# Immunology of dengue disease

# Impact on vaccine development and evaluation

Bruno Guy

Sanofi Pasteur, Lyon, France

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# Outline

- Dengue: a complex disease involving multiple arms of the immune system
  - **What should we induce and follow when developing and implementing dengue vaccines?**
- Example of the Sanofi Pasteur Vaccine
  - **Vaccine-induced responses**
  - **Clinical results: immunogenicity, safety and efficacy**
- Questions raised by clinical trials
  - **New analyses and investigations**
- A new comer: Zika
  - **Which interplay with dengue and other flaviviruses.?**
  - **Which impact on dengue vaccine development and implementation?**
- Dengue vaccine: overall safety
  - **Next steps: Post licensure plans**

# Dengue: a complex disease involving multiple arms of the immune system

**What should we induce and follow when developing and implementing dengue vaccines?**

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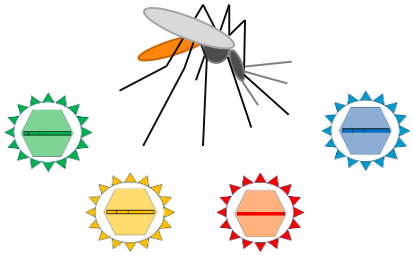
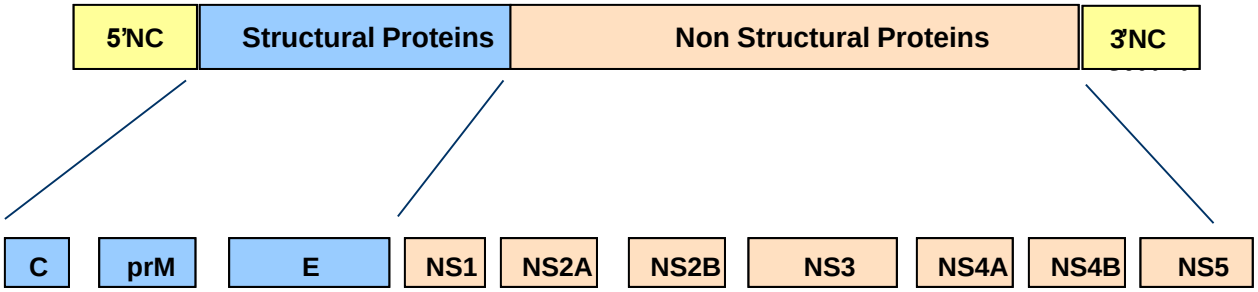
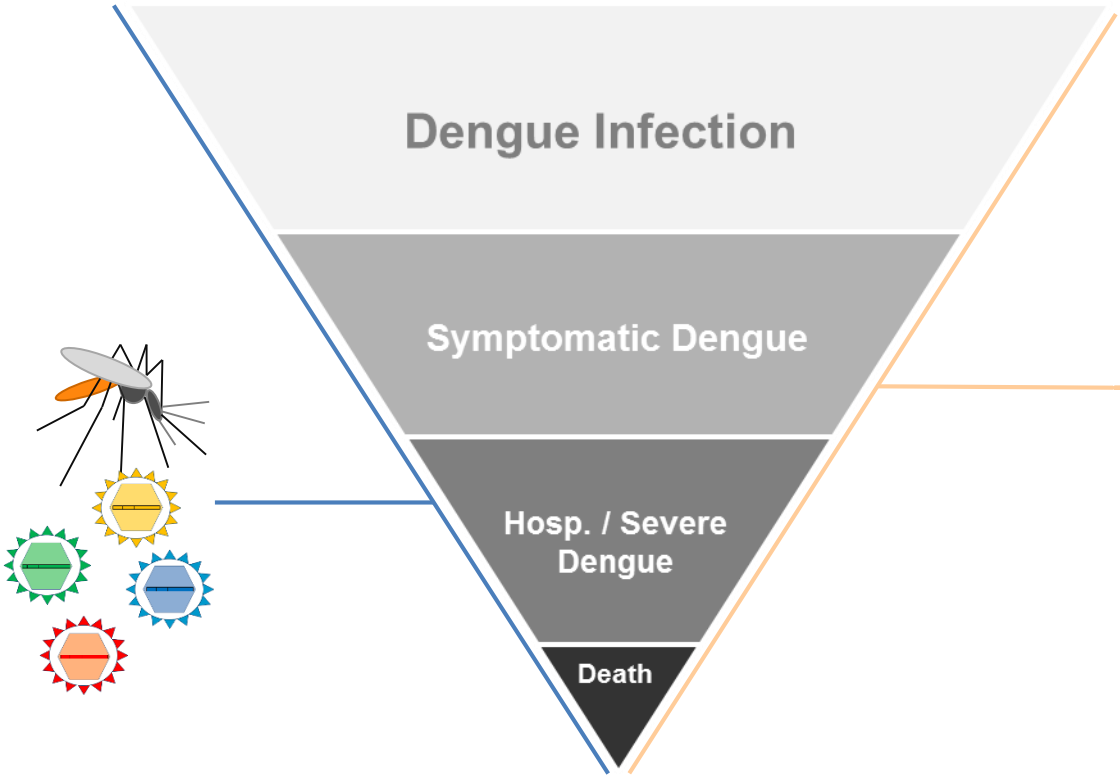
# Dengue virus

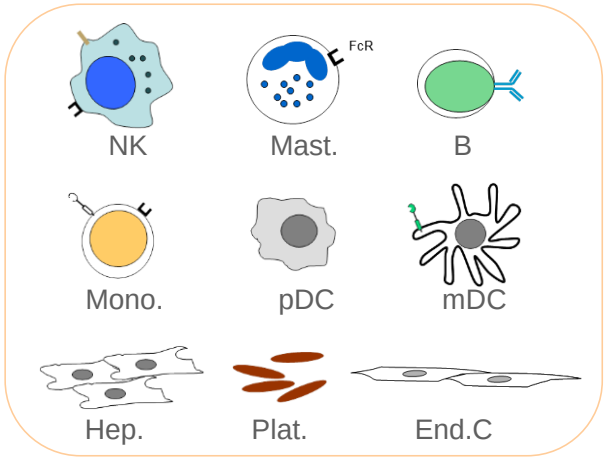
- Member of the Flaviviridae family, as other human pathogens, such as JE, WN, YF virus and some other viruses
- Four closely related, but antigenically distinct serotypes: DEN-1, DEN-2, DEN-3, DEN-4, transmitted by *Aedes* mosquitoes



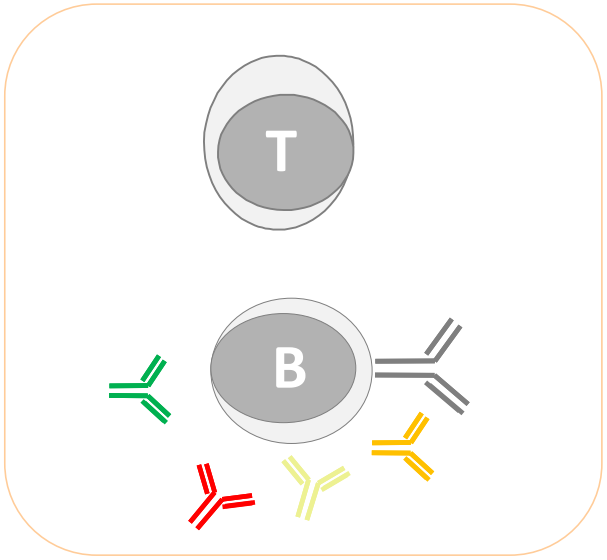
- Enveloped single-stranded RNA (~11kb) viruses
- Genome coding for 3 structural proteins and 7 non structural proteins





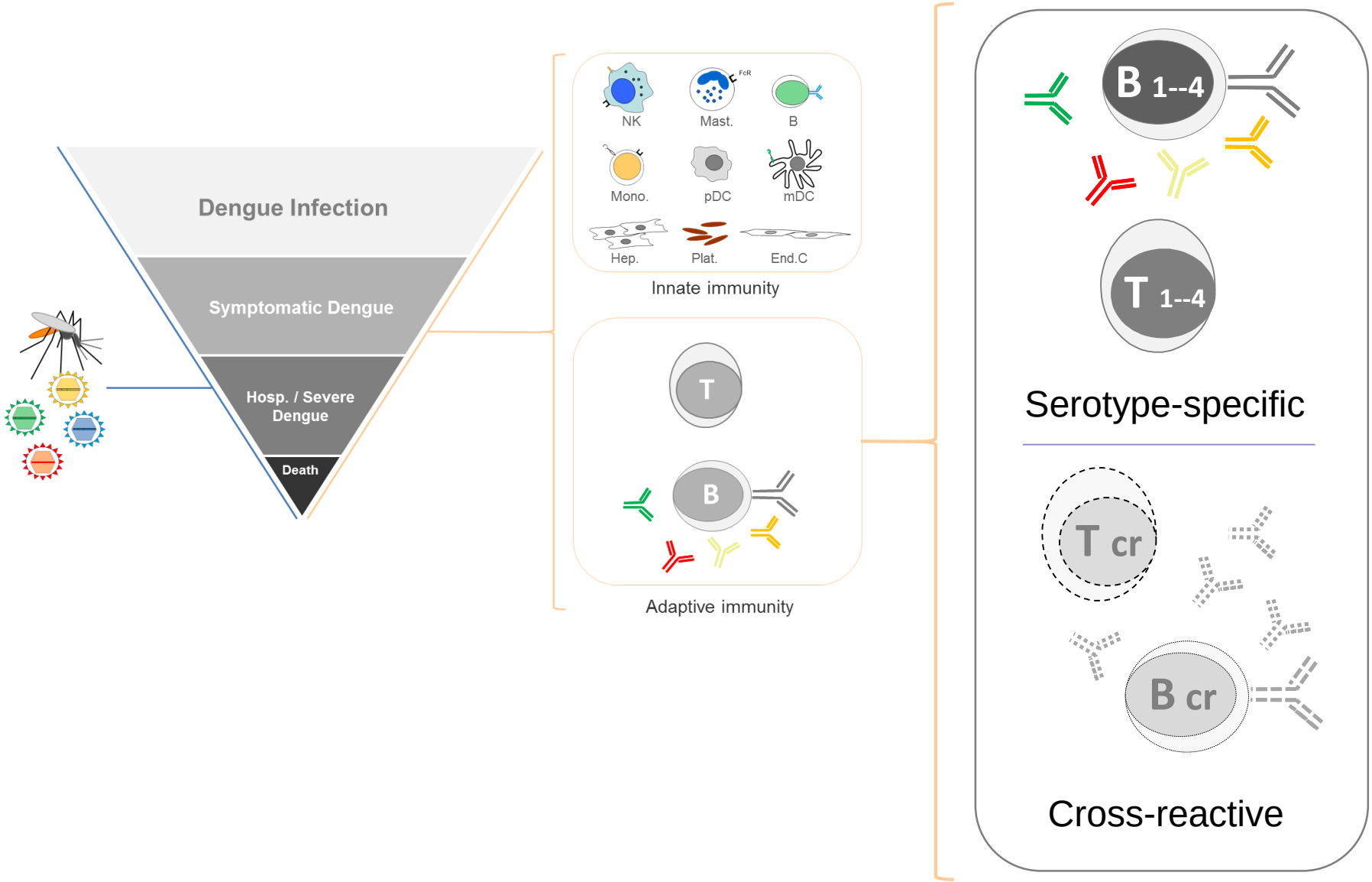




Innate immunity




Adaptive immunity

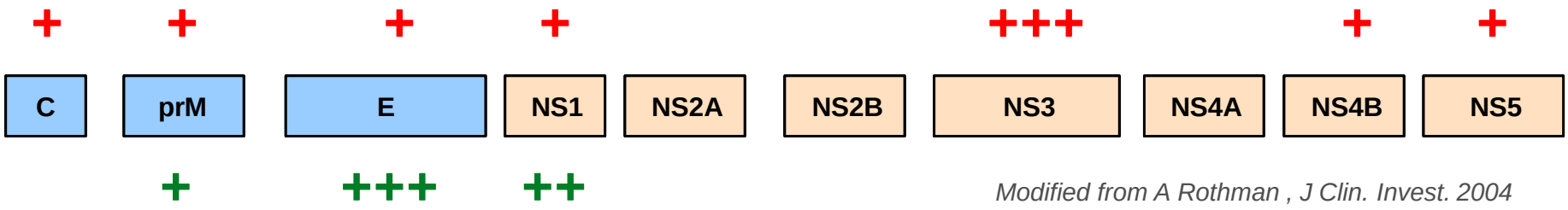
- Dengue infection can result in different disease outcome
- It triggers multiple arms of immunity, which can play a beneficial or detrimental role




- T and B cell responses can be serotype-specific or cross-reactive
- Their quality and efficacy may vary if they are triggered upon 1ary or 2ary infection

# Target antigens of specific humoral and cellular immunity

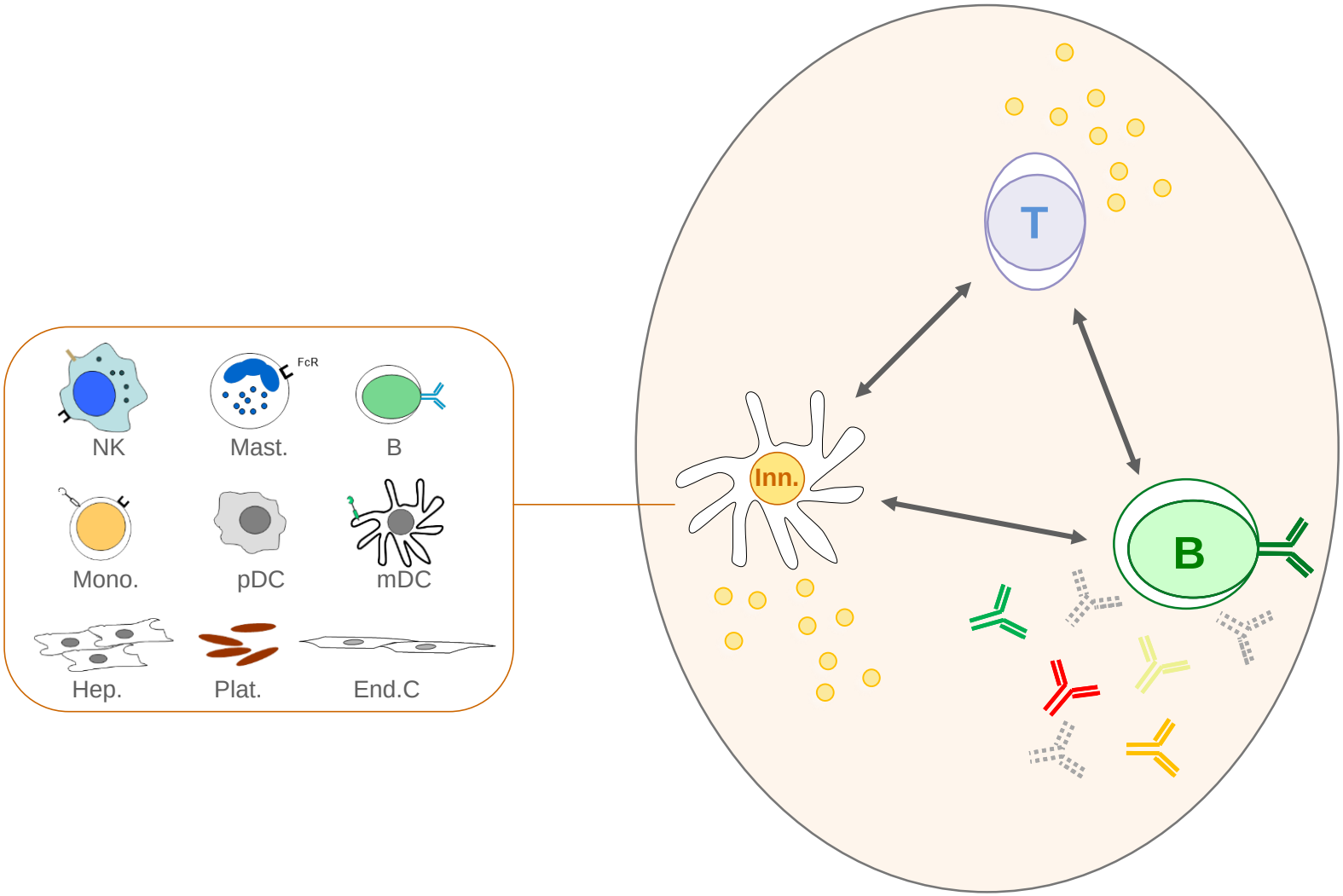
**T cells**



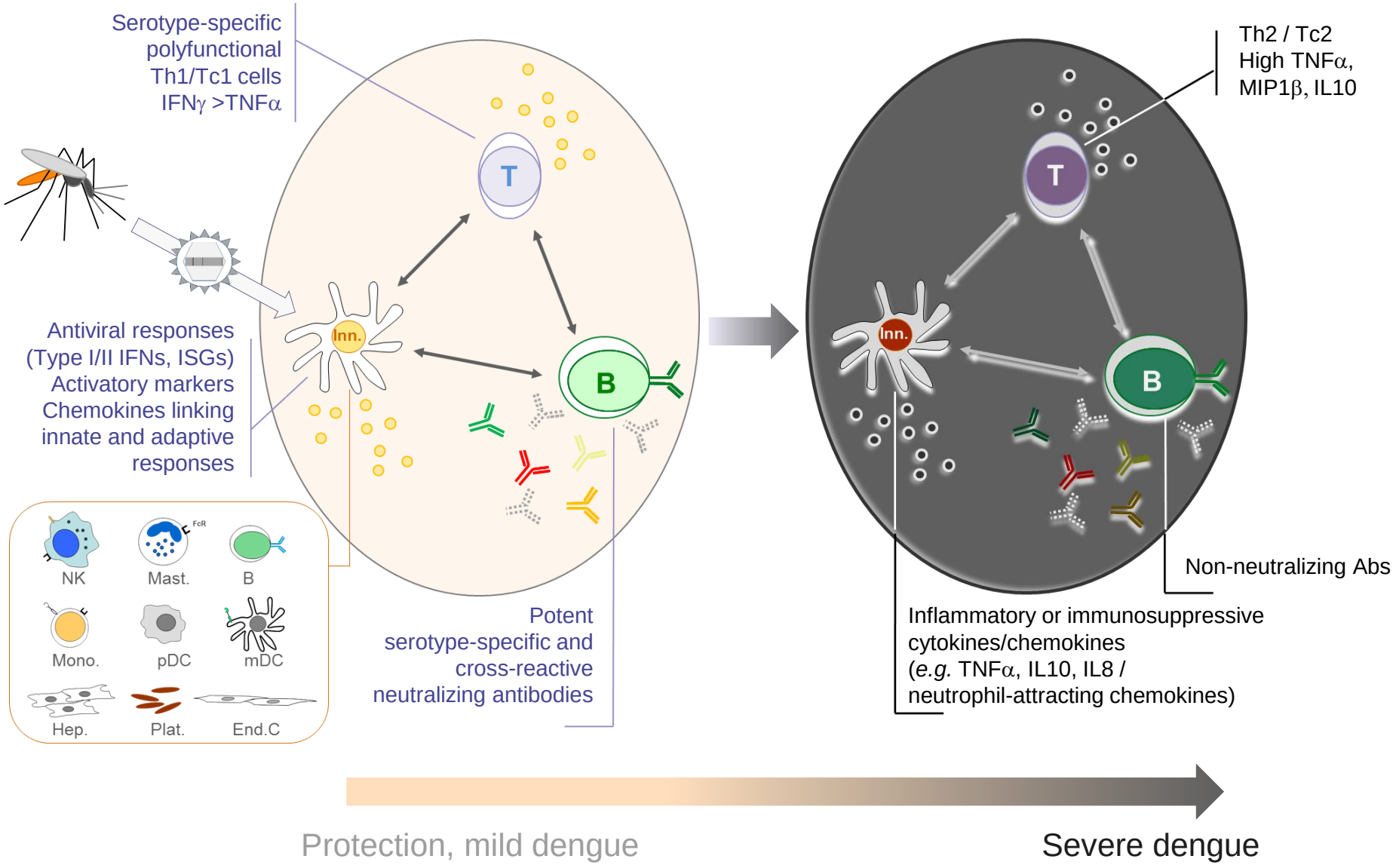

*Modified from A Rothman , J Clin. Invest. 2004*

**Antibodies**

- Antibodies are mostly triggered against prM and E structural antigens, and against NS1 (which is also acting as a virulence factor)
  - **E protein is the target of neutralizing antibodies**
- Both structural and non structural Ags trigger T cell reponses
  - **NS3 is the dominant antigen in this regard**




Innate, T and B cell /antibody responses and associated cytokines and chemokines shape the overall protective or non-protective response


Serotype-specific
polyfunctional
Th1/Tc1 cells
IFNγ >TNFα
Antiviral responses
(Type I/II IFNs, ISGs)
Activatory markers
Chemokines linking
innate and adaptive
responses
T
Inn.
B
NK
Mast.
FcR
B
Mono.
pDC
mDC
Hep.
Plat.
End.C
Potent
serotype-specific and
cross-reactive
neutralizing antibodies
Th2 / Tc2
High TNFα,
MIP1β, IL10
T
Inn.
B
Non-neutralizing Abs
Inflammatory or immunosuppressive
cytokines/chemokines
(e.g. TNFα, IL10, IL8 /
neutrophil-attracting chemokines)
Protection, mild dengue
Severe dengue

# Immunopathology and dengue

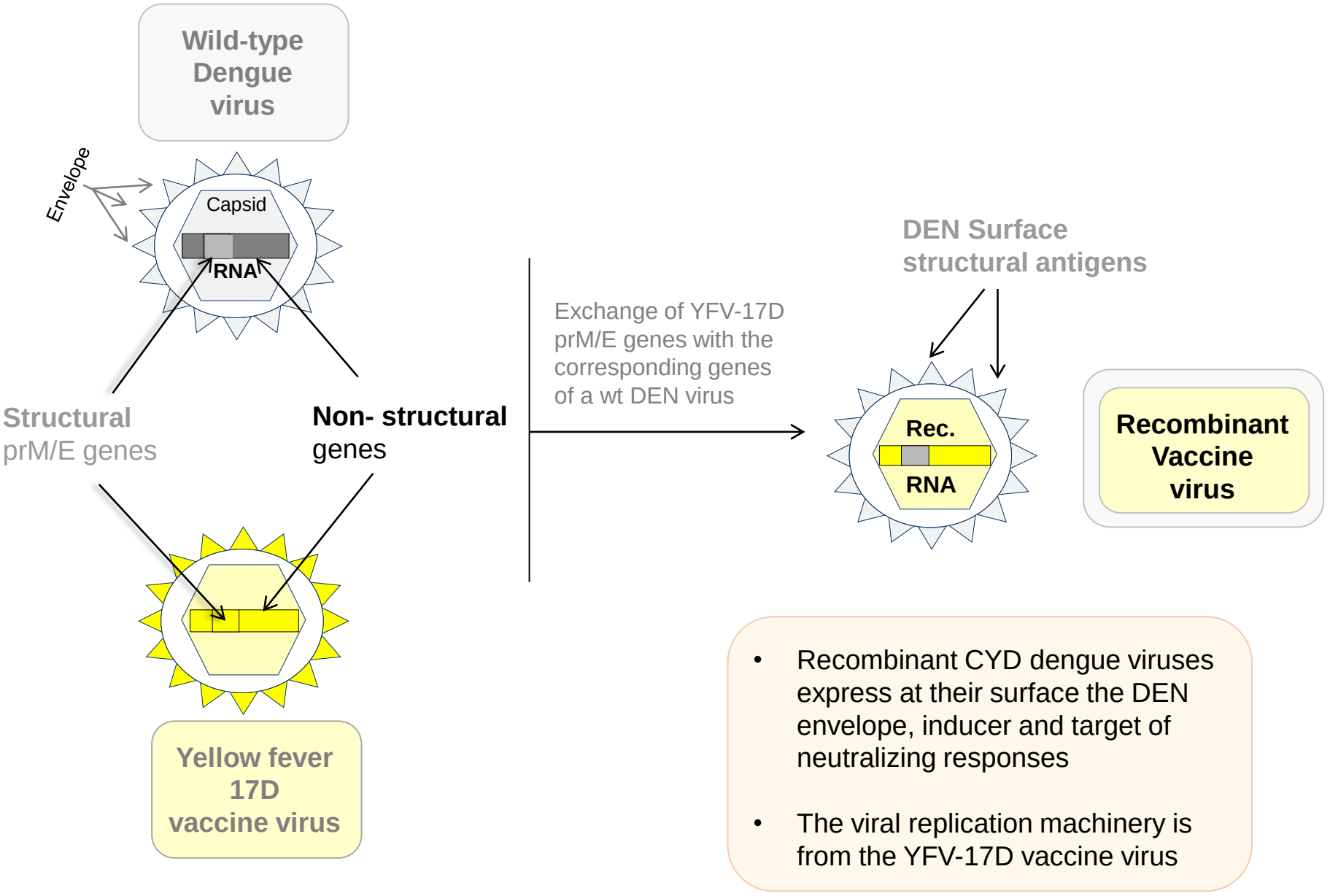
- Severe dengue has a multifactorial origin
  - **Combinations of these different factors eventually contribute to trigger immuno-pathological mechanisms most often involved in disease outcome**
- It is important to take these mechanisms into consideration when evaluating the innate and adaptive signatures of vaccine candidates
- In addition, identifying early markers of evolution towards severe disease would be beneficial to treatments already existing or under development

# Example of the Sanofi Pasteur Vaccine

**Vaccine-induced responses**

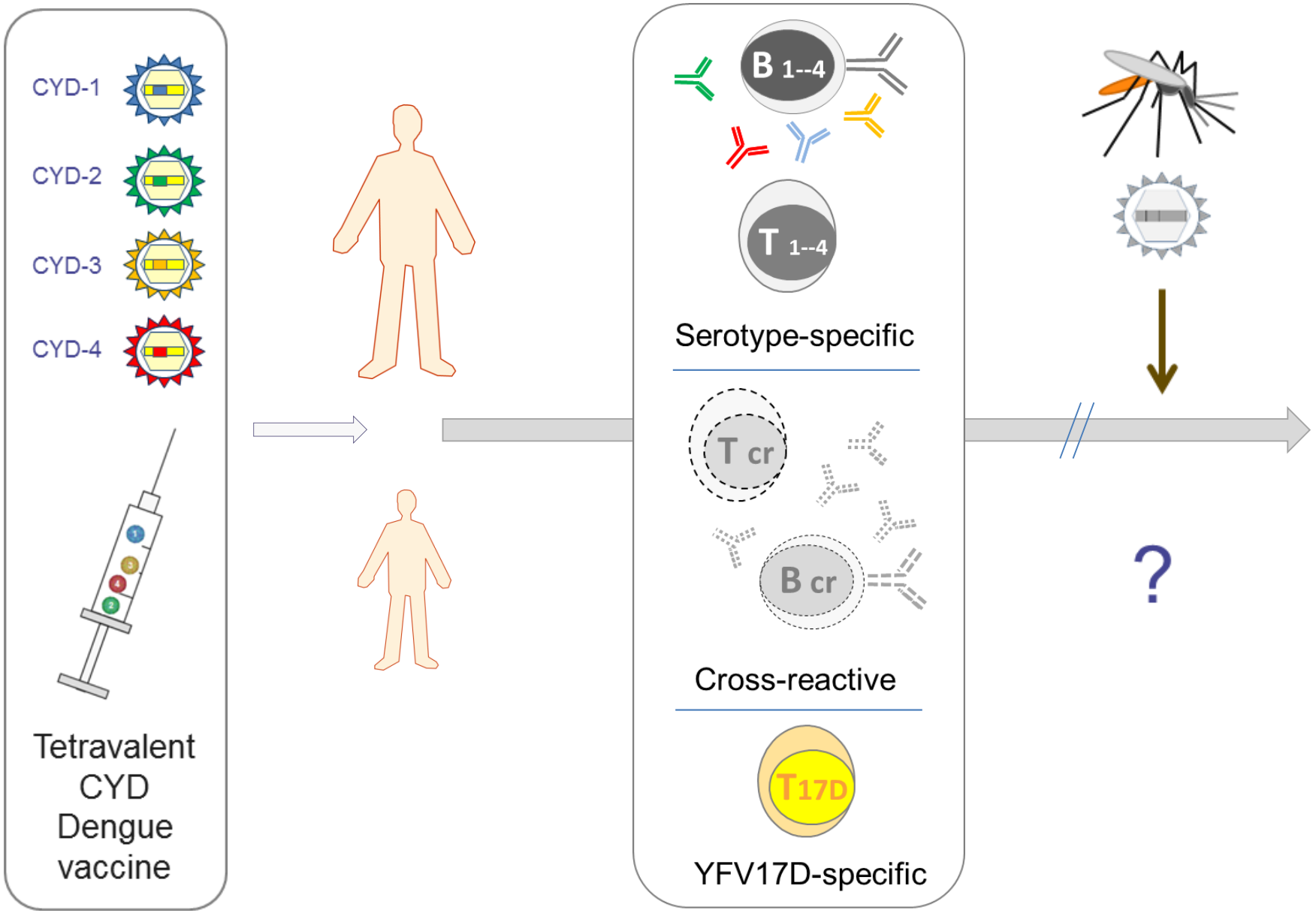
**Clinical results: immunogenicity, safety and efficacy**

# Recombinant CYD dengue viruses




- Recombinant CYD dengue viruses express at their surface the DEN envelope, inducer and target of neutralizing responses
- The viral replication machinery is from the YFV-17D vaccine virus

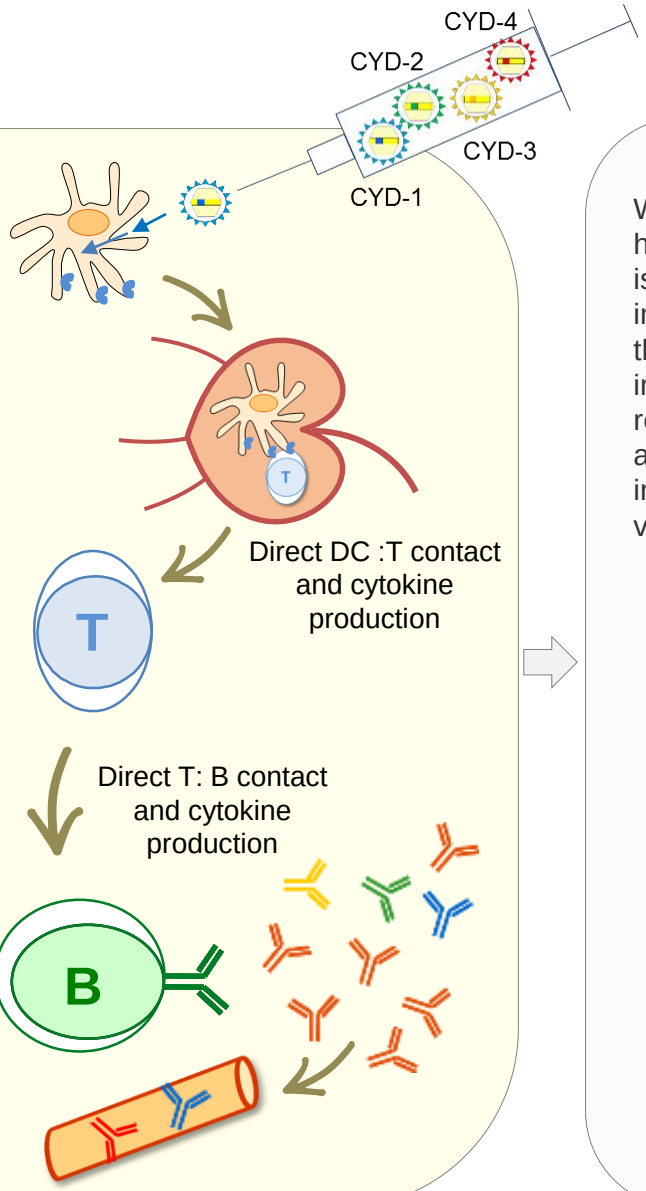
# CYD vaccine-induced responses (1)



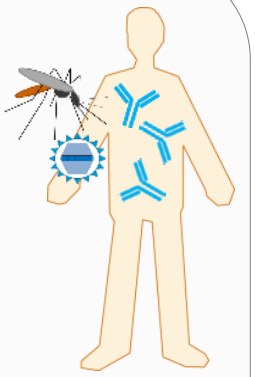
# CYD vaccine-induced responses (2)

Once in the body, the vaccine activates the immune system in three steps

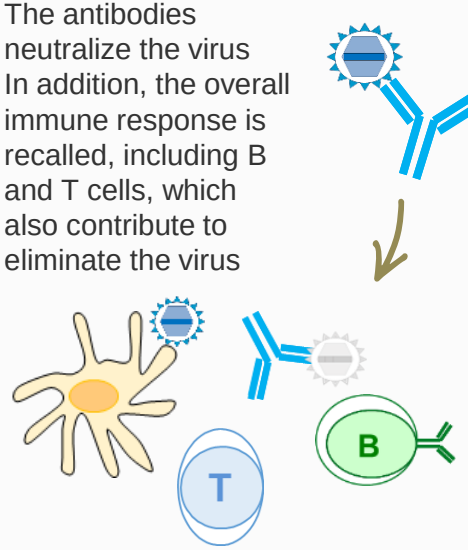
1. Dendritic cells capture and process the vaccine strains, and go through the lymphatic vessels to the lymph nodes where they interact with T and B cells
2. Dendritic cells activate dengue-specific T cells, which in turn activate dengue-specific B cells
3. B cells multiply and begin to produce antibodies against the four types of dengue virus , which are transported throughout the body via the bloodstream

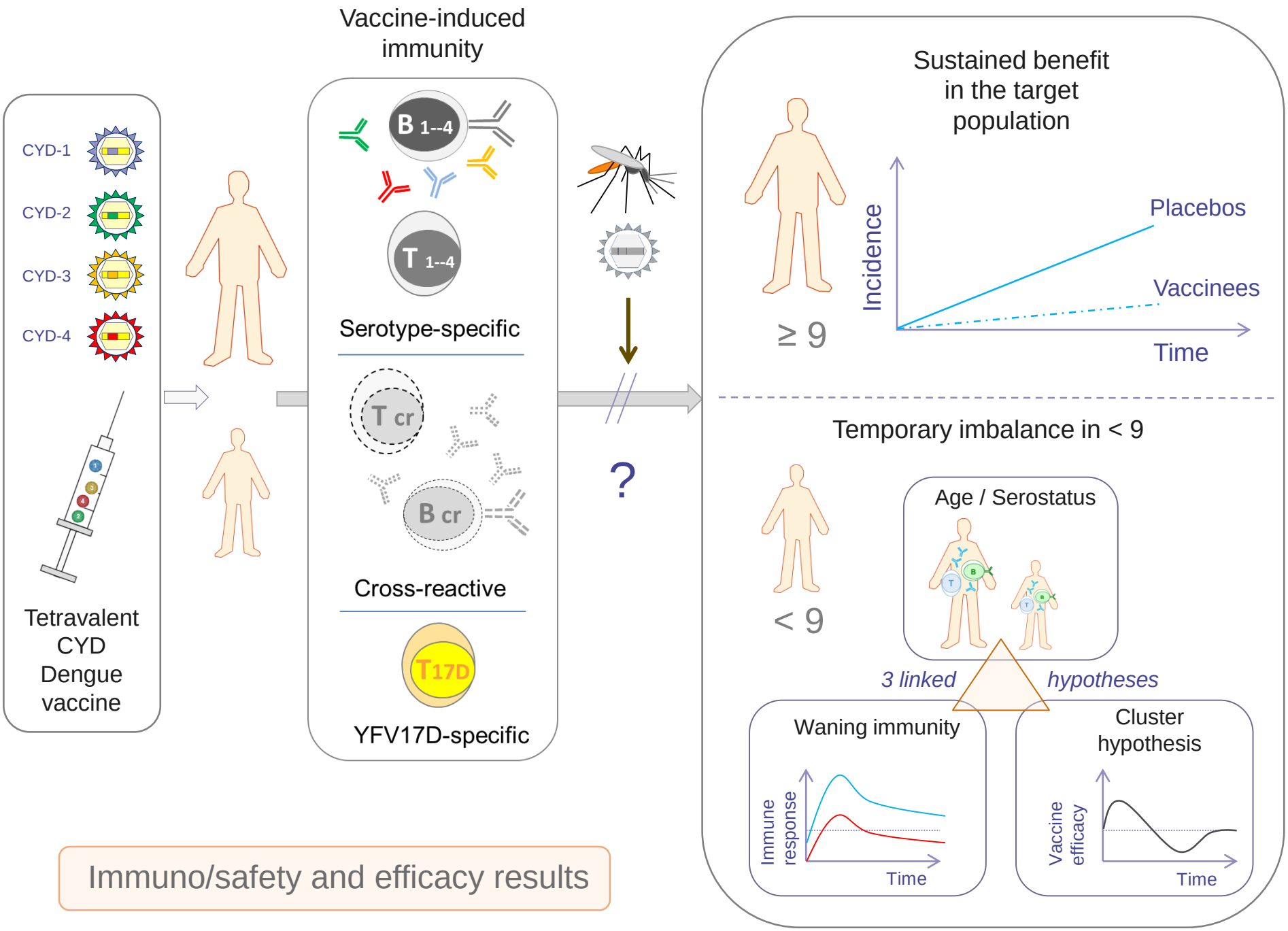



When someone who has been vaccinated is bitten by an infected mosquito, the virus is immediately recognized by the antibodies previously induced by vaccination



The antibodies neutralize the virus
In addition, the overall immune response is recalled, including B and T cells, which also contribute to eliminate the virus




Vaccine-induced immunity
CYD-1
CYD-2
CYD-3
CYD-4
Tetravalent CYD Dengue vaccine
B 1--4
T 1--4
Serotype-specific
T cr
B cr
Cross-reactive
T17D
YFV17D-specific
?
Sustained benefit in the target population
≥ 9
Incidence
Placebos
Vaccinees
Time
Temporary imbalance in < 9
< 9
Age / Serostatus
3 linked
hypotheses
Waning immunity
Immune response
Time
Cluster hypothesis
Vaccine efficacy
Time
Immuno/safety and efficacy results

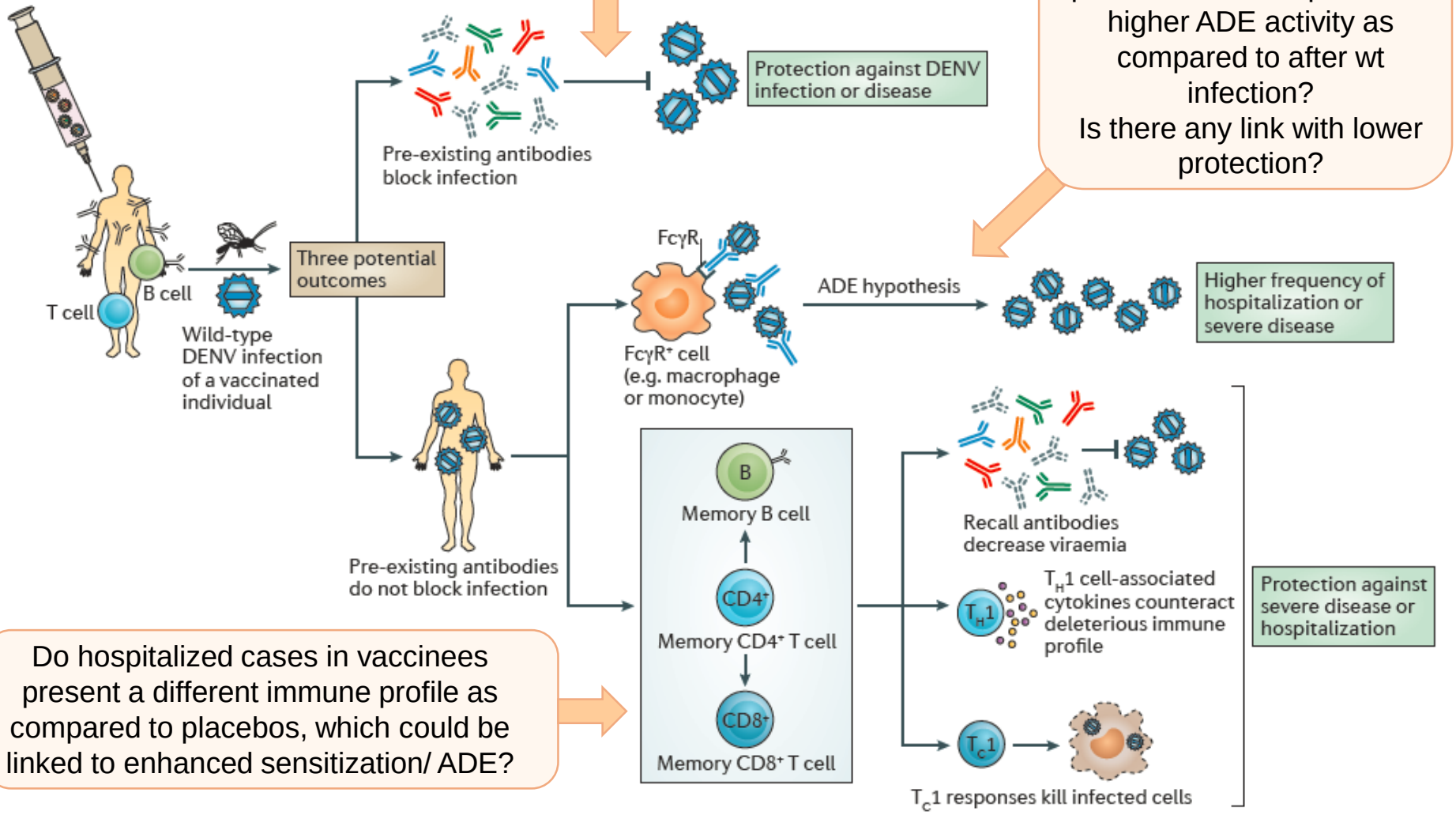
# Questions raised by clinical trials

**New analyses and investigations**

# Different levels and mechanisms of protection
# Questions raised by efficacy trials

What is the quality/specificity of the vaccine induced response in naive and primed individuals?

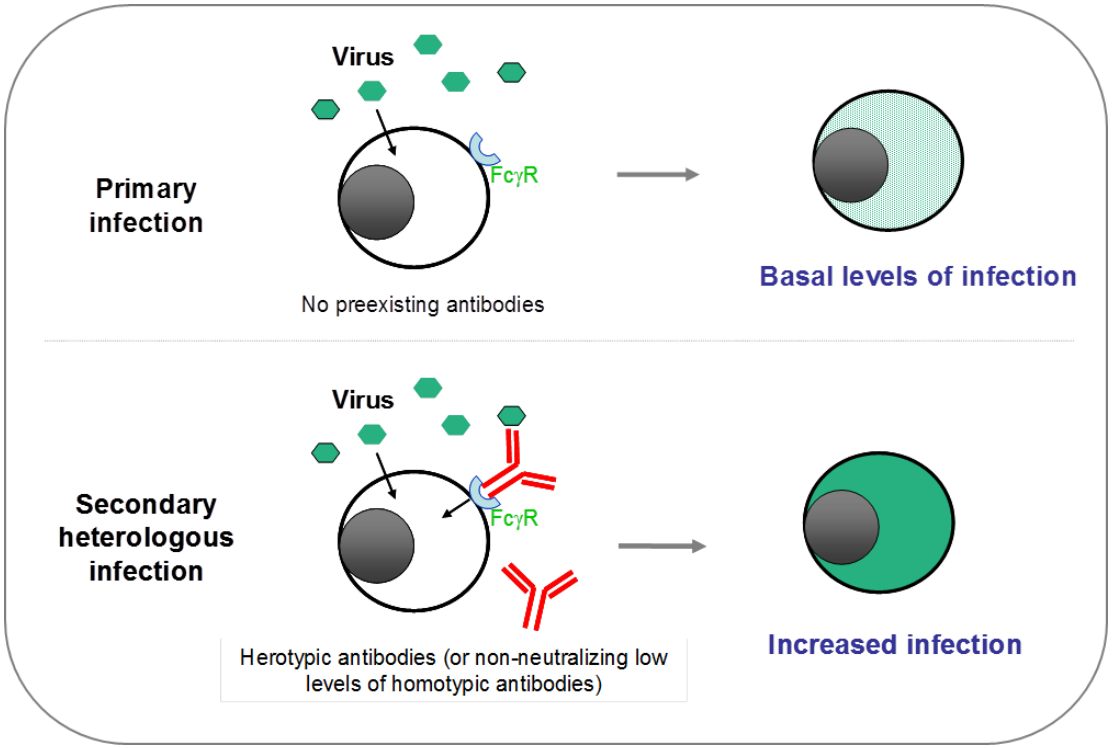
Do sera from naïve or primed vaccinees present a higher ADE activity as compared to after wt infection?
Is there any link with lower protection?




Do hospitalized cases in vaccinees present a different immune profile as compared to placebos, which could be linked to enhanced sensitization/ ADE?

Guy B and Jackson N. *Nat Rev Microbiol* 2016

# No link between differential efficacy and *in vitro* ADE



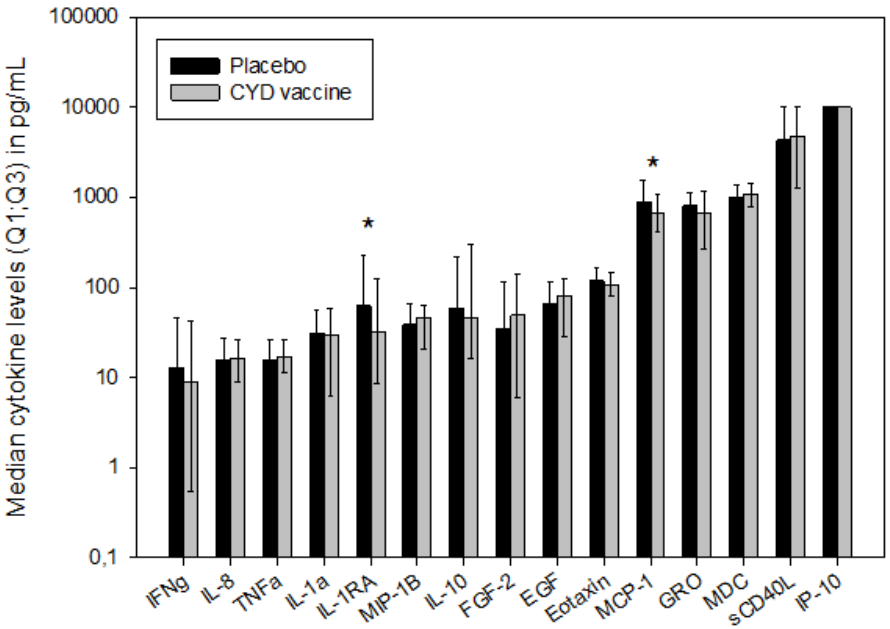

- *In vitro* neutralization assays were performed utilizing FcγR-expressing cells and vaccinee's sera
- Results indicate that observed differences in serotype-specific efficacy were not likely due to differential serotype-specific enhancement by vaccine-elicited antibodies

- This questioned the fact that ADE could be linked to lower or absent protection against symptomatic disease, and then by extension the fact that higher ADE activity could be linked to the increase in hospitalized cases in younger vaccinees

Byers AM ,et al. Open Forum Infect Dis 2015;2:ofv172

# Similar Cytokine /Chemokine / Growth Factors profile in vaccinees vs placebos in acute / hospitalized cases

- CYD-TDV (N=99) and placebos (N=108)




- In agreement with clinical findings (disease outcome and viremia) , overall cytokine/chemokine profiles in acute sera for hospitalized/severe cases show no differences, irrespective of trial, phase, severity and age
- Vaccine does not induce an overall altered profile with breakthrough disease compared with placebo

- IL-1Ra, the only factor shown to be at higher levels in severe dengue when considering all hospitalized cases, is also significantly higher in the placebo group
- On the other hand, sCD40L, shown to be at higher levels in non-severe cases is higher in the vaccine group



Harenberg A, et al. PLoS Negl Trop Dis. 2016;10(7):e0004830

# Role of ADE: present conclusions

- ADE is observed *in vitro* with multiple viruses, and its link to severe dengue cases in humans *in vivo* is still a matter of debate
- Differences in vaccine efficacy against different serotypes in our CYD dengue vaccine trials were not due to different *in vitro* enhancement by vaccine-elicited antibodies
- Other *in vivo* observations do not support a potential role for increased ADE in vaccinees
  - No differences in immune profiles between hospitalized vaccinees and placebos , no excess of deleterious cytokines, which would rule out excess ADE activity in vaccines *versus* placebos
  - Even low antibody titers induced after MV or TV CYD vaccination can have a protective activity, as seen in monkeys.
  - The pattern of hospitalized cases, including severe disease, remains similar to that observed in the control group during the active phase.
    - No increased breakthrough viremia in vaccinees compared to placebos
- *Higher ADE activity in vaccinees versus placebos would not explain the increase in hospitalized cases in younger vaccinees in CYD14 Year 3*

# Proposed hypotheses

## Signal in younger children as seen in CYD14 Y3 would be only temporary

- Hypotheses and modeling provide an array of possible inter-related explanations for CYD14 Y3 observations , involving age/serostatus, waning efficacy and “cluster” effect

- Vaccination in seronegatives may represent an attenuated subclinical primary infection, which is more likely to occur in younger children
  - These “primary infections” and subsequent “secondary” infections would be temporally clustered in younger vaccinees as compared to younger placebos
  - As a consequence, the imbalance observed in vaccinees may be only temporary, occurring during a limited period of time after which more cases would be accrued in placebo recipients
  - In addition, potential sensitization would no longer be present after a “secondary” infection has occurred thanks to the booster effect of infection, and long term benefit would then be expected to persist even in the seronegative population

Coudeville L, et al. Vaccine; in press, DOI: 10.1016/j.vaccine.2015.11.023
Guy B, Jackson N. Nat Rev Microbiol 2016;14:45-54
Gailhardou S, et al. PLoS Negl Trop Dis. 2016 Jul 14;10(7):e0004821

# Recent data and analyses support hypotheses proposed to explain the observation in younger vaccinees in CYD14 Year 3

- Additional data and analyses have been acquired and performed, which support further an independent impact of age and the fact that imbalance in vaccinees would be only temporary (cluster effect)
  - **Both age and serostatus can impact disease outcome in younger vaccinees**
    - Significant VE in seronegative individuals only ≥9 years
    - New analysis taking into consideration LTFU results (hospitalized VC) tends also to confirm the independent effect of age in seronegatives
    - Best modeling of the observed data takes into consideration an independent age-specific effect
  - **Susceptibility in vaccinated individuals is temporally clustered**
    - CYD14 Y4 data support this hypothesis: RR in children <9y lower in Y4 than in Y3.
      - Especially marked in 2-5y: RR 1.424 (0.58; 3.99) in Y4, vs 7.454 (1.15; 313.80) in Y3
      - Cumulative RR over the entire study (i.e., from Dose 1 to Year 4) was 0.79 (0.56-1.13) in children <9y
    - In ≥9y, cumulative RR of 0.39 (0.24-0.60) over 4 years
- *Additional information will be collected through the ongoing LTFU / expansion phase and post-licensure studies may allow further addressing these interconnected hypotheses*

Coudeville L, et al. Vaccine; in press, DOI: 10.1016/j.vaccine.2015.11.023
Guy B, Jackson N. Nat Rev Microbiol 2016;14:45-54
Gailhardou S, et al. PLoS Negl Trop Dis. 2016 Jul 14;10(7):e0004821
Hadinegoro SRS , et al Vaccine 2016;34: 4273–4274

# Novel Immunological Analyses and Investigations

## Quantitative and Qualitative responses

- New analyses performed on existing PRNT results
- New immunological investigations
  - ADE in vaccinee's sera
  - Immune profile in acute sera (multiplex assays)
  - Ab Affinity (Forte Bio assay)
  - Ab Specificity (homo/heterotypic; depletion studies)
  - Systems Serology (US Army group)
  - CMI / B and T cell responses / memory
    - CYD14 ancillary study / AFRIMS - URI
    - Ongoing / future trials / Booster studies

*Analyses stratified by phase, age and serostatus*

- *Possibly use the Human Dengue Infection Model / US Army – SUNY*

# A new comer: Zika

**Which interplay with dengue and other flaviviruses?**

**Which impact on dengue vaccine development and implementation?**

# Questions raised by Zika epidemics

- What are the potential impacts of Zika outbreaks on dengue vaccine immunogenicity, efficacy and effectiveness studies?
- Is there a reliable/specific diagnostic test to identify Zika? (limited specificity of clinical manifestations)
- What are the clinical spectrum of Zika disease and its immuno determinants?
  - **Does Flavivirus pre-immunity (Dengue, YF and JEV) play a role on Zika disease?**
    - Is there evidence of detrimental cross-enhancement (Dengue & Zika)?
    - Is there evidence of cross protection ?
- What are the Zika epidemiology and dynamics in terms of population at risk, time and geographical distribution?
- *These questions will be addressed in part in the ongoing CYD15 study (amendment submitted) and in future effectiveness studies*

# Zika and Dengue: cross-enhancement?

- *In vitro* enhancement with flavivirus cross-reactive Abs is expected and not correlated with clinical observations *in vivo* (1)
  - Even anti-Zika homologous Abs can induce increased Zika infection *in vitro* (2)
  - Still regarding increased Zika infection, no correlation between *in vitro* and *in vivo* preclinical data (2)
  - Recent monkey data from the U. of Puerto Rico show no increase in Zika viremia in dengue-immune monkeys (5)
- According to existing epidemiological data, pre-existing cross-reactive immunity between flaviviruses has not been associated with disease enhancement or disease severity
- Only epidemiological data from ongoing Zika outbreaks and epidemics in dengue endemic countries will provide elements of answer to this theoretical concern
  - "The ADE observed in vitro does not show that immunity to dengue virus can enhance the risk of infection with Zika virus. That conclusion would be provided only by epidemiological analyses, together with studies in validated animal models. Moreover, it remains unknown whether enhanced infection of Fc-expressing cells would influence the course of infection with Zika virus in humans" (3)
- As of today, pre-existing dengue (or flavivirus) immunity has been not associated with the severe forms of Zika disease, ie microcephaly or GBS (4)


1. Laoprasopwattana K, et al.. J Infect Dis. 2005 192(3): 510-9.
2. Stettler et al, Science. 2016 Aug 19;353(6301):823-6
3. Harrison SC. Nat Immunol. 2016 Aug 19;17(9):1010-2
4. Cao-Lormeau VM et al. Lancet. 2016 Apr 9;387(10027):1531-9

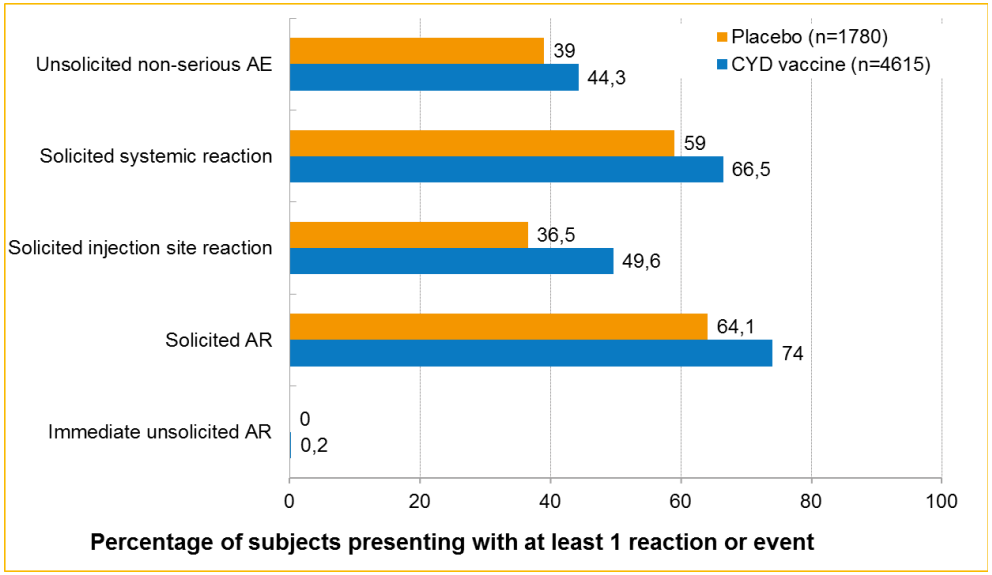
5; Petraleigh Pantoja, et al. Secondary Zika virus infection do not support evidences of Antibody-Dependent Enhancement in vivo in dengue pre-exposed rhesus macaques. 2016. p. 1-16. http://nprcresearch.org/primate/hot-topics/CPRC-Zika-Virus-Research-Page.pdf

# Dengue vaccine: overall safety

**Next steps: Post licensure plans**

# Large Integrated Safety Analyses demonstrate acceptable safety profile in the 9-60 years population

- Integrated safety analysis performed in the 9- to 60-year-old population creates significant size of safety database.
- **20,667** subjects 9–60 years of age receiving at least 1 dose of vaccine.
  - **~19,700 received all 3 doses**
- Allows detection of very common, common, and uncommon AEs in accordance with WHO guidelines.




**No safety concerns** related to the nature and frequency of unsolicited AEs

**Comparable safety** results

- Between vaccine and control groups aged 9–60 years
- Across populations (age group, sex, region)

*Integrated safety analysis pooling data from 13 studies that used the final formulation and final vaccination schedule (CYD12, 13, 22, 24, 28, 30, 47, 23, 17, 32, 14, 15, 51).
AE=adverse event; AR=adverse reaction; WHO=World Health Organization.

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Gailhardou S, et al. PLoS Negl Trop Dis. 2016 Jul 14;10(7):e0004821

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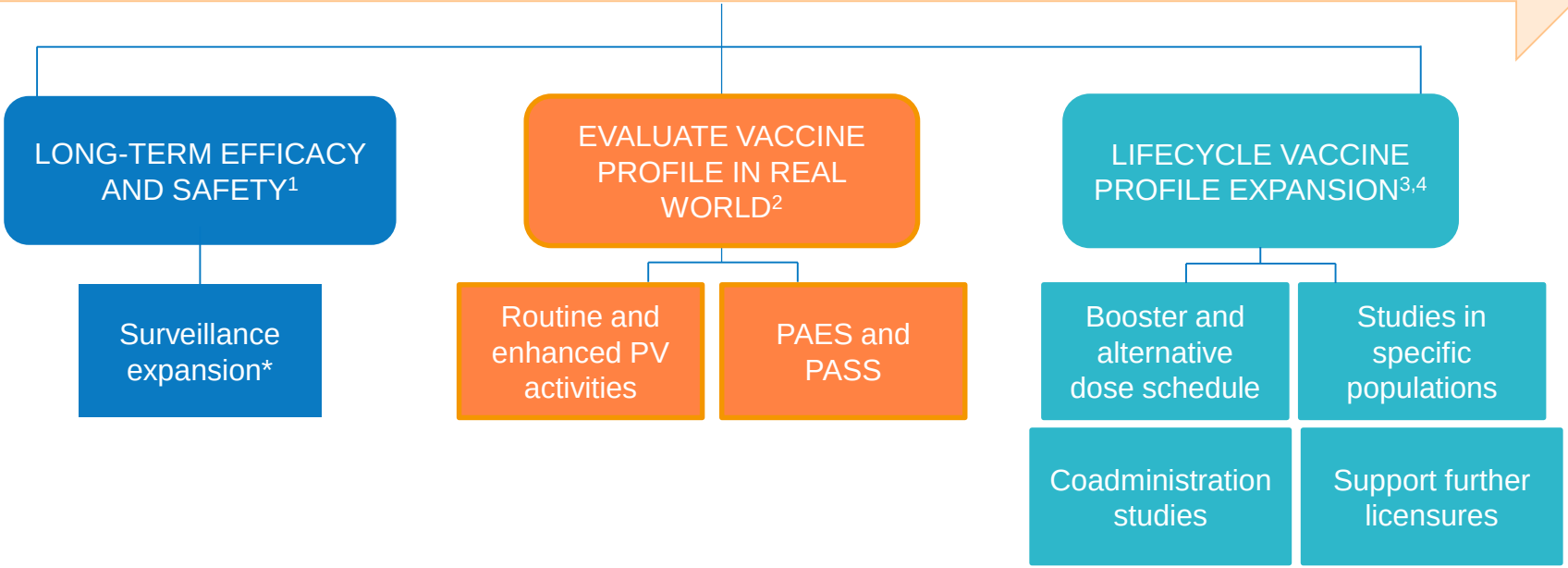
# Extensive post-licensure plan

9–60 y.o.
3 doses (0–6–12 months)
Preventive vaccine in endemic areas

Allow Benefit/Risk assessment through Risk Management Plan execution




*Active surveillance/detection of symptomatic (in addition to hospitalized) dengue cases.
PAES=postauthorization effectiveness studies; PASS=postauthorization safety studies; PV=pharmacovigilance.


1. Hadinegoro, 2015, N Engl J Med.
2. Guy, 2015, Vaccine.
3. WHO, 2011, Guidelines on the quality, safety, and efficacy of dengue tetravalent vaccines (live, attenuated).
4. Global Vaccine Safety Initiative (GVSI), 2015, fourth meeting report.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Thank you