Integral Gene Drive
Mosquito Population Replacement
& Malaria Transmission Blocking

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Mosquito population replacement
• Mosquito midgut traversal is the most critical phase
• Most parasites are eliminated by mosquito immune responses
Challenges

- Molecular design and operational complexity
  - Large and complex synthetic constructs
  - Detail spatiotemporal characterisation of promoters
  - Random integration or very little choice of loci
  - Cannot separate drive and effector for testing

- Long-term efficacy and stability of effector/drive module
  - Resistant driver (mutations or non-homologous recombination)
  - Loss of effector
  - Fitness cost of each and all components

- Identification of appropriate effector
  - No ideal effector available
  - Laboratory tests lack sensitivity and robustness
  - Proposed effectors only tested against laboratory parasites

- Regulatory hurdle
  - Driving mosquitoes cannot be easily tested in the field
Integral Gene Drive (IGD)

- Disassociate effector and driver
- Effector can drive only in the presence of driver
- Minimal genetic modifications
- Integration within any host gene of choice
- Resistance is linked to loss of host gene
- Combination of multiple drivers and effectors

Nash et al., Open Biology (2018)
Modelling efficacy of IGDs

- Substantially increased protection even in case of pre-existing resistance
- Duration of 95% protection: 81 / 15 generations (no / 10% pre-existing resistance; i.e. 5-7 / 1-2 years) and 103 / 38 generations (no / 10% pre-existing resistance; i.e. 6-9 / 2-4 years) for 1 and 2 effectors, respectively

Nash et al., Open Biology (2018)
Traditional operational design

transmission blocking test regime

gene drive test regime

endemic setting
test efficacy against wild isolates

imported strain with driving effector

deployment

regulatory burden, biological risk level and containment requirements
Gene drive – example in zpg locus

Cas9  GFP

2A  2A

wt

transgenic

zpg / vasa

Cas9  GFP

2A  2A

wt

transgenic

U6  gRNA

Scarlet  3xP3

transgenic

wt
Gene drive and pre-existing resistance

\[
\begin{align*}
\text{wt} & : 5' - \text{CAACAAATACAGCATCGAGATGCTGGAGTTTTGTAAGGCCG} - 3' \\
\text{Pre-existing R1} & : 5' - \text{CAATAAATACAGCATCAAGATGCTTTGAGTTTTGTCAGGCCG} - 3'
\end{align*}
\]

\[
\begin{align*}
gRNA1^{+/}\sigma & : +/− \\
gRNA2^{+/−}\sigma & : +/−, −/−
\end{align*}
\]

Expected = 50%
### SMFA sensitivity and robustness

<table>
<thead>
<tr>
<th>Day</th>
<th>CL3 (P. falciparum NF54)</th>
<th>Insectary (A. coluzzii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Keep continuous asexual culture at 0.5 to 1.5% trophozoite stages</td>
<td>Blood feed colony mosquitoes</td>
</tr>
<tr>
<td>2</td>
<td>Replace medium</td>
<td>Place egg dish in colony cage</td>
</tr>
<tr>
<td>3</td>
<td>Split to induce gametocytes</td>
<td>Transfer eggs to plastic tray</td>
</tr>
<tr>
<td></td>
<td>4.6% rings in asexual cultures</td>
<td>Allow L3 to hatch</td>
</tr>
<tr>
<td></td>
<td>1.15% rings in gametocytes cultures</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Replace 70% medium in gametocytes cultures</td>
<td></td>
</tr>
<tr>
<td>5 and 8</td>
<td>Replace 70% medium in gametocytes cultures</td>
<td>Provide food</td>
</tr>
<tr>
<td>11/12</td>
<td>Collect pupae</td>
<td>Experimental cage</td>
</tr>
<tr>
<td>15</td>
<td>Experimental cage</td>
<td>Pupa hatch</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>Pot 50 - 70 mosquitoes per cup</td>
</tr>
</tbody>
</table>

#### SMFA Procedure

**a)** A. coluzzii set up
- 3 hrs prior feeding
  - i. Starve mosquitoes
  - ii. Deprive mosquitoes of light
  - Move to CL3 when feeding set up is ready

**b)** Gametocytes preparation
- n° of eXflag. > 20/ml
- Gametocytes > 1%
- Transfer to a 15 ml tube
- i. Centrifuge
- ii. Remove supernatant
- iii. Add 1:1 vol of HI serum to pellet
- Feed mosquitoes for 15 min at room temp and feeders at 38°C
- Transfer to incubator at 26°C; do not feed, do not open for 48 h
- 300 μl/feeder

| 18/19 | Add 10% fructose to PF infected mosquitoes and remove foil paper from cups; refresh fructose every 2 days |
| 23/24 | i. Kill mosquitoes following the SOPs
|     | ii. Dissect mosquitoes
|     | iii. Stain and fix guts
|     |  - Mircrochrom 1%, 20 min
|     |  - PF 4%, 30 min
|     |  - 1x PBS, 5 min
|     | iv. Set up slides using 80% glycerol and coverslips
|     |  - Seal slides with nail polish
|     |  - Store at 4°C for 1 month |

Habtewold et al., Malaria J (2019)
Effectors currently tested

**Antimicrobial peptides from other species**
- Scorpine
- Mellitin
- Magainin

**Anopheles infection regulators:**
- anti-malarial: REL2
- pro-malarial: CLIPA2, CLIPA12
- Homeostasis: FN3Ds

**Plasmodium immune evasion:**
PIMMS43, P47, P230

nanobodies raised in llamas
Exotic Antimicrobial Peptides

Habitewold et al., Malaria J (2019)
Scorpine – an example in the CP locus

Carboxypeptidase locus

- CP promoter
- Scor1
- Scorpine
- Scor2
- 2A
- CP CDS

lox-out marker

X Cas9

97.2% homing rate

splicing efficiency:

- CP
- CP lox

mRNA expression

Scorpine

RBM Partnership To End Malaria
INGECLIMA (The Albian Group), Bilbao, Spain
Technical plans available via Global Access

Brian Tarimo, Sarah Moore, Fredros Okumu
Marcelina Finda, Lorenz Hofer, Theresia Nkya
Future plans for field testing

**Figure 3. Pathway to deployment of gene drive mosquitoes.**

- Laboratory studies
- Small-scale isolated releases
- Small-scale open releases
- Large-scale open releases
- Post-implementation surveillance

[Diagram showing different stages of gene drive mosquito deployment with data on release strain, regulatory risk level, and population dynamics.]
Institutions & Funding

Imperial College
London