Tick-borne disease is a major national problem

Lyme disease is the most common vector-borne illness

>300,000 cases estimated by CDC

Completely unknown 50 years ago

Other pathogens also rising

Lyme Disease Incidence, 2015
Tick-borne disease is an ecological problem

The pathogens that cause Lyme and other diseases persist by moving between mice and ticks.

Ticks pass the pathogens to humans, causing disease.
We aim to reduce the incidence of tick-borne disease by releasing mice engineered to resist disease and ticks.

How are altered mice different?

<table>
<thead>
<tr>
<th>Genetically altered mice</th>
<th>Your existing mice</th>
</tr>
</thead>
</table>
| Liver cells produce native mouse anti-Lyme and anti-tick antibodies from birth. Protection is passed on to offspring.  
*Note: Mice would be genetically altered, but still 100% mouse. *No gene drive!* | Some immune cells produce antibodies against Lyme and ticks, but they are not very effective. |
Two types of antibodies

### Anti-Lyme antibody

This antibody helps kill the bacteria that causes Lyme

**The result:**
Mice would be protected from the bacteria that cause Lyme. The main reservoir of this pathogen would likely collapse.

Antibody target:
OspA, a *Borrelia burgdorferi* surface protein

### Anti-tick antibody

This antibody prevents ticks from feeding long enough to survive

**The result:**
Many ticks would not be able to feed on white-footed mice (the most common host). The tick population should be substantially reduced - particularly infected ticks

Antibody target:
Subolesin, a tick protein
Making tick-borne disease resistant mice

Field trial 1: small island

Field trial 2: large island

Prevent disease on volunteer islands

Communities vote

Independent study of efficacy & ecological effects

Field trial 1: small island

Engineer mice expressing native mouse antibodies

Most protective
**Phase 1**

Engineer mice
- Identify protective antibodies
- Build immune mice

Prepare for field trial
- Identify appropriate islands for the field trial
- Collect baseline data

**Phase 2**

Field trial on small island(s)
Up to 3 island areas with the following interventions:
1. Release altered mice
2. Release lab reared wild-type mice
3. No change

**Phase 3**

Release mice on large island(s)

Next: Investigate opportunities for the mainland
Release immune mice in early spring

Introduced mice would increase the local mouse population to at most 300% of ‘normal’ for that time of year

For context, mouse populations often fluctuate by > 500% each year

Bait stations could be used to reduce populations near commercial and residential areas

Local reductions will not impact the spread of resistance
## Funding Status

<table>
<thead>
<tr>
<th>Institution</th>
<th>Funding purpose</th>
<th>Amount</th>
<th>Timeline</th>
<th>Start Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIT, Tufts, and Harvard</td>
<td>Researcher salaries</td>
<td>varies</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greenwall Foundation</td>
<td>Ethical review</td>
<td>175k</td>
<td>2 years</td>
<td>Jan 2017</td>
</tr>
<tr>
<td>Rainwater Foundation</td>
<td>Ecological studies</td>
<td>100k</td>
<td>1 year</td>
<td>Jan 2018</td>
</tr>
<tr>
<td>CDMRP Tick-Borne Disease Award</td>
<td>Antibody development</td>
<td>250k</td>
<td>2 years</td>
<td>Sep 2017</td>
</tr>
</tbody>
</table>

### Summer 2018: Fundraising on MV and Nantucket

Raise support for ecological research, breeding and scale-up. Additional funds will accelerate the timeline.
**Steering Committee (SC)**
Board of Health appointed representatives
- General supervision of the project
- Develops study designs and “stopping rules” in collaboration with the Data and Safety Monitoring Board (DSMB)
- Acts on recommendations of the DSMB

**Project Manager**
Reports to the Steering Committee
- Tracks project progress and budget
- Liaison with regulatory authorities and funding organizations
- Prepares regulatory documents, reports, grant requests and SC minutes

**Data Safety Monitoring Board (DSMB)**
Independent of the SC, MIT, funding agencies or representative communities. Size and makeup TBD.
- Ensures that there is no harm to the environment, animals or humans
- Defines “stopping rules” in collaboration with the SC
- Reviews and comments on all study designs
- Reviews project compliance and progress at specified intervals and ad hoc if requested by the SC
- Recommends discontinuing or altering the design of the project at predetermined times or at any time in the interest of safety

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**Example:**

**Nantucket Steering Committee**

- Dr. Howard Dickler (Chairman)
- Danica Connors
- Dr. John Goldman
- Dr. Emily Goldstein Murphy
- Dr. Malcolm MacNab (Ex officio)
- Dr. Sam Telford (Consultant)
Community Decision Points

1\textsuperscript{st} evaluation point

After immune mice have been created in the lab

- Project go/no go recommendation by DSMB
- If go, project must secure ...  
  • Regulatory approval for next phase
  • Approval for the use of an uninhabited island from the land owner/local government involved
  • Continued interest by the Boards of Health

2\textsuperscript{nd} evaluation point

After field trial on an uninhabited island

- Project go/no go recommendation by DSMB
- If go, project must secure ...  
  • Regulatory approval for next phase
  • Warrants proposed by the Boards of Health at Town Meetings
  • Warrants approved by all six towns
Community outreach

Local Presentations
6/16: Nantucket Board of Health meeting
7/16: Martha’s Vineyard health agents meeting
7/16: Edgartown Library presentation
10/16: Martha’s Vineyard All-island BoH meeting
1/17: Nantucket Board of Health meeting
3/17: Edgartown Board of Health meeting
5/17: Aquinnah Board of Health meeting
5/17: Martha’s Vineyard High School meeting
8/17: Nantucket Board of Health meeting
11/17: Nantucket Biodiversity Initiative Conference
12/17: Steering Committee meeting on MV
1/18: Steering Committee meeting on Nantucket

Project Management
7/16: Nantucket Board of Health agrees to jointly develop project management plan
7/17: Nantucket and MV Steering Committees have full membership (All 6 Martha’s Vineyard Boards of Health selected a representative for MV’s Steering Committee)
Technical Team

Dr. Kevin Esvelt
MIT

Dr. Sam Telford
Tufts

Dr. Duane Wesemann
Harvard

Dr. Linden Hu
Tufts

Dr. Jeantine Lunshof
MIT/Harvard

Dr. Teng Zuo
Harvard

Dr. Neha Chaudhary
Harvard

Dr. Rupa Kumari
Harvard

Joanna Buchthal
MIT

John Min
MIT/Harvard

Additional researchers:
Dr. Tom Watson, Arc Bio
Dr. Tanja Petnicki-Ocieja, Tufts
Byong Kang, MIT
Alison Tisdale, MIT
Glenn Paradis, MIT
Dr. Stuart Levine, MIT
Responsive Science is a way of conducting research that invites openness and community involvement from the earliest stages of each project. Real-time interaction between scientists, citizens, and broader communities allows questions and concerns to be identified before experiments are performed, fosters open discussion, and encourages research studies and new technologies to be redesigned in response to societal feedback.

Community Involvement
Transparency and societal accountability are critical for any research that involves the shared environment. Responsive Science currently focuses on applied ecological research, including gene drive systems for altering wild populations. Discussions are facilitated by PubPub, a unique collaborative tool for sharing and evaluating research, and our dedicated team.
Questions, Comments, Concerns?

esvelt@mit.edu
buchthal@mit.edu
lunshof@mit.edu

For more information:
www.responsivescience.org
By-hand vaccination reduced *Borrelia* prevalence by
- **42%** in mice
- **25%** in ticks

This study did not control for unvaccinated mice migrating into the area.

Tsao JI, Wootton JT, Bunikis J, Luna MG, Fish D, Barbour AG (2004) *PNAS*
An oral bait-delivered vaccine reduced tick infection rates by

- 25% when deployed for 2 years
- 76% when deployed for 5 years

This study did not control for unvaccinated mice migrating into the area. Increase in mouse antibody levels was barely statistically significant. This study also had issues with proper controls, suggesting high site variability.

We need to identify islands that are good proxies for MV and Nantucket

<table>
<thead>
<tr>
<th>Site</th>
<th>Deer Tick Density</th>
<th>Mouse Density</th>
<th>Tick Infection</th>
<th>Mouse Infection</th>
<th>Size</th>
<th>Ease of Study</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuckernuck</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>1.4</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Cuttyhunk</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>0.9</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Private (MA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 10</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Private (NY)</td>
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<td></td>
<td></td>
<td></td>
<td>&lt; 1</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Nantucket</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>47.8</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>MV</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>87.5</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Planned Fieldwork:** beginning spring 2018

**Baseline Data Collection**
- **Infection rates:** Test mice and ticks for infection, identify sources of past blood meals
- **Mouse demography:** Measure population size through trapping
- **Predator surveys**

**Optimization**
- **Single island feasibility:** Only young males will travel more than 500m away
- **Nest boxes:** Improve fitness and reproduction?
- **Computational modeling:** Find the number of mice to be released to reduce infected ticks by 50% or more
We need to determine the federal agency responsible for oversight.

FDA (health) vs EPA (pest control)

Guidance for mosquitoes says EPA if goal is to reduce population

Initial conversations suggest language is critical: reduce disease (FDA), reduce infected ticks (either), reduce ticks (EPA)

We plan to meet with both the FDA and EPA informally before initiating the regulatory process.
1) Inject white-footed mice with surface markers from *B. burgdorferi*
2) Isolate B cells that make antibodies that target *B. burgdorferi*
3) Isolate antibody DNA within B cells
4) Produce antibodies
5) Test antibodies...

**Test 1:** Confirm binding?

Test that antibodies bind the surface marker on a plate

**Test 2:** Kill the pathogen?

Test that antibodies kill the pathogen in a petri dish

**Test 3:** Protect mice?

Test antibodies in mice and challenge with infected ticks

**Timeline:** Over the next 12 months, we will test many hundreds of anti-Lyme and anti-tick antibodies in order to identify 4 to 8 highly protective antibodies
To make heritable changes, we need to introduce DNA into a living organism so that it is incorporated into the genomes of cells that make sperm or eggs.

<table>
<thead>
<tr>
<th>Traditional Method</th>
<th>In Vivo Method</th>
<th>Improved In Vivo Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inject DNA into embryos, then implant them</td>
<td>Introduce DNA into reproductive cells through gene therapy</td>
<td>Introduce DNA into engineered cells through gene therapy</td>
</tr>
<tr>
<td>• Costly and inefficient (lots of sacrificed animals)</td>
<td>• Inefficient: delivers to all cells</td>
<td>• Delivers only to germline cells</td>
</tr>
<tr>
<td>• Must be optimized for each species</td>
<td>• Randomly locates new DNA in the genome</td>
<td>• Integrates DNA at specific sites</td>
</tr>
<tr>
<td></td>
<td>• Amount of new DNA is limited</td>
<td>• Can deliver more DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• After one generation, animals have only the new DNA</td>
</tr>
</tbody>
</table>

Test: make white-footed mice express a known house mouse antibody against OspA