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Perspective

CRISPR/Cas9-Mediated Gene Drive to Prevent the Replication of Dengue Virus in the Mosquito Vectors to Reduce the Impact of Dengue Epidemic in Bangladesh

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Abstract: Dengue is a flavivirus transmitted by *Aedes aegypti*, leading to mosquito-borne illness causing significant morbidity and mortality each year. A majority of dengue cases around the world are caused by four serotypes-dengue virus (DENV) 1-4. The recent outbreak has broken all the previous record of infections with 101, 354 dengue cases, which has increased the urgency of finding an effective way to reduce the level of infection. CRISPR-Cas9 mediated gene drive is a novel technology that can be used to reduce the transmission by *Aedes* mosquitoes. *Ae. aegypti* can be engineered to express anti-DENV, 1C19-based, single-chain variable fragment (scFv) antibody, to provide protection against all four DENV serotypes by neutralizing them. Anti-DENV scFv-antibody transgene can be incorporated into a clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR associated (Cas); i.e., the CRISPR/Cas9-based gene drive system gene for effective suppression of dengue infection. CRISPR/Cas9-based gene drive system allows it to pass down this phenotype across the wild population in the urban area. However, the incautious release of gene drive in the environment can swipe away the entire population. This technology can greatly impact on the environment, creating an imbalance in the ecosystem if not applied carefully. Rigorous studies and mass level cooperation are needed among the scientists, local authorities and the government to make the informed decisions on the outcome of this technology.

Keywords: dengue, *Aedes aegypti*, anti-DENV scFv-antibody transgene, CRISPR-Cas9, gene drive, transgenic mosquitoes

1. Introduction

Mosquito-borne diseases such as malaria, zika, dengue and yellow fever are major global public health concerns [1]. In recent decades, dengue infections have been increasing dramatically in the world [2]. Dengue is a flavivirus transmitted among humans by *Aedes aegypti* mosquitoes. Approximately 50% of the world population is at risk of getting a viral infection [2]. Around 390 million people get infected every year, which leads to an annual economic loss of approximately $40 billion [2]. Notably, 75% of worldwide dengue cases, caused mainly by four serotypes of dengue viruses (DENV1-4), are reported in Asian countries such as India, Malaysia, Philippines, Indonesia, Thailand, and Bangladesh [1]. The biggest threat of mortality from dengue infections comes from dengue haemorrhagic fever, which

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affects around 1% of the infected individuals, and is associated with reinfection by a different dengue serotype [1, 2].

In Bangladesh, the first dengue outbreak was recorded in 2000 by the dengue virus serotype DENV 3 [3]. As reported earlier, the climatic condition in Bangladesh (regarding temperature, humidity and rainfall) is quite favorable for the transmission of the DENV through the mosquito vector *Ae. aegypti* vector (in sometimes *A. albopictus*) [1]. In 2019, Bangladesh was affected by a dengue outbreak that broke all the previous records of infection with 101,354 dengue hospitalized cases [3, 4]. The economic burden behind the treatment of each hospitalized patient is $10. The burden is increasing to facilitate awareness programs, spray insecticides, and to provide prevention drugs every year, and these strategies have proven to be less effective as much of *Aedes* mosquitoes have become resistant to the existing drugs [3]. According to research, the increase in dengue cases is driven by the unplanned urbanization, migration, and climate change [4]. As these underlying factors threaten to intensify in the coming decades, dengue and other mosquito-borne diseases will likely lead to bigger public health crises in the developing countries such as Bangladesh. Interestingly, the other major mosquito-borne viruses that have recently emerged as global threats, zika virus (ZIKV) and chikungunya virus (CHIKV), are also transmitted by the same mosquito vector [1].

Knowing the scale and possible future course of this healthcare issue, it is high time for the advent of novel methods-both of identification, treatment and prevention-to tackle it. The ongoing COVID-19 pandemic serves as an additional motivation to prevent viral diseases with effective human-to-human transmission from spiraling out of control. To that end, the novel technology of CRISPR-based gene drives can be implemented to eliminate dengue virus transmission via *Ae. aegypti* mosquitoes from Bangladesh.

Application of the clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR associated (Cas); i.e., the CRISPR-Cas9 method to induce the site-specific mutations in *Ae. aegypti* principally depends on the RNA-DNA base-pairing to spawn targeting specificity, which in turn results in effectual genome-editing [5, 6]. There is some supporting precedent for this kind of work, and successful execution at the country-level in Bangladesh would provide a roadmap for similar efforts across the globe [5, 6]. Current review accumulated the existing knowledge on the CRISPR/Cas9 genome editing method for the eradication of DENV infection, which may be applicable for the control of dengue in Bangladesh.

2. Genetic engineering and dissemination of *Ae. aegypti*

Advancements in genomics have enabled the understanding of the evolutionary mechanism underlying mosquito biology [6, 7]. The bacterial type II CRISPR-Cas9 has already been adjusted as a genome-engineering tool in array organisms as which expanded the ability to modify genomes; and the methods for the site-directed mutagenesis in *Ae. aegypti* employing the RNA-guided endonucleases (for example, the double-stranded endonuclease Cas9 derived from *Streptococcus pyogenes*) have already been implemented, which is based on the RNA-DNA base pairing to target the specific genomic locations [6]. Advances in genetic engineering technologies including the CRISPR/Cas genome editing method have been reported to generate *Ae. aegypti* mosquitoes with a decreased competence as vectors for DENV, which in turn reduces their capability to bear and transmit pathogens [2].

Interestingly, the CRISPR/Cas genes are also the constituents of an adaptive immune system widespread among bacteria and archaea [6]. *Ae. aegypti* mosquitoes can express a gene that encodes an engineered single-chain variable fragment (scFv) generated from a neutralizing DENV human monoclonal antibody (directed against each of the antigenically distinct DENV serotypes), which significantly reduces the viral infection and subsequent transmission rate [2]. When the antibody is produced and released in the posterior part of the midgut of the mosquito, it prevents the release of viral genome for replication. Dengue virus population cannot increase to the threshold level, and cannot be transmitted by the mosquito to another host [2]. For the effective suppression of dengue infection, anti-DENV scFv-antibody transgene can be incorporated into a CRISPR/Cas9-based gene drive system and allow it to pass down this phenotype across the wild population in urban area (Figure 1).

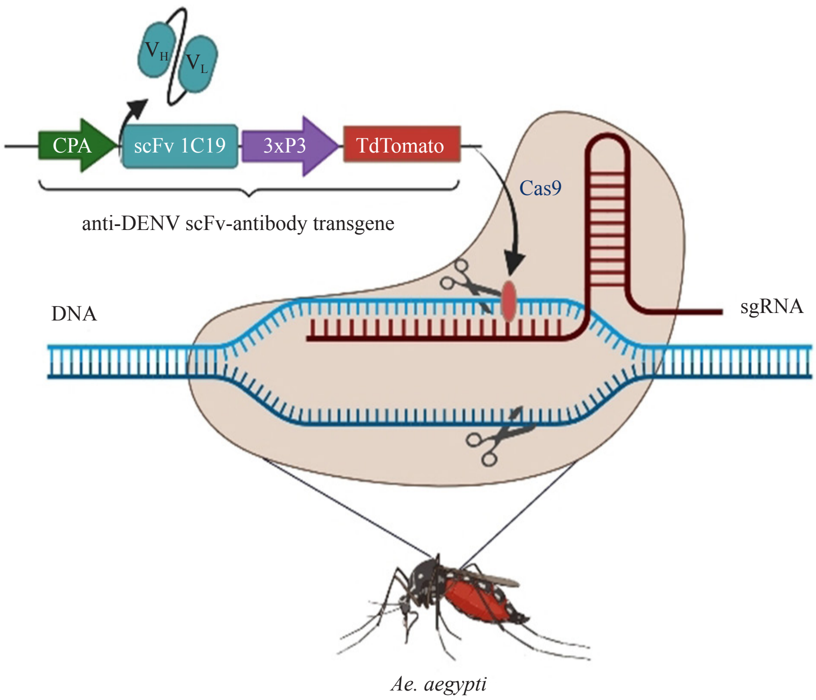

Figure 1. Incorporating anti-DENV scFv-antibody transgene using CRISPR gene drive system in transgenic mosquitoes, to push desirable traits into wild *Aedes* mosquitoes.
The application of CRISPR-Cas9 system in gene drive involve germline expression of Cas9 endonuclease to cleave targeted sequence, directed by guide RNA to the target site and insertion of anti-DENV scFv-antibody transgene [2, 7]

2.1 *Homing-based gene drives*

The gene drive was first introduced in 2003 on the basis of site-specific homing endonuclease genes [7]. Drives are linked with the gene of interest or "cargo" genes such as anti-DENV scFv-antibody transgene, which is transmitted across the wild disease-transmitting populations [2]. The homing endonuclease cuts the target sequence, and as the cell starts to repair, the homologous recombination mechanism follows and incorporates the cargo gene into the opposite DNA strand. Later, this double-stranded DNA sequence containing the anti-DENV effector genes is employed as a template. The gene of interest gets passed on to next DNA strand and the cycle gets repeated several times until the whole population integrates the gene into their genome. It is interesting to note that gene drives violate the rule of Mendelian genetics. According to Mendelian genetics, when male and female mosquitoes mate, their offspring inherit 50% of the parental chromosomes. However, the gene drive allows up to 100% of offspring to inherit the desired trait including pathogen resistance. This strategy is known as super-Mendelian inheritance [8]. It reduces the ability of mosquito to carry the pathogen, and as a result, prevents pathogen transmission from human to human [8].

3. Revolutionary gene editing tool: CRISPR-Cas9

CRISPR has recently emerged as a more efficient way to try and implement gene drives. CRISPR gene editing tools use defense mechanism of bacteria against phage invasion. It mainly consists of two components: Cas9 endonuclease and guide RNA. The application of CRISPR-Cas9 system in gene drive involves germline expression of Cas9 endonuclease to cleave targeted sequence, which is directed by guide RNA to the target site [7]. CRISPR-mediated gene drive system follows the same mechanism as homing endonuclease gene drive [7]. However, incomplete cutting was a major drawback of homing endonuclease gene drive in transgenic mosquitoes [7]. Recently many researchers are suggesting the implementation of the CRISPR gene drive system in transgenic mosquitoes, to push desirable traits into wild *Aedes* mosquitoes [9]. CRISPR gene drive exhibits certain advantages in terms of cleavage, specificity and stability. The enzyme will be guided by expressing more than two guide RNAs to increase the efficiency of Cas9 nuclease cleavage. These guide RNA sequences can be modified to target multiple target sites and prevent unstable repeats within the drive cassette [7]. Targeting multiple sites provides evolutionary stability against drive-resistance alleles. Using Cas9 nickases instead of nucleases can increase specificity and avoid off-target editing [7].

CRISPR gene drive technology leverages the sequence-targeted DNA cleavage activity and endogenous homology-directed repair mechanism to convert the heterozygous phenotypes to homozygous type against specific trait. The transgenic homozygous mosquitoes can efficiently express the DENV-targeting single-chain variable fragments (scFv) derived from previously characterized broadly neutralizing antibodies to block the infection and further transmission by these mosquitoes [2]. The other arboviruses like ZIKV and CHIKV also use the same vector for their effective transmission. The studies are being conducted to develop resistance in mosquitoes against these viruses using scFv transgene in the CRISPR gene drive system [2]. Indeed, Smith et al., identified a DENV-targeting 1C19 monoclonal antibody from human either vaccinated or naturally infected [2, 10]. The antibody was found to neutralize all DENV serotypes along with the capacity to decrease viremia in a mouse model exposed to the infection caused by DENV-1 and DENV-2 [10]. As stated above, based on this identification, *Ae. aegypti* is thus engineered to express the 1C19-based, broadly neutralizing, scFv antibody, which can neutralize all four DENV serotypes [2, 6, 10]. Therefore, *Ae. aegypti* mosquitoes can express the anti-DENV scFv that prevents them from the infection by the DENV serotypes, facilitating the remedies against DENV infection [2, 10].

4. Possible limitations of CRISPR-based approach

4.1 *Ecological effect of gene drive*

CRISPR gene drive has caught the attention of the scientific community because of its flexibility, efficiency, and ability to pass on an effector gene from a small group of pest organisms to an entire population at a faster rate. As exciting as this revolutionized technology may sound, like every new advent, there are both promises and perils to the gene drive [10, 11]. If CRISPR gene drive is linked with scFv-based transgenes, it may elicit the generation of antibodies to kill the virus inside the arthropod vector; i.e., mosquito; however, since, this is an emerging technology, more studies need to be conducted to achieve the 100% validated results *in vivo* [2, 7]. Nevertheless, this is at least, so far theoretically seems to be a very efficient and ecologically beneficial approach to suppress mosquito borne diseases like malaria, dengue, and zika [2]. If CRISPR gene drive system is released to increase the ratio of male to female, the drives can eradicate an entire population as there will be no female offspring for further sexual reproduction [7]. An important consideration should be focused on the mutagenesis and off-target editing since Cas9-induced off-target single nucleotide polymorphisms (SNPs) can be difficult to be detected precisely only by the computational algorithms [9]. Moreover, another, dreadful impact may arise due to the application of CRISPR to eradicate disease, which is the elimination of the species; i.e., the disease vectors that may cause significant environmental consequences. [11, 12]. Thus, some potential risks, as well as ethical issues, should be considered prior to the application of gene drive technology [9, 11].

4.2 *How to solve this problem?*

There are crucial factors that need to be examined carefully before releasing this technology into the wild: potentiality of gene drive for obtaining the desired outcome, the impact of the gene drive in the ecosystem, the chances of the gene drive to pass down to closely related species by gene flow [7]. In this regard, the scientist must conduct research by carefully monitoring and assessing these factors. It is not always possible to foresee any risk. That's why they should perform the initial experiment using non-native mosquitoes in a bio-containment laboratory, to prevent any undesirable outcomes if any of these species escape. It is vital to ensure transparency in disclosure of all experimentation details, and if the results obtained do not garner the majority of the public's favor, experimentation would not proceed any further [7]. Before any field trials, researchers must win the final approval from the mosquitoes control unit and the residents. They need to be reconciled and to build trust among the residents by addressing the concern for increasing dengue infections in the communities and afterwards, propose our prevention ideas. The understanding of genetics with recent major breakthroughs like CRISPR gene editing has revolutionized the field of life sciences and changed our life mostly for the better. Indeed, the CRISPR mediated gene drive is a relatively new technology and it has risks and benefits that still need to be addressed, as well as discussed broadly with the scientific community, government officials and residents. Before proceeding towards practically-implemented field tests, plenty of independent research are required to discover methods that help minimize the adverse effect of this drive [12].

4.3 *Social acceptability from Bangladesh's perspective*

From the perspective of Bangladesh, another limitation is that due to the lack of knowledge about such advanced scientific topics, people may start to resist the application of novel technologies instead of accepting them [13]. This is why good journalism is vital in correcting all the misinformation and information-gaps. Therefore, the collaboration between researchers and public health workers is essential to develop effective science communication skills in order to explain complicated scientific concepts and ideas in a simple way, distribute poster and leaflet in the community, and build trust among the public by the sharing of knowledge [10, 11].

5. Conclusion

Technology like CRISPR gene drive is complex, and it inherently comes with a lot of uncertainty and controversy based on ethical and environmental issues. Yet it is considered as an efficient and dependable tool for editing genes in an array of organisms including *Aedes aegypti* vectors carrying the DENV. Developing countries like Bangladesh with a weak healthcare infrastructure desperately requires this technology to fight mosquito-borne illnesses and reduce the already pressing economic burden. This technology may be the beginning of cultivating hope to prevent a deadly upcoming epidemic and save many people from an early death.

Conflict of interest

Authors have declared that they have no conflict of interest.

References

[1] Noor R. Re-emergence of dengue virus in Bangladesh: Current fatality and the required knowledge. *Tzu Chi Medical Journal*. 2020; 32(3): 227-233.

[2] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. *Nature Reviews Microbiology*. 2010; 8(12): S7-16.

[3] Abir T, Ekwudu O, Kalimullah NA, Nur-A Yazdani DM, Al Mamun A, Basak P, et al. Dengue in Dhaka, Bangladesh: Hospital-based cross-sectional KAP assessment at Dhaka North and Dhaka South City Corporation area. *PLoS One*. 2021; 16(3): e0249135.

[4] Sharmin S, Viennet E, Glass K, Harley D. The emergence of dengue in Bangladesh: epidemiology, challenges and future disease risk. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2015; 109(10): 619-627.

[5] Kyros K, Andrew MH, Roberto G, Nace K, Austin B, Andrea KB, et al. A CRISPR-Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nature Biotechnology*. 2018; 36: 1062-1066.

[6] Kistler KE, Vosshall LB, Matthews BJ. Genome engineering with CRISPR-Cas9 in the mosquito *Aedes aegypti*. *Cell Reports*. 2015; 11(1): 51-60.

[7] Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*. 2014; 3: e03401.

[8] Li M, Bui M, Yang T, Bowman CS, White BJ, Akbari OS. Germline Cas9 expression yields highly efficient genome engineering in a major worldwide disease vector, *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States*. 2017; 114(49): E10540-E10549.

[9] Macias VM, Ohm JR, Rasgon JL. Gene drive for msquito control: Where did it come from and where are we headed? *International Journal of Environmental Research and Public Health*. 2017; 14(9): 1006.

[10] Smith SA, de Alwis AR, Kose N, Harris E, Ibarra KD, Kahle KM, et al. The potent and broadly neutralizing human dengue virus-specific monoclonal antibody 1C19 reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein. *mBio*. 2013; 4(6): e00873-e00913.

[11] Caplan AL, Parent B, Shen M, Plunkett C. No time to waste-the ethical challenges created by CRISPR: CRISPR/Cas, being an efficient, simple, and cheap technology to edit the genome of any organism, raises many ethical and regulatory issues beyond the use to manipulate human germ line cells. *EMBO Reports*. 2015; 16(11): 1421-1426.

[12] Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. *Proceedings of the National Academy of Sciences of the United States*. 2015; 112(49): E6736-E6743.

[13] Parveen S, Islam MS, Begum M, Alam MU, Sazzad HM, Sultana R, et al. It's not only what you say, it's also how you say it: communicating nipah virus prevention messages during an outbreak in Bangladesh. *BMC Public Health*. 2016; 16: 726.