Dengue Epidemiology and Vaccine Development

ACIP
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Overview

- Dengue Viruses
- The Need for a Vaccine
  - Clinical disease burden and lack of primary prevention tools
  - Vaccines – constructs and candidates
  - Epidemiologic challenges to vaccine evaluation
  - Results of lead-candidate vaccine trial
- Considerations
Belongs to Flavivirus genus of the Flaviviridae Family

Four antigenically distinct serotypes (DENV-1, DENV-2, DENV-2, DENV-4)

Enveloped

10.7 kb ssRNA genome

3 structural proteins: E, C, M
Phylogeny of Important Flaviviruses

- **Birds**
  - Mosquito (*Culex*)
  - West Nile virus
  - Japanese encephalitis virus
  - St. Louis encephalitis virus

- **Primates**
  - Mosquito (*Aedes*)
  - Dengue virus 1
  - Dengue virus 3
  - Dengue virus 2
  - Dengue virus 4
  - Zika virus
  - Spondweni virus
  - Yellow fever virus

- **Rodents**
  - Tick
  - Tick-borne encephalitis virus
Dengue Virus Transmission

Mosquito acquires virus during feeding
virus replicates in mosquito

Mosquito infects susceptible person

Mosquito infects humans – virus in lymph nodes, other organs blood

Mosquito acquires virus during feeding
virus replicates in mosquito
Dengue Global Burden

- Emerging disease, both epidemic and endemic in tropical and sub-tropical regions
- Estimated global burden
  - 390 million infections (285M-525M)
  - 96 million clinical infections
  - 2 million severe dengue cases
  - 20,000 deaths
Severe dengue

- Shock, hemorrhage or severe organ involvement
- Shock: systemic vascular permeability leading to vascular hypovolemia and dengue shock syndrome
- Hemorrhage: bleeding manifestations due to combined effects of thrombocytopenia and deranged hemostasis
- Severe organ impairment: Encephalitis, Hepatitis, Other
Risk factors for severe dengue

- Secondary infections
- Virus strain
- Host genetics
- Co-morbidities
- Young age
- Female
Dengue pathogenesis

- Viral burden, often linked to heterologous non-neutralizing antibody
- Elevated concentration of inflammatory mediators, cytokines and chemokines
- Immune response thought to promote capillary permeability – exact mechanism unclear
- Loss of essential coagulation proteins probably plays a major role in coagulopathy
Dengue Vaccine Status

- Registered: one vaccine in several countries
- Multiple other candidates
- Vaccine types: multiple formats
- Vaccine performance: multiple trials
- Indications: pediatric and adult
- Diagnostics
  - acute disease – very good
  - vaccine antibody - need better assays
Why a Dengue Vaccine?

- Large disease and economic burden
- Primary prevention
  - vector control not effective the last 50 years
  - need effective primary prevention tool
- Secondary prevention
  - medical management of severe dengue
  - vaccine would significantly reduce health care resources required for secondary prevention
Evidence
Consensus
Absent

Present

Adapted from Bhatt, S et al Nature 2013; 496: 504-507
# 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>Global</td>
<td>96 (67.1-135.6)</td>
<td>293.9 (217.0-392.3)</td>
</tr>
</tbody>
</table>

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

- **Neutralizing antibodies**
  - 50-70 % reduction in viral plaques (PRNT\(_{50-70}\))
  - Cell culture adapted viruses
  - Non-FC receptor bearing cells used in assays

- **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 1st DENV infection in presence of passively acquired maternal antibody
  - Increased risk for DHF with 2° infections
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
Types of Dengue Vaccine Candidates

- **Present Generation** (commercial development)
  - Live attenuated
    - Cell culture adapted
    - 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

<table>
<thead>
<tr>
<th>Producer (Developer)</th>
<th>Vaccine Type</th>
<th>Clinical Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanofi Pasteur</strong></td>
<td>Live attenuated - chimera 17D yellow fever + DENV</td>
<td><img src="#" alt="Phase III" /></td>
</tr>
<tr>
<td><strong>(Acambis)</strong></td>
<td>Live attenuated - chimera DENV-2 + DENV 1,3, 4</td>
<td><img src="#" alt="Phase II" /> <img src="#" alt="Phase III" /></td>
</tr>
<tr>
<td><strong>Takeda</strong></td>
<td>DENV attenuated - mutations + DENV/DENV chimera</td>
<td><img src="#" alt="Phase II" /> <img src="#" alt="Phase III" /></td>
</tr>
<tr>
<td><strong>(CDC, Invirogen)</strong></td>
<td>Cell culture derived, inactivated</td>
<td><img src="#" alt="Phase II" /></td>
</tr>
<tr>
<td><strong>Butantan</strong></td>
<td>Envelop subunits of DENVs</td>
<td><img src="#" alt="Phase II" /></td>
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<tr>
<td><strong>(NIAID)</strong></td>
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<tr>
<td><strong>GSK</strong></td>
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<tr>
<td><strong>(WRAIR)</strong></td>
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<td><strong>MERCK</strong></td>
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<tr>
<td><strong>(Hawaii Biotech)</strong></td>
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</table>
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)

5' | prM | E | prM | E |

Chimeric cDNA -> transcribe to RNA

5' | C | Non-structural genes | 3'

Transfect mRNA

Grow virus in cell culture

Envelope = heterologous virus

RNA replicative ‘engine’ = YF 17D or DENV
Dengue Vaccine Evaluation
Lack of Good Animal Models

- Macaque model – 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 challenge model – has been developed but rarely used

- Human clinical trials required to determine performance of dengue vaccine candidates
Dengue is an acute febrile illness (AFI) syndrome
- Only defined by diagnostic testing
- Other AFI’s in dengue endemic areas: malaria, influenza, leptospirosis, meliodosis, hepatitis A

Incidence: high endemic + cyclical epidemics

Highly seasonal

Several circulating virus types (serotypes)

Peak age of incidence varies by region

Severe dengue is natural progression of disease
Dengue Epidemiology
Challenge to Vaccine Efficacy Trials

- 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

**Dengue – Diagnostic Events**

- **rRT-PCR (DENV RNA)**
- **Febrile Phase of Dengue**
- **Viremia**
- **NS1 antigen detection (immunoassay)**
- **IgM anti-DENV**

- **Incubation Period**
- **Days Post Onset of Fever**

- **0 1 2 3 4 5 6 7 8 9 10**

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**Note:** The graph illustrates the typical diagnostic events during the febrile phase of Dengue fever, including the timing of rRT-PCR for DENV RNA, NS1 antigen detection, and the rise and fall of IgM anti-DENV antibodies.
Dengue Vaccine Efficacy Trials
~3,000 children ages 3-13, annual replacement 4-5 yo

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

Fever = 37.5°C oral irrespective of duration

0.53 febrile episodes/child/ year

Clinic visits by day post fever onset = 53% day 1-2, 30% day 3-4, 14% day 5-6

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 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

- Classification by 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>Total</td>
<td>394</td>
<td>100</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
Sanofi Dengue Vaccine Efficacy Trials
WHO Guidelines*

- Randomized, blinded, placebo-controlled (2:1)
- Ages: 2-16 years (highest disease incidence)
- 3 doses: given at 0, 6 & 12 months
  - Vaccine – tetravalent, live, attenuated
  - Placebo – normal saline vaccine diluent
- End point: Symptomatic, confirmed dengue fever
  - Clinical acute febrile illness + PCR-detected viremia
- Follow-up: 25 months total (13 months after last dose)
- Longer-term follow-up: 48 months

Guidelines for the clinical evaluation of dengue vaccines in endemic areas
Capeding MR, et al Lancet 2014; 834 1358-1365
## Sanofi Dengue Vaccine Efficacy Trials (CYD)

<table>
<thead>
<tr>
<th>Site(s)</th>
<th>Design (Phase)</th>
<th>N</th>
<th>Ages (yrs)</th>
<th>Pre-existing DENV antibody (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ratchaburi, Thailand</strong></td>
<td>Phase 2B</td>
<td>4002</td>
<td>4-11</td>
<td>69.5</td>
</tr>
<tr>
<td><strong>Asia</strong> – Indonesia, Malaysia, Philippines, Thailand, Vietnam</td>
<td>Phase 3</td>
<td>10,275</td>
<td>2-14</td>
<td>67.5</td>
</tr>
<tr>
<td><strong>Latin America</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colombia, Brazil, Mexico, Puerto Rico, Honduras</td>
<td>Phase 3</td>
<td>20,869</td>
<td>9-16</td>
<td>79.4</td>
</tr>
</tbody>
</table>

## Results of Efficacy Trials

Sanofi Vaccine (per protocol results)

<table>
<thead>
<tr>
<th>DENV specific</th>
<th>Phase IIB–Thailand N= 4,002</th>
<th>Phase III–Asia N= 10,275</th>
<th>Phase III–Latin America N= 20,869</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Efficacy</td>
<td>95% CI</td>
<td>Efficacy</td>
</tr>
<tr>
<td>All DENV’s</td>
<td>30.2</td>
<td>-13–57</td>
<td>56.5</td>
</tr>
<tr>
<td>DENV 1</td>
<td>55.6</td>
<td>22–84</td>
<td>50.0</td>
</tr>
<tr>
<td>DENV 2</td>
<td>9.2</td>
<td>-75–51</td>
<td>35.0</td>
</tr>
<tr>
<td>DENV 3</td>
<td>75.3</td>
<td>-38–100</td>
<td>78.4</td>
</tr>
<tr>
<td>DENV 4</td>
<td>100</td>
<td>25–100</td>
<td>75.3</td>
</tr>
</tbody>
</table>

Capeding MR, et al Lancet 2014; 834: 1358 1365
Villar L, et al. NEJM 2015: 372 113 123
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, organ impairment
Sanofi Vaccine Trials
Other Outcomes

- No safety signals observed in short-term
  - Long-term, blinded follow-up ongoing

- Poor immunogenicity and protection in children without previous DENV infection
Conclusions

- Tetravalent, DENV – chimeric yellow fever-dengue vaccine (CYD) 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 protection against dengue with lowest protection against DENV-2, followed by DENV-1, and highest protection against DENV-3 and 4.