INSTITUTO BUTANTAN

Tetravalent Dengue Vaccine - Butantan Institute

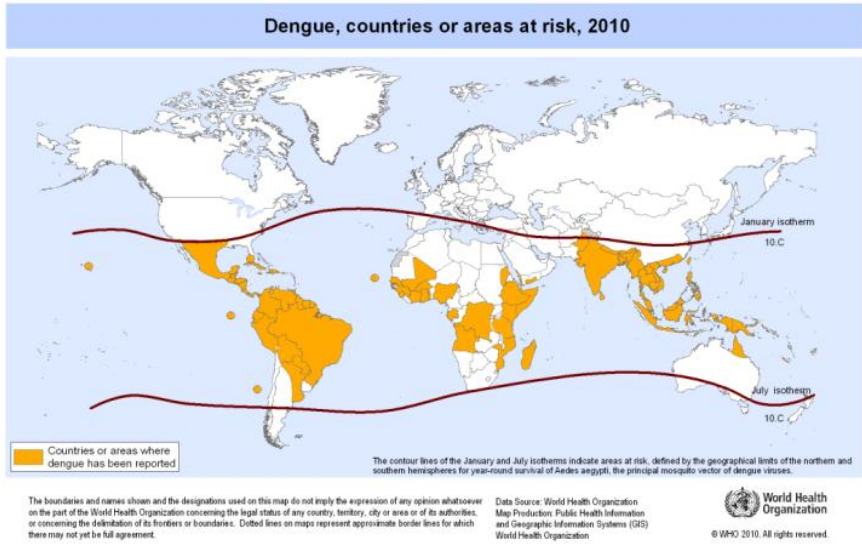
Developing Countries Vaccine Manufacturers Network

Hanoi, Vietnam

7-9th, Ocotber, 2013

Dengue Epidemiology and Clinical Disease

- **Mosquito-borne flavivirus disease - tropical and subtropical areas**
- **The incidence of dengue has increased 30-fold over the last 50 years**
- **Up to 50-100 million infections annually in over 100 endemic countries**



Dengue Vaccine Development

- **Based on the complexity of dengue immunology and pathogenesis several challanges to vaccine development have been identified;**

- **In spite of these challenges, vaccine development has made remarkable progress in recent years, and the current dengue vaccine pipeline is advanced, diverse and, in overall, promising;**

TABLE 1. Dengue vaccines in clinical development (as of November 2012)

DEVELOPER	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3
SANOFI PASTEUR				Currently conducting several Phase II trials in Australia, and in countries in Asia and Latin America on their **live attenuated chimeric tetravalent dengue vaccine**, developed using the yellow fever virus as a backbone. First results of an efficacy study in Thailand show protection against disease caused by three of the four dengue serotypes.
NAVAL MEDICAL RESEARCH CENTER (U.S.)		Has developed candidate dengue DNA vaccine constructs for each dengue serotype by incorporating pre-membrane and envelope genes into a plasmid vector in order to develop a plasmid DNA vaccine. A proof of concept Phase 1 trial for DENV1 vaccine has been completed, showing no safety signals.		
BIOLOGICAL E (India)	Made seed lots at scale of their **live attenuated tetravalent dengue vaccine** (licensed NIH vaccine) and are reviewing their clinical development strategy.			
BUTANTAN (Brazil)			First monovalents, then tetravalents of their **live attenuated tetravalent dengue vaccine** (licensed U.S. National Institutes of Health (NIH) vaccine) were tested in Phase 1. Phase 2 clinical trials are ongoing in the US and in flavivirus exposed subjects in Thailand and Brazil.	
PANACEA BIOTECH (India)	Has prepared test lots of their **live attenuated tetravalent dengue vaccine** (licensed NIH vaccine).			
VABIOTECH (Vietnam)	In the process of making seed lots of their **live attenuated tetravalent dengue vaccine** (licensed NIH vaccine).			
INVIRAGEN			Entered Phase 2 clinical trials in Puerto Rico, Colombia, Singapore and Thailand for their **live attenuated tetravalent dengue vaccine**. In addition, rapid immunization strategies and needle-less administration are being investigated.	
MERCK		Further clinically developing an adjuvanted **recombinant envelope protein vaccine** developed at Hawaii Biotech. Phase 1 studies conducted in Australia in 2012 to supplement previous Phase 1 testing with a monovalent alum adjuvanted vaccine.		
GLAXOSMITHKLINE		Currently in Phase 1 testing on a purified **inactivated vaccine** originally developed by the Walter Reed Army Institute for Medical Research (U.S.). Has signed a research agreement to develop this vaccine with Instituto Oswaldo Cruz foundation.		

PROSPECTS FOR A DENGUE VACCINE

- **Goals of Immunization**
  - **First, the vaccine must be protective against each of the four DENV serotypes;**
  - **Second, the DENV vaccine should provide lifelong protection;**
  - **Third, immunization should be safe and well tolerated;**
  - **Fourth, universal coverage should be a goal in endemic regions;**
  - **Fifth, the cost of the vaccine must be affordable to the countries most in need of it.**

Dengue Vaccine Development at Butantan

- **The production of a live attnuated tetravalent dengue vaccine in Brazil is the result of a partnership between the Laboratory of Infectious Diseases at The National Institutes of Allergy and Infectious Diseases - NIH and Butantan Institute.**

A live attenuated dengue vaccine approach was chosen by NIH for several reasons:

1) **First, live vaccines are expected to stimulate both cellular and humoral immune responses, therefore are able to induce a strong memory response and durable immune response;**

2) **Second, LA vaccines for other related flaviviruses such as yellow fever and Japanese encephalitis virus have been successfully developed;**

3) **Third, LA vaccines can be very economical to produce, helping to ensure that the countries most in need of a dengue vaccine will have access.**

LA Tetravalent Dengue Vaccine Goals

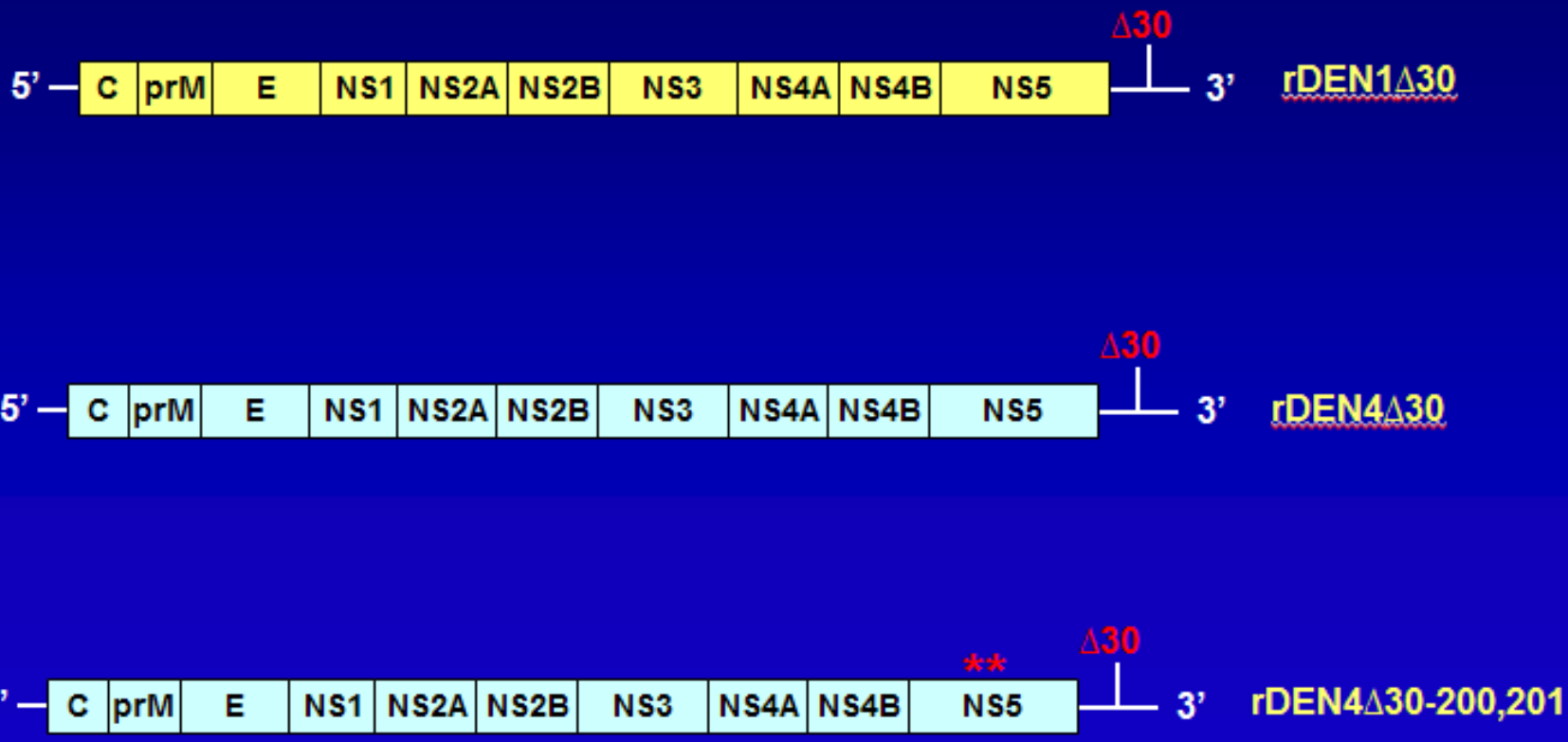
1) Immunogenic and Efficacious

- It must confer protective immunity against all four DENV serotypes (Protect against disease from ANY DENV infection)
- Elicit an appropriate immune response: Must contain, rather than promote, virus growth and not trigger excessive tissue pathology or enhance transmission

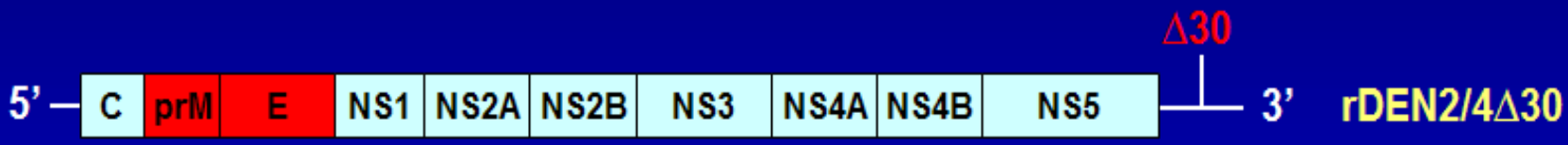
2) Safe

- For vaccinee (with minimal local and systemic reactogenicity)
- For community (not transmissible via mosquito)
- Stable (genetic and potency stability)

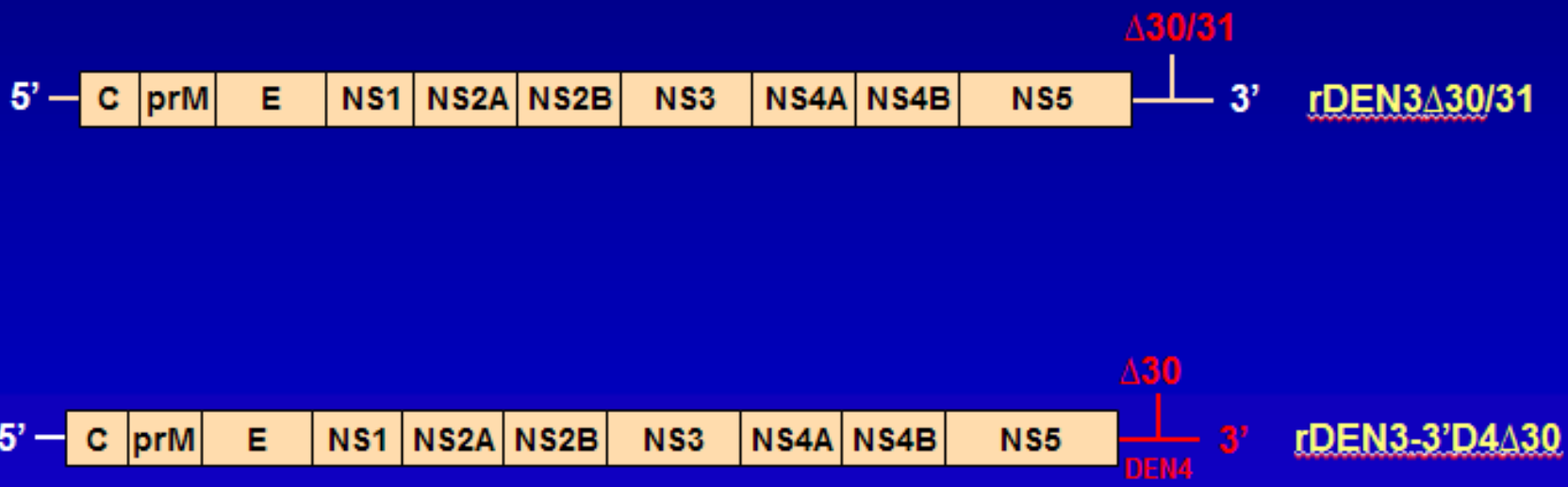
Attenuation strategies for dengue virus vaccine candidates



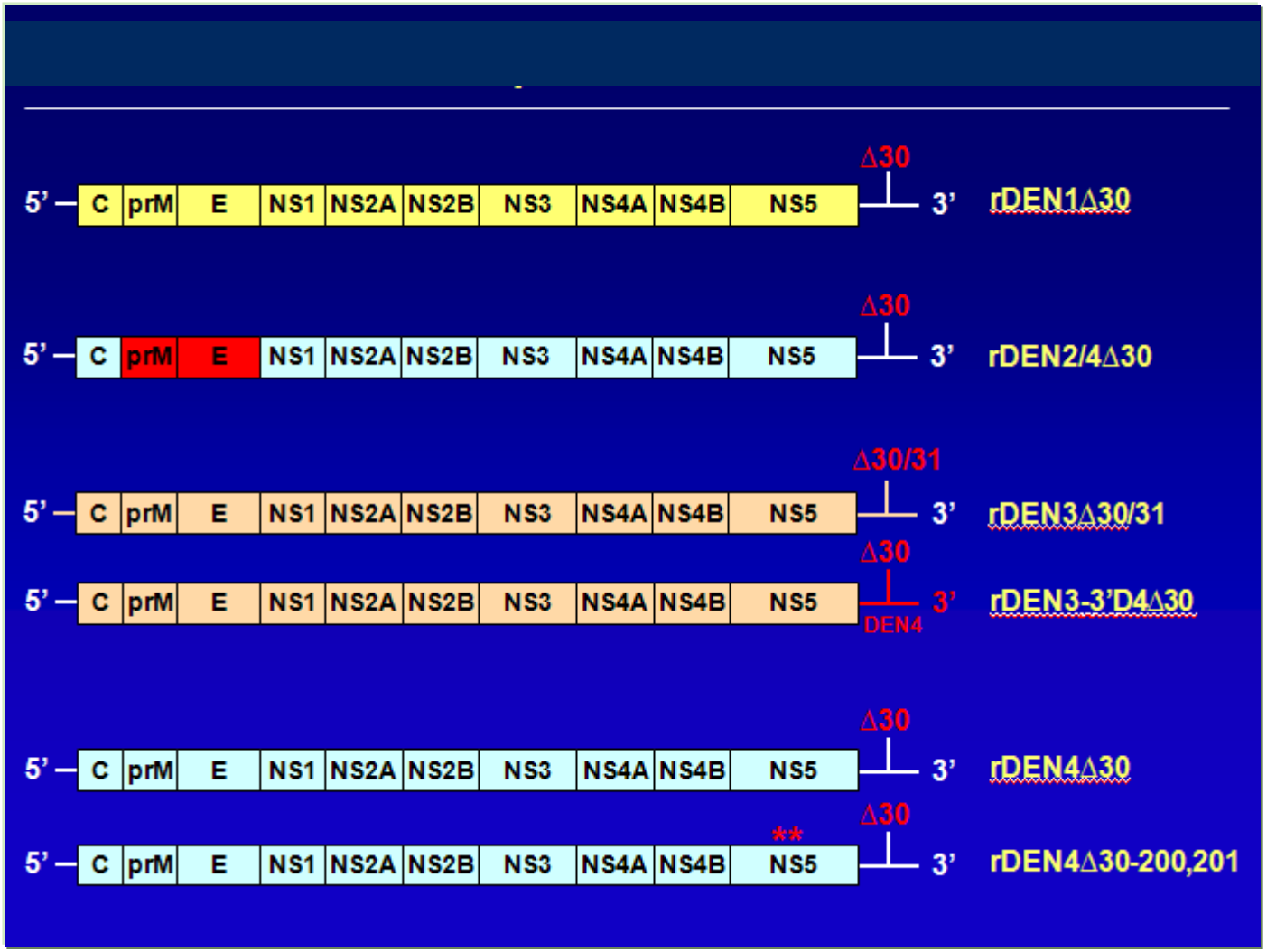
Attenuation strategies for dengue virus vaccine candidates



Attenuation strategies for dengue virus vaccine candidates



Attenuation Strategies for Dengue Viruses



Pre-Clinical Summary of the Monovalent Candidate Vaccines (US)

SCID-HuH-7 mice

- The candidate vaccine viruses were attenuated, replicating to a peak titer from $10^{0.9}$ up to $10^{2.4}$ PFU/mL

Rhesus macaques

- Low/undetectable levels of viremia ($10^{1.0}$ to $10^{1.3}$ PFU/mL)
- Immunogenic

Mosquitoes

- Vaccine viruses have restricted ability to infect the midgut and to cause a disseminated infection (*Ae. Aegypti - Toxorhynchites splendens*)
- *Ae. albopictus* fed on viremic subjects - vaccine virus was not recovered from any mosquitoes

Clinical Summary of the Monovalent Candidate Vaccines (US)

- **The monovalent candidate vaccines have been evaluated in more than 750 volunteers in US and were found to be both safe and strongly immunogenic when administered as a single subcutaneous dose of 10^3 PFU/mL**
- **Vaccinees did not develop a dengue-like illness**
- **Local reactogenicity was minimal in all subjects**
- **The most common systemic AEs observed were mild transient neutropenia, asymptomatic non-pruritic rash, and headache**

Serology Summary of the Monovalent Candidate Vaccines (US)

- Vaccine virus was recovered from the blood of subjects
- Viremia ranging from $10^{0.5}$ PFU/mL to $10^{1.6}$ PFU/mL, significantly lower than the viremia found after wild-virus infection
- Seroconversion rates were:
  - **rDEN1Δ30: 94%**
  - **rDEN2/4Δ30: 100%**
  - **rDEN3Δ30/31: 95%**
  - **rDEN3-3´4Δ30: 80%**
  - **rDEN4Δ30: 93%**
  - **rDEN4Δ30-200,201:95%**
- Seroconversion at Study Day 28 or 42 compared with Day 0.

Clinical evaluation of tetravalent admixtures

- Flavivirus-naïve adults in Baltimore, MD or Burlington, VT
- Single subcutaneous dose
- 10^3 pfu of each vaccine serotype. Potency = 3,3,3,3.
- Clinical follow-up every other day for first 16 days
- Serum for neutralizing antibody on days 28 and 42

Vaccine	Component			
TV-001	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30
TV-002	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30-200,201
TV-003	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30
TV-004	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30-200,201

Tetravalent Evaluation: flavivirus-naïve adults
Neutralizing antibody response - single dose

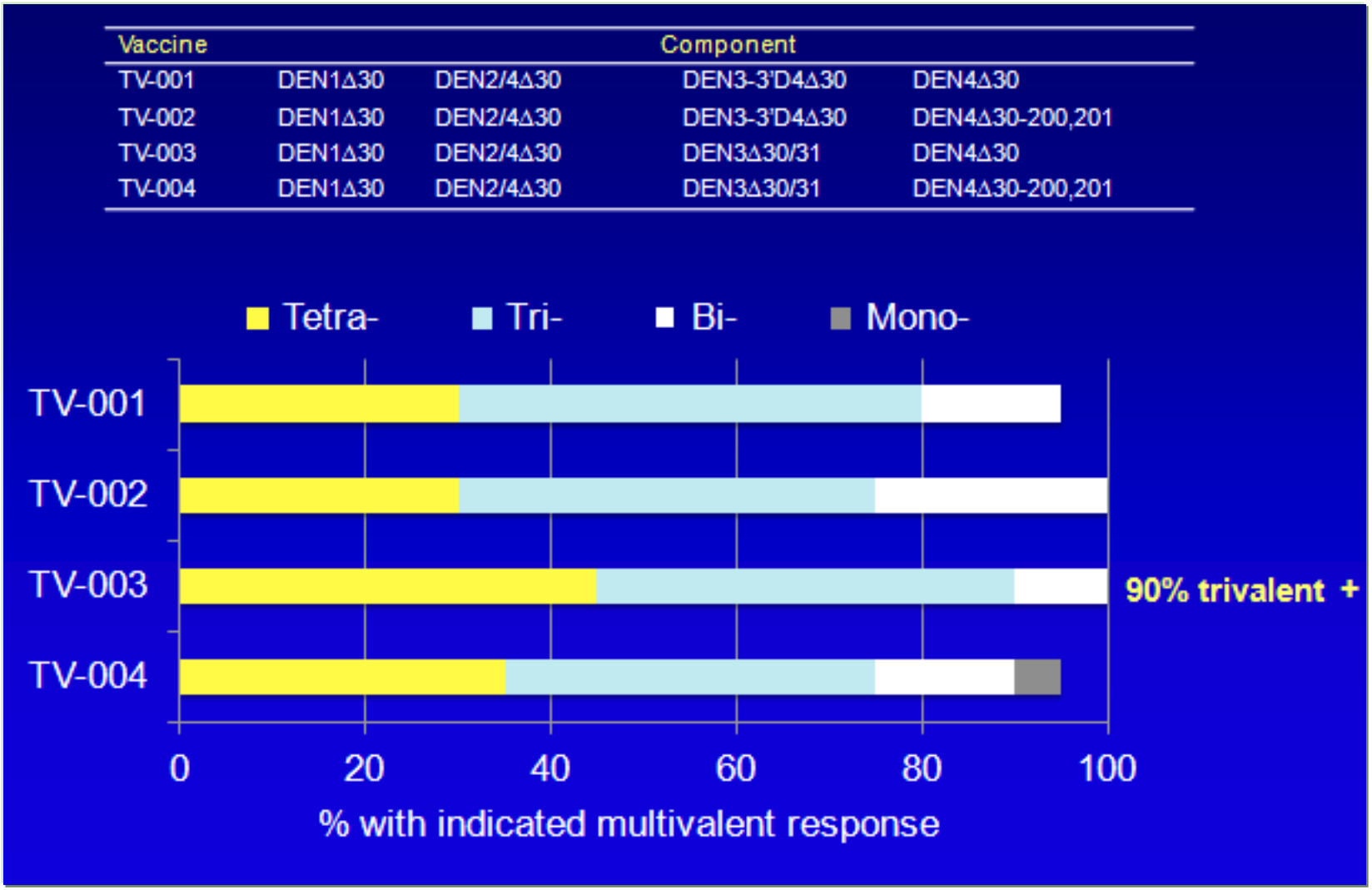
Vaccine	Component			
TV-001	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30
TV-002	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30-200,201
TV-003	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30
TV-004	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30-200,201

		% seroconverted ($PRNT_{60} \geq 10$)				Mean peak titer (GMT) ($PRNT_{60} \geq 10$)			
Vaccine	N	DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
TV-001	20	80	65	60	95	54	39	36	154
TV-002	20	80	60	75	90	118	41	31	32
TV-003	20	100	50	85	100	62	44	36	65
TV-004	20	75	50	85	85	36	17	124	32

→ Higher dose of DEN2 component?
2nd "booster" dose?
Is seroconversion later than day 42?

Tetravalent Evaluation: flavivirus-naïve adults
Neutralizing antibody response – single dose

Vaccine	Component			
TV-001	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30
TV-002	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30-200,201
TV-003	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30
TV-004	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30-200,201



Tetravalent Evaluation: flavivirus-naïve adults
Neutralizing antibody response – single dose

Vaccine	Component			
TV-001	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30
TV-002	DEN1Δ30	DEN2/4Δ30	DEN3-3'D4Δ30	DEN4Δ30-200,201
TV-003	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30
TV-004	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30-200,201
TV-005	DEN1Δ30	DEN2/4Δ30 (10X)	DEN3Δ30/31	DEN4Δ30

		% seroconverted ($PRNT_{60} \geq 10$)				Mean peak titer (GMT) ($PRNT_{60} \geq 10$)			
Vaccine	N	DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
TV-001	20	80	65	60	95	54	39	36	154
TV-002	20	80	60	75	90	118	41	31	32
TV-003	20	100	50	85	100	62	44	36	65
TV-004	20	75	50	85	85	36	17	124	32
TV-005	20	80	60	80	100	40	44	35	70

Tetravalent Evaluation: TV-003

Single dose adverse events

Adverse event	LATV (n=40)	Placebo (n=16)	*p*-value
Injection site:			
Erythema	5.0%	6.3%	1.0000
Pain	0.0%	6.3%	0.2857
Tenderness	5.0%	0.0%	1.0000
Induration	5.0%	0.0%	1.0000
Systemic:			
Fever[a]	0.0%	0.0%	n/a
Headache	45%	25%	0.2300
Rash	**55%**	**0.0%**	**< 0.0001**
Neutropenia[b]	2.5%	6.3%	0.4935
Elevated ALT[c]	5.0%	0.0%	1.0000
Myalgia	7.5%	6.3%	1.0000
Arthralgia	0.0%	6.3%	0.2857
Retro-orbital Pain	5.0%	0.0%	1.0000

[a] Oral temperature ≥ 100.4 °F

[b] Absolute neutrophil count < 1,500/mm^3

[c] Defined as > 1.25 X the clinical laboratory upper limit of normal

Tetravalent Evaluation: TV-003 expanded study Single dose

Serum collection for PRNT

TV-003: Days 28, 42

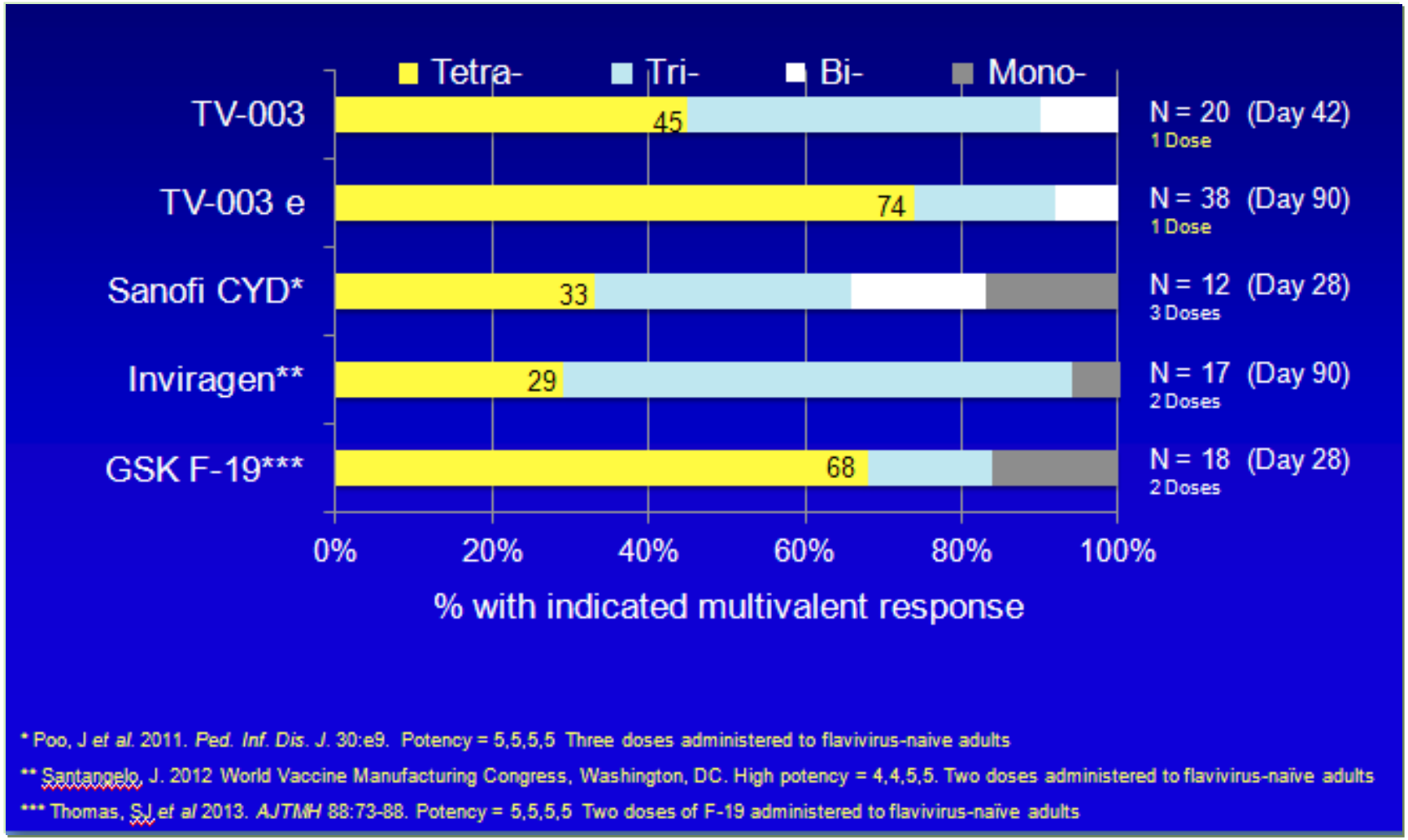
TV-003 e: Days 28, 56, 90

		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT) ($PRNT_{50} \geq 10$)			
Vaccine	N	DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
TV-003	20	100	50	85	100	106	64	42	86
TV-003 e	38	92	76	97	100	63	40	85	151
Sanofi (3 dose)*	12 adult	67	75	67	82				
GSK (2 dose)**	18 adult	83	100	83	68				

* Poo, J *et al.* 2011. *Ped. Inf. Dis. J.* 30:e9. Potency = 5,5,5,5 Three doses administered to flavivirus-naive adults

** Thomas, SJ *et al* 2013. *AJTMH* 88:73-88. Potency = 5,5,5,5 Two doses of F-19 administered to flavivirus-naïve adults

Tetravalent Evaluation: TV-003 expanded study
Neutralizing antibody response - flavivirus-naïve adults



* Poo, J *et al.* 2011. *Ped. Inf. Dis. J.* 30:e9. Potency = 5,5,5,5 Three doses administered to flavivirus-naive adults

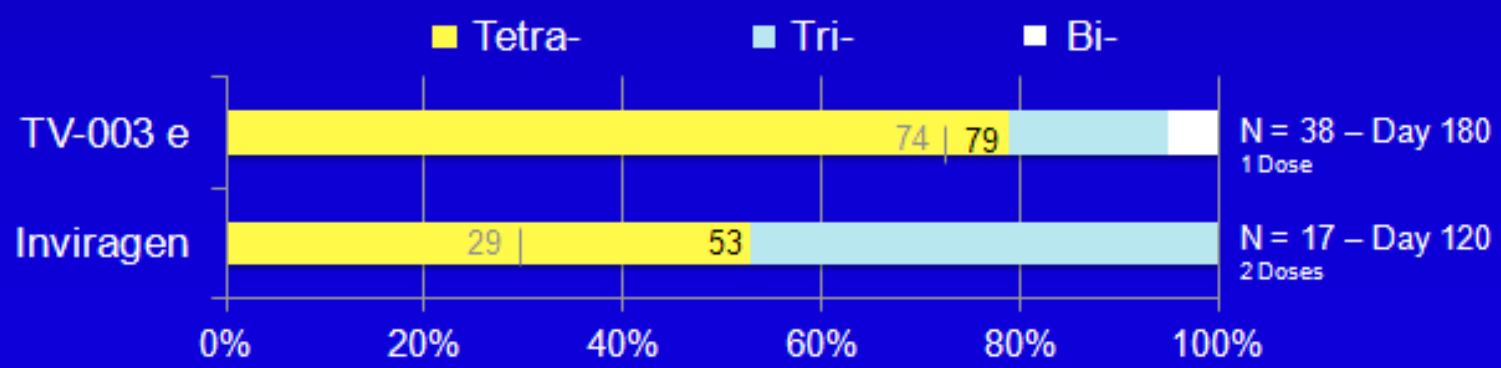
** Santangelo, J. 2012 World Vaccine Manufacturing Congress, Washington, DC. High potency = 4,4,5,5. Two doses administered to flavivirus-naïve adults

*** Thomas, SJ *et al* 2013. *AJTMH* 88:73-88. Potency = 5,5,5,5 Two doses of F-19 administered to flavivirus-naïve adults

Tetravalent Evaluation: TV-003 expanded study
Neutralizing antibody response - flavivirus-naïve adults

Significant number of TV-003 e subjects had peak antibody titers after study day 90

Serum collection	% volunteers with peak antibody titers on indicated day			
	DEN1	DEN2	DEN3	DEN4
Day 28	44	23	62	45
Day 56	25	40	24	32
Day 90	14	13	0	5
Day 150	6	3	0	10
Day 180	11	20	14	8



Tetravalent Evaluation: Two dose studies Viremia and Rash

- **91 tetravalent vaccine subjects received second dose (day 180)**
- **Second dose admixture same as primary dose**

		First dose		Second dose	
Vaccine	N	No. with viremia	No. with rash	No. with viremia	No. with rash
TV-001	12	8	6	0	0
TV-002	11	7	3	0	0
TV-003	10	8	7	0	0
TV-004	13	10	10	0	0
TV-005	11	10	5	1	1
TV-003 e	34	25	19	0	0
	91	75%	55%	1%	1%

TV-005 Subject 130: First dose: DEN4 viremia on one day (one plaque)
Second dose: DEN3 viremia on one day (one plaque) + Mild rash
Trivalent response D1, D3, D4 after first dose

Conclusions from tetravalent studies in flavivirus-naïve adults (US)

1) The tetravalent mixtures are safe.

2) Viremia remained very low in flavivirus naïve adults.

3) Up to 79% of naïve subjects had a tetravalent antibody response (1 dose)

4) Booster immunization?

   Most subjects appear to be “protected” against boost

   Vaccine viremia was not seen after second dose

   May increase antibody durability

6) Probably a one dose vaccine.

Dengue Vaccine Produced by Butantan Institute

- **The same TV-003 tetravalent vaccine has been produced by Butantan as lyophilized formulation**

Butantan Clinical Trial

- **Design and Objective**
  - **Phase II, Stepwise (A and B), Randomized, Double-blind and Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of a Lyophilized Formulation of the Dengue 1,2,3,4 (attenuated)**

Butantan Clinical Trial

- **Study Vaccine**
  - The study vaccine is the lyophilized formulation produced and formulated at Butantan Institute

- **Comparator**
  - The Butantan formulation will be compared to the liquid formulation produced and formulated according to the protocol of LID/NIAID/NIH) and to a placebo

- **Vaccine potency**
  - Both vaccine formulations contain 10^3 plaque forming units (PFU) per 0.5 mL dose, of each virus

- **Route**
  - Vaccines and placebo will be administered subcutaneously

Butantan Clinical Trial

Study Population
300 healthy male and non pregnant female / From18 to 59 years of age / With and without previous exposure to dengue

Step A

•50 without previous exposure to dengue

•Volunteers will be randomly assigned to receive either the lyophilized formulation (Butantan), or the liquid formulation (NIH), or the placebo

•Volunteers will receive a second dose, six months after first vaccination as part of an exploratory assessment

•If no safety stopping criteria is identified, then Step B will be initiated

Step B

- 250 volunteers with and without previous exposure to dengue
- Volunteers will be randomly assigned to receive either one dose of the lyophilized formulation from Butantan, or the placebo

Butantan Clinical Trial

- **Primary Endpoints**
  - The primary safety endpoint - frequency of vaccine-related AEs up to Day 21
  - The primary immunogenicity - seroconversion rate = $PRNT_{50}$ ≥1:10 for each dengue serotypes on Days 28, 56, 90, or 180
- **Secondary Endpoints**
  - Unsolicited AE after Day 21 up to Day 180 after vaccination
  - The frequency, quantity, and duration of viremia for each of the vaccine viruses
  - The frequency of monovalent, divalent, trivalent or tetravalent immune response, at Days 28, 56, 90 and 180 after vaccination
- **Exploratory Endpoint**
  - Cellular immune response
- **Follow-up**
  - Five year follow-up period after their inclusion in the study
  - Annually Immunology testing
  - Assessment of suspected and confirmed dengue cases

Butantan Clinical Trial

- **Immunology Testing**
  - **Serum plaque reduction neutralizing antibody assay using established protocols (NIH) will be performed at Adolfo Lutz Institute**
  - **Cellular immune response – Scholl of Medicine of University of São Paulo**

- **Clinical Sites (3) – University of São Paulo**

INSTITUTO BUTANTAN

GOVERNO DE SÃO PAULO

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