

SITE 1 protease (S1P) inhibitor, PF-429242 suppress dengue virus propagation *in vitro*

OLeo Uchida^{1, 3}, Shuzo Urata², Gianne Eduard L. Ulanday³, Yuki Takamatsu³, Jiro Yasuda²,
Kouichi Morita³, Daisuke Hayasaka³

¹Laboratory of Zoonotic Diseases, School of Veterinary Medicine, Rakuno Gakuen University

²Department of Emerging Infectious Disease, Institute of Tropical Medicine, Nagasaki University

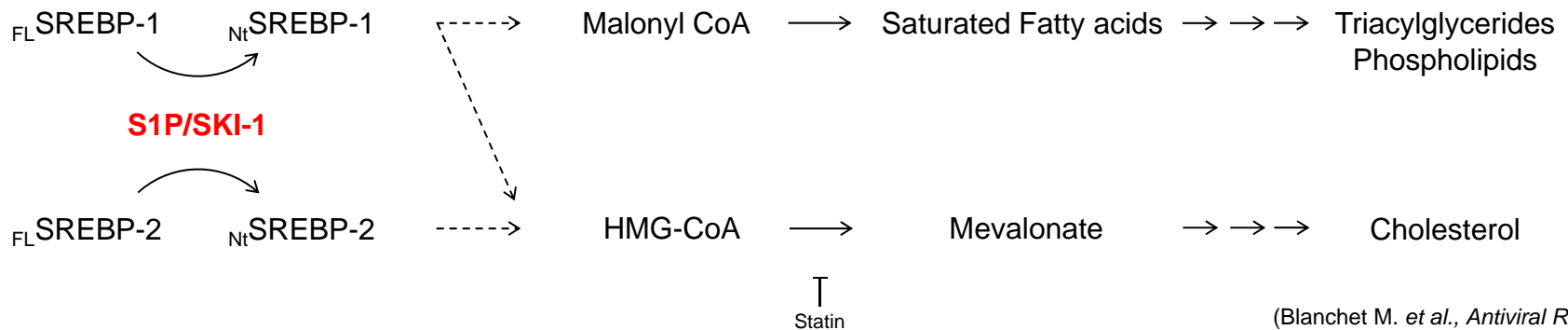
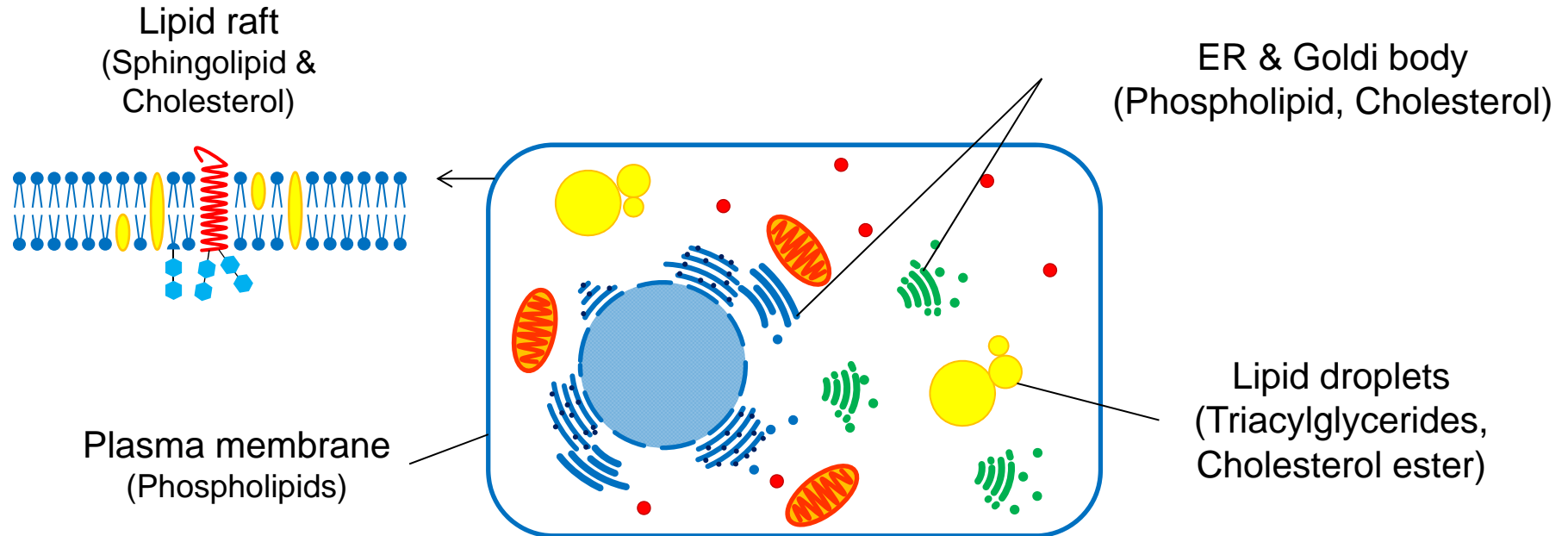
³Department of Virology, Institute of Tropical Medicine, Nagasaki University

Joint International Tropical Medicine Meeting 2017

Bangkok, Thailand

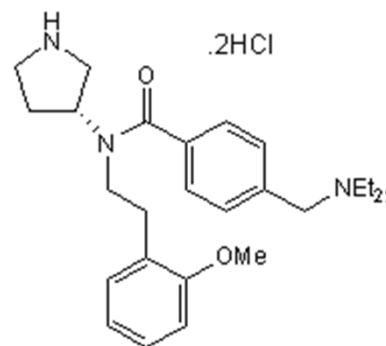
6 December 2017

Cellular lipid metabolism by SREBPs (Sterol regulatory element-binding protein)



(Blanchet M. *et al.*, *Antiviral Res.* 2012)

S1P inhibitor, PF-429242 suppresses cellular lipid levels



PF-429242

(4-[(Diethylamino)methyl]-N-[2-(2-methoxyphenyl)ethyl]-N-(3R)-3-pyrrolidinylbenzamide)

Small compound PF-429242 was developed as a **hypolipidemic** agent based on high throughput screening in a Pfizer compound library. PF-429242 inhibits the activity of **S1P** reversibly and competitively and suppresses the expression level of SREBP target genes, consequently decreasing **cellular lipid levels**.

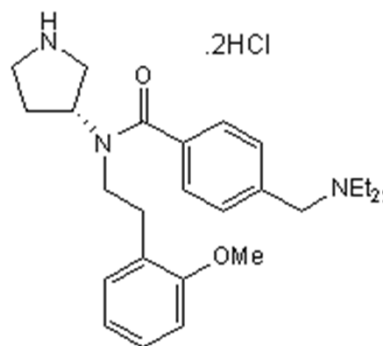
TOCRIS Bioscience
(<http://www.tocris.com/dispprod.php?ItemId=220431#.U2oXYnau4dU>)

Anti viral effects of PF-429242

<i>Arenaviridae</i>	Lassa virus	Urata S. <i>et al.</i> , <i>J Virol.</i> 2011
	Lymphocytic choriomeningitis virus	
<i>Flaviviridae</i>	Hepatitis C virus	Blanchet M. <i>et al.</i> , <i>Antiviral Res.</i> 2012
		Olmstead A. D. <i>et al.</i> , <i>PLoS Pathog.</i> 2012

S1P inhibitor, PF-429242 suppresses cellular lipid levels

TOCRIS
bioscience



PF-429242

(4-[(Diethylamino)methyl]-N-[2-(2-methoxyphenyl)ethyl]-N-(3R)-3-pyrrolidinylbenzamide)

Small compound PF-429242 was developed as a **hypolipidemic** agent based on high throughput screening in a Pfizer compound library. PF-429242 inhibits the activity of **S1P** reversibly and competitively and suppresses the expression level of SREBP target genes, consequently decreasing **cellular lipid levels**.

TOCRIS Bioscience
(<http://www.tocris.com/dispprod.php?ItemId=220431#.U2oXYnau4dU>)

Aims of this study

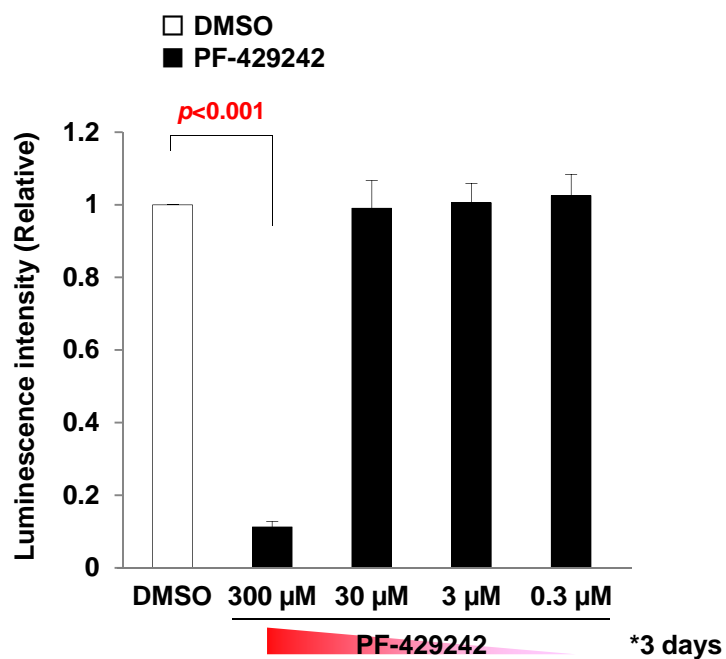
- ❑ To evaluate the **anti-DENV** effects of PF-429242 using all **four serotypes** of DENV by several **primate-derived** cell lines.
- ❑ To assess the emergence of **drug-resistant** DENV mutants.
- ❑ To evaluate the DENV **dependency on intracellular lipids** during their infection.

DENV suppression via the addition of PF-429242

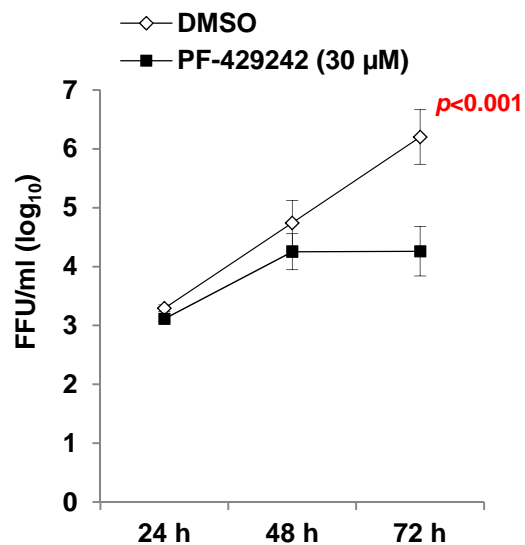
Cell viability

DENV2 strain 16681 titer

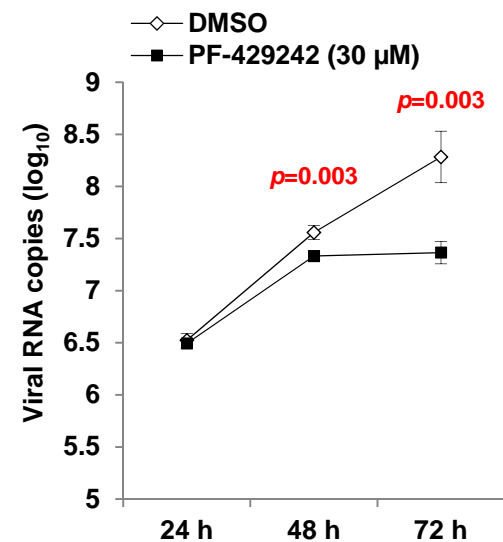
Intracellular ATP level



Focus forming assay



RT-qPCR



HeLa cell, 3 days post drug treatment

Error bars: Standard error

Statistical analysis: Arcsine transform, One-sample

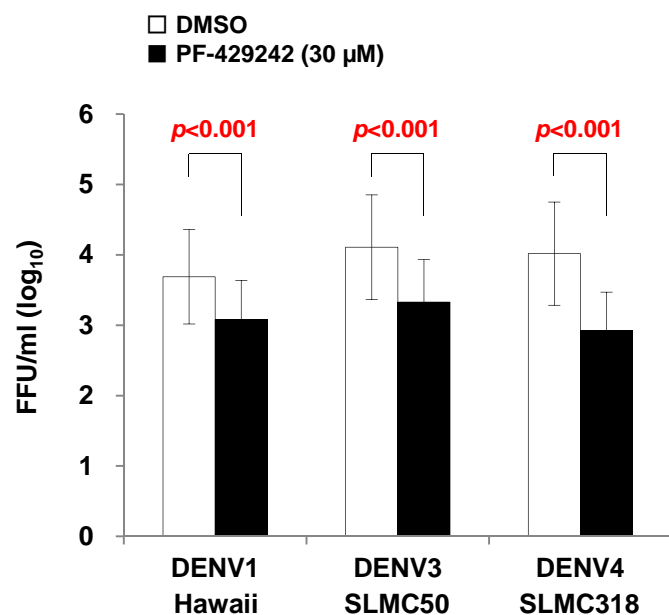
Kolmogorov-Smirnov test, Welch Two Sample t-test

(Uchida L. *et al. Viruses* 2016)

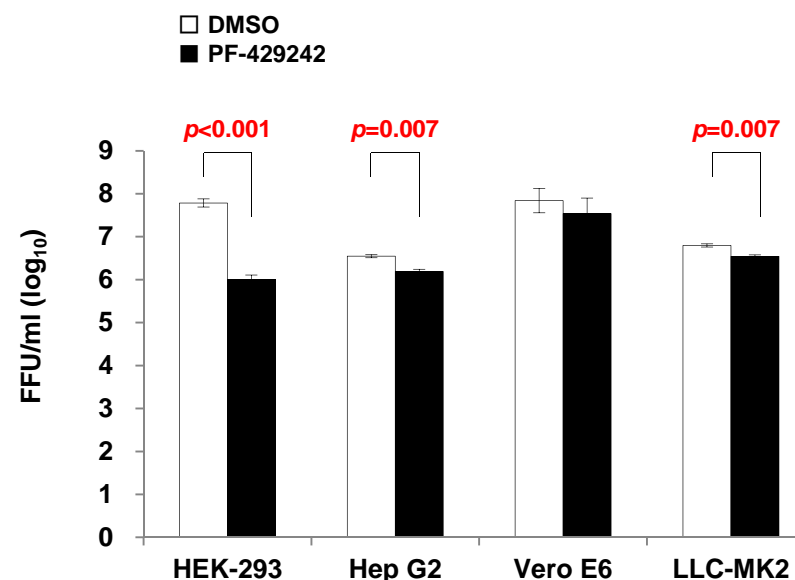
DENV suppression via the addition of PF-429242

Dengue virus titer in culture fluid

Focus forming assay

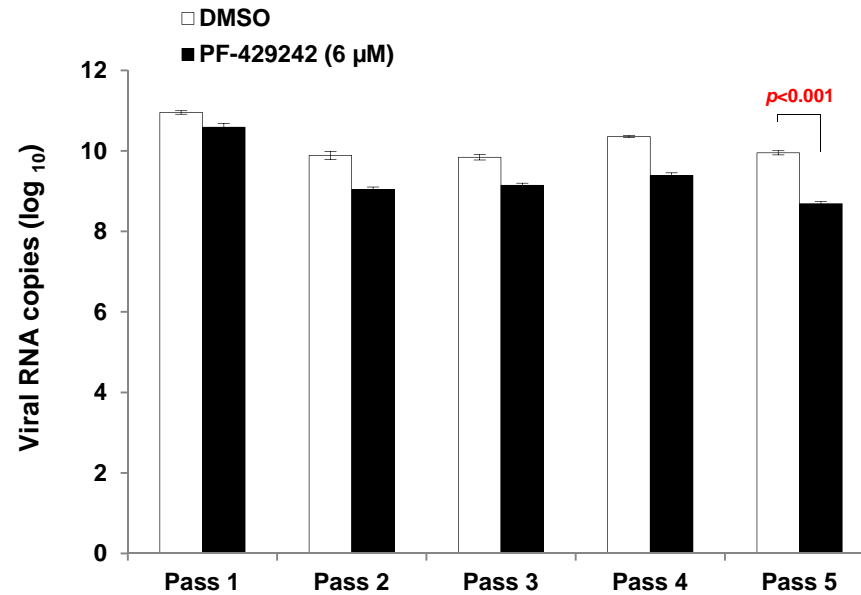


HeLa cell, 3 days p.i.
Error bars: Standard error
Statistical analysis: Arcsine transform, One-sample Kolmogorov-Smirnov test, Welch Two Sample t-test

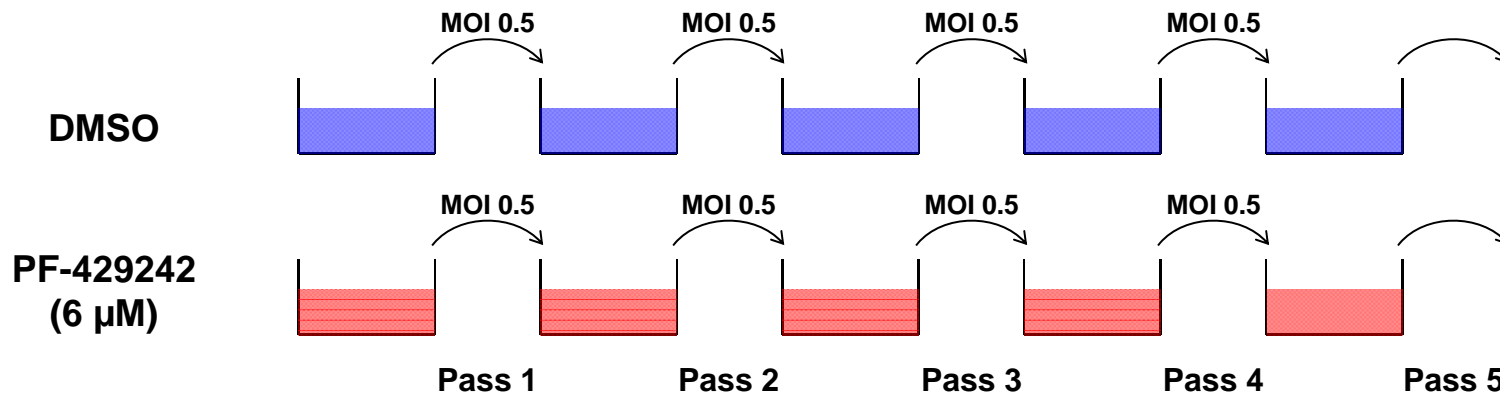


HEK-293 cell: PF-429242 (6 µM), 3 days p.i.
Hep G2 cell: PF-429242 (30 µM), 3 days p.i.
Vero E6 cell: PF-429242 (60 µM), 4 days p.i.
LLC-MK2 cell: PF-429242 (5 µM), 4 days p.i.
Error bars: Standard error
Statistical analysis: Arcsine transform, One-sample Kolmogorov-Smirnov test, Welch Two Sample t-test

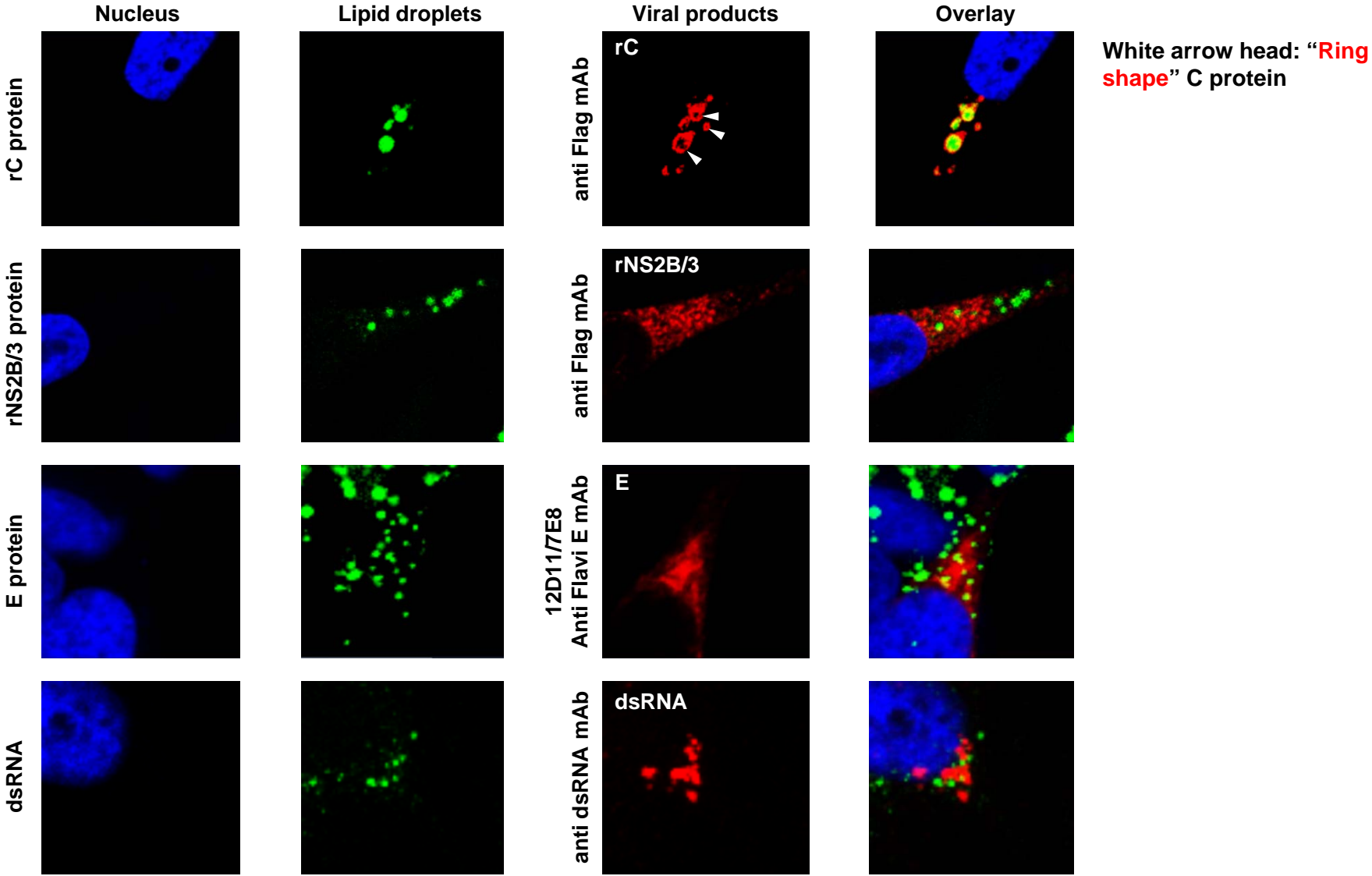
Emergence of drug-resistant mutants



*HEK293 cell, 3 days p.i.
Error bars: Standard error
Statistical analysis: Arcsine transform, One-sample Kolmogorov-Smirnov test, Welch Two Sample t-test



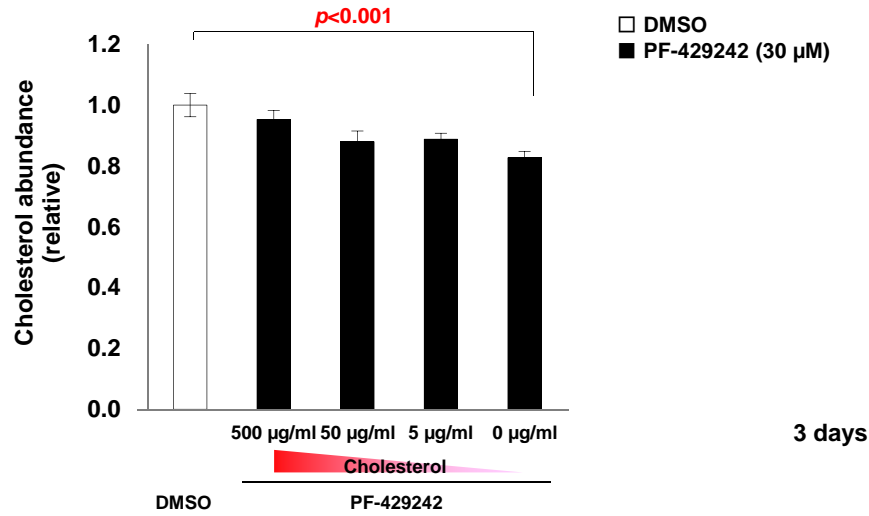
DENV C protein accumulated around Lipid droplets



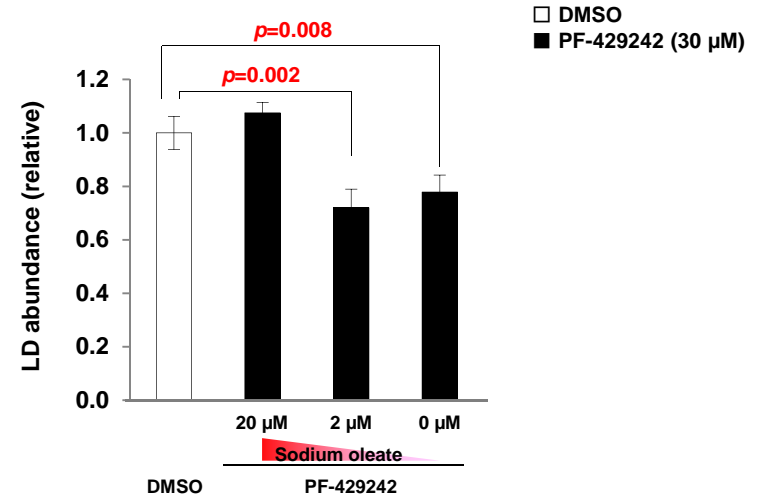
*HeLa cell, 48 h p.i. or p.t.

Exogenous lipids effects on DENV infection

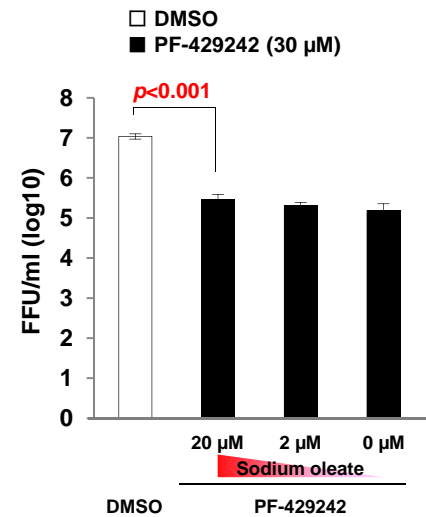
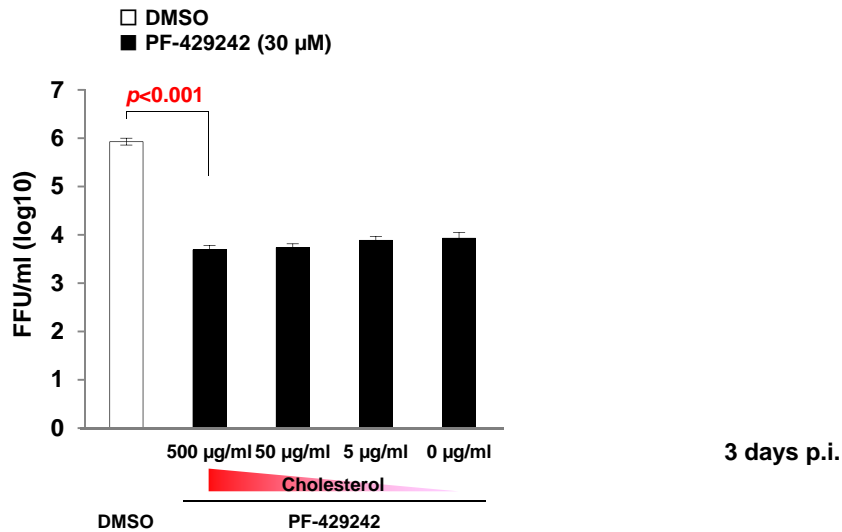
Intracellular cholesterol



Intracellular Lipid droplets



Dengue virus titer in culture fluid



*HeLa cell
Error bars: Standard error
Statistical analysis: Arcsine transform, One-sample Kolmogorov-Smirnov test, Welch Two Sample t-test

Conclusion

1. PF-429242 suppressed **four serotypes** of DENV1-4 in HeLa cells.
2. The inhibitory effect of PF-429242 was observed in **several primate-derived cell lines** infected with DENV2.
3. Drug-resistant DENV mutants **did not appear** to emerge after 5 passages.
4. DENV propagation was not restored completely by addition of exogenous fatty acids and cholesterol.

Arenavirus Suppression of cleavage of viral glycoprotein by S1P/SKI-1¹⁾

HCV Depletion of lipid droplets and LDL receptor²⁾

1) Urata S. *et al.*, *J Virol.* 2011

2) Olmstead A. D. *et al.*, *PLoS Pathog.* 2012

S1P/SKI-1 inhibitor, such as PF-429242 might be an attractive drug candidate for DENV infection.

Acknowledgement

This work was supported by a Health and Labor Sciences Research Grant on Emerging and Re-emerging Infectious Diseases from the Japanese Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants (Grants in aid H-25 Shinko-Wakate-003), KAKENHI [Grant-in-Aid for Scientific Research (B) (25304045)], AMED and J-GRID.

Department of Virology, Institute of Tropical Medicine, Nagasaki University

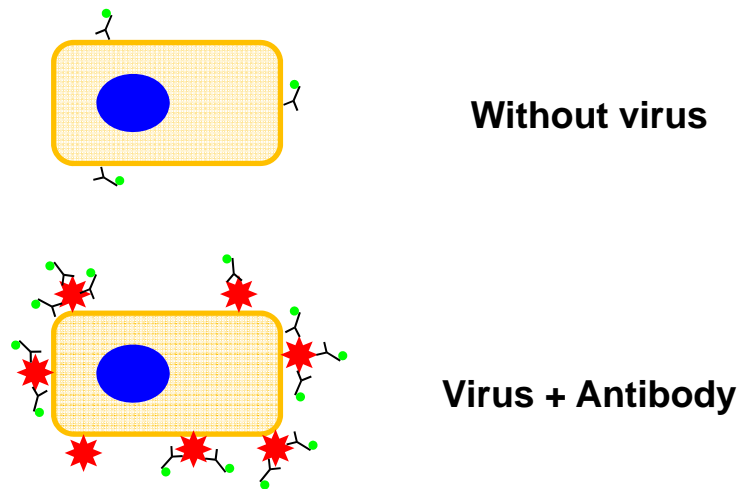
- Dr. Masanobu Agoh
- Dr. Mya Myat Ngwe Tun
- Dr. Guillermo Posadas-Herrera
- Mr. Kotaro Aoki

Department of Emerging Infectious Disease, Institute of Tropical Medicine, Nagasaki University

- Dr. Youhei Kurosaki

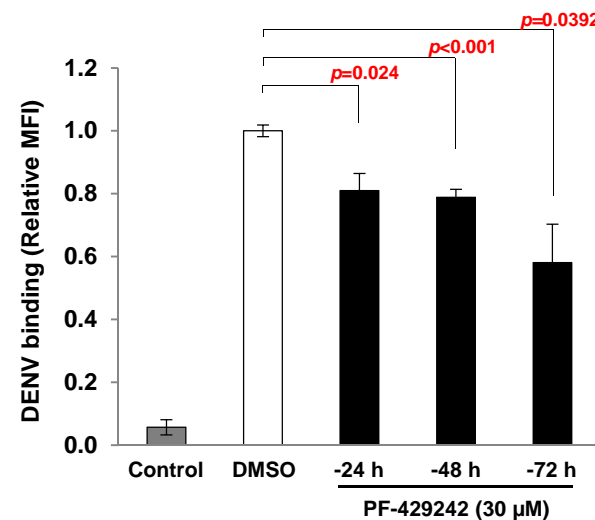
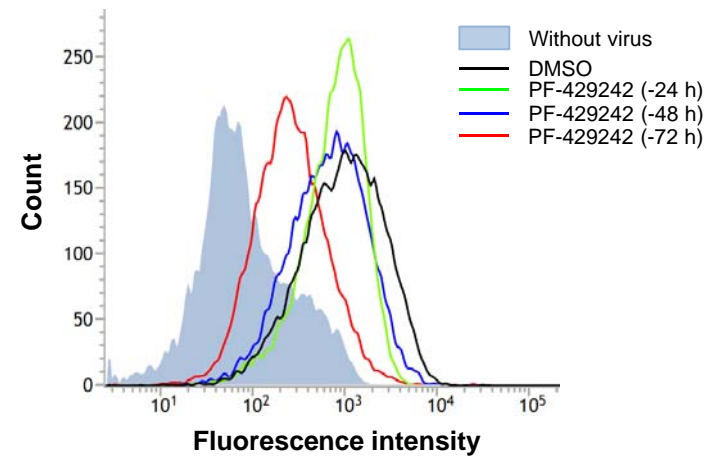
PF-429242 suppressed DENV-cell binding

DENV binding assay



DENV binding capacity

Flow cytometry



*HeLa cell
 Error bars: Standard error
 Statistical analysis: Arcsine transform, One-sample Kolmogorov-Smirnov test, Welch Two Sample t-test