# Dengue Epidemiology and Vaccine Development

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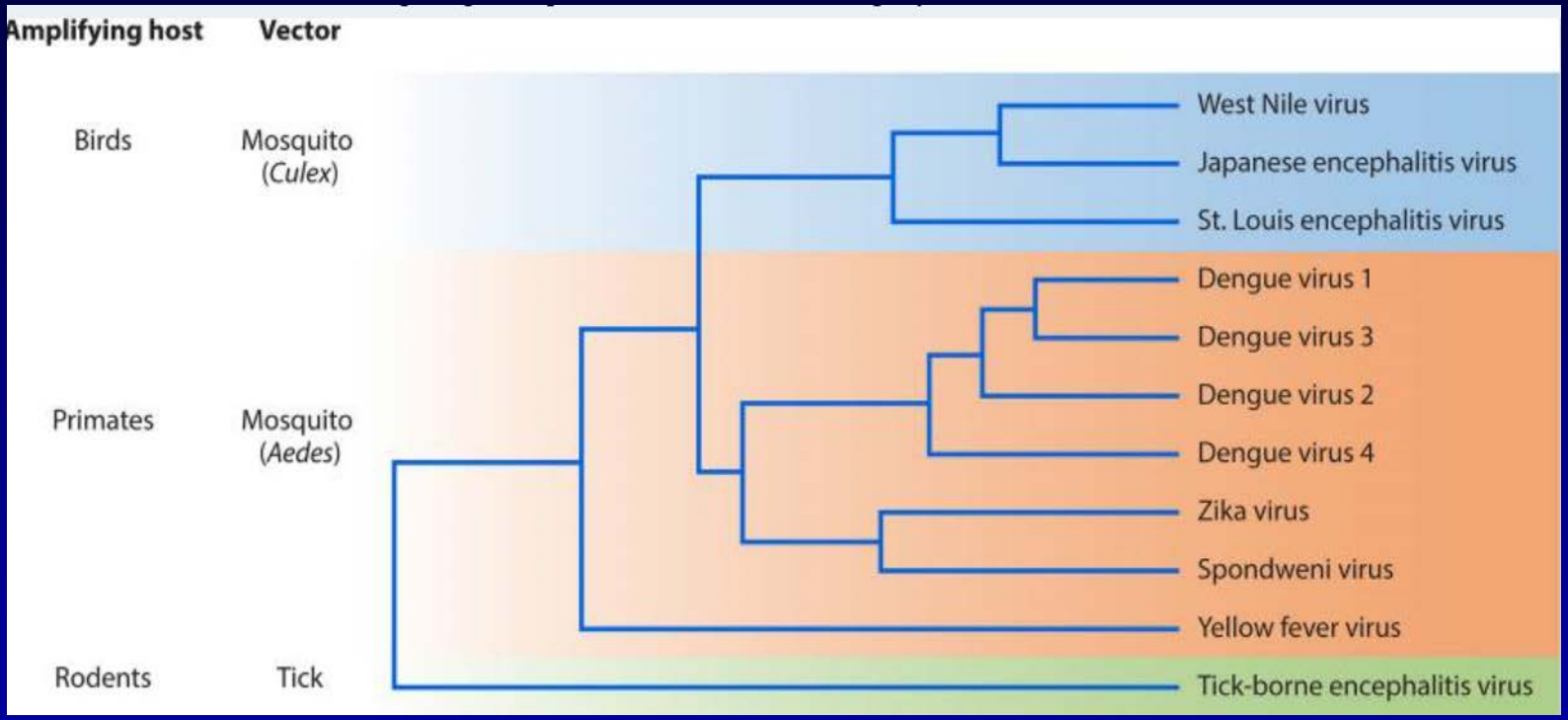
# Overview

- 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

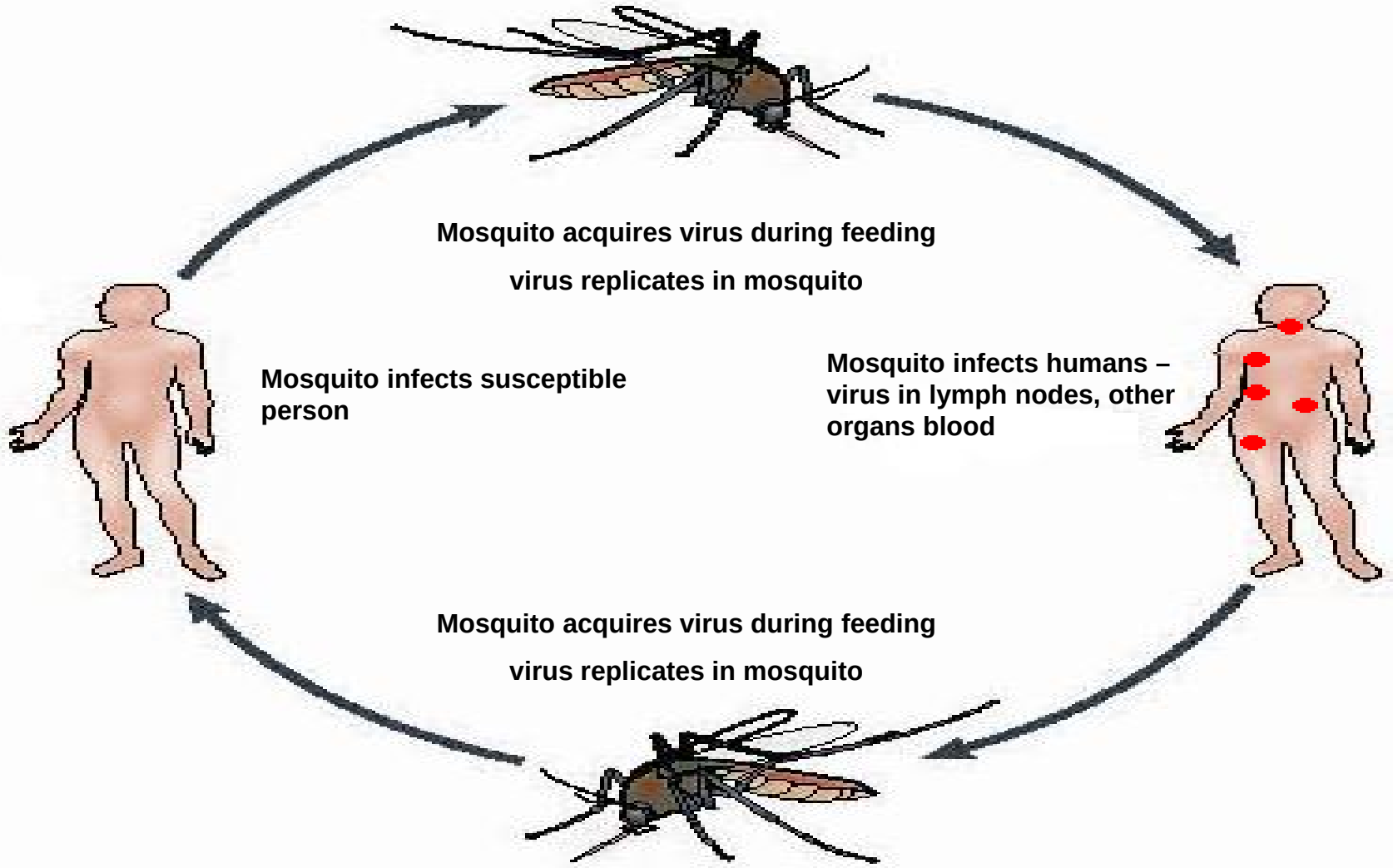
# Dengue virus

- 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



# Dengue Virus Transmission



# 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

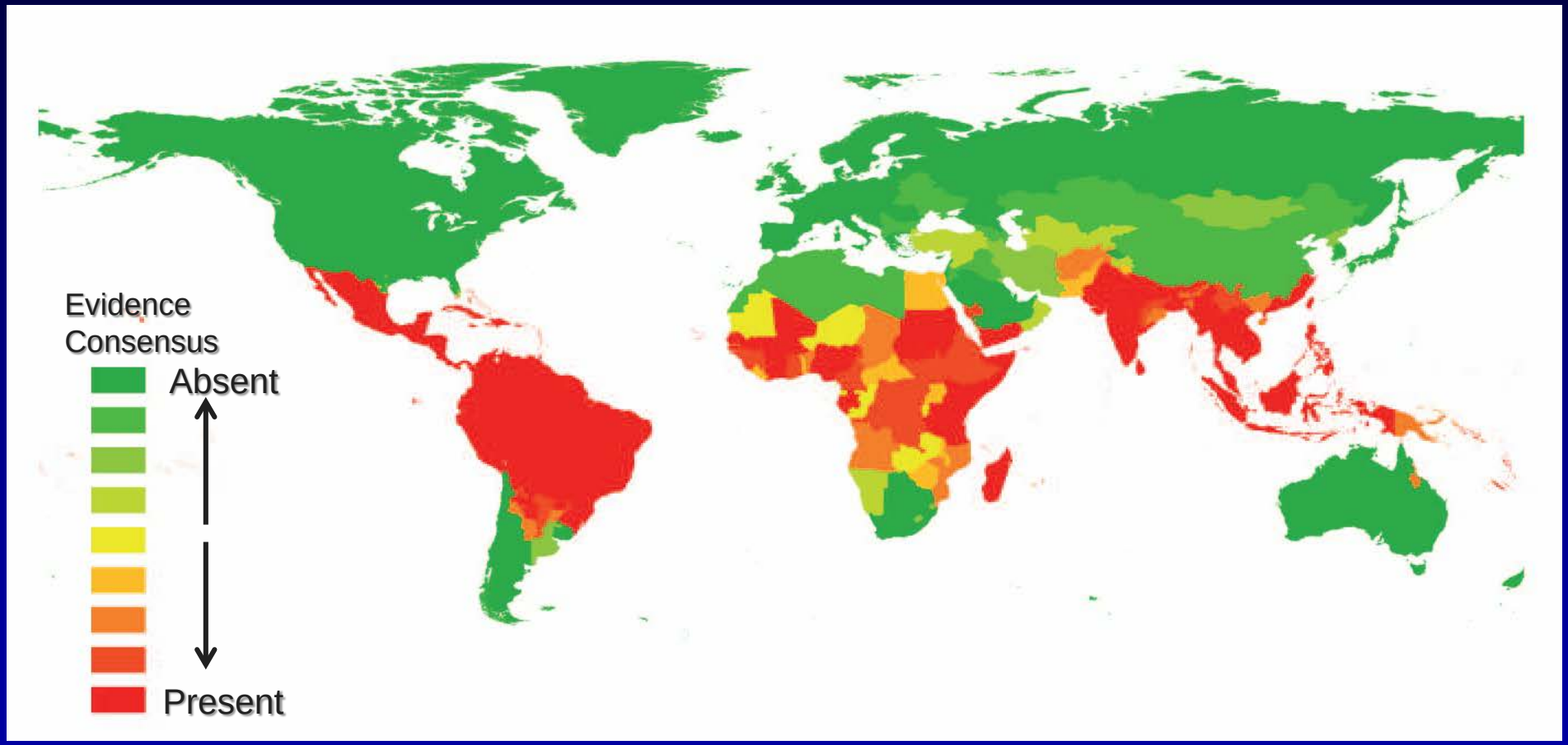
# Dengue Vaccine Status

- 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

# Dengue Globally
# Certainty of Information




Adapted from Bhatt, S et al Nature 2013; 496: 504-507

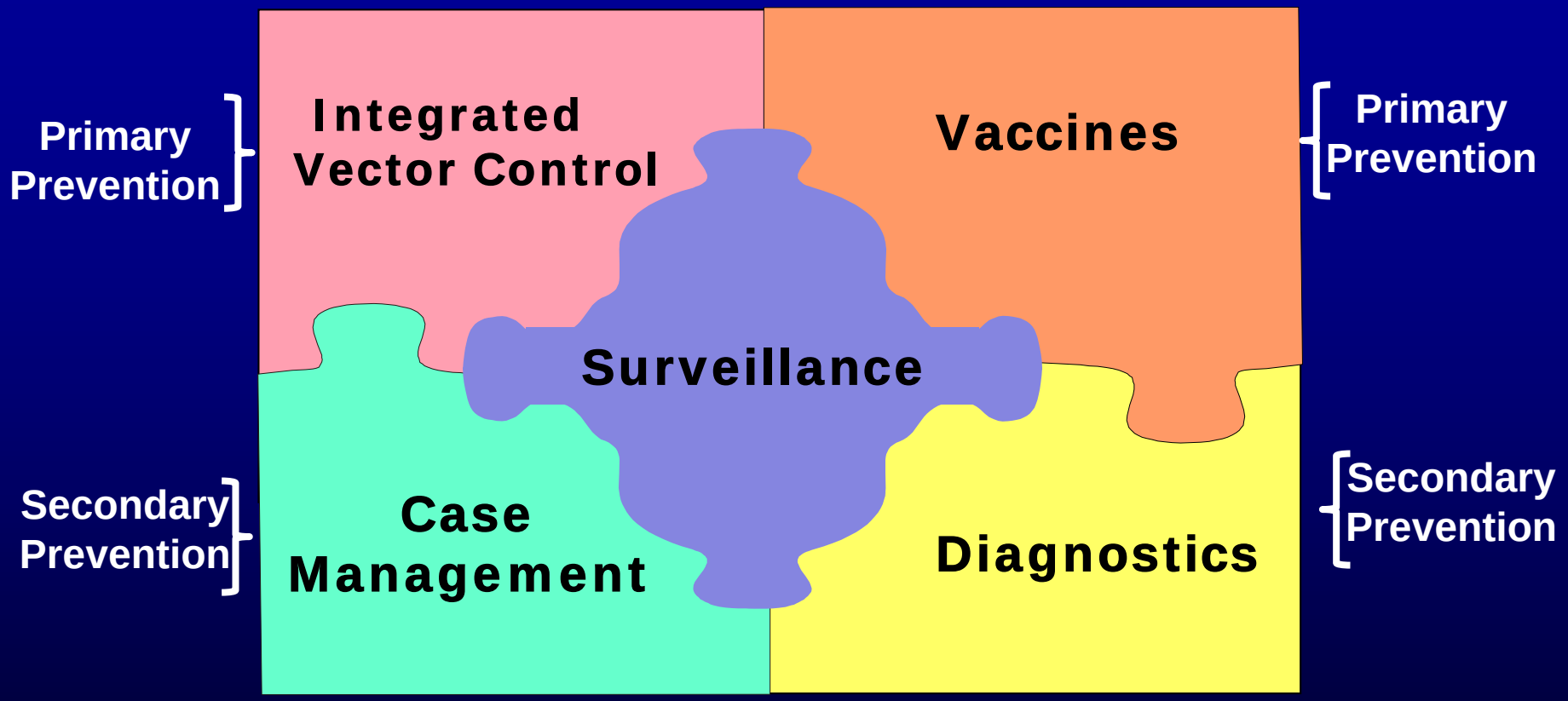
# Dengue Burden

## Estimated burden of dengue, by continent, 2010

| Continent | Dengue | Inapparent infections |
|---|---|---|
| | Millions (credible interval) | Millions (credible interval) |
| Africa | 15.7 (10.5-22.5) | 48.4 (39.3-65.2) |
| Asia | 66.8 (47.0-94.4) | 204.4 (151.8-273.0) |
| Americas | 13.3 (9.5-18.5) | 40.5 (30.5-53.3) |
| Oceana | 0.18 (0.11-0.28) | 0.55 (0.35-0.82) |
| *Global* | *96 (67.1-135.6)* | *293.9 (217.0-392.3)* |

Bhatt, S et al Nature 2013; 496: 504-507

# The Dengue Prevention Framework




Adapted from:

Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. New Edition. WHO, 2009

Global Strategy for Dengue Prevention and Control 2012-2020. WHO, 2012

# 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 1$^{st}$ DENV infection in presence of passively acquired maternal antibody
  - Increased risk for DHF with 2° infections

# The Ideal Product Profile

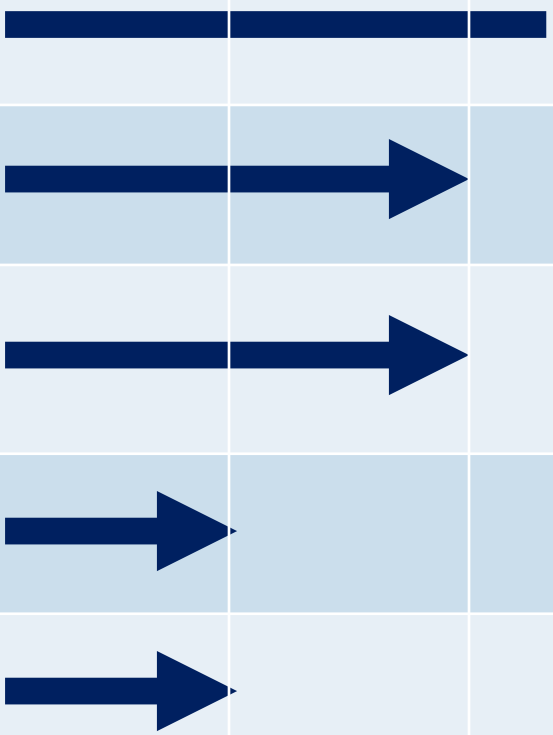
- **Formulation:** Tetravalent protection (DENV 1- 4)
- **Administration:** Delivery over 4 – 6 months and during established immunization visits
- **Storage:** off the cold chain
- **Immunogenicity:** high with ≤ 3 doses
- **Protection:** > 85% against dengue (dengue fever) ± dengue virus (DENV) infection
- **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

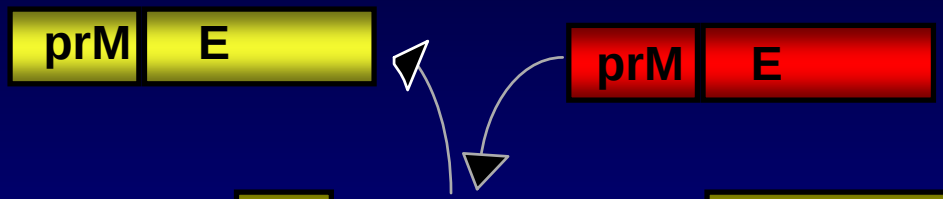
# Dengue Vaccine Candidates

| Producer (Developer) | Vaccine Type | Clinical Trial | | |
|---|---|---|---|---|
| | | Phase I | Phase II | Phase III |
| Sanofi Pasteur (Acambis) | Live attenuated - chimera 17D yellow fever + DENV | | | |
| Takeda (CDC, Invirogen) | Live attenuated - chimera DENV-2 + DENV 1,3, 4 | | | |
| Butantan (NIAID) | DENV attenuated - mutations + DENV/DENV chimera | | | |
| GSK (WRAIR) | Cell culture derived, inactivated | | | |
| MERCK (Hawaii Biotech) | Envelop subunits of DENVs | | | |



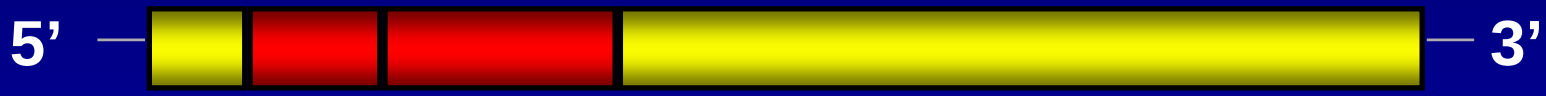
# Chimeric Flavivirus Vaccine Technology

Yellow fever 17D or Dengue genome cloned as cDNA

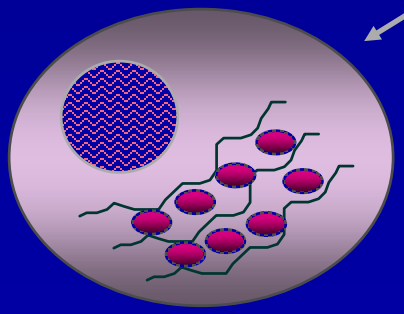




Exchange coat protein genes of dengue 1,2,3,4 (wild-type)

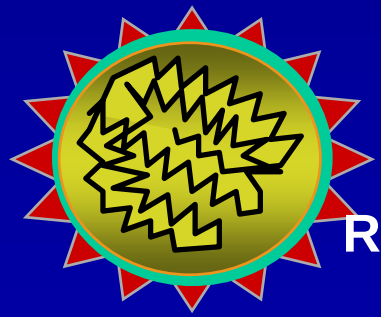

Chimeric cDNA –> transcribe to RNA




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 Epidemiology
# A Challenge to Vaccine Evaluation

- 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

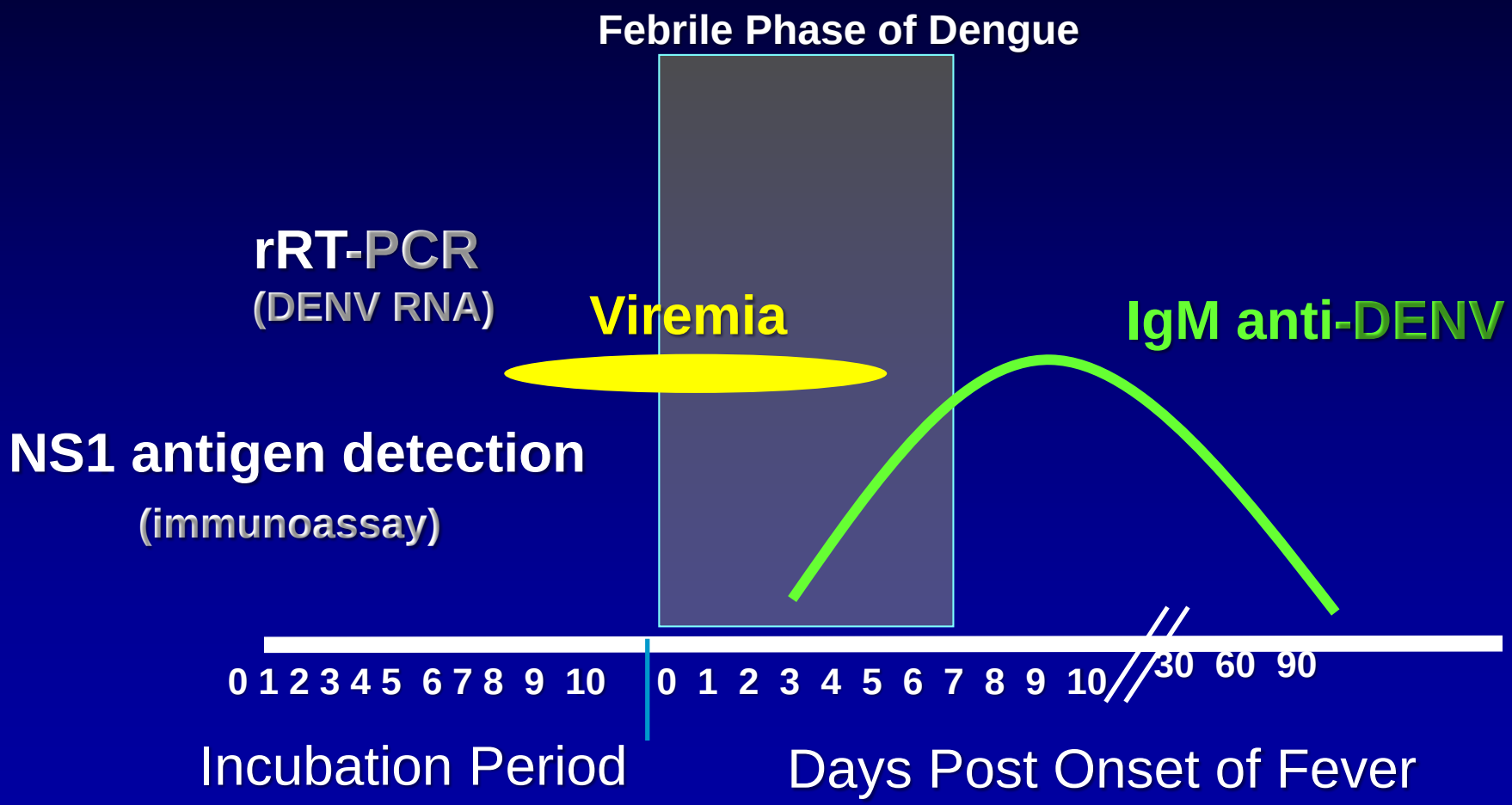
Guidelines for Clinical Evaluation of Dengue Vaccine in Dengue Endemic Areas. Vaccine 2008;26:4113-4119

# 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

Guidelines for Clinical Evaluation of Dengue Vaccine in Dengue Endemic Areas. Vaccine 2008;26:4113-4119

# Dengue – Diagnostic Events



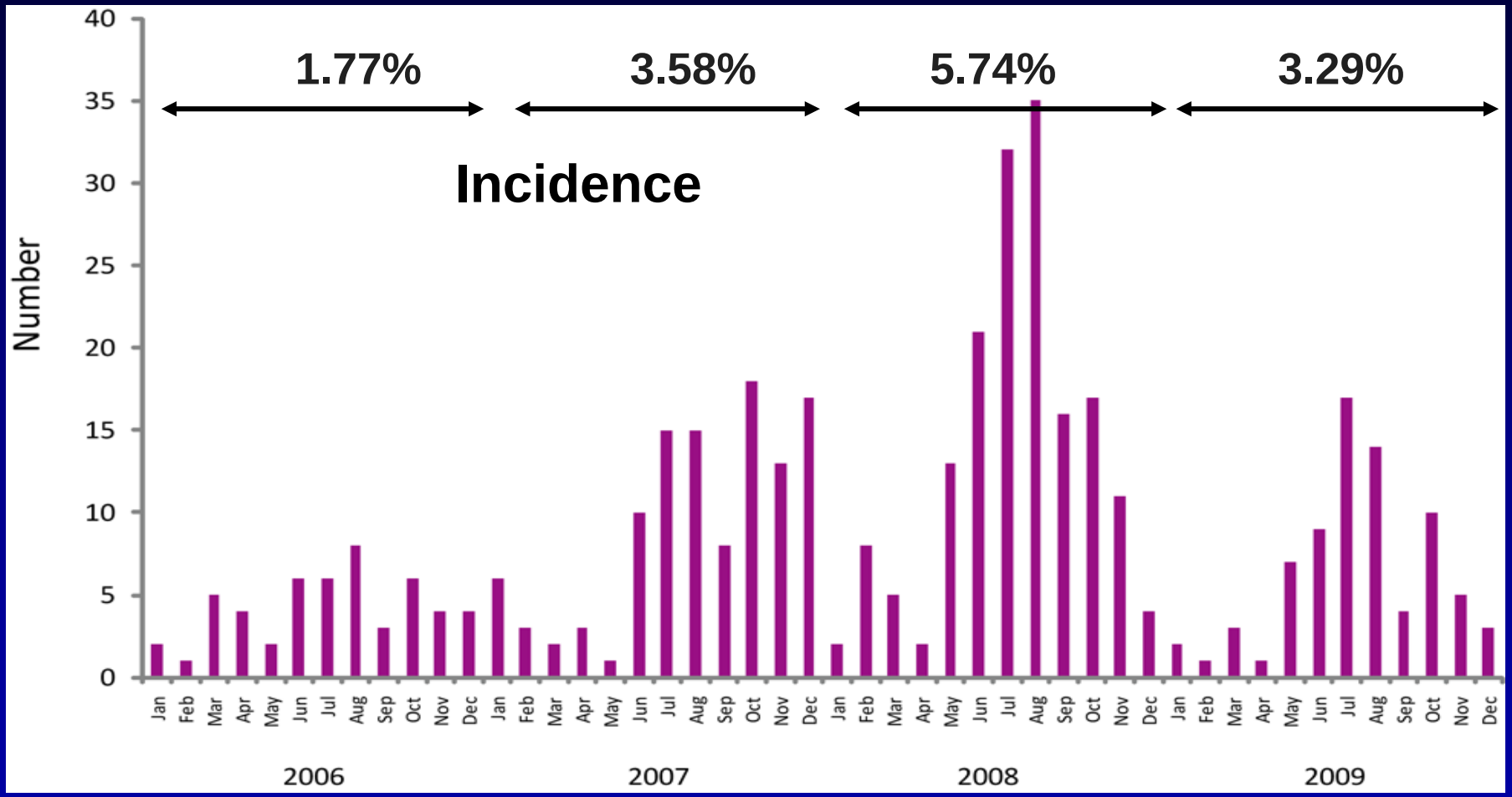
# Dengue Vaccine Efficacy Trials

# Dengue in Ratchaburi, Thailand Propsective Cohort Study, 2006 - 2009

- ~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

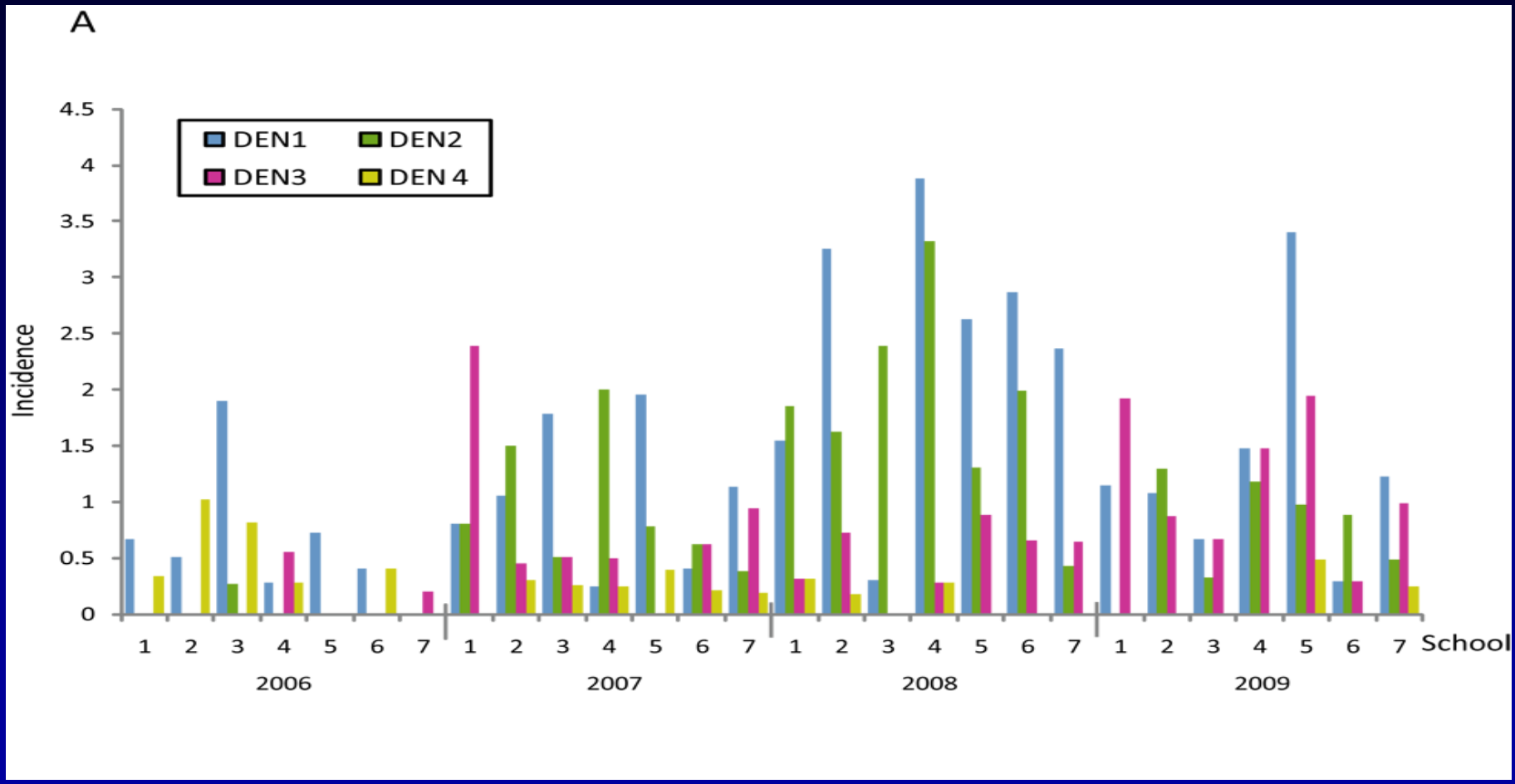
From Sabchareon, A et al. PLoS NTD 2012; 6: e1732

# 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

| Severity | Number | Percent |
|---|---|---|
| Undifferentiated Fever (UF) | 210 | 53.3 |
| Dengue Fever (DF) | 142 | 36.0 |
| Dengue Hemorrhagic Fever (DHF) | 42 | 10.7 |
| Total | 394 | 100 |

- 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
http://www.who.int/immunization/documents/WHO_IVB_08.12/en/
Capeding MR, et al Lancet 2014; 834 1358-1365

# Sanofi Dengue Vaccine Efficacy Trials (CYD)

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

**From: Sabchareon, A et al. Lancet 2012; 380:1559-1567; Capeding MR, et al Lancet 2014; 834: 1358-1365; Villar L, et al. NEJM 2015: 372 113-123.**

# Results of Efficacy Trials
# Sanofi Vaccine (per protocol results)

| DENV specific | Phase IIB–Thailand N= 4,002 | | Phase III–Asia N= 10,275 | | Phase III–Latin America N= 20,869 | |
|---|---|---|---|---|---|---|
| | Efficacy | 95% CI | Efficacy | 95% CI | Efficacy | 95% CI |
| All DENV's | 30.2 | -13–57 | 56.5 | 44–66 | 60.8 | 52–68 |
| DENV 1 | 55.6 | 22–84 | 50.0 | 25–67 | 50.3 | 29–65 |
| DENV 2 | 9.2 | -75–51 | 35.0 | -9–61 | 42.3 | 14–61 |
| DENV 3 | 75.3 | -38–100 | 78.4 | 53–91 | 74.0 | 62–82 |
| DENV 4 | 100 | 25–100 | 75.3 | 55–87 | 77.7 | 60–88 |

Sabchareon, A et al. Lancet 2012; 380:1559 1567
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