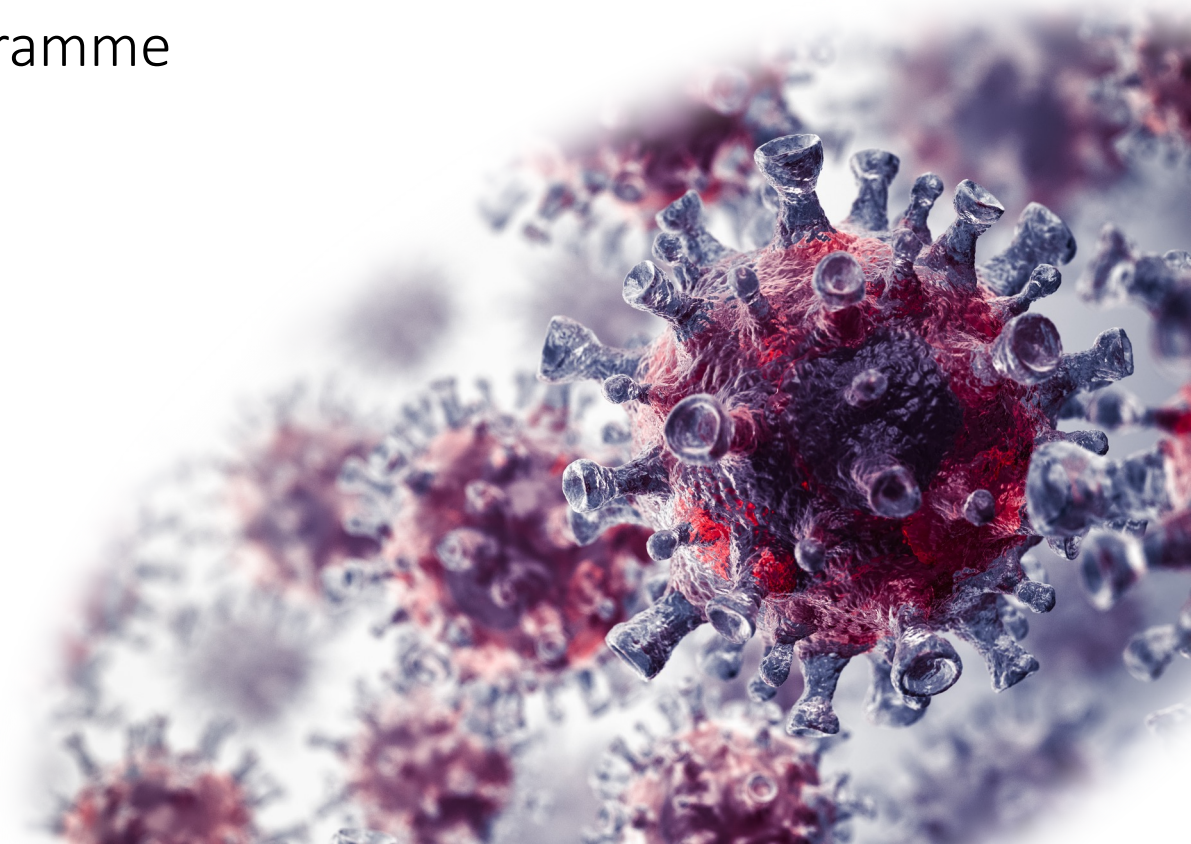


# Designing a mRNA vaccine for dengue – key considerations

31 October 2023

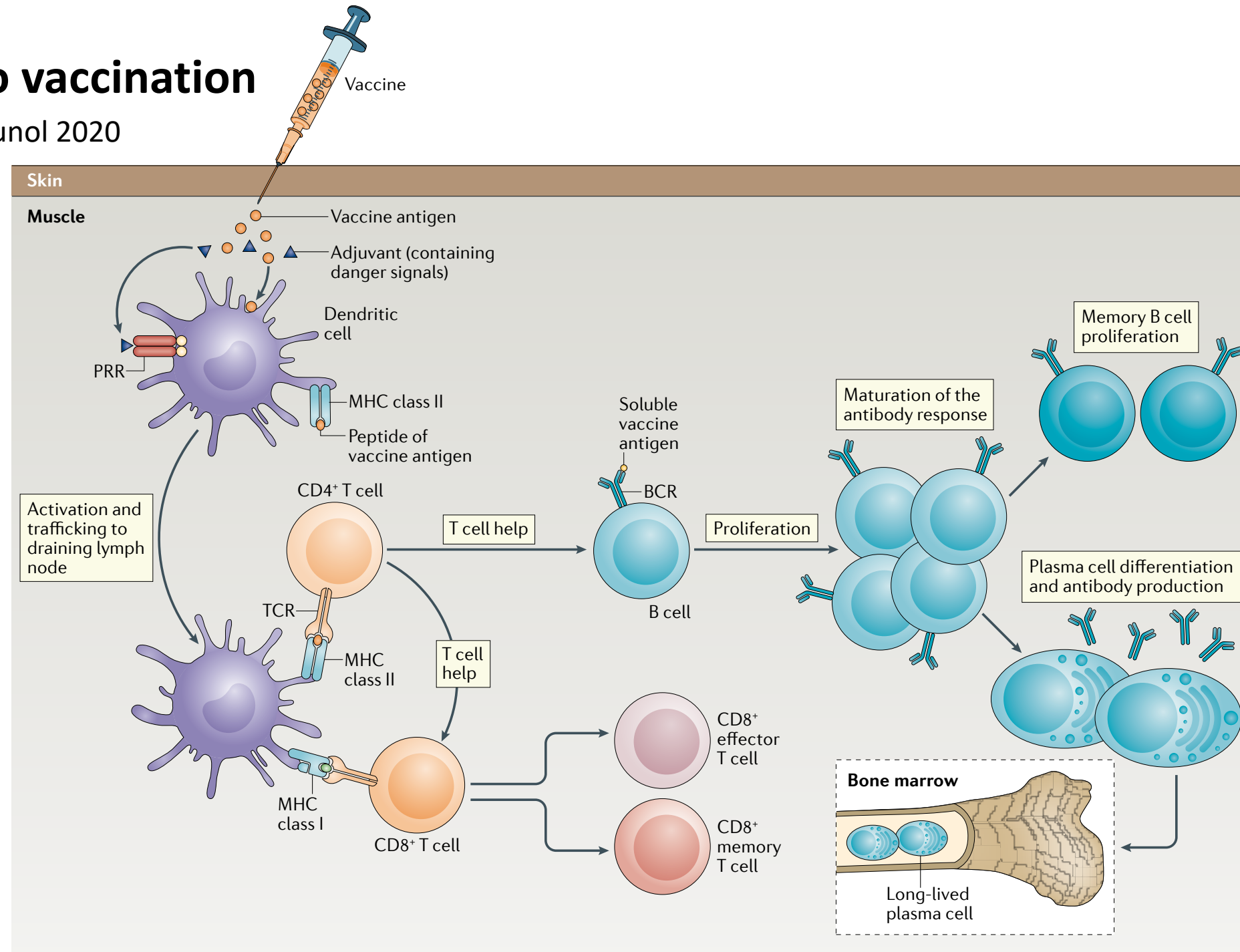
WHO/MPP mRNA Technology Transfer Programme  
Bangkok, Thailand

Dr Eugenia Ong, PhD  
Principal Research Scientist  
Viral Research and Experimental Medicine Centre  
@ SingHealth Duke-NUS (ViREMICS)  
[eugenia.ong@duke-nus.edu.sg](mailto:eugenia.ong@duke-nus.edu.sg)



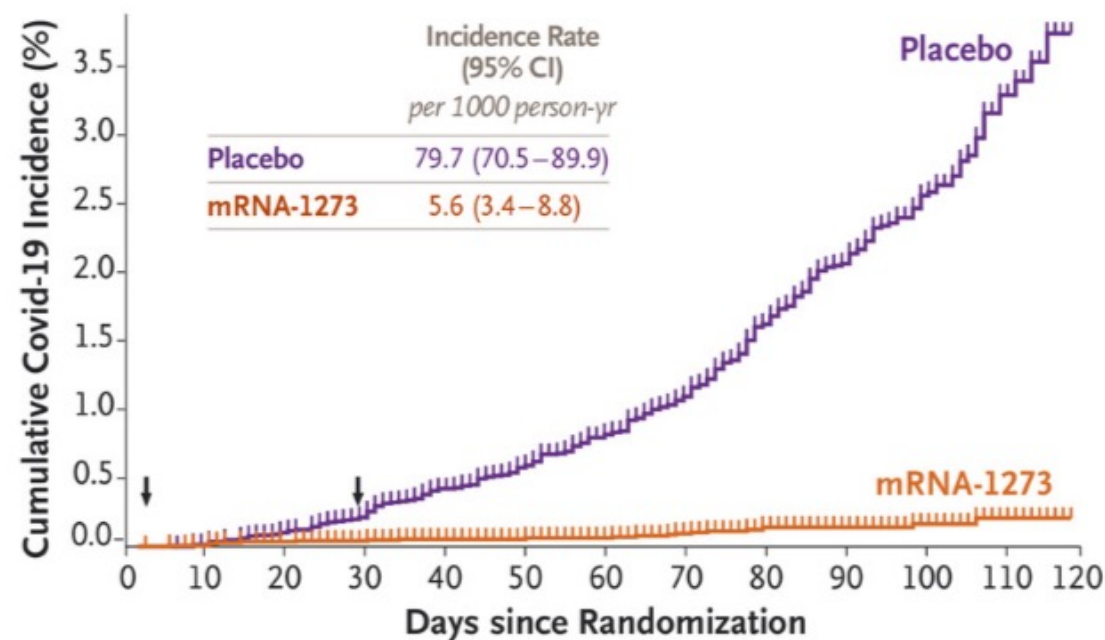
# Immune response to vaccination

Pollard and Bijker, Nat Rev Immunol 2020



# Efficacy of the SARS-CoV-2 mRNA vaccines

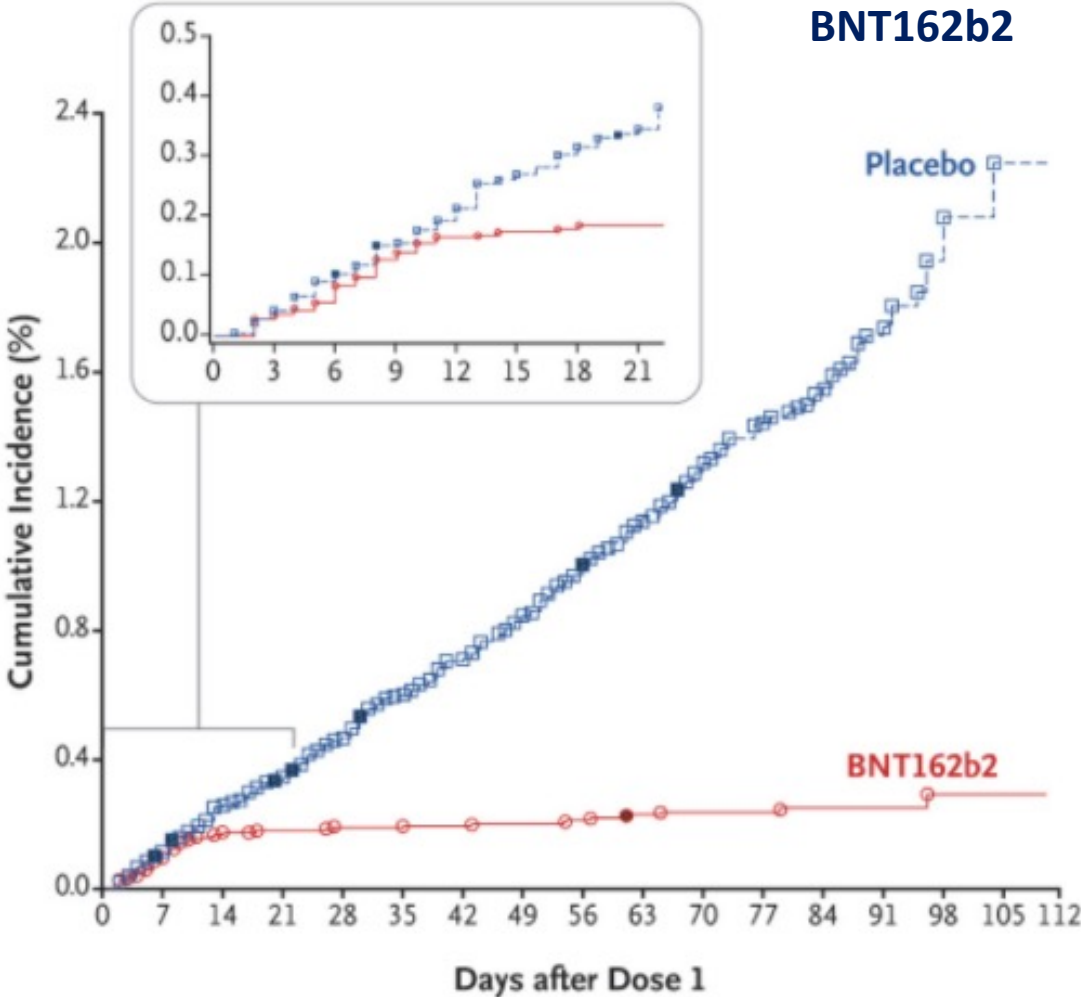
## mRNA-1273



Vaccine efficacy of 94.1% (95% CI, 89.3% - 96.8%)

Baden, NEJM, 2021

## BNT162b2



Vaccine efficacy of 95% (95% CI, 90.3% - 97.6%)

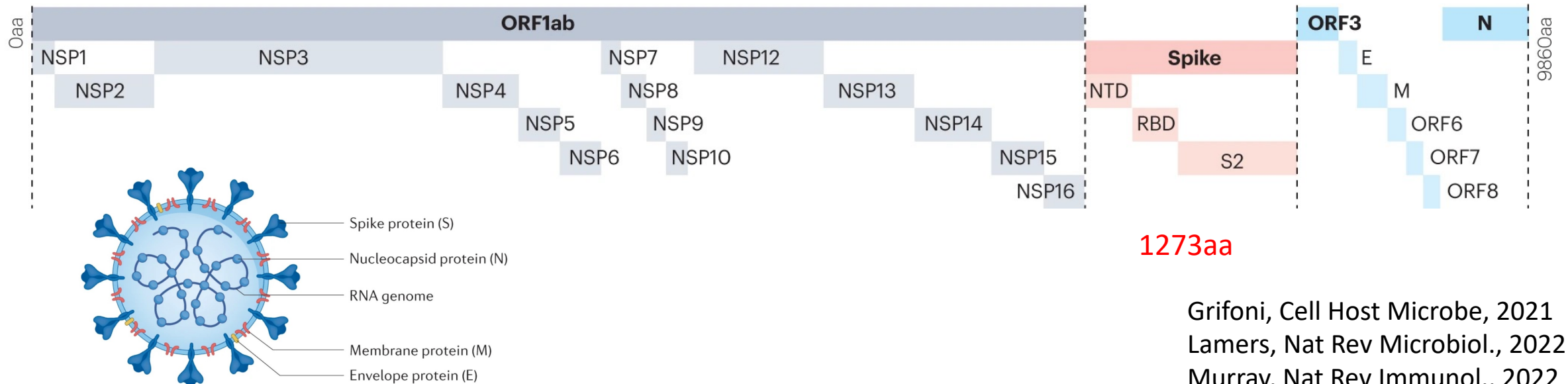
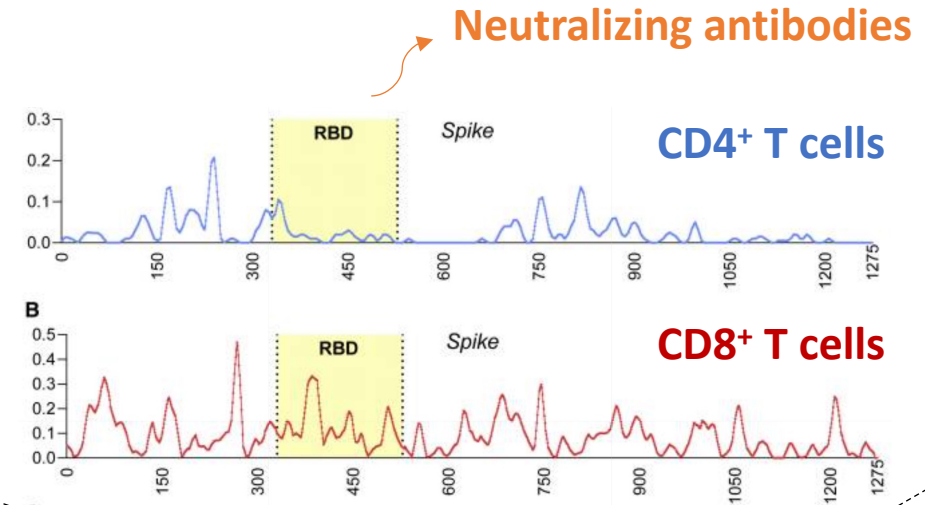
Polack, NEJM, 2020

# Antibody and T cell epitopes for SARS-CoV-2

Neutralizing antibodies are primarily directed against spike and nucleocapsid.

Structural proteins (S, M, N) are dominant targets of T cell responses

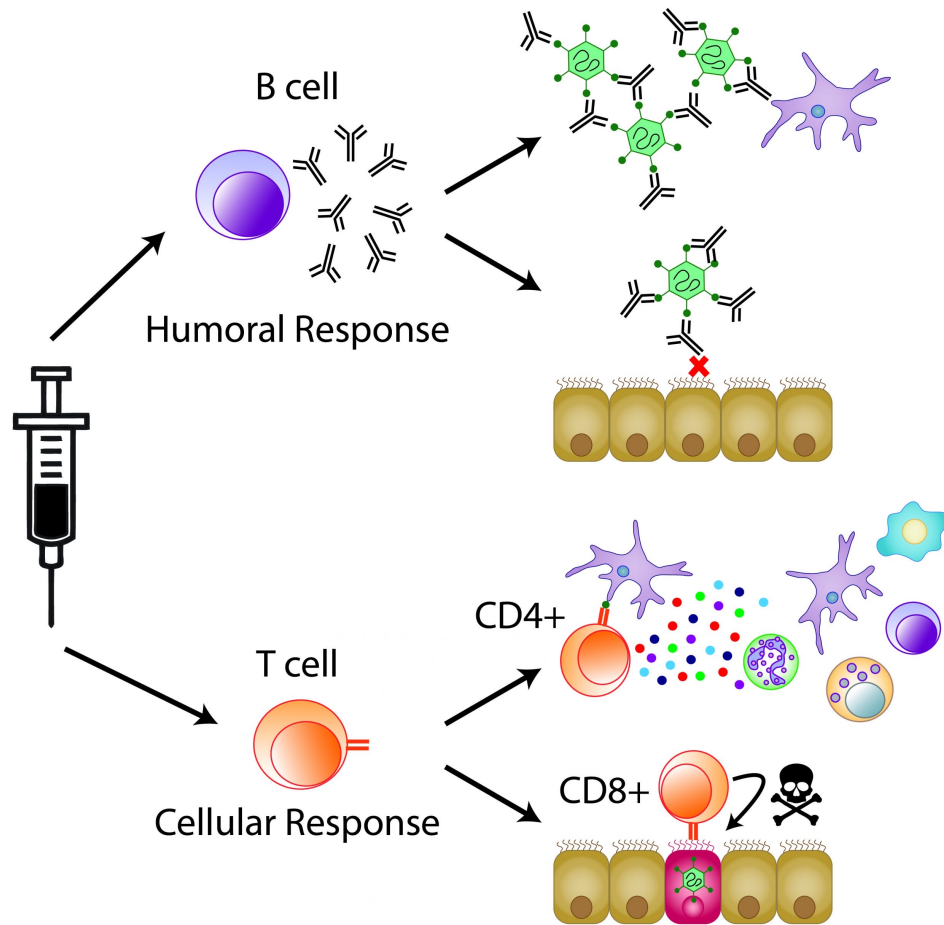
Immunodominant antigenic regions



Grifoni, Cell Host Microbe, 2021  
Lamers, Nat Rev Microbiol., 2022  
Murray, Nat Rev Immunol., 2022



# Neutralizing Ab vs. T cells: Common goal but different roles

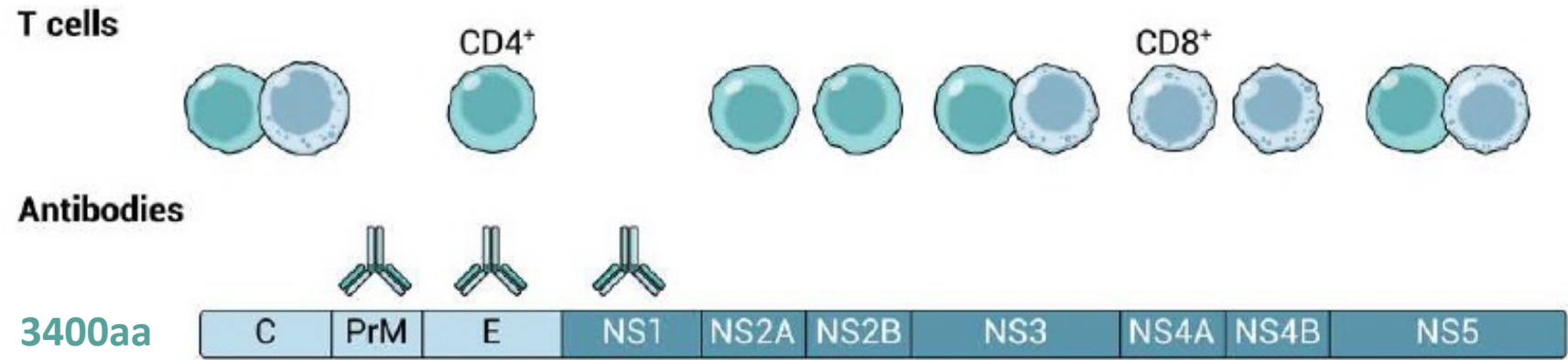


**Humoral Immunity**  
(Neutralizing) Antibodies  
***Block Infection***

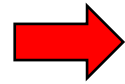
**Cellular Immunity**  
T-cells (CD4<sup>+</sup>, CD8<sup>+</sup>)  
***Viral Control***

Learning about dengue immunity  
from clinical trials

# Immunodominant proteins of DENV



Molecular constructs of 3 live attenuated tetravalent dengue vaccines



**CYD-TDV**

Dengvaxia



**TAK003**

Qdenga



**TV003**



CYD-TDV  
showed low to  
no efficacy  
against DENV2

|   | Vaccine group (N=6848) |                          |                                | Control group (N=3424) |                         |                               | Vaccine efficacy<br>(% [95% CI]) |
|---|------------------------|--------------------------|--------------------------------|------------------------|-------------------------|-------------------------------|----------------------------------|
|   | Cases*<br>(n)          | Person-years<br>at risk† | Incidence density‡<br>(95% CI) | Cases<br>(n)           | Person-years<br>at risk | Incidence density<br>(95% CI) |                                  |
| Efficacy against VCD, more than 28 days after third injection in all participants who had received three injections |                        |                          |                                |                        |                         |                               |                                  |
| Serotype 1  | 51                     | 6548                     | 0.8 (0.6 to 1.0)               | 50                     | 3210                    | 1.6 (1.2 to 2.0)              | 50.0% (24.6 to 66.8)             |
| Serotype 2  | 38                     | 6561                     | 0.6 (0.4 to 0.8)               | 29                     | 3253                    | 0.9 (0.6 to 1.3)              | 35.0% (−9.2 to 61.0)             |
| Serotype 3  | 10                     | 6613                     | 0.2 (0.1 to 0.3)               | 23                     | 3281                    | 0.7 (0.4 to 1.1)              | 78.4% (52.9 to 90.8)             |
| Serotype 4  | 17                     | 6605                     | 0.3 (0.2 to 0.4)               | 34                     | 3265                    | 1.0 (0.7 to 1.5)              | 75.3% (54.5 to 87.0)             |
| Unserotyped   | 2                      | 6634                     | <0.1 (0.0 to 0.1)              | 3                      | 3309                    | <0.1 (0.0 to 0.3)             | 66.7% (−190.3 to 97.2)           |

Capeding et al, Lancet 2014

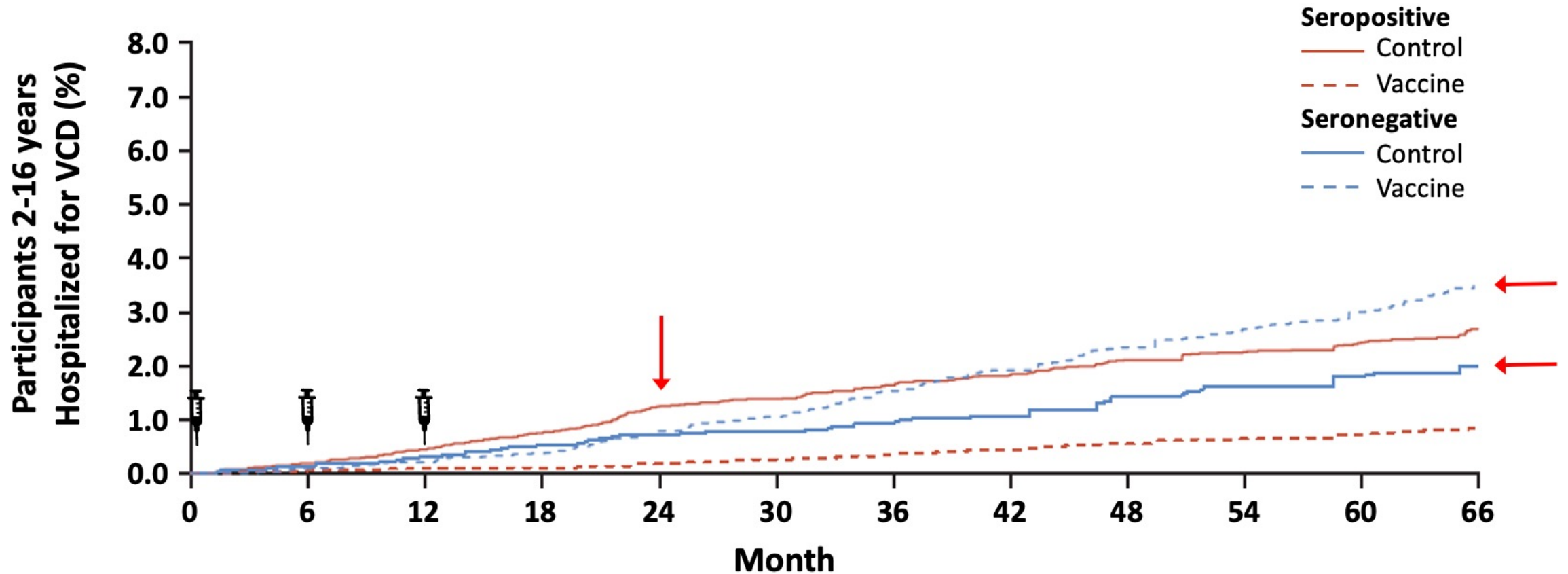
| Variable                        | Vaccine Group |                      |                               | Control Group |                      |                               | Vaccine Efficacy<br>(95% CI) |
|---------------------------------|---------------|----------------------|-------------------------------|---------------|----------------------|-------------------------------|------------------------------|
|                                 | Cases         | Person-Yr<br>at Risk | Incidence Density<br>(95% CI) | Cases         | Person-Yr<br>at Risk | Incidence Density<br>(95% CI) |                              |
|                                 | <i>no.</i>    |                      | <i>no./100 person-yr</i>      | <i>no.</i>    |                      | <i>no./100 person-yr</i>      | %                            |
| Modified per-protocol analysis* |               |                      |                               |               |                      |                               |                              |
| Serotype 1                      | 66            | 12,478               | 0.5 (0.4–0.7)                 | 66            | 6,196                | 1.1 (0.8–1.4)                 | 50.3 (29.1–65.2)             |
| Serotype 2                      | 58            | 12,495               | 0.5 (0.4–0.6)                 | 50            | 6,219                | 0.8 (0.6–1.1)                 | 42.3 (14.0–61.1)             |
| Serotype 3                      | 43            | 12,514               | 0.3 (0.2–0.5)                 | 82            | 6,213                | 1.3 (1.1–1.6)                 | 74.0 (61.9–82.4)             |
| Serotype 4                      | 18            | 12,522               | 0.1 (0.1–0.2)                 | 40            | 6,206                | 0.6 (0.5–0.9)                 | 77.7 (60.2–88.0)             |
| Unknown                         | 6             | 12,540               | <0.1 (0.0–0.1)                | 3             | 6,268                | <0.1 (0.0–0.1)                | 0.0 (–517.8–78.6)            |

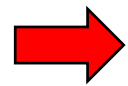
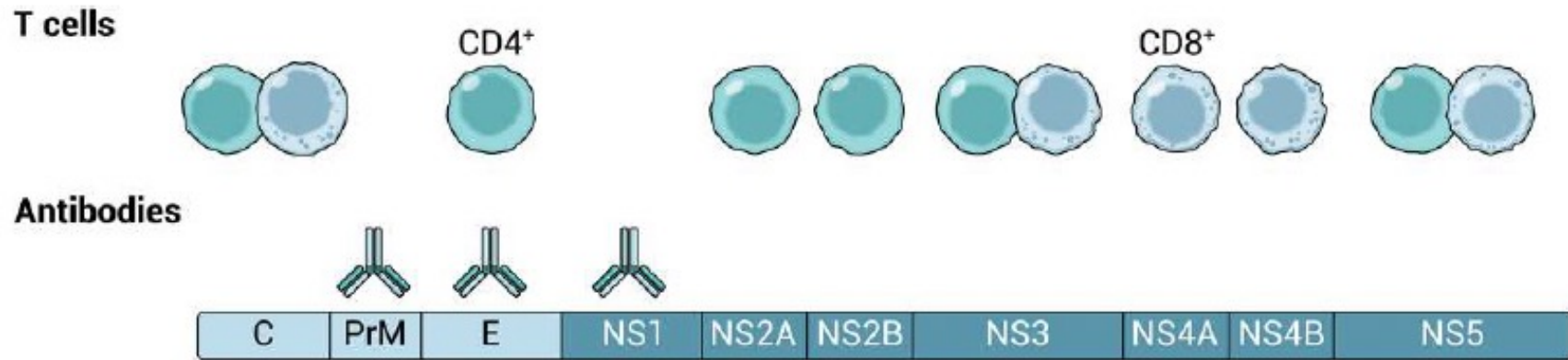
Villar et al, NEJM 2015



# CYD-TDV – increased risk of dengue hospitalization in seronegative individuals

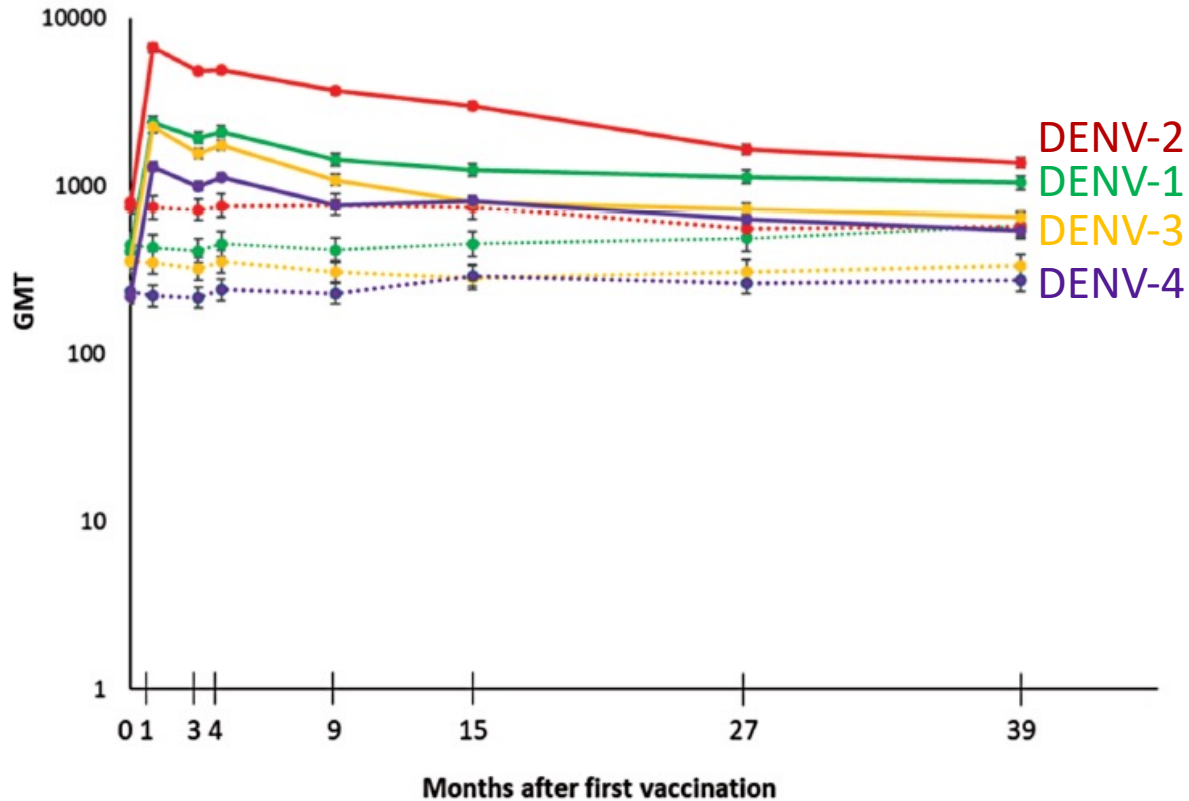
CYD-TDV is now only licensed for use in DENV-seropositive individuals



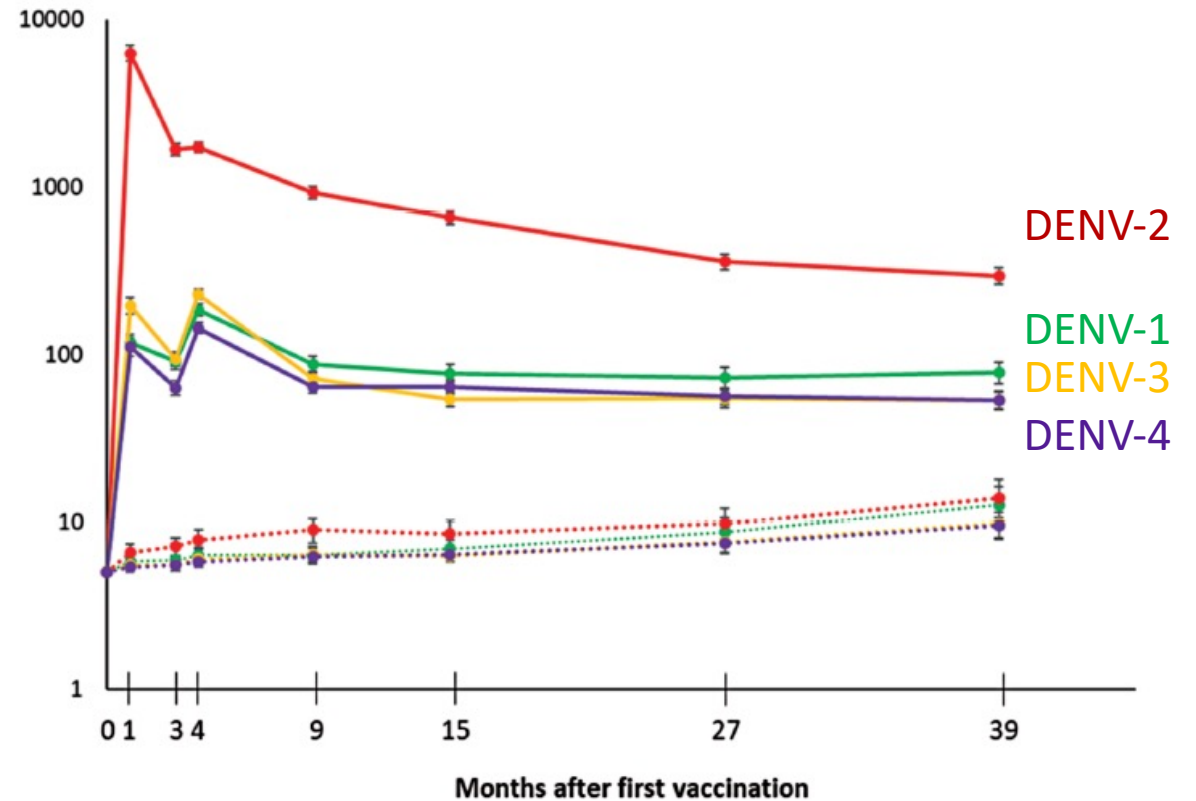


# NAb titers following TAK003 vaccination

**Baseline Seropositive**



**Baseline Seronegative**



Highest efficacy against DENV-2

## Cumulative efficacy at 3 years of follow up

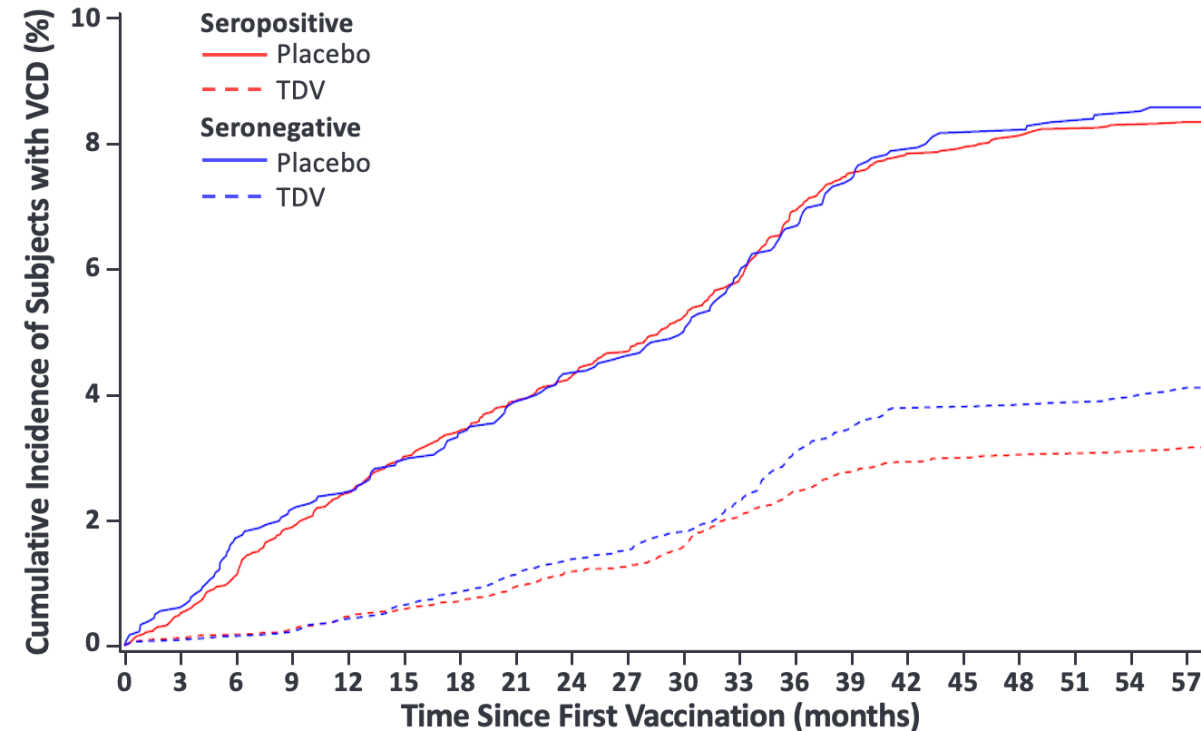
|              | Placebo   | TAK003   | Efficacy % (95% CI)     |   |
|--------------|-----------|----------|-------------------------|---|
| Seropositive | (n=4,854) | (n=9663) |                         |   |
| DENV1        | 130       | 114      | 56.2 (43.7-66.0)        |   |
| DENV2        | 124       | 42       | 83.4 (76.4-88.3)        |   |
| DENV3        | 95        | 94       | 52.3 (36.6-64.2)        |   |
| DENV4        | 15        | 12       | 60.7 (16.0-81.6)        |   |
| Overall      | 358       | 262      | 65.0 (58.9-70.1)        |   |
| Seronegative | (n=1,832) | (n=3714) |                         |   |
| DENV1        | 66        | 77       | 43.5 (21.5-49.3)        | ← |
| DENV2        | 55        | 9        | 91.9 (83.6-96.0)        |   |
| DENV3        | 15        | 36       | -23.4 (-125.3 to 32.4)  | ← |
| DENV4        | 2         | 8        | -105.5 (-867.5 to 56.4) |   |
| Overall      | 136       | 128      | 54.3 (41.9-64.1)        |   |

No efficacy against DENV-3 in seronegative individuals

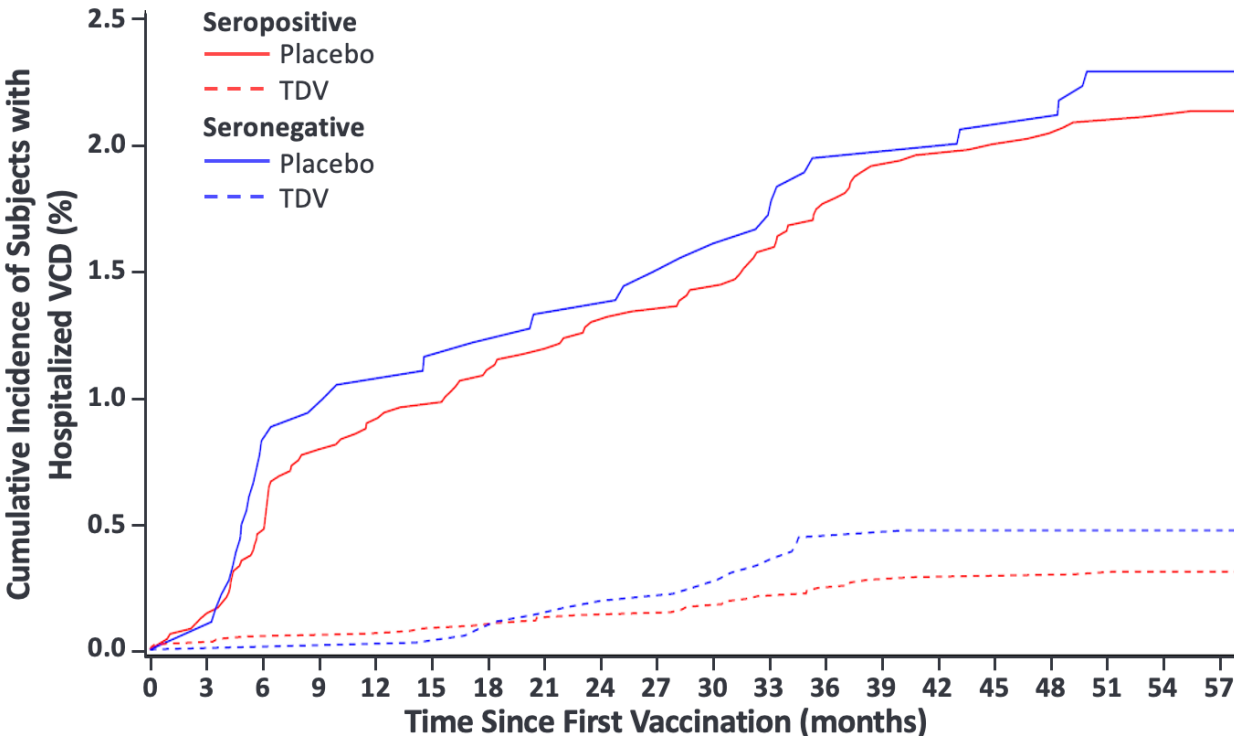


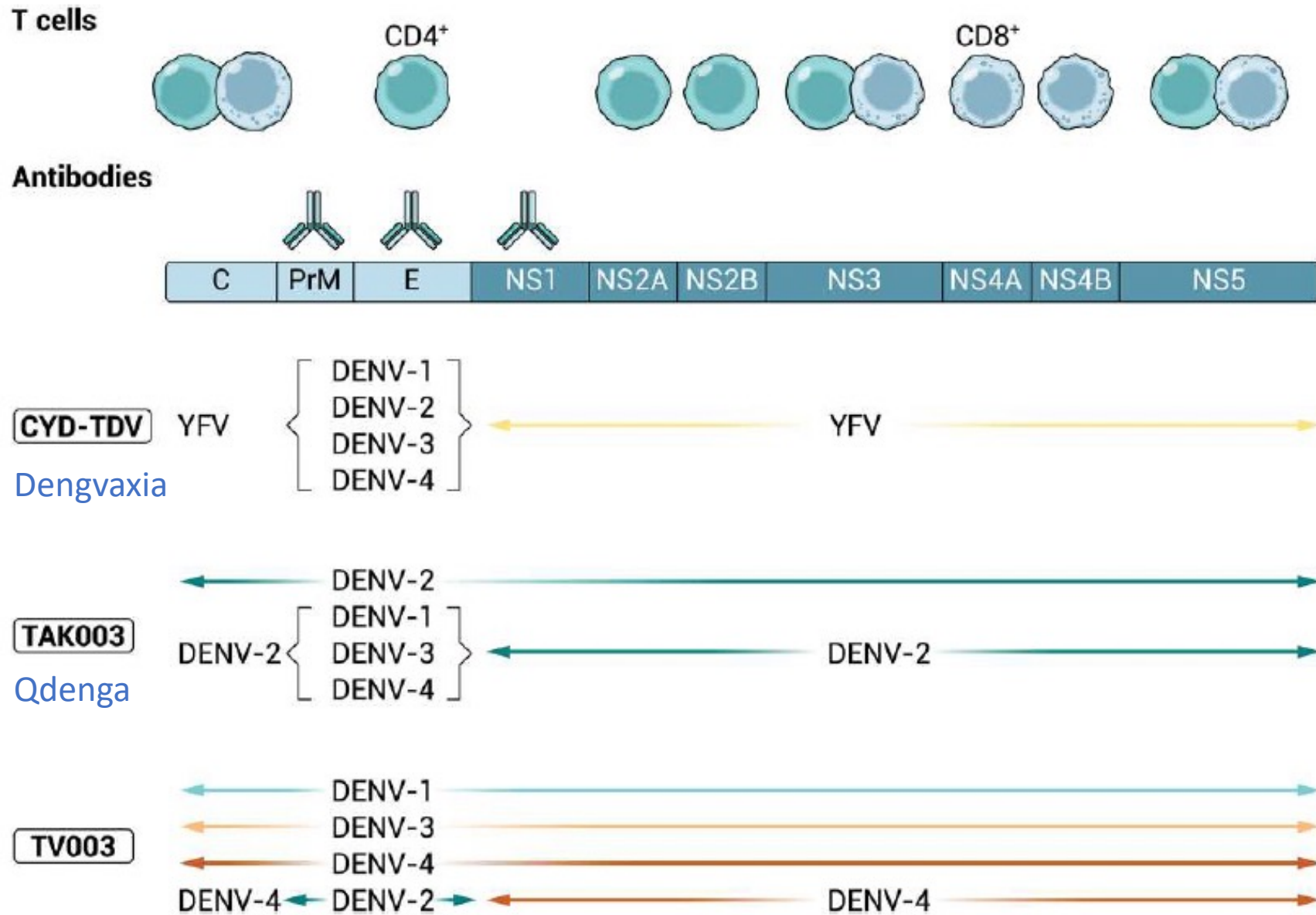
# Vaccine efficacy sustained over long-term follow up

Virologically-confirmed dengue



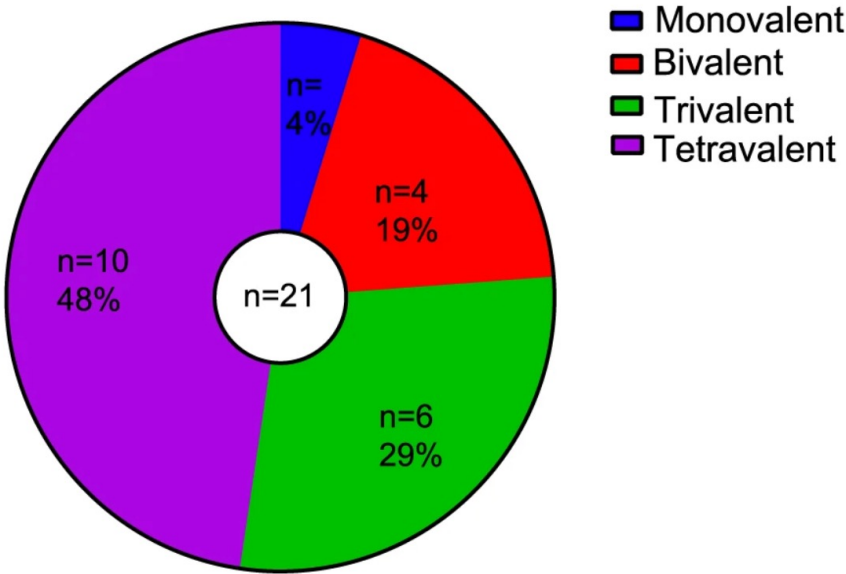
Hospitalized dengue



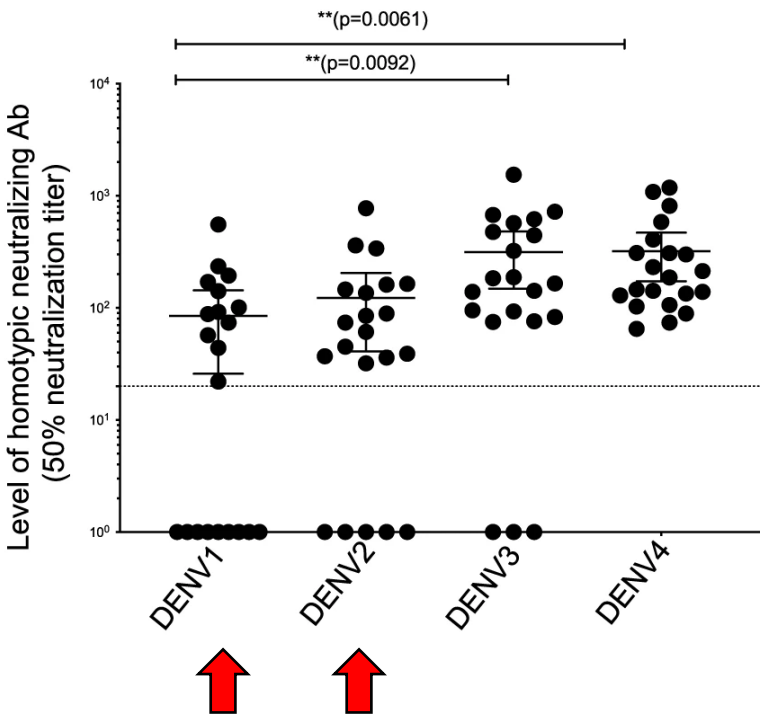


TV003 generated type specific neutralizing antibodies to all four DENVs

Nivarthi et al, Nat Commun 2021

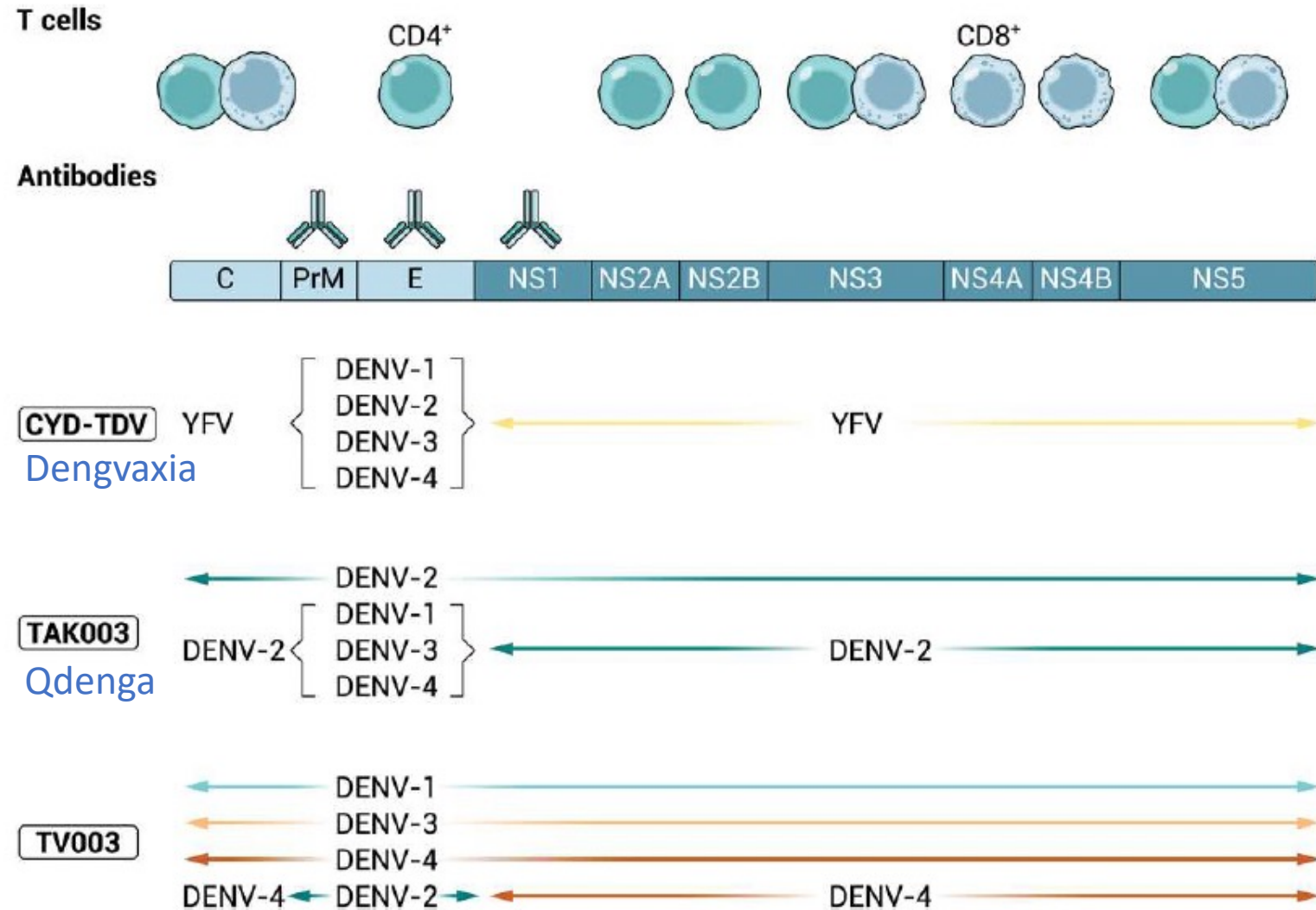


16/21 (76%) subjects had type specific nAbs to 3 or 4 DENV serotypes



| PARCIAL RESULTS PHASE<br>3<br>DENGUE VACCINE | People without<br>previous<br>infection | People with<br>previous infection | General efficacy |
|--|---|-----------------------------------|------------------|
| Efficacy                                     | 73,5%                                   | 89,2%                             | 79,6%            |
| Efficacy DENV-1                              | 85,5% ←                                 | 96,8%                             | 89,5%            |
| Efficacy DENV-2                              | 57,9% ←                                 | 83,6%                             | 69,6%            |

Neutralizing ab  
alone may not  
reliably inform  
vaccine efficacy





Different immunity thresholds  
for the 4 DENVs

Protection against  
DENV2 requires  
high nAb titers

6 DENV1 cases vs 13  
PCR neg contacts

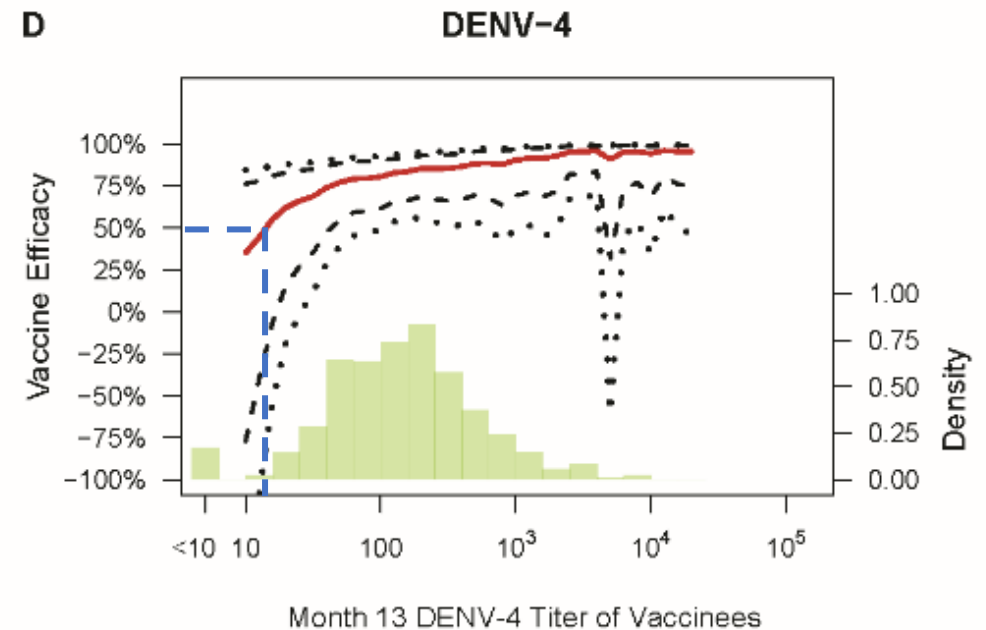
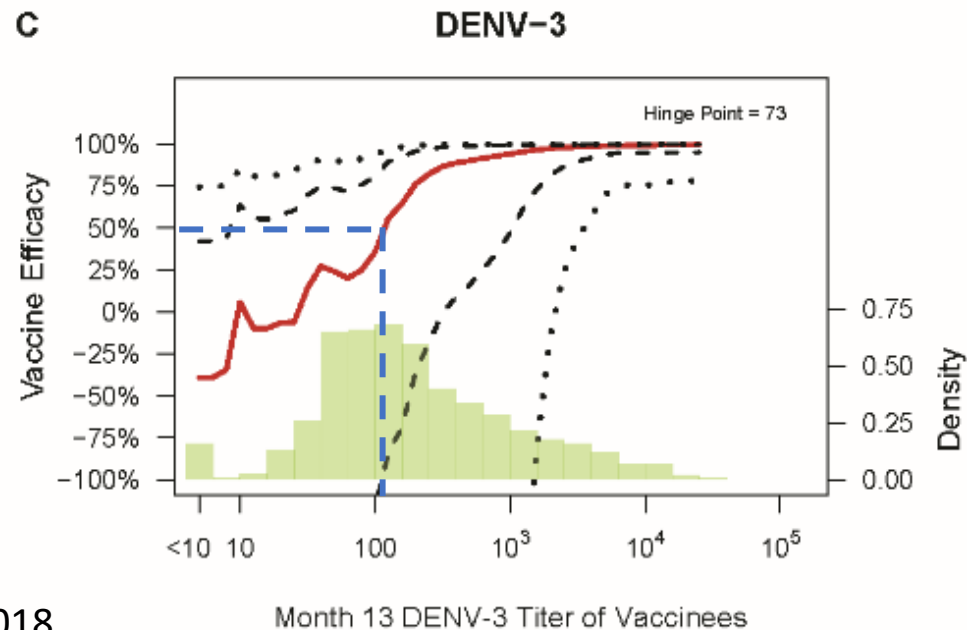
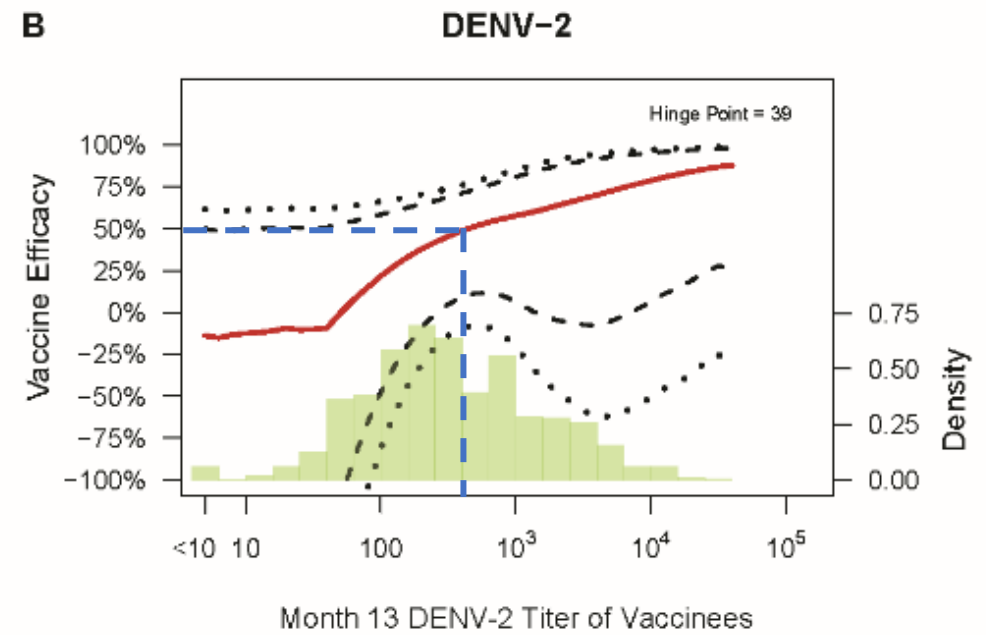
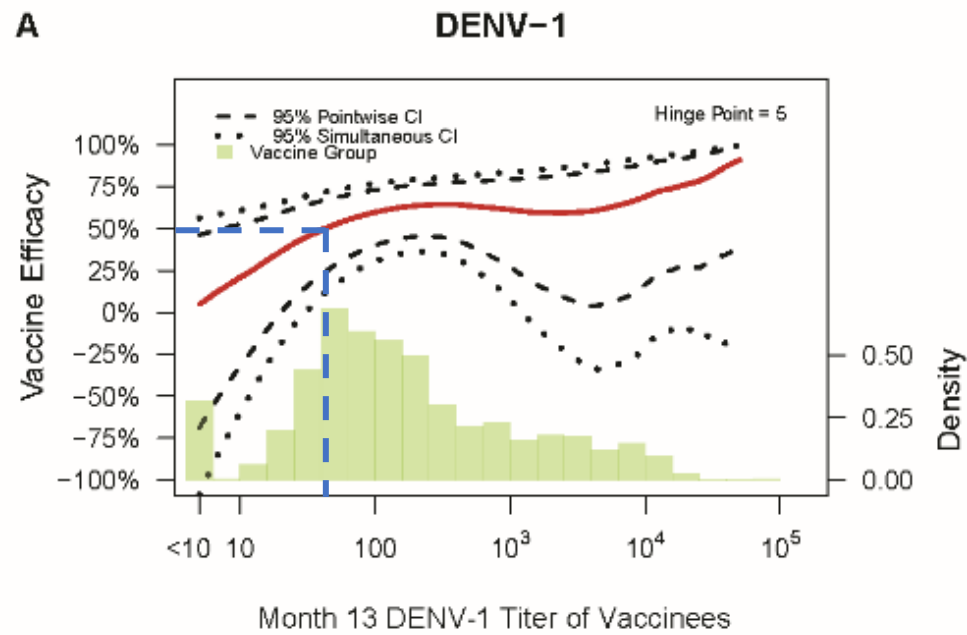
6 DENV4 cases vs 9  
PCR neg contacts

5 DENV2 cases vs 10  
PCR neg contacts

| NT cutoff status <sup>a</sup> | PCR negative, no. | PCR positive, no. |
|-------------------------------|-------------------|-------------------|
| All serotypes combined        |                   |                   |
| Homotypic NT <11              | 13                | 14                |
| Homotypic NT ≥11              | 19                | 3                 |
| DENV-1                        |                   |                   |
| DENV-1 NT <11                 | 7                 | 6                 |
| DENV-1 NT ≥11                 | 6                 | 0                 |
| DENV-4                        |                   |                   |
| DENV-4 NT <16                 | 3                 | 6                 |
| DENV-4 NT ≥16                 | 6                 | 0                 |
| DENV-2                        |                   |                   |
| DENV-2 NT <323                | 4                 | 5                 |
| DENV-2 NT ≥323                | 6                 | 0                 |



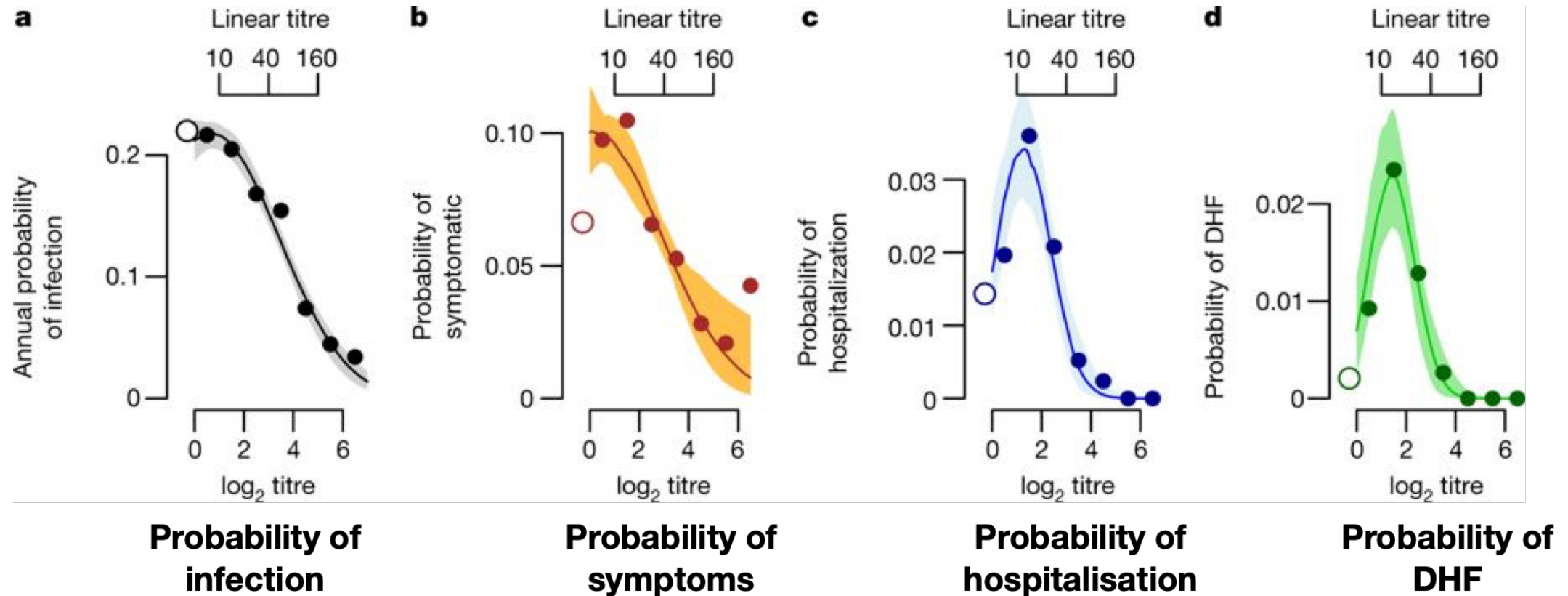
Higher post-vaccination neutralizing antibody titers needed to protect against symptomatic DENV-2 infection (CYD14 trial)



Why is the threshold for immunity  
against DENV2 the highest?



Limited range of pre-infection antibody titer is associated with risk of severe dengue

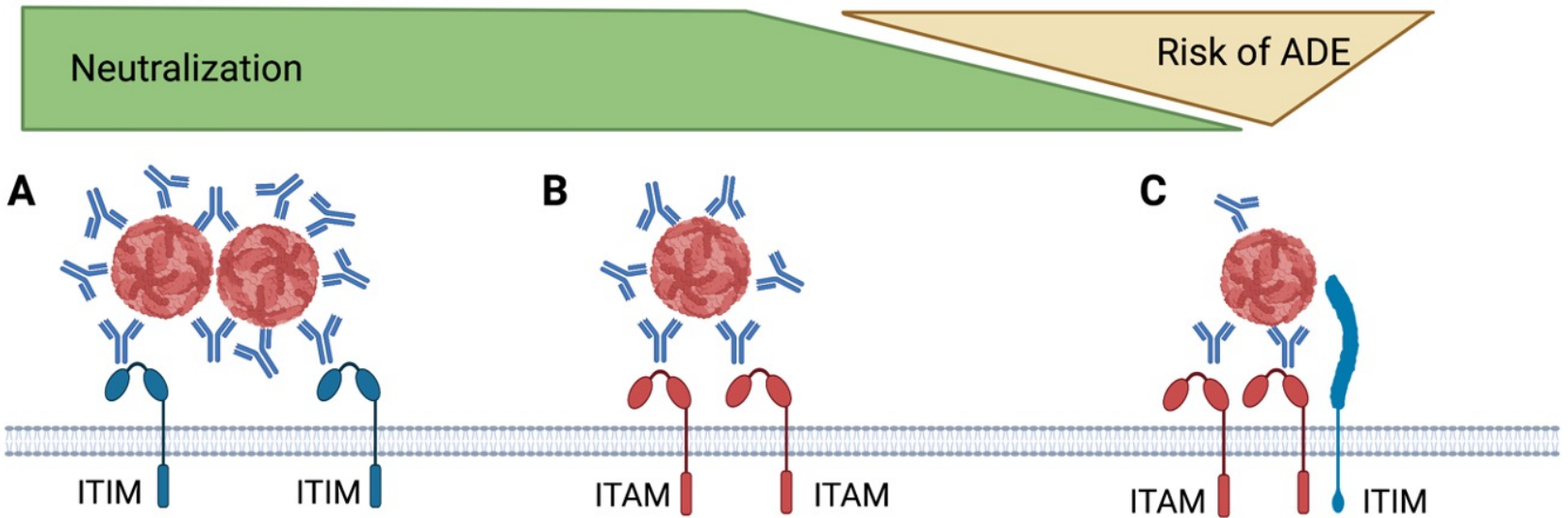


# ADE requires a Goldilocks effect

Right virus

Right amount of antibodies

Engaging the right receptors



Chan et al, PNAS 2011

Chan, Ong et al, PNAS 2014

Robinson et al, Cell 2015

Chan et al, Nat Microbiol 2016

Gan et al, EMBO J 2017

Ong et al, Sci Rep 2017

Chan C et al, mSphere 2019

Ooi and Kalimuddin, Sci Transl Med 2023

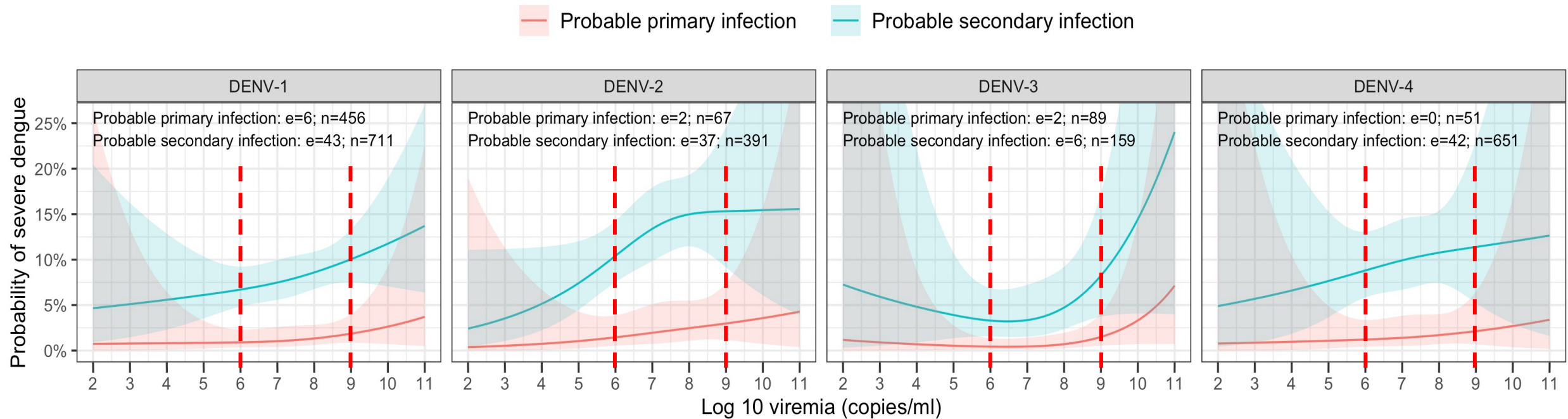
# Antibody-enhanced infection benefits DENV-2 and -4

|                         |              | 7x           | <1x          |              |        |
|-------------------------|--------------|--------------|--------------|--------------|--------|
| Group                   | DENV-1       | DENV-2       | DENV-3       | DENV-4       | Total  |
| Primary in non- infants | 0.57         | 0.05         | 0.37         | 0.01         | 1      |
|                         | [0.55, 0.60] | [0.02, 0.07] | [0.34, 0.39] | [0, 0.04]    | (1734) |
|                         | (990)        | (83)         | (639)        | (22)         |        |
| Primary in Infants      | 0.37         | 0.32         | 0.27         | 0.04         | 1      |
| [Maternal Ab]           | [0.34, 0.42] | [0.27, 0.36] | [0.23, 0.31] | [0, 0.09]    | (632)  |
|                         | (238)        | (198)        | (170)        | (26)         |        |
| Post-primary            | 0.35         | 0.31         | 0.22         | 0.12         | 1      |
| [Pre-existing Ab]       | [0.33, 0.36] | [0.30, 0.32] | [0.21, 0.24] | [0.11, 0.13] | (9717) |
|                         | (3356)       | (3014)       | (2210)       | (1137)       |        |

Table shows proportion and 95% multinomial confidence intervals in square brackets. Case numbers are in parentheses. The accompanying figure, with the data by year, is [Fig 1](#).

doi:10.1371/journal.pntd.0004262.t001

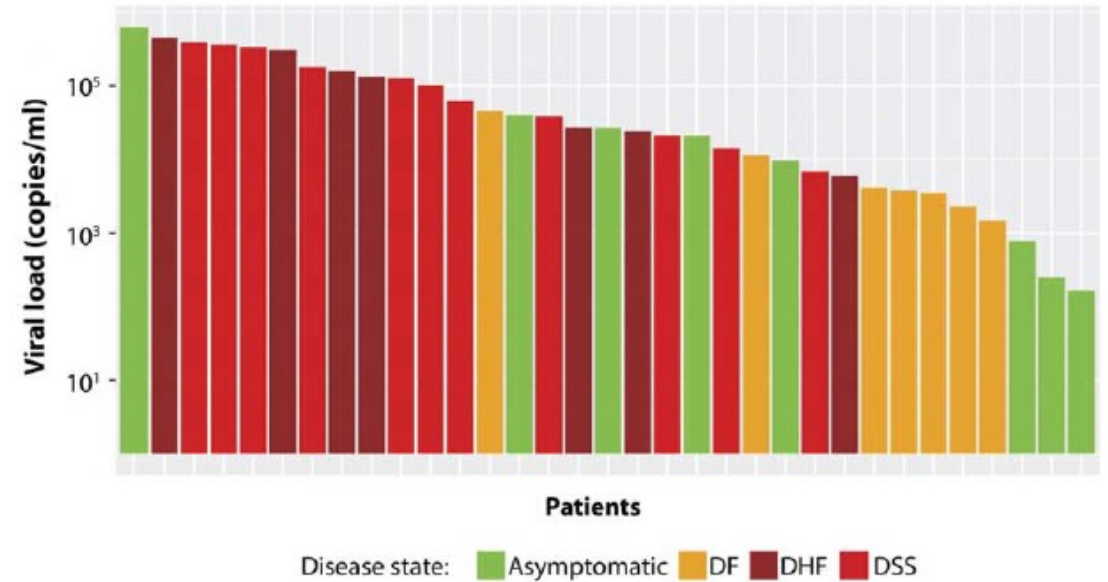
# Secondary DENV2 shows greatest increase in risk of severe dengue



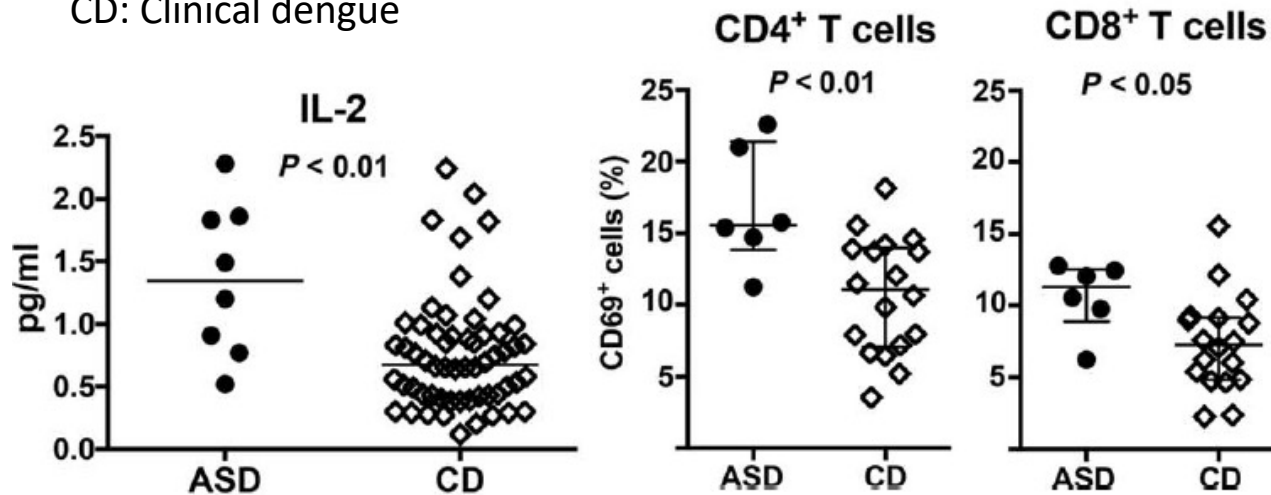
Protective role of T cells in dengue

# Increased T cell activation during viremia phase is associated with asymptomatic DENV infection

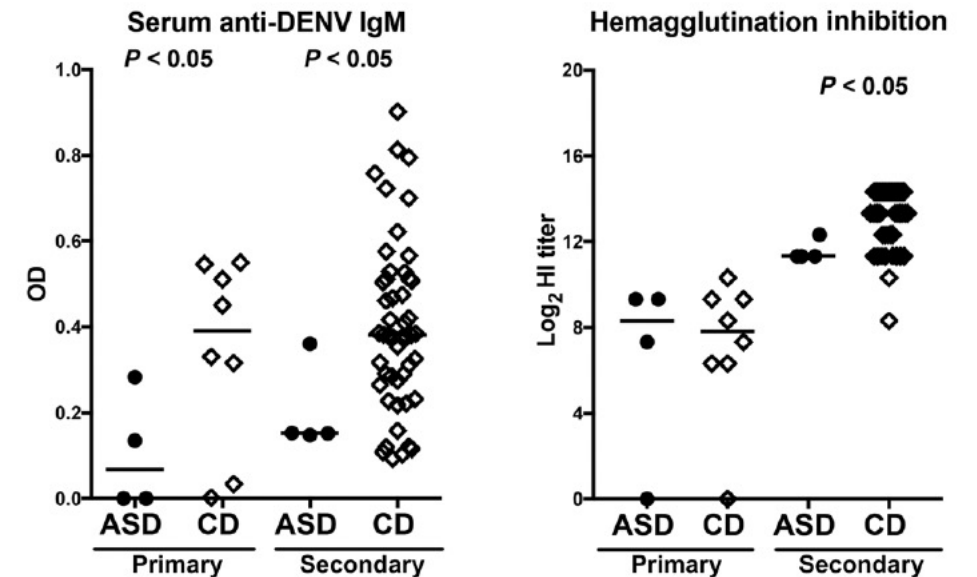
*Increased plasma cell differentiation -> clinical dengue*



ASD: Asymptomatic dengue  
CD: Clinical dengue



D





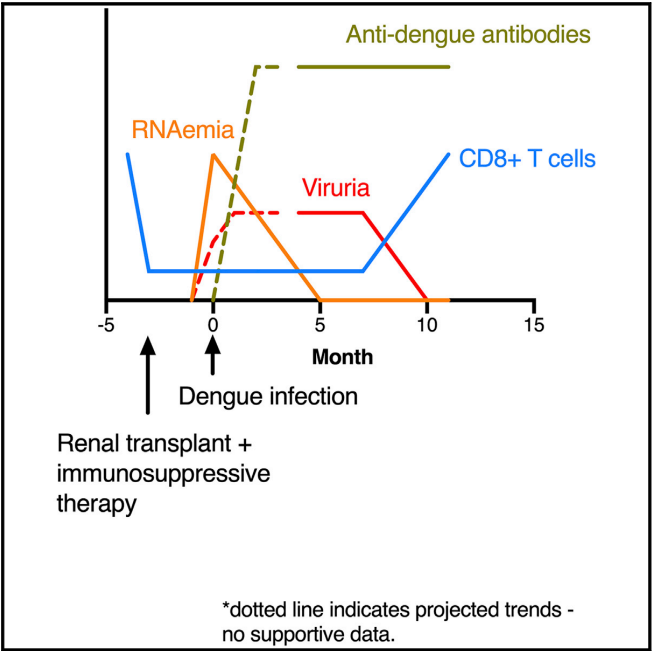
# T cells but not antibodies terminate DENV infection

## Cell Host & Microbe

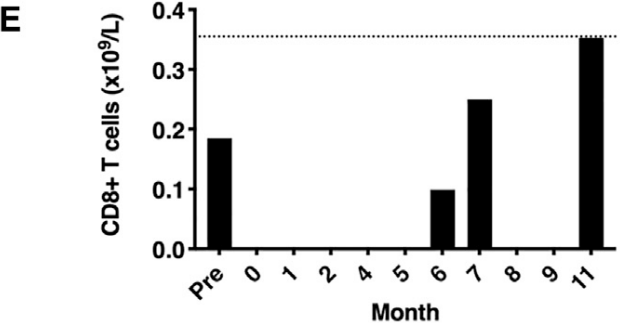
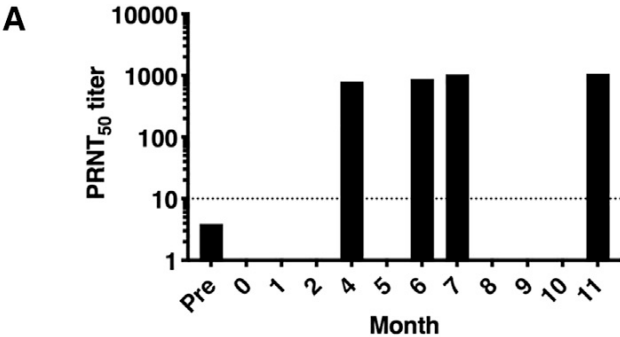
### Persistent Dengue Infection in an Immunosuppressed Patient Reveals the Roles of Humoral and Cellular Immune Responses in Virus Clearance

Kar-Hui Ng,<sup>1,2,9,\*</sup> Summer Lixin Zhang,<sup>3</sup> Hwee Cheng Tan,<sup>3</sup> Swee Sen Kwek,<sup>3</sup> October Michael Sessions,<sup>3,4</sup> Chang-Yien Chan,<sup>1</sup> Isaac Desheng Liu,<sup>2</sup> Chun Kiat Lee,<sup>5</sup> Paul Ananth Tambyah,<sup>6</sup> Eng Eong Ooi,<sup>3,4,7,8</sup> and Hui-Kim Yap<sup>1,2,8</sup>

#### Graphical Abstract



#### Renal Tx patient DENV-3 infection

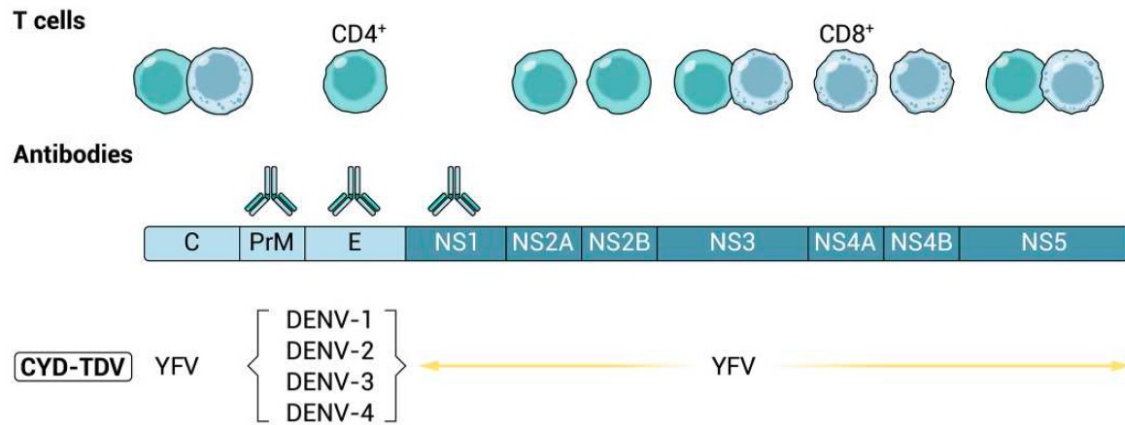


**F**

|                         |   |   |   |   |   |   |   |   |   |   |   |
|-------------------------|---|---|---|---|---|---|---|---|---|---|---|
| DENV RT-PCR (serum)     | 0 | + | + | + | + | 0 | - | - | 0 | 0 | 0 |
| Virus isolation (serum) | 0 | 0 | 0 | 0 | - | 0 | 0 | - | 0 | 0 | 0 |
| Virus isolation (urine) | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 |
| DENV RT-PCR (podocytes) | 0 | 0 | 0 | 0 | 0 | 0 | + | + | - | 0 | - |

Red arrows point to the 'Virus isolation (urine)' and 'DENV RT-PCR (podocytes)' rows, indicating persistent detection of virus in these samples.

# Lack of robust DENV-specific T cell responses may account for reduced efficacy of Dengvaxia



Majority of CD8<sup>+</sup> T cell epitopes are found on NS-proteins

Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects

Bruno Guy<sup>a,\*</sup>, Nolwenn Nougarede<sup>a</sup>, Sarah Begue<sup>a</sup>, Violette Sanchez<sup>a</sup>, Nadia Souag<sup>a</sup>, Murielle Carre<sup>a</sup>, Laurent Chambonneau<sup>a</sup>, Dennis N. Morrisson<sup>b</sup>, David Shaw<sup>c</sup>, Ming Qiao<sup>c</sup>, Rafaele Dumas<sup>a</sup>, Jean Lang<sup>a</sup>, Remi Forrat<sup>a</sup>

<sup>a</sup> Research Department, sanofi pasteur, Marcy l'Etoile, France

<sup>b</sup> Bio-Kinetic Clinical Applications, Springfield, USA

<sup>c</sup> Royal Adelaide Hospital, Adelaide, Australia

Vaccine 2008

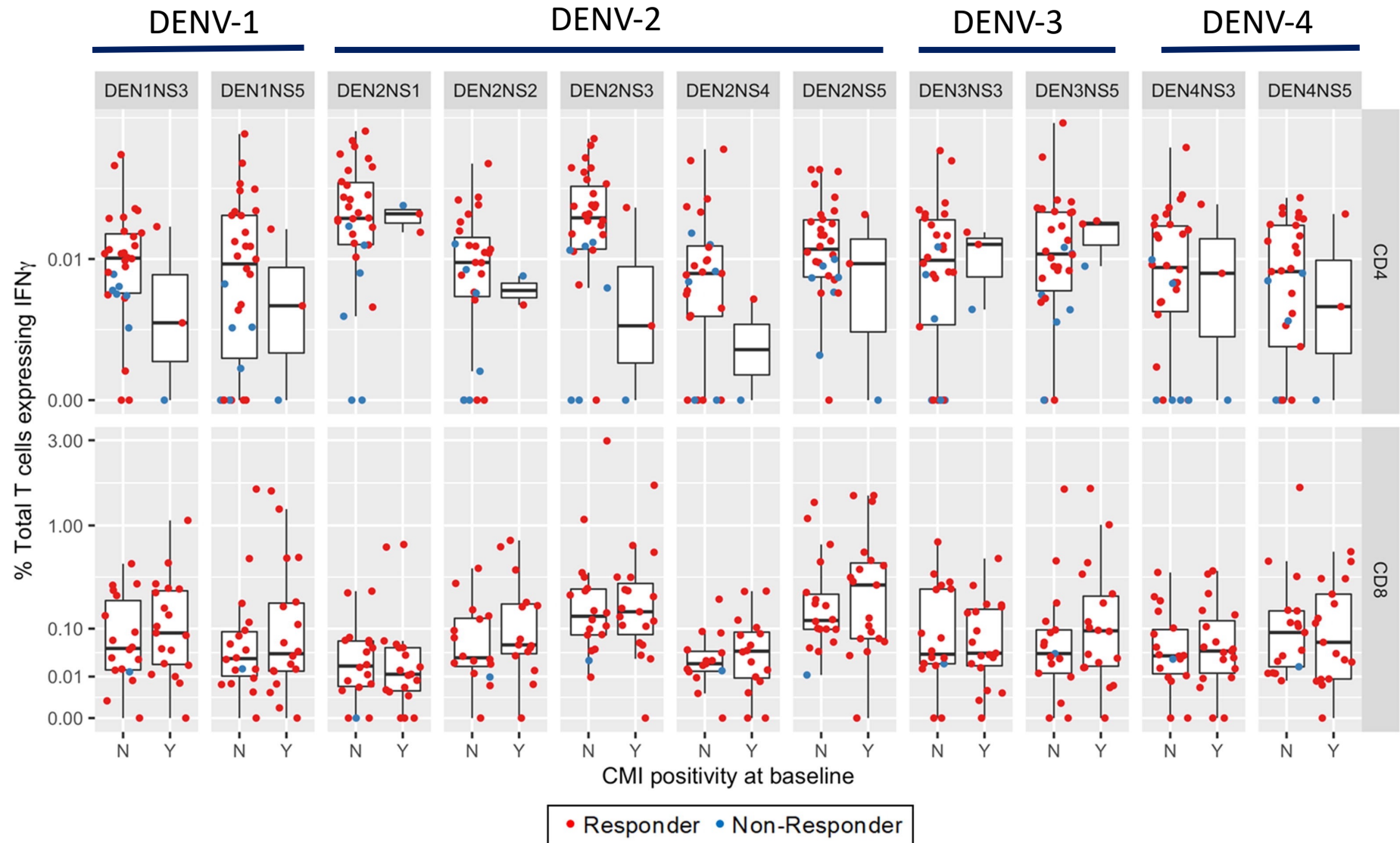
In dengue-naïve vaccinees:

- Dengvaxia induces strong CD8<sup>+</sup> T cell responses against YF17D-NS3
- Muted cross-reactive CD8<sup>+</sup> T cell responses against DENV NS3

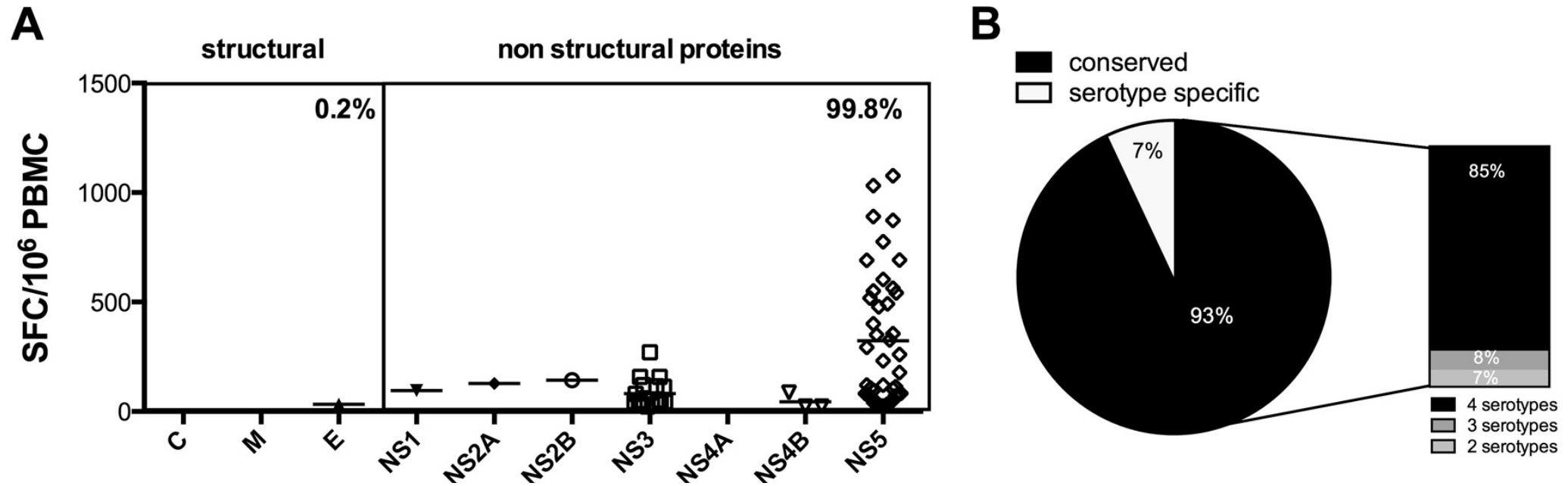
# T cells from TAK003 vaccination react against all 4 DENVs

Comparable levels of DENV-specific T cells regardless of CMI positivity at baseline

Highest frequency of T cells against DENV-2, then DENV-1



# T cells induced by TV003 vaccination target conserved epitopes on non-structural proteins



T cell responses to TV003 mirror secondary DENV infection

## Summary

- DENV1-4 are not monolithic
- Neutralizing antibodies do not reliably inform on vaccine efficacy
- T cells protect
- mRNA vaccine design?
  - Incorporate structural and NS proteins to induce B and T cell immunity
  - ?-valent formulation?
- Combination with other vaccinations (TAK003/TV003) to full protection?