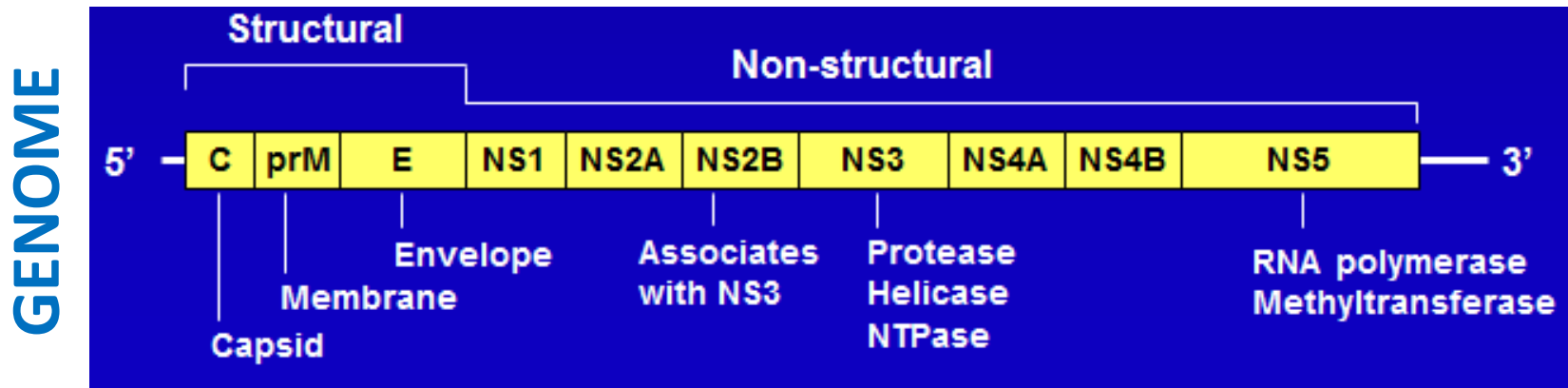


Dengue Vaccine Butantan Institute



Dengue Viruses

- Dengue is a mosquito-borne RNA virus with 4 distinct serotypes: 1,2,3,and 4



- 3 structural proteins: C, prM, and E
 - E protein is the main target of neutralizing antibody
- 7 non-structural proteins are associated with the development of cellular immune response

Dengue – Clinical Manifestations

- Each serotype is capable of causing
 - Asymptomatic disease
 - Dengue fever
 - Severe disease characterized by hemorrhagic and/or shock syndrome manifestations
- Infection by one serotype results in lifelong homotypic protection and secondary heterotypic infection has been associated with an increased risk of severe disease



Dengue is both an established disease and an emerging/re-emerging disease in many countries

- According to the WHO 2.5 billion people are at risk for acquiring dengue
- There have been from 50 to 100 million annual infections
- 500.000 hospitalizations for life-threatening conditions such as hemorrhage /shock
- Tropical and subtropical regions are the areas with the highest risk for dengue and they have circulation of all 4 serotypes

Dengue, countries or areas at risk, 2013



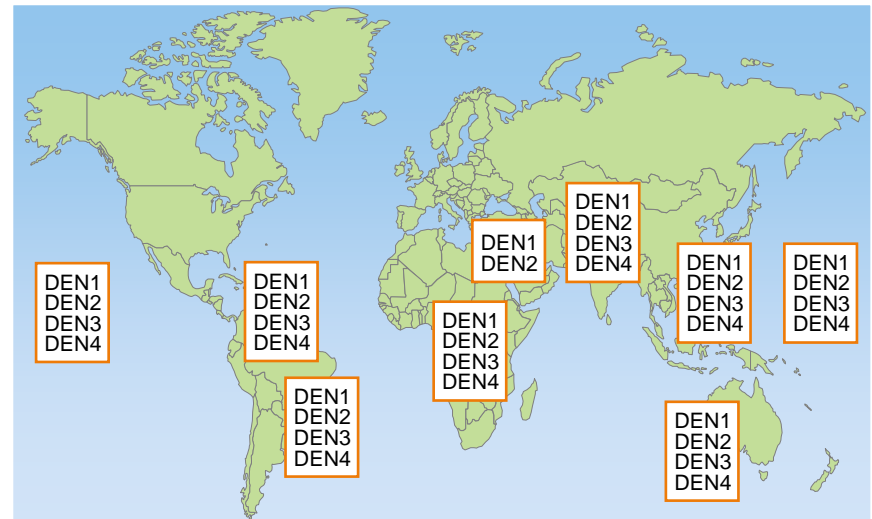
The contour lines of the January and July isotherms indicate areas at risk, defined by the geographical limits of the northern and southern hemispheres for year-round survival of *Aedes aegypti*, the principal mosquito vector of dengue viruses.

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Health Statistics and
Information Systems (HSIS)
World Health Organization



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Dengue Vaccine

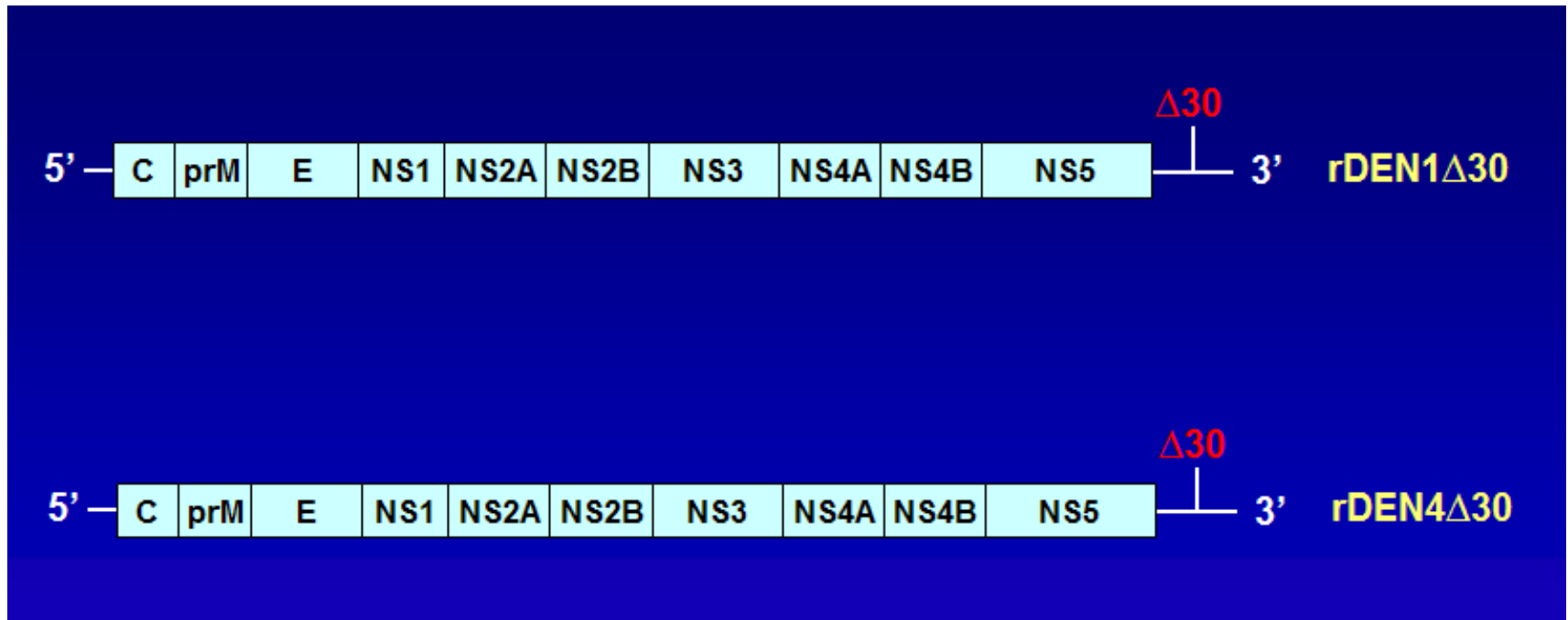
- **Butantan Institute aiming at developing and manufacturing a tetravalent dengue vaccine has established a partnership with the Laboratory of Infectious Diseases at NIH**
- **The partnership resulted in the transfer of the live attenuated dengue vaccine technology from NIH to Butantan Institute and vaccine seed viruses were subsequently acquired**

Why develop a live attenuated DENV vaccine?

- **Live attenuated vaccines have been successful for other flaviviruses: YF and JE**
- **Live attenuated vaccine**
 - **Induces both humoral and cellular immune responses**
 - **Are highly immunogenic, requiring only one or two doses**
 - **Expected to induce lifelong immunity**
 - **Can be very economical to produce and can be manufactured locally in endemic countries**

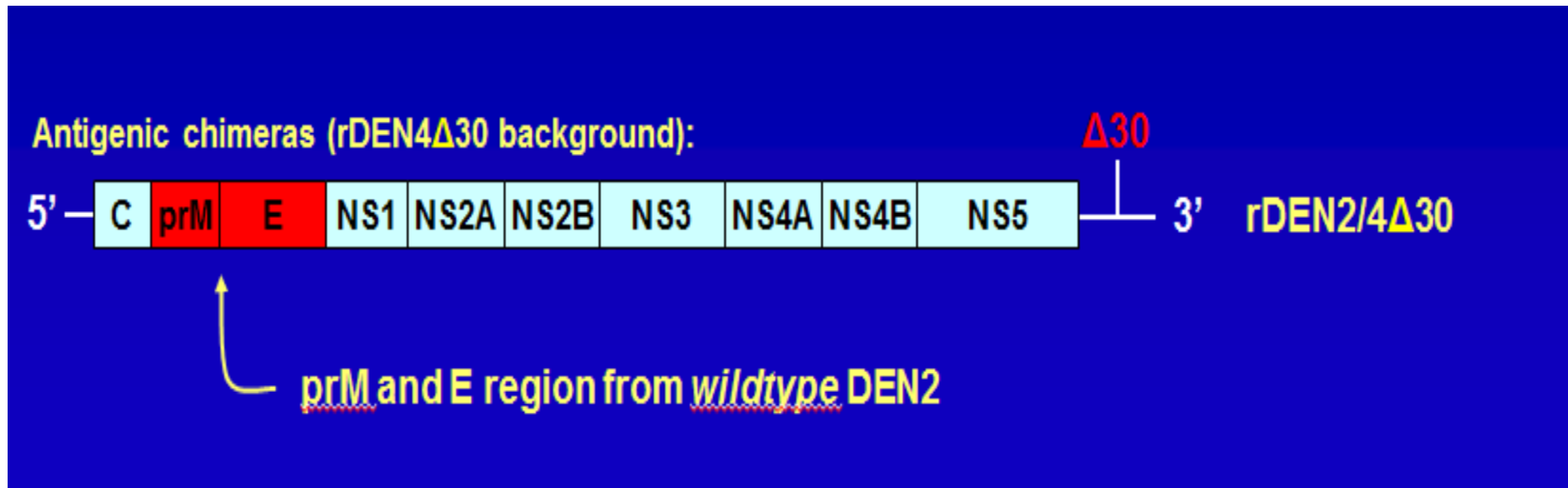
Attenuation strategies used by NIH to obtain the vaccine viruses

- Reverse genetic techniques to introduce defined attenuating deletion mutations into the 3'-untranslated region (UTR), referred to as $\Delta 30$, for DEN1 and DEN4



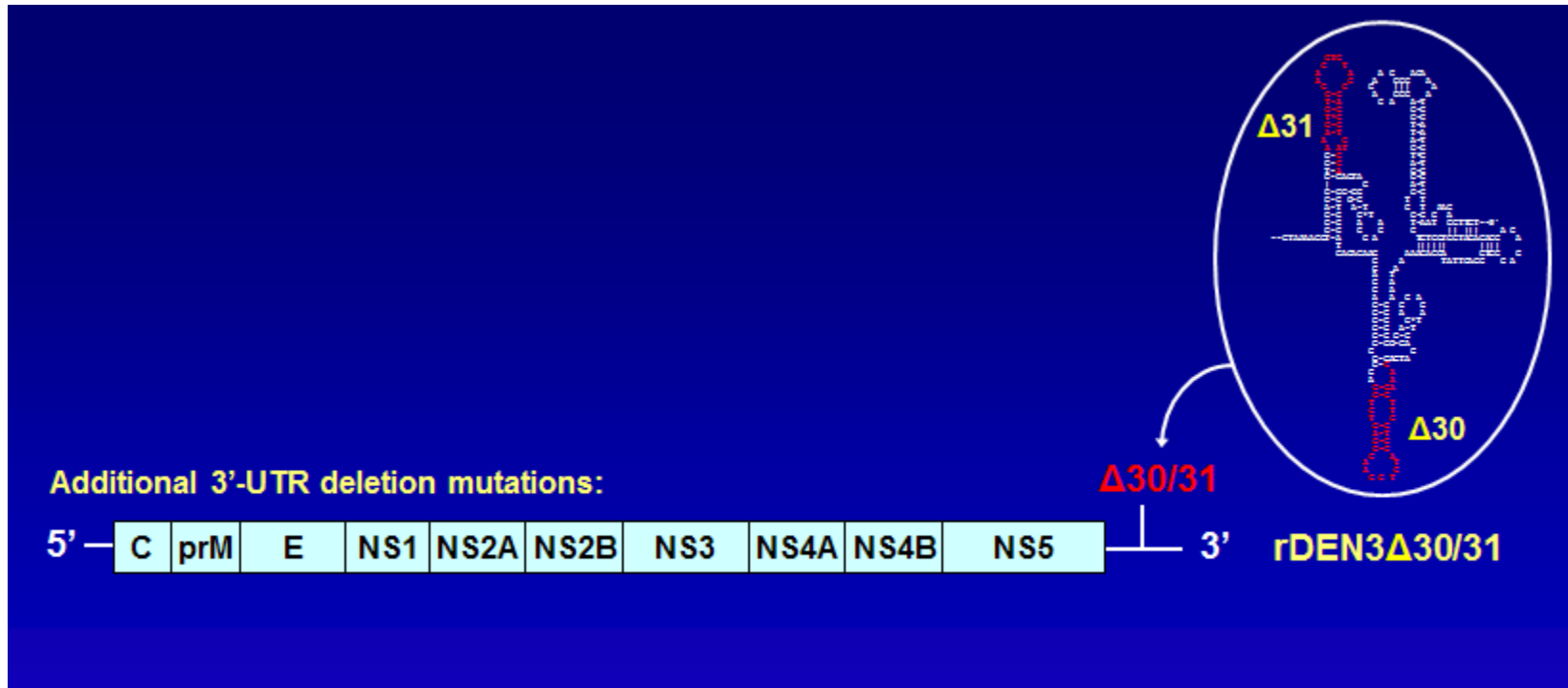
Attenuation Strategies used by NIH

- An alternative chimeric strategy based on the DEN4 Δ 30 vaccine candidate was used to create the DENV-2 vaccine
 - The membrane precursor (prM) and E proteins of the DEN4 Δ 30 genetic background were replaced by the the prM and E proteins from wildtype DEN2



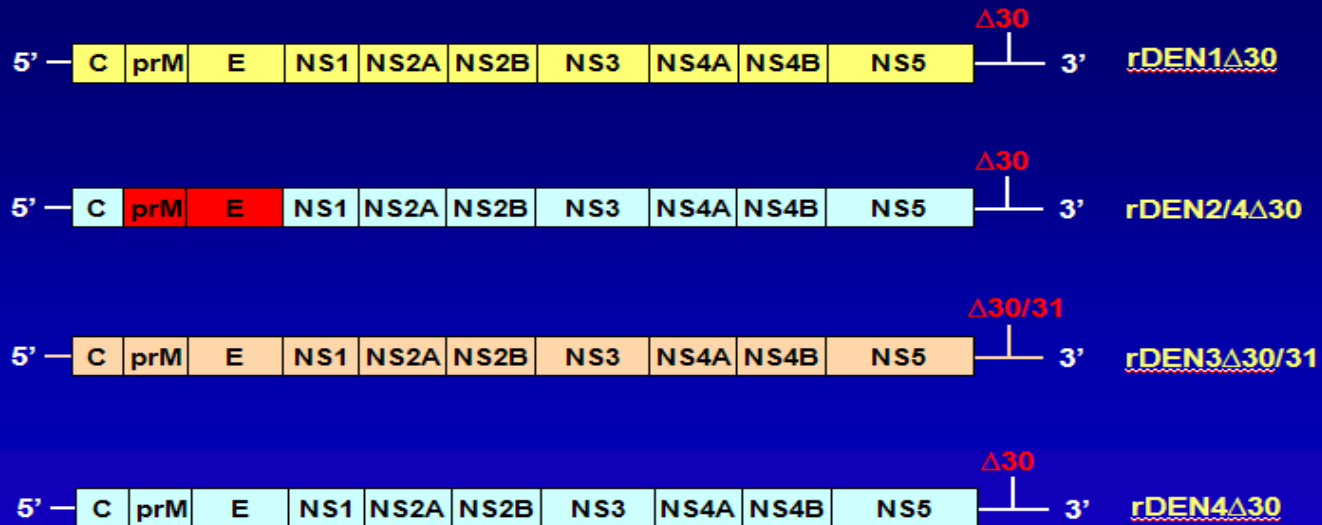
Attenuation Strategies used by NIH

- An additional 3'-UTR deletion mutation, referred to as $\Delta 31$, was applied to rDEN3 $\Delta 30$ resulting in rDEN3 $\Delta 30/31$



- As the monovalent vaccine candidates were demonstrated to be safe and immunogenic in humans they were admixture as a tetravalent liquid formulation, identified as TV003, and new phase I trials with the tetravalent formulation were conducted in the US

Tetravalent vaccine components



Summary of the clinical trials conducted with TV003 in the US

- 1) The TV003 is safe in both the flavivirus-naïve and experienced adults
 - The most common systemic AE observed was asymptomatic non-pruritic rash
- 2) Viremia remained very low in both groups
 - no increase in AR among flavivirus-experienced adults
- 3) Up to 74% of naïve subjects and up to 85% of experienced subjects had a tetraivalent antibody response after 1 dose
- 4) Vaccine-associated rash correlation with tetraivalent antibody response

The Development and Manufacturing of the Butantan Live Attenuated Tetravalent Dengue Vaccine

- The seed viruses obtained from NIH were first used to create master and working virus banks and then used in scaled-up production to generate virus fluids in Vero cells for each monovalent vaccine component
- Following mixture to generate the tetravalent formulation, the vaccine was filled, lyophilized, and shown to be stable at 2 – 8°C
- The resulting Butantan-DV lyophilized formulation is analogous to the original NIH TV-003 liquid formulation and contains rDEN1 Δ 30, rDEN2/4 Δ 30, rDEN3 Δ 30/31 and rDEN4 Δ 30 attenuated virus strains

Target Product Profile (TPP) – Butantan-DV

- **Live attenuated tetravalent dengue vaccine**
- **Potency: $10^{3\pm 0.5}$ PFU per dose/each virus**
- **Route of administration: SC**
- **Should confer at least 80% protection against any symptomatic dengue disease after a single dose**
- **Should be recommended for children and adults (2 to 59 years)**
- **Lyophilized formulation stable at 2-8°C**
- **The proposed presentation is a ten-dose vial in order to allow mass vaccination campaigns**

Butantan-DV - Clinical Trial Program

- **In Brazil we started the clinical trial program for the Butantan-DV from Phase II because the National Ethic Committee and ANVISA recognized that the NIH TV-003 liquid formulation and the Butantan lyophilized formulation are analogous vaccines and, therefore, the pre-clinical and phase I clinical trials conducted in the US could be accepted**

Butantan-DV Phase II Clinical Trial

ClinicalTrials.gov Identifier: NCT01696422

- Is a stepwise (steps A and B) randomized, multicenter, double-blind and controlled clinical trial to evaluate the safety and immunogenicity
- 300 healthy adult volunteers from 18 to 59 years of age are participating
- Primary outcomes
 - Frequency of AR up to Day 21 after vaccination
 - Seroconversion rate for each of the four vaccine viruses defined by PRNT₅₀ ≥1:10 up to Day 91 after vaccination
- Secondary outcomes
 - Investigation for vaccine strains viremia
- Exploratory outcomes
 - Cellular immune response to vaccination
- Follow-up period for all participants: 5 years
- An external safety data committee has been created and has been supporting this study

Butantan-DV Phase II Clinical Trial

ClinicalTrials.gov Identifier: NCT01696422

Step A

- 50 dengue-naïve participants
- Is a bridging study between the TV003 liquid formulation and the Butantan-DV lyophilized formulation
- Participants received either one of the vaccine formulations (20 for each formulation) or placebo (10 participants)
- A second dose, 6 months apart, was administered as an exploratory investigation

Step B

- 250 participants
- 50 dengue-naïve participants received either the Butantan-DV (n=40) or placebo (n=10)
- 200 dengue-experienced participants received either the Butantan-DV (n=150) or placebo (n=50)
- Single-dose immunization

**Vaccination in Step A and B has been completed recently
Follow-up period is still ongoing**

Butantan-DV Phase III Clinical Trial

- **Although the Phase II clinical trial is still on the follow-up period Butantan Institute has already submitted the request for ethical and regulatory approval for its Phase III based on:**
 - **the results from studies performed in the United States with TV003 formulation, including a successful challenge study with an heterotypic under-attenuated rDEN2 Δ 30 strain**
 - **the preliminary safety and immunogenicity results from the Butantan-DV phase II trial in Brazil**

Butantan-DV Phase III Clinical Trial

ClinicalTrials.gov Identifier: NCT02406729

- **Randomized, multicenter, double-blind, placebo-controlled study to evaluate the efficacy and safety of the Butantan-DV**
 - **17,000 healthy volunteers from 2 to 59 years of age**
 - **Divided in three age groups 2 to 6, 7 to 17, and 18 to 59**
 - **To receive either one dose of the Butantan-DV or placebo**
 - **14 investigational centers located in different regions of Brazil**
has been selected

Butantan-DV Phase III Clinical Trial

ClinicalTrials.gov Identifier: NCT02406729

- **An active surveillance for dengue cases will be carried out by community-based study agents to detect any symptomatic dengue case regardless severity**
- **Dengue cases will be considered for efficacy analysis after 28 days post-vaccination**
- **All participants will be followed for a 5-year period**
- **ANVISA and National Ethic Committee approvals are still pending**

Thank you



**Division of Clinical Trials and Pharmacovigilance
Butantan Institute , São Paulo, Brazil**