Immunogenicity of sanofi pasteur
Tetravalent Dengue vaccines

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• Rafaele Dumas, Jean Lang
Dengue disease

- The most important arboviral disease in humans.
- 4 serotypes transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes
- Old disease, but in the past 25 years global emergence of more frequent and larger epidemics, with more severe disease
- Dengue is found in more than 100 countries and territories
- Global strain distribution
- Supportive Human Treatment & Vector Control measures
- The development of a Dengue Vaccine is viewed as a Public Health Priority

Dengue: A Multifactorial Disease

**Epidemiological risks**
- Number of susceptible persons
- Vector density
- Endemicity

**Individual factors**
- Age
- Origin
- Health status
- Secondary Infection
- Host response
- HLA

**SEVERITY**

**Viral factors**
- Strain virulence
- Serotype

Modified from Guzmán et al., *Lancet Infectious Diseases* 2002
Immunity against dengue: Protection or Enhancement?

Early Inflammation
Th bias?
CD8 original-antigen-sin?

Primary/secondary infection/immunization

Protection?
Severe dengue?

CD4, CD8
α E, NS

T

B

ADE?

Sanofi Pasteur tetravalent (TV) Dengue vaccines

- VDV: Live Attenuated Vaccines obtained by cell passage and derived in Vero cells
- Chimeric dengue vaccines:
  - Recombinant technology replacing the genes for prM and E proteins of 17D YF virus vaccine with those of dengue viruses
  - The resulting live attenuated viruses possess the replication engine of 17D YF vaccine strain but the coat proteins of each dengue serotype

- The TV vaccine is a combination of the 4 chimeric vaccines (CYDs)
- We monitor neutralizing antibody responses triggered by dengue structural antigens, as well as cellular responses induced by both structural dengue and 17D YF non structural antigens
In vitro analysis in human m-DCs stimulated with sanofi pasteur dengue vaccines

- Changes in phenotypic markers expression observed mostly in infected cells
- Cytokines: moderate expression of inflammatory cytokines restricted to infected cells, including high type I IFN expression
- Cytokines also analyzed by RT-PCR and ELISA
Adaptive Cellular Immunity

Protection

High-affinity Th1/CD8 against each serotype
Moderate IFNγ (>TNFα)

Low affinity Th1 / CD8
High IFNγ <TNFα

Low affinity Th1/Th2 / CD8
TNFα, IL10

Which response(s) should we induce to be protected upon re-exposure in the field?

Serum cytokine variations comparable to those induced after YF vaccination

Cytokines in supernatants
Th1 profile, IL2, and IFNγ > TNFα.
E and NS-spe. CD4/CD8
IFNγ > TNFα. Serotype hierarchy? HLA?

Which type of CMI should we induce?
CMI in Dengue Clinical trials

- Quantification of serum cytokines in the first 2 weeks after immunization
- Ag-specific response measured at D0 and D28 post each vaccination
  - Quantification of secreted Th1/Th2 cytokines
    - (IL-2, IL-4, IL-5, IL-10, IFN-γ, TNF-α) in cell supernatant following in vitro stimulation with live or inactivated VDV 1 to 4 or CYD 1 to 4
  - Intra-cellular Cytokine Staining (ICS)
    - CD3/CD8/IFN-γ/TNF-α following peptides pool stimulation from YF17D-204 NS3 and/or DEN3 NS3
    - Controls for staining (lyo. IFN-γ-TNF-α+ PBMCs) and permeabilization (Perm a sure) to ensure assay consistency

Quality system for CMI monitoring in clinical trials

QUALITY MANUAL (based on GCLP)

GENERAL PROCEDURES
Personnel flow, material and waste flow
P2, P2+ and P3 working instruction, etc...

IMMUNOMONITORING PROCEDURES
Samples management, Reagents management, Data management
Equipment and facilities management
Personnel training and training record management
Technique transfer and qualification
Techniques & Instruments standard operating procedure

FACILITIES & EQUIPMENTS
TECHNIQUES
PERSONNEL
**Standardization of cellular assays for clinical trials**

**CBA (cytokine multiplex)**
- 96 well plate based protocol (FACSArray)
- Proinflammatory (serum) and Th1/Th2 (cell supernatants) kits, customized kits
- Internal control: recombinant cytokines cocktail
- Technical qualification

**ELISA**
- IFN-γ, IFN-α or other ELISA kits
- Internal control: recombinant cytokine
- Technical qualification

**ICS CD3/CD8 /IFNγ / TNFα**
- 96 well plate based protocol (HTS)
- Customized ready to use antibodies cocktail (lyophilized plate)
- Repeatability and intermediate precision study
- Internal control: lyophilized activated fixed cells
- Technical qualification

**Vaccination of flavivirus-naive / immune volunteers**

**TDV Study 1**
- Administration of Tetravalent Dengue Vaccine in flavivirus-naive volunteers
- Chimeric Dengue Vaccines, CYD 1--4
  - 5 log10 TCID50 / serotype
- Batch S4003
- 3 immunizations (dose # 1, 2 and 3): Month 0, 4 and 12
- Serum cytokine analysis: every two days after dose #1 (Day 2 to Day 20) and Day 28
- PBMCs analysis: Day 0 and Day 28

**TDV Study 2**
- Administration of Tetravalent Dengue Vaccine in flavivirus-immune volunteers from TDV study, or in flavivirus-naive volunteers
- Chimeric Dengue Vaccines, CYD 1--4
  - 5 log10 TCID50 / serotype
- Batch S4003
- 1 immunization
- PBMCs analysis: Day 0 and Day 28

**VDV Study**
- Australia
  - Administration of monovalent Dengue Vaccine 1 or 2, or YF 17D Vaccine in flavivirus-naive volunteers
  - Vero Dengue Vaccines, VDV1, VDV2
    - 4 log10 TCID50 / serotype
    - Batch S3937 and S3963
  - YF17D vaccine: Stamaril™
  - 1 immunization
  - Serum cytokine analysis: every two days after vaccination (Day 2 to Day 16) and Day 28
  - PBMCs analysis: Day 0 and Day 28

**VDV Study**
- Australia
  - Administration of monovalent Dengue Vaccine 1 or 2, or YF 17D Vaccine in flavivirus-naive volunteers
  - Vero Dengue Vaccines, VDV1, VDV2
    - 4 log10 TCID50 / serotype
    - Batch S3937 and S3963
  - YF17D vaccine: Stamaril™
  - 1 immunization
  - Serum cytokine analysis: every two days after vaccination (Day 2 to Day 16) and Day 28
  - PBMCs analysis: Day 0 and Day 28
Early changes in serum after TV CYD vaccination

No changes in serum levels of pro/anti-inflammatory cytokines induced by TV vaccination, to the difference of results obtained with reactogenic vaccines.

Which adaptive responses after TV CYD immunization?

(E) serotype-specific T and B cells

(E) cross-reactive T and B cells

17D-NS specific T (and B) cells
Which targets and stimuli for CMI responses?

Evaluation of TV CYD in Dengue-naive volunteers
IFN-γ production (pg/ml) induced by live CYD stimulation after 1 or 2 immunizations
Evaluation of TV CYD in Dengue pre-immune volunteers

IFNγ supernatant levels in DEN- or 17D-pre-immune subjects

<table>
<thead>
<tr>
<th>Medium</th>
<th>CYD1</th>
<th>CYD2</th>
<th>CYD3</th>
<th>CYD4</th>
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<td>Threshold: 20 pg/mL</td>
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</table>

- Broader response in D1- and D2-preimmune subjects
- Very low/absent TNFα levels, No Th2 cytokines
- PRNT 50: 90 to 100% seroconversion against all 4 serotypes in VDV1- and VDV2-primed vaccinees after a single dose of TV chimeric vaccine. Good priming effect of YF-17D too. No increased CYD1-4 viremia in DEN-1 or 2 pre-immune subjects

Evaluation of anti-backbone (NS3) responses in Dengue naive or pre-immune volunteers

- Peptide libraries spanning the whole DEN(3) and YF17D NS3 sequences (15mer overlapping on 11 amino acids covering the ~ 620 aa sequence)
- 7-8 pools of 20-25 peptides
- Analysis done by Intracellular Cytokine Staining (ICS)
- Negative (medium) and positive (SEB) controls
- Additional experiments with individual 9mers to identify HLA-restricted epitopes
Intracellular Cytokine Staining (ICS)
Staining and permeabilization controls

Control chart ICS
Lyophilized PBMC 342095 - Lot#BDCL3011305

Assay consistency

Example of stimulation with NS3 peptide pools in a vaccinee

<table>
<thead>
<tr>
<th>Medium</th>
<th>Pool B</th>
<th>Pool C</th>
<th>Pool D</th>
<th>Pool E</th>
<th>Pool F</th>
<th>Pool G</th>
<th>SEB</th>
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Day 0

IFNγ

TNFα

Day 28

• Usually no CD4 responses
• Up to 5% CD8 specific cells
• Class I HLA-restricted peptides can be identified in a second step
Anti NS3 CD8 response after TV CYD vaccination in naive or flavivirus-immune volunteers

<table>
<thead>
<tr>
<th>Pre-vaccination status in the CYD trial</th>
<th>Pre-existing responses obtained in the LAV Trial</th>
<th>Responses in the CYD Trial</th>
<th>Responses in the CYD Trial</th>
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<td>Induction of DEN-specific Th1 immune responses in the majority of vaccinees, as expected for a live vaccine</td>
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<td>A broader Th response including all 4 serotypes was observed after the second immunization, in agreement with a broader PRNT 50 response</td>
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<td></td>
<td>Such a broader response was seen in pre-immune subjects after the first vaccination</td>
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<td>In naive or 17D-preimmune subjects, anti-17D NS3 CD8 responses were induced (IFNγ &gt; TNFα)</td>
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<td></td>
<td>In Dengue-preimmune subjects, persistent anti-Dengue NS3 CD8 responses were unchanged after CYD vaccination (IFNγ &gt; TNFα)</td>
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</table>
• Cross-reactive DEN NS-specific CD8 responses have been linked to immuno-pathological responses

• However, as far as NS backbone is concerned, there generally seems not to exist significant cross-reactivity between 17D and DEN NS(3) antigens

• This would prevent in CYDs vaccinees a boost of (unwanted?) cross-reactive NS-specific T cell responses triggered by subsequent DEN infection in the field

• The situation is different for E-specific B and T cells, as they are triggered specifically by all live vaccine approaches

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**NS3 HLA-restricted peptides**

• In further experiments, we have identified several Class I -restricted peptides in DEN and YF17D NS3

• Numerous Class I - restricted peptides have already been identified in DEN NS3 by several groups

  ➔ One could define a « positive control » by pooling these known peptides (one per serotype), which corresponding HLA may cover 30 to 60% of the considered population

• If more peptides are identified in 17DNS3, one could define similarly a « positive control » pool that could be used whichever the vaccinee’s HLA
Children: which assays with a limited amount of blood? (max 3-5 ml)

Blood collected on Heparin
(to be processed within a few hours)

Ficoll separation

Assays on whole blood

- Peptide stimulation (2 pools in DEN NS3, 2 pools in 17D NS3, 2 controls) for subsequent ICS analysis (in sanofi pasteur)
- 6 stimulation conditions

Assays on separated cells

- Vaccine stimulation (CYD1 to 4, + 2 controls) and collection of supernatants for cytokine analysis (in sanofi pasteur)
- 6 stimulation conditions

sanofi pasteur

- ICS analysis (CD4, CD8)
- CBA / Luminex analysis of cytokine content (Th1/Th2,...)

Conclusions

- Results obtained from pre-clinical and clinical evaluation of sanofi pasteur dengue vaccines are in agreement with their short-term safety and immunogenicity

- These data would also be consistent with their long-term safety and immunogenicity, as judged by the immune profile induced upon immunization

- Long-term surveillance will nevertheless establish how durable is the vaccine-induced immunity and how frequent booster immunization would be required, if needed

- The situation might be different for people leaving in endemic areas where "natural boost" would occur, compared to travelers leaving in non-endemic areas