Developing and Using Dengue Virus Strains for Human Challenge Trials

Stephen Whitehead, 28 Sept 2017
Laboratory of Infectious Diseases

The NIAID Dengue Vaccine

- Live attenuated
- Tetravalent
- Single dose
- Tested in over 1000 subjects (Phase 1 & 2) in 3 countries
  - Challenge studies to evaluate vaccine protection (de-risking)
- Phase III efficacy study in Brazil

Clinical Endpoints for HCT

Infection Model vs. Disease Model

Natural Infection: Most dengue infections are asymptomatic or infected persons do not present for care (80%)

Dengue fever: Fever, rash, headache, myalgia, retro-orbital pain
Severe dengue: Hemorrhage, thrombocytopenia, vascular leak, shock

Phase III trial: Efficacy against virologically-confirmed dengue disease of any severity
Powered to capture symptomatic disease N = 10000 - 20000

Infection Model: Sampling for viremia – labor intensive
Highly infectious virus = smaller number of subjects
Probably more stringent than disease model
Safer – avoids need to overdose to ensure disease outcome

Potential Challenge Strains

- Safe
- High infectivity → Reproducibility
- Consistent clinical endpoints
- Wildtype surface glycoproteins
- Genotype different from vaccine virus / antigens
- DENV Serotype 2

Potential Challenge Strains

Naturally occurring strains with low virulence?

Am. J. Trop. Med. Hyg., 27(3), 1978, pp. 581–589

EPIDEMIOLOGIC, CLINICAL, AND VIROLOGIC OBSERVATIONS ON DENGUE IN THE KINGDOM OF TONGA

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Abstract. An outbreak of dengue type 2 infection occurred in the Pacific island Kingdom of Tonga in 1974 and an outbreak of dengue type 1 occurred there in 1975. The 1974 outbreak was characterized by relatively mild clinical disease with few hemorrhagic manifestations, a low attack rate, and relatively low viremia levels. The 1975 outbreak was characterized by relatively severe disease with frequent hemorrhagic manifestations and a high attack rate. The differences between the outbreaks could not be attributed to differences in abundance of, or susceptibility to infection of, mosquito vectors or to the prior immune status or other characteristics of the human population. It appeared that a difference in viral virulence was the most likely explanation.

Tonga

1974 DEN2
Mild

1975 DEN1
Severe

Potential Challenge Strains

Naturally occurring strains with low virulence?

explosive, associated with mild illness, and low viremia. The dengue virus isolation rate from serologically confirmed patients was only 32% compared to 65% for an epidemic in Bantul a year earlier. Neither dengue hemagglutination-inhibition antibody titers nor day of illness on which specimens were collected accounted for this difference. These data suggest that some naturally occurring strains of dengue virus (endemic strains) are associated with low viremia and generally cause only mild illness in man.

In late 1976, explosive epidemics of dengue hemorrhagic fever (DHF) occurred almost simultaneously in West, Central, and East Java, Indonesia, with subsequent epidemics occurring in other parts of the country.[1] The predominant virus isolated in all of those epidemics studied was dengue 3.[1]

Of particular interest were two epidemics which occurred in rural Central Java. The first, which occurred in Bantul during November 1976 to March 1977, was very explosive and characterized by severe and fatal disease and high viremia.[2–4] The second, which occurred a year later in Sleman, an ecologically similar area about 40 km north of Bantul (see Fig. 1), was less explosive the period of peak transmission (January 1978) by the same methods used a year earlier in Bantul.[2,3,5] Here we report virologic data which may help explain the lower transmission and milder disease associated with the outbreak in Sleman.

MATERIALS AND METHODS

Description of the area

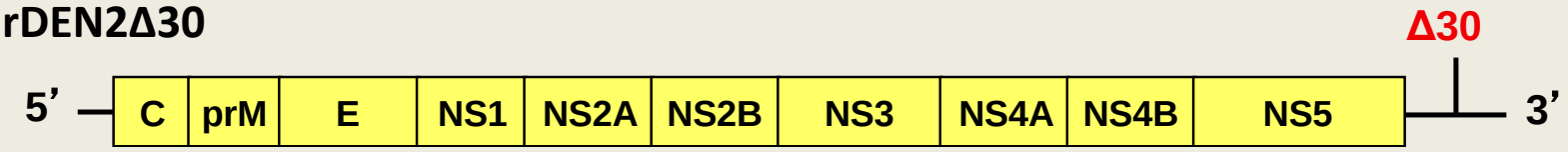
A detailed description of rural Central Java (Bantul) has already been given.[2] Sleman (Fig. 1) is about 40 km north and is similar ecologically to Bantul. It has a population of approximately

Indonesia

1978 DEN3
Mild

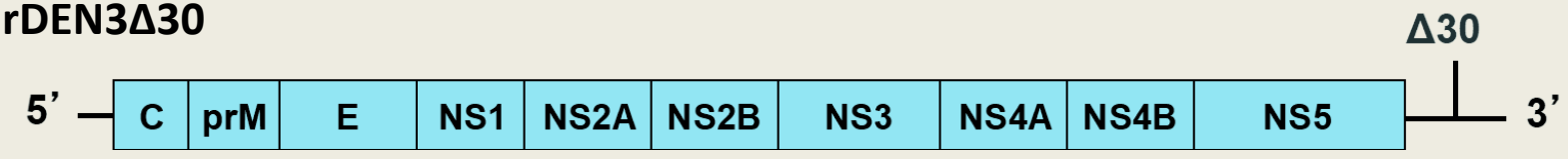
DENV Challenge strains

rDEN2Δ30



DENV2 Tonga/74

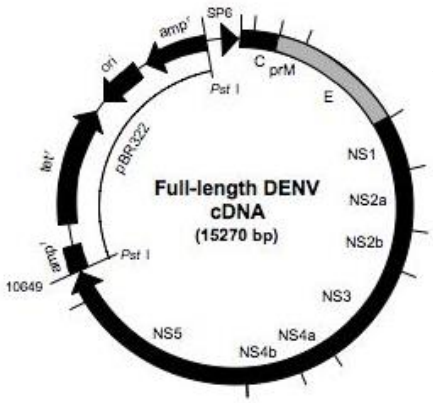
rDEN3Δ30



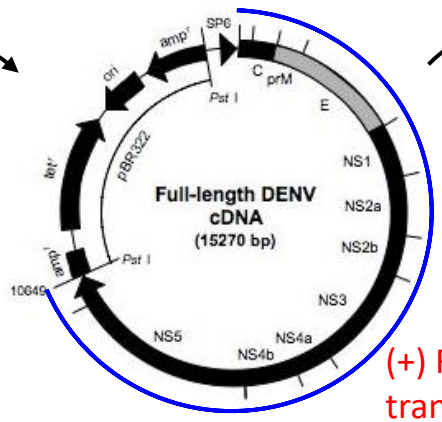
DENV3 Indonesia/Sleman/78

Dengue virus reverse genetics system

1. Construct cDNA plasmid



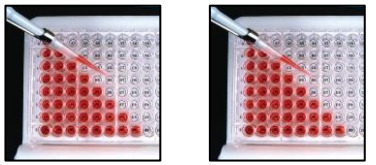
2. *In vitro* RNA transcription



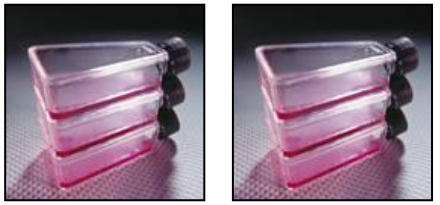
(+) RNA transcript

3. Transfect RNA into Vero cells

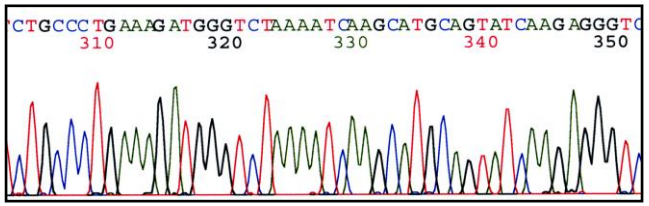
4. Terminal dilutions



5. Amplification in Vero



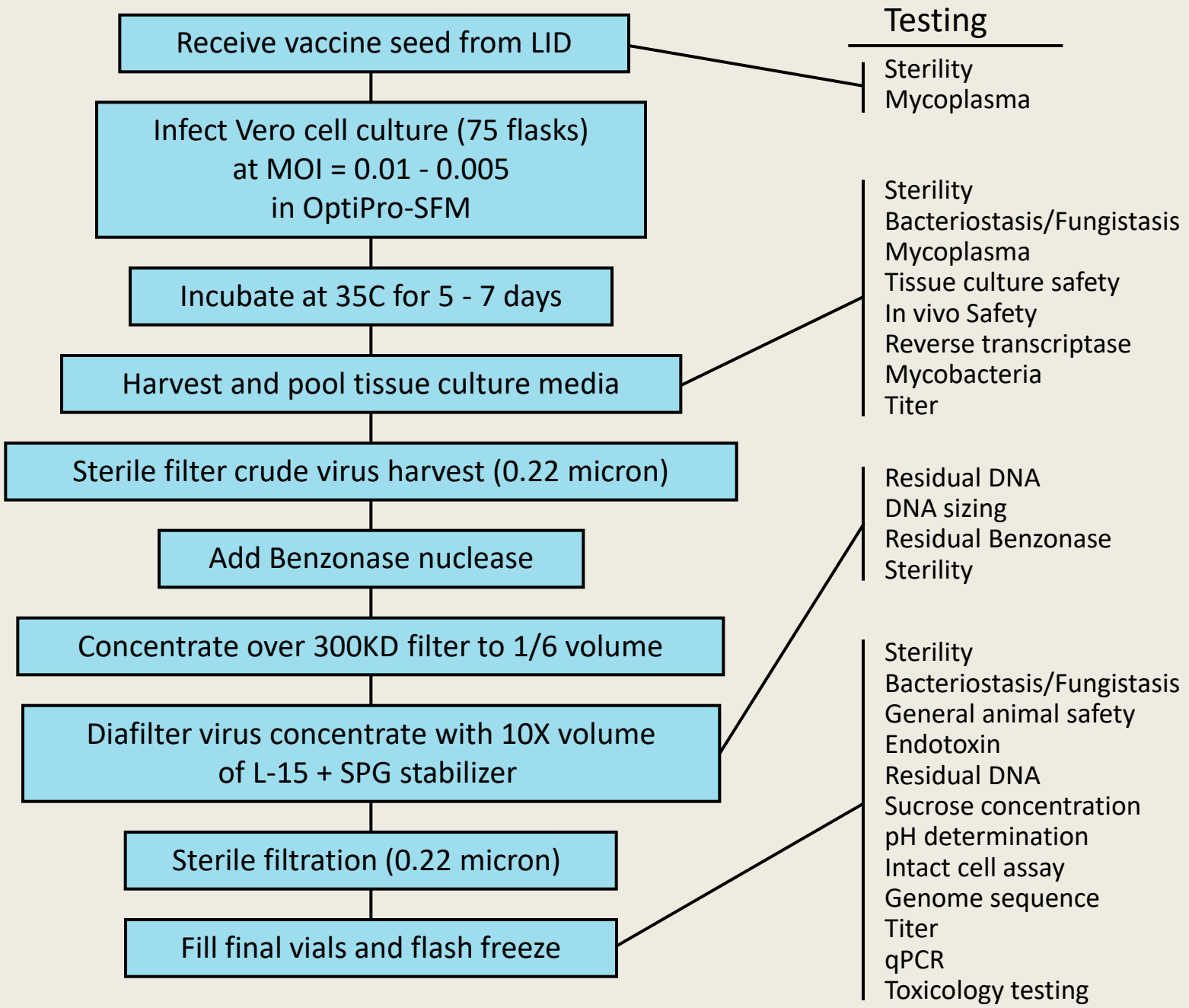
6. Verify sequence



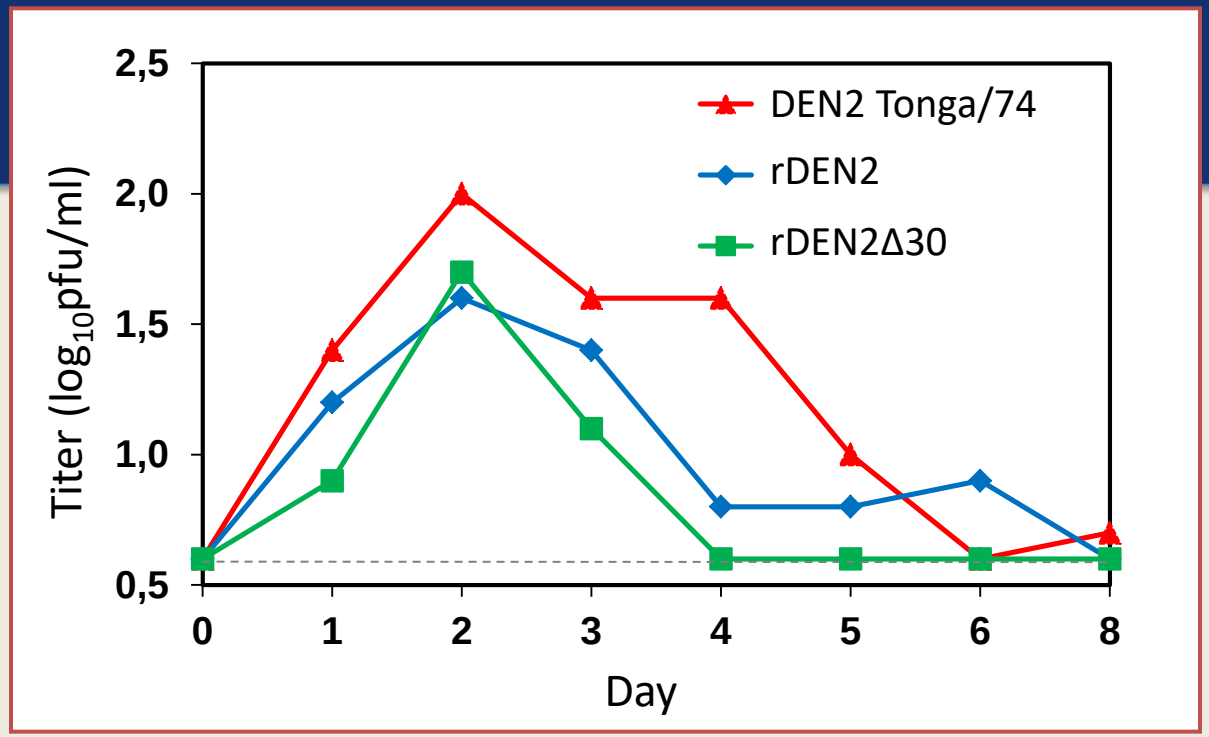
7. NHP evaluation

8. cGMP lot production

GMP manufacture of DENV challenge strains



rDEN2Δ30

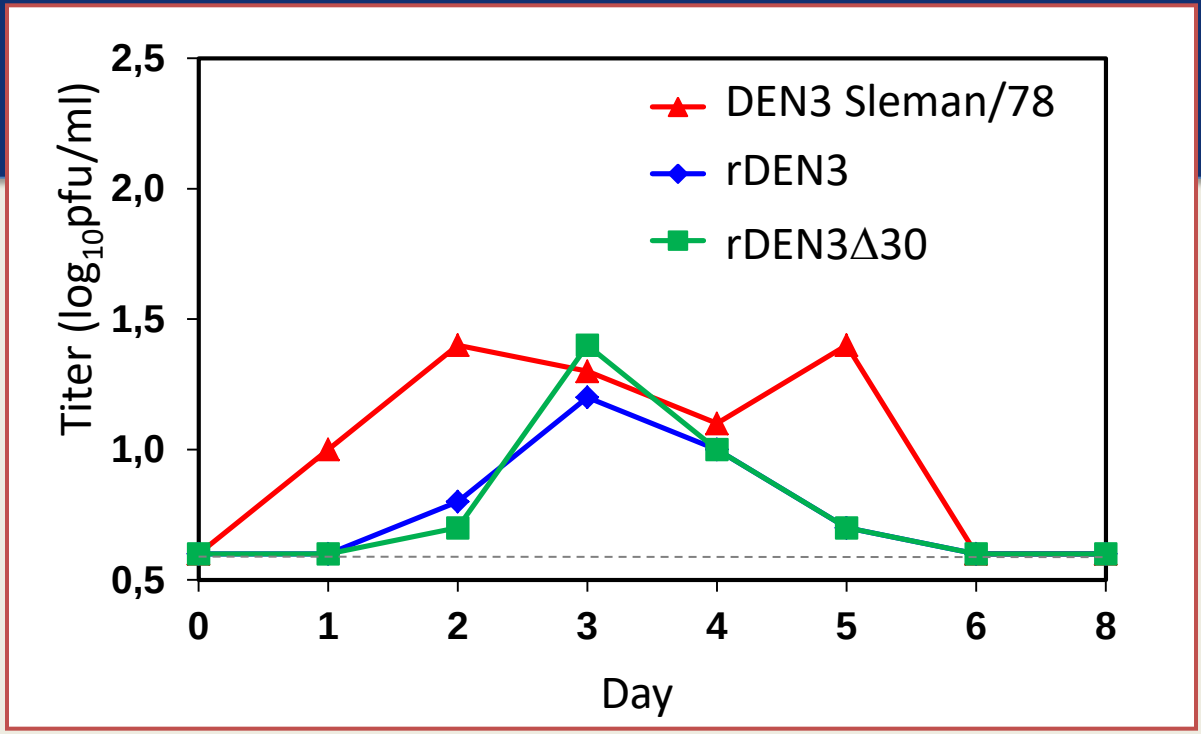


Virus[1]	No. of monkeys	% with viremia	Mean no. days with viremia	Mean peak virus titer (log_{10}pfu/ml)	Mean serum neutralizing titer[2]
DEN2 Tonga/74	4	100	4.5	2.1 ± 0.3	311
rDEN2	4	100	4.0	1.9 ± 0.1	173
rDEN2Δ30	4	100	2.3	1.7 ± 0.2	91

[1] Monkeys were inoculated subcutaneously with 5.0 log_{10} PFU of virus.

[2] Geometric mean serum neutralizing titer (reciprocal dilution) on day 28.

rDEN3Δ30

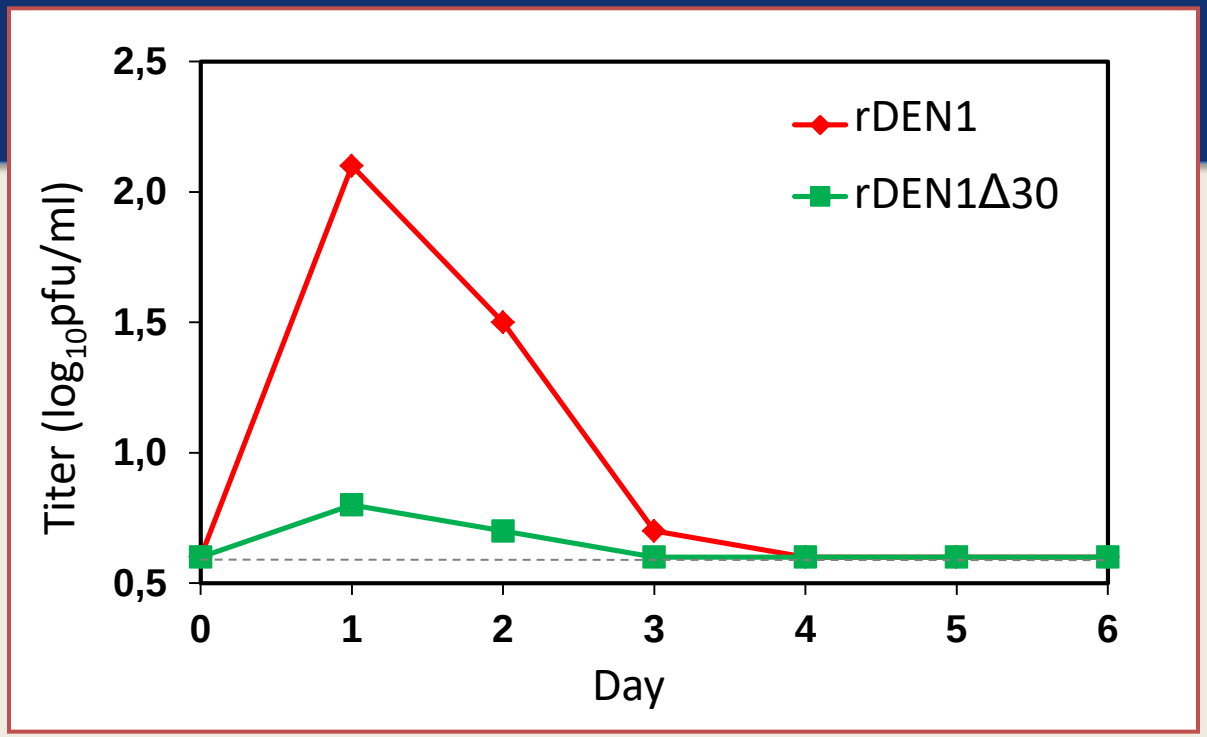


Virus[1]	No. of monkeys	% with viremia	Mean no. days with viremia	Mean peak virus titer (log_{10}pfu/ml)	Mean serum neutralizing titer[2]
DEN3 Sleman/78	4	75	2.8	1.8 ± 0.4	325
rDEN3	4	100	2.3	1.3 ± 0.3	363
rDEN3Δ30	4	100	2.5	1.5 ± 0.1	265

[1] Monkeys were inoculated subcutaneously with 5.0 log_{10} PFU of virus.

[2] Geometric mean serum neutralizing titer (reciprocal dilution) on day 28.

rDEN1Δ30



Virus[1]	No. of monkeys	% with viremia	Mean no. days with viremia	Mean peak virus titer (log_{10}pfu/ml)	Mean serum neutralizing titer[2]
rDEN1	4	100	2.8	2.1 ± 0.1	1230
rDEN1Δ30	4	50	0.5	0.8 ± 0.1	780

[1] Monkeys were inoculated subcutaneously with 5.0 log_{10} PFU of virus.

[2] Geometric mean serum neutralizing titer (reciprocal dilution) on day 28.

DEN2Δ30 in healthy volunteers

Subjects received 10^3 pfu of rDEN2Δ30:

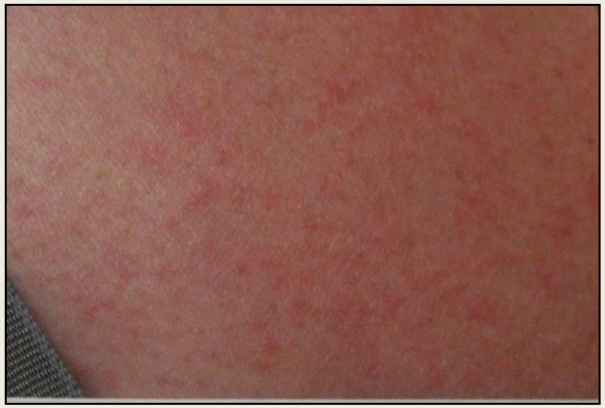
- 100% of subjects have viremia:
  - Mean peak titer = 300 pfu/ml serum (Vaccine yields 3 – 6 pfu/mL)
- 80% of subjects present with diffuse rash
  - Half were moderate in intensity
- 40% of subjects experience transient neutropenia
  - Half were Moderate: ANC nadirs = 592 - 695/mm^3
  - Half were Mild: ANC nadirs = 806 - 961/mm^3
- No subject developed fever, elevated LFTs, or signs of vascular leak

DEN3Δ30 in healthy volunteers

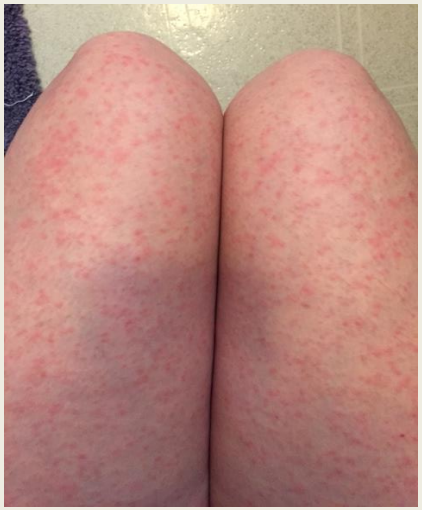
Subjects received 10^3 pfu of rDEN3Δ30:

- 100% of subjects have viremia:
  - Mean peak titer = 60 pfu/ml serum (Vaccine yields 3 – 6 pfu/mL)
- 80% of subjects present with diffuse rash
  - Half were moderate in intensity
- 10% of subjects experience transient neutropenia
- 20% of subjects experience transient thrombocytopenia
- No subject developed fever, elevated LFTs, or signs of vascular leak

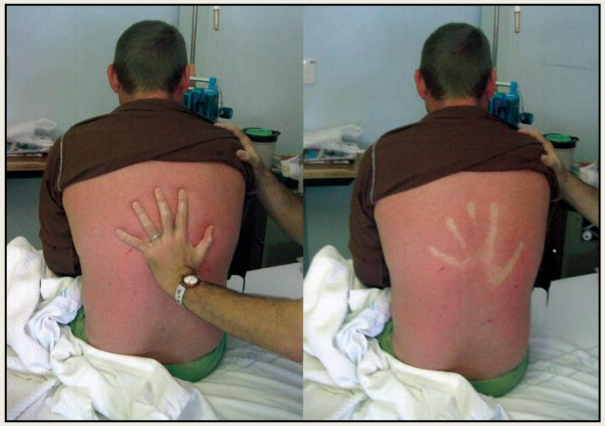
DENV challenge rash



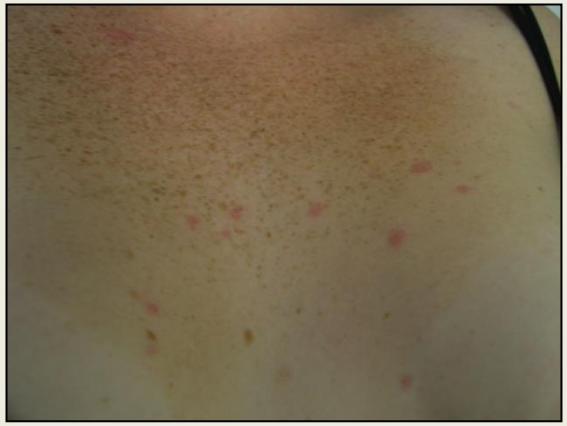
DEN2Δ30



DEN3Δ30



Typical wt dengue

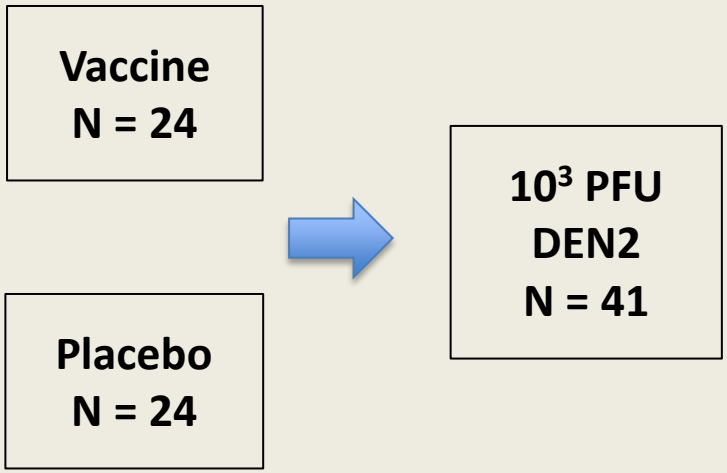


NIAID vaccine (US)

Evaluation of vaccine efficacy

Day 0
Vaccination

Day 180
Challenge



Efficacy endpoints:

Primary: Protection against viremia

Secondary: Protection against rash
Protection against neutropenia

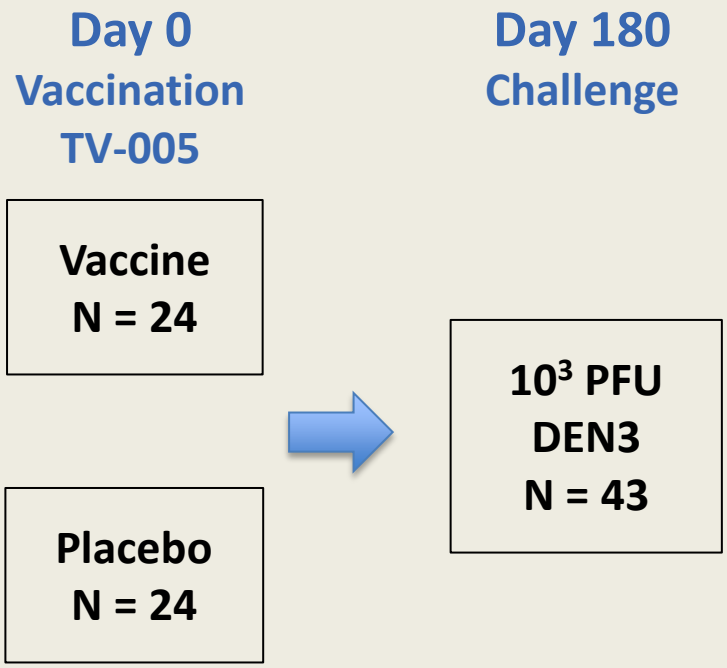
DENV-2 Vaccine Challenge Study

		Viremia post-challenge with DEN2			Rash presentation	
Cohort	N	Frequency of viremia	Mean peak Viremia	Mean duration (days)	Frequency of rash	Mean duration (days)
Placebo	20	100%	200 pfu/mL	5.6	80%	8.1
TV-003	21	0%	n/a	n/a	0%	n/a

		Viremia post-challenge with DEN2			Rash presentation	
Cohort	N	Frequency of viremia	Mean peak Viremia	Mean duration (days)	Frequency of rash	Mean duration (days)
Placebo	21	100%	170 pfu/mL	4.6	100%	9.2
TV-005	21	0%	n/a	n/a	0%	n/a

- **NIAID vaccines provide 100% efficacy against DEN2 challenge viremia and rash**

Evaluation of vaccine efficacy



Efficacy endpoints:

Primary: Protection against viremia

Secondary: Protection against rash
Protection against thrombocytopenia

DENV-3 Vaccine Challenge Study

Adverse event	Post-challenge		p-value 1-sided
	Placebo recipient (n=20)	TV-005 recipient (n=23)	
Viremia	**85.0%**	**0.0%**	**<0.0001**
Systemic:			
Fever	5.0%	13.0%	0.9282
Headache	65.0%	56.5%	0.4004
Rash	**95.0%**	**0.0%**	**<0.0001**
Neutropenia	25.0%	8.7%	0.1518
Elevated ALT	0.0%	4.4%	1.0000
Myalgia	35.0%	21.7%	0.2655
Arthralgia	10.0%	4.4%	0.4465
Retro-orbital Pain	**40.0%**	**8.7%**	**0.0187**
Thrombocytopenia	15.0%	0.0%	0.0924

DENV Vaccine Challenge Studies

What do the challenge studies tell us?

Vaccine is 100% efficacious against DEN2 & DEN3 infection

Because of the high level of efficacy, definition of a correlate of protection may not be possible.

Conclusions

- DENV challenge studies can be performed safely
- Challenge strains can be derived from natural isolates
- Challenge viremia is a remarkably reproducible clinical endpoint and is relevant for viruses that predominantly cause asymptomatic infections
- Challenge trials can provide the opportunity to down-select candidates prior to larger trials
- Challenge trials can provide a treasure trove of samples for scientific investigations

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