibrium
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Self-Limiting
- Transgenes spread to fixation
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Linked Gene Drive Design
- Cas9
- gRNA
- Effector

Split-Drives
- gRNA
- Effector
- X
- Cas9
We have engineered Split Homing Based Drive systems in *Ae. Aegypti*

~84% Homing
100% Cleavage

Progeny generation

Li. et al. Under review @ eLI
**Aedes aegypti** split drive is self-limiting and is confineable.

10 releases of homozygous males at 1:1 total population

Will we see this technology in the field in the future?? Will persistence be a problem?
STERILE INSECT TECHNIQUE (SIT)
A method of biological mosquito control

1. Mass Rear
2. Separate and Sterilize Males
3. Deploy Sterile Males
4. Sterile Males Mate
5. No Progeny

Major Limitations:
1. Irradiation reduces fitness of released males
2. Does not work well for mosquitoes
3. Must release adults – difficult to scale.

North and central America
Mex. Fruit Fly
Medfly

North and central America
Northern Mexico
Peru, Mexico
Existing Technologies

A. Many factories, manual sexing, manual release of fragile adults
CRISPR SIT system to combat mosquitoes

- Logistically scalable
- Environmentally friendly
- Evolutionary stable
- Safe
- Effective
- Controllable
Exciting Frontiers - Transgenic is the future!

1960s

Next several years

2019

Apple iPhone 11
Thank You!!

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Adler Dillman (UCR)
Thank You!!
Major Limitations
- Sexing not efficient,
- Manual sexing and release difficult to scale
- Tetracycline is toxic – ablates microbiota,
- Males not as competitive (need to release a lot)
- Difficult to truly scale
Major Limitations

- Sexing not efficient,
- Manual sexing and release - difficult to scale
- If females released - SIT effect will diminish
- Do not understand the mechanism for incompat
- Impossible to remove from environment
- Published work indicates that in some cases *wolbachia* infection can *enhance* vector competence
Cas9 SIT system: guide RNAs

(1) Female-specific lethality
Sex-determination genes

Sex lethal (Sxl)

(2) Male-specific sterility

βTub85D, a sperm specific tubulin

βTub85D, a sperm specific tubulin
pgSIT system: Single gRNA Phenotypes

- **Cas9 line**: gRNA line
  - Panel: Sxl or Dsx
  - bTub

  - Female lethality
  - Males sterile

  - Egg

  - Males sterile

- 100% dead females
- Fertile males

- 100% sterile
- Intersexes

- 100% sterile
- Intersexes

- Fertile females
- 100% sterile
- Males with normal mating behavior
pgSIT system: Double gRNA Phenotypes
pgSIT system: Double gRNA Phenotypes

![Diagram showing different protein expression in female and male flies with various genetic modifications.](Image)
pgSIT mechanism depends on Lethal Biallelic Mosaicism throughout development.

- Not all phenotypes were penetrant if using a weak Cas9 that was not inherited as a gene.
- A stronger Cas9 could ensure complete penetrance of some genes but not all when Cas9 was not inherited as a gene.
- When both Cas9 and gRNA are inherited complete penetrance was observed.
The future of pgSIT - Agriculture

- Cross-species portable SIT
- Environmentally friendly closed system
- Logistically scalable system: easy to make and move large numbers of eggs
- Evolutionary stable system

Sterile Males hatch and mate with wild females.
Unfertilized eggs don’t hatch
Wild pest population crashes
A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes

Kyros Kyrou\textsuperscript{1,2}, Andrew M Hammond\textsuperscript{1,2,6}, Roberto Galizi\textsuperscript{1,6}, Nace Kranjc\textsuperscript{1,6}, Austin Burt\textsuperscript{1}, Andrea K Beaghton\textsuperscript{1}, Tony Nolan\textsuperscript{1,6} & Andrea Crisanti\textsuperscript{1}
Going Forward

• Continue developing antiviral effectors
  – CRISPR-RNA targeting systems in mosquitoes
• Gene Drive in *Aedes aegypti*
  – Link effectors to split-drives
  – Develop Suppression Drives
• **Precision Guided SIT (pgSIT)**
  – Develop in *Aedes aegypti*