Flavivirus Vaccines
Japanese Encephalitis and Dengue

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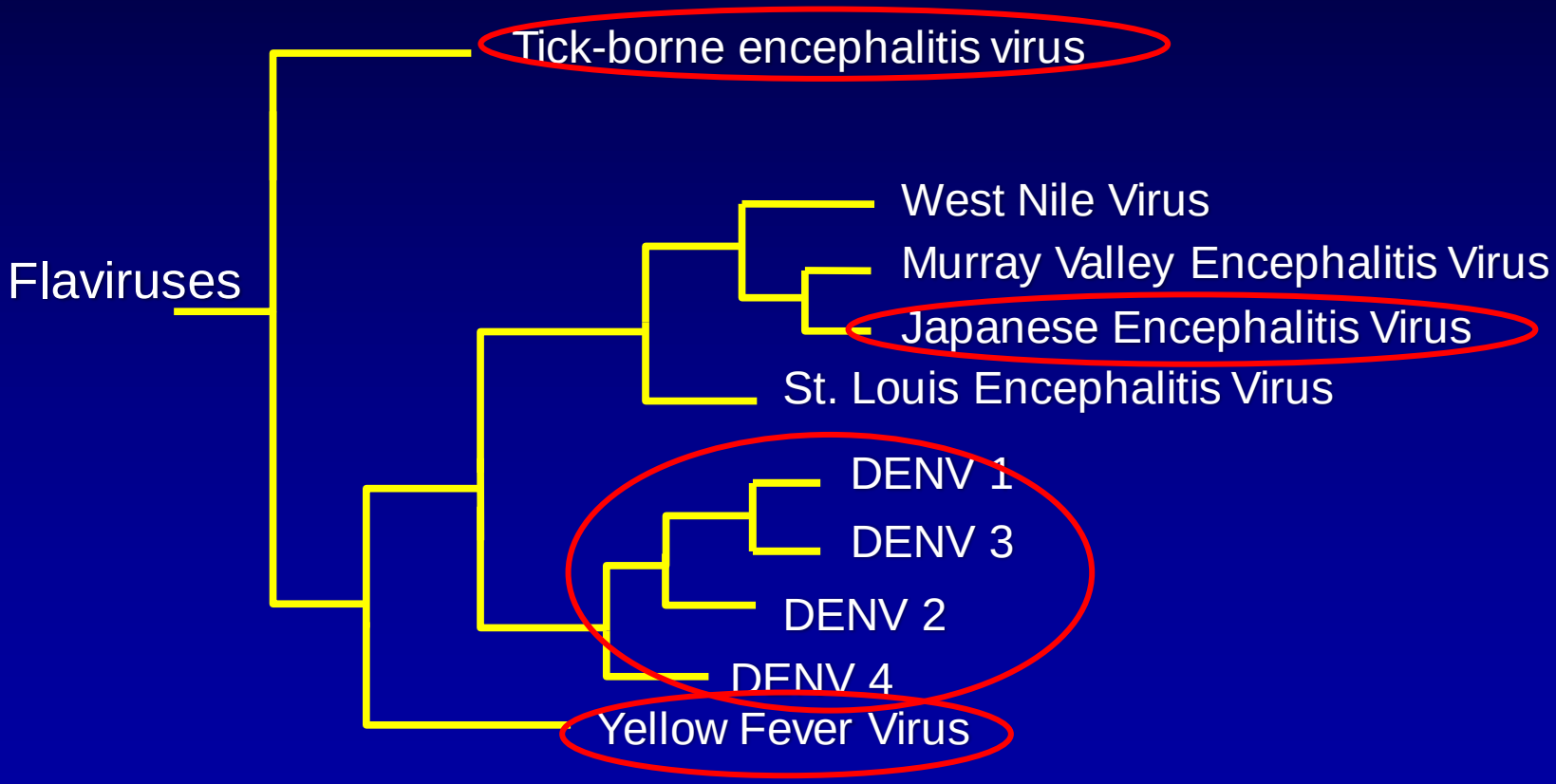
The Presentation

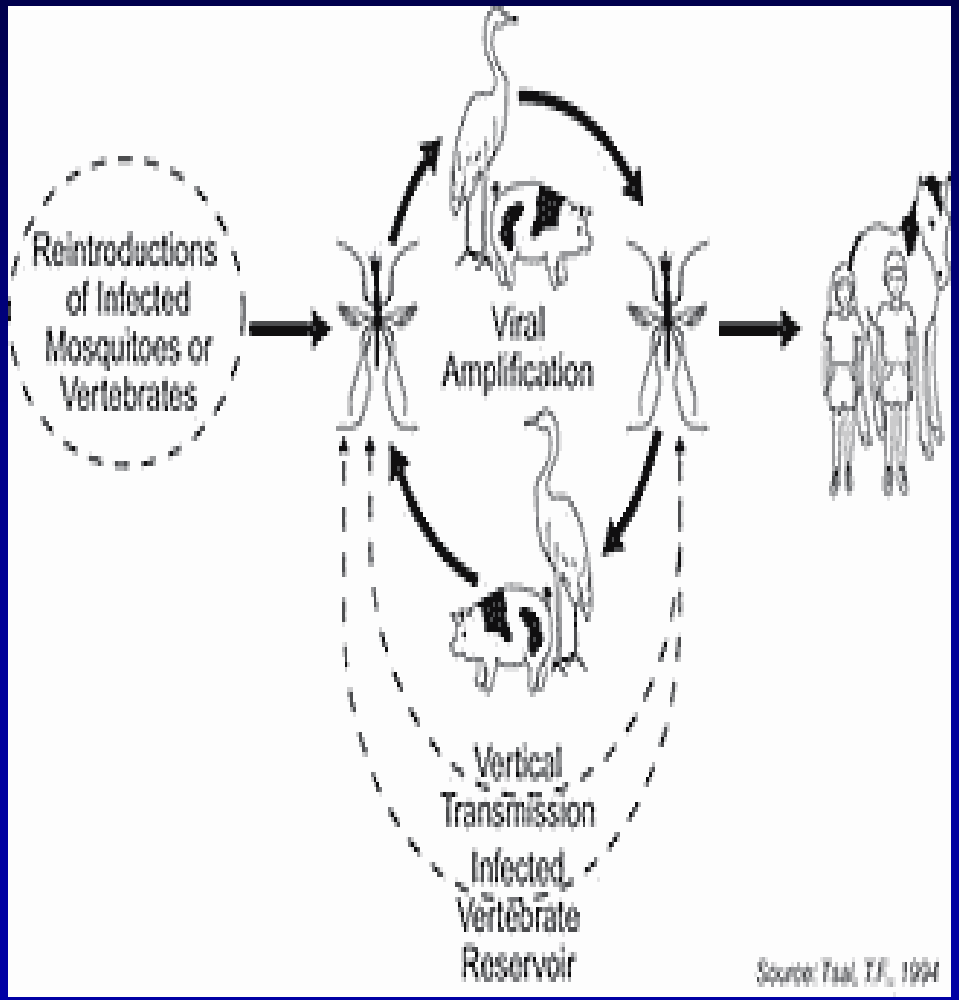
- Comparisons
- Japanese Encephalitis Vaccine
  - The need - disease burden and distribution
  - WHO and GAVI perspectives
  - Status of vaccines
- Dengue Vaccines
  - The need – burden and lack of primary prevention tools
  - Vaccines – constructs and candidates
  - Epidemiologic challenges to vaccine evaluation
  - Lead-candidate vaccine trial

Flaviviruses

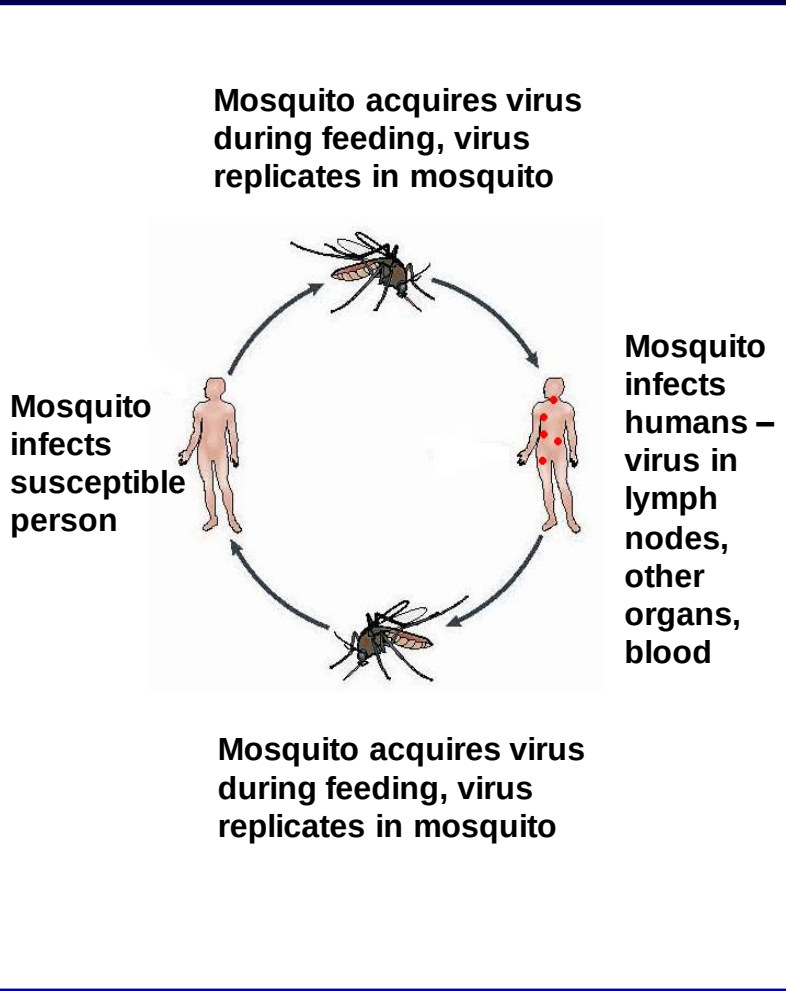


Japanese Encephalitis and Dengue Life-cycles

Japanese Encephalitis



Dengue



JE and Dengue Vaccine Status

JE Vaccine

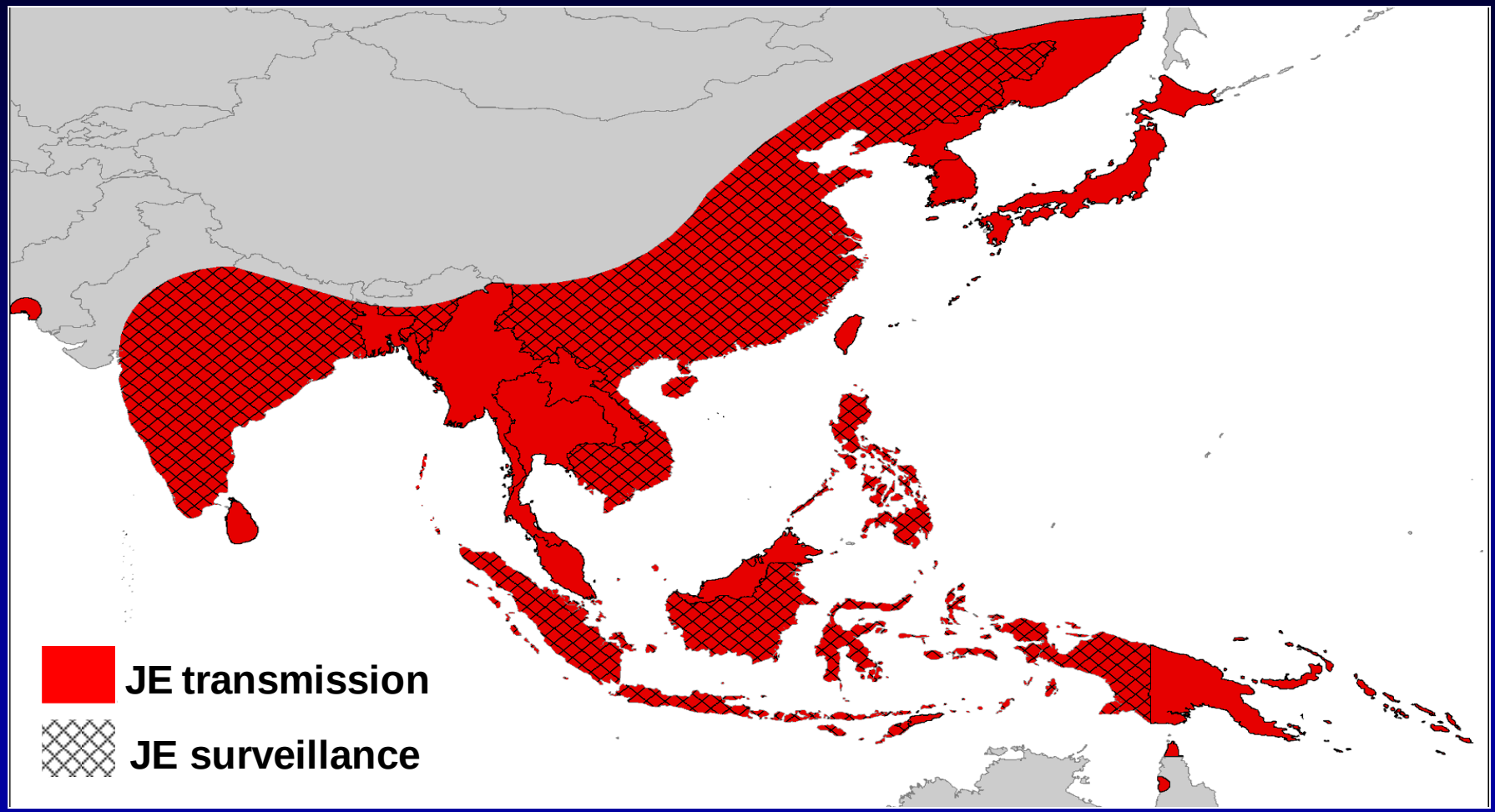
- Multiple licensed products
- WHO Prequalified vaccine
- Vaccine types: live attenuated, inactivated, chimeric live attenuated
- Indications: pediatric and adult
- Need better diagnostics
- Need to increase usage

Dengue Vaccine

- No licensed product
- Multiple vaccines in trials
- Strong pipeline
- Chimeric attenuated, inactivated, subunit
- Indications: pediatric and adult
- Need data - vaccine performance
- Need better diagnostics

Japanese Encephalitis Vaccines

Japanese Encephalitis Surveillance



Source: J. Hombach, WHO-IVR

Japanese Encephalitis Disease Burden

The WHO Perspective

- Need for increased JE awareness and for vaccination in areas where a public health problem
- Most effective immunization strategy
  - one time campaign in target population, as defined by epidemiological data, followed by inclusion into routine immunization programme.
- SAGE supported JE immunization and recognized JE vaccine to be underutilized

Sources Weekly Epidemiological Record, 25 August 2006
SAGE 2008

The GAVI Perspective

- Prioritized JE vaccine (2008)
- Included JE in pledging conference (2011)
- Work Group identified issues, options and technical elements to guide countries to prepare applications
- October 2013 – WHO added SA 14-14-2 live, attenuated vaccine developed by China's Chengdu Institute of Biological Products to its list of prequalified vaccines
- 2014 – invited applications for support
- 2015 – expect first country JE vaccination campaigns

JE Surveillance
Countries with Transmission Risk

National	Risk Areas / Sentinel	None
▪ Burma	▪ Australia	▪ Brunei
▪ China	▪ Bangladesh	▪ Indonesia
▪ Japan	▪ Bhutan	▪ North Korea
▪ Laos	▪ Cambodia	▪ Pakistan
▪ Malaysia	▪ India	▪ Timor Leste
▪ South Korea	▪ Nepal	
▪ Taiwan	▪ Papua New Guinea	
▪ Thailand	▪ Philippines	
▪ Sri Lanka		
▪ Vietnam		

Adapted from: MMWR 2013; 62: 658-62

JE Immunization
Countries with Transmission Risk

National	Subnational / Risk Areas	None
▪ China	▪ Australia	▪ Bangladesh
▪ Japan	▪ Cambodia	▪ Bhutan
▪ South Korea	▪ India	▪ Burma
▪ Taiwan	▪ Malaysia	▪ Brunei
▪ Thailand	▪ Nepal	▪ Indonesia
▪ Sri Lanka	▪ North Korea	▪ Laos
	▪ Vietnam	▪ Philippines
		▪ Timor Leste
		▪ Pakistan
		▪ Papua New Guinea

Adapted from: MMWR 2013; 62: 658-62

JE Vaccines - Inactivated

Type	Strain	Producer	Doses	Status
Inactivated mouse-brain (JE - VAX)	Nakayama Beijing	Biken	3	No longer produced
Inactivated Vero cell (IC51, IXIARO, JE-VC)	SA 14-14-2	Intercell /Novartis Biological E	2-3	Licensed - US, Canada, EU (travellers); endemic area trials underway
Inactivated Vero cell	Beijing 1	Biken Kaketsuken	3	In development Japan use only
Inactivated Vero Cell	Indian JEV strain	Bharat	2	Serologic correlate studies, under consideration for Indian license

JE Vaccines – Attenuated

Type	Strain	Producer	Doses	Status
Attenuated, YF chimera Vero cell (ChimeriVAX JE)	SA14-14-2	sanofi pasteur	1	Serologic correlates, licensed in Thailand, Australia, India
Attenuated PHK cell	SA14-14-2	Chengdu Institute of Biological Products	1-2	Efficacy studies WHO prequalified 2013

Clinical trials of SA 14-14-2 vaccine sponsored by PATH

- Non-inferiority of concurrent LJEV and measles administration: Philippines.
- Long-term antibody to LJEV: Philippines.
- Immunogenicity and safety among children who have and have not already received mouse-brain vaccine: Sri Lanka.
- Lot-to-lot consistency: Bangladesh.

Immune Response to Concomitant or Sequential Administration of Measles and JEV

		Group 1 (LJEV then MV) (N=88)		Group 2 (concomitant) (N=222)*		Group 3 (MV then LJEV) (N=180)†	
		% seropositive (95% CI)	GMC (mIU/mL) or GMT	% seropositive (95% CI)	GMC (mIU/mL) or GMT	% seropositive (95% CI)	GMC (mIU/mL) or GMT
Measles vaccine response (anti-measles IgG)	Day 0	1.1 (0.0-6.2)	12.8 (10.2-16.2)	0.0 (0.0-1.7)	7.4 (6.3-8.8)	0.0 (0.0-2.1)	7.0 (5.8-8.5)
	Day 28	88.6 (80.1-94.4)	318.9 (273.0-372.6)	91.8 (87.3-95.1)	301.9 (269.0-338.9)	86.5 (80.6-91.2)	262.5 (222.2-310.2)
JE vaccine response (anti-JE neutralizing antibody)	Day 0	3.4 (0.7-9.6)	5.7 (4.9-6.5)	5.4 (2.8-9.3)	5.7 (5.2-6.1)	6.1 (3.1-10.7)	5.9 (5.3-6.6)
	Day 28	92.1 (84.3-96.7)	202.8 (140.5-292.9)	90.5 (85.9-94.1)	155.0 (123.5-194.5)	90.6 (85.3-94.4)	139.4 (109.5-177.5)

Gatchalian S, Yao Y, Zhou B, Zhang L, Yoksan S, Kelly K, Neuzil KM, Yaïch M, Jacobson J. Comparison of the immunogenicity and safety of measles vaccine administered alone or with live, attenuated Japanese encephalitis SA 14-14-2 vaccine in Philippine infants. Vaccine 2008; 26(18):2234-41.

Victor JC , Gatchalian S, Yao Y, Zhou B, Zhang L, Yoksan S, Yaïch M, Neuzil KM. Corrigendum to "Comparison of the immunogenicity and safety of measles vaccine administered alone or with live, attenuated Japanese encephalitis SA 14-14-2 vaccine in Philippine infants" [Vaccine 26 (2008) 2234–2241]. Vaccine. 32 (2014) 306-308

JE Vaccination in Lao PDR, April 2014





Earlier this month, JE vaccination campaigns launched in Lao PDR with the goal of vaccinating **170,000 kids.** PATH and our partners have been working for more than a decade to identify and accelerate the delivery of a safe, effective, and affordable JE vaccine. Now, with prequalification and upcoming GAVI support, more countries are moving forward with JE vaccinations to ensure their children are protected.




Summary

- Routine childhood JE immunization with available vaccines is effective in high incidence areas
- Expanding efforts to provide JE immunization in high risk areas
- JE vaccines appear to have good safety profiles
- Need to improved JEV diagnostics to obtain better disease burden estimates, improve surveillance and determine vaccine effectiveness

Dengue

A Vaccinology Perspective

Why a Dengue Vaccine ?

- Large disease and economic burden
- Need for effective primary prevention tool
  - Present tool = vector control, does not work
- Would significantly reduce health care resources required for secondary prevention
  - Medical care has significantly reduced dengue mortality

Dengue Burden

Estimated burden of dengue, by continent, 2010

Continent	Dengue	Inapparent infections
	Millions (credible interval)	Millions (credible interval)
Africa	15.7 (10.5-22.5)	48.4 (39.3-65.2)
Asia	66.8 (47.0-94.4)	204.4 (151.8-273.0)
Americas	13.3 (9.5-18.5)	40.5 (30.5-53.3)
Oceana	0.18 (0.11-0.28)	0.55 (0.35-0.82)
Global	*96 (67.1-135.6)*	*293.9 (217.0-392.3)*

Bhatt, S et al Nature 2013; 496: 504-507

Dengue in Africa

- **An old disease - a new recognition**
- **24 countries with local transmission or dengue in returning travelers**
- ***Aedes aegypti* originated in Africa – present in 66% of countries**
- **Recent outbreaks: East Sudan; Mogadishu; Mandera, Kenya; Mombasa, Kenya; Luanda, Angola; Dar es Salaam, Tanzania**

From: Emer Infect Dis 2011; 17:1349-54: Am J Trop Med Hyg 2012; 86: 171–77 Trav Med Infect Dis 2011; 9:246-48; J Med Virol 2012; 84:500-03; Kyobe Bosa H, et al; ICEID 2012 poster; MMWR 2013; 62: 504-507; CDC Unpublished

Dengue Vaccines

Post-Infection Antibodies Protect Natural History Studies

- **Neutralizing antibodies**
  - **50-70 % reduction in viral plaques ($PRNT_{50-70}$)**
  - **Cell culture adapted viruses**
  - **Non-FC receptor bearing cells used in assays**
- **Homotypic Antibodies**
  - Protect against homologous DENV disease / infection (Sabin 1952; Halstead 1974)
  - Cohorts followed over multiple years
- **Heterotypic Antibodies**
  - Cross protection against disease ~ 6 months (Sabin, 1952)
  - Cross protection against infection may last longer

Problems with Antibodies
Antibody Dependent Enhancement of Infection (ADE)

- **Enhanced infection in presence of heterotypic (non-neutralizing) antibodies**
  - *In vitro* observations
  - Chimpanzee studies with passively transferred antibodies
  - AG129 interferon deficient mouse model
- **Severe dengue (DHF) – epidemiologic observations**
  - DHF among infants with 1st DENV infection in presence of passively acquired maternal antibody
  - Increased risk for DHF with 2° infections

The Ideal Product Profile

- **Formulation:** Tetravalent protection (DENV 1- 4)
- **Administration:** Delivery over 4 – 6 months and during established immunization visits
- **Storage:** off the cold chain
- **Immunogenicity:** high with ≤ 3 doses
- **Protection:** > 85% against dengue (dengue fever) ± dengue virus (DENV) infection
- **Long-term protection:** w/o booster doses

Types of Dengue Vaccine Candidates

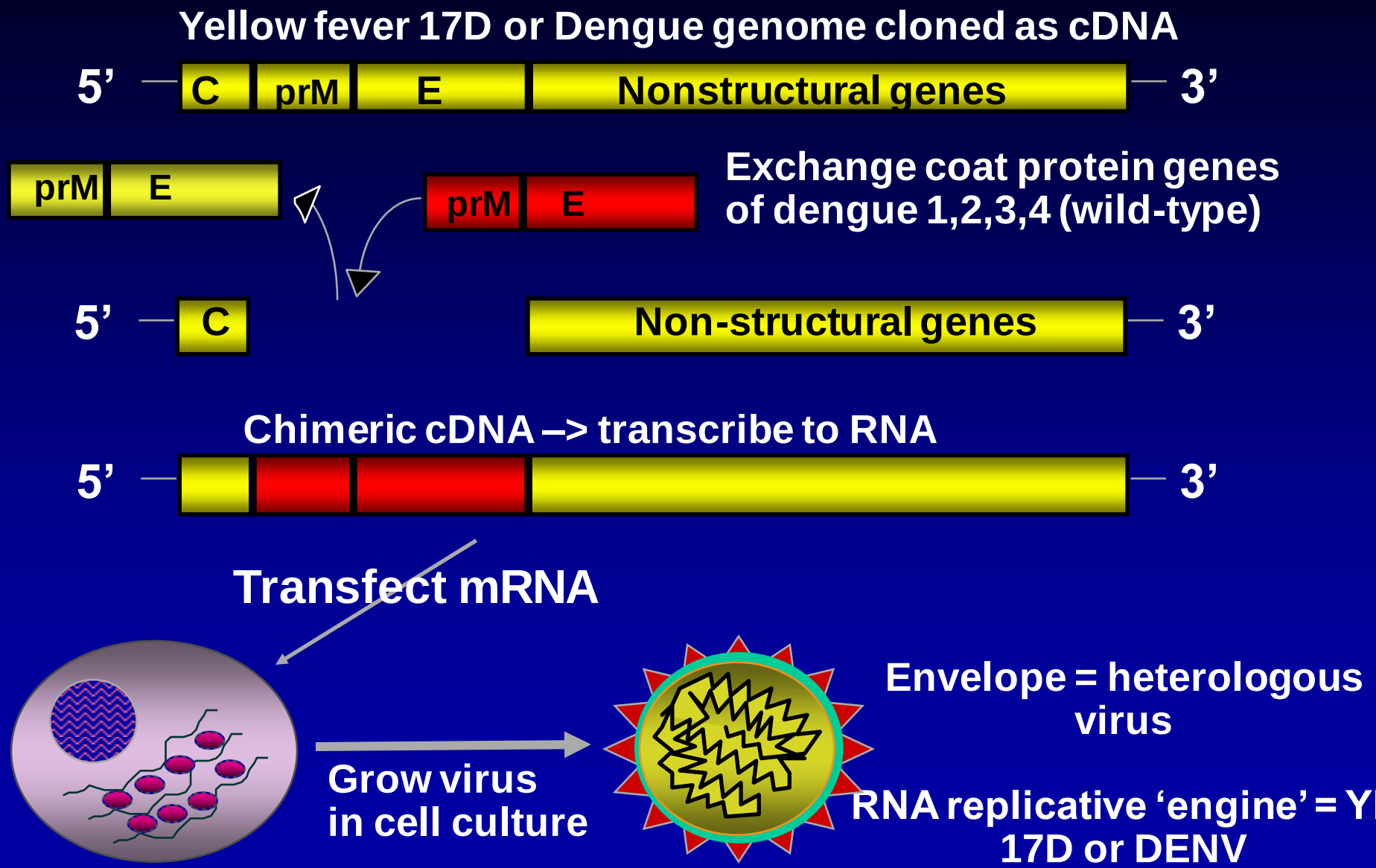
- **Present Generation** (commercial development)
  - ~~Cell culture adapted, live attenuated viruses~~
  - Infectious clones
    - chimeric viruses
    - attenuation by site directed mutagenesis
  - Recombinant subunits of DENV envelope proteins
  - Inactivated dengue viruses

- **Next Generation** (in development)
  - Viral vectored subunits
  - VLPs
  - Peptide chimeras
  - DNA

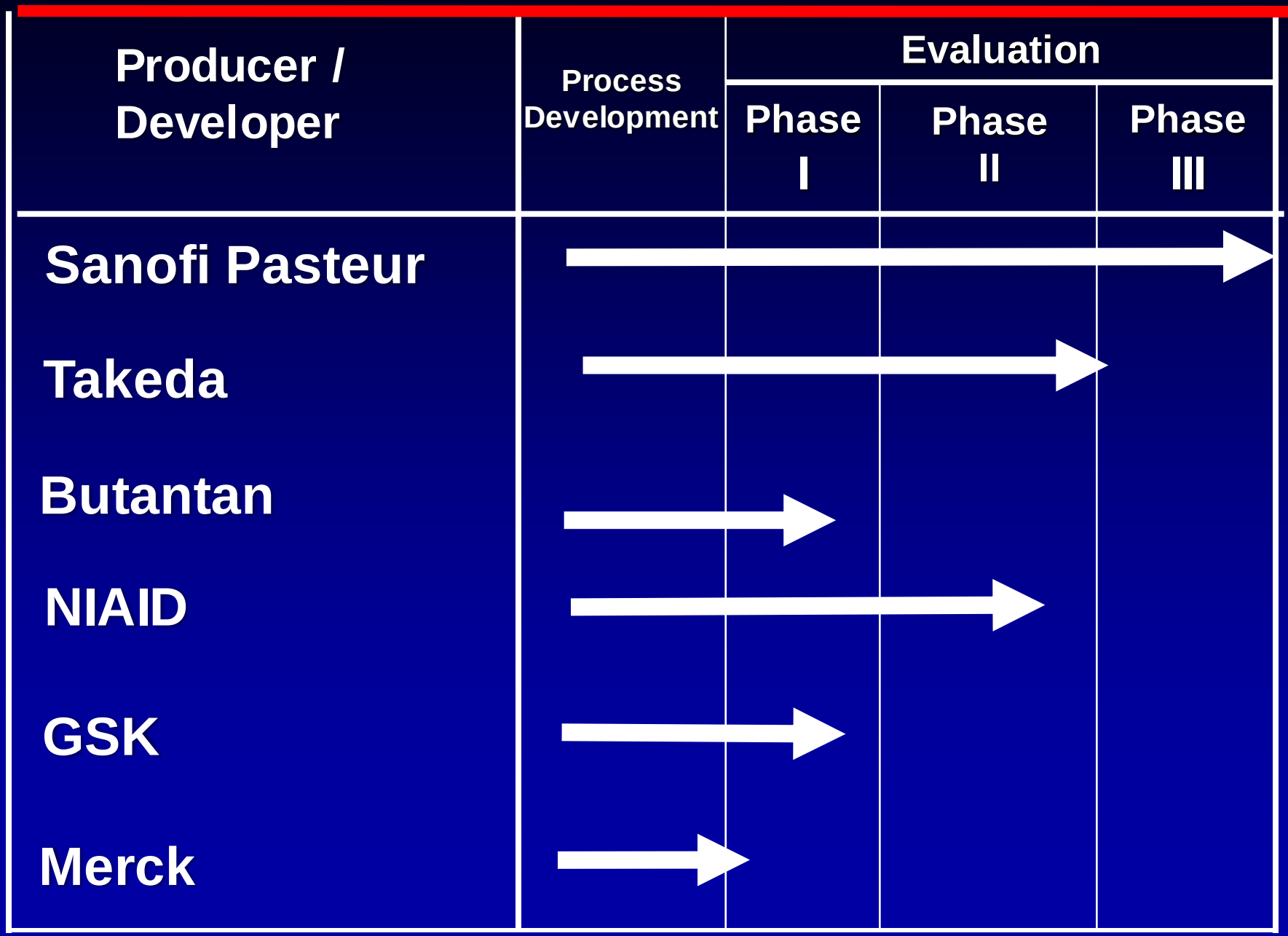
Dengue Vaccine Candidates, Tetravalent (Commercial)

Producer	Approach
Sanofi Pasteur	Live attenuated chimeric vaccine 17D yellow fever virus non-structural genes + respective DENV 1,2,3 or 4 envelope genes
GSK (WRAIR)	Switching from cell culture derived live attenuated vaccine to cell culture derived inactivated vaccine
Takeda (InViragen, CDC)	Live attenuated chimeric vaccine Attenuated DENV-2 + chimeras of DENV-2 non-structural genes + DENV 1,3, or 4 envelope genes
Butantan (NIAID)	Engineered mutations in 3' NTR and non - structural genes of DENV-1, 2, 4 & DENV-4/DEN-3 chimera
Merck (Hawaii Biotech)	Subunits of DENV 1,2,3,4 envelope protein expressed in Drosophila S2 cell lines + alum adjuvant

Chimeric Flavivirus Vaccine Technology



Status of Dengue Vaccines - 2014



Dengue Vaccine Evaluation

Lack of Good Animal Models

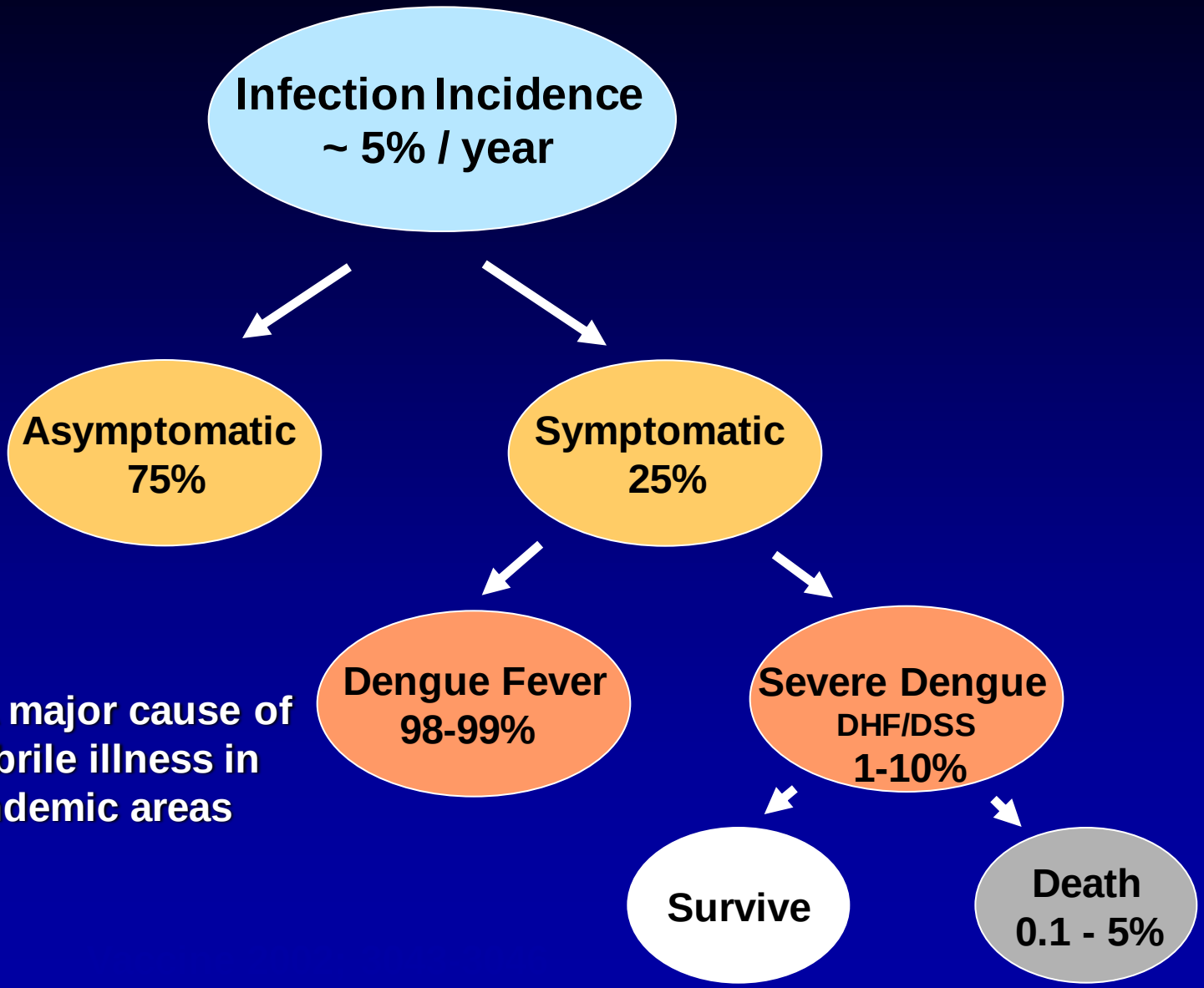
- Macaque model – short incubation period, infection only, no disease, does not readily predict immunogenicity in humans
- AG 129 interferon deficient mouse model – short incubation period, infection, disease (DHF)
- Human challenge model – has been developed but rarely used
- Human clinical trials required to determine performance of dengue vaccine candidates

Dengue Epidemiology
A Challenge to Vaccine Evaluation

- Dengue is an acute febrile illness (AFI) syndrome
  - Only defined by diagnostic testing
  - Other AFI's in dengue endemic areas: malaria, influenza, leptospirosis, meliodosis, hepatitis A
- Incidence: high endemic + cyclical epidemics
- Highly seasonal
- Several circulating virus types (serotypes)
- Peak age of incidence varies by region
- Severe dengue is natural progression of disease

Guidelines for Clinical Evaluation of Dengue Vaccine in Dengue Endemic Areas. Vaccine 2008;26:4113-4119

Dengue Virus Infection – Natural History



- A major cause of febrile illness in endemic areas

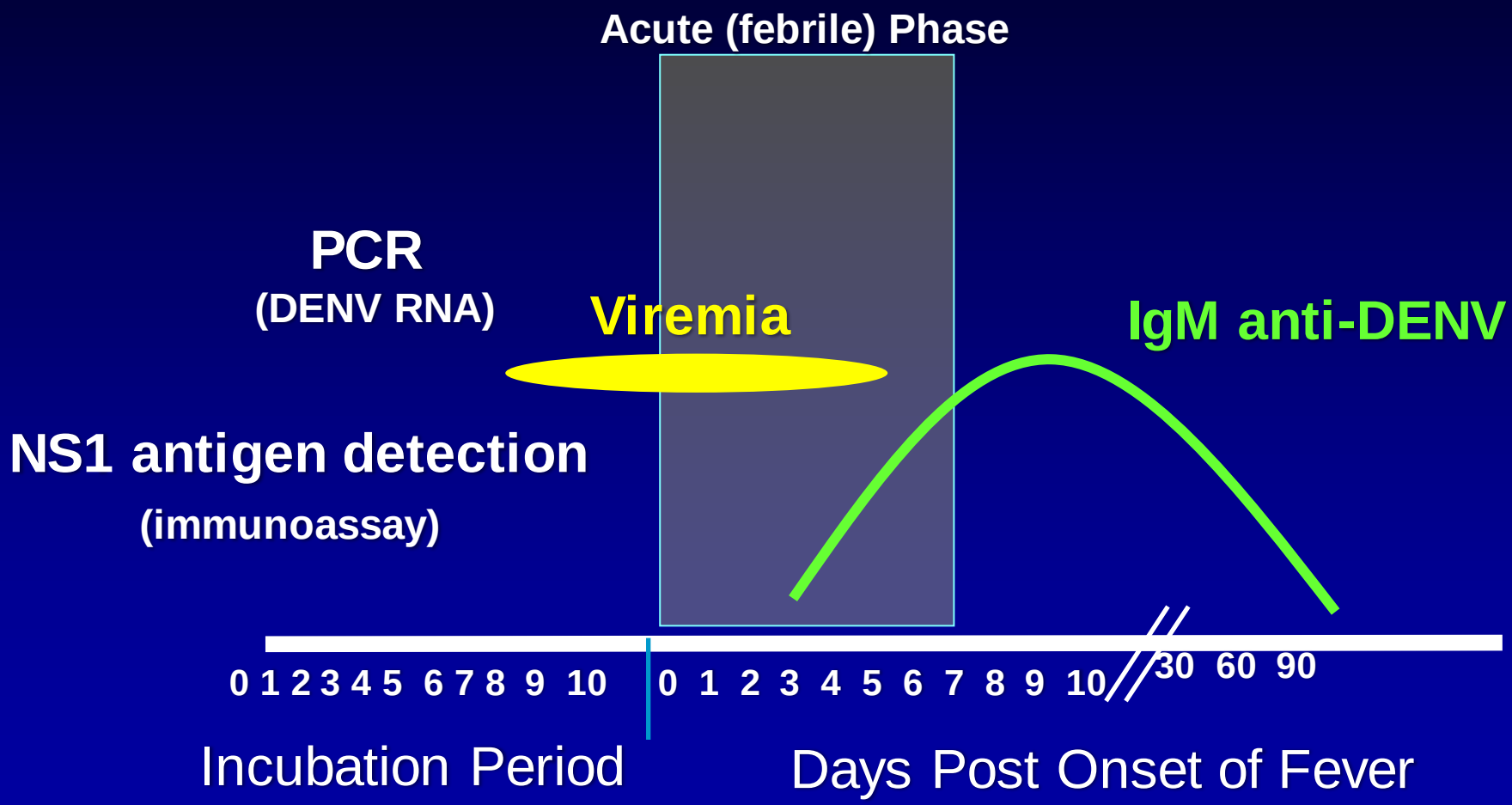
Adapted from Vaccine 2004; 22: 1275-1280

Dengue Vaccine Efficacy Trials Challenges

- Need for **large population base** because of focal nature of dengue
- **Febrile illness surveillance** to identify DF cases and determine:
  - Age-specific disease incidence
  - Determine variation in incidence over several seasons (~3 yrs)
- **Molecular and immuno–diagnostic** testing for dengue (DF) = febrile illness ≥2 days + DENV viremia detected by PCR or NS1 antigen

Guidelines for Clinical Evaluation of Dengue Vaccine in Dengue Endemic Areas. Vaccine 2008;26:4113-4119

Dengue – Diagnostic Events



First Dengue Vaccine Efficacy Trial (Phase IIB)

Dengue in Ratchaburi, Thailand 2006 - 2009

- Prospective study cohort - acute febrile illness
  - 3,013 children ages 3-13 with annual replacement with 4-5 year olds
  - Active surveillance for absences / febrile episodes in schools and home visits during vacations
  - Fever = 37.5°C oral irrespective of duration
  - Clinic evaluation = blood draw + follow-up blood draw
  - Diagnostic testing = DENV by PCR, IgM anti-DENV

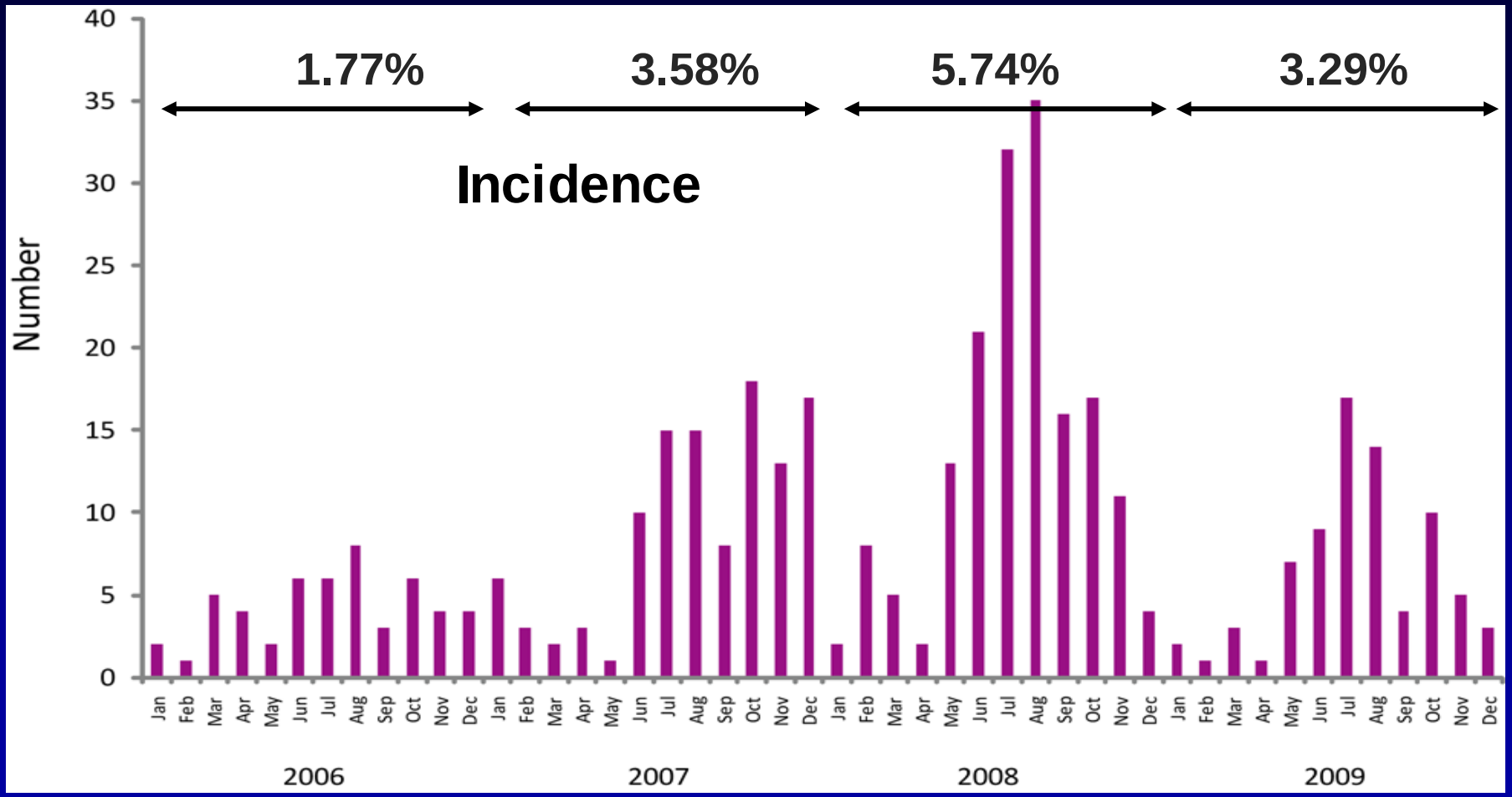
From Sabchareon, A et al. PLoS NTD 2012; 6: e1732

Dengue in Ratchaburi, Thailand 2006 - 2009

- Cohort dropout rate ~4% (2008 = 14% due to enrollment in CYD 23 vaccine trial)
- 3.39 absences / child, 0.53 febrile episodes
- Clinic visits by day post fever onset = 53% day 1-2, 30% day 3-4, 14% day 5-6
- Hospitalizations by respective years: 18%, 10%, 8%, 8%

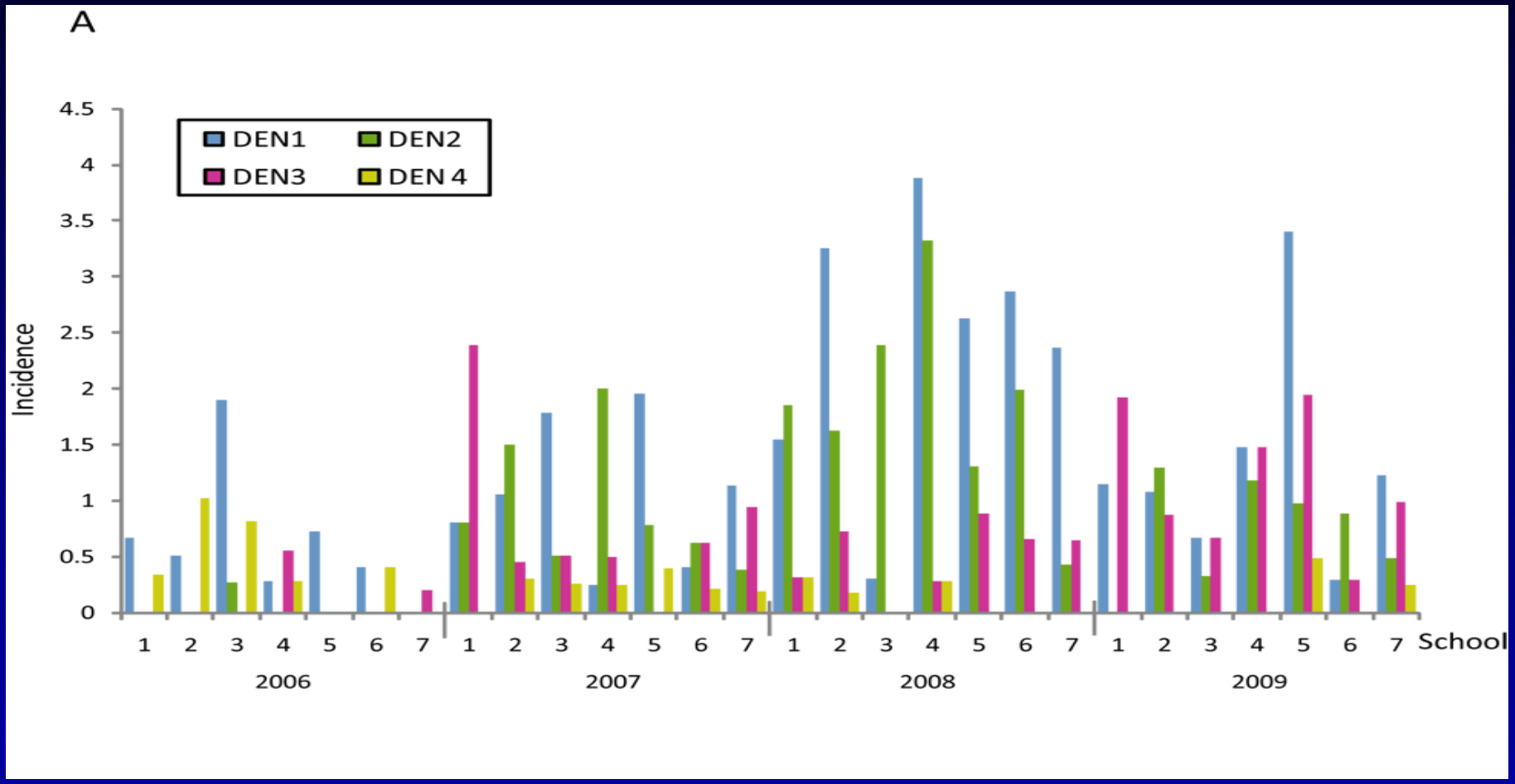
From Sabchareon, A et al. PLoS NTD 2012; 6: e1732

Dengue Cases by Month, Ratchaburi, 2006 - 2009



Adapted from Sabchareon, A et al. PLoS NTD 2012; 6: e1732

Dengue Virus Serotypes, Ratchaburi 2006 - 2009



All years (%): DENV-1 (43); DENV-2 (29); DENV-3 (20); DENV-4 (8)

Adapted from Sabchareon, A et al. PLoS NTD 2012; 6: e1732

Disease Severity, Ratchaburi, Thailand 2006 - 2009

- Classification by 1997 WHO Case Definitions

Severity	Number	Percent
Undifferentiated Fever (UF)	210	53.3
Dengue Fever (DF)	142	36.0
Dengue Hemorrhagic Fever (DHF)	42	10.7
Total	394	100

- Hospitalization: UF= 15%; DF = 84%; DHF = 100%
- 86.3% = 2° infections, no association with severity
- No association of DENV serotype and severity

From Sabchareon, A et al. PLoS NTD 2012; 6: e1732

Dengue Vaccine Efficacy Trial (CYD 23) Ratchaburi, Thailand, 2009 - 2012

- Blinded, placebo-controlled, 2:1 individual randomization (Phase IIB)
- Vaccines
  - Dengue - tetravalent, live attenuated 17D YF- DENV chimera
  - Placebo – vaccine diluent (initially rabies vaccine)
- Sample size: 4002 children ages 4-11 years
- End-point: dengue fever (acute febrile illness + DENV viremia by PCR or NS1)
- Follow-up: 13 months after 3rd vaccine dose

Adapted from Sabchareon, A et al. Lancet 2012; 380:1559-1567

CYD23 Vaccine Trial, Ratchaburi, Thailand
The Participants

	Vaccine (n=2669)		Placebo (n=1333)	
For Per Protocol Analysis				
Characteristic	N	%	N	%
	2452		1221	
Age	8.18 yrs		8.23 yrs	
Male	1187	48	583	48
From the Immunogenicity Subset (n=300)				
	197		99	
Anti-DENV (≥1 serotype)	138	70	68	69
Anti-JEV	157	80	77	78

Adapted from Sabchareon, A et al. Lancet 2012; 380:1559-1567

Safety Results - CYD 23 Trial

Adverse Event	Dengue Vaccine		Control	
	N	%	N	%
Analysis set	2666		1281	
SAE – any (anytime)	315	11.8	168	13.1
SAE - vaccine related	0	0	1	0.1
Analysis set	697		300	
AE - 30 minutes of injection	0	0	0	0
AE injection site (solicited within 7 days)	426	62	189	63
AE systemic (solicited within 14 days)	538	78	142	47
Discontinued study	0		0	

Sabchareon, A et al. Lancet 2012; 380:1559-1567

Clinical Outcomes of Dengue

- No differences between vaccine and placebo groups in clinical features or severity of dengue
  - Duration of clinical syndrome, fever or hospitalization
  - Bleeding, plasma leakage, thrombocytopenia, shock (n=0), organ impairment (n=1)

Serotype Specific and Overall Efficacy CYD 23 Trial

Per protocol	Dengue Vaccine		Control		Efficacy	
	Person Years Risk	Cases	Person Years Risk	Cases	%	95% CI
Total	**2522**	**45**	**1251**	**32**	**30.2**	**-13.4 – 56.6**
DENV 1	2436	9	1251	10	55.6	21.6 - 84
DENV 2	2510	31	1250	17	9.2	-75 - 51.3
DENV 3	2541	1	1263	2	75.3	-37.5-100
DENV 4	2542	0	1265	4	100	24.8 - 100

Sabchareon, A et al. Lancet 2012; 380:1559-1567

Immune Response in Trial Participants CYD 23 Trial

Per protocol	Dengue Vaccine		Control	
	N	Seropositive $PRNT_{50}$ >10 (%)	N	Seropositive $PRNT_{50}$ >10 (%)
28 days post last dose	**N=95**		**N=49**	
DENV 1	90	95	22	46
DENV 2	94	99	27	56
DENV 3	95	100	26	54
DENV 4	93	98	26	54
1 year post last dose	**N=95**		**N=48**	
DENV 1	73	77	22	46
DENV 2	81	85	27	56
DENV 3	85	89	26	54
DENV 4	89	94	26	54

Sabchareon, A et al. Lancet 2012; 380:1559-1567

Conclusions

- Tetravalent, DENV – YF chimeric vaccine (CYD23) shown to be safe when administered to children living in dengue endemic area and high background of previous DENV infection
- However, vaccine showed only partial (low) protection against dengue due to almost no protection against DENV – 2 infection

Possible Explanations - Phase IIB

- Statistical outliers - study not designed to look at serotype-specific results but …..
- Interference in immune response due to administration of multiple live vaccine viruses
- WT virus (DENV), vaccine virus mismatch
- Lack of stimulation of T- cells since DENV non-structural proteins NOT in vaccine (YF –backbone)
- Present way to measure IgG anti-DENV. $PRNT_{50}$ is not measuring the right (protective) antibody

SANOFI PASTEUR

The World's First, Large-Scale Dengue Vaccine Efficacy Study Successfully Achieved Its Primary Clinical Endpoint

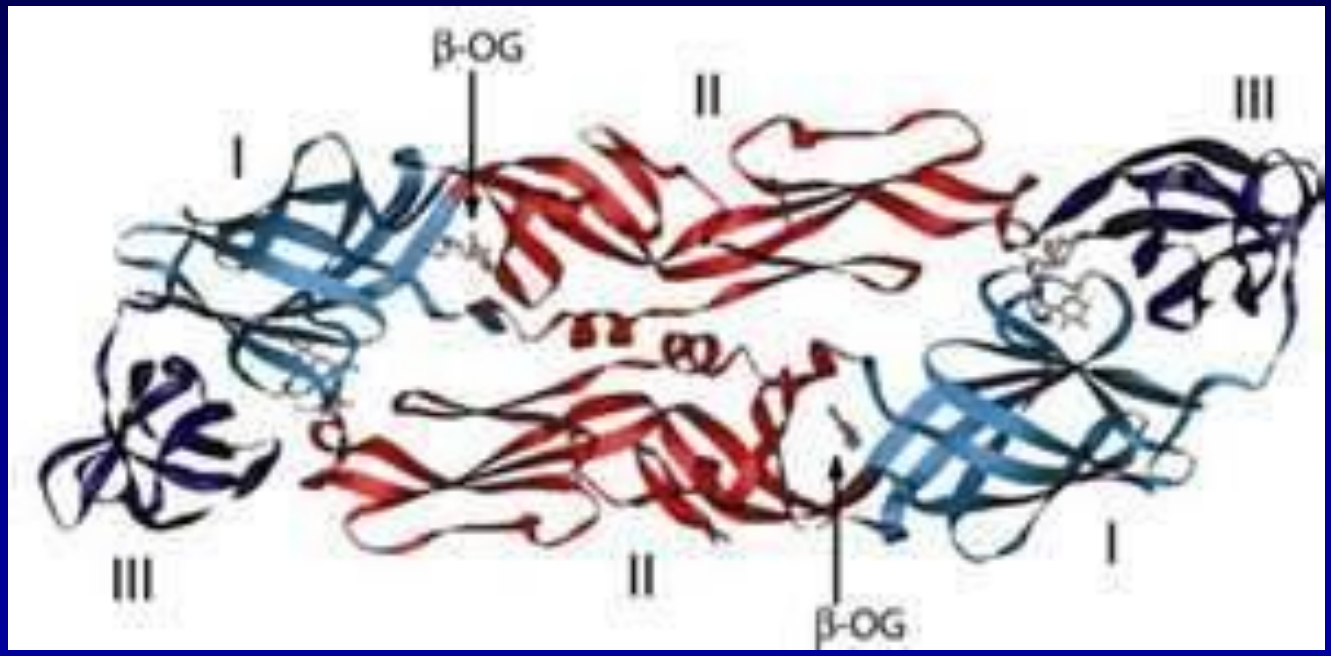
- *First available data demonstrated a 56% reduction of dengue disease cases in a study of more than 10,000 volunteers from Asia*
- *Initial safety data are consistent with the good safety profile observed in previous studies*

Lyon, France - April 28, 2014 - Sanofi Pasteur, the vaccines division of Sanofi (EURONEXT: SAN and NYSE: SNY), today announced that the first of two pivotal Phase III efficacy studies with its dengue vaccine candidate has achieved its primary clinical endpoint. The efficacy study showed a significant reduction of 56% of dengue disease cases. Initial safety data are consistent with the good safety profile observed in previous studies. Full analysis of the data will be undertaken in the coming weeks and reviewed by external experts prior to disclosure at an upcoming international scientific congress and publication in a peer-reviewed journal later this year.

Dengue is a threat to nearly half the world's population[1, 2] and is a pressing public health priority in many countries in Asia and Latin America where epidemics occur. The annual incidence rate of 4.7% observed in the control group demonstrates the very high burden of disease in Asia.

"This achievement is the result of more than 20 years of work in the field of dengue, collaborating with investigators, volunteers, authorities, scientific experts and international organizations," said Olivier Charmeil, President and CEO of Sanofi Pasteur. *"Developing a dengue vaccine for the benefit of children and their parents is at the heart of our mission. Our goal is to make dengue the next vaccine-preventable disease and to support the WHO's ambition to reduce dengue mortality by 50% and morbidity by 25% by 2020."*

Dengue Virus



We still don't know what we need to know