# Innovation on Therapeutic & diagnostic of Dengue virus

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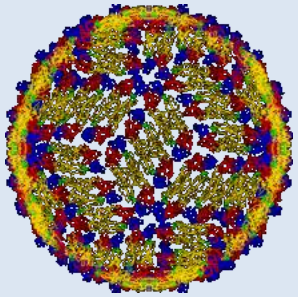
International Research Network (IRN)

Thailand Research Fund

# Dengue virus

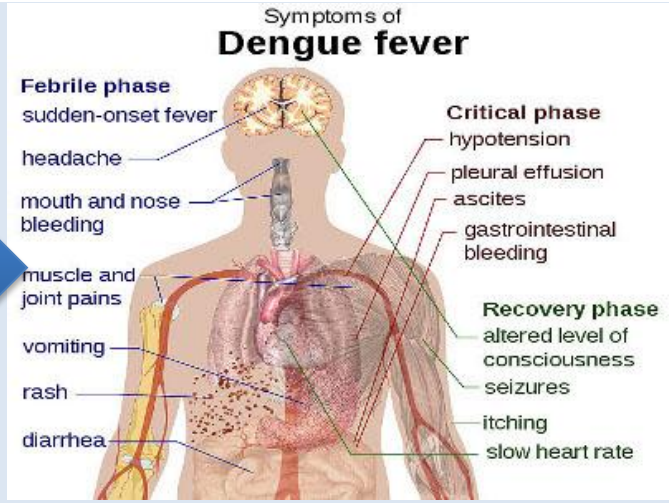
- Transmitted from mosquito to humans causing fatal disease
- WHO reported 100 Million cases/ year



Dengue virus

Infection

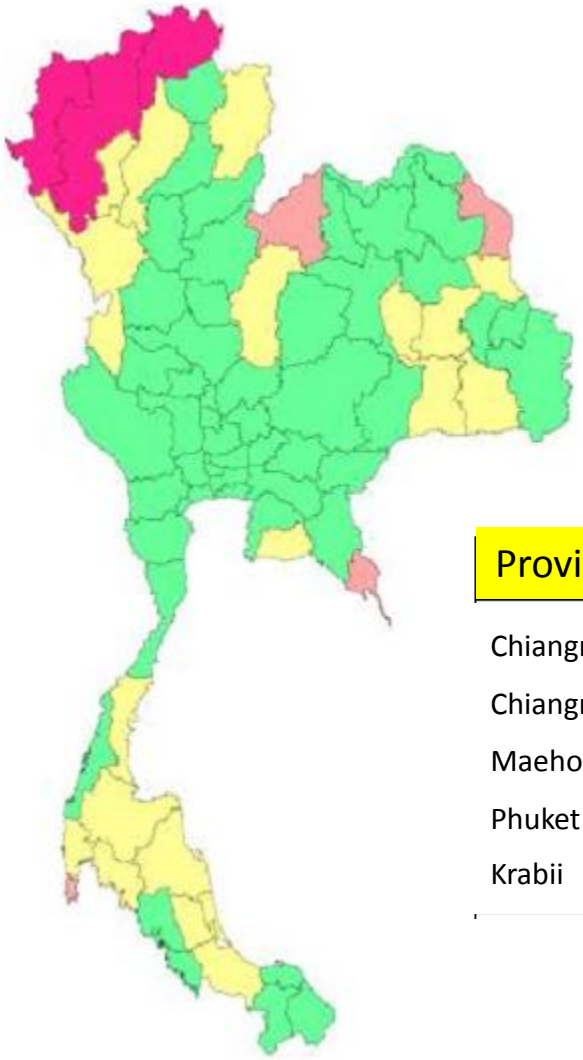

Virus transmission




- Family *Flaviviridae,* genus Flavivirus
- Single stranded non-segmented RNA virus
- 4 distinct serotypes: DENV-1 to -4

# DF/DHF/DSS Situation in Thailand, 2013

(As of 3 September 2013)



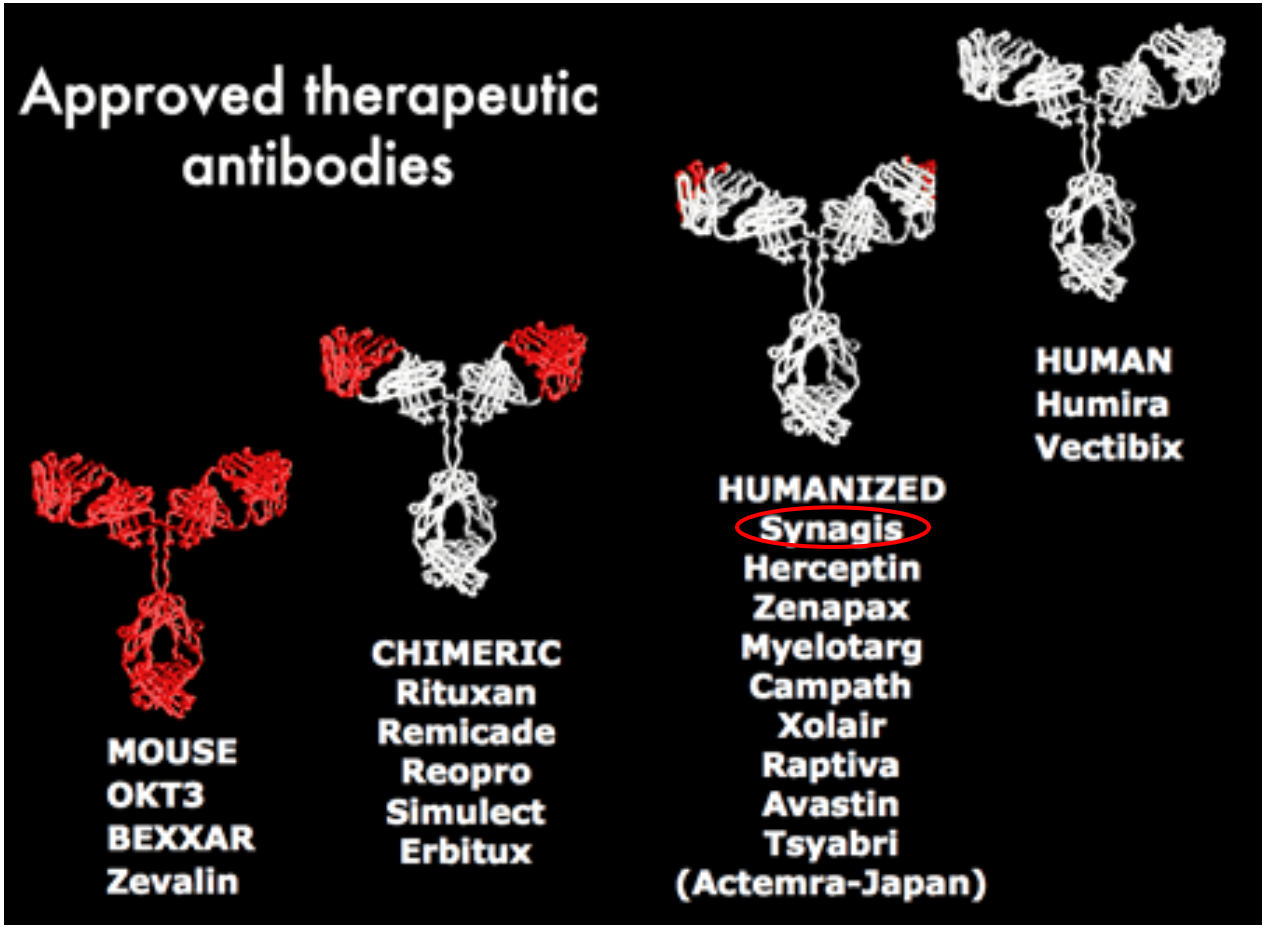

No. of cases: 115,840 cases
Morbidity rate: 180.3 per 100,000 pop
No. of deaths: 107 cases
CFR: 0.09%

| Province | Cases | Deaths | Case rate | Death rate | CFR |
|---|---|---|---|---|---|
| Chiangrai | 8,613 | 8 | **718.03** | 0.67 | 0.09 |
| Chiangmai | 10,465 | 8 | **633.90** | 0.48 | 0.08 |
| Maehongson | 1,327 | 0 | **543.40** | 0.00 | 0.00 |
| Phuket | 1,830 | 1 | **512.07** | 0.28 | 0.05 |
| Krabii | 1,840 | 2 | **416.76** | 0.45 | 0.11 |

Source: Bureau of Epidemiology, MoPH, Thailand

- Until present, there have no effective vaccine & therapeutic products against all 4 serotypes of DENV.

- Therapeutic antibody have been increasingly developed.

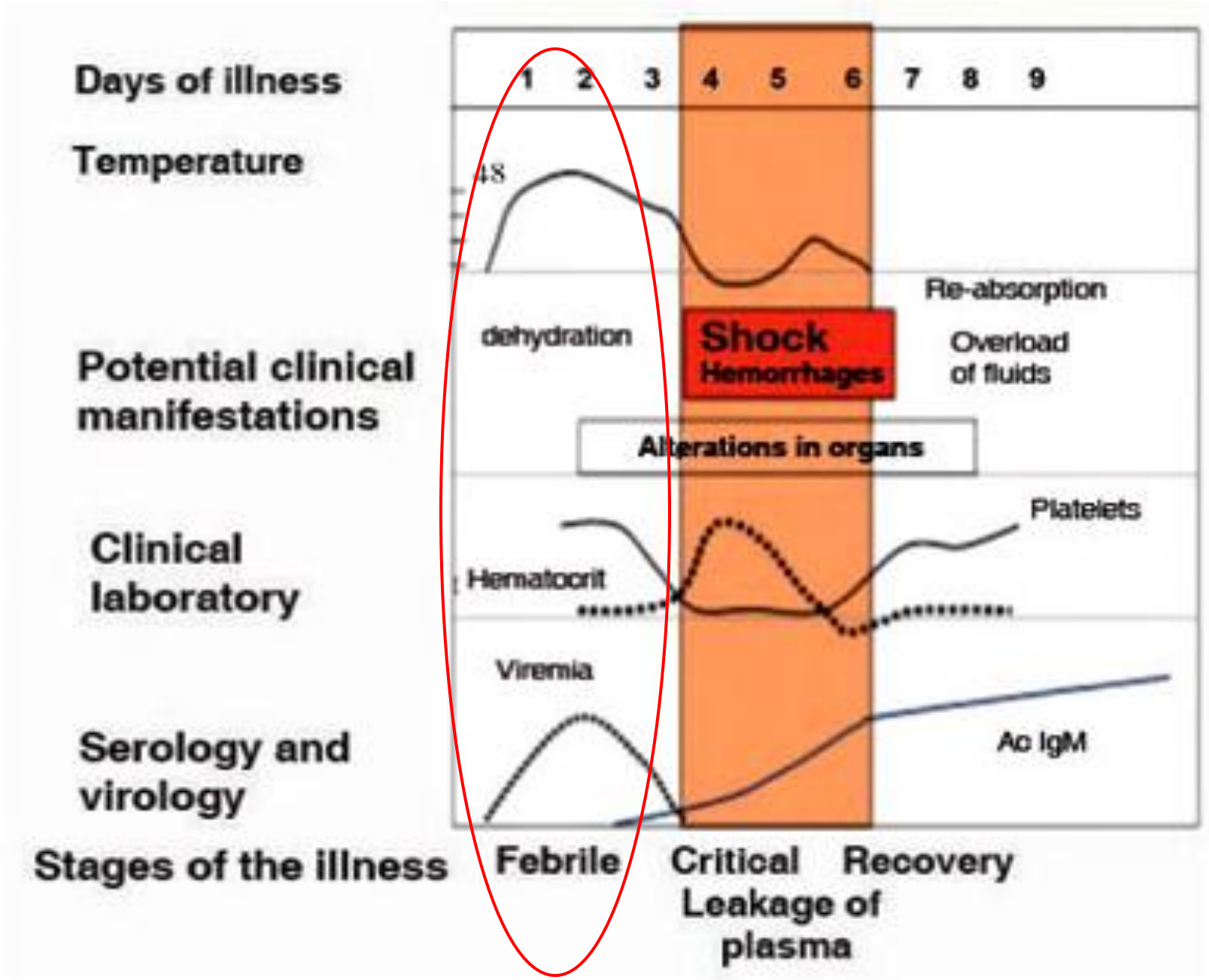



# The World's most expensive drug

**Soliris; Eculizumab** (Alexion Pharmaceutical, USA) The monoclonal antibody drug treats a rare disorder of the immune system destroys red blood cells at night. "Paroxysymal nocturnal hemoglobinuria" (PNH), hits 8,000 Americans. Treatment Cost 410,000 US$ / year.

In 2009, Soliris sales were 295 million $

http://en.wikipedia.org/wiki/Eculizumab


Days of illness
1 2 3 4 5 6 7 8 9
Temperature
48
Potential clinical manifestations
dehydration
Shock
Hemorrhages
Re-absorption
Overload of fluids
Alterations in organs
Clinical laboratory
Hematocrit
Platelets
Viremia
Serology and virology
Ac IgM
Stages of the illness
Febrile
Critical
Leakage of plasma
Recovery

# Thailand Japan Research Collaboration on Development of Therapeutic human MAbs

# against Dengue virus (2009-2013)

Research collaboration project between;

1. Research Institute for Microbial Diseases, Osaka U., Japan

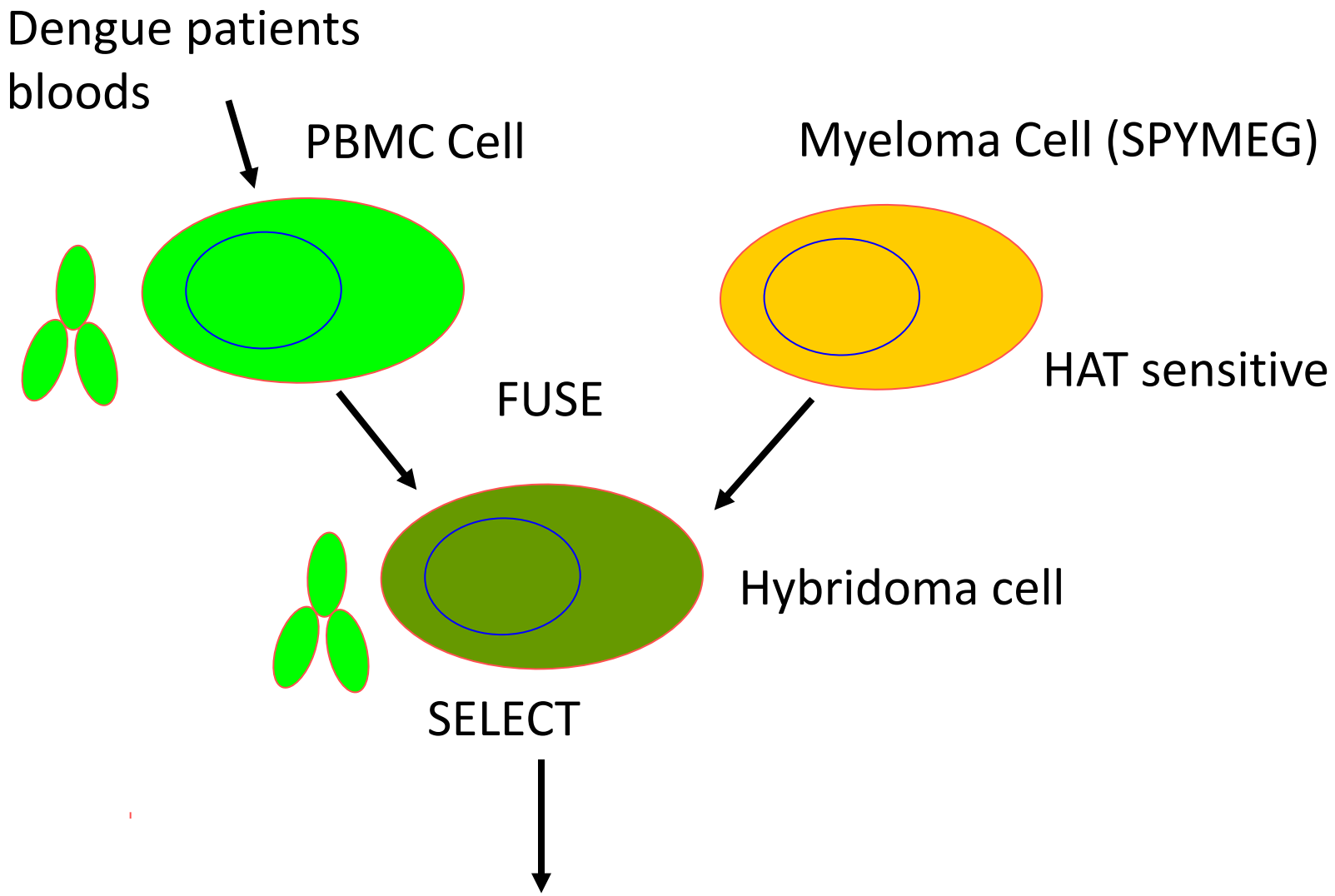
2. DMSc, MOPH, 3. Mahidol University, Thailand

- Research project ”Production of Therapeutic human Monoclonal Antibody against Dengue virus (2009-2013)
- Equipments and Technological supported by JICA; **3 Million US$**

JICA

JAPAN
Official Development Assistance

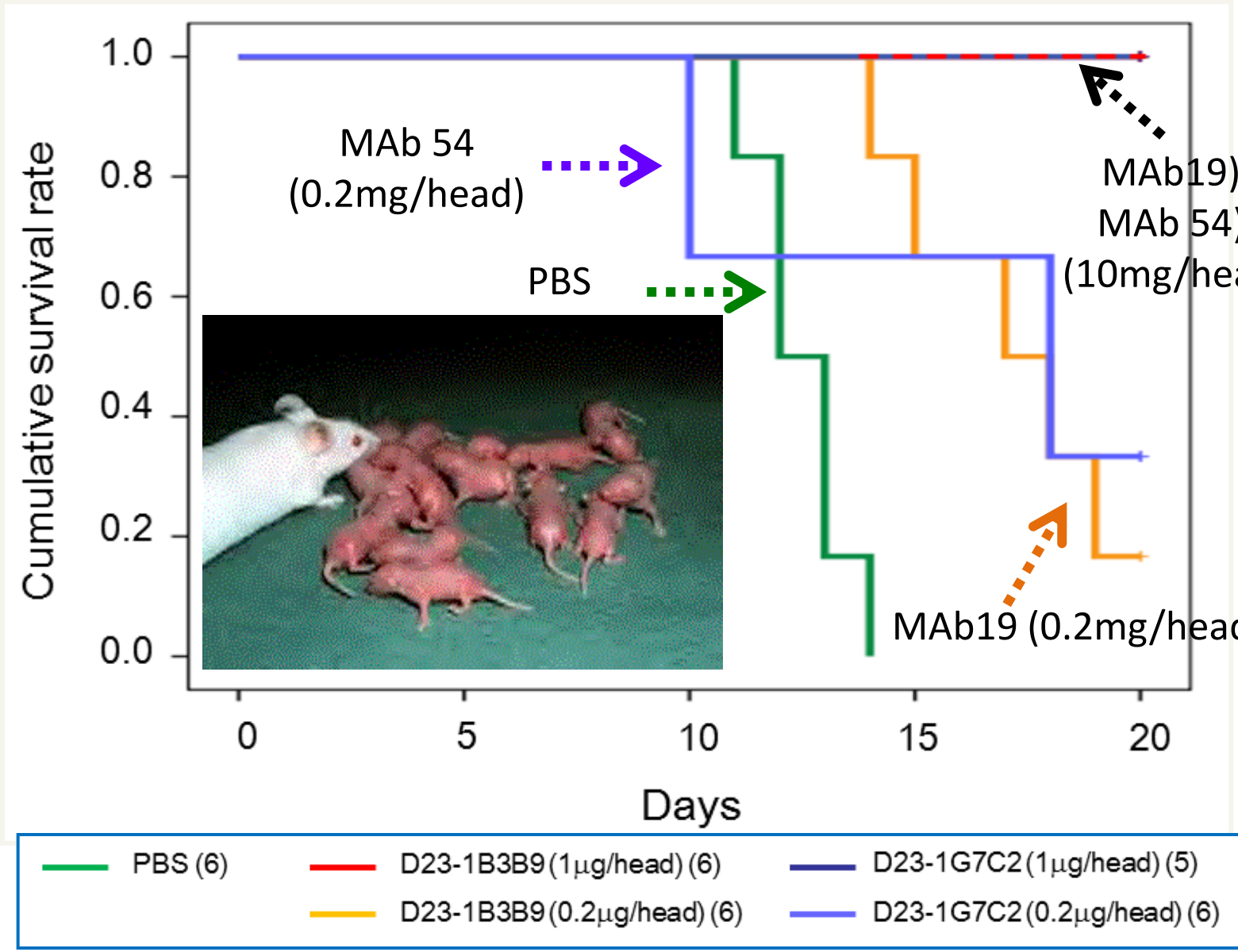
# HuMAbs against 4 serotypes of DENV were developed using Hybridoma;SPYMEG (Setthapramote et al, 2012)




3 best cells that produce neutralize Ab against all 4 serotype of DENV

# *In vivo* evaluation of NhuMAbs using suckling mouse (SM)

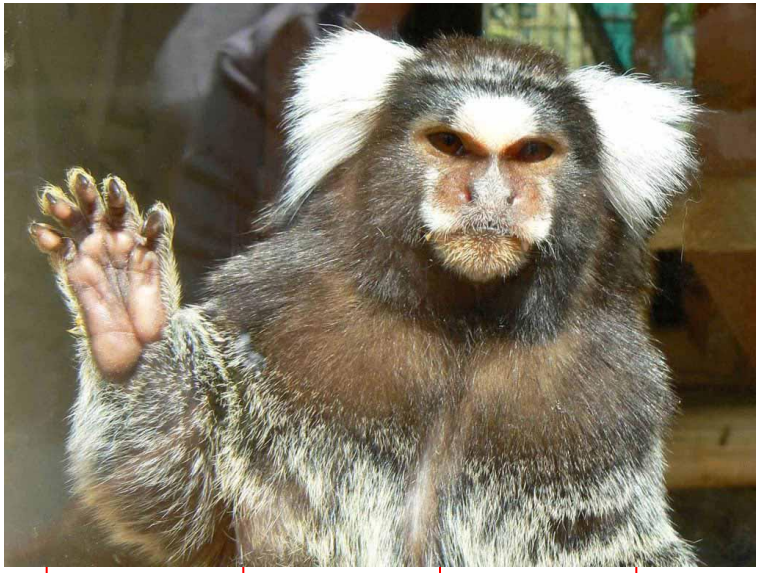
2 days Balb/c SM were IC with 20 ul 20,000 FFU DV2 + 0.2-10 ug of each NhuMAbs

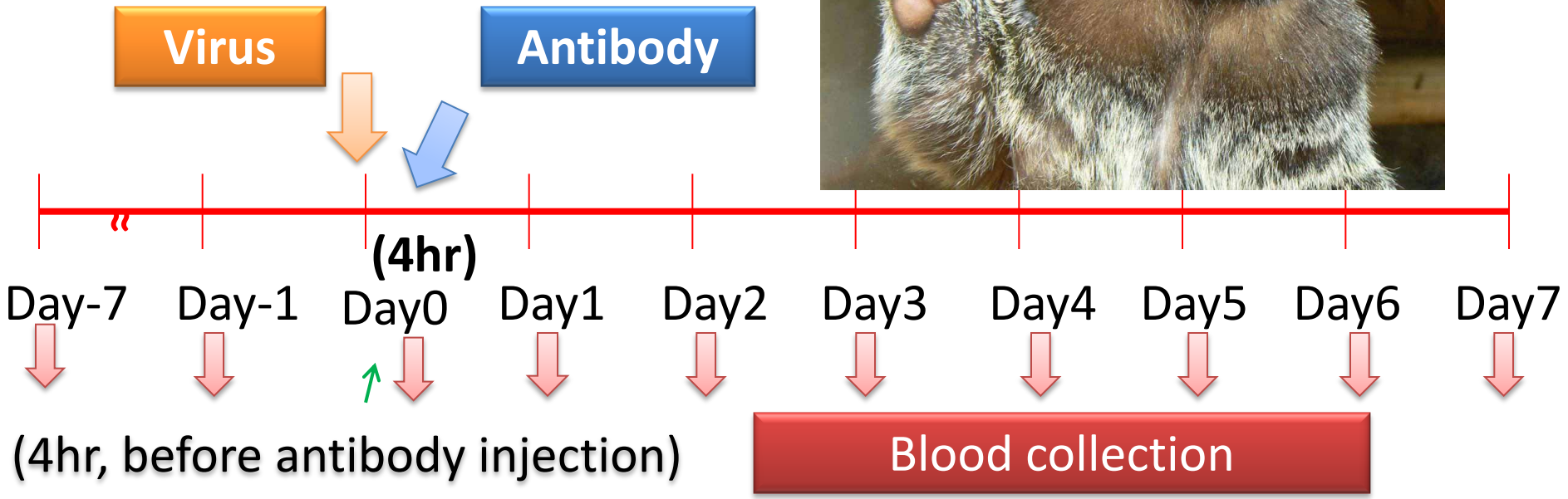


# In vivo evaluation test for NhuMAbs using marmosets (Post-treatment)

- **Antibodies**: 20mg/kg (ip)
  huMAb 19, huMAb 54, huMAb 36, Human IgG (Commercial products, PBS as negative control)

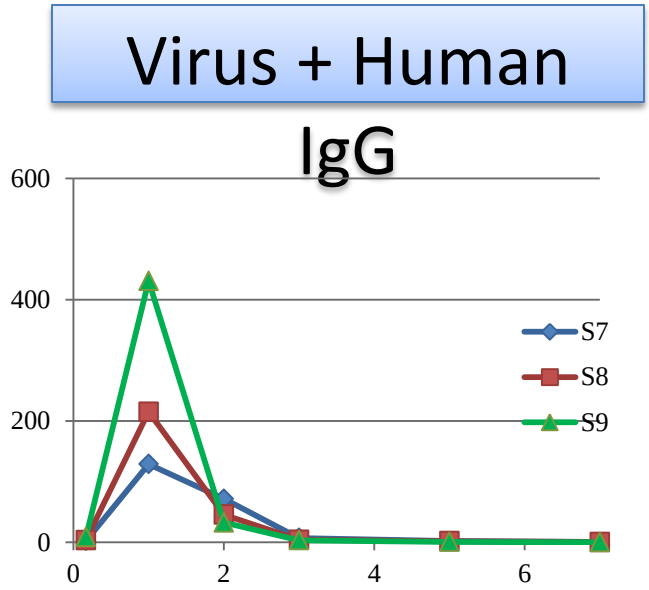
- **Virus**: DENV-2 16681 strain
  (1.0 x $10^7$ FFU/head (ip))

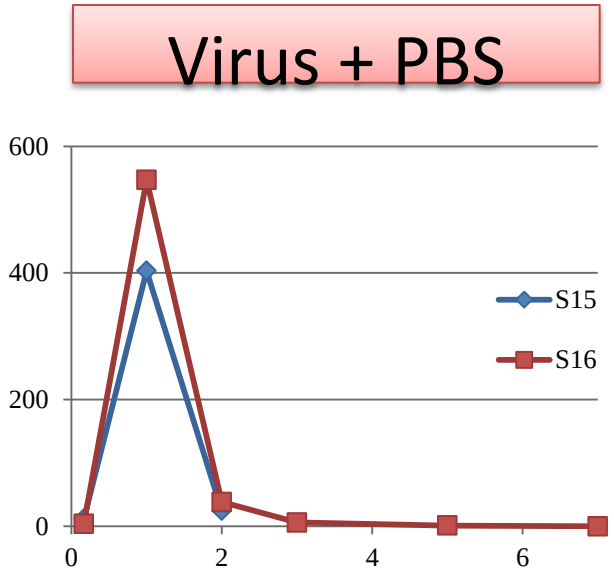


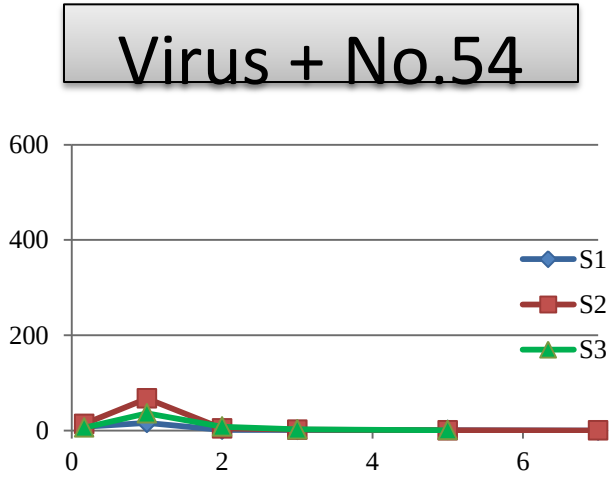


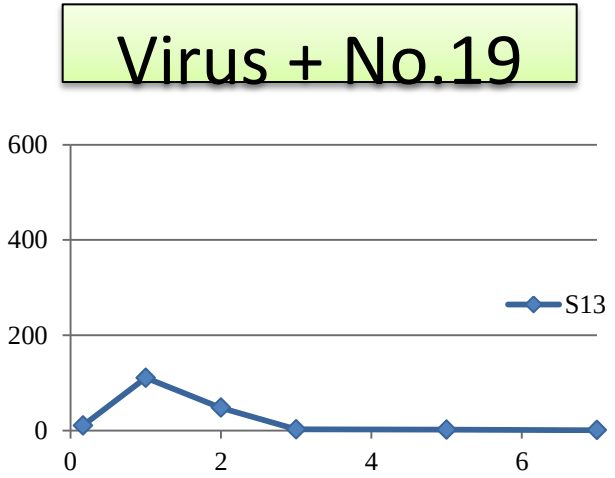
# Results of post-treatment

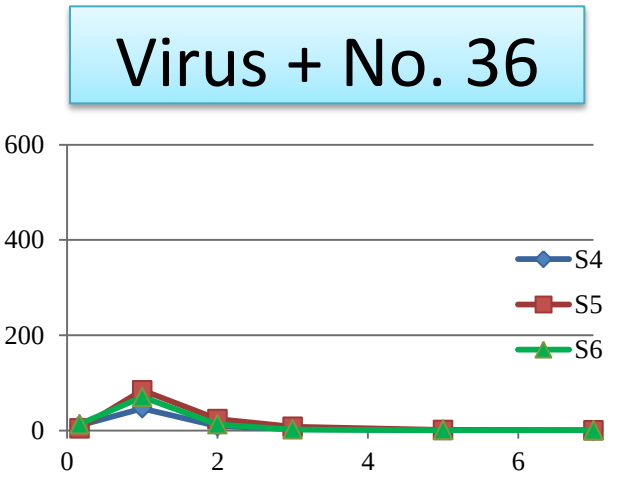
Copy No. of dengue virus gene



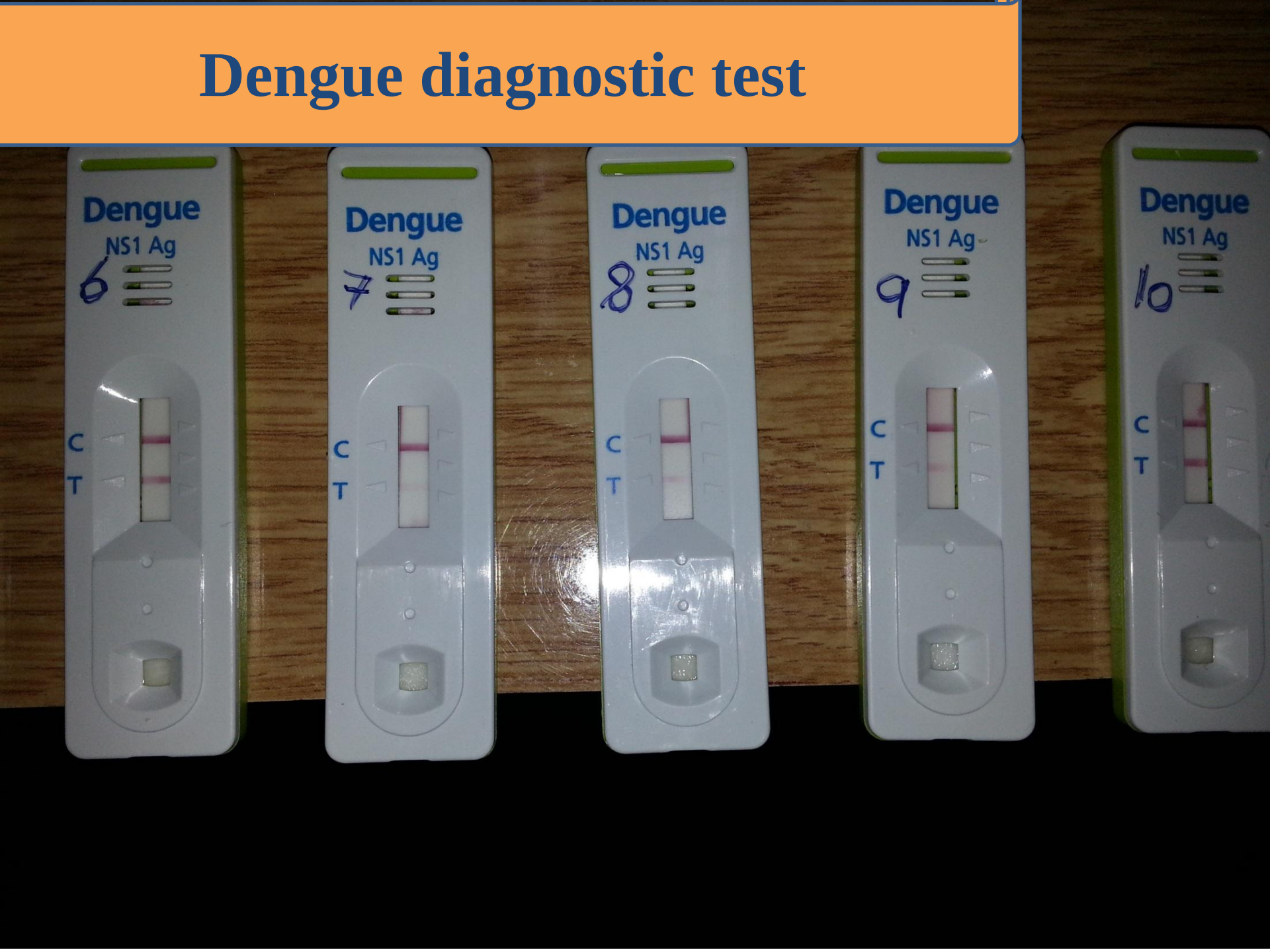



# Ongoing works; Stable expression of rIgG

- rIgG expressed from CHO cells under GMP condition, further used in human clinical trial **(Under negotiation with Industry)**

# Dengue diagnostic test



# Dengue-virus serotype neutralizing antibodies

WO 2013035345 A3

## ABSTRACT

Materials and methods are provided for treating dengue infections. Human monoclonal antibodies against all serotypes of dengue virus are also provided. Methods of using human monoclonal antibodies to neutralize all dengue-virus serotypes are provided using patients' peripheral blood lymphocytes.

| | |
|---|---|
| Publication number | WO2013035345 A3 |
| Publication type | Application |
| Application number | PCT/JP2012/005699 |
| Publication date | Sep 6, 2013 |
| Filing date | Sep 7, 2012 |
| Priority date | Sep 9, 2011 |
| Also published as | WO2013035345A2 |
| Inventors | Chayanee Setthapramote, Tadahiro Sasaki, Motoki Kuhara, Pongrama Ramasoota, Aree Thattiyaphong, Surapee Anantapreecha, Pathom Sawanpanyalert, Yoshinobu Okuno, Kazuyoshi Ikuta, Atchareeya A-nuegoonpipat, Panadda Dhepakson, Apichai Prachasuphap, Less « |
| Applicant | Osaka University, The Research Foundation For Microbial Diseases Of Osaka University, Medical And Biological Laboratories Co., Ltd, Mahidol University, Department of Medical Sciences (DMSc), Less « |
| Export Citation | BiBTeX, EndNote, RefMan |

Classifications (6), Legal Events (2)

External Links: Patentscope, Espacenet

# Antigenic peptide derived from dengue virus

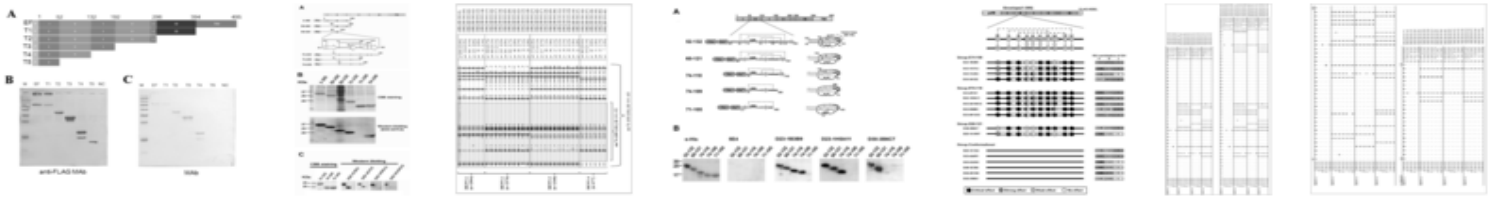
**WO 2014064943 A1**


| | |
|---|---|
| หมายเลขการตีพิมพ์ | WO2014064943 A1 |
| ประเภทการตีพิมพ์ | คำขอจดทะเบียน |
| หมายเลขคำขอสิทธิบัตร | PCT/JP2013/006333 |
| วันที่ตีพิมพ์ | 1 พ.ค. 2014 |
| วันที่ยื่น | 25 ต.ค. 2013 |
| วันยื่นคำขอ | 25 ต.ค. 2012 |
| ผู้ประดิษฐ์ | Kazuyoshi Ikuta, Tadahiro Sasaki, Mitsuhiro Nishimura, Takeshi Kurosu, Itaru HIRAI, Akifumi Yamashita, Shota Nakamura, Norihito Kawashita, Chonlatip PIPATTANABOON, Pannamthip PITAKSAJJAKUL, Tamaki OKABAYASHI, Ken-Ichiro Ono, Yoshinobu Okuno, Pongrama Ramasoota, น้อยลง « |
| ผู้ขอรับสิทธิบัตร | Osaka University, The Research Foundation For Microbial Diseases Of Osaka University, Medical And Biological Laboratories Co., Ltd, Mahidol University, น้อยลง « |
| ส่งออกการอ้างอิง | BiBTeX, EndNote, RefMan |
| การจัดหมวดหมู่ (4) | |
| ลิงก์ภายนอก: | Patentscope, Espacenet |


## บทคัดย่อ

Materials and methods are provided for detecting, preventing, and treating dengue virus infections and symptoms. Antigenic peptides, isolated nucleic acids encoding such peptides, reagents containing such peptides, reagent kits, and method of detections are provided. Vaccines are provided that contain one or more antigenic peptides based on the first domain II of a dengue virus (DENV) envelope protein (EDII). These vaccines are capable of stimulating a dengue virus immunological response in a subject previously infected with a dengue virus. Methods of manufacturing such vaccines are also presented. Further provided are methods of administering such vaccines to vaccinate a subject that has or has not been previously infected with a dengue virus.

## ภาพ (7)



## คำอธิบาย

ANTIGENIC PEPTIDE DERIVED FROM DENGUE VIRUS

The present invention relates to materials and methods for detecting,

## การอ้างสิทธิ์ (38)

1. An isolated antigenic peptide comprising at least 8 consecutive amino acid residues of amino acid residues 52-132 in the first domain II of a dengue virus (DENV) envelope protein (EDII).

Biochemical and Biophysical Research Communications 423 (2012) 867–872

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Human monoclonal antibodies to neutralize all dengue virus serotypes using lymphocytes from patients at acute phase of the secondary infection

Chayanee Setthapramote[a,k,1], Tadahiro Sasaki[b,k,1], Orapim Puiprom[c], Kriengsak Limkittikul[d,k], Pannamthip Pitaksajjakul[e,k], Chonlatip Pipattanaboon[a,k], Mikiko Sasayama[c], Pornsawan Leuangwutiwong[a,k], Weerapong Phumratanaprapin[f,k], Supat Chamnachanan[f,k], Teera Kusolsuk[g,k], Akanitt Jittmittraphap[a,k], Azusa Asai[b,k], Juan Fernando Arias[b,k], Itaru Hirai[h,k], Motoki Kuhara[i,k], Yoshinobu Okuno[j], Takeshi Kurosu[b,c,k], Pongrama Ramasoota[e,k,*], Kazuyoshi Ikuta[b,c,k,*]

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Antiviral Research 98 (2013) 423–431

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

# Dengue virus neutralization and antibody-dependent enhancement activities of human monoclonal antibodies derived from dengue patients at acute phase of secondary infection

Tadahiro Sasaki [a,j,1], Chayanee Setthapramote [b,j,1], Takeshi Kurosu [a,j], Mitsuhiro Nishimura [a,j], Azusa Asai [a,j], Magot D. Omokoko [a,j], Chonlatip Pipattanaboon [b,j], Pannamthip Pitaksajjakul [c,j], Kriengsak Limkittikul [d,j], Arunee Subchareon [d], Panjaporn Chaichana [e], Tamaki Okabayashi [e], Itaru Hirai [a,f,j], Pornsawan Leaungwutiwong [b,j], Ryo Misaki [g,j], Kazuhito Fujiyama [g,j], Ken-ichiro Ono [h,j], Yoshinobu Okuno [i], Pongrama Ramasoota [c,j,*], Kazuyoshi Ikuta [a,j,*]

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Biologics: Targets and Therapy

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ORIGINAL RESEARCH

# Cross-reactivity of human monoclonal antibodies generated with peripheral blood lymphocytes from dengue patients with *Japanese encephalitis virus*

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Number of times this article has been viewed

Chonlatip Pipattanaboon[1,2,8,*]
Tadahiro Sasaki[2,8,*]
Mitsuhiro Nishimura[2,8]
Chayanee Setthapramote[1,8]
Pannamthip Pitaksajjakul[1,4,8]
Pornsawan Leaungwutiwong[1,2,8]
Kriengsak Limkittikul[5,8]
Orapim Puiprom[6]
Mikiko Sasayama[6]
Panjaporn Chaichana[6]
Tamaki Okabayashi[6]
Takeshi Kurosu[2,8]
Ken-ichiro Ono[7,8]
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**Background:** Hybridomas that produce human monoclonal antibodies (HuMAbs) against *Dengue virus* (DV) had been prepared previously using peripheral blood lymphocytes from patients with DV during the acute and convalescent phases of a secondary infection. Anti-DV envelope glycoprotein (E) 99 clones, anti-DV premembrane protein (prM) 8 clones, and anti-DV nonstructural protein 1 (NS1) 4 clones were derived from four acute-phase patients, and anti-DV E 2 clones, anti-DV prM 2 clones, and anti-DV NS1 8 clones were derived from five convalescent-phase patients.

**Methods and results:** In the present study, we examined whether these clones cross-reacted with *Japanese encephalitis virus* (JEV), which belongs to the same virus family. Forty-six of the above-described 99 (46/99) anti-E, 0/8 anti-prM, and 2/4 anti-NS1 HuMAbs from acute-phase, and 0/2 anti-E, 0/2 anti-prM, and 5/8 anti-NS1 HuMAbs from convalescent-phase showed neutralizing activity against JEV. Thus, most of the anti-E and anti-NS1 (but not the anti-prM) antibodies cross-reacted with JEV and neutralized this virus. Interestingly, 3/46 anti-E HuMAbs derived from acute-phase patients and 3/5 anti-NS1 HuMAbs from convalescent-phase patients showed particularly high neutralizing activity against JEV. Consequently, the HuMAbs showing neutralization against JEV mostly consisted of two populations: one was HuMAbs recognizing DV E and showing neutralization activity against all four DV serotypes (complex-type) and the other was HuMAbs recognizing DV NS1 and showing subcomplex-type cross-reaction with DV.

**Conclusion:** Anti-DV E from acute phase (46/99) and anti-DV NS1 (7/12) indicate neutralizing activity against JEV. In particular, three of 46 anti-DV E clones from acute phase and three of five anti-NS1 clones from convalescent phase showed strong neutralizing activity against JEV.

**Keywords:** *Dengue virus*, *Japanese encephalitis virus*, viral neutralization, human monoclonal antibody, envelope, nonstructural protein 1

## Introduction

*Dengue virus* (DV) encodes capsid protein (C), premembrane protein (prM), and envelope glycoprotein (E), in addition to seven nonstructural proteins (NS).[1] There are four antigenically distinct serotypes (DV1–DV4), which share major antigens with each other and with other mosquito-borne and tick-borne flaviviruses, including *Japanese encephalitis virus* (JEV).[2–8] DV and JEV are closely related, belonging to the same virus family, Flaviviridae. Both viruses are cocirculating in areas of Southeast

# Output

1. Eight publications
2. Two US. Patents and 9 countries
3. Graduate students; 4 Ph.D., 3 M.Sc.
4. Training in Japan; 6 staffs, 6 students
4. International conference presentations; 25
5. Well equiped COE; Faculty staffs could utilized high tech equipments.
6. Outstanding Thailand Research Awards 2010, 2013 and 5 international Awards 2014.

# Supporting factors

- Strong collaboration & friendship; Japanese & Thai
- Researchers; Clinician, Biomedical & Company
- Research grants; JST for Japanese, TRF for Thai
- Tech transfer; from JICA experts
- Equipments; fully supported by JICA ,
- COE Lab space: fully supported by MU, Thailand
- Ph.D. students & Post-doc; TRF scholarship + JICA
- Institution, leader & policy; support translation grant (670,000 US$) for;
  - Industrial scale production & clinical trial

# terima kasih