Flavivirus Vaccines
Japanese Encephalitis and Dengue

14th Advanced Vaccinology Course
Veyrier du Lac, France
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The Presentation

- Comparisons
- Japanese Encephalitis Vaccine
  - The need - disease burden and distribution
  - WHO and GAVI perspectives
  - Status of vaccines
- Dengue Vaccines
  - The need – burden and lack of primary prevention tools
  - Dengue -
  - Vaccines – constructs and candidates
  - Lead-candidate vaccine trial
Flaviviruses

- Tick-borne encephalitis virus
  - West Nile Virus
  - Murray Valley Encephalitis Virus
  - Japanese Encephalitis Virus
  - St. Louis Encephalitis Virus

- DENV 1
- DENV 2
- DENV 3
- DENV 4

Yellow Fever Virus
Japanese Encephalitis and Dengue Life-cycles

Japanese Encephalitis

- Virus in lymph nodes, other organs, blood
- Vertical Transmission
- Vertical Transmission
- Mosquito infects susceptible person
- Mosquito infects susceptible person
- Mosquito acquires virus during feeding, virus replicates in mosquito
- Mosquito acquires virus during feeding, virus replicates in mosquito
- Reintroductions of infected mosquitoes or vertebrates
- Viral Amplification

Dengue

- Mosquito infects humans – virus in lymph nodes, other organs, blood
- Mosquito acquires virus during feeding, virus replicates in mosquito
- Mosquito acquires virus during feeding, virus replicates in mosquito
- Mosquito acquires virus during feeding, virus replicates in mosquito
- Mosquito acquires virus during feeding, virus replicates in mosquito

Source: Yu, T.F., 1994
### JE and Dengue Vaccine Status

<table>
<thead>
<tr>
<th>JE Vaccine</th>
<th>Dengue Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple licensed products</td>
<td>No licensed product</td>
</tr>
<tr>
<td>New / replacement vaccines</td>
<td>Multiple vaccines in trials</td>
</tr>
<tr>
<td>Strong pipeline</td>
<td>Strong pipeline</td>
</tr>
<tr>
<td>Inactivated, live attenuated, chimeric attenuated</td>
<td>Chimeric attenuated, inactivated, subunit</td>
</tr>
<tr>
<td>Indications: pediatric and adult</td>
<td>Indications(?): pediatric and adult</td>
</tr>
<tr>
<td>Need better data</td>
<td>Need data - vaccine performance</td>
</tr>
<tr>
<td>Need better diagnostics</td>
<td>Need better diagnostics</td>
</tr>
<tr>
<td>Need to increase usage</td>
<td></td>
</tr>
</tbody>
</table>
Japanese Encephalitis Vaccines
Japanese Encephalitis Surveillance

Source: J. Hombach, WHO-IVR
Japanese Encephalitis Disease Burden

3 billion people living in at-risk areas

50,000 cases reported annually

~30% of cases with neurologic deficits

10 - 15,000 deaths / yr (estimated)
The WHO Perspective

- Need for increased JE awareness and for vaccination in areas where a public health problem

- Most effective immunization strategy
  - one time campaign in target population, as defined by epidemiological data, followed by inclusion into routine immunization programme.

- SAGE supported JE immunization and recognized JE vaccine to be underutilized

Sources Weekly Epidemiological Record, 25 August 2006
SAGE 2008
The GAVI Perspective

- Prioritized JE vaccine (2008)
- Included JE in pledging conference (2011)
- Work Group has identified issues and options to guide countries to prepare applications for support
- Reviewed technical elements for applications e.g., disease burden data, target population, implementation strategy, vaccine status, critical gaps
- 2011 – decided to open funding window once WHO prequalified vaccine is available
### Status of JE Vaccination Programs

#### Endemic Countries

<table>
<thead>
<tr>
<th>Comprehensive*</th>
<th>Expanding</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>India</td>
<td>Bhutan</td>
</tr>
<tr>
<td>Japan</td>
<td>Malaysia</td>
<td>Brunei</td>
</tr>
<tr>
<td>South Korea</td>
<td>Cambodia</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Taiwan</td>
<td>North Korea</td>
<td>Myanmar</td>
</tr>
<tr>
<td>Thailand</td>
<td>Bangladesh</td>
<td>Philippines</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Laos (pilot)</td>
<td>Timor Leste</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nepal (initiating)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- No surveillance for JE

*Used in EPI or annual campaigns on a national or broad regional basis*
<table>
<thead>
<tr>
<th>Type</th>
<th>Strain</th>
<th>Producer</th>
<th>Status / WHO Prequalification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inactivated mouse-brain (JE - VAX)</strong></td>
<td>Nakayama Beijing</td>
<td>No longer produced</td>
<td>No new production WHO = no</td>
</tr>
<tr>
<td><strong>Inactivated Vero cell (IXIARO)</strong></td>
<td>SA 14-14-2</td>
<td>Intercell /Novartis Biological E</td>
<td>Licensed - US, Canada, EU (travellers); endemic area trials underway; India license WHO ?</td>
</tr>
<tr>
<td><strong>Inactivated Vero cell</strong></td>
<td>Beijing 1</td>
<td>Biken Kaketsuken</td>
<td>In development Japan use only</td>
</tr>
<tr>
<td><strong>Attenuated chimera, Vero cell (IMOJEV)</strong></td>
<td>SA14-14-2 / YF chimera</td>
<td>sanofi pasteur</td>
<td>Approved – Thailand, Australia, India</td>
</tr>
<tr>
<td><strong>Attenuated PHK based</strong></td>
<td>SA14-14-2</td>
<td>Chengdu Institute of Biological Products</td>
<td>Individual country registrations ? WHO 2013</td>
</tr>
</tbody>
</table>
## Current JE Vaccines

<table>
<thead>
<tr>
<th>Type</th>
<th>Doses</th>
<th>Booster Doses</th>
<th>Ages</th>
<th>Initial Efficacy studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated Vero cell (IXIARO)</td>
<td>2 (0, 28 days)</td>
<td>After 1-2 yrs, studies ongoing</td>
<td>≥17 yrs Peds under study</td>
<td>Immunogenicity (non-inferiority)</td>
</tr>
<tr>
<td>Attenuated chimera, Vero cell (IMOJEV)</td>
<td>1</td>
<td>None studies ongoing</td>
<td>≥12 months</td>
<td>Immunogenicity (non-inferiority)</td>
</tr>
<tr>
<td>Attenuated PHK based</td>
<td>1</td>
<td>After 1 yr, studies ongoing</td>
<td>≥ 9 months</td>
<td>Case-control Immunogenicity (non-inferiority)</td>
</tr>
</tbody>
</table>
Summary

- Routine childhood JE immunization with available vaccines is effective in high incidence areas.
- Expanding efforts to provide JE immunization in high risk areas.
- JE vaccines appear to have good safety profiles.
- Need to improve JEV diagnostics to obtain better disease burden estimates, improve surveillance and determine vaccine effectiveness.
Dengue
A Vaccinology Perspective
Dengue Virus Infection – Natural History

Infection Incidence
~ 5% / year

Asymptomatic
75%

Symptomatic
25%

Dengue Fever
98-99%

Severe Dengue
DHF/DSS
1-5%

Survive

Risk factors:
– Viral titer
2° Infection

Death
0.1 - 5%

• A major cause of febrile illness in endemic areas

Adapted from Vaccine 2004; 22: 1275-1280
Clinical Course of Dengue

Mosquito bite
- Range: 3 to 14 d; usually 4 to 7 days

Incubation
- Range: 2 to 7 days; usually 3 to 5 days

Viremia

Febrile Phase
- Muscle, joint, and/or bone pain, headache, eye pain, rash
- Range: 2 to 7 days; usually 3 to 5 days

Critical Phase
- 1 to 3 days; usually <48 hrs

Convalescent Phase
- Usually 3 to 5 days

Day of Illness
-2 0 2 4 6 8 10 12
Dengue – Diagnostic Events

Acute (febrile) Phase

Viremia

PCR (DENV RNA)

NS1 antigen detection (immunoassay)

IgM anti-DENV

0 1 2 3 4 5 6 7 8 9 10
Incubation Period

Days Post Onset of Fever

0 1 2 3 4 5 6 7 8 9 10
30 60 90

Days Post Onset of Fever
Why a Dengue Vaccine?

- Large burden - disease and economic
- Need for effective primary prevention tool
  - Present = vector control, does not work
- Would complement secondary prevention
  - Medical care has significantly reduced dengue mortality
## Dengue Burden

### Estimated burden of dengue, by continent, 2010

<table>
<thead>
<tr>
<th>Continent</th>
<th>Dengue (Millions (credible interval))</th>
<th>Inapparent infections (Millions (credible interval))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>15.7 (10.5-22.5)</td>
<td>48.4 (39.3-65.2)</td>
</tr>
<tr>
<td>Asia</td>
<td>66.8 (47.0-94.4)</td>
<td>204.4 (151.8-273.0)</td>
</tr>
<tr>
<td>Americas</td>
<td>13.3 (9.5-18.5)</td>
<td>40.5 (30.5-53.3)</td>
</tr>
<tr>
<td>Oceana</td>
<td>0.18 (0.11-0.28)</td>
<td>0.55 (0.35-0.82)</td>
</tr>
<tr>
<td><strong>Global</strong></td>
<td><strong>96 (67.1-135.6)</strong></td>
<td><strong>293.9 (217.0-392.3)</strong></td>
</tr>
</tbody>
</table>

Bhatt, S et al Nature 2013; 496: 504-507
Dengue Vaccines
Post-Infection Antibodies Protect Natural History Studies

- **Homotypic Antibodies**
  - Protect against homologous DENV disease / infection
    - (Sabin 1952; Halstead 1974)
  - Cohorts followed over multiple years

- **Heterotypic Antibodies**
  - Cross protection against disease ~ 6 months (Sabin, 1952)
  - Cross protection against infection may last longer
Problems with Antibodies
Antibody Dependent Enhancement of Infection (ADE)

- Enhanced infection in presence of heterotypic (non-neutralizing) antibodies
  - *In vitro* observations
  - Chimpanzee studies with passively transferred antibodies
  - AG129 interferon deficient mouse model

- Severe dengue (DHF) – epidemiologic observations
  - DHF among infants with 1\textsuperscript{st} DENV infection (passively acquired maternal antibody)
  - Increased risk for DHF with 2\textdegree infections
Types of Dengue Vaccine Candidates

- **Present Generation** (commercial development)
  - Cell culture adapted, live attenuated viruses
  - Infectious clones
    - chimeric viruses
    - attenuation by site directed mutagenesis
  - Recombinant subunits of DENV envelope proteins
  - Inactivated dengue viruses

- **Next Generation** (in development)
  - Viral vectored subunits
  - VLPs
  - Peptide chimeras
  - DNA
# Dengue Vaccine Candidates, Tetravalent (Commercial)

<table>
<thead>
<tr>
<th>Producer</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanofi Pasteur</strong></td>
<td>Live attenuated chimeric vaccine</td>
</tr>
<tr>
<td></td>
<td>17D yellow fever virus non-structural genes + respective DENV 1,2,3 or 4</td>
</tr>
<tr>
<td></td>
<td>envelope genes</td>
</tr>
<tr>
<td><strong>GSK</strong> <em>(WRAIR)</em></td>
<td>Switching from cell culture derived live attenuated vaccine to cell culture</td>
</tr>
<tr>
<td></td>
<td>derived inactivated vaccine</td>
</tr>
<tr>
<td><strong>Takeda</strong> <em>(InViragen, CDC)</em></td>
<td>Live attenuated chimeric vaccine</td>
</tr>
<tr>
<td></td>
<td>Attenuated DENV-2 + chimeras of DENV-2 non-structural genes + DENV 1,3, or 4</td>
</tr>
<tr>
<td></td>
<td>envelope genes</td>
</tr>
<tr>
<td><strong>Butantan</strong> <em>(NIAID)</em></td>
<td>Engineered mutations in 3’ NTR and non-structural genes of DENV-1, 2, 4 &amp; DENV-4/DEN-3 chimera</td>
</tr>
<tr>
<td><strong>Merck</strong> <em>(Hawaii Biotech)</em></td>
<td>Subunits of DENV 1,2,3,4 envelope protein expressed in Drosophila S2 cell lines + alum adjuvant</td>
</tr>
</tbody>
</table>
### Status of Dengue Vaccines (tetravalent)

<table>
<thead>
<tr>
<th>Producer / Developer</th>
<th>Process Development</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanofi Pasteur</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(YF-DENV chimeras)</td>
<td></td>
<td>2009 - 12</td>
</tr>
<tr>
<td><strong>GSK</strong></td>
<td></td>
<td>2013</td>
</tr>
<tr>
<td>(inactivated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NIAID</strong></td>
<td></td>
<td>2013</td>
</tr>
<tr>
<td>(DENV chimeras + engineered mutations)</td>
<td></td>
<td>2011</td>
</tr>
<tr>
<td><strong>InViragen</strong></td>
<td></td>
<td>2012</td>
</tr>
<tr>
<td>(attenuated DENV chimera)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Merck</strong></td>
<td></td>
<td>2012</td>
</tr>
<tr>
<td>(recombinant subunit)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **NIAID**: Phase I, Phase II, Phase IIB-III
- **Sanofi Pasteur**: Phase II
- **GSK**: Phase I
- **InViragen**: Phase I
- **Merck**: Phase I
Chimeric Flavivirus Vaccine Technology

Yellow fever 17D or Dengue genome cloned as cDNA

5' C prM E Nonstructural genes 3'

Exchange coat protein genes of dengue 1,2,3,4 (wild-type)

prM E

prM E

5' C prM E Non-structural genes 3'

Chimeric cDNA -> transcribe to RNA

5' C prM E Non-structural genes 3'

Transfect mRNA

Grow virus in cell culture

Envelope = heterologous virus

RNA replicative ‘engine’ = YF 17D or DENV
The Ideal Product Profile

- **Formulation:** Tetravalent protection (DENV 1-4)
- **Administration:** Delivery over 4 – 6 months and during established immunization visits
- **Storage:** off the cold chain
- **Immunogenicity:** high with ≤ 3 doses
- **Protection:** > 85% against dengue (dengue fever) ± dengue virus (DENV) infection
- **Long-term protection:** w/o booster doses
Dengue Vaccine Evaluation
Lack of Good Animal Models
Dengue

- Macaque models – short incubation period, infection only, no disease, does not readily predict immunogenicity in humans
- AG 129 interferon deficient mouse model – short incubation period, infection, disease (DHF)
- Human clinical trials required to determine performance of dengue vaccine candidates
Dengue Epidemiology
A Challenge to Vaccine Evaluation

- Disease presentation – acute febrile illness
- Incidence: high endemic + cyclical epidemics
- Highly seasonal
- Several circulating virus types (serotypes)
- Peak age of incidence varies by region
- Severe dengue, potential adverse event, is natural progression of disease

Dengue Vaccine Efficacy Trial Sites

- **Need for large population base** because of focal nature of dengue

- **Febrile illness surveillance** to identify DF cases and determine:
  - Age-specific disease incidence
  - Determine variation in incidence over several seasons (~3 yrs)

- **Molecular and immuno-diagnostic testing** for dengue (DF) = febrile illness ≥2 days + DENV viremia detected by PCR or NS1 antigen

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First Dengue Vaccine Efficacy Trial (Phase IIB)
Prospective study of cohort for acute febrile illnesses

- 3,013 children ages 3-13 with annual replacement with children 4-5 years of age

- Active surveillance for absences / febrile episodes in schools and home visits during vacations

- Fever = 37.5°C oral irrespective of duration

- Hospital clinic evaluation + blood draw + follow-up blood draw

- Diagnostic testing = DENV by PCR, IgM anti-DENV

From Sabchareon, A et al. PLoS NTD 2012; 6: e1732
Dengue in Ratchaburi, Thailand 2006 - 2009

- Cohort dropout rate ~4% (2008 = 14% due to enrollment in CYD 23 vaccine trial)
- 3.39 absences / child, 0.53 febrile episodes
- Dengue clinic visit - day post fever onset = 53% day 1-2, 30% day 3-4, 14% day 5-6
- Hospitalizations: 18%, 10%, 8%, 8%, respective yrs

From Sabchareon, A et al. PLoS NTD 2012; 6: e1732
Dengue Cases by Month, Ratchaburi, 2006 - 2009

Adapted from Sabchareon, A et al. PLoS NTD 2012; 6: e1732
Dengue Virus Serotypes, Ratchaburi 2006 - 2009

All years (%): DENV-1 (43); DENV-2 (29); DENV-3 (20); DENV-4 (8)

Adapted from Sabchareon, A et al. PLoS NTD 2012; 6: e1732
### Disease Severity, Ratchaburi, Thailand 2006 - 2009

- Used 1997 WHO Case Definitions

<table>
<thead>
<tr>
<th>Severity</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undifferentiated Fever (UF)</td>
<td>210</td>
<td>53.3</td>
</tr>
<tr>
<td>Dengue Fever (DF)</td>
<td>142</td>
<td>36.0</td>
</tr>
<tr>
<td>Dengue Hemorrhagic Fever (DHF)</td>
<td>42</td>
<td>10.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>394</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

- Hospitalization: UF= 15%; DF = 84%; DHF = 100%
- 86.3% = 2° infections, no association with severity
- No association of DENV serotype and severity

From Sabchareon, A et al. PLoS NTD 2012; 6: e1732
Dengue Vaccine Efficacy Trial (CYD 23) Ratchaburi, Thailand, 2009 - 2012

- Blinded, placebo-controlled, 2:1 individual randomization (Phase IIB)
- Vaccines
  - Dengue - tetravalent, live attenuated 17D YF- DENV chimera
  - Placebo – vaccine diluent (initially rabies vaccine)
- Sample size: 4002 children ages 4-11 years
- End-point: dengue fever (acute febrile illness + DENV viremia by PCR or NS1)
- Follow-up: 13 months after 3\textsuperscript{rd} vaccine dose

Adapted from Sabchareon, A et al. Lancet 2012; 380:1559-1567
## CYD23 Vaccine Trial, Ratchaburi, Thailand

### The Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vaccine (n=2669)</th>
<th>Placebo (n=1333)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td>8.18 yrs</td>
<td>8.23 yrs</td>
</tr>
<tr>
<td>Male</td>
<td>1187</td>
<td>48</td>
</tr>
</tbody>
</table>

### From the Immunogenicity Subset (n=300)

<table>
<thead>
<tr>
<th></th>
<th>Vaccine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-DENV (≥1 serotype)</td>
<td>138</td>
<td>68</td>
</tr>
<tr>
<td>Anti-JEV</td>
<td>157</td>
<td>77</td>
</tr>
</tbody>
</table>

Adapted from Sabchareon, A et al. Lancet 2012; 380:1559-1567
## Safety Results - CYD 23 Trial

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Dengue Vaccine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Analysis set</strong></td>
<td>2666</td>
<td></td>
</tr>
<tr>
<td>SAE – any (anytime)</td>
<td>315</td>
<td>11.8</td>
</tr>
<tr>
<td>SAE - vaccine related</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Analysis set</strong></td>
<td>697</td>
<td></td>
</tr>
<tr>
<td>AE - 30 minutes of injection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AE injection site (solicited within 7 days)</td>
<td>426</td>
<td>62</td>
</tr>
<tr>
<td>AE systemic (solicited within 14 days)</td>
<td>538</td>
<td>78</td>
</tr>
<tr>
<td>Discontinued study</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Clinical Outcomes of Dengue

- No differences between vaccine and placebo groups in clinical features or severity of dengue
  - Duration of clinical syndrome, fever or hospitalization
  - Bleeding, plasma leakage, thrombocytopenia, shock (n=0), organ impairment (n=1)
**Serotype Specific and Overall Efficacy CYD 23 Trial**

<table>
<thead>
<tr>
<th>Per protocol</th>
<th>Dengue Vaccine</th>
<th>Control</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person Years Risk</td>
<td>Cases</td>
<td>Person Years Risk</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2522</td>
<td>45</td>
<td>1251</td>
</tr>
<tr>
<td><strong>DENV 1</strong></td>
<td>2436</td>
<td>9</td>
<td>1251</td>
</tr>
<tr>
<td><strong>DENV 2</strong></td>
<td>2510</td>
<td>31</td>
<td>1250</td>
</tr>
<tr>
<td><strong>DENV 3</strong></td>
<td>2541</td>
<td>1</td>
<td>1263</td>
</tr>
<tr>
<td><strong>DENV 4</strong></td>
<td>2542</td>
<td>0</td>
<td>1265</td>
</tr>
</tbody>
</table>

# Immune Response in Trial Participants

**CYD 23 Trial**

<table>
<thead>
<tr>
<th>Per protocol</th>
<th>Dengue Vaccine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Seropositive PRNT&lt;sub&gt;50&lt;/sub&gt; &gt;10 (%)</td>
</tr>
<tr>
<td><strong>28 days post last dose</strong></td>
<td>N=95</td>
<td></td>
</tr>
<tr>
<td>DENV 1</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>DENV 2</td>
<td>94</td>
<td>99</td>
</tr>
<tr>
<td>DENV 3</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>DENV 4</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td><strong>1 year post last dose</strong></td>
<td>N=95</td>
<td></td>
</tr>
<tr>
<td>DENV 1</td>
<td>73</td>
<td>77</td>
</tr>
<tr>
<td>DENV 2</td>
<td>81</td>
<td>85</td>
</tr>
<tr>
<td>DENV 3</td>
<td>85</td>
<td>89</td>
</tr>
<tr>
<td>DENV 4</td>
<td>89</td>
<td>94</td>
</tr>
</tbody>
</table>

Conclusions

- Tetravalent, DENV – YF chimeric vaccine (CYD23) shown to be safe when administered to children living in dengue endemic area and high background of previous DENV infection.

- However, vaccine showed only partial (low) protection against dengue due to almost no protection against DENV – 2 infection.
Possible Explanations

- Statistical outliers - study not designed to look at serotype-specific results but ..... 
- Interference in immune response due to administration of multiple live vaccine viruses 
- WT virus (DENV), vaccine virus mismatch 
- Lack of stimulation of T-cells since DENV non-structural proteins NOT in vaccine (YF - backbone) 
- Present way to measure IgG anti-DENV. PRNT_{50} is not measuring the right (protective) antibody
Dengue Virus