Vaccines difficult to develop

Jorge Kalil MD PhD Dr h c

DCVMN 2014
VACCINE DEVELOPMENT CHALLENGES

• USING AN ANTIGEN THAT TRIGGERS AUTOIMMUNITY – Rheumatic Heart Dis.

• USING EPITOPES THAT TRIGGER THE ACTUAL DISEASE - HIV

• USING THE VIRUS WHICH CAN CAUSE ENHANCED DISEASE - Dengue
DEVELOPMENT OF A VACCINE AGAINST STREPTOCOCCUS PYOGENES TO AVOID RHEUMATIC HEART DISEASE
Streptococcus pyogenes

Peptide

Cross reactive Antibody

Cross reactive heart tissue antigen

Throat

Myocardium
N-acetyl β-D glucosamine
Goldstein, Zabriskie 1967

S. pyogenes

Kaplan, 1964
“Biological Mimicry”

M Protein

Human Protein Cross-reactive Epitopes
Several authors

Molecular Mimicry Mechanism

Cellular and Humoral Immune Responses

HLA/Peptide

T Cell

APC

Self-protein peptide

M protein peptide

APC
M protein- C-terminal Region: T and B cell Epitopes

T Epitope (22aa) 253-279
260 individuals

B Epitope (25aa) 288-312
620 individuals

Identical Region

253 KGLRRDLASREAKKQLEAEQQ 279
T epitope

288 EASR KGLRRDLASREAKKQVEKA 312
B epitope

PepVac/Rc.Prot – StreptInCor 55 aa

253 KGLRRDLASREAKKQLEAEQQKLEEQNKISEASRKGLRRDLASREAKKQVEKA 312

Patents INPI – 0501290 / 0604997-4, PCT-BR07/000184
Adhesion/Inhibition Assays

*Streptococcus pyogenes* - Hep 2 Cells and sera

<table>
<thead>
<tr>
<th>Control</th>
<th>Adhesion/Invasion inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (N=5)</td>
<td>95.0 %</td>
</tr>
<tr>
<td>C57BL6 (N=7)</td>
<td>92.0 %</td>
</tr>
<tr>
<td>Swiss (N=3)</td>
<td>98.5 %</td>
</tr>
<tr>
<td>CFU without sera</td>
<td>&gt; 200,000</td>
</tr>
</tbody>
</table>

**Notes:**
- CFU: Colony Forming Units
S. pyogenes: Survival post-vaccination (StreptInCor + ALUM)

1x10^7 bacteria I.P

Log rank test

P = 0.05

Swiss (n=15)

10 µg of
StreptInCor Controls.

Plos One, 2013
Clinical Phase I Assays / Design of the Study

• Clinical Phase I: randomized, double-blind, placebo controlled, sequential dosing of StreptInCor (50 µg, 100µg, 200 µg - 2 doses with 28d interval); 6 months boost.

• Healthy Volunteers: individuals without confirmed disease diagnosis or infection that would compromise the immune response, with ages between 18 and 45 years old.
Prof. Luiza Guilherme, Pharm, PhD, Rheumatic Fever Group's Leader

**RF/RHD Mechanism of Pathogenesis**
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Luiz R Mundel
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3. Prof Pablo Pommerantzeff, MD, Carlos Brandão, MD
4. Lea Demarchi, MD, Prof Vera D Aiello, MD; Paulo S Gutierrez

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**Saint Louis Hospital, Paris, France – TCR, Treg Cells**
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**Oklahoma University, Oklahoma, USA – Cardiac Myosin Studies**
Prof Madeleine W Cunningham, PhD

**Finlay Institute, Havana, Cuba – Mucosal Adjuvant**
Prof Oliver Perez Martin, MD; Miriam Lastre, MD; Caridad Zayas

**Mayo Clinic Rochester, USA, HLA-class II Transgenic Mice**
Prof Chella David, MD, PhD
DEVELOPMENT OF A T CELL VACCINE AGAINST HIV
Potential impact of an HIV vaccine - Brazil

Thousands of new Infections/year

Baseline scenario:
Expanded prevention and treatment efforts but no vaccine — about 956,700 adult HIV infections from 2016 to 2050

<table>
<thead>
<tr>
<th>Vaccine scenarios</th>
<th>Efficacy</th>
<th>Percentage of population given vaccine</th>
<th>New infections averted, 2016-50</th>
<th>Percentage reduction in new infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>40%</td>
<td>80%</td>
<td>617,647</td>
<td>73%</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>70%</td>
<td>80%</td>
<td>775,481</td>
<td>92%</td>
</tr>
</tbody>
</table>

International AIDS Vaccine Initiative, 2010
Fonseca et al., 2010
Previous HIV-1 vaccines taken to efficacy trials have failed

Randomized, Double-Blind, Placebo-Controlled Efficacy Trial of a Bivalent Recombinant Glycoprotein 120 HIV-1 Vaccine among Injection Drug Users in Bangkok, Thailand

Punnee Pitisutthithum,1 Peter Gilbert,1 Marc Gurwith,1 William Heyward,2 Michael Martin,¹ Fritz van Griensven,3 Dale Hu,4 Jordan W. Tappero,4 and Kachit Choopanya,2 for the Bangkok Vaccine Evaluation Group

1Department of Clinical Tropical Medicine, Mahidol University, and 2Bangkok Metropolitan Administration, Bangkok, and 3Thailand Ministry of Public Health–US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand; 4Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, Washington; 5VaxGen, Inc., Brisbane, California; *US Centers for Disease Control and Prevention, Atlanta, Georgia

Articles

Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial


HVTN 505, 2013

Summary

Background Observational data and non-human primate challenge studies suggest that cell-mediated immune
Main HIV vaccine concept test to now: whole proteins aiming CD8 responses

2007: phase IIb trial, **STEP** (Merck): Ad5GagPolNef

- only 25% CD4 and CD8 T cell responses
- Few epitopes recognized per vaccinee

2009: Phase III **RV 144**, Thailand: ALVAC+GP120, 30% efficacy

- 40% displayed a CD4 T cell response (Env V2), no CD8+
- Only binding antibodies (no broadly neutralizing)

✓ Insufficient efficacy
✓ Incomplete immunological coverage (especially CD4, less than 40% in both studies)
✓ Recognition of few epitopes
✓ Failed to cope with variability
✓ Whole HIV proteins: evolutionary escape?
Induction of CD4 T cell responses has been overlooked in HIV vaccine design

Why?

1. Activation of CD4+ T cells could fuel viral replication

2. Antibody and CD8 responses are *necessary* and *sufficient* *

“Textbook” information on cellular immunology says

- anti-HIV CD8 and antibody responses are CD4 dependent
- Anti-HIV CD4 may have direct effector functions
Rational approach: *in silico* prediction of CD4 epitopes binding to multiple HLA-DR molecules in HIV-1 clade B

HIV-1 whole proteome: clade B *conserved* consensus protein sequences

- gag
- env
- pol
- nef
- tat
- rev
- vif
- vpr
- vpu

**TEPITOPE algorithm:**

- binding to multiple HLA-DR molecules
- Sturniolo 1999,
- Our group: Proteins from ca 15 different pathogens

Potential multiple HLA-DR-binding conserved T cell epitopes

Fonseca et al. AIDS 2006
18 predicted conserved CD4+ T cell epitopes in 8 major HIV proteins (gag, pol, env, rev, vpr, vif, vpu, nef)

- Recognized by over 90% HIV-1 infected patients
- Each patient recognized an average of 5 peptides
- No HLA association
- Polyepitopic/polyallelic vaccine candidate?

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>p1773–89</td>
<td>EELRSLYNTVATLYCVH</td>
</tr>
<tr>
<td>p2433–45</td>
<td>SPEVIPMFSALSE</td>
</tr>
<tr>
<td>p24131–150</td>
<td>KRWIILGLNKRMRYSPTSI</td>
</tr>
<tr>
<td>p632–46</td>
<td>DKELYPLASLRSLFG</td>
</tr>
<tr>
<td>pol63–77</td>
<td>QRPLVTIKGGQLKE</td>
</tr>
<tr>
<td>pol136–150</td>
<td>TPVNIIGRNLNTQIG</td>
</tr>
<tr>
<td>pol785–799</td>
<td>GKIILVAVHVAGSYI</td>
</tr>
<tr>
<td>gp41261–276</td>
<td>RDLLLIVTRIVELLGR</td>
</tr>
<tr>
<td>gp16019–31</td>
<td>TMLLGMLMCSAA</td>
</tr>
<tr>
<td>gp160174–185</td>
<td>ALFYKLDVVPID</td>
</tr>
<tr>
<td>gp160188–201</td>
<td>NTSYRLISCNTSVI</td>
</tr>
<tr>
<td>gp160481–498</td>
<td>SELLYLYVKVKEPLGVAP</td>
</tr>
<tr>
<td>rev11–27</td>
<td>ELLKTVRLIKFLYQSNP</td>
</tr>
<tr>
<td>vpr58–72</td>
<td>EAIIIRILQQLLFIHF</td>
</tr>
<tr>
<td>vpr65–82</td>
<td>QQLLFIHFRIGCRHSRIG</td>
</tr>
<tr>
<td>vif144–158</td>
<td>SLQYLALVALVAPKK</td>
</tr>
<tr>
<td>vpu6–20</td>
<td>VLAIVALVVATIIAI</td>
</tr>
<tr>
<td>nef180–194</td>
<td>VLEWRFPSRLAFHHV</td>
</tr>
</tbody>
</table>
Construction of a CD4 multiepitope HIV vaccine

**Artificial gene**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Start</th>
<th>End</th>
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<tbody>
<tr>
<td>p17</td>
<td>73-89</td>
<td></td>
</tr>
<tr>
<td>p24</td>
<td>33-45</td>
<td>150</td>
</tr>
<tr>
<td>p24</td>
<td>(131-150)</td>
<td></td>
</tr>
<tr>
<td>p6</td>
<td>32-46</td>
<td></td>
</tr>
<tr>
<td>pol</td>
<td>63-77</td>
<td>150</td>
</tr>
<tr>
<td>pol</td>
<td>(136-150)</td>
<td></td>
</tr>
<tr>
<td>pol</td>
<td>785-799</td>
<td></td>
</tr>
<tr>
<td>gp41</td>
<td>(261-276)</td>
<td></td>
</tr>
<tr>
<td>gp160</td>
<td>(19-31)</td>
<td></td>
</tr>
<tr>
<td>gp160</td>
<td>(174-185)</td>
<td></td>
</tr>
<tr>
<td>gp160</td>
<td>(188-201)</td>
<td></td>
</tr>
<tr>
<td>gp160</td>
<td>(481-498)</td>
<td></td>
</tr>
<tr>
<td>rev</td>
<td>11-27</td>
<td></td>
</tr>
<tr>
<td>vpr</td>
<td>58-72</td>
<td></td>
</tr>
<tr>
<td>vpr</td>
<td>(65-82)</td>
<td></td>
</tr>
<tr>
<td>vif</td>
<td>(144-158)</td>
<td></td>
</tr>
<tr>
<td>vpu</td>
<td>(6-20)</td>
<td></td>
</tr>
<tr>
<td>nef</td>
<td>(180-194)</td>
<td></td>
</tr>
</tbody>
</table>

**GPGPG Spacers**

**Codon optimization**

**Novel epitopes**

**Insertion into pVAX1 plasmid**
HIVBr18 can elicit CD4+ T cell responses in HLA class II-transgenic mice

Tg mice: 16/18 epitopes recognized, 11 by CD4+ T cells
Avg: 5 pept recognized by CD4+Tcells/Tg strain
Humans bear 3-8 HLA class II molecules

Ribeiro et al. 2010
Immunization of Rhesus macaques vs. mouse strains:
3 doses of HIVBr18 DNA IM

IFN-g ELISPOT assay

<table>
<thead>
<tr>
<th>Rhesus (individual)</th>
<th>Mouse strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh #33</td>
<td>DR2</td>
</tr>
<tr>
<td>Rh #41</td>
<td>DR4</td>
</tr>
<tr>
<td>Rh #43</td>
<td>DQ6</td>
</tr>
<tr>
<td>Rh #45</td>
<td>DQ8</td>
</tr>
<tr>
<td>Balb/c</td>
<td></td>
</tr>
</tbody>
</table>
Summary

-The HIVBr18 DNA vaccine induced broad, strong polyfunctional CD4+ T cell responses in conventional or HLA class II-transgenic mice as well as Rhesus macaques
-Preimmunization with HIVBr18 augments CD8+ T cell activity and switches IgG antibody subclass to subsequent vaccination with whole HIV genes/proteins

Perspectives

-Selection of viral vectors for a Phase I clinical trial
-SHIV challenge after immunization with SHIV epitope vaccccine
University of São Paulo School of Medicine

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Funding: FAPESP, CNPq, Brazilian Ministry of Health, ICGEB
Heart Institute (InCor) University of São Paulo, Brazil
Dengue Epidemiology and Clinical Disease

- Dengue is a mosquito-borne flavivirus disease
- Prevalent in most tropical and many subtropical areas;
- The incidence has increased 30-fold over the last 50 years;
- Up to 50-100 million infections in over 100 endemic countries,
- Half of the world’s population at risk.
Dengue Epidemiology and Clinical Disease

- Four closely related virus DEN-1, DEN-2, DEN-3, and DEN-4

- Clinical manifestations:
  - asymptomatic infection
  - dengue fever (DF)
  - dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS);

- Serious disease can occur during the primary infection

- More frequent following the secondary infection with a different serotype

- Third and fourth infections leading to hospitalization are rare.

Antibody-Dependent Enhancement (ADE)

Figure 4

Model of antibody-dependent enhancement (ADE) of DENV replication and disease. ADE of virus replication is thought to occur when heterotypic, nonneutralizing antibody present in the host from a previous DENV infection binds to the virus during a subsequent heterotypic infection but is unable to neutralize the virus. Instead, the newly formed antibody-virus complex gains access to circulating monocytes via binding to the Fcγ receptors (FcγR), thereby facilitating the infection of FcγR cell types not readily infected in the absence of antibody. The overall result is an increase in virus replication and the level of viremia, which is associated with an increase in disease severity.

Note: DENV replicates poorly in FcγR-bearing cells in absence of heterotypic antibody

Increased access to FcγR-bearing cells leads to increased virus load and disease
Live Attenuated Vaccine – NIH /Butantan

Why develop a live attenuated vaccine?

1) Successful for other flaviviruses: YFV and JE;
2) It Induces both humoral and cellular immune responses;
3) Vaccination mimics the natural route of infection;
4) It is highly immunogenic;
5) Can induce lifelong immunity;
6) It is very economical to produce.
Dengue Vaccine Development at Butantan

- Live attenuated tetravalent dengue vaccine in Brazil:

  Laboratory of Infectious Diseases at NIAID/NIH and

  Butantan Institute
Target Product Profile (TPP)

- Live attenuated tetravalent dengue vaccine

- Single-dose vaccine
- All ages groups
- Lyophilized formulation
- $10^3$ PFU per dose of
- Stable 2-8°C
- Multi and/or uni-dose

Vaccine target: Prevention of Symptomatic Dengue
Immunogenicity Evaluation (USA) – After 1 dose $10^3$ PFU

**TV003 in flavivirus-experienced adults**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>% seroconverted ($\text{PRNT}_{50} \geq 10$)</th>
<th>Mean peak titer (GMT) ($\text{PRNT}_{50} \geq 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEN1</td>
<td>DEN2</td>
</tr>
<tr>
<td>TV003 (FV+)</td>
<td>40</td>
<td>93</td>
</tr>
<tr>
<td>TV003 (FV-)</td>
<td>40</td>
<td>92</td>
</tr>
</tbody>
</table>

- **TV-003 (+)**
  - Tetra-: 85%
  - Tri-: 32%
  - Bi-: 13%
  - Mono: 2%

- **TV-003 e**
  - Tetra-: 74%
  - Tri-: 23%
  - Bi-: 11%
  - Mono: 2%

98% with trivalent or better response after single dose
### TABLE 5: Seropositivity frequencies following one or two doses of the live attenuated tetravalent dengue vaccine with a lower (TV003) or higher (TV005) dose of the DENV-2 component

<table>
<thead>
<tr>
<th>Study/Admixture</th>
<th>Dose</th>
<th>N</th>
<th>DENV-1 (%)</th>
<th>DENV-2 (%)</th>
<th>DENV-3 (%)</th>
<th>DENV-4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIR279 TV003</td>
<td>1</td>
<td>38</td>
<td>35 (92)</td>
<td>29 (76)</td>
<td>37 (97)</td>
<td>38 (100)</td>
</tr>
<tr>
<td>CIR279 TV005</td>
<td>1</td>
<td>39</td>
<td>36 (92)</td>
<td>38 (97)</td>
<td>38 (97)</td>
<td>38 (97)</td>
</tr>
<tr>
<td>CIR279 TV003</td>
<td>2</td>
<td>34</td>
<td>33 (97)</td>
<td>32 (94)</td>
<td>34 (100)</td>
<td>34 (100)</td>
</tr>
<tr>
<td>CIR279 TV005</td>
<td>2</td>
<td>33</td>
<td>31 (94)</td>
<td>33 (100)</td>
<td>33 (100)</td>
<td>33 (100)</td>
</tr>
</tbody>
</table>

1. In CIR268, subjects were considered seropositive if they became seropositive at any time point out to study day 42. In CIR279, subjects were considered seropositive if they became seropositive at any time point out to study day 90.

2. Once a subject became seropositive, the subject was considered to be seropositive for all remaining analyses.

3. $P = 0.039$ (Fisher’s Exact Test)
Dengue Vaccine Produced by Butantan Institute

- TV-003 tetravalent vaccine has been produced by Butantan as lyophilized formulation contain $10^3$ plaque forming units (PFU) per 0.5 mL dose, of each virus

- Design: Phase II, Stepwise (A and B), Randomized, Double-blind and Controlled Clinical Trial

- Objective: to Evaluate the Safety and Immunogenicity of the Lyophilized Tetravalent Formulation
Phase II Clinical Trial

- University of São Paulo
  - School of Medicine, São Paulo
  - Child’s Institute
  - School of Medicine, Ribeirão Preto
- 300 volunteers, 18-59 y-o

**STEP A**
- 50 subjects, all vaccinated
  - Seronegative, by PRNT
  - 50 subjects: 20 liquid (NIH), 20 lyophilized (Butantan), 10 placebo
  - Intensive sample collection

**STEP B**
- 250 subjects, August 2014
  - Seropositive, by PRNT
  - 40 subjects: ~23 lyophilized (Butantan), 17 placebo
  - Intensive sample collection

- Any serostatus
  - 210 subjects: 140 lyophilized (Butantan), 70 placebo
Butantan Phase II Clinical Trial – Endpoints

- **Primary safety endpoint**
  - Frequency of vaccine-related AEs up to Day 21

- **Primary immunogenicity endpoint**
  - Seroconversion rate = $\text{PRNT}_{50} \geq 1:10$ 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 Phase II Clinical Trial – Step A
Solicited adverse events (n=50)

<table>
<thead>
<tr>
<th>Local Adverse Reactions*</th>
<th>N (%)</th>
<th>Systemic Adverse Reactions §</th>
<th>N (%)</th>
<th>Grade 1</th>
<th>Grade 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>4 (8%)</td>
<td>Nausea</td>
<td>1 (2%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Erythema</td>
<td>0</td>
<td>Vomiting</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swelling</td>
<td>0</td>
<td>Fever</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induration</td>
<td>0</td>
<td>Chills</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (2%)</td>
<td>Headache</td>
<td>25 (50%)</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retro-orbital pain</td>
<td>3 (6%)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Photophobia</td>
<td>1 (2%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malaise</td>
<td>4 (8%)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myalgia</td>
<td>5 (10%)</td>
<td>4</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Arthralgia</td>
<td>2 (4%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rash</td>
<td>34 (68%)</td>
<td>31</td>
<td>3</td>
</tr>
</tbody>
</table>

* All of them are Grade 1

§ No Grade 3 or 4 reactions were reported
Identification of clinical sites for epidemiological studies and for phase III trial

Potential – 24 sites

Dengue serotypes circulation in Brazil

Challenge: Financial support for the epidemiological studies – US$ 3,5 millions
Start of the epidemiological studies October 2014
Preparedness for the phase III trial
Dengue cases per age group
Brazil, 2007-2012

Fonte: SINAN.
Phase III trial: 2015-2019

- Design: June to October 2014 followed by presentation to ANVISA
- Sample size: ~15,000 (naive and experienced dengue participants)
  - Clinical sites from 15 to 20
  - 1 dose-vaccine
  - Follow-up period for active surveillance cases: 5 years
  - Efficacy - Prevention of Symptomatic Dengue
  - Safety and immunogenicity
  - Laboratory analysis
    - Screening
    - PRNT ~ 20%
    - Viremia (PCR) / Culture
    - Differential diagnostic
- Training / Monitoring
- Travelling to clinical sites
- Vaccine and samples transportation
- Statistician / Data analysis
Sanofi Pasteur Dengue Vaccine – criticisms on recent data release

- 3 doses required for a live attenuated vaccine
- Efficacy
  - Low overall efficacy 56.5%
  - Unbalanced efficacy: 50% - DEN1, 35% - DEN 2, 78.4% - DEN 3 and 75.3% - DEN 4
  - Dissociation between PRNT results and efficacy – data can not be extrapolated to other age groups
  - PRNT assay seems not fit to evaluate Sanofi Pasteur vaccine
- Hospitalization and hemorrhagic data by the serotype has not been provided
- Longer period of follow-up is necessary to evaluate the durability of antibody response, if there is any, and the potential risks associated to it – severe disease due to antibody enhancement
- Protocol had as primary endpoint prevention against dengue (any case confirmed by serology) and it was not designed to allow conclusions for severe cases as rate of hospitalization
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