

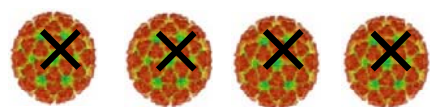
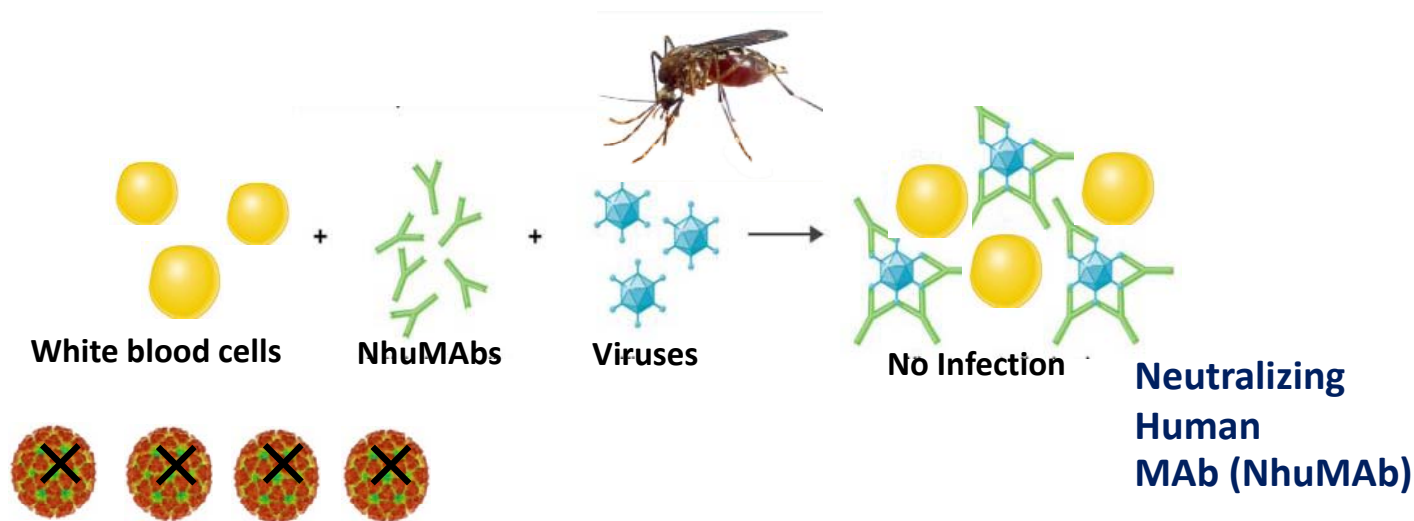


Center of Excellence for Antibody Research

CEAR



Neutralizing human MAb against Dengue virus; towards industrial production and commercialization



90-100% killing activity DENV1-4

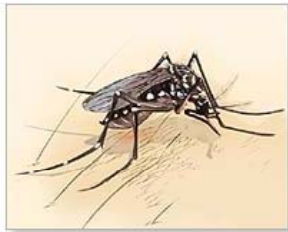


Pongrama Ramasoota

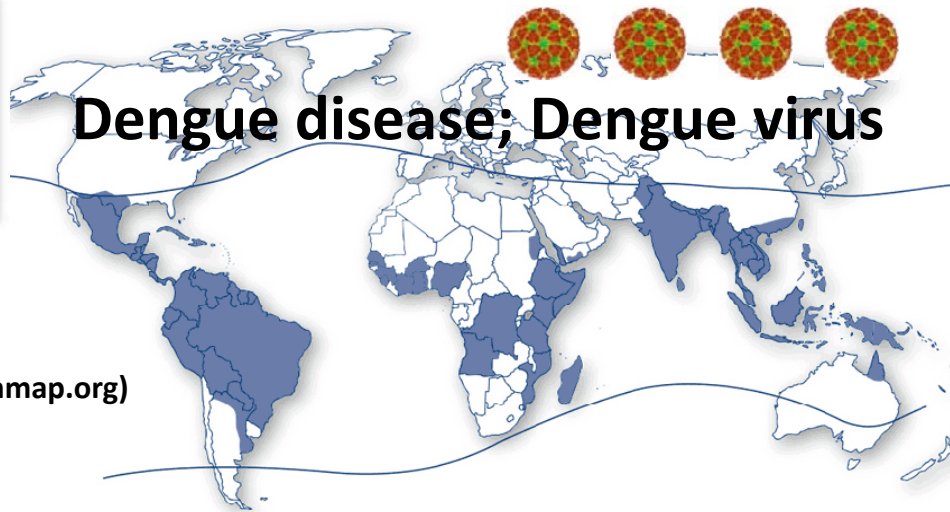
DVM, M.Sc., Ph.D. (Uppsala, Sweden)

Faculty of Tropical Medicine, Mahidol University

Collaborative Professor, Osaka University



Aedes aegypti mosquito



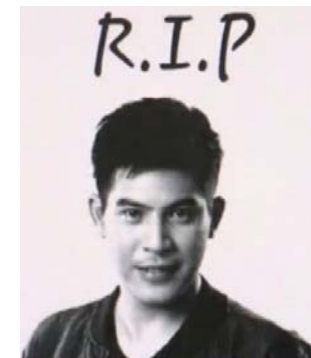
Dengue disease; Dengue virus

(<http://www.healthmap.org>)

■ Countries or areas where dengue has been reported

Note: Lines define the boundaries of year-round survival of the dengue mosquito vector,

Health Organization.



3.9 billion peoples live in dengue endemic countries / year

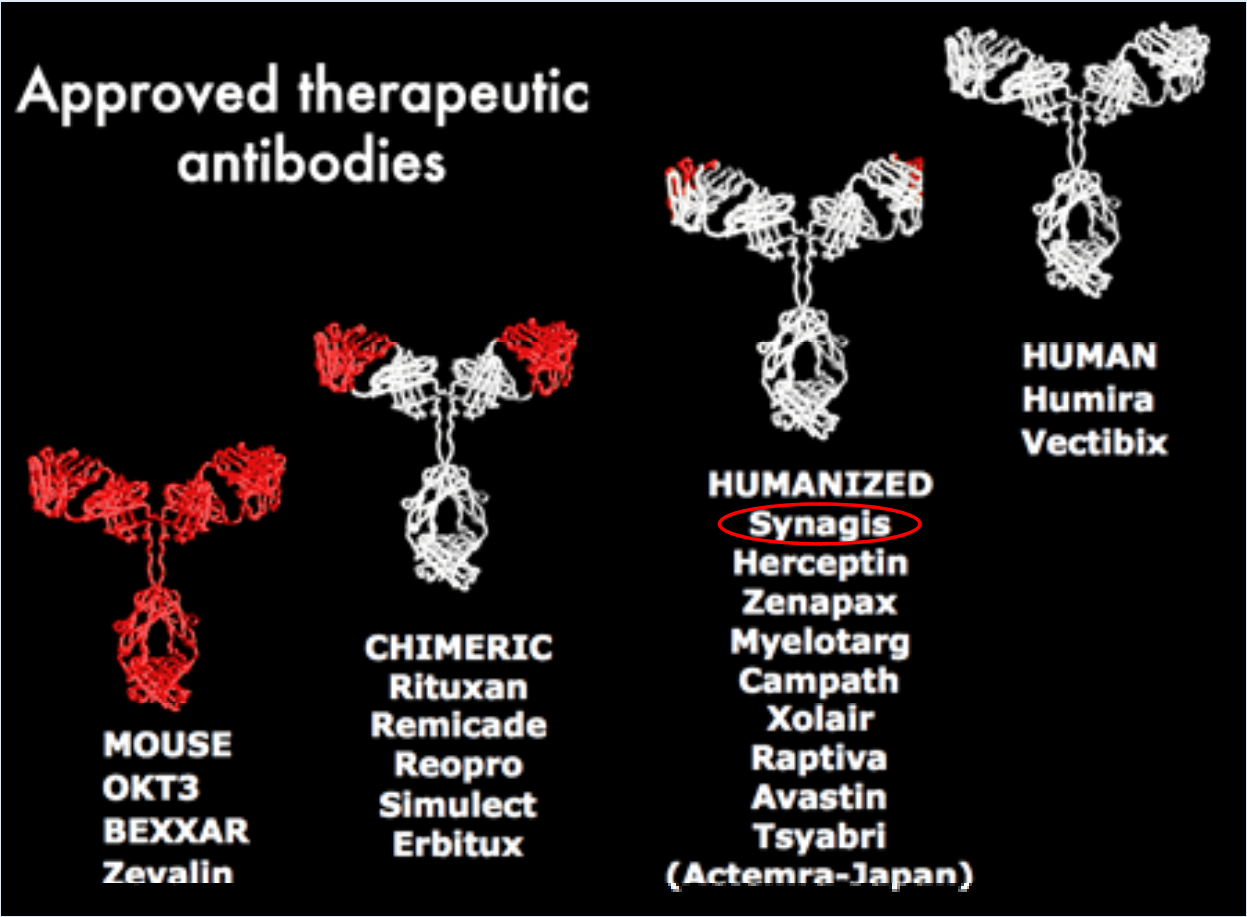
100 million dengue symptomatic cases / year

30,000 cases die from severe dengue / year

No specific drugs available



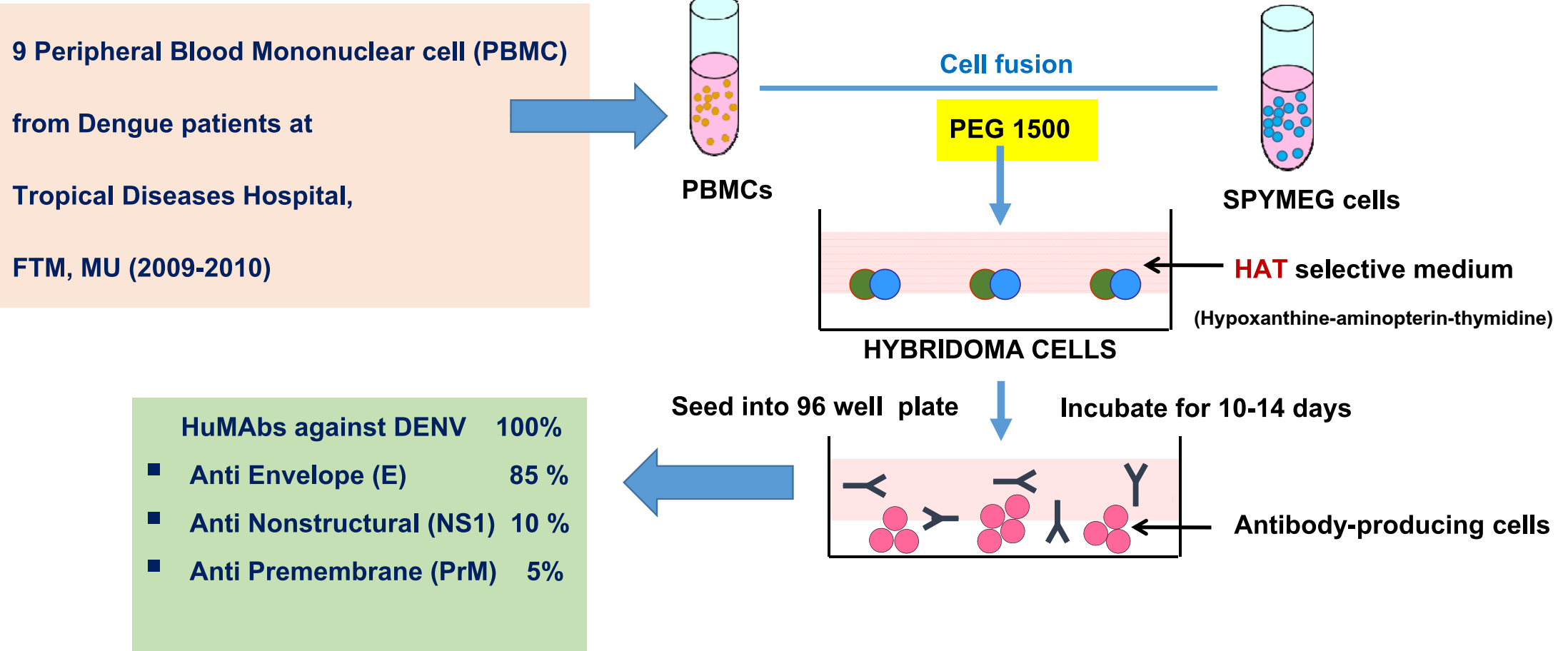
Therapeutic antibody have been increasingly developed for the treatment of Cancer, Immune, viral diseases.



can be alternative DENV treatment (Chan et al, 2013, Fabrienza and Lok, 2015)

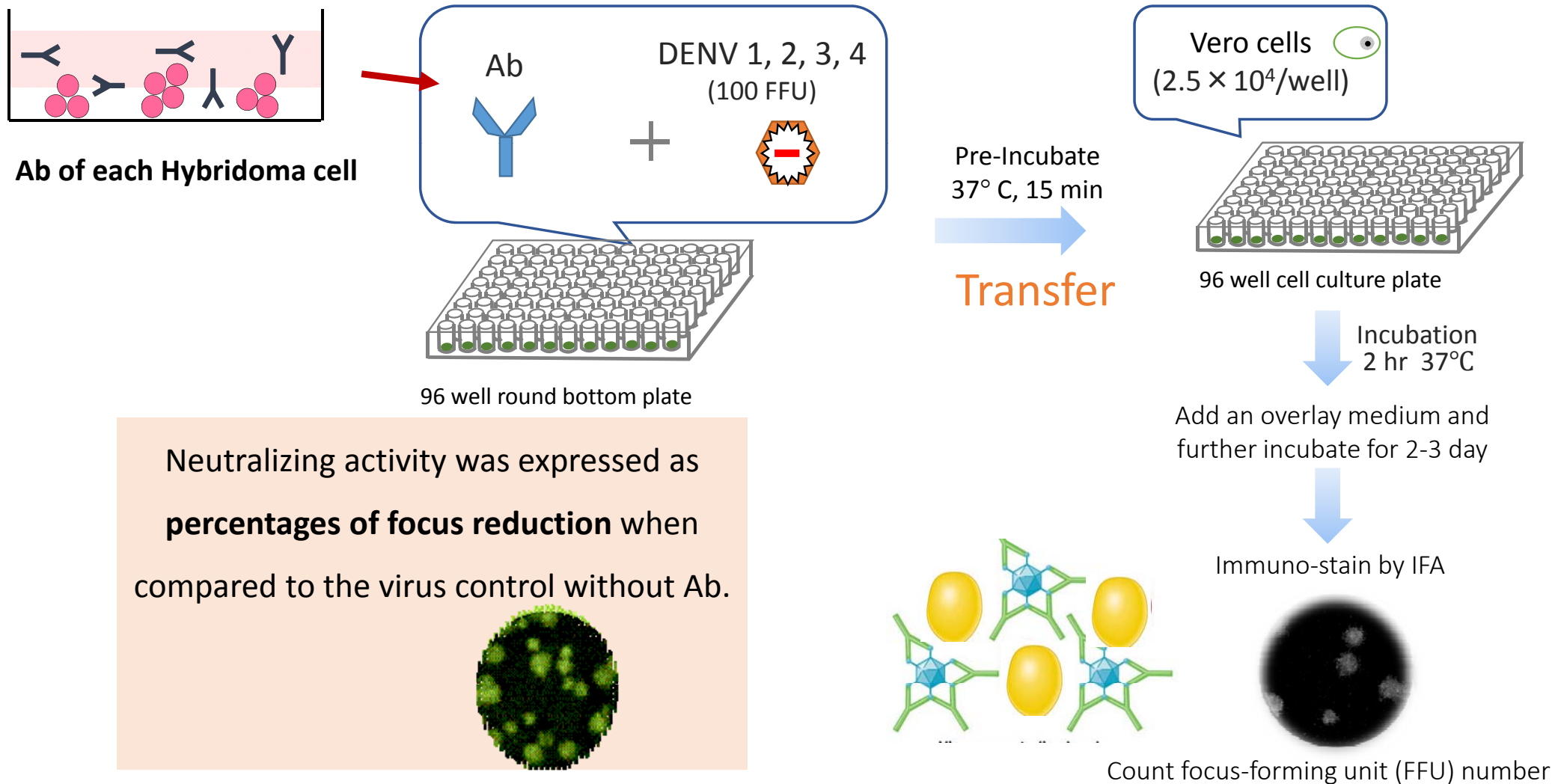
Fully Human monoclonal antibodies (HumAbs) against DENV

(Setthapramote et al, 2012; Sasaki et al, 2013)



All HuMAbs were tested for their Neutralization against 4 serotypes of DENV

Neutralization assay



20 Anti E NhuMAbs with 90-100 % NT

Code	Sample	1st	2nd	DV1	DV2	DV3	DV4	Vero	NT (DV1) Reduction Rate	NT (DV2) Reduction Rate	NT (DV3) Reduction Rate	NT (DV4) Reduction Rate
19	D23-1	B3	B9	1	1	1	1	0	89.7%	100.0%	90.5%	94.3%
54	D23-1	G7	C2	1	1	1	1	0	97.4%	100.0%	89.1%	93.4%
106	D23-4	A6	F9	1	1	1	1	0	87.2%	100.0%	93.4%	90.5%
118	D23-4	D10	E9	1	1	1	1	0	94.9%	100.0%	89.1%	92.4%
131	D23-4	F5	E1	1	1	1	1	0	92.3%	100.0%	89.8%	91.5%
135	D23-4	H12	C8	1	1	1	1	0	87.2%	100.0%	92.0%	87.7%
178	D23-5	G2	D2	1	1	1	1	0	92.3%	100.0%	85.4%	92.4%
411	D30-3	B6	C7	1	1	1	1	0	87.07%	93.31%	85.16%	94.93%
533	D32-2	H8	G1	1	1	1	1	0	86.31%	93.31%	93.64%	94.20%



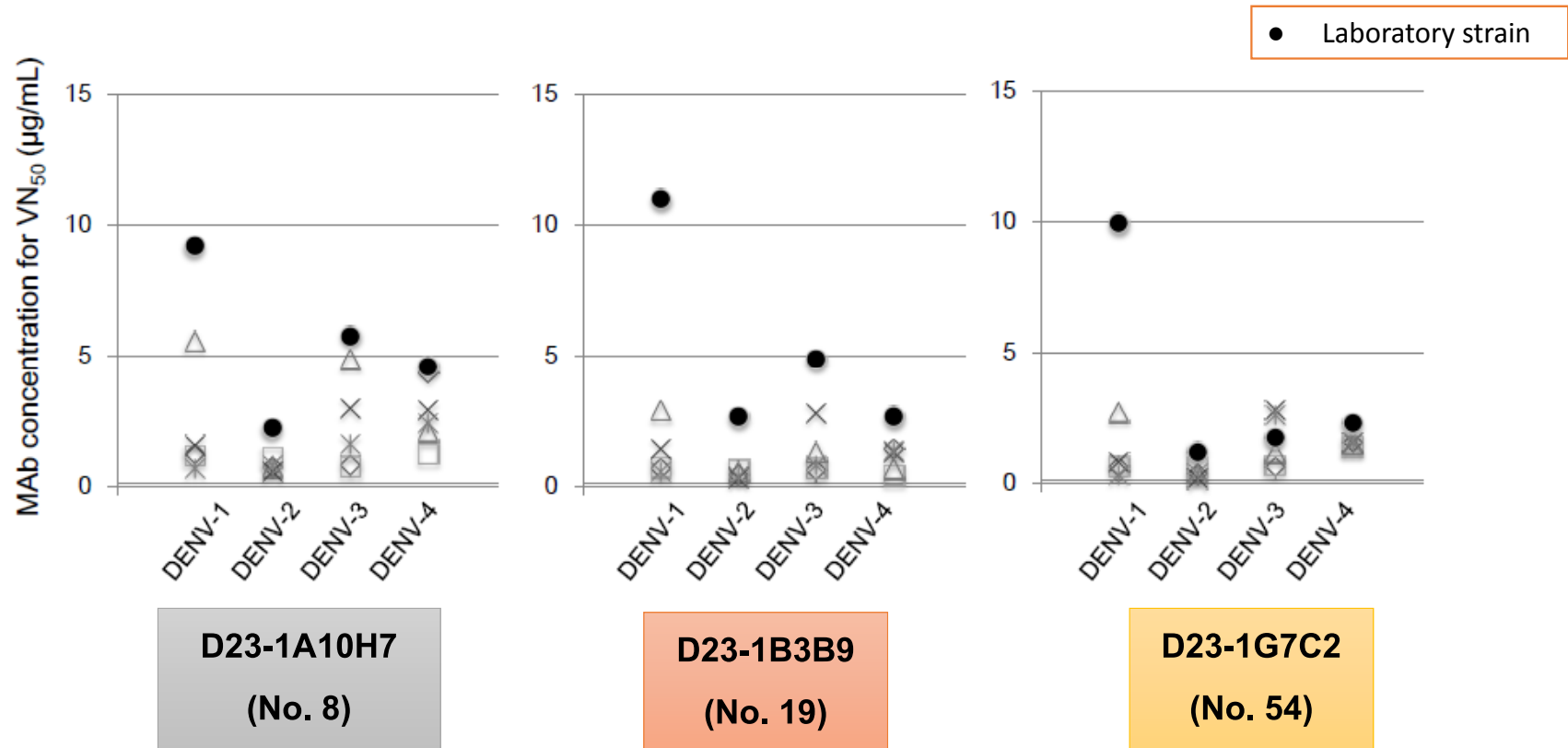
3 best mAbs (# 8, #19, #54) were selected for Nt studies
in vitro (clinical isolates)
 & *in vivo* (suckling mice & Marmoset) (Sasaki et al, 2013)



Clinical isolated DENV for *in vitro* test

Serotype	Our code	Year of Isolation	Titer (FFU/ml)	Serotype	Our code	Year of Isolation	Titer (FFU/ml)
DENV-1	D1-1	2008	4.71×10^6	DENV-3	D3-1	2008	2.83×10^6
	D1-2	2008	3.58×10^6		D3-2	2008	5.90×10^6
	D1-3	2008	7.88×10^5		D3-3	2008	2.99×10^6
	D1-4	2007	1.80×10^6		D3-4	2009	3.40×10^6
	D1-5	2008	1.92×10^5		D3-5	2010	2.40×10^6
DENV-2	D2-1	2008	4.20×10^6	DENV-4	D4-1	2007	9.47×10^4
	D2-2	2008	9.27×10^5		D4-2	2008	2.30×10^4
	D2-3	2008	3.07×10^5		D4-3	2007	3.20×10^3
	D2-4	2008	2.66×10^6		D4-4	2008	1.97×10^6
	D2-5	2009	6.14×10^5		D4-5	2008	3.58×10^4

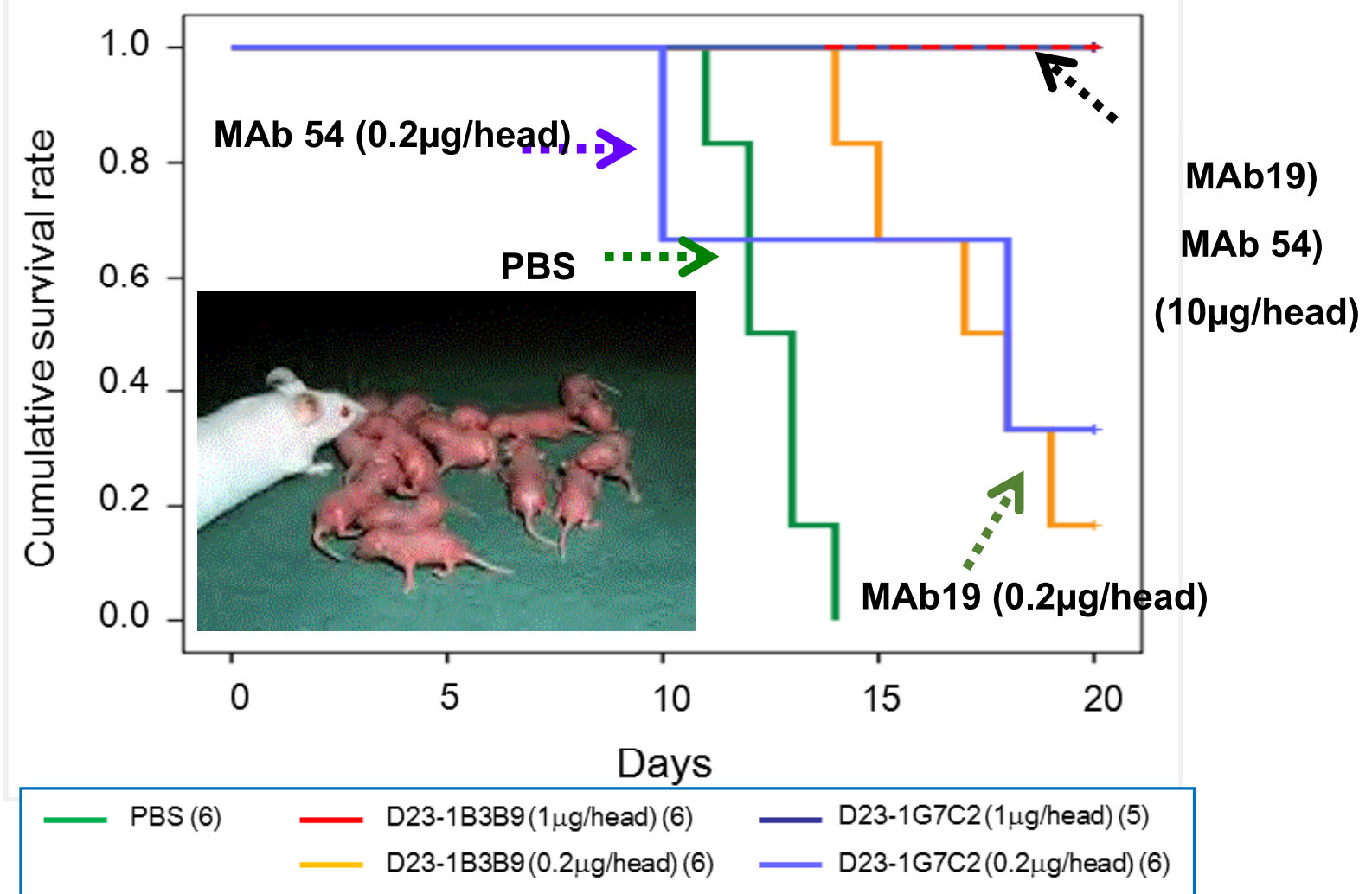
NT activities of 3 candidate NhuMAbs to 20 clinical isolates DENV



Representative HuMAbs, D23-1A10H7, D23-1B3B9, and D23-1G7C2, were examined for the VN50 to a total of 20 clinical isolates (5 isolates of each serotypes isolated from Thai patients between 2007 and 2010) and laboratory strains. The VN50 to the laboratory strains in four serotypes are shown by closed circle.

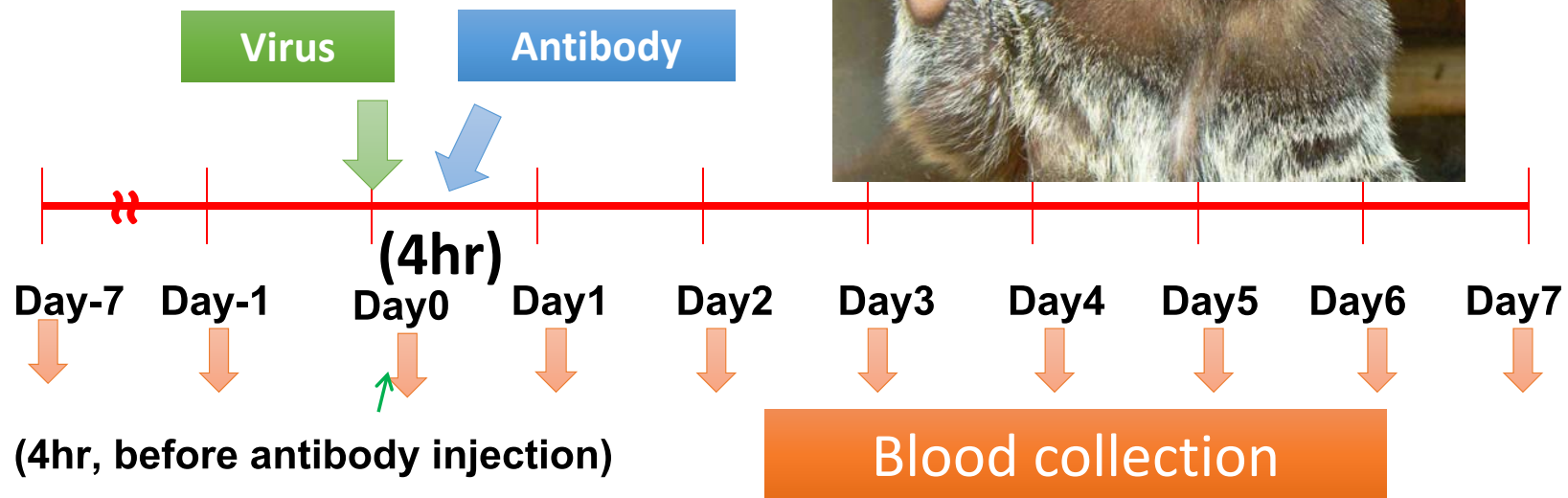
In vivo evaluation of NhuMAbs using suckling mouse (SM)

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

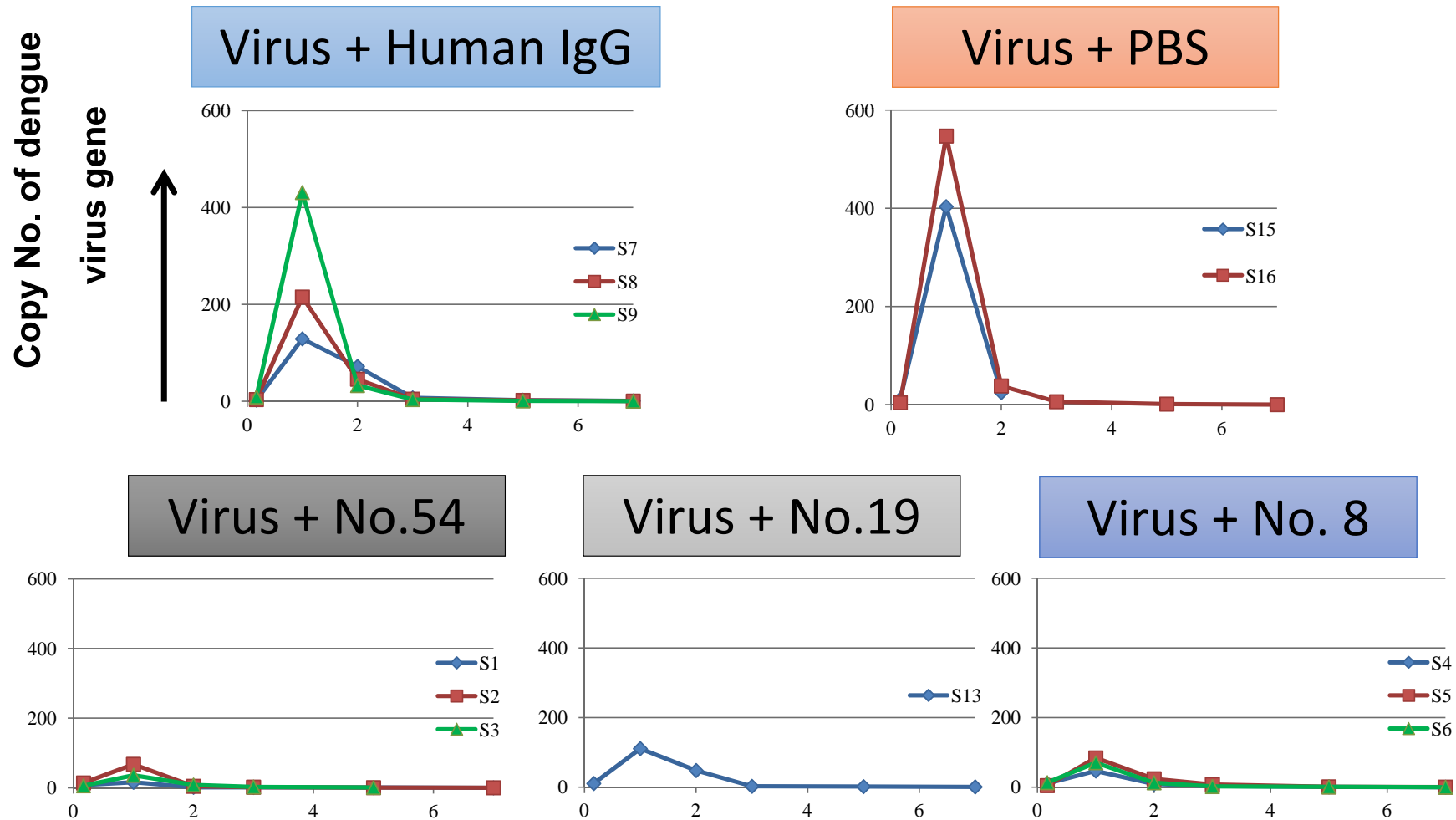


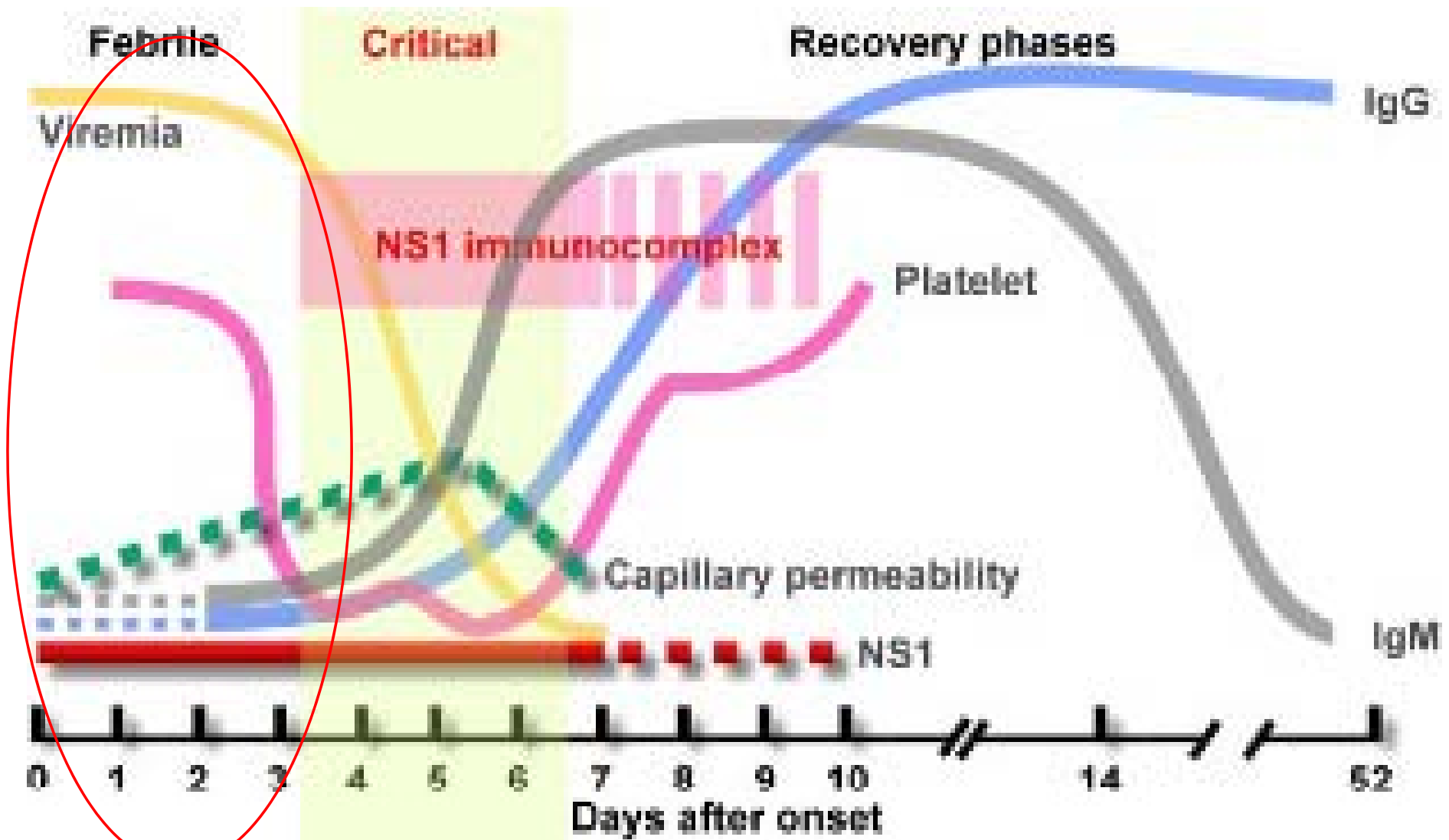
In vivo evaluation test for NhuMAbs using marmosets (Post-treatment)

- **Antibodies:** 20mg/kg (ip) NhuMAb 54, NhuMAb 19, NhuMAb 8 (Human IgG Commercial products, PBS as negative control)
- **Virus:** DENV-2 16681 strain (1.0 x 10⁷ FFU/head (ip))



Results of post-treatment





High DENV viremia in PBMC associated with severe dengue (Srikiatkachorn et al, Plos ONE 2012)



Ramasoota



Patents

English

French

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.

Patented in 11 countries; USA, Australia, Japan, India, Indonesia, Singapore, Malaysia, Philippine, Vietnam, Laos and Thailand

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)

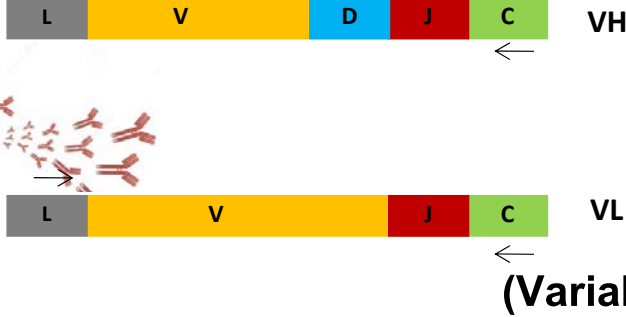
External Links: [Patentscope](#), [Espacenet](#)

Therapeutic NhuMAb to be used in human

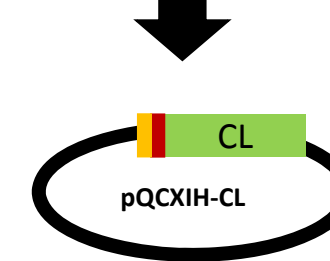
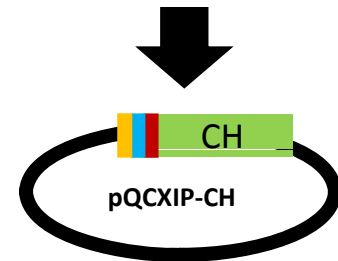


Food and Drug Administration (FDA) accepted Therapeutic MAb expressed from Chinese Hamster Ovary (CHO) cell

Antibody genes amplified by PCR (Variable Heavy)



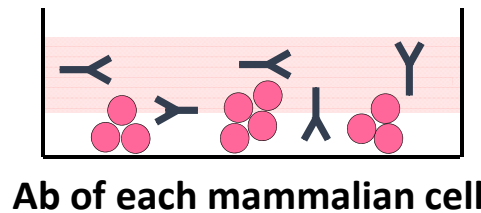
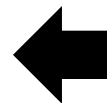
Cloning vector



Mammalian expression vector

Co-transfection

- Reactivity check by IFA
- NT activity compared with those from hybridoma cell



Ab of each mammalian cell



CHO cells

Binding activity of rIgG compared with IgG from hybridoma cells by IFA

Biochemical and Biophysical Research Communications 446 (2014) 475–480

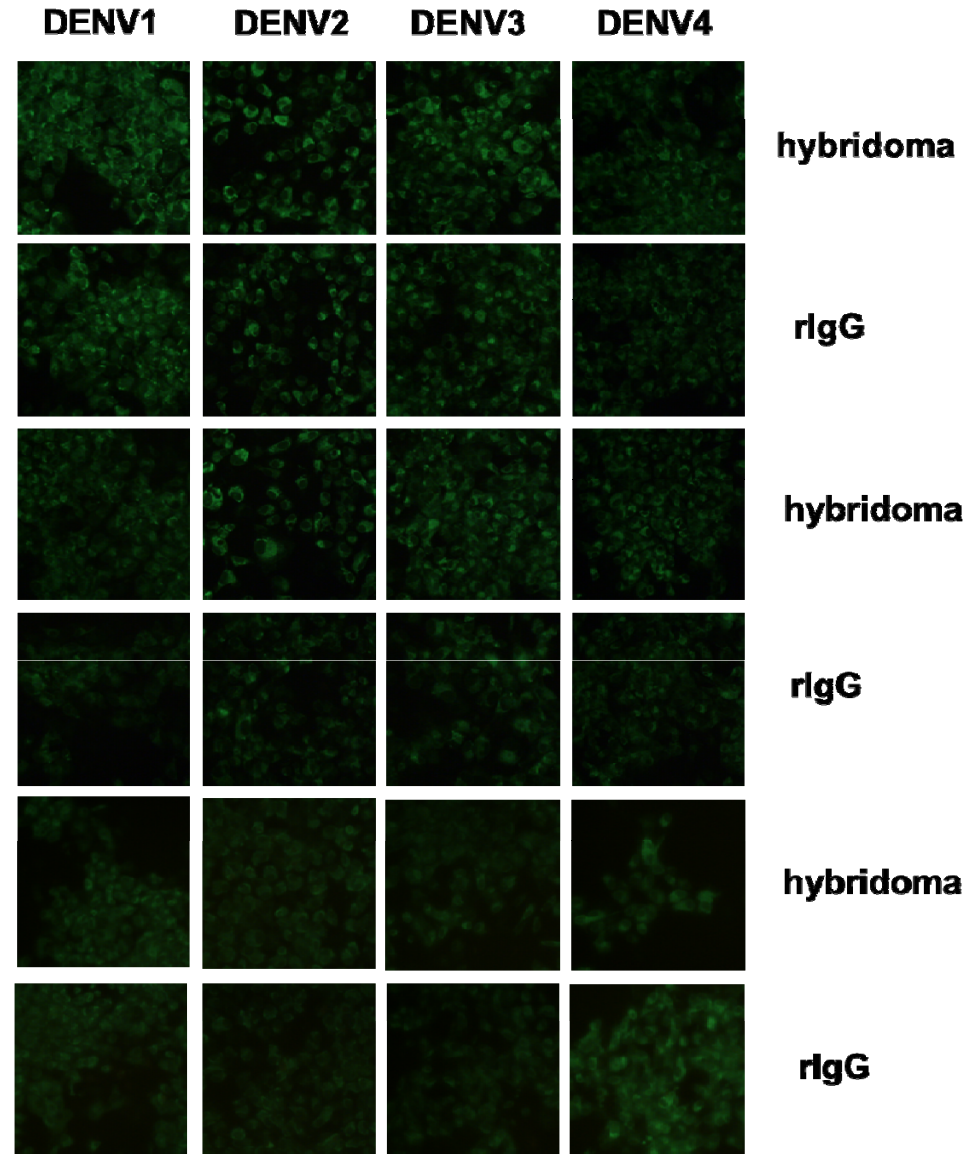
Contents lists available at ScienceDirect

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



D23-1A10H7
(No. 8)



D23-1B3B9
(No. 19)

D23-1G7C2
(No. 54)



CrossMark

Antibody germline characterization of cross-neutralizing human IgGs against 4 serotypes of dengue virus

Pannamthip Pitaksajjakul ^{a,b}, Surachet Benjathummarak ^a, Chonlatip Pipattanaboon ^a, Waranya Wongwit ^b, Tamaki Okabayashi ^{c,e}, Motoki Kuhara ^e, Ryo Misaki ^d, Kazuhito Fujiyama ^d, Pongrama Ramasoota ^{a,b,*}

^aCenter of Excellence for Antibody Research (CEAR), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

^bDepartment of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

^cMahidol Osaka Center for Infectious Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

^dInternational Center for Biotechnology, Osaka University, Japan

^eMedical & Biological Laboratories Corporation, Ltd., Ina, Nagano, Japan

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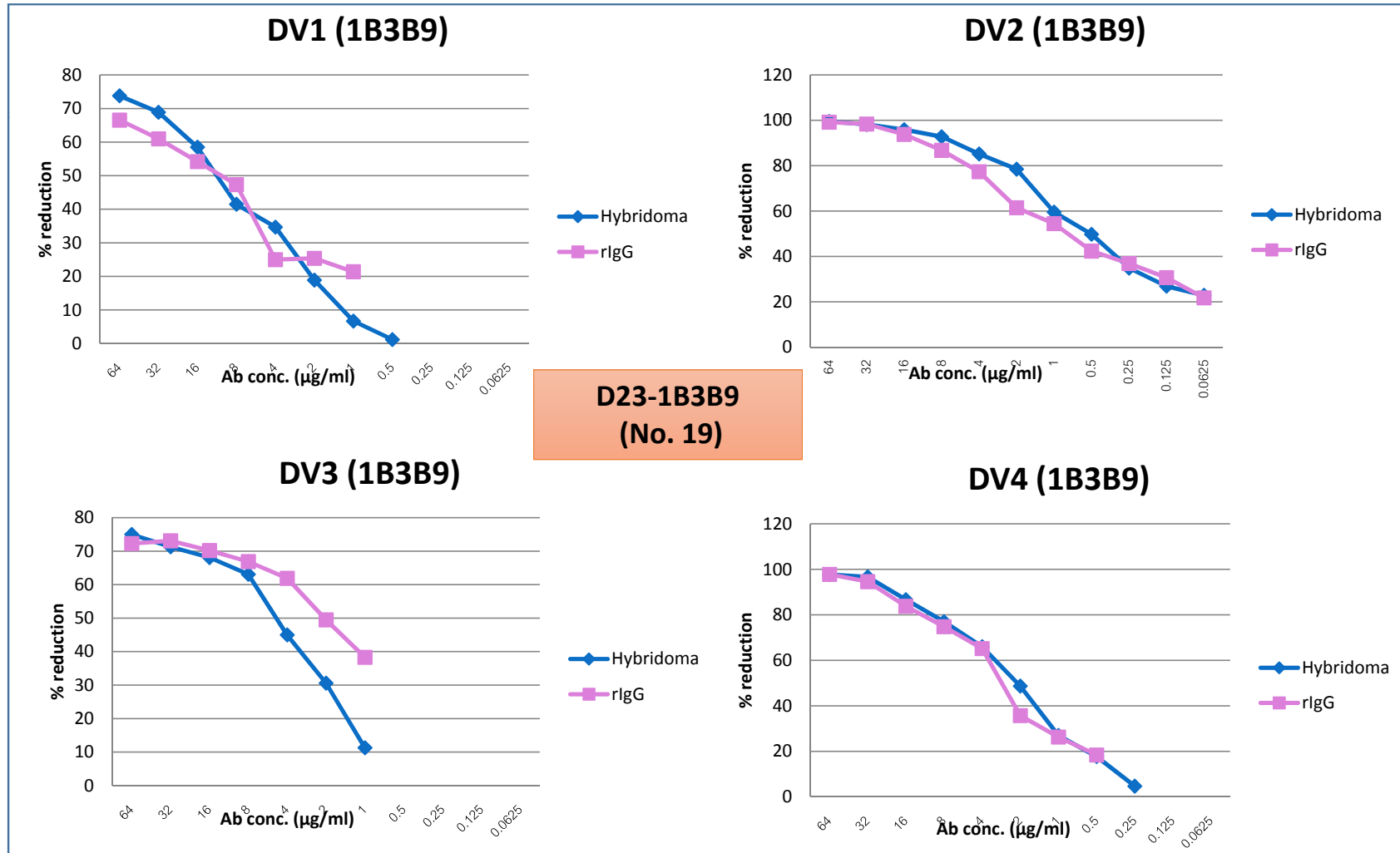
Keywords:
Recombinant IgG
Neutralization
Dengue virus
Cross-neutralizing
Germline

ABSTRACT

Dengue virus (DENV), a re-emerging virus, constitutes the largest vector-borne disease virus, with 50–100 million cases reported every year. Although DENV infection induces lifelong immunity against viruses of the same serotypes, the subsequent infection with the heterologous serotypes can cause more severe form of the disease, such as Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). However, there is neither approved vaccine nor specific drugs available to treat this disease. In this study, previously developed 19 human monoclonal antibodies (HuMAbs) showing strong to moderate cross neutralizing activity were selected. Most of them (13/19) were targeted to domain II of envelop glycoprotein. To understand and clarify the recognition properties, the maturation mechanisms comprising Variable/Diversity/Joining (VDJ) recombination, Variable Heavy (VH)/Variable Light (VL) chain pairing, variability at junctional site, and somatic hypermutation (SHM) of those antibodies were studied and compared with their predecessor germline sequences. IMGT/V-QUEST database was applied to analyze the isolated VH and VL sequences. To confirm the correction of isolated VH/VL, 3 HuMAbs (1A10H7, 1B3B9, 1G7C2) was transiently expressed in HEK293T cell. All three clones of the expressed recombinant IgG (rIgG) showed the same binding and neutralizing activity as same as those from hybridomas. The data obtained in this study will elucidate the properties of those HuMAbs for further genetic modification, and its binding epitopes.

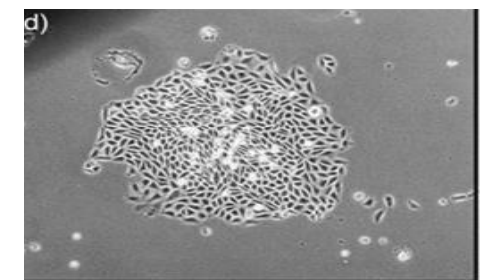
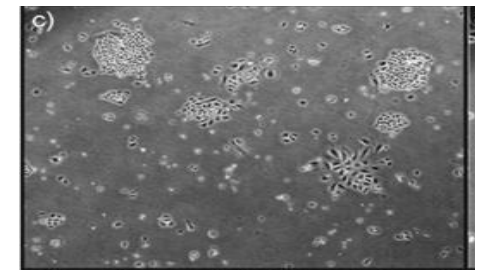
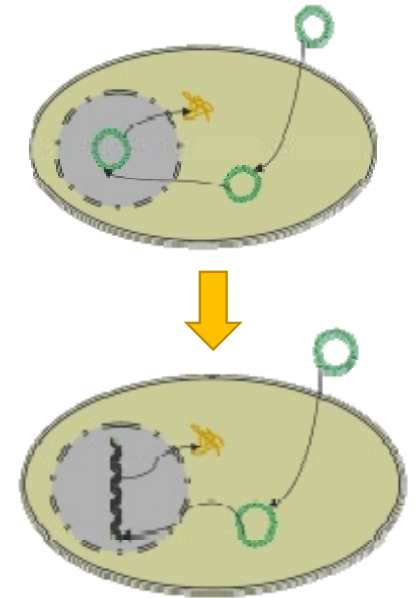
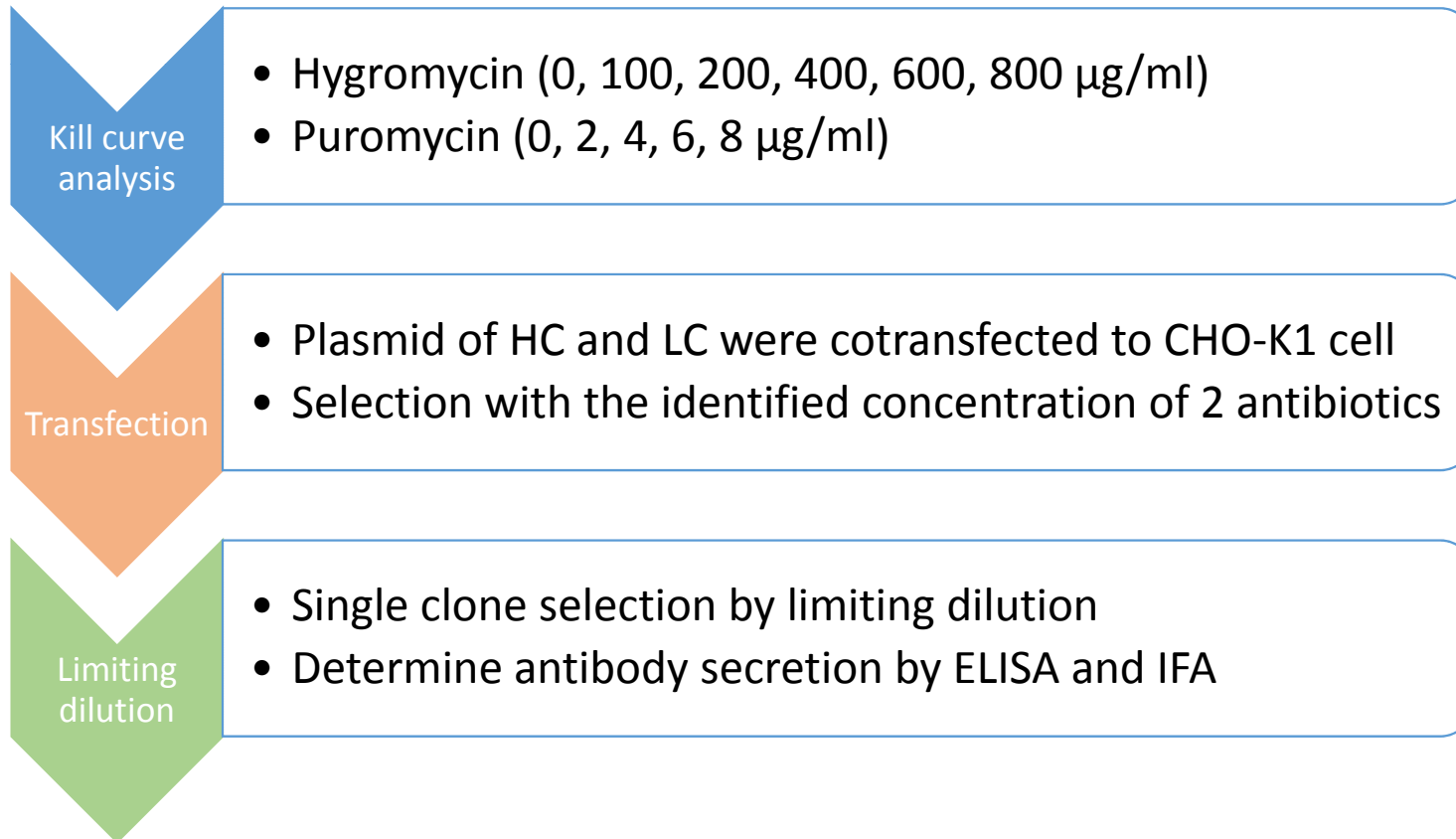
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Focus Reduction Neutralization test of 1 HuMAb rIgG compared with hybridoma-secreting MAb with antibody concentration started at 64 µg/ml



Production of stable expressed CHO cells

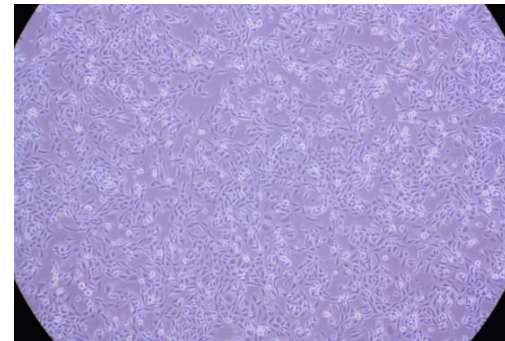
Antibiotics selection system



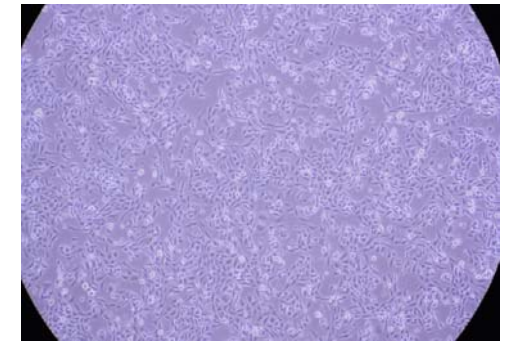
Adaptation to suspension cell, and Serum-free medium

Reducing ratio of serum supplemented medium with serum-free

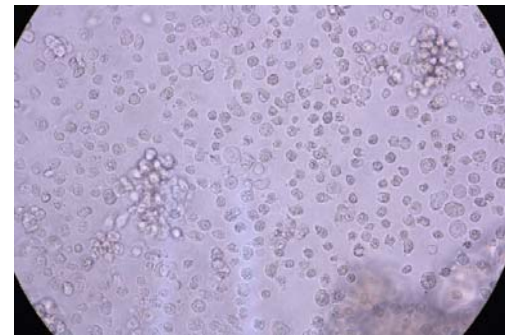
% serum	10% Fetal Bovine serum ratio	Serum-free medium ratio
10	100	0
7.5	75	25
5	50	50
2.5	25	75
1	10	90
0	0	100



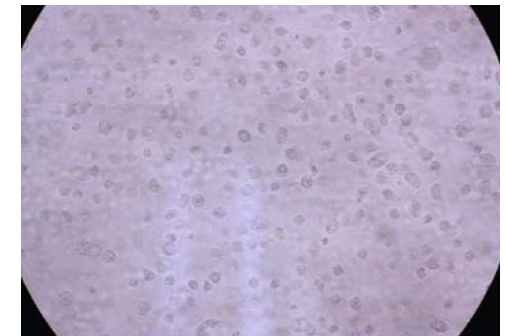
10% serum



7.5% serum

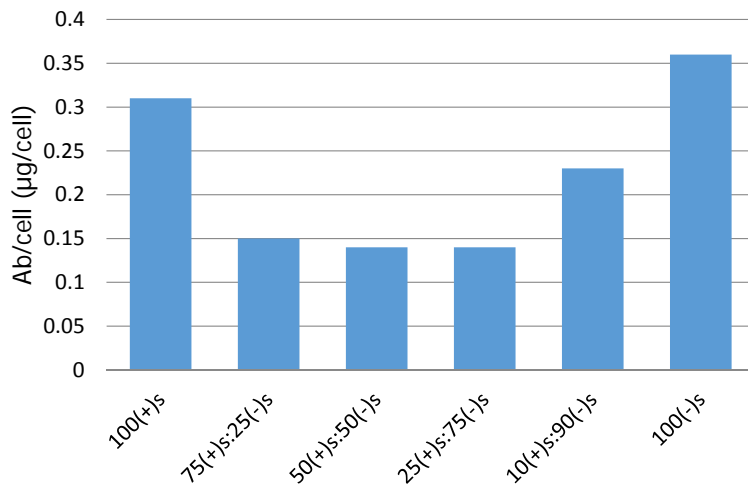


5% serum



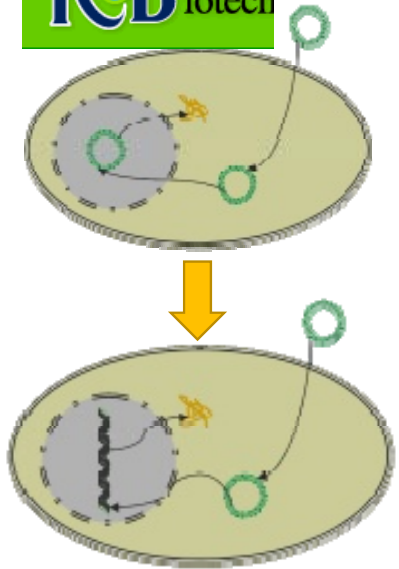
2.5% serum

mAb CHO19#1 stable cell



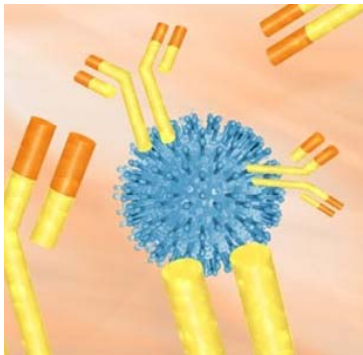
4 mg / Litre

Industrial scale production of NhuMAbs by stable expressed CHO cell

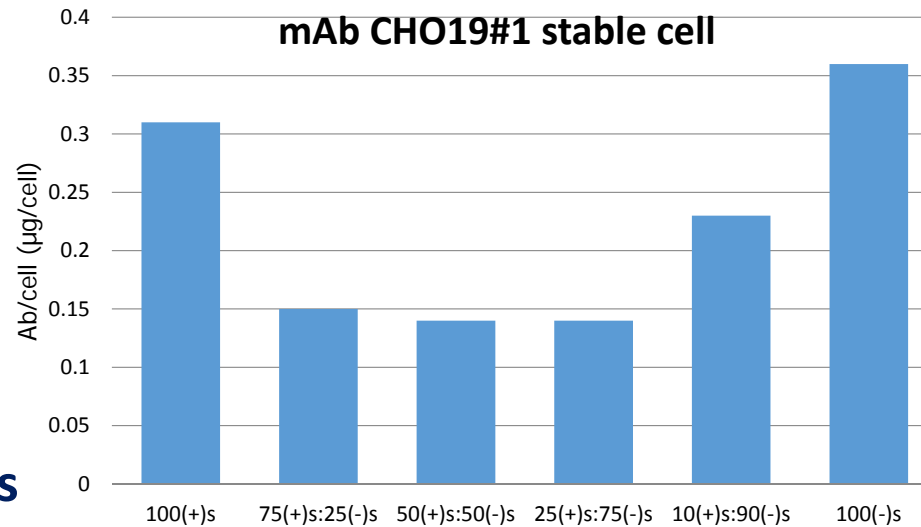


Ready for GMP Production;

- Safety & toxicity test
- Clinical trial Phase 1 - 3



Stable expressed CHO cells
in serum free media



0.36 µg/cell/ 3 days

120 ng/cell/day

> 10 pg/cell/day



5 years NRCT Innovation Awards



National Research Council Of Thailand (NRCT)





2014年台北國際發明暨技術交易展
2014 TAIPEI INT'L INVENTION SHOW & TECHNOMART

Mr. Pongrama RAMASOOTA, Miss Pannamthip Pitaksajjakul and Miss Chonlatip Pipattanaboon

之

Therapeutic human monoclonal antibodies against 4 serotypes of Dengue virus

榮獲「2014年台北國際發明暨技術交易展」—發明競賽

金牌獎

特頒此狀·以茲表揚

發明競賽評審委員會主任委員 張進福
 2014年9月20日於臺北市

This
Gold Medal Award
 is presented to

Mr. Pongrama RAMASOOTA, Miss Pannamthip Pitaksajjakul and Miss Chonlatip Pipattanaboon

In recognition for the invention of

Therapeutic human monoclonal antibodies against 4 serotypes of Dengue virus

2014 Taipei International Invention Show & Technomart
 Invention Contest

J. Chang
 Award Committee Chair
 September 20, 2014, Taipei City

B0431



**Gold Medal
 Award**



2014 Taipei Int'l
 Invention Show & Technomart

Honor of Invention

Presented to

Mahidol University

Therapeutic Human Monoclonal Antibodies against 4 Serotypes of Dengue Virus

in recognition of

Mr. Pongrama Ramasoota, Ms. Pannamthip Pitaksajjakul, and Ms. Chonlatip Pipattanaboon

exhibited at

Taipei Int'l Invention Show & Technomart

Taipei, Taiwan

18-21 September, 2014



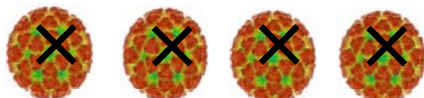
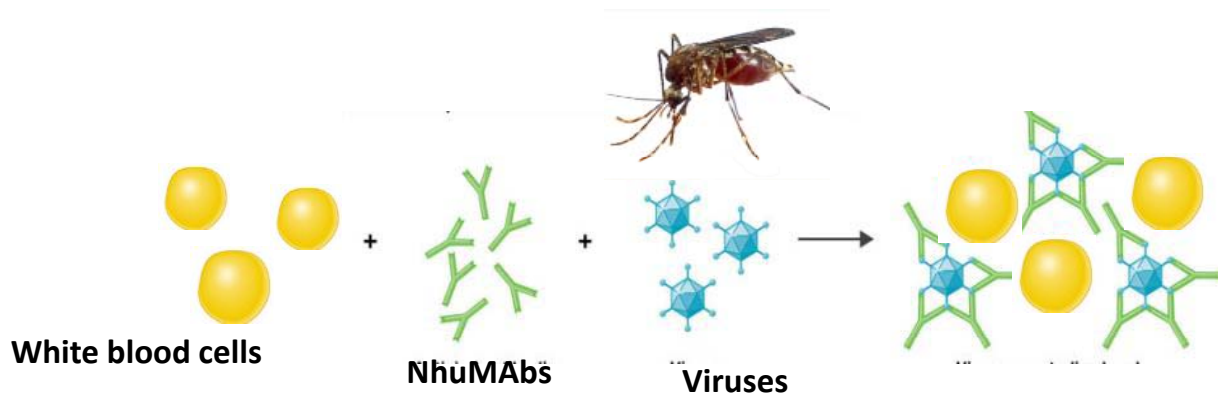
Hsieh Hsin-Ming

Hsieh, Hsin-Ming
 President

World Invention Intellectual Property Associations



Neutralizing human MAb (NhuMAb) against Dengue virus



90-100% neutralizing against DENV1-4



Pre-clinical tested in mice and monkey

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, Tadahi 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

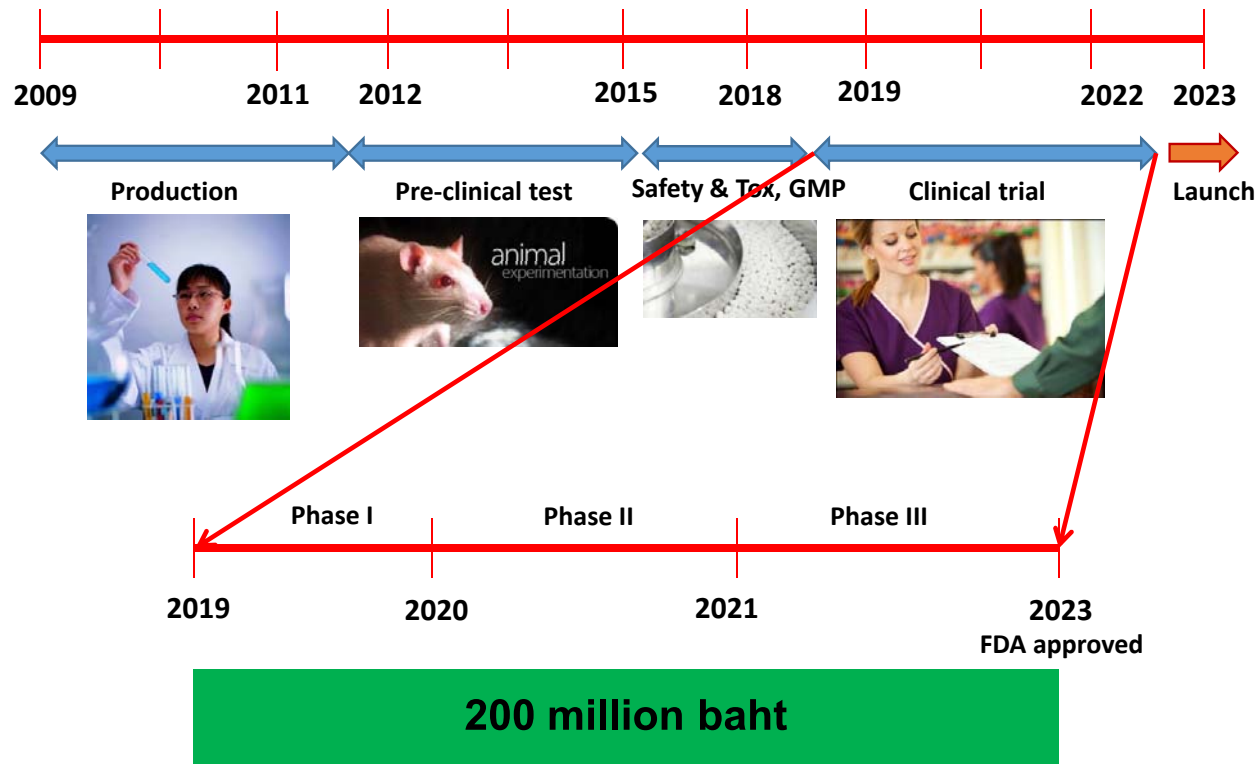
Dengue-virus serotype neutralizing antibodies
WO 2013035345 A3

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, Tadahi Sasaki, Mitsuhiro Nishimura, Takeshi Kurosu, Itaru HIRAI, Akifumi Yamashita, Shota Nakamura, Norihito Kawashita, Chonlatip PIPATTANABOON, Pannamthip PITAKSAJAKUL, 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 «

Antigenic peptide derived from dengue virus
WO 2014064943 A1



Milestones



<http://aspe.hhs.gov/report/examination-clinical-trial-costs-and-barriers-drug-development>

Dr. John Kaundinya, President, BSV BioSciences Inc. signed MTA to produce & test our NhuMAbs before further licensing & clinical trials.



Bharat Serums & Vaccines Ltd.



John Kaundinya PhD
President & Chief Operating Officer

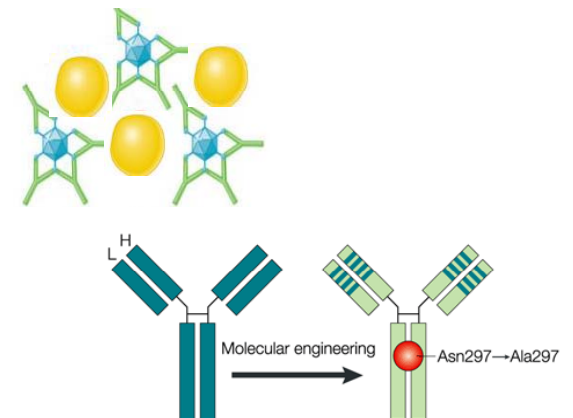
380 Woodview Avenue
Morgan Hill CA 95037
USA

Phone : 408-722-1776

Email : john.k@bsvbio.com

NhuMAbs;

- 1. Anti E clone 19**
- 2. Anti E clone 54**
- 3. Fc modified 19**
- 4. Fc modified 54**



Thank you

