Animal Models in Preclinical Evaluation of sanofi pasteur Flavivirus Vaccines Dengue Tetravalent Vaccine Application

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Dengue Disease: A Major Public Health Concern

- Mosquito-borne disease
- 2nd most widespread tropical disease after Malaria; 1st in Latin America and Asia Pacific
- Almost 50% of world population is at risk
- Each year:
  - 230 million infected
  - 2 million severe disease (90% children)
  - 25 000 deaths
- Increasing geographic spread and burden of disease

Global Prevalence of Dengue

- Areas infested with *Aedes aegypti*
- Areas with *Aedes aegypti* and dengue epidemic activity

(2) WHO 2009
# Dengue Disease Prevention: High Unmet Medical Need

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WHO</th>
<th>Sanofi Pasteur</th>
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<tbody>
<tr>
<td>- No effective drug treatments</td>
<td>- Prevention through vaccination is an important objective*</td>
<td>- Tetravalent dengue vaccine</td>
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<tr>
<td>- No effective prevention</td>
<td>- Guidelines for clinical evaluation, production, quality control, and antibody testing</td>
<td>- Compliance with WHO guidelines</td>
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<tr>
<td></td>
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<td>- Will be made available to endemic areas first</td>
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* World Health Organization, 2009 Fact Sheet #117
Developing a Safe and Effective Tetravalent Vaccine against Dengue

A Balancing Act

ATTENUATED

POTENT

FOR 4 SEROTYPES SIMULTANEOUSLY
Innovative Approach
Designed to Maximize Safety

- YF vaccine: Live attenuated
- High genetic stability

- Dengue virus: Wild Type
- Serotype differences linked to protein differences at surface
- Envelope E proteins trigger production of neutralizing antibodies
CYD Dengue Vaccine: Tetravalent Combination of Chimeric LAV

- Four genetic constructs are created, one for each serotype
- All are based on same YF 17D backbone
- Insertion of E and prM genes, isolated from each serotype

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>prM</th>
<th>E</th>
<th>YFV17D Non-Structural genes</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>prM</td>
<td></td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>prM</td>
<td></td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>prM</td>
<td></td>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

CYD1  CYD2  CYD3  CYD4
**In vitro models**
- PBMCs, DCs,
- Monocytes,
- Endothelial cells
- Hepatic cells/lines

**Mosquito vectors**
- Replication
- Transmission
- Genetic stability

**Animal models**
- Monkeys
- Mice
- Hamsters

**Clinical Immunogenicity / Safety**
- Antibody (Abs)
- Cytotoxic T lymphocyte (CMI)
- Viremia
- Safety
- Protection

**Infectivity**
- Tropism
- Phenotype modification
- Cytokines induction
- Viscerotropism

**Replication**
- Transcription
- Genetic stability

**Immunogenicity / Safety**
- Abs
- CMI
- Viremia
- Safety
- Protection

**Guy et al. Vaccine. 2010, 2011**
Vaccine Development Pathway

**Exploratory**
- Identifying new antigens

**Pre-clinical**
- Assessing antigens’ safety in animals & developing the product process

**Clinical development**
- Testing the NV in humans and improving the product process

**Registration**
- Obtaining the approval from health authorities

**License**
- Product monitoring

- **Discovery**
  - 2-4 years
  - New Ag/Techno identification

- **Research**
  - 1-2 years
  - Immunological characterization

- **Process Development**
  - 6-8 years
  - Toxicology assessment

- **AS&AD**
  - 6-8 years
  - Vaccine characterization

- **NCS**
  - 6-8 years
  - Toxicology assessment

- **Clinical**
  - 1-1.5 years
  - Immunological characterization

- **PV**
  - 1-1.5 years
  - GMO risk assessment

- **Regulatory Affairs**
  - 1-1.5 years
  - GMO risk assessment
PreClinical Dengue Vaccine Development

Mouse
Neurovirulence Immunogenicity

Monkey
Viremia Neurovirulence Immunogenicity

Man
Reactogenicity Immunogenicity Protection
Primate species and Immunology protocols

● Macaques: Rhesus and cynomolgus
  • Due to species availability, cynomologus were the most used

● Same design for most of protocols. Dosing scheme and end-points tailored based on study objectives
  • Same dose as clinical trials (passage, formulation): 3 to 5 log
  • 1 to 3 SC administrations, usually 8 wks apart
  • 4 to 6 flavivirus-negative monkeys per group
    - Animal screening prior to the test. Depend on the origin

● Ethical review process includes species justification
General Toxicology

- Repeated dose-toxicity study, GLP

- Study Objective: to assess the systemic safety and the local tolerance of CYD1-4 vaccine candidates

- Monovalent vs tetravalent
  - Well tolerated
  - No synergistic effect when Tetravalent

- No major direct or indirect toxic effects
  - No concerns identified
  - Supportive of the clinical trials
Neurotropism

- **Guidelines**
  - WHO n°872, 1998, A4.1.3, Monkey safety test (neurotropism test) or Eur. Ph. 5th Ed: Yellow fever vaccine (live)
  - Comparator is parental YF-17D
  - 0.25 ml in frontal lobe (~5 Log CCID50)

- **Every CYD serotype at master seed level**

- **Mono and tetravalent CYDs are less neurovirulent than YF17D**
  - Target score ranged from 0.03 to 0.15 vs 1.1 for YF-17D

- **Suckling mouse model (4 days old) is more discriminating**
  - Done at Master & Working seed levels
  - Less Neurovirulent than licensed YF 17D 204 licensed vaccine
ChimeriVax™-DENTV Monkey safety test Histopathology:
Group mean histological lesion scores in Brain & spinal cords

<table>
<thead>
<tr>
<th>Test article</th>
<th>N</th>
<th>Target area Mean (± SD)</th>
<th>Discriminator areas Mean (± SD)</th>
<th>Combined Mean (± SD)</th>
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<tbody>
<tr>
<td>ChimeriVax™-DENTV</td>
<td>11</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
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<tr>
<td>YF-VAX®</td>
<td>11</td>
<td>0.43</td>
<td>0.35</td>
<td>0.39</td>
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<tr>
<td>P value (ANOVA)</td>
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<td>0.0023</td>
<td>0.0015</td>
<td>0.0018</td>
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</table>
Biodistribution, viral shedding

- **Study objectives**: assess the distribution, the persistence or elimination, as well as the shedding of the vaccine
- **Scheduled protocol**
  - One SC administration with final formulation
  - 3 time points: peak of viremia, end of viremia, late point
  - About 20 tissues per animal will be tested (RT-PCR): liver, lung, heart, blood, kidney, testes
  - Viral shedding: faeces, urine, saliva, injection site, brain, lymphoid tissues
- **Results**
  - Limited distribution/replication mainly at the injection site and draining lymph nodes and in a limited number of other tissues such as liver or lymphoid tissues, without viral persistence.
  - No associated changes in clinical pathology parameters, and no findings at the histopathology examination
Kinetic and distribution of CYD 1-4 viruses
(only positive tissues are shown, in bracket: highest virus levels in positive tissues \(\log_{10} \text{copies/g}\))

- **Injection site**: Day 4 (8.5), Day 10 (6.0)
- **Auxiliary lymph**: Day 4 (6.8), Day 10 (5.5), Day 22 (BLQ)
- **Liver**: Day 10 (5.0), Day 4 (BLQ)
- **Mesentric lymph**: Day 4 (6.5), Day 10 (5.0), Day 22 (BLQ)
- **Spleen**: Day 10 (5.6), Day 4 (5.1)
- **Thymus**: Day 22 (5.6)
- **Adrenal**: Day 22 (5.6)
- **Bone marrow**: Day 22 (BLQ)
- **Skeletal muscle**: Day 22 (BLQ)

BLQ: below the limit of quantification
Liver Tropism Monkey Model

- **DEN4 strain 1228 and Asibi used as controls**
  - Model characterization
  - Background data

- **Comparison of pathology and biodistribution with CYD and vaccinal YF17D**
  - Little (YF17D) to no infection foci (CYD), no pathology

- **Lack of pathology associated with DEN4 strain 1128**
  - No clinical signs, or biomarker of infection, enlarged spleen, adenomegalia

*Lefeuvre A. Thèse Univ Lyon, 2005*
In vitro hepatotropism summary

- Viscerotropism is characteristic of wild-type flavivirus infection in humans

- Human cell lines have been used to evaluate in vitro hepatotropism of CYD candidates

- CYD do not replicate or at very low titers in HepG2 cells, compared YF17D
  - Similar study with same conclusions performed at Acambis on HepG2 and ThLe cells

- On going: evaluation of primary hepatocytes derived from human stem cells
Human Predictivity of Monkey Models

- Negative predictivity of primate model
  - ‘Hot’ strains in NHP are hot in man
  - Low viremia strain does not predict attenuation in man

- Main criteria for in vivo attenuation evaluation is viremia (one marker of viscerotropism)

- Similar immune response (% of response after immunization, mean titer) but with different serotype dominance

- Lack of disease with wild-type DEN viruses

Sanchez V et al. Vaccine, 2006
Monkey Immunology assessment

- **Viremia**
  - Short lasting
    - Onset: Day 6, mean duration: 2-3 days
  - Low level
    - Mean peak level: 1 to 2 log pfu/ml
  - Almost only with CYD4. Interaction between serotypes
  - Lower or no viremia after 2nd immunization

- **Neutralizing Ab (PRNT)**
  - Variable according to serotype (4 > 1 > 2-3)
  - Boost is required
  - Convenient model to test alternate schedule of immunization

- **One year boost** induces strong responses against all 4 serotypes

- **Challenge models**
  - Protective immunity against all 4 serotypes 6 months post immunization
  - Viremia as marker of protection; no detectable viremia

Monkey Model & Interference

- Interaction between serotypes
  - Different viremia if monovalent or tetravalent
  - Most predominant serotype might change depending on the species
    - In NHP, CYD4 > 1 > 2-3
    - In Man, CYD4 > 2-3 > 1

- But no synergistic effects
  - Tetravalent vaccine has the same pattern of the ‘hottest’ monovalent (infectivity pattern)

*Guy B et al. AJTMH, 2009*
Mouse models for preclinical dengue vaccine evaluation

- In most cases, KO mouse models (type I/II IFNR, such as A129 or AG129)
  - Non immunocompetent mice, unsuitable to assess immunogenicity/protection

- Humanized mice
  - Complex, human donor variability

- WHO TRS932 Recommendation (part B.4.3)
  - “At present, the AG129 mouse seems most suitable for safety studies, but National regulatory authorities should be aware of the pitfalls of interpreting results, since these animals do not possess an intact innate immune response. For this same reason, as mentioned earlier, it would not be advisable to use AG129 mice for classic toxicology studies”
Conclusions: Key Elements of the sp Dengue Vaccine
Non-Clinical Development Risk Management Strategy

The vaccine is not transmitted by mosquitoes
Reversion to virulence is almost impossible

The vaccine neutralizes all circulating strains tested so far
Tetravalent vaccination will address the ADE risk

Natural recombination is highly unlikely
Gene transfer is impossible

Excellent genotypic and phenotypic stability


Presentation Contributors

- Sanofi pasteur
  - Bruno Guy, Director, Discovery
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