Dengue Vaccine Development: The Role of the WHO South-East Asia Regional Office

World Health Organization
Regional Office for South-East Asia

SEA-DEN-9
Distribution: General

Dengue Vaccine Development: The Role of the WHO South-East Asia Regional Office

World Health Organization
Regional Office for South-East Asia

Printed in India

Contents

Page

Acknowledgements

This document was prepared by Sutee Yoksan of the Department of Pathology, Mahidol University at Salaya, Center for Vaccine Development, Institute of Science and Technology for Development, Nakornpathom, Thailand, and Duane J. Gubler,Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii.

History

A pandemic of dengue fever (DF) occurred in South-East Asia during and in the aftermath of World War II (Gubler, 1997). Both the virus and the mosquito vector spread over a large geographical area during the war due to the Allied and Japanese soldiers who traversed vast stretches of terrain. This left most countries in the Region with multiple dengue virus serotypes co-circulating (hyperendemicity) in the period after the war ended.

Subsequently, an economic boom in South-East Asia began in the 1950s and has more or less continued to this day. This has triggered a spurt in urbanization and demographic movement in most countries of the Region. Uncontrolled urbanization has created mega-cities where millions of people live in crowded conditions with poor housing, inadequate sewage and improper waste management. This, combined with a conspicuous lack of effective mosquito control, resulted in dramatically increased dengue transmission and the emergence of epidemic dengue haemorrhagic fever (DHF) in most countries of Asia (Gubler, 2006). Modern transportation and globalization spurred the spread of both the virus and the vector mosquito. By the early 1970s, DF/DHF had become an important tropical emerging infectious disease, and was one of the leading causes of hospitalization and death among children in South-East Asian countries (WHO, 1997). Epidemic DHF spread within Asia and to the Pacific in the 1970s and to the Americas in the 1980s.

There is no vaccine for DF/DHF. Prevention and control depends on controlling the principal vector mosquito, *Aedes aegypti*. Unfortunately, mosquito control has failed in most countries because of lack of resources and placing too much emphasis on adult mosquito control (Gubler, 2005). It was in this setting that dengue researchers and public health officials began to discuss the possibility of developing dengue vaccines in the early 1970s. The decision to support dengue vaccine research at that time (WHO, 1978) provided the stimulus that, 30 years later, has resulted in the development of several excellent candidate vaccines that were in pre-clinical, Phase I and Phase II trials in 2008 (Whitehead *et al.*, 2007). Although the development of dengue vaccines has been far more complicated, and progress has been much slower than originally anticipated, the current crop of candidate vaccines in the pipeline looks very promising.

History of the role of SEARO in dengue vaccine development

Early discussions on whether a dengue vaccine was a feasible method of preventing DHF were held in 1971 by the United States Armed Forces Epidemiology Board, which initiated a programme to develop a dengue vaccine in cooperation with the Department of Tropical Medicine and Microbiology, University of Hawaii at Mānoa in Honolulu (Halstead, 2003). This latter group, under the leadership of Scott B. Halstead, conducted the initial research, screening primary cells and cell lines that had been used to prepare licensed vaccines in the United States. Of the cells tested (WI-38 human fibroblasts, primary chick and duck embryo, kidney cells from rabbit, dog and African Green monkeys), only the primary dog kidney (PDK) and African Green monkey kidney (GMK) cells supported the growth of all four dengue serotypes (Halstead & Marchette, 2003).

Based on the progress of this early work by Halstead and his group(Halstead, *et al.*, 1984a-d; Halstead & Marchette, 2003) and the United States Army (Eckels *et al*. 1976; Harrison *et al*. 1977), the SEARO/WHO Research Study Group proposed in early February 1977 that vaccine research be initiated. The proposal was endorsed by the South-East Asia Regional Advisory Committee on Medical Research at their meeting in Colombo, Sri Lanka, later that same month (WHO, 1977). Further detailed discussions on dengue vaccine development took place at the "Conference on Dengue Haemorrhagic Fever: New Development and Future Research" in Singapore on 24-28 October 1977. At that meeting, it was suggested that field trials with candidate vaccines could be achieved in ten years (Halstead, 1978; Russell, 1978). A number of important research questions were identified as prerequisites to developing a dengue vaccine. The SEARO Steering Committee for the Research Study Group then met on 29-30 October 1977 following the Singapore meeting, and proposed a follow-up meeting at SEARO in February 1978 to make specific recommendations. The recommendation made by the US Armed Forces Epidemiology Board in 1971, that the first priority should be development of live attenuated candidate vaccine strains for all four dengue virus serotypes, was endorsed at this meeting (WHO, 1978). It was also proposed that to begin the programme, virologists from the South-East Asia and Western Pacific Regions be trained in the United States of America in research on the development of dengue vaccines, after which they should return to their respective countries and be directly involved in the dengue vaccine development programme. In order to shorten the period needed to develop a vaccine programme, they further recommended that there be an immediate transfer of technology as well as an already developed candidate dengue vaccine strain from the University of Hawaii,

for use in the Asia Pacific region (WHO, 1978). The meetings and participants involved in the early SEARO discussions and decisions are presented in Table 1.

It was this group of scientists, under the stewardship of SEARO and with the help of short-term consultants such as Scott B. Halstead and Philip K. Russell, that provided the initiative to develop the dengue WHO vaccine programme. The list of key WHO staff who were involved in this effort is presented in Table 2. It should be noted that the development of this research programme was almost exclusively a decision of the WHO Regional Office for South-East Asia in New Delhi (WHO, 1978). WHO headquarters in Geneva, while being involved throughout the duration of the programme, played only a nominal role both in terms of guidance and funding. The bulk of the funds to support the programme came from the SEARO research budget, the governments of Thailand and Italy, and from the Rockefeller Foundation (Table 3).

Development of the programme

Once the decision had been made by Dr V.T.H. Gunaratne, the Regional Director for South-East Asia at the time, to develop the dengue vaccine research programme, SEARO staff proceeded to select a country and institution in the Region most suited to develop the programme. The Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, in Bangkok, Thailand, was selected. Under the direction of Professor Natth Bhamarapravati, the Center for Vaccine Development (CVD) was formed in Thailand to undertake dengue vaccine research and development. This centre was later moved to the Salaya Campus of Mahidol University in 1987.

Following the recommendations of the Advisory Committee on Medical Research (WHO, 1978), SEARO, staff from the CVD, Mahidol University, were sent to the Department of Tropical Medicine and Medical Microbiology, John A. Burns School of Medicine, University of Hawaii at Mānoa, Honolulu, for training in dengue virology and vaccinology. The first SEARO grant was awarded to the CVD in April 1980, and the centre itself was fully equipped and operational in November 1980.

The viruses selected for attenuation were all low-passage, originally isolated from DHF cases in Thailand (DENV-1 and DENV-2) and the Philippines (DENV-3) by Dr S.B. Halstead in 1964, and from a DF case in Indonesia (DENV-4) by Dr D.J. Gubler in 1976 (Halstead, *et al.*1984a). Before being inoculated into PDK cells, all viruses were passed twice in *Aedes albopictus* and/or *Toxorynchites* mosquitoes by

intrathoraic inoculation at the University of Hawaii.(Rosen & Gubler, 1974). The initial passage of the four candidate dengue vaccines was then carried out in Hawaii. In 1980, a Rockefeller Foundation–supported collaborative dengue vaccine technology transfer project was initiated between the University of Hawaii, SEARO and Mahidol University. A training laboratory was equipped and all virus passages in Hawaii were performed under conditions that would permit use in humans (Halstead & Marchette, 2003). Scientists from Thailand were trained in Hawaii and a set of PDK passage 15 viruses (DENV-1, -2 and -4) and GMK passage 5 (DENV-3) were transferred to the SEARO-supported programme in Thailand for further vaccine development (Halstead & Marchette, 2003).

In Thailand, each lot of PDK cells was subjected to a series of safety tests to ensure that they were free of adventitious agents, including mycoplasma and other bacteria, fungi, and cytopathic and haemabsorbing agents. At every fifth virus passage level, a virus seed pool was prepared and tested for plaque size in LLC-MK2 cells, temperature sensitivity, monkey and mouse neurovirulence, and growth in human monocytes (Yoksan *et al*. 1986). The candidate vaccine was tested for safety by inoculating into suckling mice, guinea pigs, rabbits and several tissue culture systems. Since there was no good animal model to measure virulence in the passaged viruses, the following attenuation markers were used:

- Pinpoint plaques in LLC-MK2 cells
- Lack of neurovirulence in suckling mice
- Temperature sensitivity
- The absence of CPE in LLC-MK2 cells

If all of these attenuation markers were present, the candidate vaccine virus was then tested for neurovirulence in monkeys by intracerebral inoculation at the University of Hawaii, and master and production seeds of the candidate vaccine were prepared (Yoksan and Bhamarapravati, 1990; Angsubhakorn *et al*. 1987a, 1987b, 1988).

International peer review of dengue vaccine development

As development of candidate dengue vaccines for each of the virus serotypes progressed, it became obvious that the CVD required an international peer review of development methods and procedures, and evaluation of results. Accordingly, in

1983, WHO-SEARO appointed an international panel of experts to review progress on an annual basis. The first meeting of this group was in August 1983. The principal focus of that meeting was to review the results of the immunological and safety tests and the biological characteristics of the DENV-2 candidate vaccines, which had been passaged 53 times in PDK cells (WHO, 1983). Careful attention was given to the attenuation markers, especially temperature sensitivity, plaque size, and suckling mouse and monkey neurovirulence. This candidate was judged to be ready for safety and immunogenicity trials in humans, which was planned for Lampun Province, Thailand in January 1984 (WHO, 1983). This attention to detail was followed in all subsequent peer review meetings (12 in all), the last one of which was held in 1994 (WHO, 1984–1994). In each review, the expert panel made recommendations to WHO-SEARO, CVD and the Ministry of Public Health of Thailand. The members of the expert panels and other participants are presented in Table 4.

In 1993, the candidate tetravalent dengue vaccine was licensed to Pasteur Merieux (now Sanofi Pasteur). This agreement provided for technology transfer by CVD to Pasteur Merieux and gave the latter full rights to develop the tetravalent vaccine. It also gave Pasteur Merieux first right of refusal on any second-generation vaccine developed from the original Mahidol candidate.

Clinical trials

Phase 1 safety and immunogenicity trials with the monovalent candidate vaccines in non-immune adult males in 1984–85 showed mixed results (Bhamarapravati & Yoksan, 1985; Bhamarapravati *et al*. 1986, 1987). The DENV-2-PDK53 virus proved to be an ideal live attenuated vaccine candidate, with no severe adverse reactions and 100% seroconversion with a very low virus dose (5 to 7 PFU). Neutralizing antibodies were still detectable one year after vaccination with one dose. This monovalent vaccine is probably as good as yellow fever 17D vaccine in terms of safety and immunogenicity.

The DENV-1 candidate was found to be over-attenuated at all passages initially tested (PDK20, PDK30 and PDK43). Ultimately, the PDK13 virus was selected as the candidate DENV-1 vaccine.

The DENV-3 vaccine development was plagued with difficulties from the beginning. First, the virus strain selected (16562) did not grow well in PDK cells. As a result, the virus was passaged 50 times in GMK cells. After testing several passages, the GMK33 virus was selected as the best candidate, and was passed into

fetal rhesus lung (FRhL) cells for two additional passages before preparing the naster seed. Monovalent safety and immunogenicity trials showed equivocal results. Although the peer review panel discussed developing a new candidate DENV-3 vaccine using another virus, this was never done.

The DENV-4 virus was passed in PDK cells 48 times, after which attenuation markers suggested it was an acceptable candidate. Monovalent safety and immunogenicity trials showed acceptable results.

Over a period of 12 years, a series of bivalent, trivalent and tetravalent trials were carried out in Thailand in young adults and in children, both immune and non-immune (Bhamarapravati & Yoksan, 1989, 1990, 1997, 2000; Bhamarapravati, *et al*. 1993; Vaughn *et al*. 1996). While results were generally acceptable, considerable effort was directed towards determining the best formulation of the four attenuated candidate vaccine viruses to provide the best immunogenicity for all four serotypes. The DEN3 GMK33-FRhL-2 virus, however, remained problematic; producing low neutralizing antibody titres in most recipients of the tetravalent formulation. Moreover, not all vaccine recipients seroconverted to all four vaccine serotypes. In the end, however, the tetravalent vaccine was judged to be safe and of acceptable immunicity in children 5 to 12 years of age.

Licensing

The Mahidol tetravalent dengue vaccine was licensed to Pasteur Merieux (now Sanofi Pasteur) and transferred to them in February 1993; the CVD at Mahidol provided technical assistance. Master seeds of the monovalent candidate vaccines were produced under GMP conditions. These seeds all had surprisingly high titres, and the tetravalent vaccine formulation was made by mixing equal volumes of the four virus serotypes. This tetravalent formulation was tested in non-immune adult volunteers in the United States in 1995, with a high rate of reactogenicity. This increased reactogenicity was due to the DEN3-GMK33-FRhL-2 virus overgrowing the other serotypes; the neutralizing antibodies in these volunteers were primarily DENV-3, with low or undetectable titres against the other three serotypes. Interestingly, a subsequent trial of the tetravalent vaccine in Thailand using different formulations of the four monovalent candidates resulted in an improved safety profile. In a two-dose vaccination schedule, there was 71% seroconversion to all four serotypes with acceptable reactogenicity (Sabchareon *et al*. 2002).

Despite these results, in 2000 Sanofi Pasteur contracted with the Division of Vector-Borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado, United States, to re-derive the DENV-3 candidate vaccine. It was found that the DEN3-GMK33-FRhL-2 vaccine virus did not revert to a virulent form; instead, this strain contained two subpopulations, one of them attenuated and one still the wild type virulent virus. Interestingly, the peer review panel had speculated that this might be the case in 1991 (WHO, 1991). For reasons that are not fully understood, the wild type virulent population overgrew the attenuated population when it was put into non-immune humans. The attenuation markers used in the original development (temperature sensitivity, small plaque size, lack of CPE in mammalian cells, and deceased neurovirulence in mice) were used by the CDC group to derive a new candidate vaccine strain of DENV-3 from the original Mahidol candidate vaccine. This monovalent candidate was tested for safety and immunogenicity in 2004 among medical students in Hong Kong SAR. Unfortunately, it was still highly reactogenic and was deemed unacceptable as a vaccine. At this point, Sanofi Pasteur decided to shelve the tetravalent Mahidol live attenuated dengue vaccine.

Second-generation recombinant vaccines

In the early 1990s, there was much interest in the Mahidol candidate dengue vaccine, and many researchers approached Professor Natth about obtaining the monovalent vaccines for sequencing and their use in the development of second-generation recombinant vaccines. The DEN2-PDK53 virus was first sequenced by Dr Jan Blok in Brisbane, Australia (Blok *et al*. 1992) but the decision to sign an agreement with Dr Duane J. Gubler at the Division of Vector-Borne Infectious Diseases (DVBID), CDC, in Fort Collins, Colorado, was made in 1993. A Cooperative Research and Development Agreement (CRADA) was signed between Mahidol University and DVBID, CDC in August, 1994. The original vaccine candidate vaccine viruses derived at Mahidol CVD were shipped to Fort Collins and each of the vaccine viruses and the parental wild type strains were sequenced, with the help of a CVD scientist from Thailand assigned to the project.

An infectious CDNA clone of the DEN2-PDK53 candidate, which had been determined at CVD in Thailand to be an ideal monovalent vaccine, was made and used as the backbone for construction of three chimeric viruses in which the PrM and envelope genes were replaced in the DEN2-PDK53 virus by those genes from the parental wild type strains of DENV-1, -3 and -4 (Kinney *et al*. 1997; Butrapet *et al*. 2000; Huang *et al*. 2000; Huang *et al*. 2003). This second-generation vaccine has been licensed to Invirogen, Inc. and is currently being prepared for safety and

immunogenicity trials. The intellectual property rights of this vaccine are jointly owned by CDC and Mahidol University CVD.

Summary and conclusions

While the original tetravalent vaccine candidate developed at Mahidol University with the support of SEARO and the University of Hawaii was not successful, the initiative and the leadership provided by SEARO in the early days was directly responsible for the subsequent progress that has been made in developing a tetravalent vaccine for this complicated disease. Without that leadership, which was responsible for the early successes, there would not be the excellent pipeline of vaccine candidates available today. Thus, while the initial candidate failed, the concept and the overall programme was an unequivocal success, to the point today that two of the largest pharmaceutical companies in the world are involved in dengue vaccine development, and the leadership of WHO is being shouldered by the Organization's headquarters in Geneva. Currently, there are at least five candidate vaccines that are in or ready to be taken into clinical and safety trials (Whitehead *et al*. 2007). Phase III efficacy trials on at least one of these candidates in 2008. Finally, the Bill and Melinda Gates Foundation has funded the Pediatric Dengue Vaccine Initiative to facilitate the development and introduction of a safe, effective and economical dengue vaccine (Edelman, 2007).

Another measure of success of the programme is that the original Mahidol monovalent DEN2-PDK53 vaccine was an excellent vaccine and is currently being used as the backbone to produce a second-generation chimeric tetravalent vaccine that shows great promise (Huang *et al.*, 2003). The original investment by WHO-SEARO during from 1970s tthroughthe 1990s has thus been more than justified. It is anticipated that the ultimate outcome will be the effective control of DF/DHF on a global scale.

References

Angsubhakorn S, Moe JB, Marchette NJ, Palumbo NE, Yoksan S, Bhamarapravati N. (1987). Neurovirulence effects of dengue-2 viruses on the rhesus (MACACA MULATTA) brain and spinal cord. *Southeast Asian Journal of Tropical Medicine and Public Health*. *18 (1): 52-5*.

Angsubhakorn S, Moe JB, Marchette NJ, Latendresse JR, Palumbo NE, Yoksan S, Bhamarapravati N. (1987). Neurovirulence detection of dengue virus using rhesus and cynomolgus monkeys. *Journal of Virological Methods.18 (1): 13-24*.

Angsubhakorn S, Yoksan S, Bhamarapravati N, Moe JB, Marchette NJ, Pradermwong A, Sahaphong S. (1988). Dengue-4 vaccine: neurovirulence, viruaemia and immune responses in rhesus and cynomolus monkeys. *Transactions of the Royal Society of Tropical Medicine and Hygiene. 82 (5): 746-9.*

Anonymous (1978). Progress toward dengue vaccines – panel and general discussion. *Asian Journal of Infectious Diseases. 2:121-7.*

Bhamarapravati N. Yoksan S. (1985). Dengue vaccine development, reactogenicity and immunogenicity of dengue 2 candidate vaccine (16681-PDK53). In: *Proceedings of the Asia and Pacific Conference on Malaria, HawaII,USA. 21-27 April 1985.* pp. 433-438.

Bhamarapravati N, Yoksan S. (1989). Study of bivalent dengue vaccine in volunteers. *Lancet.* 13; 1 (8646): 1077.

Bhamarapravati N, Yoksan S. (1990). The clinical trial of trivalent dengue vaccine. *Southeast Asian Journal of Tropical Medicine and Public Health. 21(4): 709.*

Bhamarapravati N, Yoksan S. (1997). Live attenuated tetravalent dengue vaccine. In: Gubler DJ and Kumo G, eds. *Dengue and dengue hemorrhagic fever*. Oxford: CAB International. pp 367-377.

Bhamarapravati N, Yoksan S. (2000). Live attenuated tetravalent dengue vaccine. *Vaccine*. 26;18 (Suppl 2): 44-7.

Bhamarapravati N, Yoksan S, Chayaniyayothin T, Angsubhakorn S, Bunyaratvej A. (1986). Dengue virus vaccine development 2, clinical, immunological and biological responses in flavivirus immune and non-immune human volunteers inoculated with dengue 2 (16881) passaged 53 times in primary dog kidney cells. *Arbovirus Research in Australia – Proceedings Fourth Symposium, 6-9 May 1986*: 51-56.

Bhamarapravati N, Yoksan S, Chayaniyayothin T, Angsubphakorn S, Bunyaratvej A. (1987). Immunization with a live attenuated dengue-2 virus candidate vaccine (16681-PDK53): clinical, immunological and biological response in adult volunteers. *Bulletin of the World Health Organization. 65 (2):189-95.*

Bhamarapravati N, Yoksan S, Angsubhakorn S, Khin MM & Darakul T. (1993). Clinical trials of live attenuated dengue vaccine current status. *International Symposium on Current Situation of Dengue Virus Infection and its Control, 10-12 October 1993*, Nagasaki, Japan.

Blok J, McWilliam SM, Butler HC, Gibbs AJ, Weiller G, Herring BL, Hemsley AC, Aaskov JG, Yoksan S, Bhamarapravati N. (1992). Comparison of a dengue-2 virus and its candidate vaccine derivative: sequence relationships with the flaviviruses and other viruses. *Virology. 187 (2): 573-90.*

Butrapet S, Huang CYH, Pierro DJ, Bhamarapravati N, Gubler DJ, Kinney RM. (2000). 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. *Journal of Virology. 74(7): 3011-9*.

Eckels KH, Brandt WE, Harrison VR, McCowan JM, Russell PK. (1976). Isolation of a temperature-sensitive dengue-2 virus under conditions suitable for vaccine development. *Infection and Immunity. 14(5): 1221-7*.

Edelman R. (2007). Dengue vaccines: approach to the finish line. *Clinical Infectious Diseases. 15; 45 (Suppl 1): S56-60*.

Gubler, DJ. (1997). Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, eds. *Dengue and Dengue Hemorrhagic Fever*. London: CAB International. pp 1-22.

Gubler, DJ. *(*2005). The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. *Pan American Journal of Public Health. 17(4): 221-4*.

Gubler DJ. (2006). Dengue/dengue haemorrhagic fever: history and current status. In: Novartis Foundation. *New treatment strategies for dengue and other flaviviral diseases*. No. 277. John Wiley & Sons, pp 3-22.

Halstead, Scott B. (1978). Studies on the attenuation of Dengue 4. *Asian Journal of Infectious Diseases. 2: 112-7*.

Halstead SB, Marchette NJ. (2003). Biologic properties of dengue viruses following serial passage in primary dog kidney cells: studies at the University of HawaII. *American Journal of Tropical Medicine and Hygiene*. 69 (6 Suppl): 5-11.

Halstead SB, Diwan AR, Marchette NJ, Palumbo, NE, Srisukonth L. (1984). Selection of attenuated dengue 4 viruses by serial passage in primary kidney cells. I. Attributes of uncloned viruses at different passage levels. *American Journal of Tropical Medicine and Hygiene. 33(4): 654-65*.

Halstead SB, Marchette NJ, Diwan AR, Palumbo NE, Putvatana R. (1984). Selection of attenuated dengue 7 viruses by serial passage in primary kidney cells. II. Attributes of virus cloned at different dog kidney passage levels. *American Journal of Tropical Medicine and Hygiene. 33(4): 666-71*.

Halstead SB, Marchette NJ, Diwan AR, Palumbo NE, Putvatana R, Larsen K. (1984). Selection of attenuated dengue 7 viruses by serial passage in primary kidney cells. III. Reversion to virulence by passage of cloned virus in fetal rhesus lung cells. *American Journal of Tropical Medicine and Hygiene*. 33(4): 672-8.

Halstead SB, Eckels KH, Putvatana R, Larsen LK, Marchette NJ. (1984). Selection of attenuated dengue 7 viruses by serial passage in primary kidney cells. IV. Characterization of a vaccine candidate in fetal rhesus lung cells. *American Journal of Tropical Medicine and Hygiene*. 33(4): 679-83.

Harrison VR, Eckels KH, Sagartz JW, Russell PK. (1977). Virulence and immunogenicity of a temperature-sensitive dengue-2 virus in lower primates. *Infection and Immunity*. 18(1)*: 151-6*.

Huang CYH, Butrapet S, Pierro DJ, Chang GJ, Hunt AR, Bhamarapravati N, Gubler DJ, Kinney RM. (2000). Chimeric dengue type 2 (vaccine strain PDK-53)/ dengue type 1 virus as a potential candidate dengue type 1 virus vaccine. *Journal of Virology*. 74 (7): 3020-8.

Huang CY, Butrapet S., Tsuchiya KR, Bhamarapravit N, Gubler DJ, Kinney RM. (2003). Dengue 2 PDK-53 virus as a chimeric carrier for tetravalent dengue vaccine development. *Journal of Virology*. 77 (2):11436-47.

Kinney RM, Butrapet S, Chang GJ, Tsuchiya KR, Roehrig JT, Bhamarapravati N, Gubler DJ**.** (1997). Construction of infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK-53. *Virology*. *230(2): 300-8*.

Rosen L, Gubler D. (1974). The use of mosquitoes to detect and propagate dengue viruses. *American Journal of Tropical Medicine and Hygiene*. 23(6): 1153-60.

Russell, Philip K. (1978). Progress toward dengue vaccines. *Asian Journal of Infectious Diseases*. *2: 118-120.*

Sabchareon A, Lang J, Chanthavanich P, Yoksan S, Forrat R, Attanath P, Sirivichayakul C, Pengsaa K, Pojjaroen-Anant C, Chokejindachai W, Jagsudee A, Saluzzo JF, Bhamarapravati N. (2002). Safety and immunogenicity of tetravalent live-attenuated dengue vaccines in Thai adult volunteers: role of serotype concentration, ratio, and multiple doses. *American Journal of Tropical Medicine and Hygiene*. 66(3): 264-72

Vaughn DW, Hoek CH, Yoksan S, LaChance R, Innis BL, Rice RM, Bhamarapravati N. (1996). Testing of a dengue-2 live-attenuated vaccine (strain 16681 PDK53) in ten American volunteers. *Vaccine*. 14(4): 329-36.

Whitehead SS, Blaney JE, Durbin AP, Murphy BR. (2007). Prospects for a dengue virus vaccine. *Nature Review Microbiology*. 5(7): 518-28.

WHO (1978). *Research in the development of appropriate technology for the improvement of environmental health at the village level: fourth session of Regional Advisory Committee on Medical Research, 3-6 April 1978*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/RACMR/78.1.

WHO (1983). *Report on dengue virus vaccine development peer review meeting, Bangkok, 1-5 August 1983*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/45.

WHO (1985). *Report to the Regional Director on the development of dengue virus vaccine, second peer review meeting, Bangkok, 23-25 August 1984*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/48.

WHO (1985). *Report to the Regional Director on the development of dengue virus vaccine, third peer review meeting, Bangkok, 31 July -2 August 1985*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/49.

WHO (1988). *Development of dengue virus vaccine: report of the sixth peer review meeting, Bangkok, 1-5 August 1988*. New Delhi: World Health Organization, Regional Office for South-East Asia.. Document SEA/Haem-Fever/57.

WHO (1989). *Development of dengue virus vaccine: report of the seventh peer review meeting, Bangkok, 7-11 August 1989*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/58.

WHO (1990). *Development of dengue virus vaccine: report of the eighth peer review meeting, Bangkok, 29-30 September 1990*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document No. SEA/Haem-Fever/59.

WHO (1991). *Development of dengue virus vaccine: report of the ninth peer review meeting, Bangkok, 26-28 August 1991*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/60.

WHO (1993). *Development of dengue virus vaccine: report of the tenth peer review meeting, Bangkok, 24-26 August 1992*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/62.

WHO (1994). *Dengue vaccine development: Report of the eleventh WHO peer review meeting, Bangkok, 29-31 August 1993*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/63.

WHO (1995). *Dengue vaccine development: Report of the twelfth WHO peer review meeting, Bangkok, 29-31 August 1994*. New Delhi: World Health Organization, Regional Office for South-East Asia. Document SEA/Haem-Fever/64.

WHO (1997). *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control*. 2nd ed. Geneva: World Health Organization.

Yoksan S, Bhamarapravati N, Halstead SB. (1986). Dengue virus vaccine development: study on biological markers of uncloned dengue 1-4 viruses serially passaged in primary kidney cells. Arbovirus Research in Australia. *Proceedings of the Fourth Symposium, 6-9 May 1986*. 35-38.

Yoksan S, Bhamarapravati N. (1990). Evaluation of biological markers for live attenuated dengue vaccines. *Southeast Asian Journal of Tropical Medicine and Public Health. 21(4): 708-9*.

Table 1: *SEARO sponsored meetings and principal persons involved in early discussions on Dengue Vaccine Development.*

Meeting	Dates	Participants
SEARO Research Study Group	6-8 February 1977 New Delhi	Dr Chaiyan K. Sanyahom Dr B. Jayaweera Dr G. Tonigiani Dr P. Bres Dr D.M. McFadyen Dr V. Chan Dr Natth Bhamarapravati Dr Suchitra Nimmanitya Dr S.B. Halstead Dr P.K.Russell Dr A.C. Allison
Conference on DHF	24-28 October 1977 Singapore	Dr S. B. Halstead Dr P.K. Russell Dr J. Sulianit Saroso Dr R.M. Scott Dr L. Rosen Dr D. J. Gubler Dr V. Chan Dr K Pavri Dr A. Rudnick Dr Y.C. Chan Dr P. Bres
SEARO Research Study Group	6-8 February 1978 New Delhi	Dr S.B. Halstead Dr P.K. Russell Dr A.C. Allison Dr Natth Bhamarapravati Dr Suchitra Nimmanitya Dr Chaiyan K. Sanyahorn

Table 2: *World Health Organization Staff Responsible for Initiating and Overseeing the SEARO Dengue Vaccine Development Programme.*

Name	Position	WHO
Dr.V.T.H. Gunaratne	Regional Director	SEARO
Dr U. Ko Ko	Regional Director	SEARO
Dr Chaiyan K. Sanyakorn	Director, Dis. Control & Pres.	SEARO
Dr N. K. Shah	Director, Dis. Control & Pres.	SEARO
Dr F. A. Assad	Director, Div. Comm.Dis.	SEARO
Dr S. Pattanayak	Regional Adviser. Comm. Dis.	SEARO
Dr Sujarti Jatasen	Regional Adviser. Comm. Dis.	SEARO
Dr.Aung Than Batu	Director, Research	SEARO
Dr S. P. Tripathy	Director,Research	SEARO
Dr.A. G. Andjaparidze	Regional Adviser. Comm. Dis.	SEARO
Dr N. K. Okabe	Regional Adviser. Comm. Dis.	WPRO
Dr T. Umenai	Reg. Adviser. Comm. Dis.	WPRO
Dr.G. Torrigiani	Director, CDS/HQ	WPRO
Dr T. Bektimirov	Chief, Virology	HQ
Dr P. Bres	Chief, Virology	HQ
Dr.J. Esparza	Microbiology Support Services	HQ
Dr.J. Meegan	Medical Officer	HQ
Dr. J. Le Duc	Medical Officer	HQ
Dr H. Suzuki	Key Adviser Comm. Dis.	HQ

Table 3: *Agencies providing funds for the SEARO Dengue Vaccine Development Programme*

Agency	Approximate Funding
South-East Regional Office, WHO	$3 000 000
Government of Thailand	$2 200 000
Government of Italy	$2 000 000
Rockefeller Foundation	$ 550 000
Government of Australia	$ 58 000
WHO headquarters	$ 25 000
Total	$7 833 000

Table 4: *SEARO/WHO Dengue Vaccine Development Peer Review Panel members.**

Name	Affiliation	Dates of participation
Dr R.E. Shope	Yale University School of Medicine	1983 – 1993
Dr K. Fukai	Osaka University	1983,1986,1987,1989,1990
Dr. P.C. Doherty	Australia National University	1983,1984,1986,1987
Dr P.K. Russell	Walter Reed Army Inst. of Research	1983,1991,1992
Dr W.H. Bancroft	Walter Reed Army Inst. of Research	1984, 1986, 1988,1989
Dr D.J. Gubler	Division of Vector Borne Infectious Diseases, CDC	1991,1992,1993,1994
Dr. S.B. Halstead		1992,1993,1994

* In addition to the Peer Review Panel members, several others attended these meetings as resource persons and representatives of various agencies, including experts from WHO headquarters and the Western Pacific Regional Office.

Table 5: *Meeting to Review the History of Dengue Vaccine Development in the South-East Asia Region, Yangon, Myanmar, 16-17 February 2007.*

List of Participants	Affiliation
Dr Duane Gubler	Director, Asia-Pacific Institute of Tropical Medicine and Infectious Diseases; Professor & Chair, Dept. of Tropical Medicine and Medical Microbiology, University of Hawaii, USA
Dr Sujarti Jatanasen	Former Regional Adviser, Communicable Diseases, WHO/SEARO
Dr Suchitra Nimmanitya	Adviser, Ministry of Public Health, Thailand
Dr Prayura Kunasol*	Former Director-General, Dept of Communicable Disease Control, Ministry of Public Health, Thailand
Dr N. K. Shah	Former Director, Prevention and Control of Diseases, WHO/SEARO & former WR India
Prof. U Ko Ko	Former Regional Director, WHO/SEARO
Prof. U Aung Than Batu	Former Director, Research and Family Health, WHO/SEARO
Dr Kyaw Lin*	Director, Food and Drug Administration, Dept of Health

WHO Secretariat	Affiliation
Prof. Adik Wibowo	WHO Representative, Myanmar
Dr Alexander Andjaparidze	WHO Representative, Timor-Leste
Dr Chusak Prasittisuk	Communicable Disease Control Coordinator, (CDC/SEARO)
Dr Leonard Ortega	WHO, Myanmar Medical Officer (Malaria)
Phyu Phyu Khin	WHO, Myanmar Secretary to Medical Officer (Malaria)

*Unable to attend

World Health Organization
Regional Office for South-East Asia
World Health House
Indraprastha Estate,
Mahatma Gandhi Marg,
New Delhi-110002, India
Website: www.searo.who.int

SEA-DEN-9