Rev Saúde Pública 2015;49:60

Comentário

DOI:10.1590/S0034-8910.2015049006146

Emiliana Pereira Abrão

Danillo Lucas Alves Espósito

Flávio Lauretti

Benedito Antonio Lopes da Fonseca

Dengue vaccines: what we know, what has been done, but what does the future hold?

ABSTRACT

Dengue, a disease caused by any of the four serotypes of dengue viruses, is the most important arthropod-borne viral disease in the world in terms of both morbidity and mortality. The infection by these viruses induces a plethora of clinical manifestations ranging from asymptomatic infections to severe diseases with involvement of several organs. Severe forms of the disease are more frequent in secondary infections by distinct serotypes and, consequently, a dengue vaccine must be tetravalent. Although several approaches have been used on the vaccine development, no vaccine is available against these viruses, especially because of problems on the development of a tetravalent vaccine. Here, we describe briefly the vaccine candidates available and their ability to elicit a protective immune response. We also discuss the problems and possibilities of any of the vaccines in final development stage reaching the market for human use.

DESCRIPTORS: Dengue Vaccines. Dengue, prevention & control. Scientific Research and Technological Development. Communicable Disease Control.

Departamento de Clínica Médica. Faculdade de Medicina de Ribeirão Preto. Universidade de São Paulo. Ribeirão Preto, SP, Brasil

Correspondence:
Benedito Antonio Lopes da Fonseca
Departamento de Clínica Médica – FMRP/USP
Avenida dos Bandeirantes, 3900
14049-900 Ribeirão Preto, SP, Brasil
E-mail: baldfons@fmrp.usp.br

Received: 1/24/2015
Approved: 3/26/2015

INTRODUCTION

Dengue is an acute febrile illness caused by the infection with any of dengue viruses. These arthropod-borne viruses belong to the genus *Flavivirus* from the family Flaviviridae, and are serologically classified into four distinct serotypes, dengue-1 to -4 (DENV-1 to DENV-4). These viruses are transmitted to humans by the bite of infected females of *Aedes* mosquitos, mainly by *Aedes aegypti*,[35] and generate a lifelong protective immune response against homologous dengue strains and transient cross-protection against the other serotypes for a few months after the primary infection.[35]

Dengue viruses cause an important and rapidly spreading arboviral disease and are widely distributed in tropical and subtropical regions of the world.[2] Dengue is endemic in more than 100 countries including Southeast Asia, South and Central America, the Caribbean and South Pacific regions and, among them, more than 60 countries have now reported dengue hemorrhagic fever (DHF).[35] The World Health Organization (WHO) estimates that 50 to 100 million dengue infections occur annually and that approximately 2.5 billion people live in endemic areas where 500,000 to 1,000,000 dengue infections evolve to DHF/dengue shock syndrome (DSS), resulting in approximately 25,000 deaths.[35] However, these numbers could be underestimated, and this hypothesis has been corroborated by a recent study by Bhatt et al,[2] who used a formal modeling framework and suggest that approximately 390 million humans are infected every year, more than three times the previously reported by WHO.

A considerable portion of dengue virus infections result in asymptomatic infections, but also in clinical manifestations that include mild undifferentiated fever, dengue fever, and many more severe disease manifestations, the most studied one being DHF/DSS, in which the increased vascular permeability is the hallmark.[35]

Up to now, no animal model has allowed to conclude which are the precise mechanisms involved in the pathogenesis of dengue virus infection. Therefore, the pathophysiologic processes on the causation of severe dengue infections are not completely understood in spite of several studies that have addressed this issue. Several hypotheses that include mechanisms involving both viral and host factors have been proposed, such as the virulence of different virus strains,[24] host genetics,[5,27] the multifactorial interaction,[17] the immune-mediated mechanisms,[4,8] and the antibody-dependent enhancement.[14] The latter phenomenon, mainly observed in epidemiological studies, may have an important role in the pathogenesis of DHF/DSS and is related to an enhancement of the disease severity after a secondary infection. The non-neutralizing heterotypic antibodies from a first infection enhance the viral entry into Fc gamma receptor (FcγR)-bearing cells, thus enhancing the viral load and consequently increasing the immune response.[13,29]

Currently, there is no specific drug for dengue treatment, and mosquito control is mandatory to contain the disease in affected countries. In this context, a preventive dengue vaccine is essential to control the disease and has been a priority for the WHO for several decades. The challenges to vaccine development lay on the complex immunopathogenesis of the disease, especially the antibody-dependent enhancement of infection phenomenon, requiring the development of tetravalent vaccines. These vaccines should provide long-term protection against all virus serotypes, an essential requirement to vaccine approval by regulatory agencies.[35] Dengue vaccine research ranges from traditional methods of virus inactivation to advanced molecular biology strategies. Here we discuss these strategies briefly and point the problems with each one and the perspectives for a licensed dengue vaccine.

Dengue vaccine candidates under development and on clinical trials

DNA vaccines

In recent decades, DNA vaccination has been used to induce cellular immune responses against various antigens, using *in vitro* or animal models, some of them being evaluated on clinical trials. This technology is based on cloning a specific fragment or gene into a bacterial plasmid containing a strong promoter for optimal expression in mammalian cells. Several preclinical DNA vaccine candidates were engineered against DENV but, up to this present publication, only two have been approved to be tested in humans.

A monovalent plasmid DNA vaccine against DENV-1 ($D1ME^{100}$) that encodes the premembrane (prM) and envelope (E) proteins was tested in 22 healthy adults by intramuscular injection. Phase 1 trial results showed this vaccine candidate to be safe, but only five subjects that received higher doses (5 mg) had detectable neutralizing antibody responses (about 20.0%). An increase in IFN-γ expression and DENV-1 serotype cross-reactivity immune responses to DENV-2, -3 and -4 were also reported.[1]

Another DNA vaccine candidate approved for phase I of clinical trial, produced by the U.S. Naval Medical Research Center and Vical Incorporated, is a Tetravalent DENV Vaccine (TVDV) that also encodes prM and E genes, but from all serotypes (1-4), in a backbone plasmid VR1012 (Vical Incorporated). Preclinical studies immunizing Indian rhesus monkeys with this tetravalent vaccine associated with Vaxfectin® adjuvant (Vical Incorporated) showed lower viremia in animals

inoculated with the TVDV + Vaxfectin® when challenged with live DENV-2 than control animals. The authors also reported a 2- to 10-fold increase in the neutralizing antibody response against DENV-1, -3 and -4.[22]

Although DNA vaccines seem to be an excellent approach to the development of dengue vaccines, the tests with these vaccine candidates are in their infancy, compared with other strategies.

Live attenuated and inactivated virus vaccines

Live attenuated virus vaccines have been successfully developed for other viruses and this strategy has also been used to develop a dengue vaccine candidate. These vaccines, made of weakened viruses, are excellent immunogens because they can induce humoral and cellular immune responses similar to a natural infection, but viral replication is insufficient to produce disease. The attenuated vaccine for yellow fever virus, another virus from the genus *Flavivirus*, is one of the most effective available in the market.[23] The production of an attenuated vaccine against dengue virus was not as successful as for yellow fever virus. The Walter Reed Army Institute of Research (WRAIR) and GlaxoSmithKline (GSK) have infected primary dog kidney (PDK) cells with all four DENV serotypes to produce a tetravalent DENV vaccine expecting these vaccines to produce low viremia in recipients.[33] The vaccine, which is in clinical phase II, was tested in flavivirus-naive Thai adults and showed to be safe and immunogenic. After two immunization doses, almost all subjects became seropositive to all DENV types.[33] This approach, tested in adults and infants (by WRAIR and Mahidol University, respectively), did not show the same seroconversion success.[28,32]

On the other hand, inactivated vaccines are made from viruses that become noninfectious by heating or formaldehyde inactivation. They are safer than attenuated virus candidates because of the inability of reversion to virulence and do not represent any harm to immunocompromised individuals. A tetravalent dengue inactivated vaccine (DPIV), developed by Fiocruz (Brazil) in collaboration with GSK and WRAIR, is on phase I clinical trial, and showed to be protective and immunogenic in nonhuman primates in a preclinical test.[11]

Recombinant protein vaccines

Recombinant subunit vaccines are widely developed for several pathogens because of their safety profile, but they do not produce the same immunological responses to pathogens as live attenuated vaccines since they contain only one or few viral proteins. The DENV envelope protein is the most immunogenic and generally used for vaccine production.[7] Hawaii Biotech/Merck developed a subunit vaccine candidate against all dengue serotypes containing a truncated E protein (DEN-80E) expressed in Drosophila S2 cells.[9] Preclinical results showed an induction of high-titer neutralizing antibodies in both mice and nonhuman primates when administered with a specific adjuvant (ISCOMATRIX™ – CSL).[9] This vaccine is currently in phase I clinical trial in healthy young Australian adults, and three doses will be administered at monthly intervals.

The chimeric dengue virus vaccines

The yellow fever/dengue virus (CYD) vaccine is the one in the most advanced stage on dengue vaccine development, being evaluated on large-scale clinical trials around the world and tested in more than 10,000 children in five Southeast Asian countries and in more than 20,000 subjects in five countries in Latin America, including Brazil.[20] This vaccine candidate is based on a yellow fever virus backbone (vaccine strain 17D) with the yellow fever virus prM and E genes replaced by DENV type-specific prM and E genes.

The results of the phase IIb clinical trial that took place in Thailand were released recently.[26] In this study, 4,002 healthy Thai schoolchildren aged 4 to 11 years were randomly assigned to receive three injections of dengue vaccine or control (rabies vaccine or placebo) at zero, six and 12 months to assess its protective efficacy against virologically confirmed, symptomatic dengue occurring one month or longer after the third dose. Dengue disease occurred in 134 children during the study. Overall efficacy for all four serotypes was 30.2%, but only 9.0% for DENV-2, although neutralizing antibodies, measured shortly after the last doses, had relatively high mean titers.

The most recent results, obtained in a phase III study carried out in the Asia-Pacific region that evaluated 10,275 healthy children aged 2 to 14 years, were consistent with the phase IIb in Thailand, and the overall efficacy was 56.5%, but only 35.0% for DENV-2.[6] Perhaps the best news of this vaccine was a decrease in cases of dengue hemorrhagic fever, the most severe form of the disease, by 88.5%. Of greater concern is the relative vaccine inefficacy in dengue virus-naive participants (35.5%), suggesting that this vaccine boosts and broadens preexisting immunity rather than primarily raising protective immunity, which might also explain the better efficacy in older children exposed to the virus.[34] Another issue of concern is the lower efficacy against DENV-2 disease, although it is hard to explain why the presence of specific neutralizing antibodies is unprotective against dengue. Viral interference poses a probable problem in live tetravalent formulations resulting in an unequal immune response to each of the four serotypes.[15]

It had already been observed in early preclinical studies with CYD vaccine in monkeys that the simultaneous administration of tetravalent formulations produces a hierarchy of immune responses, with DENV-4 showing

the best and DENV-2 the worst ones.[12] Also, the concept that the infection with one serotype will confer lifelong protection to subsequent infections against serotypes from different regions of the world is under scrutiny. Most cohort studies on this concept were done in areas where each serotype is represented by a single circulating genotype and the role of genotypic variation of a serotype in repeated infections is less certain.[31]

Sabchareon et al[26] hypothesized that the poor level of protection of CYD vaccine could be due to a mismatch between the vaccine strain and the genetically divergent circulating DENV-2 Asian strain. Studies with mouse and human convalescent sera show that the neutralizing activity against DENV-3 from different genotypes of the same serotype varies in the ability to neutralize the virus infection *in vitro*.[19,25,30] If true, this hypothesis has an enormous impact in future dengue vaccines, because instead of a universal vaccine, each vaccine candidate will have to be designed to specific circulating strains around the world.

Lastly, the lack of DENV T-cell immunity in CYD vaccine is the most likely reason for the lack of multitypic protective immunity.[15] Data from mouse models and recent studies in humans suggest that T-cell immunity contributes importantly to protection against infection by different serotypes of DENV. In this regard, the role of nonstructural proteins (NS) in eliciting cellular immunity is being considered in dengue vaccine approaches. Studies with DENV-infected patients or volunteers receiving candidate vaccines evidenced CD4+ and CD8+ T-cell epitopes throughout NS3.[18] It is plausible that vaccines presenting yellow fever virus but not DENV nonstructural protein antigens, such as the CYD vaccine, do not mount a strong and protective anti-DENV T-cell response.[15]

The chimeric live attenuated DENV-2/DENV vaccine (DENVax)

The DENVax was constructed using the backbone of the cell culture attenuated DENV-2 (PDK-53 strain), in which the prM and E genes of DENV-2 PDK-53 were substituted with those of wild-type DENV-1, -3, or -4.[16] DENVax is undergoing extensive safety and tolerability tests on phase I and II clinical trials in Colombia, Puerto Rico, Singapore and Thailand. In Colombia, 96 healthy individuals aged 18 to 45 years were assigned to receive two doses of tetravalent DENVax 90 days apart. Results show that the vaccine was well tolerated and immunogenic. Thirty days after a second dose, 62.0% of patients seroconverted to all four dengue serotypes.[21] The next steps will concentrate in the efficacy trials. Although promising results are expected, the lessons learned with Chimerivax vaccine draw our attention to the fact that protection against dengue disease should not rely solely in humoral immunity but also in the cellular immune responses. Thus, additional markers of protection such as T-cell activation, interferon-gamma (INF-g) and tumor necrosis factor-alpha (TNF-a) production should be used on immunogenicity assays.[15]

The chimeric live attenuated DENV/DENV vaccine (TetraVax-DV)

Another approach was used to produce a different tetravalent DENV vaccine (TetraVax-DV vaccine). Attenuation was achieved by a deletion of 30 nucleotides (Δ30) on 3' untranslated region of wild-type DENV-1 and -4. Since this approach did not result in attenuation for the other two viruses, DENV-2 chimeric vaccine candidate was generated by replacing prM and E genes on the DEN4Δ30 backbone with those of the wild-type viruses and DENV-3 vaccine candidate by additional deletion of the 3' untranslated region, respectively. These vaccine candidates retain wild-type structural proteins to maximize infectivity, thereby decreasing the potential for virus interference, and also to increase the magnitude and extension of the neutralizing antibody response.[3,7]

TetraVax has been tested for safety and immunogenicity in flavivirus-naive subjects in the United States. All vaccines were safe and immunogenic and one formulation was shown to induce tetravalent immune response in 79.0% of subjects after only a single dose.[7,10] The vaccine is being tested for its safety and immunogenicity profile in phase II Clinical Trial in several sites around the world.

CONCLUSION

As mentioned above, several candidate vaccines are undergoing clinical trials. Most of them elicit good profiles of immunogenicity and reactogenicity when administered as a single vaccine, but none have achieved a good percentage of protection against dengue infections when used as a tetravalent vaccine. The reasons for this low efficacy are the possible viral interference among virus strains combined in the vaccines, the uncertainty about the levels of protective titers of neutralizing antibodies, the possible lack of protection against strains different from those contained in the vaccines, and the evidence for the need of nonstructural proteins to elicit an adequate immune response. All candidate vaccines will have to deal with these questions before they are approved for human use. Finally, the vaccine candidate in the most advanced stage of development has just completed a phase III clinical trial and has induced an efficacy of a little less than 60.0%, and this efficacy has been achieved only after three doses of the vaccine. Even though this efficacy would result in a considerable reduction in the number of dengue cases, the questions are concerned to whom is going to use this vaccine and if there will be vaccines available for the whole world, in particular for developing countries, where dengue represents an important public health problem.

REFERENCES

1. Beckett CG, Tjaden J, Burgess T, Danko JR, Tamminga C, Simmons M, et al. Evaluation of a prototype dengue-1 DNA vaccine in a Phase 1 clinical trial. *Vaccine*. 2011;29(5):960-8. DOI:10.1016/j.vaccine.2010.11.050

2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature*. 2013;496(7446):504-7. DOI:10.1038/nature12060

3. Blaney JE Jr, Matro JM, Murphy BR, Whitehead SS. Recombinant, live-atenuated tetravalent dengue virus vaccine formulations induce a balanced, broad, and protective neutralizing antibody response against each of the four serotypes in rhesus monkeys. *J Virol*. 2005;79(9):5516-28. DOI:10.1128/JVI.79.9.5516-5528.2005

4. Bozza FA, Cruz OG, Zagne SMO, Azeredo EL, Nogueira RMR, Assis EF, et al. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC Infect Dis*. 2008;8:86. DOI:10.1186/1471-2334-8-86

5. Bravo JR, Guzmán MG, Kouri GP. Why dengue hemorrhagic fever in Cuba? 1. Individual risk factors for dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). *Trans R Soc Trop Med Hyg*. 1987;81(5):816-20.

6. Capeding MR, Tran NH, Hadinegoro SRS, Ismail HI, Chotpitayasunondh T, Chua MN, 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(9951):1358-65. DOI:10.1016/S0140-6736(14)61060-6

7. Cassetti MC, Halstead SB. Consultation on dengue vaccines: progress in understanding protection, 26-28 June, Rockville, Maryland. *Vaccine*. 2014;32(26):3115-21. DOI:10.1016/j.vaccine.2014.04.017

8. Chen LC, Lei HY, Liu CC, Shiesh SC, Chen SH, Liu HS, et al. Correlation of serum levels of macrophage migration inhibitory factor with disease severity and clinical outcome in dengue patients. *Am J Trop Med Hyg*. 2006;74(1):142-7.

9. Coller BA, Clements DE, Bett AJ, Sagar SL, Ter Meulen JH. The development of recombinant subunit envelope-based vaccines to protect against dengue virus induced disease. *Vaccine*. 2011;29(42):7267-75. DOI:10.1016/j.vaccine.2011.07.021

10. Durbin AP, Kirkpatrick BD, Pierce KK, Elwood D, Larsson CJ, Lindow JC, et al. A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive adults: a randomized, double-blind clinical trial. *J Infect Dis*. 2013;207(6):957-65. DOI:10.1093/infdis/jis936

11. Fernandez ST, Thomas SJ, Barrera R, Imerbsin R, Jarman RG, Baras B, et al. Immunogenicity and protection elicited by adjuvanted tetravalent dengue virus purified inactivated vaccine (TDENV PIV) in rhesus macaques [poster]. In: 61st Annual Meeting of the American Society of Tropical Medicine and Hygiene; 2012 Nov 11-15; Atlanta (GA), USA. Poster Session 128 - Poster Session C.

12. Guirakhoo F, Pugachev K, Arroyo J, Miller C, Zhang ZX, Weltzin R, et al. Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine: genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. *Virology*. 2002;298(1):146-59. DOI:10.1006/viro.2002.1462

13. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med*. 1970;42(5):311-28.

14. Halstead SB. Neutralization and antibody-dependent enhancement of dengue viruses. *Adv Virus Res*. 2003;60:421-67. DOI:10.1016/S0065-3527(03)60011-4

15. Halstead SB. Identifying protective dengue vaccines: guide to mastering an empirical process. *Vaccine*. 2013;31(41):4501-7. DOI:10.1016/j.vaccine.2013.06.079

16. Huang CYH, Butrapet S, Tsuchiya KR, Bhamarapravati N, Gubler DJ, Kinney RM. Dengue 2 PDK-53 virus as a chimeric carrier for tetravalent dengue vaccine development. *J. Virol*. 2003;77(21):11436-47. DOI:10.1128/JVI.77.21.11436-11447.2003

17. Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. *Postgrad Med J*. 2004;80(948):588-601. DOI:10.1136/pgmj.2004.019638

18. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. *Immunol Rev*. 2008;225:300-13. DOI:10.1111/j.1600-065X.2008.00678.x

19. Messer WB, Yount B, Hacker KE, Donaldson EF, Huynh JP, Silva AM, et al. Development and characterization of a reverse genetic system for studying dengue virus serotype 3 strain variation and neutralization. *PLoS Negl Trop Dis*. 2012;6(2):e1486. DOI:10.1371/journal.pntd.0001486

20. Normile D. Dengue vaccine trial poses public health quandary. *Science*. 2014;345(6195):367-8. DOI:10.1126/science.345.6195.367

21. Osorio JE, Velez ID, Thomson C, Lopez L, Jimenez A, Haller AA, 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(9):830-8. DOI:10.1016/S1473-3099(14)70811-4

22. Porter KR, Ewing D, Chen L, Wu SJ, Hayes CG, Ferrari M, et al. Immunogenicity and protective efficacy of a vaxfectin-adjuvanted tetravalent dengue DNA vaccine. *Vaccine*. 2012;30(2):336-41. DOI:10.1016/j.vaccine.2011.10.085

23. Pugachev KV, Guirakhoo F, Monath TP. New developments in flavivirus vaccines with special attention to yellow fever. *Curr Opin Infect Dis*. 2005;18:387-394.

24. Rico-Hesse R. Microevolution and virulence of dengue viruses. *Adv Virus Res*. 2003;59:315-41.

25. Russell PK, McCown JM. Comparison of dengue-2 and dengue-3 virus strains by neutralization tests and identification of a subtype of dengue-3. *Am J Trop Med Hyg*. 1972;21(2):97-9.

26. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet*. 2012;380(9853):1559-67. DOI:10.1016/S0140-6736(12)61428-7

27. Sierra BC, Kouri G, Guzman MG. Race: a risk factor for dengue hemorrhagic fever. *Arch Virol*. 2007;152(3):533-42. DOI:10.1007/s00705-006-0869-x

28. Sun W, Edelman R, Kanesa-Thasan N, Eckels KH, Putnak JR, King AD, et al. Vaccination of human volunteers with monovalent and tetravalent live-attenuated dengue vaccine candidates. *Am J Trop Med Hyg*. 2003;69(6 Suppl):24-31.

29. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitiya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis*. 2000;181(1):2-9. DOI:10.1086/315215

30. Wahala WM, Donaldson EF, Alwis R, Accavitti-Loper MA, Baric RS, Silva AM. Natural strain variation and antibody neutralization of dengue serotype 3 viruses. *PLoS Pathog*. 2010;6(3):e1000821. DOI:10.1371/journal.ppat.1000821

31. Wahala WM, Silva AM. The human antibody response to dengue virus infection. *Viruses*. 2011;3(12):2374-95. DOI:10.3390/v3122374

32. Watanaveeradej V, Simasathien S, Nisalak A, Endy TP, Jarman RG, Innis BL, et al. Safety and immunogenicity of a tetravalent live-attenuated dengue vaccine in flavivirus-naive infants. *Am J Trop Med Hyg*. 2011;85(2):341-51. DOI:10.4269/ajtmh.2011.10-0501

33. Watanaveeradej V, Gibbons RV, Simasathien S, Nisalak A, Jarman RG, Kerdpanich A, et al. Safety and immunogenicity of a rederived, live-attenuated dengue virus vaccine in healthy adults living in Thailand: a randomized trial. *Am J Trop Med Hyg*. 2014;91(1):119-28. DOI:10.4269/ajtmh.13-0452

34. Wilder-Smith A. Dengue vaccines: dawning at last? *Lancet*. 2014;384(9951):1327-9. DOI:10.1016/S0140-6736(14)61142-9

35. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New ed. Geneva; 2009.

The authors declare no conflict of interest.