Accuracy And Efficacy Of Dengue CYD-TDV Pre-vaccination Screening With Five Existing IgG Serotests: Retrospective Analysis Of Phase III Trials

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INTRODUCTION

- The tetravalent dengue vaccine CYD-TDV (Dengvaxia®) is indicated for use in those 9 years of age and older living in dengue endemic countries with evidence of prior dengue infection, based on findings from two pivotal Phase III trials[1-4]
- CYD-TDV was found to confer durable protection against dengue hospitalization and severe dengue in subjects with prior dengue infection (PDI), while seronegative vaccinees experienced an increased risk of these outcomes following natural dengue exposure[4]
- WHO has recommended pre-vaccination screening to identify those with PDI who could be offered vaccination[5]
- Several marketed dengue IgG-detecting rapid diagnostic tests (RDT) and IgG Enzyme-linked immunosorbent assays (ELISA) were shown to be highly specific, while ELISAs exhibited higher sensitivity than the RDTs in identifying PDI[6-7]
- In this study, we leveraged existing CYD-TDV Phase III trial data to evaluate:
  - Effectiveness of a 'test & vaccinate' approach against symptomatic and hospitalized dengue, using existing dengue serotests; and
  - Performance of existing dengue serotests in settings consistent with areas where CYD-TDV is licensed

OBJECTIVES

Primary objectives:

- Assess vaccine efficacy (VE) for symptomatic virologically-confirmed dengue (VCD) regardless of severity and serotype in all test(+) subjects 2-16 years of age during Active Phase follow-up (Month 0 to Month 25)
- Assess VE against hospitalized VCD regardless of serotype in all test(+) subjects 2-16 years of age over the entire 6 years of follow-up (M0 to M72)
- Determine sensitivity, specificity of each assay in all subjects (2-16 yrs)

Sensitivity analyses included the following outcomes:

- VE for symptomatic VCD (M0 to M25) in test(+) subjects, by age strata*
- VE for hospitalized VCD (M0 to M72) in test(+) subjects by age strata*
- VE against severe VCD regardless of serotype in all test(+) subjects 2-16 years of age over entire study period (M0 to M72)

*Sensitivity analyses included subgroup analyses in the following age strata: ≥9 yrs, and ≥6 yrs of age

METHODS

Study Design and Participants

- The CYD-TDV Phase III trials were randomized, placebo-controlled studies conducted in 5 Asia-Pacific countries in 2-14 year old participants (CYD14) and 5 Latin American countries in 6-16 year olds (CYD15), who were randomized (2:1) to receive 3 injections of CYD-TDV or placebo at six month intervals[1,2]
- The present analyses were performed in the immunogenicity subsets, comprising a random subset of 20% (1,983/10,275) and 10% (2,000/20,869) of subjects in CYD14 and CYD15, respectively, who had pre-vaccination blood samples and received at least one injection

Efficacy Outcomes

- Efficacy analyses compared subjects randomized to receive CYD-TDV vs. placebo group using pooled data from the immunogenicity subsets of CYD14 and CYD15
- VE, defined as (1-Hazard Ratio)*100, were estimated in test(+) subjects using a Cox regression model with the treatment group and study as fixed effects, with corresponding 95% confidence intervals calculated by the exact binomial method (Clopper-Pearson method)

Test Performances

- Analyses related to performance of each dengue immunoassay were done in:
  - The full Immunogenicity Subset of CYD14 and CYD15 with samples that had valid readouts for both the evaluated serotest and the reference tests ($PRNT_{50}$, $PRNT_{90}$, anti-dengue NS1 IgG ELISA)
- The performance of each immunoassay was estimated as
  - Sensitivity: (Immunoassay test(+) ÷ All reference dengue seropositives)×100
  - Specificity: (Immunoassay test(-) ÷ All reference dengue seronegatives)×100
  - 95% CI were estimated by the exact binomial method
- Five dengue IgG immunoassays were used (**Table 1**) to test pre-vaccination sera in the Immunogenicity Subset of CYD14 and CYD15 (n ≥ 3,841)
- Testing was performed in accordance with manufacturers' recommended protocols
- Classification of reference dengue serostatus by subject was based on results of $PRNT_{50}$, $PRNT_{90}$, and anti-dengue NS1 IgG ELISA (**Table 2**)[8,9]

Table 1. Characteristics of dengue immunoassays used in this study.

Dengue Serotest	OnSite IgG/IgM RDT[1,2]	SD Bioline IgG/IgM RDT[1,3,4]	TellMeFast IgG/IgM RDT[1,2]	Panbio® IgG Indirect ELISA[3]	Euroimmun IgG ELISA[2]
Manufacturer	CTK Biotech	Alere (Abbott)	Biocan	Euroimmun	Euroimmun
Catalog number	R0061C	11FK20	B803C	EI 266b-9601 G	EI 266b-9601 G
Assay format	Lateral flow	Lateral flow	Lateral flow	ELISA	ELISA
Dengue antigen	Recombinant envelope protein	Recombinant envelope antigen	Recombinant dengue antigen	Purified DEN virus antigen	Purified viral particles DEN2
Zika cross-reactivity, % (no. positive/no. tested)[5]	0 (0/41)	7 (3/41)	3 (1/38)	34 (14/41)	8 (2/26)

[1]Readout for the IgG band only was considered in determining the test result for the IgG/IgM RDTs.

Testing was performed at the [2]Global Clinical Immunology Laboratory, Sanofi Pasteur, Swiftwater, PA USA or the [3]Central Virology Laboratory, Israel Ministry of Health, Ramat Gan, Israel.

[4]Two readers were used, with pre-defined plan to score discordant readings (+/-) as a positive test result.

[5]Cross-reactivity sample selection and results for all tests except Euroimmun IgG ELISA were previously published (refs. 6, 7).

Table 2. Algorithm for classification of reference dengue serostatus.

Group	Reference test results			Interpretation
	$PRNT_{90}$[1]	$PRNT_{50}$[1]	DV NS1 IgG ELISA[2]	
1	Negative	Negative	Negative	Reference seronegative
2	Negative	Positive	Negative	Reference seronegative
3	Negative	Negative	Low positive	Reference seronegative
4	Negative	Positive	Low positive	Reference seropositive
5	Negative	Any[3]	High positive	Reference seropositive
6	Positive	Positive	Any[3]	Reference seropositive

[1]Positive $PRNT_{50}$ and $PRNT_{90}$ were defined by a titer ≥10 (1/dil) against ≥1 dengue serotype.

[2]Anti-dengue NS1 IgG ELISA results were classified as negative (titer <9 EU/ml), low positive (≥9 to <50 EU/ml) and high positive (≥50 EU/ml).

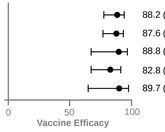
[3]Any = positive or negative.

RESULTS

- Estimates of VE against symptomatic VCD in test(+) subjects similarly high with all five serotests

Figure 1. CYD-TDV efficacy against symptomatic VCD up to M25 in test(+) subjects 2-16 years of age.

Immunoassay	Density Incidence* (95% CI)		Vaccine Efficacy (95% CI)
	CYD-TDV	Placebo	
Euroimmun-ELISA	**0.4** (0.2, 0.6)	**3.0** (2.2, 4.0)	88.2 (77.3, 93.9)
Panbio-ELISA	**0.4** (0.2, 0.6)	**3.0** (2.2, 3.9)	87.6 (76.7, 93.4)
TELL ME FAST-RDT	**0.2** (0.1, 0.6)	**2.0** (1.2, 3.0)	88.8 (67.0, 96.2)
SD BIOLINE-RDT	**0.5** (0.2, 0.8)	**2.7** (1.9, 3.8)	82.8 (66.9, 91.1)
OnSite RDT	**0.2** (0.0, 0.5)	**1.7** (1.0, 2.8)	89.7 (64.6, 97.0)

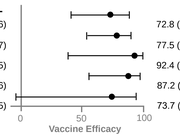


Data pooled from CYD14 and CYD15 in all ages (2-16 years).
VCD symptomatic virologically-confirmed dengue.
*per 100 person-years at risk.

- Point estimates consistent with protection against hospitalized VCD in test(+) subjects for each immunoassay
  - Lower bound of 95% CI was above the null for all but one test (*OnSite* RDT)

Figure 2. CYD-TDV efficacy against hospitalized VCD up to 6 years in test(+) subjects 2-16 years of age.

Immunoassay	Density Incidence* (95% CI)		Vaccine Efficacy (95% CI)
	CYD-TDV	Placebo	
EUROIMMUN-ELISA	**0.1** (0.0, 0.2)	**0.4** (0.2, 0.6)	72.8 (38.9, 87.9)
Panbio-ELISA	**0.1** (0.1, 0.2)	**0.5** (0.3, 0.7)	77.5 (52.8, 89.3)
TELL ME FAST-RDT	**<0.1** (0.0, 0.1)	**0.3** (0.1, 0.5)	92.4 (37.8, 99.1)
SD BIOLINE-RDT	**<0.1** (0.0, 0.1)	**0.3** (0.2, 0.6)	87.2 (54.5, 96.4)
OnSite-RDT	**<0.1** (0.0, 0.2)	**0.2** (0.1, 0.5)	73.7 (−5.1, 93.4)

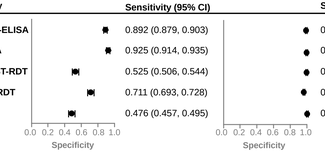


Data pooled from CYD14 and CYD15 in all ages.
VCD symptomatic virologically-confirmed dengue
*per 100 person-years at risk.

- Very high specificity (>98.5%) for four of the five assays
- IgG ELISAs exhibited significantly higher sensitivity compared to the RDTs

Figure 3. Immunoassay sensitivity and specificity in all subjects 2-16 years of age.

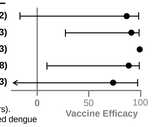
Immunoassay	Sensitivity (95% CI)	Specificity (95% CI)
EUROIMMUN-ELISA	0.892 (0.879, 0.903)	0.988 (0.979, 0.993)
Panbio-ELISA	0.925 (0.914, 0.935)	0.992 (0.985, 0.996)
TELL ME FAST-RDT	0.525 (0.506, 0.544)	0.990 (0.983, 0.995)
SD BIOLINE-RDT	0.711 (0.693, 0.728)	0.960 (0.948, 0.971)
OnSite-RDT	0.476 (0.457, 0.495)	0.995 (0.989, 0.998)



- Pooled estimates are consistent with protective effect against severe VCD
- Imprecision of estimates due to low number of events

Figure 4. CYD-TDV efficacy against hospitalized VCD up to 6 years in test(+) subjects 2-16 years of age.

Immunoassay	Density Incidence* (95% CI)		Vaccine Efficacy (95% CI)
	CYD-TDV	Placebo	
EUROIMMUN-ELISA	**<0.1 (0.0, 0.1)**	**<0.1 (0.0, 0.2)**	86.9 (−16.8, 98.5)
Panbio-ELISA	**<0.1 (0.0, 0.1)**	**0.1 (0.0, 0.3)**	91.3 (27.6, 99.0)
TELL ME FAST-RDT	**<0.1 (0.0, 0.1)**	**<0.1 (0.0, 0.3)**	100 (NC, 100)
SD BIOLINE-RDT	**<0.1 (0.0, 0.1)**	**0.1 (0.1, 0.8)**	89.4 (9.6, 98.8)
OnSite-RDT	**<0.1 (0.0, 0.1)**	**<0.1 (0.0, 0.3)**	73.4 (−193.7, 97.6)



Data pooled from CYD14 and CYD15 in all ages (2-16 years).
NC, not calculable; VCD symptomatic virologically-confirmed dengue
*per 100 person-years at risk.

Table 3. VE against symptomatic VCD in test(+) subjects by different age cutoffs.

Immunoassay	CYD-TDV group		Placebo group		Vaccine Efficacy[2] [95% CI]
	n/N	Density Incidence[1] [95% CI]	n/N	Density Incidence[1] [95% CI]	
Participants ≥9 years					
Euroimmun ELISA	5/1336	0.2 [0.1, 0.4]	33/679	2.5 [1.7, 3.5]	92.4 [80.6, 97.0]
Panbio ELISA	8/1387	0.3 [0.1, 0.6]	34/698	2.5 [1.7, 3.5]	88.3 [74.8, 94.6]
TELL ME FAST RDT	3/827	0.2 [0.0, 0.5]	15/437	1.7 [1.0, 2.9]	89.3 [63.0, 96.9]
SD Bioline RDT	9/1104	0.4 [0.2, 0.8]	25/560	2.3 [1.5, 3.4]	81.8 [60.9, 91.5]
OnSite RDT	1/759	<0.1 [0.0, 0.4]	10/388	1.3 [0.6, 2.4]	94.9 [60.0, 99.3]
Participants ≥6 years					
Euroimmun ELISA	8/1446	0.3 [0.1, 0.5]	40/738	2.8 (2.0, 3.7)	89.8 [78.3, 95.2]
Panbio ELISA	11/1503	0.4 [0.2, 0.7]	42/768	2.8 (2.0, 3.8)	86.6 [74.1, 93.1]
TELL ME FAST RDT	4/874	0.2 [0.1, 0.6]	16/461	1.8 (1.0, 2.9)	86.6 [60.0, 95.5]
SD Bioline RDT	11/1186	0.5 [0.2, 0.8]	31/604	2.6 (1.8, 3.7)	81.8 [63.9, 90.9]
OnSite RDT	2/804	0.1 [0.0, 0.4]	11/413	1.3 (0.7, 2.4)	90.6 [57.5, 97.9]

Data pooled from CYD14 and CYD15.
n, number of cases; N, number of test-positive participants; VCD, virologically-confirmed dengue.
[1]Density incidence is presented as cases per 100 person-years. [2]VE from M0 to M25.

Table 4. VE against hospitalized VCD in test(+) subjects by different age cutoffs.

Immunoassay	CYD-TDV group		Placebo group		Vaccine Efficacy[2] [95% CI]
	n/N	Density Incidence[1] [95% CI]	n/N	Density Incidence[1] [95% CI]	
Participants ≥9 years					
Euroimmun ELISA	6/1336	<0.1 (0.0, 0.2)	8/679	0.2 (0.1, 0.4)	61.0 (−12.5, 86.5)
Panbio ELISA	7/1387	<0.1 (0.0, 0.2)	12/698	0.3 (0.2, 0.5)	70.3 (24.6, 88.3)
TELL ME FAST RDT	0/827	0.0 (0.0, 0.1)	4/437	0.2 (0.0, 0.4)	100 (NC, 100)
SD Bioline RDT	1/1104	<0.1 (0.0, 0.1)	6/560	0.2 (0.1, 0.4)	91.4 (28.2, 99.0)
OnSite RDT	2/759	<0.1 (0.0, 0.2)	2/388	<0.1 (0.0, 0.3)	45.8 (−285.1, 92.4)
Participants ≥6 years					
Euroimmun ELISA	7/1446	<0.1 (0.0, 0.2)	14/738	0.3 (0.2, 0.6)	73.7 (34.8, 89.4)
Panbio ELISA	8/1503	<0.1 (0.0, 0.2)	19/768	0.4 (0.3, 0.7)	78.0 (49.7, 90.4)
TELL ME FAST RDT	0/874	0.0 (0.0, 0.1)	5/461	0.2 (0.1, 0.5)	100 (NC, 100)
SD Bioline RDT	2/1186	<0.1 (0.0, 0.1)	9/604	0.3 (0.1, 0.5)	88.3 (45.7, 97.5)
OnSite RDT	2/804	<0.1 (0.0, 0.2)	4/413	0.2 (0.0, 0.4)	72.9 (−47.8, 95.0)

Data pooled from CYD14 and CYD15.
n, number of cases; N, number of test-positive participants; VCD, virologically-confirmed dengue.
[1]Density incidence is presented as cases per 100 person-years. [2]VE from M0 to M72.

STUDY LIMITATIONS

- The sparsity of severe VCD events in test(+) subjects with all immunoassays limited precision of estimates for VE against severe VCD
  - The same is also true for some of the sensitivity/subgroup analyses
- The impact of Zika exposure on test performance was not evaluable, since CYD15 baseline sampling (2011-2) occurred before Zika introduction to Latin America (2015)
  - However, the three RDTs and Euroimmun ELISA have previously shown no (*OnSite*) or low Zika cross-reactivity[6,7] (Euroimmun ELISA data unpublished)

CONCLUSIONS

- This study represents the first evaluation of CYD-TDV vaccine efficacy against dengue disease outcomes in test(+) subjects as identified by currently available dengue IgG serotests
- For each of the 5 serotests, there was evidence that CYD-TDV conferred high efficacy in test(+) subjects against VCD over 2 years and hospitalized VCD over 6 years of follow-up
  - Similar findings were observed in those ≥9 years of age, for which the vaccine is currently indicated, as well as in those ≥6 years of age
- Estimates of VE against severe VCD over 6 years in test(+) were highly favorable
- For all but one of the serotests, specificity was very high (>98.5%), which would be expected to greatly limit vaccination of false-positive individuals
- Sensitivity of the two ELISAs was significantly higher than that for the RDTs, offering the advantage of identifying a larger proportion of true seropositive individuals who would benefit from vaccination
- These findings indicate that use of these existing serotests for pre-vaccination screening would enable the safe and effective use of CYD-TDV and could thus serve as suitable temporizing tools to inform vaccination decisions until more sensitive, point-of-care tests become available

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Disclosures

CD, MB, HW, MZ, SH, YA-Ö, and SJS are employees of Sanofi Pasteur. YL and ES have nothing to declare.

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