Accepted: 3 February 2020

Dengue: Status of current and under-development vaccines

Marianna Redoni[1] | **Sophie Yacoub**[2,3] | **Laura Rivino**[4] | **Daniele Roberto Giacobbe**[5] | **Roberto Luzzati**[1] | **Stefano Di Bella**[1]

[1]Infectious Diseases Department, University Hospital of Trieste, Trieste, Italy

[2]Department of Medicine, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

[3]Centre for Tropical Medicine and Global health, University of Oxford, Oxford, UK

[4]School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK

[5]Dipartimento di Scienze della Salute (DISSAL), University of Genoa, Genoa, Italy

Correspondence
Stefano Di Bella, Infectious Diseases Department, University Hospital of Trieste, Piazza dell'Ospitale, 1, 34129 Trieste, Italy.
Email: stefano932@gmail.com

Summary

Dengue is an emerging mosquito-borne viral infection with increasing reports of outbreaks. The clinical picture ranges from a benign febrile illness through to severe and potentially fatal manifestations. No specific anti-viral treatment exists, and therapy only consists of supportive care. During the last three decades, several attempts to develop an effective vaccine have been made. The first dengue vaccine to obtain licensure was Dengvaxia, which was authorized in 2015 and is currently available in over 20 countries. Its use has been approved with strict limitations regarding age and serostatus of the recipients, highlighting the necessity for a more safe and efficacious vaccine. At present several vaccine, candidates are undergoing clinical and pre-clinical trials. The most advanced candidates are TDV and TDV 003/005, two live-attenuated vaccines, but another 15 vaccines are under development, introducing novel immunization strategies to the traditional dengue vaccine scenario. This work reviews the current research status on dengue vaccines.

KEYWORDS

dengue, vaccination, vaccine

1 | INTRODUCTION

The burden of dengue has become a major public health concern over the last few decades. It has been identified as one of the 10 threats to global health in 2019 by WHO,[1] underlining the urgent need for a safe and effective vaccine. Although there have been numerous attempts to produce a valid candidate, the development of a vaccine for dengue still remains a challenging task, because there are several critical issues that have to be considered.[2]

A major limitation is that, despite decades of research, many aspects of the dengue immunopathogenesis remain unknown, in part owing to the lack of an appropriate experimental model.[3] Improving the understanding of the immune response against DENV infections would allow a more targeted approach in the development of a successful vaccination strategy.[4] The pursuit for an immune correlate of protection for dengue is a priority objective, as defining the most relevant viral epitopes, the antibodies with the highest neutralizing activity and the sufficient titer to protect from infection, would help to identify the most efficacious vaccine candidates.[5,6]

Another useful tool for this purpose is the development of dengue human infection models, such as those performed using rDEN2Δ30, that would allow us to test the efficacy of vaccine candidates before submitting them to clinical trials involving large cohorts of patients.[7]

Dengue belongs to the family *Flaviviridae*, genus *Flavivirus*, species *Dengue virus*. It consists of four distinct virus serotypes (DENV1-4); the unpredictable and simultaneous circulation of all four viral strains in hyperendemic areas makes it necessary to have a tetravalent vaccine, to provide a balanced immunity against all four serotypes.[8] Indeed, if an adequate immune response to all of the dengue serotypes is not provided, vaccinated patients would still be at risk of contracting the disease. Moreover, the chances of developing more

List of abbreviations: AS01E, adjuvant system 01E; AS03B, adjuvant system 03B; CYD-TDV, chimeric yellow fever dengue - tetravalent dengue vaccine; DENV, dengue virus; DENV-2 PDK53, dengue virus 2 primary dog kidney cells; E, envelope gene; LAVΔ30, live attenuated vaccine; MSD, Merck, Sharp & Dohme; NHP, non-human primates; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institute of Health; prM, pre-membrane gene; RIDL, release of insects carrying dominant lethal genes; SAGE, strategic advisory group of experts; TDENV-PIV, tetravalent dengue purified inactivated vaccine; TDV, tetravalent dengue vaccine; TV003/TV005, tetravalent vaccine; WHO, World Health Organization.

severe disease would be paradoxically higher precisely because of the prior immunization, as a result of the antibody-dependent enhancement of disease and possibly original antigenic sin.[9,10] Therefore, not only can a vaccine that provides incomplete immunity fail to prevent the disease, but it can also place those given the vaccine at higher risk of developing a severe form.[11]

2 | CURRENTLY LICENSED VACCINE

After decades of extensive studies, the first vaccine for dengue has been licensed: CYD-TDV, commercialized under the brand name Dengvaxia by Sanofi-Pasteur. It was initially released in December 2015 in Mexico and is now licensed in over 20 dengue endemic countries, with an age indication limited to subjects of 9-45 years of age.

2.1 | Dengvaxia - CYD-TDV

CYD-TDV is a tetravalent live-attenuated virus vaccine; therefore, it is supposed to provide a balanced immunity against all of the four serotypes of the virus. CYD-TDV incorporates four chimeric viruses, which were created by using the backbone of another flavivirus, the yellow fever virus vaccine 17D (Figure 1A).[12,13]

Phase I clinical trials showed that CYD-TDV was well tolerated (no serious adverse events were reported) and manifested a high seroconversion rate, as 100% of the participants seroconverted to all four serotypes after receiving three doses.[14,15] However, phase IIb and III clinical trials showed limited efficacy against DENV-2 (34.7% efficacy against virologically confirmed dengue), which is unfortunately most frequently associated with severe dengue cases. This suggests that in many cases the DENV-2 antibody titer might not be sufficient to guarantee a solid protective immunity against that particular serotype. Efficacy against DENV-1, 3, and 4 was 54.5%, 65.2%, and 72.4%, respectively.[16,17]

A large phase III clinical trial recruited over 10 000 children aged 2-14 years in five Asian-Pacific countries. The primary objective was to evaluate the efficacy of the vaccine. Overall, a 56.5% (95% CI 43.8-66.4) reduction of PCR-confirmed dengue cases was observed in the vaccine recipients compared to the control group.[17]

Nevertheless, the long-term follow-up analyses showed a worrying trend: vaccines under 9 years of age presented a 1.58 relative risk for dengue-related hospitalization compared to the control group. Moreover, the efficacy of the vaccine seems to be consistently lower in children under 9 years compared to that of older participants (44.6% vs 67.8%),[18] because as these children are younger in age they are less likely to be seropositive. For these reasons, CYD-TDV has been licensed for use only in individuals above 9 years of age.[19]

One additional limitation to the use of Dengvaxia is that the vaccine candidate appears to sensitize dengue naïve participants, which acts like a natural primary infection, therefore putting them at a higher risk of developing a secondary infection which is associated with more severe forms of dengue. The reason behind this phenomenon is not fully understood yet; however, it might be explained by the mechanism of antibody-dependent enhancement, combined with an imbalanced immunogenicity against the different serotypes.[20]

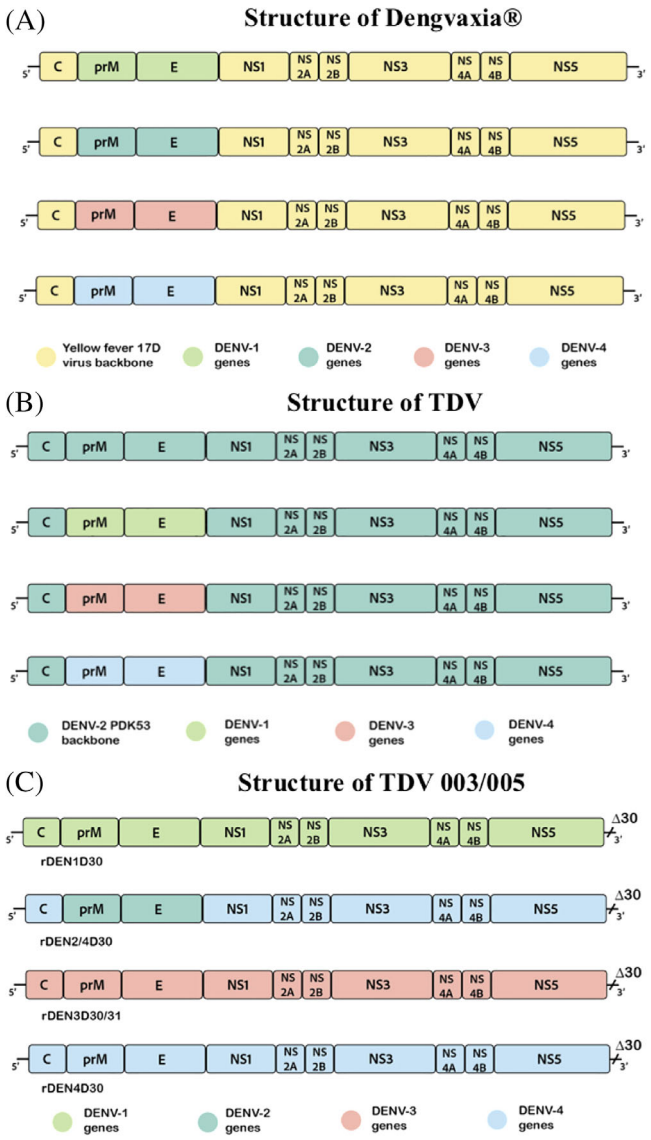

FIGURE 1 (A) Structure of Dengvaxia®, (B) Structure of TDV, (C) Structure of TV003/TV005

Lower efficacy for seronegatives at baseline was observed for all participants and not only the younger group. CYD-TDV has been proven to be safe and effective in patients with antibodies against at least one dengue serotype; however, its administration is potentially dangerous in seronegative individuals. Given this fact, the Strategic Advisory Group of Experts (SAGE) on immunization from WHO recommends a prevaccination screening to assess the serostatus of the recipients, to immunize exclusively the people who had a previous DENV infection. In environments where this screening is not feasible, the vaccine should be administered only in those countries where the seroprevalence has been demonstrated to be greater or equal to 80% over the age of 9.[21]

Dengvaxia is currently undergoing postmarketing surveillance studies, to monitor any rare or long-term adverse event and to collect additional data about its efficacy.[22–24]

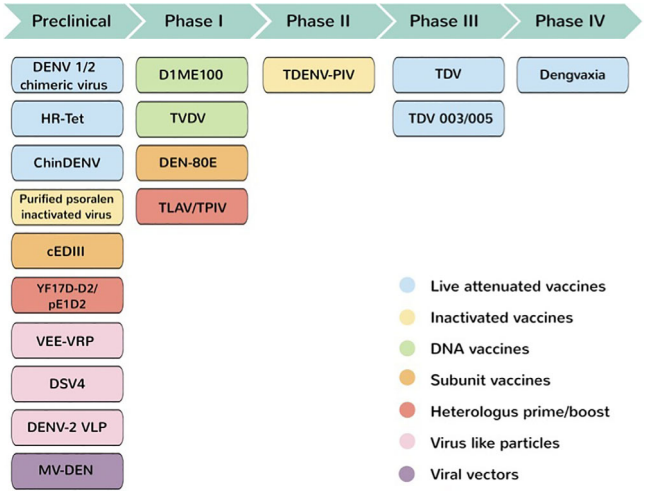

FIGURE 2 Dengue vaccine pipeline

After a mass vaccination campaign, the vaccine was suspended in 2017 in the Philippines, as a precautionary measure, in response to a higher hospitalization rate in children over the age of 9 who contracted dengue. In high seroprevalence regions, overall public health benefit is still favorable despite the negative press that has surrounded the dengue vaccination campaign in the Philippines. However this event confirms, once again, the urgent need for an alternative vaccine strategy. The ideal vaccine has to be safe and effective against all of the serotypes, and it is necessary to find a candidate which ensures a solid protective immunity from DENV infection regardless of the age or serological status of the recipients.[25]

3 | FUTURE VACCINE CANDIDATES PIPELINE

The increasing global burden of dengue in the last decades has motivated researchers and pharmaceutical companies to invest a growing amount of resources in the development of new vaccines. Thanks to these investments, there are now several vaccine candidates under development involving various approaches (Figure 2).

The most advanced candidates are the two live-attenuated vaccines: *TDV* and *TDV 003/005*, which are both currently in phase III clinical trials. *TDENV-PIV* is a purified inactivated vaccine; it is the only candidate which is currently in phase II clinical trial. The candidates which are undergoing phase I and preclinical trials are listed and briefly described in Tables 1 and 2, respectively.[49,50]

3.1 | Tetravalent dengue vaccine—DENVax

TDV (Tetravalent Dengue Vaccine) or DENVax is a live attenuated chimeric vaccine manufactured by Takeda Vaccines (Singapore). It is based on the backbone of the live attenuated dengue serotype 2 viral strain DENV-2 PDK53,[51] which was first tested as a monovalent vaccine in two small phase I clinical trials enrolling 10 adult volunteers each. In these trials, the live attenuated vaccine was found to be safe, with no reported severe adverse reactions, as well as highly immunogenic, with neutralizing antibodies persisting for at least 12-15 months.[52,53] DENV-2 PDK53 has been combined with the pre-membrane and envelope genes from DENV-1, DENV-3, and DENV-4, to create TDV, a tetravalent formulation made up of four different chimeric viruses (Figure 1B).[54]

TDV has been administered to dengue naïve individuals in phase I and II clinical trials, enrolling 96 and 148 volunteers, respectively. The vaccine candidate was well tolerated by recipients, who only manifested mild adverse events such as injection site pain, itching, and erythema. In these trials, TDV elicited seroconversion for all four serotypes in 62% of the cases, for three or more serotypes in 96% of the cases, with the strongest immune response against DENV-2 (>95% seropositivity after two doses) and the poorest against DENV-4 (87.5% seropositivity after two doses).[55,56]

This vaccine candidate is currently undergoing a large phase III clinical trial. This is a double-blind, multicenter, controlled, randomized clinical trial which aims to enroll over 20 000 children from 4 to 16 years of age in Asia and Central/South America. Its primary objective is to verify the vaccine efficacy of two doses of TDV in preventing virologically confirmed dengue. Primary efficacy data published in November 2019 showed encouraging results, with 80.2% overall vaccine efficacy, 95.4% efficacy in preventing severe forms of dengue, and 74.9% efficacy in dengue seronegative patients.[57] One secondary objective is to assess the long-term safety of the vaccine (by analyzing the percentage of participants and severity of solicited adverse events), with results expected by 2021.[58]

3.2 | TV003/TV005—LAVΔ30

TV003/TV005 also known as LAVΔ30 or TetraVax-DV is a live attenuated tetravalent vaccine candidate for dengue developed by the U.S. National Institute of Health (NIH). It was generated by using three wild-type dengue viral strains attenuated through the deletion of a series of 30/31 nucleotides from the 3′ untranslated region: rDENV1Δ30, rDENV3Δ30/31, and rDENV4Δ30. As rDENV2Δ30 did not result in a sufficiently attenuated strain, the DENV-2 component of this vaccine was obtained by substituting prM and E genes from DENV-2 into the rDENV4Δ30 backbone to generate the chimeric strain rDENV2/4Δ30 (Figure 1C). TV003 and TV005 are two different vaccine formulations, which only differ in the dose for the DENV2 component.[59]

Seven phase I and II clinical trials were conducted to verify the safety and efficacy of TV003/TV005. The vaccine candidate had an acceptable safety profile, the most common adverse event being a mild maculo-papular rash in 61%-79% of the recipients. The rash was classified as mild and asymptomatic. Less common adverse events were mild and temporary neutropenia and raised alanine transaminase serum levels. Tetravalent and trivalent antibody responses were detected in 74% and 92% of the cases, respectively, after a single dose. A two-dose regimen of administration was excluded, as it did not solicit any significant antibody boost.[60,61]

TABLE 1 Phase I vaccine candidates

Vaccine type	Candidate name	Features
DNA vaccine	D1ME[100]	Circular, double-stranded, monovalent DNA plasmid that expresses prM and E genes from DENV-1. Pre-clinical studies have shown that monovalent dengue DNA vaccine candidates are able to elicit a solid immune response in mice and NHP[26–28]; DENV-1 DNA vaccine in particular provided 80%-95% protection during dengue live virus challenge in apes.[29] Given these results, D1ME[100] has been tested in a phase I clinical trial to assess its safety and reactogenicity in humans. The 22 participants to the trial were randomized in two groups to receive 1.0 mg or 5.0 mg of D1ME[100] vaccine. The vaccine was found to be safe; however, the results were overall disappointing, because anti-dengue neutralizing antibodies were detected in 0% of the recipients in the low-dose group and only 41.6% of the recipients in the high-dose group. By contrast, IFN-γ T cell response was far more significant, with 50%-83% of individuals responding.[30]
	TVDV - TVDV[VAX]	Tetravalent DNA vaccine that comprises four distinct monovalent DNA plasmids, encoding prM and E genes from the four serotypes. Combination with the adjuvant Vaxfectin stimulates a stronger immune response. Pre-clinical trials on NHP showed an improved immune response comparing to that of D1ME[100], along with a robust protection in a DENV-2 live virus challenge.[31] TVDV[VAX] was well-tolerated in a phase I trial, but immunogenicity remained poor, as only 10% of the study participants manifested a tetravalent antibody response. As seen in D1ME[100], TVDV[VAX] stimulates a pronounced T cell IFN-γ response in a dose-related manner.[32] Although these first attempts to develop a DNA vaccine for dengue might have been discouraging, this technique still has potential and should be developed further. New DNA vaccine delivery strategies, such as electroporation, are being evaluated at present as potential ways to promote a targeted immune response.[33]
Subunit vaccine	DEN-80E - V180	Tetravalent subunit vaccine that focuses on the expression of dengue envelope (E) genes from each of the four serotypes. The recombinant structural proteins are truncated at the carboxy-terminal portion of E (DEN-80E), and they are produced through Drosophila S2 cell expression system, which allows to obtain high-quality proteins that maintain a native-like conformation.[34] After showing promising preclinical data, DEN-80E underwent the first-in-man phase I clinical trial, in which dengue-naïve individuals were randomized to receive the pure vaccine, formulations with a variety of adjuvants or placebo. All the formulations were well-tolerated and ISCOMATRIX formulated DEN-80E demonstrated the strongest immunogenicity (tetravalent responses were observed in 71-88% after receiving two doses), although it was more often related to mild AEs. In all formulations, neutralizing antibody titers waned after 6 months.[35]
Heterologous prime/boost	TLAV prime / TPIV boost	This strategy involves the use of two phase II vaccine candidates: the live-attenuated vaccine TDENV-LAV and the purified inactivated vaccine TDENV-PIV. TLAV is used to prime the study participants and TPIV serves as a booster, or vice versa. Using multiple types of vaccines may help to overcome the critical issues of a single category. Indeed, a combined approach allows to achieve more balanced and solid immune response.[36] TLAV/TPIV and vice versa combinations are currently being tested in different regimens of administration in two phase I clinical trials.[37,38]

DENV-2 manifested the poorest rate of seroconversion (76%), in comparison with the other serotypes (92% for DENV-1, 97% for DENV-3, 100% for DENV-4); for this reason, it appeared necessary to verify its immunogenicity. Immunogenicity of TV003 was tested in a dengue human infection model which used the DENV-2 strain rDEN2Δ30 as a challenge virus. rDEN2Δ30 was administered to TV003 recipients 6 months after immunization and to dengue naïve subjects as a control; as a result, none of the vaccines manifested viremia or other clinical features, whereas the controls manifested viremia in 100%, rash in 80% and neutropenia in 20% of the cases. TV003 therefore has been proven to be highly protective against infection with rDEN2Δ30.[7,61]

TV003 has been licensed independently to different companies for different geographical regions; it has been tested in a phase I clinical trial with MSD and NIAID[62] and in a phase II clinical trial with Medigen Vaccine Biologics Corp.[63] TV003 has also been licensed by Butantan Institute in Brazil and is currently undergoing a double blind, controlled, multicenter, phase III randomized clinical trial which is expected to enroll 16 944 subject from 2 to 59 years of age. Participants receive a single dose of TV003 or placebo in a 2:1 ratio, and the primary objective is to compare the incidence of virologically confirmed dengue infections in the two study groups; results are expected in 2025.[64]

3.3 | Purified formalin-inactivated vaccine—TDENV-PIV

TDENV-PIV is a tetravalent purified inactivated vaccine developed by the Walter Reed Army Institute of Research and GlaxoSmithKline. It

TABLE 2 Preclinical vaccine candidates

Vaccine type	Candidate name	Features
Live-attenuated vaccines	DENV-1/2 chimeric virus	Mutant chimeric virus created by replacing DENV-1 prM and E genes in a live attenuated DENV-2 viral strain; enhanced prM cleavage apparently increases the virus specific infectivity.[39]
	HR-Tet	Tetravalent vaccine for dengue that comprises four viral strains carrying host-range (HR) mutations, generated by truncating a transmembrane segment of the envelope protein.[40]
	ChinDENV	Chimeric flavivirus based on the backbone of a Japanese encephalitis virus LAV, with the addition of prM and E structural genes from DENV-2; it elicited a strong immune response in NHP in a dose-related manner.[41]
Inactivated vaccine	Purified psoralen-inactivated virus	DENV-1 WP74 viral strain has been attenuated by using psoralen, a photoreactive compound that inactivates dengue virus after exposure to UVA radiation. The vaccine has been administered in a preclinical trial on NHP in combination with alum adjuvant.[42]
Subunit vaccine	cEDIII	Consensus envelope (E) domain III is a recombinant protein antigen that has been produced through the expression system of *E. coli*. The administration of cEDIII combined with alum adjuvant induces E-specific neutralizing antibodies in NHP.[43]
Heterologous prime/boost	YF17D-D2 prime / pE1D2 boost	YF17D-D2 (a chimeric yellow fever and DENV-2 live-attenuated vaccine) and pE1D2 (a DNA vaccine) are combined in different regimens of administration with the prime/boost strategy or simultaneously, to achieve a solid and balanced immune response.[44]
Virus like particles	VEE-VRP	Venezuelan equine encephalitis (VEE) virus replicon particle (VRP) hosts a recombinant genome that expresses the prME and E85 antigens of all four viral strains; it showed a rapid and effective seroconversion rate in mice and NHP.[45]
	DSV4	This non-infectious virus like particle (VLP) carries EDIII antigens from the four dengue serotypes; VLP vaccine platforms are a safe and inexpensive strategy to induce a protective immune response against DENV infections.[46]
	DENV-2 VLP	DENV-2 VLP is a monovalent candidate that expresses prM and E antigens; it aims to boost the immune response against DENV-2 after immunization with Dengvaxia, a live-attenuated vaccine that needs an improvement in the seroconversion rate against this viral serotype.[47]
Viral vector vaccine	MV-DEN	The envelope domain III (EDIII) antigens from the four dengue serotypes are delivered through a recombinant, live-attenuated measles vaccine (MV), which serves as a replicating viral vector.[48]

involves four viral strains which have been chemically inactivated through formalin. In this way, it is possible to suppress the infectivity of the viral strains, while at the same time maintaining their structure and antigenicity.[36]

The first-in-man, phase I clinical trial that tested a monovalent PIV candidate, showed that DENV-1 PIV has an acceptable safety profile—with only mild adverse events occurring such as minimal injection site reactions, and induced seroconversion in all of the study participants.[65] The high safety level of inactivated vaccines comes, however, with a lower immunogenicity compared to that of live attenuated vaccines; however, the immune response can be enhanced through co-administration with an adjuvant compound. Two phase I clinical trials were conducted to evaluate the safety and efficacy of the tetravalent formulation TDENV-PIV combined with different adjuvants: aluminum hydroxide, AS01E, or AS03B; all the formulations were well tolerated by the recipients and induced a balanced immune response against all of the four serotypes, with the highest mean antibody titers reached with AS01E and AS03B.[66,67] A phase II clinical trial is currently evaluating TDENV-PIV with AS03B to determine the most effective injection schedule (0-1, 0-1-6, or 0-3 months).[68]

4 | DISCUSSION

The burden of dengue has increased considerably over the last 50 years, and its incidence has risen 30-fold from the 1970s, turning it from a neglected tropical disease to a global public health concern.[2]

Despite decades of extensive studies, the ideal vaccine candidate for dengue has not been found yet, due to the multiple obstacles that render the development of a vaccine for this disease extremely challenging: insufficient knowledge of the dengue immunopathogenesis, lack of an adequate experimental model, need for a tetravalent immunity, and risk of enhanced disease.[2–4]

Dengvaxia is the only candidate that has been licensed so far; however, the severe limitations in its use highlight the demand for a more safe and versatile vaccine for dengue. Indeed, Dengvaxia can only be given to children older than 9 years of age, leaving younger

individuals uncovered. Children are at increased risk of developing dengue shock syndrome; therefore, it is essential that any vaccine against dengue should be efficacious and safe in both children and adults. Immunization with Dengvaxia may increase the risk of severe disease in dengue naïve individuals; therefore, testing the recipient's serostatus before administration of the vaccine is now recommended. This would make the roll out of a widespread vaccination campaign difficult and too costly for many endemic countries.[17]

TDV might be a viable alternative to Dengvaxia, due to its efficacy in preventing severe forms of dengue (95.4%) and its strong protective immune response against DENV-2,[54] whereas the usage of TV003/TV005 is likely to be hampered by the significant incidence of mild adverse events.[57,58]

However, live-attenuated vaccines tend to induce a strong and long-lasting immunogenicity; but, they are subject to viral interference, which can cause an imbalanced immune response and expose the recipients to a higher risk of contracting severe dengue. For several years, they were the main investigated vaccine platform; but, now a wide range of different dengue vaccination strategies are emerging, such as inactivated, DNA, or subunit vaccines.[22]

Clinical trials for TDENV-PIV, for the DNA vaccine TVDV^{VAX} [57,58] and for the subunit vaccine DEN80E[35] showed very encouraging results: although these novel techniques may be less immunogenic than live-attenuated vaccines, they manifest significantly lower viral serotype interference, along with a higher safety profile, possibly allowing the administration in infants and seronegative individuals as well.

The most appealing perspective for the future is the heterologous prime/boost approach, which combines different vaccine platforms (LAV with DNA/subunit/inactivated vaccines) to overcome one single vaccine category's drawbacks, to achieve a more balanced and solid protective immunity.[36,44]

Vaccines are certainly one fundamental resource in preventing the spread of dengue infection among the human population; however, given their current limited efficacy, it is necessary to implement diverse techniques to reduce the impact of this rapidly expanding disease.

Vector control programs remain the main way to prevent the transmission of dengue. The control of *Aedes* mosquito vectors is accomplished through a number of different techniques, which can be mainly divided into environmental, chemical, and biological methods. Environmental control consists in eliminating potential mosquito breeding sites and requires the education and involvement of community members. The implementation of preventive measures has been shown to be very effective in limiting mosquito propagation in urban areas.[69]

Chemical methods involve the development of new insecticide products and prevention of mosquito resistance to these substances. Traditionally, the use of insect repellents and insecticides has had limited effectiveness, as it is difficult to maintain these programs in large populations living in endemic countries. One emerging approach consists of creating textile fabric impregnated with long-lasting insecticides.[70] Biological control is an exciting and promising area, which involves the use of Wolbachia—an intracellular bacterium that lives in a symbiotic relationship with insects. Infecting female *Aedes* mosquitoes with Wolbachia reduces their ability to transmit dengue and other arboviruses.[71] The roll out of Wolbachia infected mosquitoes is underway in Vietnam, Indonesia, and parts of Australia and Brazil through the World Mosquito Program with encouraging results.[72] A different approach is the Release of Insects carrying Dominant Lethal genes (RIDL), which aims to create genetically modified male mosquitoes that diffuse infertility genes among the mosquito population.[73]

Although vector control strategies alone will not be sufficient, their integration with an efficient prophylactic vaccine is the key strategy to prevent the global spread of dengue.[5]

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ORCID

Stefano Di Bella https://orcid.org/0000-0001-6121-7009

REFERENCES

1. https://www.who.int/emergencies/ten-threats-to-global-health-in-2019
2. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature*. 2013;496:504-507.
3. Na W, Yeom M, Choi IK, Yook H, Song D. Animal models for dengue vaccine development and testing. *Clin Exp Vaccine Res*. 2017;6:104-110.
4. McArthur MA, Sztein MB, Edelman R. Dengue vaccines: recent developments, ongoing challenges and current candidates. *Expert Rev Vaccines*. 2013;12:933-953.
5. Tsai WY, Lin HE, Wang WK. Complexity of human antibody response to dengue virus: implication for vaccine development. *Front Microbiol*. 2017;8:1372.
6. Srikiatkhachorn A, Yoon IK. Immune correlates for dengue vaccine development. *Expert Rev Vaccines*. 2015;15:455-465.
7. Kirkpatrick BD, Durbin AP, Pierce KK, et al. Robust and balanced immune responses to all 4 dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, Flavivirus-naive adults. *J Infect Dis*. 2015;212:702-710.
8. World Health Organization. *Special Programme for Research and Training in Tropical Diseases. Dengue: guidelines for diagnosis, treatment, prevention and control: new edition*. Geneva: *WHO*; 2009:14-16.
9. Guzman MG, Harris E. Dengue. *Lancet*. 2015;385:453-465.
10. Mongkolsapaya J, Dejnirattisai W, Xu XN, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat Med*. 2003;9:921-927.
11. Shrivastava A, Tripathi NK, Dash PK, Parida M. Working towards dengue as a vaccine-preventable disease: challenges and opportunities. *Expert Opin Biol Ther*. 2017;17:1193-1199.
12. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine*. 2011;29:7229-7241.
13. Chambers TJ, Nestorowicz A, Mason PW, Rice CM. Yellow fever/Japanese encephalitis chimeric viruses: construction and biological properties. *J Virol*. 1999;73:3095-3101.
14. Morrison D, Legg TJ, Billings CW, Forrat R, Yoksan S, Lang J. A novel tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in flavivirus-naive adults. *J Infect Dis*. 2010;201:370-377.

15. Capeding RZ, Luna IA, Bomasang E, et al. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in The Philippines. *Vaccine*. 2011;29:3863-3872.
16. Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomized, controlled phase 2b trial. *Lancet*. 2012;380:1559-1567.
17. Capeding MR, Tran NH, Hadinegoro SRS, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer- masked, placebo-controlled trial. *Lancet*. 2014;384:1358-1365.
18. Hadinegoro SR, Arredondo-Garcìa JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med*. 2015;373:1195-1206.
19. Villar L, Dayan GH, Arredondo-García JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med*. 2015; 372:113-123.
20. Halstead SB, Russell PK. Protective and immunological behavior of chimeric yellow fever dengue vaccine. *Vaccine*. 2016;34:1643-1647.
21. World Health Organization. Dengue vaccine: WHO position paper – September 2018. *Weekly epidemiological record*. Vol 36. Geneva, Switzerland: *WHO*; 2018:457-476.
22. ClinicalTrials.gov. Identifier: NCT02948933. Post-Authorization Safety Study: Cohort Event Monitoring for Dengvaxia®, CYD-TDV Dengue Vaccine.
23. ClinicalTrials.gov. Identifier: NCT03803618. Effectiveness of the Tetravalent Dengue Vaccine, CYD-TDV (Dengvaxia®) in the Philippines.
24. ClinicalTrials.gov. Identifier: NCT03465254. Effect of Baseline Dengue Serostatus Among Tetravalent Dengue Vaccine CYDTDV (Dengvaxia®) Recipients on Subsequent Virologically Confirmed Dengue in the Philippines.
25. Bos S, Gadea G, Despres P. Dengue: a growing threat requiring vaccine development for disease prevention. *Pathog Global Health*. 2018; 112:1-12.
26. Raviprakash K, Ewing D, Simmons M, et al. Needle-free Biojector injection of a dengue virus type 1 DNA vaccine with human immunostimulatory sequences and the GM-CSF gene increases immunogenicity and protection from virus challenge in Aotus monkeys. *Virology*. 2003;315:345-352.
27. Putnak R, Fuller J, Vander Zanden L, Innis BL, Vaughn DW. Vaccination of rhesus macaques against dengue-2 virus with a plasmid DNA vaccine encoding the viral pre-membrane and envelope genes. *Am J Trop Med Hyg*. 2003;68:469-476.
28. Blair PJ, Kochel TJ, Raviprakash K, et al. Evaluation of immunity and protective efficacy of a dengue-3 pre-membrane and envelope DNA vaccine in Aotus nancymae monkeys. *Vaccine*. 2006;24:1427-1432.
29. Kochel TJ, Raviprakash K, Hayes CG, et al. A dengue virus serotype-1 DNA vaccine induces virus neutralizing antibodies and provides protection from viral challenge in Aotus monkeys. *Vaccine*. 2000;18: 3166-3173.
30. Beckett CG, Tjaden J, Burgess T, et al. Evaluation of a prototype dengue-1 DNA vaccine in a phase 1 clinical trial. *Vaccine*. 2011;29: 960-968.
31. Porter KR, Ewing D, Chen L, et al. Immunogenicity and protective efficacy of a vaxfectin-adjuvanted tetravalent dengue DNA vaccine. *Vaccine*. 2012;30:336-341.2.
32. Danko JR, Kochel T, Teneza-Mora N, et al. Safety and immunogenicity of a tetravalent dengue DNA vaccine administered with a cationic lipid-based adjuvant in a phase 1 clinical trial. *Am J Trop Med Hyg*. 2018;98:849-856.
33. Prompetchara E, Ketloy C, Keelapang P, Sittisombut N, Ruxrungtham K. Induction of neutralizing antibody response against four dengue viruses in mice by intramuscular electroporation of tetravalent DNA vaccines. *PLoS One*. 2014;9:e92643. https://doi.org/10.1371/journal.pone.0092643.
34. Manoff SB, George SL, Bett AJ, et al. Preclinical and clinical development of a dengue recombinant subunit vaccine. *Vaccine*. 2015;33: 7126-7134.
35. Manoff SB, Sausser M, Falk Russell A, et al. Immunogenicity and safety of an investigational tetravalent recombinant subunit vaccine for dengue: results of a phase I randomized clinical trial in flavivirus-Naïve adults. *Hum Vaccines Immunother*. 2018;3:1-10.
36. Simmons M, Burgess T, Lynch J, Putnak R. Protection against dengue virus by non-replicating and live attenuated vaccines used together in a prime boost vaccination strategy. *Virology*. 2010;396: 280-288.
37. ClinicalTrials.gov. Identifier:NCT02239614. A Phase 1, Randomized, Open-label, Single-center, Study of TDENV-PIV and LAV Dengue Vaccine Platforms as Part of a Heterologous Prime-boost Strategy in Healthy Adults in a Nonendemic Region.
38. ClinicalTrials.gov. Identifier:NCT03141138. A Phase 1, Randomized, Open-label, Single-center, Comparison of Heterologous Prime-Boost Vaccination Schedules of Tetravalent Dengue Virus Purified Inactivated Vaccine (PIV) and Tetravalent Dengue Virus Live Attenuated Vaccine (LAV) in Healthy Adults in a Nonendemic Region.
39. Keelapang P, Nitatpattana N, Suphatrakul A, et al. Generation and preclinical evaluation of a DENV-1/2 prM + E chimeric live attenuated vaccine candidate with enhanced prM cleavage. *Vaccine*. 2013; 31:5134-5140.
40. Briggs CM, Smith KM, Piper A, et al. Live attenuated tetravalent dengue virus host range vaccine is immunogenic in African green monkeys following a single vaccination. *J Virol*. 2014;88:6729-6742.
41. Li XF, Deng YQ, Yang HQ, et al. A chimeric dengue virus vaccine using Japanese encephalitis virus vaccine strain SA14-14-2 as backbone is immunogenic and protective against either parental virus in mice and nonhuman primates. *J Virol*. 2013;87:13694-13705.
42. Maves RC, Oré RMC, Porter KR, Kochel TJ. Immunogenicity and protective efficacy of a psoralen-inactivated dengue-1 virus vaccine candidate in *Aotus nancymaae* monkeys. *Vaccine*. 2011;29:2691-2696.
43. Chen HW, Liu SJ, Li YS, et al. A consensus envelope protein domain III can induce neutralizing antibody responses against serotype 2 of dengue virus in non-human primates. *Arch Virol*. 2013;158:1523-1531.
44. Azevedo AS, Goncalves AJS, Archer M, Freire MS, Galler R, Alves AMB. The synergistic effect of combined immunization with a DNA vaccine and chimeric yellow fever/dengue virus leads to strong protection against dengue. *PLoS One* 2013;8:1-10. doi: https://doi.org/10.1371/journal.pone.0058357
45. White LJ, Sariol CA, Mattocks MD, et al. An alphavirus vector-based tetravalent dengue vaccine induces a rapid and protective immune response in macaques that differs qualitatively from immunity induced by live virus infection. *J Virol*. 2013;87:3409-3424.
46. Ramasamy V, Arora U, Shukla R, et al. A tetravalent virus-like particle vaccine designed to display domain III of dengue envelope proteins induces multi-serotype neutralizing antibodies in mice and macaques which confer protection against antibody dependent enhancement in AG129 mice. *PLoS Neglected Trop Dis*. 2018;12:e0006191. https://doi.org/10.1371/journal.pntd.0006191.
47. Suphatrakul A, Yasanga T, Keelapang P, et al. Generation and preclinical immunogenicity study of dengue type 2 virus-like particles derived from stably transfected mosquito cells. *Vaccine*. 2015;33:5613-5622.
48. Brandler S, Ruffie C, Najburg V, et al. Pediatric measles vaccine expressing a dengue tetravalent antigen elicits neutralizing antibodies against all four dengue viruses. *Vaccine*. 2010;28:6730-6739.
49. Khetarpal N, Khanna I. Dengue fever: causes, complications, and vaccine strategies. *J Immunol Res*. 2016;2016:1-14. https://doi.org/10.1155/2016/6803098.

50. Prompetchara E, Ketloy C, Thomas SJ, Ruxrungtham K. Dengue vaccine: global development update. *Asian Pac J Allergy Immunol*. 2019. https://doi.org/10.12932/AP-100518-0309.
51. Butrapet S, Huang CYH, Pierro DJ, Bhamarapravati N, Gubler DJ, Kinney RM. Attenuation markers of a candidate dengue type 2 vaccine virus, strain 16681 (PDK-53), are defined by mutations in the 5′ noncoding region and nonstructural proteins 1 and 3. *J Virol*. 2000; 74:3011-3019.
52. Bhamarapravati N, Yoksan S, Chayaniyayothin T, Angsubphakorn S, Bunyaratvej A. Immunization with a live attenuated dengue-2-virus candidate vaccine (16681- PDK 53): clinical, immunological and biological responses in adult volunteers. *Bulletin WHO*. 1987;65: 189-195.
53. Vaughn DW, Hoke CH Jr, Yoksan S, et al. Testing of a dengue 2 live-attenuated vaccine (strain 16681 PDK 53) in ten American volunteers. *Vaccine*. 1996;14:329-336.
54. Osorio JE, Brewoo JN, Powell TD, et al. Efficacy of a tetravalent chimeric dengue vaccine (DENVax) in cynomolgus macaques. *Am J Trop Med Hyg*. 2011;84:978-987.
55. Osorio JE, Velez ID, Thomson C, et al. Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study. *Lancet Infect Dis*. 2014;14:830-838.
56. Sirivichayakul C, Barranco-Santana EA, Esquilin-Rivera I, et al. Safety and immunogenicity of a tetravalent dengue vaccine candidate in healthy children and adults in dengue-endemic regions: a randomized, placebo-controlled phase 2 study. *J Infect Dis*. 2016;213:1562-1572.
57. Biswal S, Reynales H, Saez-Llorens X, et al. Efficacy of a tetravalent dengue vaccine in healthy children and adolescents. *N Engl J Med*. 2019;381:2009-2019.
58. ClinicalTrials.gov. Identifier: NCT02747927. Efficacy, Safety and Immunogenicity of Takeda's Tetravalent Dengue Vaccine (TDV) in Healthy Children (TIDES).
59. Whitehead SS, Falgout B, Hanley KA, Blaney JE Jr, Markoff L, Murphy BR. A live, attenuated dengue virus type 1 vaccine candidate with a 30-nucleotide deletion in the 3′ untranslated region is highly attenuated and immunogenic in monkeys. *J Virol*. 2003;77:1653-1657.
60. Kirkpatrick BD, Whitehead SS, Pierce KK, et al. The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. *Sci Transl Med*. 2016;8:330-336.
61. Larsen CP, Whitehead SS, Durbin AP. Dengue human infection models to advance dengue vaccine development. *Vaccine*. 2015;33: 7075-7082.
62. ClinicalTrials.gov. Identifier: NCT02450838. A Phase I Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Immunogenicity of a Tetravalent Recombinant Subunit Dengue Vaccine (V180) in Healthy Adults Who Previously Received a Live-Attenuated Tetravalent Vaccine (TV003 or TV005)
63. ClinicalTrials.gov. Identifier: NCT03485144. A Phase II Randomized, Double-blinded, Placebo-controlled Clinical Study to Evaluate the Immunogenicity and Safety of TetraVax-DV in Healthy Adults in Taiwan.
64. ClinicalTrials.gov. Identifier: NCT02406729. Phase III Trial to Evaluate Efficacy and Safety of a Tetravalent Dengue Vaccine.
65. Martinez LJ, Lin L, Blaylock JM, et al. Safety and immunogenicity of a dengue virus serotype-1 purified-inactivated vaccine: results of a phase 1 clinical trial. *Am J Trop Med Hyg*. 2015;93:454-460.
66. Schmidt AC, Lin L, Martinez LJ, et al. Phase 1 randomized study of a tetravalent dengue purified inactivated vaccine in healthy adults in the United States. *Am J Trop Med Hyg*. 2017;96:1325-1337.
67. Diaz C, Lin L, Martinez LJ, et al. Phase I randomized study of a tetravalent dengue purified inactivated vaccine in healthy adults from Puerto Rico. *Am J Trop Med Hyg*. 2018;98:1435-1443.
68. ClinicalTrials.gov. Identifier: NCT02421367. Study of Varying Injection Schedules of TDENV-PIV Vaccine With AS03B Adjuvant and Placebo in Healthy US Adults.
69. Louis VR, Montenegro Quiñonez CA, Kusumawathie P, et al. Characteristics of and factors associated with dengue vector breeding sites in the City of Colombo, Sri Lanka. *Pathog Glob Health*. 2016;110: 79-86.
70. Kittayapong P, Olanratmanee P, Maskhao P, Byass P, Logan J, Tozan Y, Louis V, Gubler DJ, Wilder-Smith A. Mitigating diseases transmitted by aedes mosquitoes: a cluster-randomised trial of permethrin-impregnated school uniforms. *PLoS Negl Trop Dis* 2017;11 (1):1-12.
71. Ndii M, Allingham D, Hickson R, Glass K. The effect of Wolbachia on dengue outbreaks when dengue is repeatedly introduced. *Theor Popul Biol*. 2016;111:9-15.
72. O'Neill SL, Ryan PA, Turley AP, et al. Scaled deployment of Wolbachia to protect the community from dengue and other *Aedes* transmitted arboviruses. *Gates Open Res*. 2019;2:36.
73. Qsim M, Ashfaq UA, Zubair Yousaf M, et al. Genetically modified *Aedes aegypti* to control dengue: a review. *Crit Rev Eukaryot Gene Expr*. 2017;27:331-340.