26 Dengue Vector Control: New Approaches

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Introduction

Scope

In a little over a decade, the human population world-wide has climbed from 6 billion in 1999 to over 7 billion in 2012 (Bloom, 2011). This massive population growth, as well as a shift in demographics towards urbanization and an increase in international trade and travel, has created ideal circumstances for an explosive rise in the number of dengue virus infections (Gubler, 1998). Disease incidence has increased 30-fold in the last 50 years and the viruses are now endemic in more than 100 countries.[1,2] An estimated 390 million infections with 96 million symptomatic cases occur annually and over half of the world's population is at risk of infection (Beatty *et al*., 2009; Bhatt *et al*., 2013).

Dengue

Dengue is a disease caused by four closely related but serotypically distinct viruses spread to humans through the bite of infected female mosquitoes. The primary vector is *Aedes aegypti*, but *Ae. albopictus, Ae. mediovittatus, Ae. scutellaris* and *Ae. polynesiensis* also contribute to regional transmission (Rosen *et al*., 1954; Ooi *et al*., 2006; WHO/TDR, 2006). *Ae. aegypti* is primarily responsible for epidemic transmission throughout most of the world; however, in certain regions, *Ae. albopictus* can support epidemic-level transmission (Lambrechts *et al*., 2010; Wu *et al*., 2010). There are no dengue-specific prophylactic or therapeutic drugs and licensed vaccines are not yet available. The only available approach to controlling dengue infection is prevention of contact with infected vector mosquitoes.

Contemporary vector control

Unfortunately, there is little to no effective vector control in many dengue-endemic countries. Even where robust programs are implemented, the disease remains an ongoing risk (Ballenger-Browning and Elder, 2009). Vector control tools are limited in number and not as efficacious as needed. The logistical challenges associated with insecticide applications (adulticides and larvicides), the primary tools used for vector control, result from the need for these agents to come in direct contact with the target vector. In large urban settings, the scale and timing of applications are impractical, especially where resources are limited. Furthermore, many protocols show no benefit to disease control. For example, adulticide fogging is a

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cosmetic but ineffective approach used following the detection of dengue outbreaks, usually well after the epidemic has taken hold (Gubler, 1989; Newton and Reiter, 1992; Reiter and Gubler, 1997). The ineffectiveness of these efforts is due in part to insufficient monitoring and a lack of well-designed programs, but is exacerbated by the rise in the level of insecticide resistance (Georghiou and Taylor, 1986). Agricultural uses of insecticides also have been suggested to sustain pressure to maintain resistance traits in mosquito populations in or near areas of virus transmission (Breeland *et al.*, 1970; Khan *et al.*, 2011; Fane *et al.*, 2012).

Community-based source reduction by treating mosquito breeding sites has been tried in a number of locations (Erlanger *et al.*, 2008; Ballenger-Browning and Elder, 2009; Al-Muhandis and Hunter, 2011). However, even the most site-specific community-based campaigns have limitations. These strategies require education of local people on how dengue is spread, the threat of the mosquito vector, how to identify both the adult and juvenile forms of *Aedes* mosquitoes, and how to find and manage breeding sites. Resource-intensive education is needed to reach the large number of people necessary to wage an effective campaign. A lack of full participation and a breakdown in protocol compliance defeats these efforts (Phuanukoonnon *et al.*, 2006; Shirayama *et al.*, 2007; Toé *et al.*, 2009; Atkinson *et al.*, 2010). Not only is it difficult to convince people to take ownership of the problem (i.e. be willing to accept that their property is a source of the problem), it can be difficult to convince them to take ownership of the solution (i.e. be willing to accept that it is their responsibility and not the role of the government). Compliance wanes from the lack of sustained control activities over long periods of time and adherence to protocol activities with the frequency required to prevent the emergence of adults from juvenile stages (~10 days, depending on nutrient and temperature conditions). Finally, community-based campaigns struggle with campaign fatigue. It is especially challenging to remain vigilant in the absence of an outbreak and to remain steadfast in the face of non-compliance by neighbors or the apparent ineffectiveness of the campaign.

Example of challenge

Large-scale and sustained control of dengue virus transmission has been difficult to achieve in certain regions of the world (Ballenger-Browning and Elder, 2009). The dengue control program of the Singapore Government is a good example of the challenges in sustaining dengue prevention. Control efforts in this country arguably have enjoyed the highest standards in terms of resources *per capita* and community compliance backed by a sound legal system with rigorously enforced laws (Seow, 2001; Ooi *et al.*, 2006). Despite these advantages, dengue transmission remains an ongoing problem (Fig. 26.1). Current tools can achieve a premises index (a measurement of mosquito-positive homes) of just 2%. Yet despite this laudable reduction, dengue transmission continues (Ooi *et al.*, 2006). After years with few cases, epidemic dengue resumed in 1990, with periodic episodes increasing in intensity in subsequent years. The reasons for this re-emergence are many and complex (Ooi *et al.*, 2006), but at least two confounding factors have contributed. Singapore's geographic location and status as an economic hub in Southeast Asia fosters importation of new dengue cases from surrounding countries. Furthermore, in the years following the successful vector control campaign, there has been a reduction in the level of herd immunity, resulting in a significant increase in the population susceptible to infection (Goh, 1995, 1998). The residual populations of *Ae. aegypti* are sufficient to initiate dengue transmission under these circumstances.

New Tools

Need

Vector control agencies need new affordable, efficacious tools that are safe for people and the environment. Ideally, these tools should

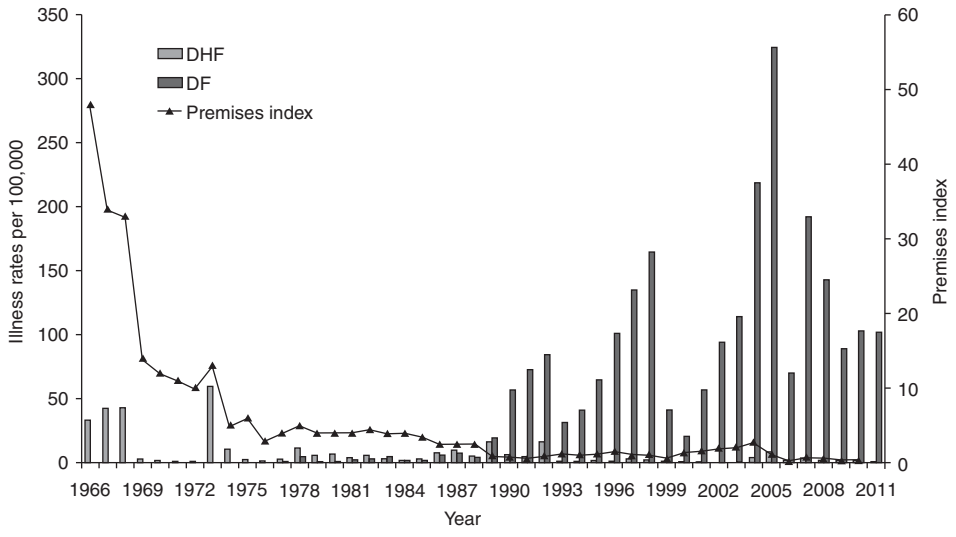

Fig. 26.1. Annual incidence of dengue fever (DF), dengue hemorrhagic fever (DHF) and the premises index for *Aedes* mosquitoes in Singapore from 1966 to 2010. The incidence of DF and DHF are reported also for 2011. In 1966, DHF was made a notifiable disease in Singapore and DF became a notifiable disease in 1977. Incidences were calculated from the annual number of reported cases. The annual premises index is expressed as a percentage of the premises positive for *Aedes aegypti* or *Ae. albopictus* larvae divided by the number of premises visited by environmental health officers. (This figure is adapted from Ooi *et al.* (2006) with permission.)

be scalable from small villages to large cities, and be socially acceptable and economically and politically sustainable. Furthermore, they should be compatible with current and developing control tools, including vaccines, anti-viral drugs and new insecticides. Genetic-based strategies targeting the vector mosquitoes have the potential to fill these needs and are poised to contribute to future vector control efforts.

Genetic-based tools

Genetic strategies are based on the widely accepted theory that disruption of the vector phase of the pathogen life-cycle will reduce or eliminate transmission to humans. Disruption can be achieved by eliminating or reducing mosquito densities below transmission thresholds or by making the mosquitoes refractory to virus infection (Milani, 1967; Curtis, 1968; Collins and James, 1996; James, 2000; James *et al.*, 2006). Novel strategies are being developed based on genetically engineered strains of mosquitoes with design features that maximize utility and safety profiles (Braig and Yan, 2002; James, 2005; Alphey *et al.*, 2010). Some strategies are designed to be resilient to the immigration of wild mosquitoes originating temporally (from aestivating eggs) and spatially (from neighboring populations or from global-trade stowaways). Others have the potential to lower population densities below the transmission threshold of one or more vector-borne pathogens. Importantly, most genetic-based strategies are anticipated to become more efficacious as the sizes of wild mosquito populations dwindle, working synergistically with conventional strategies that become increasingly less cost effective under these circumstances. Finally, these tools offer access to mosquito breeding sites that would otherwise be inaccessible or cryptic using conventional tools.

Genetic approaches in principle should be safer than vector control strategies employing

insecticides. Engineered strains are species-specific, and because *Ae. aegypti* and *Ae. albopictus* are invasive species throughout most regions in the world and are preyed upon opportunistically (i.e. no birds, bats, fish or any other insects feed exclusively on the species), deployment of these strategies is anticipated to have a negligible impact on ecosystems. Strains can be designed to lower or minimize vector competence[3] for all known pathogens transmitted by the targeted species and increase insecticide susceptibility. Species-specific genetic mechanisms mitigate potential impact in the unlikely event that a gene (or part of a gene) is transferred to a non-targeted species (horizontal gene transfer). Adopting any of these strategies will require site-specific risk assessment prior to release, and monitoring and surveillance during and following releases. Design criteria should exclude selective traits (e.g. antibiotic resistance).

Successful genetic strategies will have to be affordable to have a meaningful impact on dengue transmission. The cost benefits have the potential to transform reactive, ineffective policies to those that are proactive and preventative. For governments that have the resources to support ongoing vector control programs, these strategies offer a highly cost-effective way to reach the last vestiges of remaining mosquito populations (Knipling, 1955; Dyck *et al.*, 2005; Atkinson *et al.*, 2007; Alphey *et al.*, 2011). For non-governmental agencies and philanthropic organizations looking to maximize the health impact per investment dollar, supporting product discovery and development offsets up-front costs for strategies with the potential to have long-lasting benefits. Additional value can be drawn from the fact that these tools offer egalitarian public health, improving circumstances for all treatment-area residents regardless of income, education or social status.

Design characteristics of genetically engineered mosquitoes

A genetically engineered mosquito should be thought of as a product and defined in terms of a target product profile (TPP) (Curry and Brown, 2003; Simmerman and Donnelly, 2005; Okumu *et al.*, 2010; Killeen *et al.*, 2011; malERA Consultative Group on Vaccines, 2011). The TPP states clearly what the product is, how it is to be used and how it should perform. To this end, the TPP should identify design attributes of the strain and the ideal and minimally acceptable performance characteristics that must be met. These performance characteristics are used to evaluate the product during development with an emphasis on identifying 'fatal flaws' (those attributes that do not meet minimal performance criteria) at the earliest stage possible. The TPP also may identify key hurdles for uptake and adoption of the strategy and can be instrumental in trial design. Attributes might not always be quantifiable nor may the minimum attributes be known precisely, but this exercise of defining and evaluating performance characteristics combined with robust modeling is critical for both product viability and efficient resource allocation.

Strategies and properties

Genetics-based vector control tools are categorized in ways that emphasize the intended strategy to impact target populations (population suppression/population replacement) or by the intended properties these tools would have in the field following release (self-limiting/self-sustaining) (Benedict and Robinson, 2003; Benedict *et al*., 2008; Marshall, 2009). Population suppression strategies are anticipated to control or eliminate dengue by reducing vector population densities to levels unable to sustain epidemic transmission. Population-replacement strategies are designed to create mosquito populations refractory to disease transmission by the introgression at a high frequency of an anti-virus effector gene. Either one of these strategies can have the property of being self-limiting (lost by design from the environment once mosquito releases terminate) or self-sustaining (have lower recurring requirements for maintaining the benefits). Strategy impact and field properties can be combined as follows:

- Self-limiting population suppression – periodic releases of genetically engineered mosquitoes suppress population size

followed by the elimination of the engineered insects at the cessation of releases.
- Self-sustaining population suppression – fewer periodic releases needed to suppress population sizes, requires a gene-drive mechanism that results eventually in the collapse of the target mosquito population.
- Self-limiting population replacement – eliminates ability of the mosquitoes to transmit the virus, requires a transitory gene-drive system to introgress a dengue-refractory effector gene to a high frequency within the targeted population.
- Self-sustaining population replacement – eliminates ability of the mosquito to transmit the virus, requires a gene-drive mechanism and refractory effector gene and is expected to persist for long periods.

Features of genetics-based tools

Self-limiting population suppression is broadly analogous to sterile insect techniques (SIT) where infertile males are released to mate with wild females, resulting in a reduction of the targeted insect population. Historically, sterility was achieved by random genetic mutations caused by irradiation or chemical treatment (Dame *et al.*, 2009; Black *et al.*, 2011). However, these non-specific mutations have off-target physiological consequences leading to lower mating competitiveness of males in the field. These off-target issues can be mitigated by engineering mosquitoes to carrying a conditional dominant lethal gene. The conditional dominant lethal phenotype is suppressible under laboratory and manufacturing conditions allowing rearing of large numbers of insects. Suppression of wild populations can be achieved through the release of insects carrying a dominant lethal (RIDL; Thomas *et al.*, 2000). Strictly speaking, RIDL is not SIT because it uses lethality instead of sterility as the conferred phenotype (Black *et al.*, 2011). RIDL has more favorable risk-assessment characteristics than SIT because the basis for the phenotype is explicit and can be studied for non-target or unintended consequences.

Self-sustaining population replacement strategies are expected to spread a dengue-refractory gene into a high proportion of the target population when the refractory gene is associated with a gene drive system (Sinkins and Gould, 2006). These systems serve a number of needs, including the ability to spread favorable traits into a target population on a time-scale that is meaningful to public health (James, 2005). The mechanisms on which these systems are based derive from the behavior of broad classes of selfish genetic elements that propagate independently of the fitness of the whole organism or establish both a fitness penalty and provide mitigation to that penalty. Drive systems are defined by whether they are designed to be established indefinitely (until the components fail due to genetic drift) and how easily they are established in the populations they invade. Alternative designs have been modeled extensively and the results used to make research-investment and product-design decisions (Braig and Yan, 2002; Gould and Schleikelman, 2004; Sinkins and Gould, 2006; Marshall, 2009; Marshall and Hay, 2012).

Genetic drive mechanisms associated with self-sustaining strategies can be regarded as being permanent or transitory and can have the properties of being invasive or non-invasive. Permanent drive systems are designed to be stable, while transitory alternatives have finite life spans and are designed eventually to be lost from target populations. Properties of specific systems include the release ratio necessary to spread throughout a target population. An invasive drive system spreads into populations at low introductory ratios. Non-invasive gene drive systems must be introduced at ratios high enough to overcome population genetic barriers (an unstable equilibrium point; Marshall, 2009; Marshall and Hay, 2012). A drive mechanism designed to be permanent and invasive is anticipated to be maintained indefinitely in the target population and capable of moving beyond inoculated populations. Mechanisms for these types of gene drive systems include homing endonuclease genes (HEGs) and transposable elements (Braig and Yan, 2002; Windbichler *et al.*, 2007; Marshall, 2009). Permanent and non-invasive systems are maintained indefinitely in

the target population and are refractory to the effects of immigration, but are not anticipated to emigrate and become established in populations outside of the treatment areas. Examples include *Medea* (with high fitness costs), inverse *Medea, Semele*, underdominance and infection with *Wolbachia* species (Marshall, 2009; Marshall and Hay, 2012). Drive systems that are transitory but still considered invasive will be capable of moving beyond the targeted region, but eventually will be lost from all primary and ancillary populations. A drive system that results in a male-gender bias such as a HEGs X-shredder (Deredec *et al.*, 2008) could have the ability to emigrate into new, non-targeted populations, but would have to do so within a narrow time as the strategy will eventually lead to a population crash and subsequent loss of the drive system. Finally, transitory and noninvasive gene drive systems drive a transgenic element to a high frequency in a target population, but lose drive properties by a designed uncoupling of transgenic components (e.g. by not being linked chromosomally). These systems are expected eventually to be lost from the target populations without further intervention or mitigating activities, and would not be refractory to immigration of wild mosquitoes. An example is the killer-rescue system (Gould *et al.*, 2008).

There are advantages to field-testing self-limiting population replacement strategies before releasing self-sustaining strains (Benedict and Robinson, 2003; Benedict *et al.*, 2008; Marshall, 2009). Self-limiting strains allow the characteristics of drive mechanisms and dengue-refractory genes to be measured in open-field conditions without the risk of unplanned spread. The killer-rescue system is an untested example of this type of system (Gould *et al.*, 2008). A cytotoxic killer gene (K) unlinked to a rescue gene (R) that in turn is linked tightly to a dengue-refractory gene would drive the refractory gene into the target population for a limited time. Once K becomes separated from R by independent assortment, progeny carrying K will not survive and K will eventually be lost from the population. The R gene, along with the anti-dengue refractory effector, will persist in the target population as a function of the fitness cost of carrying the transgene. The rate at which R and the anti-dengue effector are driven into the target populations and their frequency is a function of the frequency of K. Therefore, the frequency of R in the target population can be boosted by subsequent releases of K.

A number of approaches are being taken in the development of effector components for both pathogen refractoriness and as parts of gene-drive systems (Nirmala and James, 2003; Franz *et al.*, 2006; Chen *et al.*, 2007; Carter and Hurd, 2010). These include cytotoxic-, antidote- and antiviral-gene products and under certain circumstances the same component could function in multiple capacities. Cytotoxic genes can be induced as part of a toxin/antidote system for gene drive or can be induced upon infection as an anti-viral agent. Dominant-lethal cytotoxic genes include those that induce apoptosis or cause the misregulation of normal cellular function. Alternatively, cytotoxicity can be induced through the reduction or elimination of the expression of an essential gene (e.g. expression of engineered nucleases or induction of RNAi). Expression of genes encoding true toxins or venoms is not recommended because of potential risks associated with off-target effects and environmental accumulation.

Some effector genes can complement or neutralize cytotoxicity and function as an antidote as part of a gene-drive system, and/or function as an anti-viral agent. Effector genes also can complement cytotoxic effects through alternately encoded essential gene expression. In addition, effectors can act to neutralize cytotoxic gene products by repressing their expression. Anti-pathogen effector genes can be artificial and completely novel to the host genome and natural defenses (e.g. expression of antibody fragments), or can stimulate innate immune mechanisms in the mosquito (e.g. induction of RNAi or *Rel2* pathways; Dong *et al.*, 2011). Dengue-specific anti-pathogen effectors are currently under development (Franz *et al.*, 2006; Mathur *et al.*, 2010).

Effector genes need to have the proper sex-, tissue- and temporal-expression patterns to block virus transmission within the mosquito, establish a sex-specific phenotype or affect meiotic tissue to establish a pattern of inheritance. In principle, patterns of effector

gene expression can be manipulated by adapting specific gene control elements (promoters) found naturally in the mosquito (Chen *et al*., 2008). No new genetic information is added to the mosquito genome, it is just redefined contextually. These control sequences are influenced strongly by the position within the genome where they are reintroduced. Site-specific recombination and DNA insulator systems can be used to mitigate some of these influences (Franz *et al*., 2011; Carballar *et al*., 2013).

Progress in genetic-based tools

The first open-field release of genetically engineered *Ae. aegypti* involved an RIDL product in the Cayman Islands (Phuc *et al*., 2007; Harris *et al*., 2011). These trials measured how successfully males from one RIDL line find and mate with wild females. The numbers released in the initial trial were not expected to have an impact on the population density. Manufacturing and release numbers were increased, and in a landmark study, an 80% decrease in *Ae. aegypti* population densities was observed following a ~6-month release regimen (Harris *et al*., 2012).

The RIDL product released in the Cayman Islands has a dominant conditional lethal gene that affects both sexes (bi-sex). Mosquitoes must be sexed manually to assure that only non-biting males are released. A next-generation RIDL technology with a conditional female-specific flightless phenotype (fsRIDL) has the potential to lower the cost of manufacturing by avoiding the need to sex mosquitoes before release (Fu *et al*., 2010; Labbé *et al*., 2012). In addition, fsRIDL has logistical advantages associated with delivery over both SIT and bi-sex RIDL. This product can be released into a target population as genetically sexed male adults, non-sexed pupae or by distributing egg papers into local breeding sites. In indoor cage trials, non-sexed fsRIDL pupae were released into target population at a ~9 fsRIDL male:1 wild-type male ratio, eliminating those populations in 10–20 weeks (Wise de Valdez *et al*., 2011). Since both RIDL and fsRIDL strains carry genes that are lethal under wild conditions, they are self-limiting and removed rapidly from the environment following cessation of releases. Large cage trials of an fsRIDL strain identified challenges in male competitiveness that must be mitigated for this technology to move forward (Facchinelli *et al*., 2013).

Good progress has been made in the area of designing and developing a self-sustaining gene-drive system for population replacement. HEGs can be used in this capacity if linked tightly to an effector gene (Deredec *et al*., 2008; Windbichler *et al*., 2011). HEGs used for self-sustaining population suppression strategies encode sequence-specific DNA endonucleases that recognize cleavage sites 20–30 base pairs in length (Stoddard, 2005). If a cleavage site in the mosquito genome is at the same location as the endonuclease-encoding gene on the homologous chromosome in hemizygous individuals, breaks repaired by homologous recombination will result in the mosquito vector becoming homozygous for the HEG (Deredec *et al*., 2008). A proof-of-principle synthetic HEG-drive system was demonstrated in the human malaria vector, *Anopheles gambiae* (Windbichler *et al*., 2011). HEGs could be used to decrease the overall fitness of a population or decrease population densities to the point of elimination. Alternatively, if the HEG is linked to a male-determining factor and the endonuclease recognizes an essential female-specific gene, the result would be a sex-ratio bias that could eliminate the mosquito population. The molecular basis of the genetics of sex determination in *Aedes* species is not sufficiently understood at this time to initiate the development of this strategy for the dengue vectors.

A milestone in development of gene-drive products was reached when a proof-of-principle for the concept of an engineered meiotic drive, *Medea* (maternal-effect, dominant embryonic arrest), was demonstrated in the fruit fly, *Drosophila melanogaster* (Chen *et al*., 2007). *Medea* takes advantage of the fact that every sibling embryo, regardless of its genotype, inherits the same maternally derived transcripts. If a cytotoxic gene product (RNA or protein) is delivered to all of a female's embryos, development will arrest, except for those embryos carrying a gene encoding an antidote that rescues it. If the

cytotoxic, rescue and anti-dengue effector genes are linked closely, they form a gene drive system capable of introgressing a dengue-refractory phenotype at high levels within a wild mosquito population. Modern gene cloning tools make this achievable. Advancement of this system is particularly exciting due to the safety characteristics of *Medea*, which if carrying a fitness cost is not predicted to invade non-target populations and can be removed or replaced through the release of a mitigating (recall) line (Marshall and Hay, 2012). However, finding the specific functional components for mosquitoes so far has been challenging.

Alternative self-sustaining population replacement approaches are under consideration. Underdominance exploits two engineered genes (or two sets of genes) that confer lowered fitness individually, but wild-type or near wild-type fitness when together (Davis *et al.*, 2001). Inverse *Medea* depends on inheritance of maternal gene products, but under conditions where the maternal transcripts serve as an antidote to a cytotoxic gene expressed in the embryo (Marshall and Hay, 2011). *Semele* is akin to cytoplasmic incompatibility in that there is a semen-based toxin and a female antidote, but beyond effects in the embryo, considers a toxin/antidote relationship in the adult mosquitoes as well (Marshall and Hay, 2011). Transposable elements are mobile genetic elements known to invade species and are used as the basis for many insect transgenesis protocols (Adelman *et al.*, 2002). Autonomous (self-mobilizing) class II elements are being developed as the basis of an invasive self-sustaining strategy (Adelman *et al.*, 2007). While all of these systems are well-described in the literature and some have been studied in other insects, none has demonstrated proof-of-principle in mosquitoes.

Significant advancements also have been made in the development of engineered *Ae. aegypti* refractory to dengue serotype 2 virus (Franz *et al.*, 2006; Mathur *et al.*, 2010). Anti-dengue effector genes expressed in the midguts and salivary glands in some cases produced 100% refractoriness as measured by viral titers in saliva. A hammerhead ribozyme suppresses dengue infection in cultured cells (Nawtaisong *et al.*, 2009) and an anti-dengue effector showed efficacy in tissue culture using trans-splicing group I introns (Carter *et al.*, 2010). Studies of *Wolbachia*, a symbiotic organism, and transcriptome profiling of *Ae. aegypti* strains with differing vector competence support a hypothesis that a general induction of the mosquito immune system might have potent anti-dengue properties (Walker *et al.*, 2011; Bonizzoni *et al.*, 2012).

Wolbachia spreads into naïve populations through maternal inheritance and cytoplasmic incompatibility (see Chapter 27, this volume). Open-field trials demonstrated the viability of a population replacement strategy with *Ae. aegypti* infected with the *wMel* strain (Hoffmann *et al.*, 2011). The expectation is that the dengue-resistance phenotype will persist for a reasonable length of time to have an impact on virus transmission. Additional efforts are underway to develop *Wolbachia* paratransgenesis approaches using prophages as potential genetic transformation tools (Fujii *et al.*, 2004; Tanaka *et al.*, 2009).

Points to consider

Each genetic strategy offers a unique profile of challenges based on where they are used. These include the ongoing costs, perceived risks and commitments to mitigation contingencies, persistence of the strategy at the target site, garnering regulatory permits, and gaining acceptability among the stakeholders (Fig. 26.2).

All of the genetics-based strategies are predicted to be highly cost-effective. Regardless of the strategy employed, the bulk of the costs associated with deployment of any of these strategies are anticipated to be in support of monitoring and surveillance. Coordinating directed and efficient monitoring and surveillance of entomological and epidemiological parameters related to dengue transmission is an ongoing challenge for all vector control programs; conventional or innovative (Hemmingway *et al.*, 2006; Morrison *et al.*, 2008). Regardless, there are differential anticipated costs for each strategy over the long term. Invasive self-sustaining strategies are anticipated to have the lowest deployment cost, while

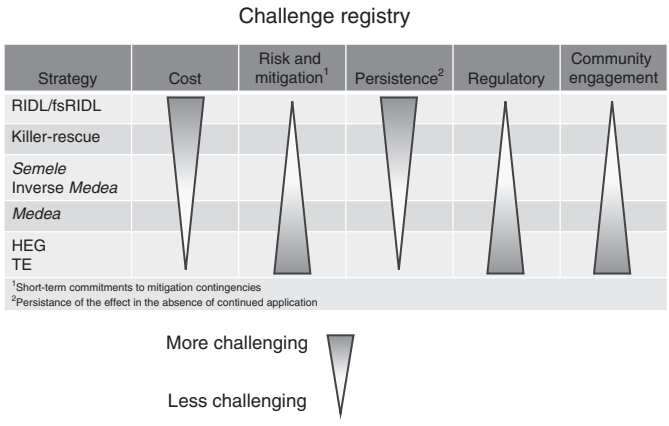

Fig. 26.2. Registry of some challenges impacting the feasibility and acceptability of various genetic strategies for control of dengue virus transmission. A generalization of the level of challenge each category is anticipated to present is depicted for a number of genetic strategies. The level of challenge is represented as a continuum from more (triangle base) to less (triangle tip) challenging for strategy adoption and implementation. The level of challenge is relative to the other strategies and may be significantly less or more than current conventional vector control strategies. Stakeholders may gauge their willingness to accept and take on challenges within each category while considering a strategy that is a 'best fit' for a particular intervention site.

population suppression strategies may cost more. Noninvasive self-sustaining strategy costs will vary depending on their unstable equilibrium point.

Transgene frequencies will go to zero when releases cease in self-limiting strategies, and the perceived risks and commitments to short-term mitigation efforts ostensibly pose a lesser challenge than self-sustaining approaches. Noninvasive self-sustaining strategies require a greater commitment for mitigation contingencies and may engender a greater perceived risk. Designs for mitigation efforts include target-site population suppression (probably via an aggressive insecticide treatment strategy) and also may include intentional reintroduction of wild populations to drive the engineered mosquitoes below an unstable equilibrium point. The greatest challenge to mitigation efforts comes from those strategies that are self-sustaining and potentially invasive. Area-wide mitigation contingencies would be necessary to cover regions outside the target site.

A consideration for regulators and policy makers is the persistence of a strategy. This characteristic reflects how long in real time the desired benefits remain in the intervention site in the absence of continued application. The shorter the persistence, the more often the strategy would have to be deployed. Persistence should not be confused with the durability of the vector control program, that is, whether or not it has the resources and stable backing of political will to maintain it.

While there are efforts underway to establish best-practice guidelines (Benedict *et al.*, 2008; Mumford *et al.*, 2009; WHO/TDR, 2010), each country will have its own regulatory pathways and sets of criteria to be met. Those strategies perceived to have the highest risk profile will have the greatest challenges navigating regulatory approval processes. Furthermore, invasive self-sustaining strategies must address the possibility of transgenes crossing international borders. Notwithstanding a multilateral agreement, releases of mosquitoes of this type face regulatory challenges in countries adhering to the Cartagena Protocol on Biosafety (Marshall, 2010).

Significant efforts are needed to communicate with relevant stakeholders to secure community authorization (Lavery *et al.*, 2010). As with regulatory pathways, the procedures

and requirements for community engagement likely will be specific to individual countries. Frameworks are proposed for approaching community engagement as it pertains to scientific research of innovative technologies developed for global health (Benedict *et al.*, 2008; Lavery *et al.*, 2008, 2010; Mumford *et al.*, 2009; WHO/TDR, 2010). While standard practices for community engagement are not yet codified, we anticipate that challenges facing more invasive and self-sustaining strategies will be greater than for noninvasive, self-limiting and transitory strategies.

Phased Pathways

Genetically engineered mosquito product design, testing and deployment move forward in phased and progressive steps. This is a key theme adopted by researchers making these tools (WHO/TDR, 2010). The phased approach includes design (populating product pipeline), testing of safety and efficacy, and product development.

Product design – filling in the pipeline

Product design and roll-out will be influenced strongly by the stakeholder's assessments of acceptable risks, dengue transmission dynamics, characteristics of the vector biology in the treatment area, attributes of the treatment area (size, logistics, human population densities, other vectored diseases and isolation) and available resources (infrastructure, financial, intellectual) (Marshall and Hay, 2012). The first generation of tools effect self-limiting population suppression and emphasize safety and acceptability among stakeholders (Benedict and Robinson, 2003; Benedict *et al.*, 2008). The next generation of products introduces dengue-refractory genes into a mosquito population. These genes should be lost from the population over a number of generations (depending on the associated fitness cost). A large-scale (inundative) release of mosquitoes with multiple unlinked refractory genes could be used to test and monitor the refractory genes under field conditions, while having the added potential benefit of protecting (or partially protecting) local residents (Rasgon, 2009). This could be followed by the introduction of self-limiting population replacement products using a transitory gene-drive system. The next steps progress toward self-sustaining population replacement products designed to have a permanent but noninvasive gene-drive system. The final and most aggressive strategies incorporate effector genes in permanent and invasive drive systems. This strategy allows for relatively small releases of mosquitoes to move a gene throughout the treatment area and possibly beyond into areas outside of the treatment area. Even here, the types of gene drive strategies used may be generational. An invasive but transitory strategy would precede an invasive and permanent strategy. It is possible that several approaches may be used simultaneously or in support of one another. For example, population suppression strategies could be used in preparation with non-invasive drive strategies to help overcome an unstable equilibrium point for permanent establishment.

Testing – a phased approach

Testing of the strategies will be an ongoing and iterative process (WHO/TDR, 2010). Strategies may have to undergo progressive and sometimes redundant testing to satisfy the requirements of regulators, collaborators and other program stakeholders. Testing protocols focus on safety, efficacy and design criteria as stipulated in the TPP.

Initial tests will begin in laboratories where the components of the design products are developed. The earliest tests will screen for desired phenotypic characteristics in small cage trials. Laboratory tests also will be performed to measure key safety features such as insecticide resistance and vector competency to serotype- and strain-specific dengue viruses, as well as other relevant flaviviruses and alphaviruses. As candidate products are identified, large indoor cage trials will test for efficacy and continued monitoring for safety (Benedict *et al.*, 2008; Wise de Valdez *et al.*, 2011). Large cage trials allow for a more advanced look at impacts on population genetics as well

as measuring competitive mating fitness. Candidate products validated by laboratory trials are considered further in field trials.

Laboratory conditions usually lack influences found in the wild, such as temperature fluctuations, humidity, wind and light as well as exposure to indigenous microbial flora (Facchinelli *et al.*, 2011). Depending on stakeholder assessments of acceptable risks, product testing will continue in contained-field trials (e.g. large cages in an outdoor setting) or in confined-field trials (sites that offer geographical, environmental or biological confinement) (WHO/TDR, 2010; Facchinelli *et al.*, 2013). These tests measure performance against mosquitoes from the local population under more natural conditions. While field cages can approximate environmental influences, they cannot faithfully replicate the entire spectrum of conditions in the open field. The only true measurement of performance under natural conditions must occur in open-field trials.

Open-field trials are preceded by studies that first determine the biology of the mosquitoes at the target site. Initial trials may consist of releasing wild-type mosquitoes marked with a dye for later recapture and analysis (mark, release, recapture) to determine flight range characteristics and the relative number of mosquitoes in the target population (Valerio *et al.*, 2012). Releases of a relatively small number of engineered mosquitoes will identify how well the engineered male mosquitoes disperse and mate with the wild females (Harris *et al.*, 2011). Data collected are used to design small-scale release trials. These trials have entomological criteria as endpoints and are not expected to have an impact on dengue transmission. Data are used to redesign the trial protocols or to develop the protocol for the next testing phase. Large-scale trials test the ability to scale-up production with continued measuring of entomological endpoints as well as assessing ecological impacts (Harris *et al.*, 2012). At this scale, these trials may have an effect on dengue transmission; however, that would not be the intent of the trials nor would it likely be practical or feasible to measure epidemiological impact at this stage.

Once products are tested in the open field and the logistics and manufacturing scaled-up successfully, large-scale open-release trials test the capacity of the engineered products to have an impact on both entomological and epidemiological endpoints. These studies would establish a proof-of-principle for the capacity of the engineered product to disrupt area-wide dengue transmission. In addition, the trials determine the feasibility of moving toward wide-scale implementation. Efforts continue to assay safety and efficacy of the product in the field and these are coupled with the development and testing of standard operating procedures (SOP) as well as good manufacturing practices (GMP) for large scale roll-out. In addition, the scale-up of activities allows for a careful economic analysis as costs models are supplied with real numbers.

Significant challenges exist in measuring epidemiological endpoints (James *et al.*, 2011; Wolbers *et al.*, 2012). Proper experimental controls will be critical for defining successes. Control sites will be determined both geographically (matched locations) and temporally (historical data). Because these trials could impact the behavior of the residents in the target site(s), field trials may have to be blinded and/or randomized to the extent possible. Unfortunately, trials of these types cannot borrow from pharmaceutical or vaccine trial designs as these products are designed to impact public health on an individual level. In contrast, engineered strategies are designed to have an area-wide impact on public health. Thus, trial design presents ongoing and novel challenges.

Applications

Adoption and implementation of genetic control strategies will depend on many factors, including cost. Countries that have the means to support ongoing vector control may choose to test and implement self-limiting population suppression strategies where appropriate. As knowledge of the use of genetically modified mosquitoes accrues and as new products become available, these countries may wish to switch to more potentially cost-effective self-sustaining population replacement strategies. For countries that do not have the means to test and support ongoing vector control efforts, there may be a willingness to use

self-sustaining tools as soon as they have established a proven track record elsewhere.

Site selection

Many factors in addition to the presence of the mosquito vector need to be considered before any genetic control strategies are adopted. Identifying and evaluating criteria by which to select an intervention site is a process intimately intertwined with product development. A thorough and rigorous site-selection process should include the active participation of stakeholders and emphasize ethical, social and cultural (ESC) considerations (Lavery *et al*., 2008, 2010). In the end, the final decision for where to test these strategies will be the shared responsibility of the developers/researchers and the collaborators of the disease-endemic country. The ultimate success or failure of a product can be influenced significantly by the actions and relationships forged during site selection.

Topics to be considered during site selection include biological/environmental specifications, technical/logistical considerations, regulatory frameworks and the stakeholders (ESC considerations) (Brown *et al*., 2014). The process involves evaluation of essential criteria representing 'go/no go' decision points and other criteria that are weighed on balance with other considerations.

Biological considerations include local entomological, human and pathogen characteristics, while environmental criteria consider seasonal patterns of weather and the potential for disruptive events (natural and man-made disasters). Considerations of technical criteria include information about the number and size of potential sites, information on the quantity and quality of local infrastructure, current vector control practices and capacities, an assessment of enthusiasm of potential collaborators, institutional leaders and political structures, administrative life cycles and local resources that are available for cost- and/or work-load sharing. Regulatory considerations include specific oversight of importing and working with genetically engineered mosquitoes, research activities including institutional oversight of working with pathogens and recombinant DNA, vertebrate animal usage and human subjects. Regulatory agencies will play an important role in each phase of development, but may have the biggest impact on experimental design of field trials. A range of regulatory bodies (institutional, local, county, state, provincial, national and international) may have input (Marshall, 2010; WHO/TDR, 2010). Therefore, aspects of trial design will be site-specific and may have unique sets of regulatory oversight endpoints to satisfy. Mitigation plans also will be both product- and site-specific and will be influenced by the regulatory framework. Trials will undoubtedly raise questions of jurisdiction, intellectual property and liability. These issues are not unique to this technology; pharmaceutical and vaccine trials grapple with similar concerns.

Product maturation

Innovations go through phases of maturation as they move from being an idea to a viable product. The initial phase begins in the laboratory, where a researcher makes a basic discovery or conceptualizes a functional relationship that could lead to the disruption of dengue transmission. Proof-of-principle is usually the objective and these efforts often are not made with much foresight as to how an end-product would be adopted. The next phase considers the end-product and initiates the process of transforming an idea into an end product that may eventually go to market. Products are defined and evaluated against performance criteria, end-users and stakeholder requirements, and anticipated production costs. This phase also explores the feasibility of scale-up and manufacturing for area-wide treatment. The final phase includes aspects of how to get the product to the end user. Activities include construction of manufacturing facilities, defining distribution logistics, establishing pricing and promoting the product. While there is no well-developed pathway at this time for the developers of the new control tools to follow, we encourage critical thinking at the proof-of-principle stage about how an approach ultimately would translate into a final product.

Funding

Research scientists often are neither trained nor called upon to guide a product through the initial to final stages of development and application. The development of insecticides, pharmaceuticals or vaccines depends on well-established industries that have expert knowledge of this process. However, for products designed to impact public health in developing nations, there are few financial incentives for industry to invest the time, money and expertise necessary to move ideas from bench-top to delivery.

Basic research funding provides the resources for the innovation of product ideas, but in order for a product to mature, additional support is needed from industry and/or philanthropic groups. Philanthropic or NGO investments can de-risk product development by absorbing costs associated with moving through the phases with the expectation of enticing private entities to lend their resources toward product development and delivery. This model has promise for products closely matching well-established industry profiles (chemicals, drugs, vaccines), but remains a challenge for products that have neither financial incentives nor established pathways to product maturation. Genetic-based strategies depend on products so unique that for now the standard operating procedures, good manufacturing practices and road maps for developing the expertise on how these products would move to market remain in the realm of basic research.

Community engagement

No matter how efficacious the product, no matter how well intentioned the researchers, the use of genetically engineered products will never be realized unless community engagement is embraced (Lavery *et al.*, 2010). Community engagement promotes communication so that people feel empowered to ask 'tough' questions. While education is valued highly, it is unlikely that all stakeholders will have a detailed understanding of the science behind the trials. Trust is earned over time by engaging people from the outset, making information available, and opening and maintaining lines of communication.

Future

The coming years are sure to bring a variety of reports of the development of both novel anti-dengue effector genes and advancements in gene drive systems. Scientists are working earnestly to establish guidelines for the development of these tools (Benedict *et al.*, 2008; Lavery *et al.*, 2008, 2010; Mumford *et al.*, 2009; WHO/TDR, 2010; Reeves *et al.*, 2012). Population suppression tools are blazing a pathway for field trials of more ambitious tools. Undoubtedly, each new development will foster further conversation and debate regarding the role these tools should and will take in public health.

One of the strengths of the potential for genetically engineered products is the great variety of ideas that can be brought to bear on the problem of vector management for disease control. However, this variety remains a challenge for establishing a one-size-fits-all best-practices regimen. Furthermore, challenges remain in the development of a viable business model where precedents have yet to be established. Unless small start-up companies are able to survive, the field may have to wait for the development and adoption of genetically engineered arthropod products for agricultural pests before an industry can be established to provide technical and business resources necessary to bring these technologies to market.

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Notes

[1] WHO Global Alert and Response, Dengue/dengue hemorrhagic fever, Impact of Dengue; URL: http://www.who.int/csr/disease/dengue/impact/en/index.html (accessed 30 January 2012).
[2] WHO Fact sheet N°117, January 2012.
[3] Vector competence is defined as the genetically determined ability of a vector to support the development and propagation of a pathogen (Hardy *et al.*, 1983).

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