The Dengue disease

‘Vaccinology 2008’
Cartagena, Colombia
June 4, 2008

Alain Bouckenooghe MD MPH DTMH
sanofi pasteur
Clinical Development
Traveler’s and Endemic diseases
Swiftwater, PA, USA
Overview of the presentation

- Dengue virus and disease
- Live attenuated vaccines and second generation
- Product
- Preclinical data
- General overview of studies
- Clinical study results
  - Results of Ph I trials (CYD01/02)
  - Partial results of Ph II trials (CYD04/05/06/10)
  - Efficacy trials and further development
- Conclusions
**Dengue virus**

- Flavivirus (YF, JE)
- Single stranded RNA genome
  - 3 structural proteins
  - 7 non structural proteins

**4 Dengue virus serotypes**

- Den 1
- Den 2
- Den 3
- Den 4

**Clinical diagnosis**
- Sub clinical infections
- Fever
- Classic Dengue
- Hemorrhagic Dengue Fever

**Serological diagnosis**
- Primary infection
- Secondary infection

Transmission by Aedes aegypti
Dengue Fever and Dengue Hemorrhagic Fever are a threat to more than 2.5 billion people in tropical and subtropical regions.
Resurgence ex: reinfestation of Aedes aegypti in South and Latin America
Serotypes distribution, 1970

Serotypes distribution, 2004

Dengue

Flavivirus
4 serotypes

Mosquito Transmission

Pathogenesis

- Target tissues include monocytes / macrophages and dendritic cells are permissive to infection with dengue virus
- Homologous (same serotype) immunity is probably lifelong
- Infection with one serotype does not provide lifelong cross-protective immunity
- Most clinically overt illness probably occurs during primary or secondary infections (ADE controversial theory, unproven in-vivo in humans)
Viremia, Fever and Antibody in Secondary Dengue Natural Infection

Ref 15- adapted from CDC slide kit viremia, 2008 and
Ref 7 : Vaughn, J Infect Dis; 1997, Aug 176 (2) 322-30
Comparison of clinical features of dengue fever and dengue hemorrhagic fever

<table>
<thead>
<tr>
<th>Feature</th>
<th>DF</th>
<th>DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myalgias</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rash</td>
<td>0/+</td>
<td>0/+</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0/+</td>
<td>0/+</td>
</tr>
<tr>
<td>Plasma leakage</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>
Haematological findings in DHF:

- Low platelet count <100X10^9/l
- Haemoconcentration (rise in the packed cell volume >20% of basal level)
- Leucopenia early in the illness
- Atypical lymphocytosis (>15%)
- Abnormal coagulation profile

Biochemical investigations

- Low albumin levels
- Electrolytes disturbances
- Elevated liver enzymes
- Acidosis

Frequency of dengue syndromes among Thai children aged 1-14 yrs

Halstead SB, 1980

Ref 2: Malavige GN, Postgrad Med J. 2004 Oct;80(948):592
Dengue Hemorrhagic Fever: Hypotheses

Racial/genetic host factor

HUMAN

IMMUNE RESPONSE

VIRUS

Virus load

Virulence of some strains

mosquito

Subneutralizing antibody
Cellular immune response
Cytokines

mosquito
Management of Dengue infections

- Mainly symptomatic (no specific drugs against dengue virus)
  - Temperature control with paracetamol and tepid sponging
  - Light diet
  - Early identification of leakage phase
  - Proper maintenance of fluid balance
  - Monitor platelet count, packed cell volume, coagulation parameters
  - Adequate fluid administration according to severity

Dengue Vaccine development

- Important public health need for vaccines, there are no approved therapeutics
- Different vaccine approaches
  - Inactivated virus
  - Live attenuated virus
    - Traditional culture techniques
      - reactogenicity and stability problems
    - Recombinant vaccines
- Others: DNA, proteins
Dengue Vaccine Developments

- **Butantan**
  - NIH licensee

- **Biological E.**
  - NIH licensee

- **Panacea**
  - NIH licensee

- **Fiocruz**
  - Chimeric

- **Hawaii Biotech/NIH/WRAIR/GSK**
  - Subunit

- **Inviragen/CDC/Shantha**
  - Live attenuated
  - DEN/DEN Chimeric

- **WRAIR/Crucell**
  - Whole virus
  - Inactivated

- **NMRC/WRAIR**
  - DNA vaccine
    - (monovalent)

- **Sanofi pasteur Chimeric**
  - Live attenuated

- **NIH/Univ. Of Maryland**
  - DEN/DEN Chimeric

- **GSK/WRAIR**
  - Live attenuated

- **Preclinical**

- **Phase I**

- **Phase II**

- **Phase III**
PDK Mahidol / sanofi pasteur First Generation
Tetravalent Dengue Vaccine

Establishing The Dengue Tetravalent Live Attenuated Vaccine Proof of Concept in Thai Adults and Children

- Live-attenuated whole virion vaccine
- Tetravalent = Combined vaccine of the 4 polyclonal monovalents pre-MS Issued from the Thai Mahidol University
  - DEN 1 (parent 16007/ PDK-13),
  - DEN 2 (parent 16681/ PDK-53),
  - DEN 3 (parent 16562/ PGMK-30/FRhL-3)
  - DEN 4 (parent 1036/ PDK-48)
- GMP manufacture at Master, Working & Bulk levels in PDK certified cells
- Lyophylised & stabilized clinical lots
- Subcutaneous route
- 0.5mL
GMTs of neutralizing antibody to DEN 1-4 in children recipients of dengue vaccine (Study-2) without Wt dengue infection (A, n=74) and with Wt dengue infection (B, n=8)

Antibodies to dengue viruses of dengue vaccine recipients were well persisting. GMT values of neutralizing antibodies for den-1 and den-2 of dengue vaccine recipients with Wt dengue were significantly higher than those of vaccine recipients without Wt dengue infection and surpassed that for den-3.
Common Systemic Reactions and Rash after 1<sup>st</sup>, 2<sup>nd</sup> and Booster dose of Dengue vaccine F1 and F2

- **Fever**
  - Dose 1: F1 (3212) (n=40, 39, 33)
  - Dose 2: F2 (3313) (n=42, 40, 38)

- **Headache**

- **Myalgia**

- **Nausea**

- **Rash**

The diagram shows the percentage distribution of common reactions and rash after different doses of the Dengue vaccine.
Second generation: Attenuated dengue construction

17D YF genome cloned as cDNA

Exchange dengue envelope protein genes

Recombinant cDNA

*Chimerivax™ technology, Acambis
Sanofi pasteur second generation
dengue vaccine

Recombinant technology replacing the genes for pre-M and E proteins of 17D YF virus vaccine with those of other flaviviruses

The resulting live attenuated viruses containing replication engine of 17D YF vaccine strain but the coat proteins of each dengue serotypes
INDICATION

- Prevention of symptomatic dengue disease i.e. covering the spectrum from Dengue Fever to severe Dengue cases due to serotypes 1, 2, 3 or 4.

- Priority: children to adults in all endemic countries (Asia, Latin America, and US Caribbean areas)

- Travelers indication for children and adults from non-endemic areas
Basic Preclinical data

Chimeric Tetravalent Dengue Vaccine

- Genetically stable
- Not neurovirulent in 3-4 week old mice (IC route)
- Less neurovirulent than YF 17D in suckling mouse and monkey models (IC route)
- Does not become more neurovirulent upon extensive in vitro passages
- Lower replication rate than YF 17D in liver cells
- Does not infect mosquitoes by insect’s oral route
- Replicates in mosquitoes by IT route similarly to the YF 17D virus and significantly lower than their WT parent viruses
- Protects monkeys upon a single dose vaccination against heterologous WT challenge
- Induces low viremia and high neutralizing (last > 1 year) response when administered as a single SC monovalent dose to monkeys
- Lack of negative interference with YF vaccine
<table>
<thead>
<tr>
<th>Code</th>
<th>Dengue Vaccine</th>
<th>Populations</th>
<th>Country</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYD01</td>
<td>Chimerivax Monovalent D2 (3&amp;5 log10 PFU)</td>
<td>Adults (18-40 yo) n=56</td>
<td>US</td>
<td>Completed (2002)</td>
</tr>
<tr>
<td>CYD02</td>
<td>Chimerivax Tetravalent (4 log10 TCID50/serotype)</td>
<td>Adults (18-40 yo) n=99</td>
<td>US</td>
<td>Completed (2005)</td>
</tr>
<tr>
<td>CYD04</td>
<td>Chimerivax Tetravalent (5 log10 TCID50/serotype)</td>
<td>Adults (18-45 yo) n=66</td>
<td>US</td>
<td>Completed</td>
</tr>
<tr>
<td>CYD05</td>
<td>Chimerivax Tetravalent (5 log10 TCID50/serotype)</td>
<td>Adults (18-45 yo) Adolescents (12-17 yo), Children (2-11 yo) n=126</td>
<td>Philippines</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CYD06</td>
<td>Chimerivax Tetravalent (5 log10 TCID50/serotype)</td>
<td>Adults (18-45 yo) Adolescents (12-17 yo), Children (2-11 yo) n=126</td>
<td>Mexico</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CYD10</td>
<td>Chimerivax Tetravalent (5 log10 TCID50/serotype)</td>
<td>Adults (18-40 yo) (DIV12 Trial subjects, VDV1, VDV2 or YF primed) Max n=48</td>
<td>Australia</td>
<td>Completed</td>
</tr>
</tbody>
</table>
Ph I completed
Two Ph I studies

CYD01: monovalent (DEN-2) vaccine was given to 56 healthy US adults
- Good reactogenicity profile in YF naïve and YF vaccinated
- Good immune response at 1 month, and maintained up to 12 months
- Log 5 seemed better than log 3 dose
- Guirakhoo et al. Hum Vaccin. 2006;2(2):60-7

CYD02: tetravalent (log 4) study with 99 healthy US adults
- AE rates equal to placebo and trend to lower rate compared to YF
- Injection site reactions lowest in TV group
- No increase in AE with second dose
CYD02 Trial: Safety profile post dose 1 and 2 (% Subjects with adverse reactions)

Group ChimeriVax TV - 2 doses
- Severe: 15.2% (Dose 1), 0% (Dose 2)
- Moderate: 34.5% (Dose 1), 34.5% (Dose 2)
- Mild: 57.6% (Dose 1), 0% (Dose 2)

Group YFV - ChimeriVax TV
- Severe: 42.4% (YFV), 0% (Dose 1)
- Moderate: 26.9% (YFV), 57.7% (Dose 1)
- Mild: 34.5% (YFV), 0% (Dose 1)

Group Placebo - ChimeriVax TV
- Severe: 0% (Placebo), 0% (Dose 1)
- Moderate: 18.2% (Placebo), 37.5% (Dose 1)
- Mild: 54.5% (Placebo), 41.7% (Dose 1)
CYD02: post dose 1 systemic reactogenicity profile

- **ChimeriVax** (n=33)
  - Headache
  - Myalgia
  - Malaise
  - Fatigue
  - Pyrexia

- **YFV** (n=33)
  - Headache
  - Myalgia
  - Malaise
  - Fatigue
  - Pyrexia

- **Placebo** (n=33)
  - Headache
  - Myalgia
  - Malaise
  - Fatigue
  - Pyrexia

**Graph Details**:
- **Axes**:
  - X-axis: Percentage
  - Y-axis: Symptoms
- **Legend**:
  - **Mild**
  - **Moderate**
  - **Severe**
- **Legend Position**: Top right

**Data Breakdown**:
- **ChimeriVax**:
  - **Headache**: Mild (30), Moderate (40), Severe (50)
  - **Myalgia**: Mild (20), Moderate (30), Severe (40)
  - **Malaise**: Mild (20), Moderate (30), Severe (40)
  - **Fatigue**: Mild (20), Moderate (30), Severe (40)
  - **Pyrexia**: Mild (5), Moderate (10), Severe (15)
- **YFV**:
  - **Headache**: Mild (30), Moderate (40), Severe (50)
  - **Myalgia**: Mild (20), Moderate (30), Severe (40)
  - **Malaise**: Mild (20), Moderate (30), Severe (40)
  - **Fatigue**: Mild (20), Moderate (30), Severe (40)
  - **Pyrexia**: Mild (5), Moderate (10), Severe (15)
- **Placebo**:
  - **Headache**: Mild (10), Moderate (20), Severe (30)
  - **Myalgia**: Mild (10), Moderate (20), Severe (30)
  - **Malaise**: Mild (10), Moderate (20), Severe (30)
  - **Fatigue**: Mild (10), Moderate (20), Severe (30)
  - **Pyrexia**: Mild (5), Moderate (10), Severe (15)
CYD02: Seroconversion Post-dose 1 & 2
Neutralizing antibody response (% subjects with antibody level ≥1:10)

**Serotype 1**

- Pre-dose 1
- Pre-dose 2
- Post-dose 2

**Serotype 2**

- Pre-dose 1
- Pre-dose 2
- Post-dose 2

**Serotype 3**

- Pre-dose 1
- Pre-dose 2
- Post-dose 2

**Serotype 4**

- Pre-dose 1
- Pre-dose 2
- Post-dose 2
• Ph I studies: acceptable reactogenicity profile, good safety, low viremia, good immune responses

Ph II studies
CYD 04
CYD05
CYD06
CYD10
### Three phase II observer-blind randomized controlled trials

<table>
<thead>
<tr>
<th>Study:</th>
<th>USA CYD04</th>
<th>Philippines CYD05</th>
<th>Mexico CYD06</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
<td>66 Adults 18-40yr</td>
<td>18 Adults 18-45yr 36 Adolescents 12-17yr 72 Children 2-11yr</td>
<td>18 Adults 18-45yr 36 Adolescents 12-17yr 72 Children 2-11yr</td>
</tr>
<tr>
<td><strong>FV Immune status at baseline</strong></td>
<td>3%</td>
<td>80.1 %</td>
<td>7.9 %</td>
</tr>
<tr>
<td><strong>Protocol</strong></td>
<td>--------3 injections DV or control: Months 0, 3-4, 12--------</td>
<td>DV, DV, DV</td>
<td>DV, DV, DV</td>
</tr>
<tr>
<td><strong>Group 1:</strong></td>
<td>DV*, DV, DV</td>
<td>DV, DV, DV</td>
<td>DV, DV, DV</td>
</tr>
<tr>
<td><strong>Group 2:</strong></td>
<td>*Placebo, DV, DV</td>
<td>*TyphimVi®, DV, DV</td>
<td>*Stamaril®, DV, DV</td>
</tr>
<tr>
<td><strong>Objective</strong></td>
<td>To describe: safety, viremia and humoral immune responses, after each vaccine injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Viremia testing:</strong></td>
<td>1) Screening of samples with a YF NS5 qRT-PCR method 2) Testing of positive samples by plaque assay, and by four RT-PCRs, specific to each dengue vaccine strain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*DV= Dengue Vaccine a log 5555doses
**based on neut antibodies (dengue and JE for the Philippines)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYD05 Trial</strong></td>
<td>N=18</td>
<td>N=36</td>
<td>N=36</td>
<td>N=36</td>
<td>N=126</td>
</tr>
<tr>
<td>(dengue endemic area)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavivirus positive</td>
<td>100%</td>
<td>97.2%</td>
<td>66.6%</td>
<td>66.6%</td>
<td>80.1%</td>
</tr>
<tr>
<td>(presence of neutralizing antibody against dengue and/or JE at baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYD06 Trial</strong></td>
<td>N=18</td>
<td>N=36</td>
<td>N=36</td>
<td>N=36</td>
<td>N=126</td>
</tr>
<tr>
<td>(non-dengue endemic area)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavivirus positive</td>
<td>16.6%</td>
<td>8.3%</td>
<td>8.3%</td>
<td>2.7%</td>
<td>7.9%</td>
</tr>
<tr>
<td>(presence of neutralizing antibody against dengue at baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ph II safety
Post-dose 1 Reactogenicity of tetravalent dengue vaccine vs placebo, typhoid or yellow fever vaccines

Compared to a placebo, there is a trend in the FV naïve population to a higher proportion of subjects experiencing systemic reactions, in terms of asthenia, and myalgia.

Compared to active control, there is no difference in the proportion of subjects experiencing systemic reactions.

Overall the majority of adverse events were mild-moderate and transient.
Reactogenicity of each dose of CYD vaccine in CYD04/CYD05/CYD06 Trials (% Subjects with adverse events)

<table>
<thead>
<tr>
<th></th>
<th>CYD04 Trial</th>
<th>CYD05 Trial</th>
<th>CYD06 Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose 1 (n=33)</td>
<td>Dose 2 (n=30)</td>
<td>Dose 3 (n=23)</td>
</tr>
<tr>
<td>Any Adverse event</td>
<td>84.8</td>
<td>80.0</td>
<td>65.2</td>
</tr>
<tr>
<td>Any Adverse reaction</td>
<td>81.8</td>
<td>70.0</td>
<td>43.5</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>18.2</td>
<td>36.7</td>
<td>17.4</td>
</tr>
<tr>
<td>Systemic reaction</td>
<td>78.8</td>
<td>66.7</td>
<td>43.5</td>
</tr>
<tr>
<td>Severe Injection site reaction</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Severe Systemic reaction</td>
<td>15.2</td>
<td>3.3</td>
<td>0s</td>
</tr>
</tbody>
</table>

➢ No increase of the incidence of AE after a 2nd or a 3rd dose in comparison to a 1st dose and even a decrease after the 3rd dose in CYD04 trial and after the 2nd dose in CYD05 trial.
Effect of age and dengue endemicity on post-dose 1 reactogenicity of dengue vaccine

**Philippines (endemic)**
80% Den/JE neut ab positive at baseline

**Mexico (non-endemic)**
8% Den neut ab positive at baseline

**Fewer systemic reactions reported for children, except fever but**
- Incidence of Fever in DV recipients was similar to the incidence in Typhim Vi\textsuperscript{®} recipients
- Episodes were transient (1-3 days), mild/moderate (temperature \( \leq 38.9^\circ\text{C} \))

**No observed effect of baseline flavivirus immune status**

**Systemic adverse events frequently associated with concomitant illness (e.g. pharyngitis)**

R. Forrat, ASTM&H presentation 2007
SAEs in Ph II

- No vaccine related SAEs reported
- So far 12 SAEs reported (5 in CYD4, 3 in CYD5, and 4 in CYD6; none in CYD10)
  - Exposure of approximately 350 subjects to dengue vaccine in Ph II
- 2 fatalities, not vaccine related (as assessed by investigator and IDMC)
Summary of Fatal Serious Adverse Events

CYD 04 Study – Ischemic stroke

- 46-year-old female subject
- medical history of heavy smoking, essential hypertension, seizures and alcohol abuse in the past
- Onset of event: 37 days after 2nd dose of ChimeriVax™ vaccine
- Diagnosis: extensive right middle cerebral artery distribution ischemic stroke due to carotid artery stenosis (100% of right and 80% of left carotid artery)
- Developed cerebral edema and respiratory failure
- Was taken off the ventilator and subsequently died 12 days after onset of the event

CYD 06 Study – Car accident

- 17-year-old male subject
- car accident on high way 5 months after receiving vaccine
- multiple head and abdominal contusions led to hepatic hilum and aorta system laceration that produced internal hemorrhage
- Despite life support the subject died 3 hours later
Ph II safety:

- mild reactogenicty
- AE profile mild; myalgia/asthenia observed a little more than placebo but same as in control
- low SAEs and so far no related SAEs
<table>
<thead>
<tr>
<th></th>
<th>ChimeriVax-Dengue n=33</th>
<th>Placebo n=33</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST ↑</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>ALT ↑</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total Bilirubin ↑</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine ↑</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CPK ↑</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC ↓</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Neutrophils ↓</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes ↓</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
CYD04: WBC changes after dose 1 and 2

![Graphs showing WBC changes after dose 1 and 2 for Group 1 (Chimerivax) and Group 2 (Control Group).]
<table>
<thead>
<tr>
<th></th>
<th>ChimeriVax-Dengue Dose 1 n=33</th>
<th>ChimeriVax-Dengue Dose 2 n=30</th>
<th>ChimeriVax-Dengue Dose 3 n=23</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST ↑</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ALT ↑</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total Bilirubin ↑</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Creatinine ↑</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CPK ↑</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC ↓</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Neutrophils ↓</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes ↓</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Platelet ↓</td>
<td>1</td>
<td>42</td>
<td>0</td>
</tr>
</tbody>
</table>

(Out of normal range values among all subjects with normal value at baseline)
These clinical trials are not closed; no vaccine-related SAE have been observed to date.

**Biological safety Evaluation**

- Transient decreases of White Blood cell count were occasionally observed one week after dengue vaccination.
  - They occurred more frequently in TV than after a placebo vaccine but not more than after an active control vaccine.
  - If noted, typically after dose 1.
  - They were of mild to moderate intensity, transient (≤1 week) and not part of a clinical syndrome.
  - They were not increased in dengue endemic subjects.

- Biochemistry parameters were comparable between DV recipients and control vaccine recipients.
Ph II vaccine viremia
CYD04/CYD05/CYD06 Trials – Viremia Testing

Timing

- CYD04: every two days from Day 0 to Day 20
- CYD05 and CYD06: 0, 7 and 14 days after vaccination

Method (testing at GCI lab)

2 steps

- 1-Screening of samples with a YF-RT-PCR method
- 2-Positive samples are then tested by a serotype-specific ChimeriVax RT-PCR and plaque assay
CYD04 Trial: Post-dose 1 & 2 viremia data

**Post-Dose 1&2 Viremia - Grp1 (YF-RT-PCR)**

- **Viremia mainly post dose 1**
- **Viremia level low**
  - <1.4 log10 pfu/mL (LLOQ)
  - None + by plaque assay

**Post-Dose 1-ChimeriVax Dengue Serotype-specific RT-PCR**

- **Viremia mainly serotype 4**
- **less 3 and rarely 2**

After the 2nd dose, among 9 positive samples in YF-RT-PCR, only serotype 2 was identified in 2 samples.
Effect of age and dengue endemicity on dengue vaccine viremia

Philippines (endemic)
80% Den/JE neut ab at baseline

Mexico (non-endemic)
8% Den neut ab positive at baseline

- 2 (ado group) vaccinated subjects had antigenemia levels above the LLOQ (max 2.3 log10 pfu/mL)
- None were positive in Plaque assay
- Antigenemia was predominantly serotype 4, then 3

- 6 vaccinated subjects had antigenemia levels above the LLOQ (max 2.1 log10 pfu/mL)
- 4 were positive in plaque assay (max 2.0 log10 pfu/mL)
- Antigenemia was predominantly serotype 4, then 3

R. Forrat, ASTM&H presentation 2007
Ph II viremia:

- expected and common observation
- peak day 7
- more common in flavi-naïve
- viral load very low

Ph II immune response
CYD04 Trial – Neutralizing Antibody Results for each Serotype in Group receiving 3 doses of dengue vaccine –

**Serotype 1**
- Post-Vacc 1: 12.1%
- Post-Vacc 2: 70%
- Post-Vacc 3: 100%

**Serotype 3**
- Post-Vacc 1: 27.3%
- Post-Vacc 2: 73.3%
- Post-Vacc 3: 100%

**Serotype 2**
- Post-Vacc 1: 66.7%
- Post-Vacc 2: 93.3%
- Post-Vacc 3: 100%

**Serotype 4**
- Post-Vacc 1: 63.6%
- Post-Vacc 2: 86.7%
- Post-Vacc 3: 100%
CYD04 Trial – Neutralizing Antibody Results for Serotypes 1 & 2 in Group receiving 3 doses of dengue vaccine –

% Seropositivity - Serotype 1 - Grp 1 CYD-CYD-CYD

% Seropositivity - Serotype 2 - Grp 1 CYD-CYD-CYD

GMT - Serotype 1 - Grp 1 CYD-CYD-CYD

GMT - Serotype 2 - Grp 1 CYD-CYD-CYD

+95% IC
CYD04 Trial – Neutralizing Antibody Results for Serotypes 3 & 4 in Group receiving 3 doses of dengue vaccine –

% Seropositivity - Serotype 3 - Grp 1 CYD-CYD-CYD

% Subjects

CVD-WHO

Post-Vacc 1: 27.3%
Post-Vacc 2: 73.3%
Post-Vacc 3: 100%

% Seropositivity - Serotype 4 - Grp 1 CYD-CYD-CYD

% Subjects

CVD-WHO

Post-Vacc 1: 63.6%
Post-Vacc 2: 86.7%
Post-Vacc 3: 100%

GMT - Serotype 3 - Grp 1 CYD-CYD-CYD

1/dil

CVD-WHO

Post-Vacc 1: 10
Post-Vacc 2: 100
Post-Vacc 3: 1000

GMT - Serotype 4 - Grp 1 CYD-CYD-CYD

1/dil

CVD-WHO

Post-Vacc 1: 1
Post-Vacc 2: 10
Post-Vacc 3: 100

+95% IC
CYD05 - Neutralizing antibody results in endemic population – % Seropositive (Abs level >=10 1/dil) per Serotype after **Two doses** of TV Dengue Vaccine -

Adults (TV ➔ TV)

![Graph showing % Seropositivity-Adults (n=12)]

Adolescents (TV ➔ TV)

![Graph showing % Seropositivity-Adolescents (n=24)]

Children [6-11y] (TV ➔ TV)

![Graph showing % Seropositivity-Children [6-11y] (n=24)]

Children [2-5y] (TV ➔ TV)

![Graph showing % Seropositivity-Children [2-5y] (n=24)]
CYD06 - Neutralizing Antibody Results for each serotype in Children after 2 doses or 1 dose post-priming

Children [6-11y] in Grp 1 (TV ➔ TV)

- 2 CYD doses
- YF primed

Children [2-5y] in Grp 1 (TV ➔ TV)

- 2 CYD doses
- YF primed

Children [6-11y] in Grp 2 (YF vaccine ➔ TV)

- Children [6-11y] (n=12)

Children [2-5y] in Grp 2 (YF vaccine ➔ TV)

- Children [2-5y] (n=24)
CYD06 - Neutralizing Antibody Results for each serotype in Adults & Adolescents after 2 doses or 1 dose post-priming

Adulst in Grp 1 (TV ➔ TV)

Adulst in Grp 2 (YF vaccine ➔ TV)

Adolescents in Grp 1 (TV ➔ TV)

Adolescents in Grp 2 (YF vaccine ➔ TV)
CYD06 Trial – (2-45years): neutralizing antibody results for each serotype in group receiving 3 doses of dengue vaccine or 1 dose of YF vaccine followed by 2 doses of dengue vaccine.

Neut antib response after three doses of dengue vaccine

Neut antib response after two doses of dengue vaccine in YF-primed subjects
CYD 10 results
### Trial Design CYD 10

<table>
<thead>
<tr>
<th>Trials</th>
<th>Design</th>
<th>Population</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYD 10</td>
<td>One injection (1 year after vaccination in DIV12 trial), 6 months follow-up</td>
<td>Adults 18-40y (n=36 from DIV12 trial) (n=12 FV naïve)</td>
<td>1 inject completed: 35 subjects 23 subjects from DIV12 trial 12 subjects FV naïve 6 month follow-up ongoing</td>
</tr>
<tr>
<td>grp 1:</td>
<td>monovalent VDV1 ➔ CYD TV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grp 2:</td>
<td>monovalent VDV2 ➔ CYD TV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grp 3:</td>
<td>Stamaril ® ➔ CYD TV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grp 4:</td>
<td>CYD TV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Objective:** explore effect of pre-exposure

**CYD TV:** ~5 log10 TCID50 per serotype

**Australia**
CYD 10 (Australia) immunologic response after 1 dose of TV dengue

**CYD10 - % Seropositivity - FV naive grp (n=12)**

- Serotype 1: 0%
- Serotype 2: 25%
- Serotype 3: 58.3%
- Serotype 4: 66.7%

**CYD10 - % Seropositivity - Den1 grp (n=7)**

- Serotype 1: 42.9%
- Serotype 2: 0%
- Serotype 3: 57.1%
- Serotype 4: 71.4%

**CYD10 - % Seropositivity - Den2 grp (n=8)**

- Serotype 1: 87.5%
- Serotype 2: 87.5%
- Serotype 3: 100%
- Serotype 4: 100%
PH II conclusions
Conclusions on Safety

- No SAE related to vaccination
- No mild dengue like syndrome as previously observed with whole virion live-attenuated dengue vaccine
- Reactogenicity profile (clinical & biological) comparable to control registered vaccine
- No increase of reactogenicity in FV immune subjects (dengue or YF) in comparison to FV naive subjects
- No increase of reactogenicity when moving to younger subjects
- No increase of reactogenicity after a 2\textsuperscript{nd} or a 3\textsuperscript{rd} dose
Conclusions on viremia

- Low level of viremia observed (<LLOQ)
- Serotype 4 the most detected then serotype 3
- Age effect on viremia results (less in younger children)
- Less viremia in dengue immune subjects compared to dengue naive subjects
- Decrease of viremia occurrence after a 2nd dose vs 1st dose
- No increase of viremia occurrence in YF primed subjects in comparison to YF naive subjects
Conclusions on immunogenicity

In non-endemic population,

- Increase in seroconversion after the 2\textsuperscript{nd} and a 3\textsuperscript{rd} dose of dengue vaccine
- Priming effect of previous YF vaccination: similar response of a 1\textsuperscript{st} dose in YF primed subjects compared to two doses in YF naive subjects

In endemic population

- Substantial Increase in seroconversion rates against each serotype, after two-doses of dengue vaccine
Moving towards efficacy trials and Ph III
From the Phase II trials to Phase III Efficacy Trials

Phase II trials

- S&I Adults-ado
  - Children JE+/-, Den+/- Vietnam

- S&I Ado-Adults
  - Den+/- Singapore

- S&I Adults
  - FV naive US

- S&I Adolescents
  - Den+/- Latin Am

- S&I Children
  - YF+, Den+/- Peru

POC Efficacy
- Children JE+/-, Den+/- Thailand

Phase III trials

- Efficacy + Large Scale S&I
  - Children Asia

- Efficacy + Large Scale S&I
  - Adolescents Latin America
Challenges with the Ph III

- Changing epidemiology year-by-year
- Different epidemiology by country/sub-region
- Peak incidence in different age groups for various countries
- Infrastructure build up for large scale clinical trial, while maintaining pace of additional Ph II trials
- Definition of study endpoints
- Validation and scaling up of lab assays
Acknowledgements

Acambis, Cambridge, USA

Mahidol University, Thailand

CDC Puerto Rico, USA

Pediatric Dengue Vaccine Initiative
Seoul, South Korea

Clinical Trial Teams in Mexico, Philippines, and USA.
<table>
<thead>
<tr>
<th>Department</th>
<th>Name</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASAD</td>
<td>M. Boaz</td>
<td>Marketing</td>
</tr>
<tr>
<td></td>
<td>S. Hildreth</td>
<td>Dengue GCI Team</td>
</tr>
<tr>
<td>Business Development</td>
<td>D. Julien</td>
<td>Non Clinical Safety</td>
</tr>
<tr>
<td></td>
<td>R. Forrat / D. Crevat</td>
<td>Public Policy</td>
</tr>
<tr>
<td>Clinical</td>
<td>G Dayan/ A Bouckenooghe</td>
<td>QC Dev.</td>
</tr>
<tr>
<td></td>
<td>+Dengue Clinical Team</td>
<td></td>
</tr>
<tr>
<td>Development</td>
<td>C. Navarro</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td></td>
<td>Dengue Development Team</td>
<td></td>
</tr>
<tr>
<td>GMA</td>
<td>C. Luxemburger</td>
<td>Regulatory</td>
</tr>
<tr>
<td></td>
<td>L. Pollissard</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dengue GMA Team</td>
<td></td>
</tr>
<tr>
<td>Industrial Operations</td>
<td>C Malinowski</td>
<td>Research</td>
</tr>
<tr>
<td></td>
<td>Dengue IO Team</td>
<td></td>
</tr>
<tr>
<td>Intellectual Property</td>
<td>N Schaeffer</td>
<td></td>
</tr>
<tr>
<td>Legal</td>
<td>S. Bauer</td>
<td>Program Office</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>International</td>
<td>A Warthel (Asia)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Zambrano (Latin America)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*sanofi pasteur Dengue Global Team*
Gracias

Thank you