

# Antiviral therapy, a lot has been achieved, yet far to go

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[www.antivirals.be](http://www.antivirals.be) & [www.facebook.com/NeytsLab](https://www.facebook.com/NeytsLab)

KU LEUVEN



GVN Annual Meeting, Annecy,  
France, November 29<sup>th</sup>, 2018



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# What do we have today ....

## Herpesvirus



## HBV



## HIV



## HCV



## What do we not have today ...?

Antivirals against paramyxo, entero/rhino, arena, bunya, corona, flavi, alpha, noro, rabies, filo, HEV....

## Influenza





### The Neyts-lab of Virology, Antiviral Drug & Vaccine Research

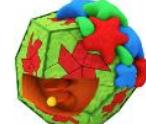
Our Mission : The development of (i) antiviral strategies against a range of (RNA) viruses and (ii) a game-changing novel vaccine technology platform



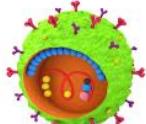
[Flaviviridae](#)



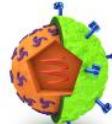
[Picornaviridae](#)



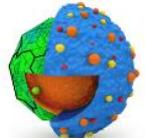
[Norovirus](#)



[Respiratory syncytial virus](#)



[Chikungunya virus](#)



[Hepatitis B virus](#)



[Bunyaviridae](#)



[Hepatitis E virus](#)



[Coronavirus](#)



[Rabies virus](#)

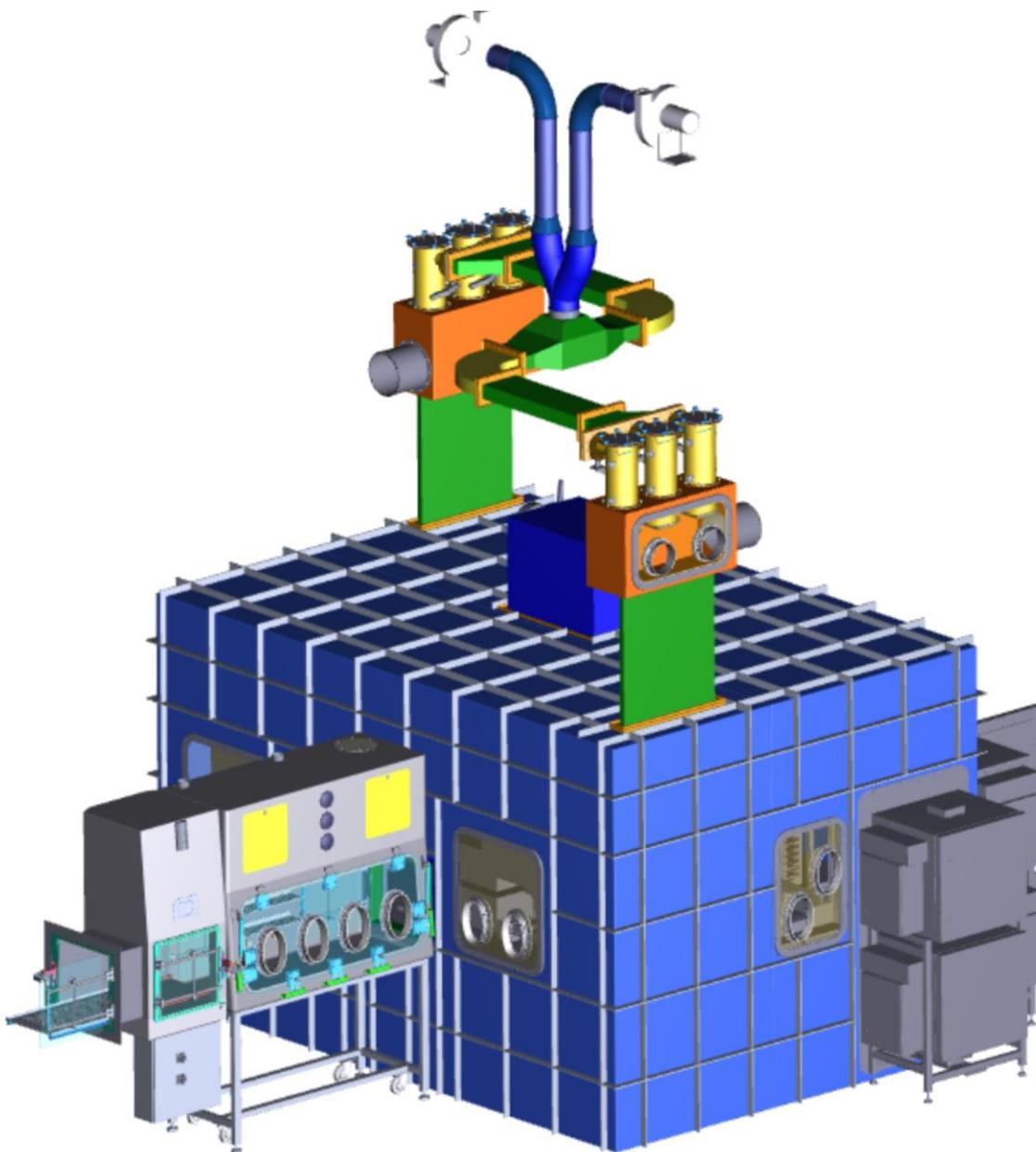
# Using cell based/phenotypic antiviral screens



\*\* the needle = a promising hit  
# the haystack = the infected cell



# Caps-It a robotized lab-in-a-box...



... and a  
*human-out-  
of-the-box*  
system

Biosafe handling and full  
containment at all times



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# Caps-It an automated platform in a BSL3+ environment





High-throughput  
research and  
screening

Video see [www.antivirals.be](http://www.antivirals.be)

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Safe import, export  
and handling of  
materials containing  
live pathogens



Chlorine dioxide  
decontamination

Video see [www.antivirals.be](http://www.antivirals.be)

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Replacement of  
double HEPA H14  
filters without system  
decontamination



caps-it@kuleuven.be

Video see [www.antivirals.be](http://www.antivirals.be)

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# Dengue

Second-most prevalent mosquito-borne infection after malaria.

~360 million infections and ~96 million cases/ year.

~ 2/5<sup>th</sup> of the world's population at risk [WHO]

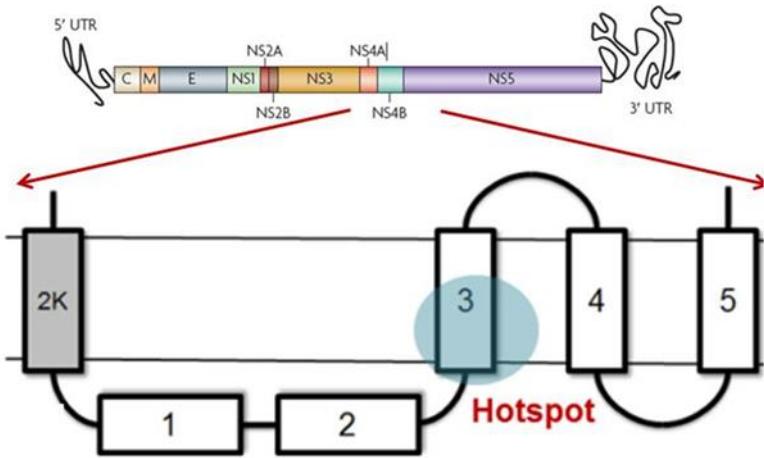
One hospitalization every minute around the globe

## Dengue patients overwhelm hospitals



LAHORE: Dengue outbreak in Lahore is taking a serious turn, as cases are pouring in at such an overwhelming rate that hospitals are forced to lay three patients on one bed, Geo News reported.

# Potent NS4b targeting pan-serotype inhibitors



DENV-2 hit

Med ↓ Chem

Pan-serotype  
Low nM to pM  
activity  
Clinical isolates

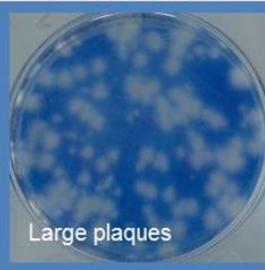
## DENV NS4B

No human homologue – no homologue of HCV NS4B

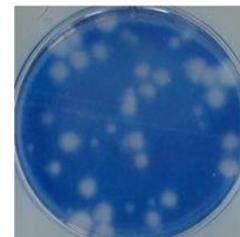
97% conserved intra-serotype, 78% conserved inter-serotype

Critical for viral replication

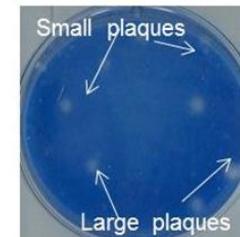
WT DENV P1



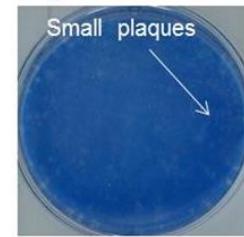
DENV<sub>CIM032160</sub> P15



DENV<sub>CIM032160</sub> P20



p30



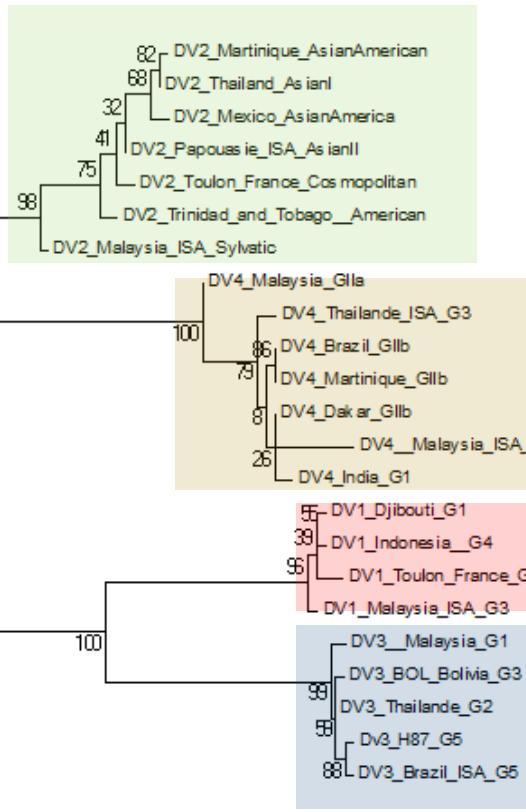
Dengue + placebo



Dengue + inhibitor



# Potent pan-serotype DENV activity



| Antiviral activity against DENV: RT-qPCR assay<br>EC <sub>50</sub> (nM) |                    |                |                    |                        |                            |                            |                          |                    |                      |                     |
|---|--------------------|----------------|--------------------|------------------------|----------------------------|----------------------------|--------------------------|--------------------|----------------------|---------------------|
| DENV-1  |                    |                |                    |                        | DENV-2                     |                            |                          |                    |                      |                     |
| Djibouti (G1)   | Malaysia* ISA (G3) | Indonesia (G4) | Toulon France (G5) | Mexico (Asian America) | Martinique (Asian America) | Trinidad/Tobago (American) | Toulon France (Cosmopol) | Thailand (Asian I) | Papouasie (Asian II) | Malaysia (Sylvatic) |
| 7.5   | 10                 | 6.9            | 9.6                | 6.5                    | 11                         | 0.5                        | <0.04                    | 9.7                | 0.036                | <0.04               |

| Antiviral activity against DENV: RT-qPCR assay<br>EC <sub>50</sub> (nM) |               |              |          |                 |            |                 |              |                   |               |               |               |
|---|---------------|--------------|----------|-----------------|------------|-----------------|--------------|-------------------|---------------|---------------|---------------|
| DENV-3  |               |              |          |                 | DENV-4     |                 |              |                   |               |               |               |
| Malaysia (G1)   | Thailand (G2) | Bolivia (G3) | H87 (G5) | Brasil ISA (G5) | India (GI) | Malaysia (GIIa) | Dakar (GIIb) | Martinique (GIIb) | Brasil (BIIb) | Thailand (G3) | Malaysia (GS) |
| 10  | 27            | 37           | 29       | 27              | <0.04      | 9.8             | 88           | 10                | 6.5           | >33           | 8.8           |

# KU Leuven, the Wellcome Trust and Janssen are together joining the fight against Dengue fever (breakbone fever).

Report to the  
**Community** 2013

Economic Sustainability

janssen 



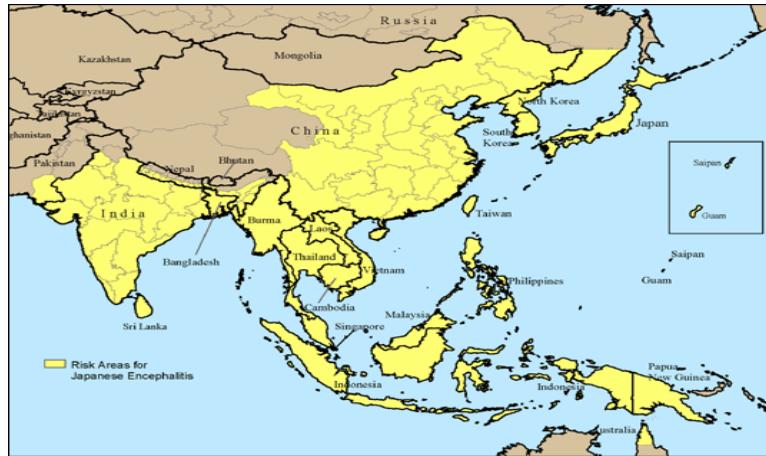
Collaboration in the fight against Dengue fever

**KU LEUVEN**

# Need for pan-flavivirus antivirals

## Japanese encephalitis

~ 50.000  
cases/year  
(30% mortality)



## Yellow fever



200.000 cases/year, 30.000 deaths

## Zika virus

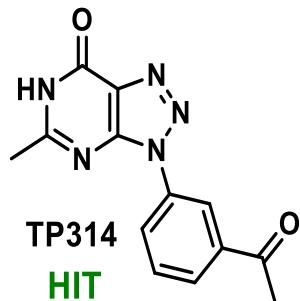


# Chikungunya



# A novel class of CHIKV inhibitors ....

## MADTP



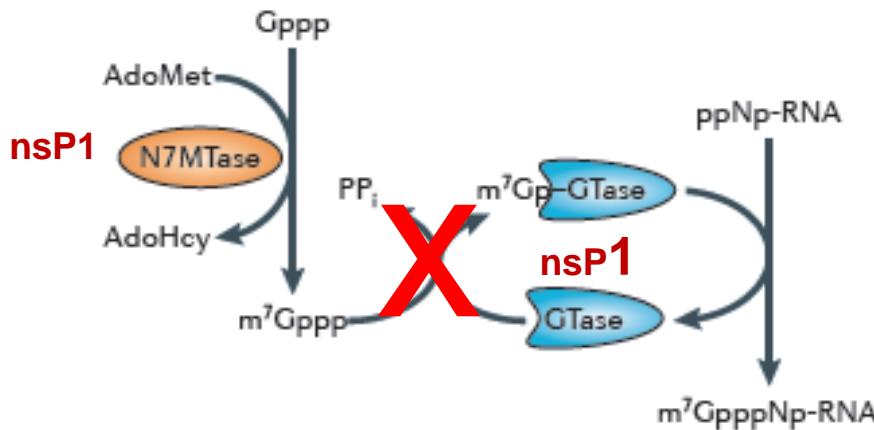
| Virus (Strain)         | EC <sub>50</sub> ( $\mu$ M) |
|------------------------|-----------------------------|
|                        | MADTP-372                   |
| 899 (lab)              | 2.6                         |
| Venturini (Italy 2008) | 1.4                         |
| Congo 95 (2011)        | 0.75                        |
| St Martin (2013)       | 4.3                         |
| VEEV                   | 6.2                         |

# .... that target viral capping

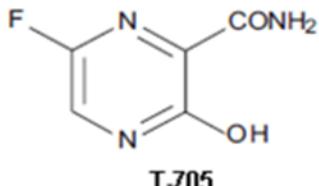
Resistant CHIKV clones : P34S mutation in NS1

Introduction in WT background confirms resistant phenotype

Decroly et al, Nature Reviews Microbiology 2012

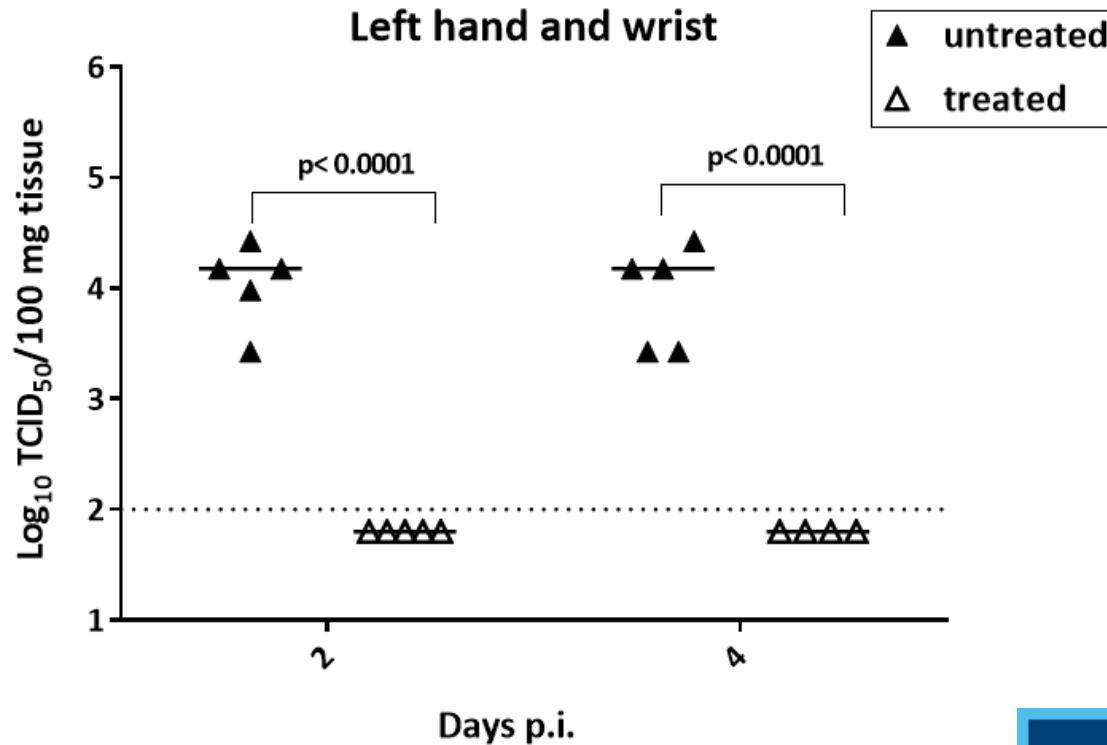
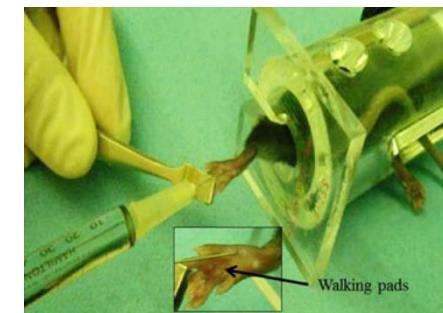


# Favipiravir (T-705) protects against CHIKV

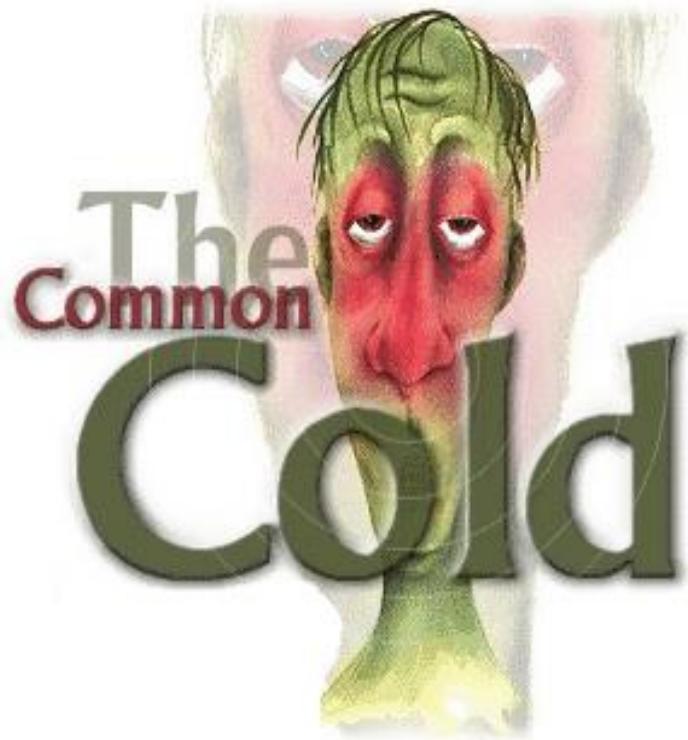


Toyama Pharm.

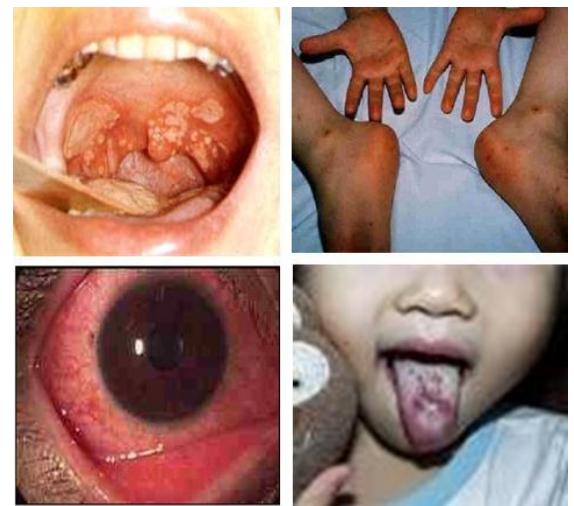
Flu inhibitor also active  
against flavi-, arena-,  
bunya-, filoviruses



# Rhino & enteroviruses



Exacerbations of asthma and COPD



18 July 2012 Last updated at 14:06 GMT

BBC

## Cambodia shuts schools to stop EV71 virus spreading

Cambodia has closed schools weeks ahead of the summer holidays to prevent the spread of a virus that has killed dozens of children since April.

Officials say that they closed kindergarten and primary schools in reaction to parental concern about the spread of the EV71 virus.

EV71 is one of the triggers of hand, foot and mouth disease.



Villagers lined up for medical checks outside a children's hospital in Phnom Penh

I think I need  
antibiotics for my  
col...

IT'S A VIRUS!





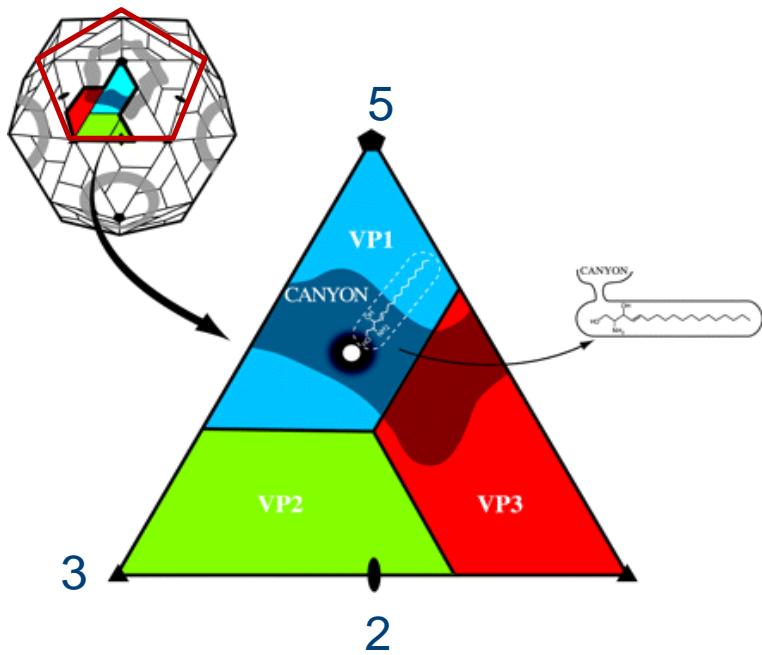
TOOLS AND LIBRARY → RESEARCH + INNOVATION → ANTIVIRALS

## ANTIVIRALS

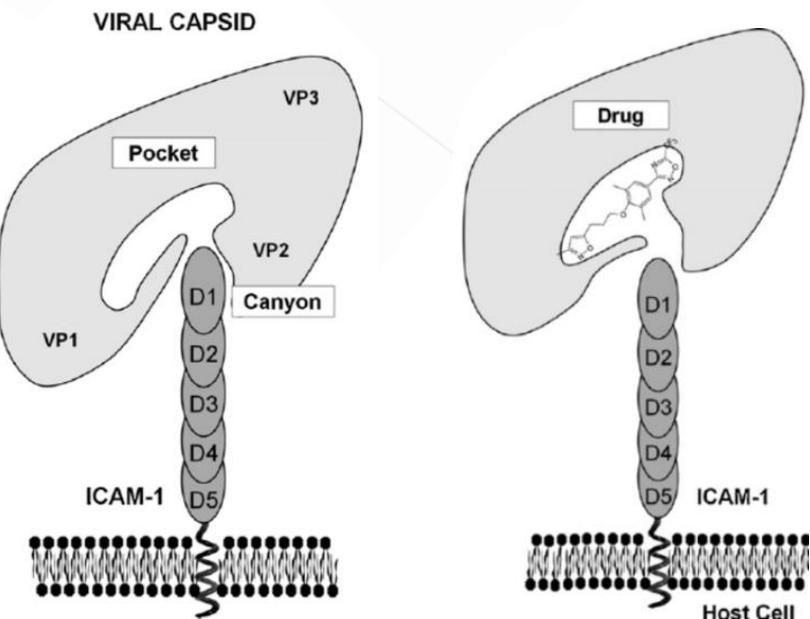
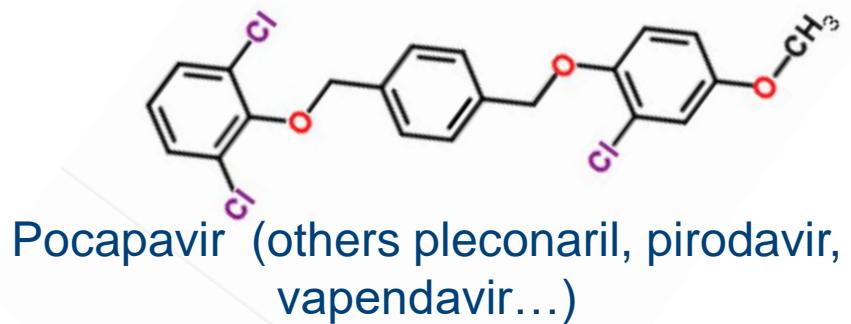
### Exploring the role of antiviral drugs before and after eradication

- treatment of immunodeficient people who are excreting poliovirus;
- preventative treatment for people exposed to poliovirus, for example through unintentional laboratory exposure;
- use in communities exposed to circulating vaccine-derived poliovirus (cVDPV) outbreaks in the post-eradication era (likely in conjunction with inactivated polio vaccine).

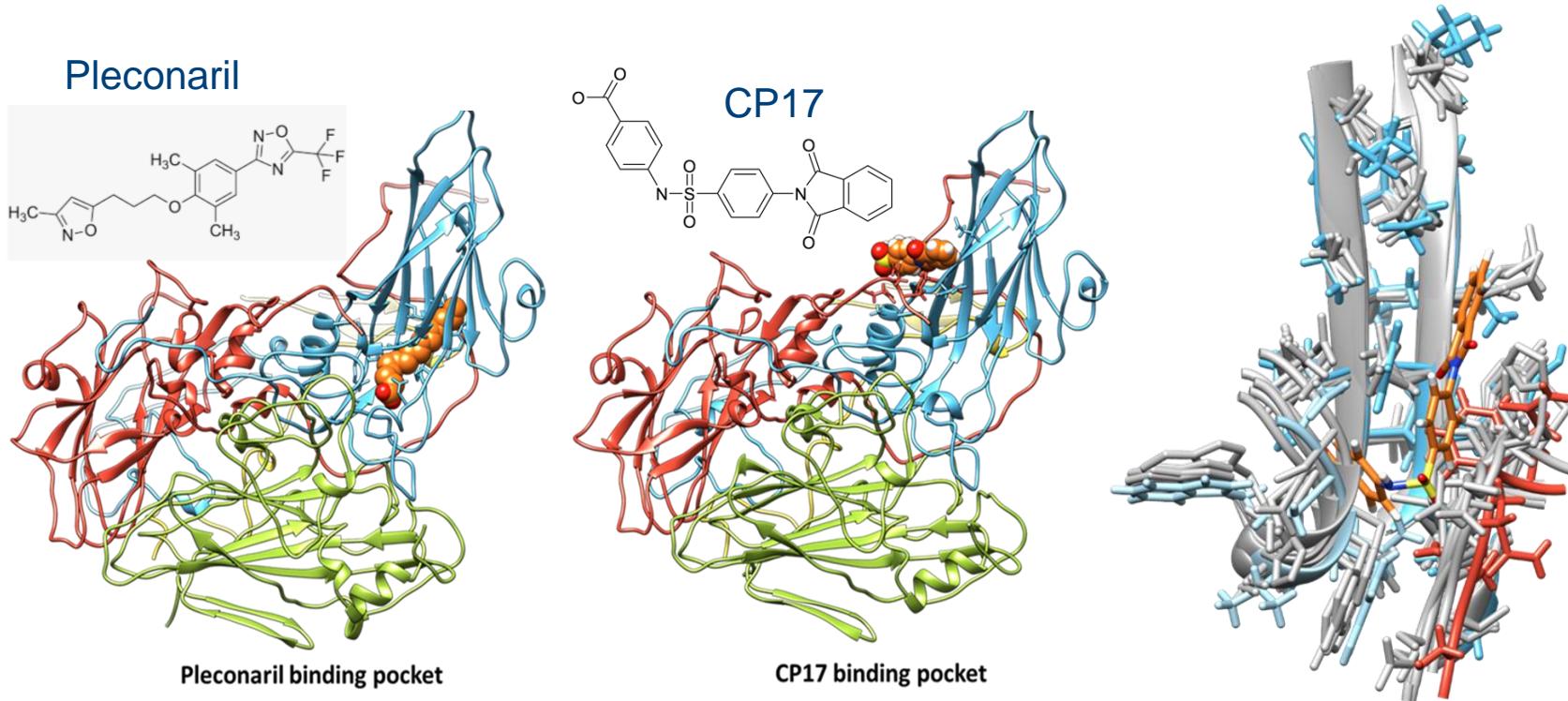
# Entero/rhinovirus capsid binders



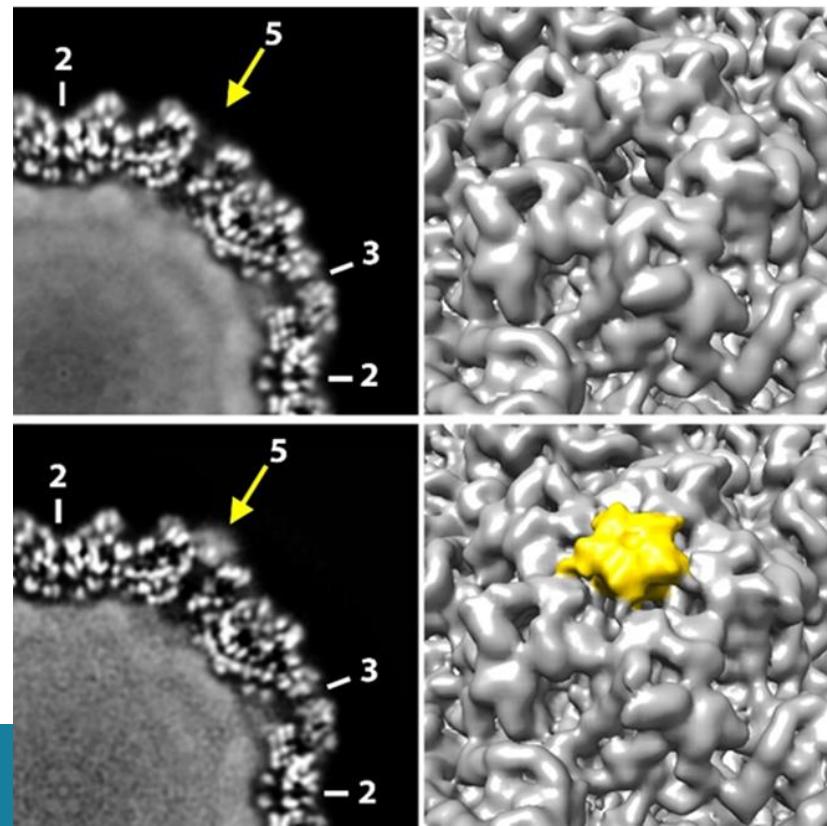
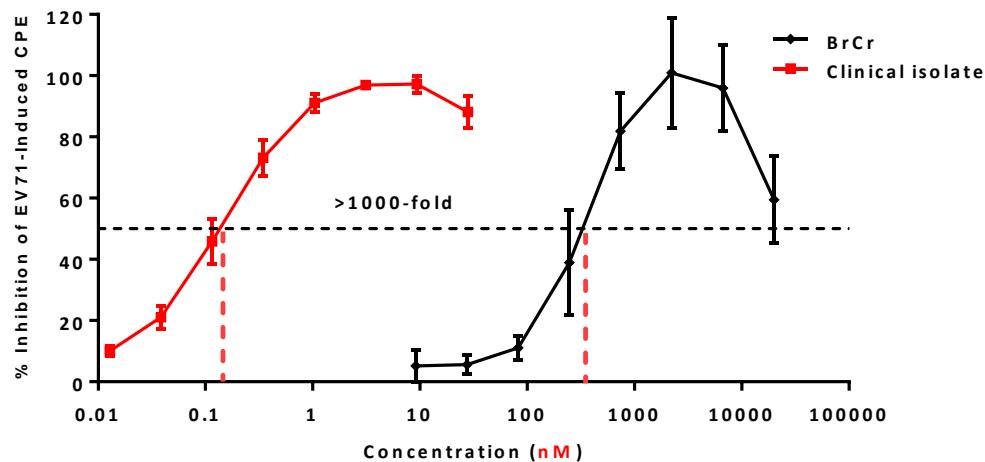
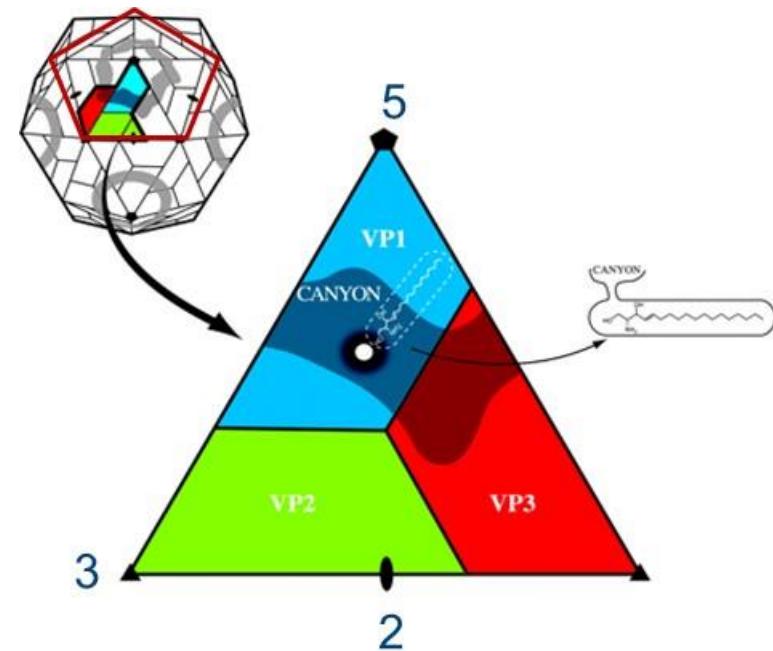
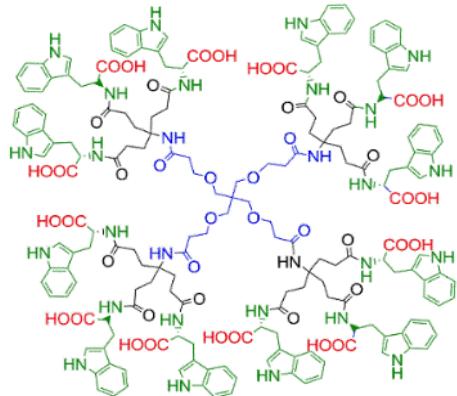
Pocapavir (V-073), originally discovered @ Schering-Plough; licensed by ViroDefense Inc.



# A novel enterovirus inhibitor with different binding pocket than capsid binders



# Tryptophan dendrimers as highly potent inhibitors of EV-A71



# Novel enterovirus/rhinovirus inhibitors



Korean Research Institute for Chemical Technology

| Compound  | EV71  | CVB3  | PV1  | HRV-2 (A) | HRV-14 (B) |
|-----------|-------|-------|------|-----------|------------|
| # 1 (hit) | 0.9   | 0.2   | 4    | >100      | >100       |
| # 56      | 0.01  | 0.02  | 0.7  | >100      | >100       |
| # 438     | 0.008 | 0.001 | 0.05 | 4.3       | 0.05       |

$EC_{50}$  in  $\mu M$

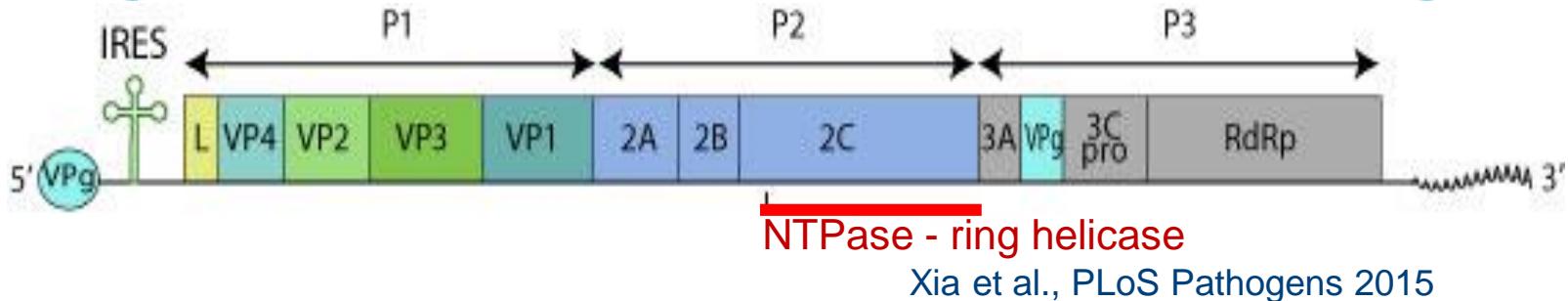
Compound # 696

|                     |     |            |
|---------------------|-----|------------|
| HRV-A (19 isolates) | 0.1 | [0.01-0.7] |
|---------------------|-----|------------|

|                    |       |                |
|--------------------|-------|----------------|
| HRV-B (5 isolates) | 0.004 | [0.001- 0.005] |
|--------------------|-------|----------------|

$EC_{50}$  in  $\mu M$

# High barrier to resistance and 2C as target

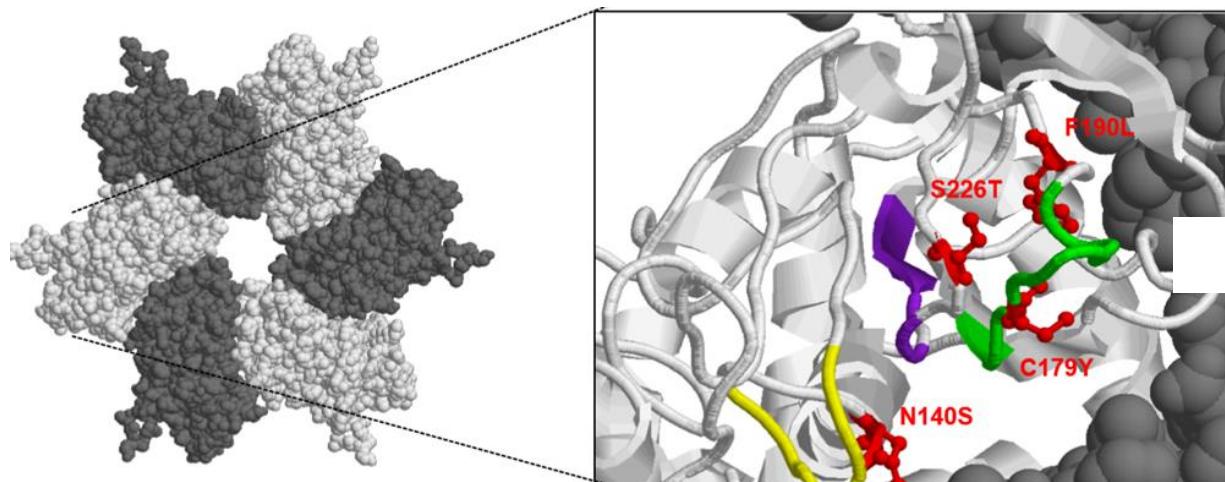


Resistance selection > 20 passages

At least 4 mutations in 2C

Reverse genetics : multiple mutations needed for resistance

No effect on 2C NTPase

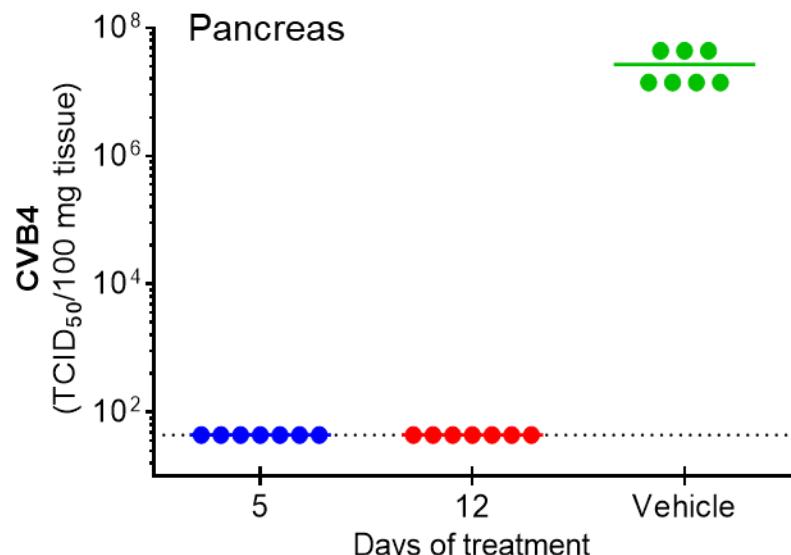
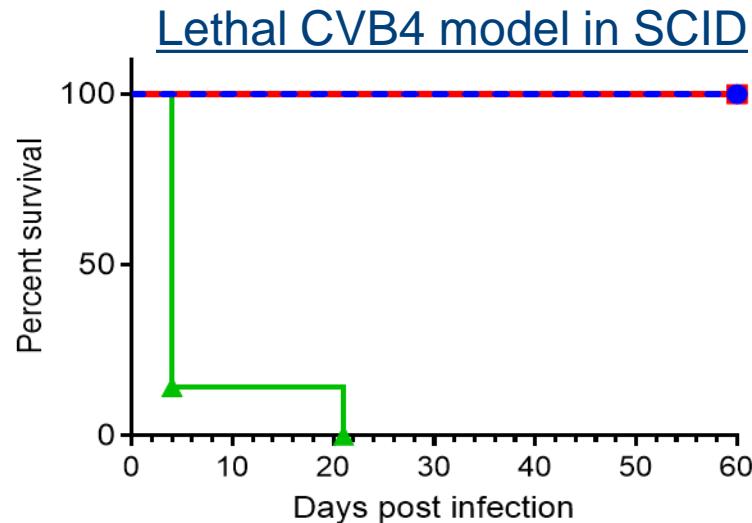
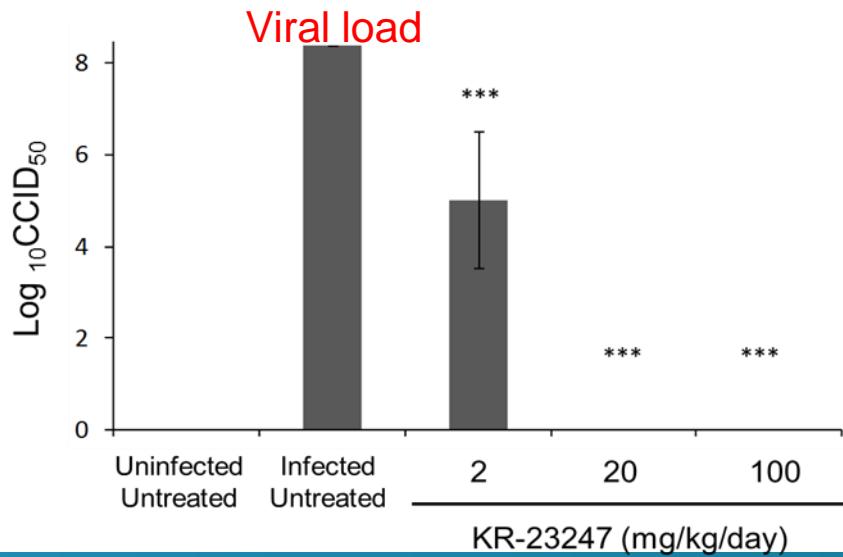
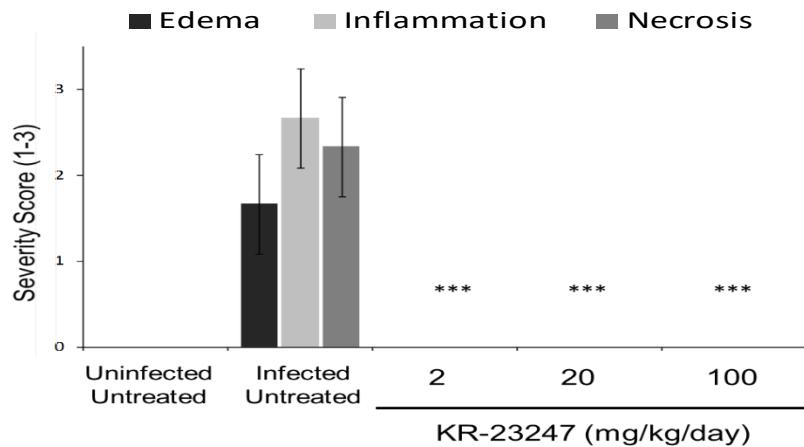


Hexameric model of the 2C protein of Echovirus 30

# Highly effective in enterovirus infected mice

## CVB4 Pancreatitis model

### Histopathology Markers

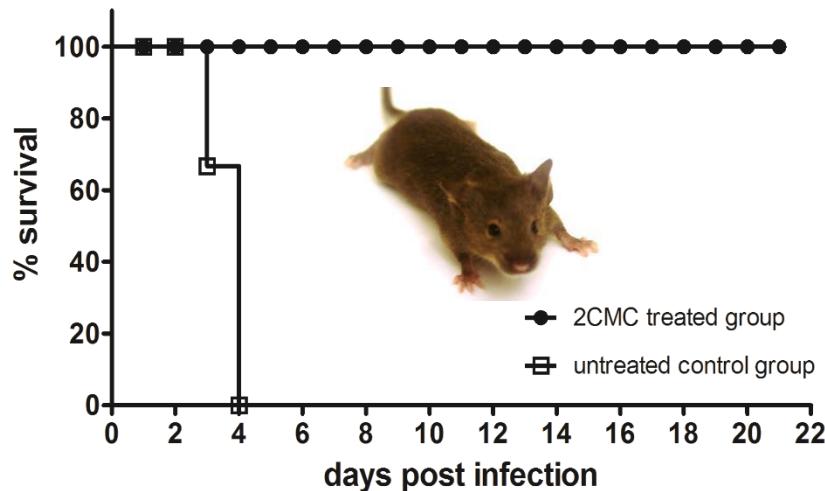


# (VIRAL) DIARRHEA

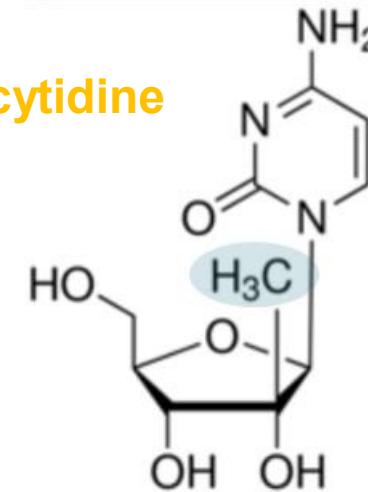
**4 min flow of the Niagara Falls**

~ 4 billion cases of **diarrhea** worldwide/year ([www.who.int](http://www.who.int))  
3 days with each 4 “events” of 200 ml = 9.600.000.000 liter

# Antiviral treatment of mouse norovirus infection

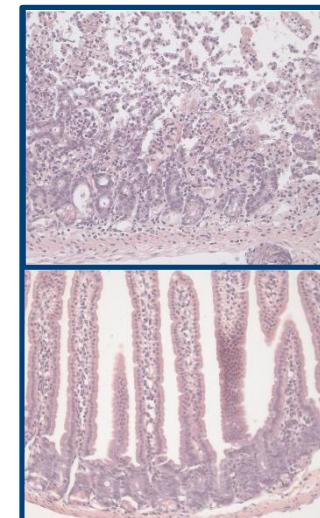
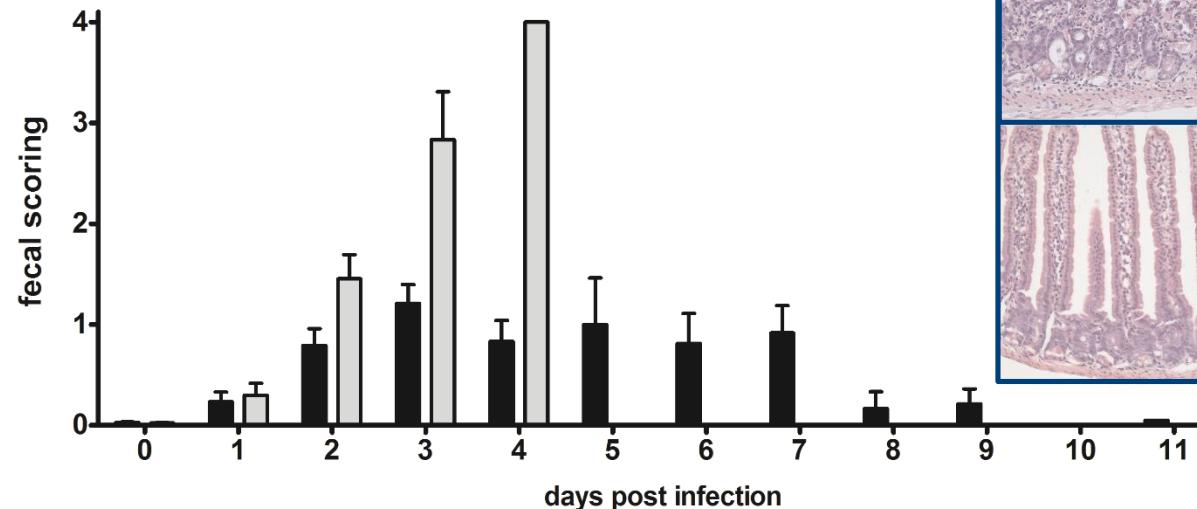


2'C methylcytidine  
(2CMC)

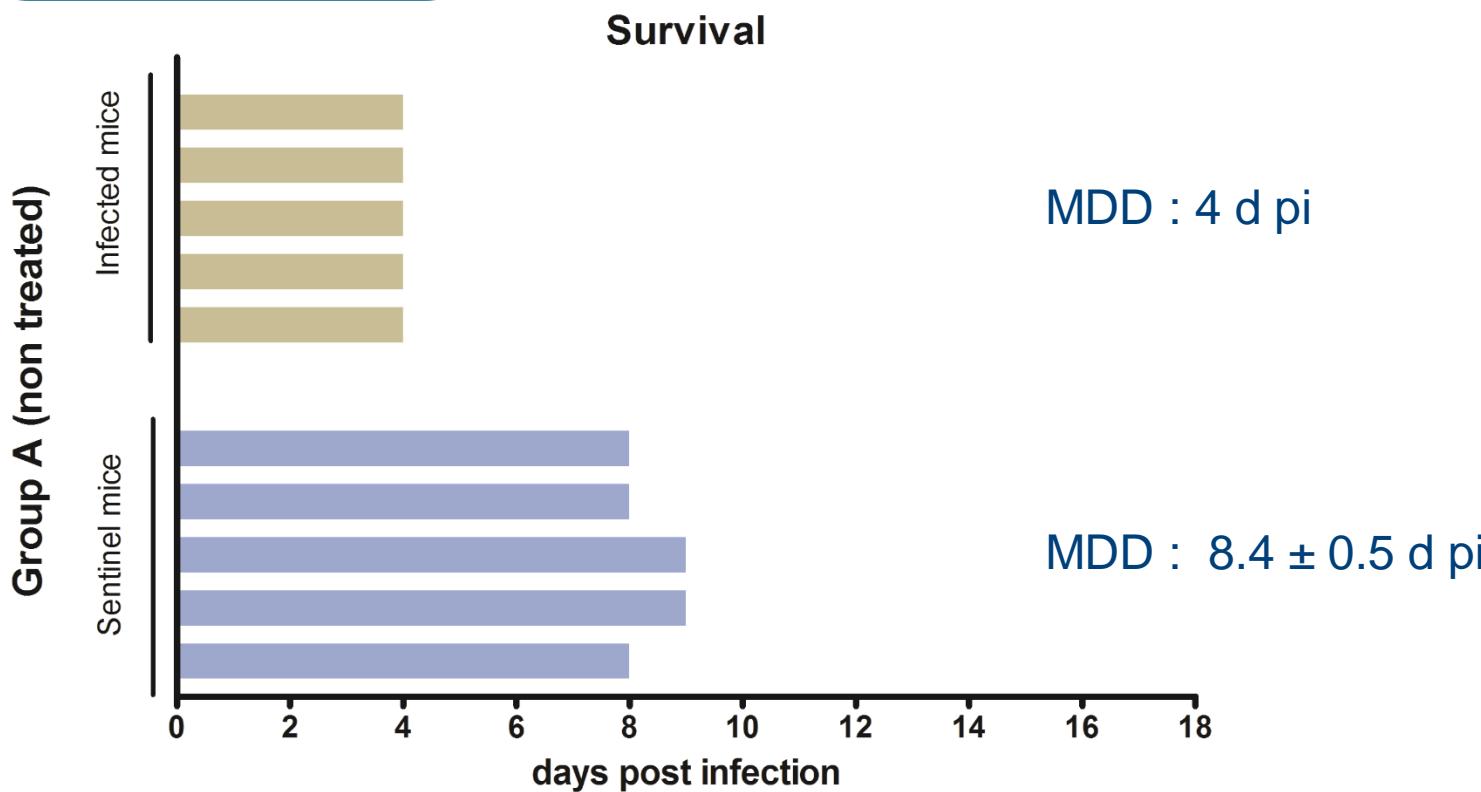
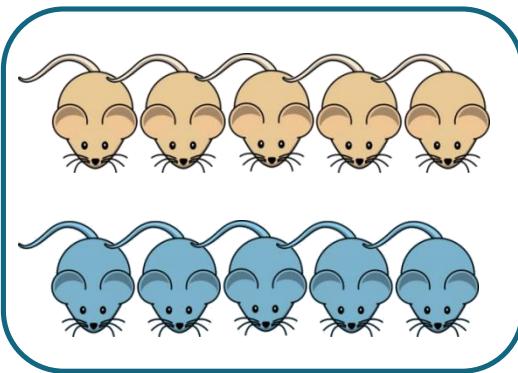


## Fecal consistency

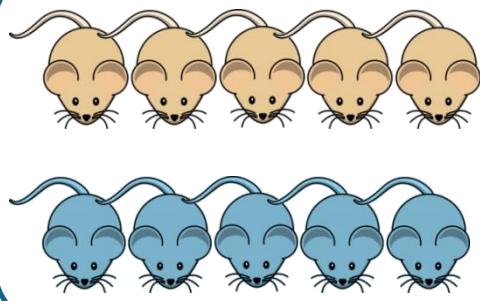
$P=.0314$     $P=.0097$     $P<.0001$



# Transmission of mouse norovirus



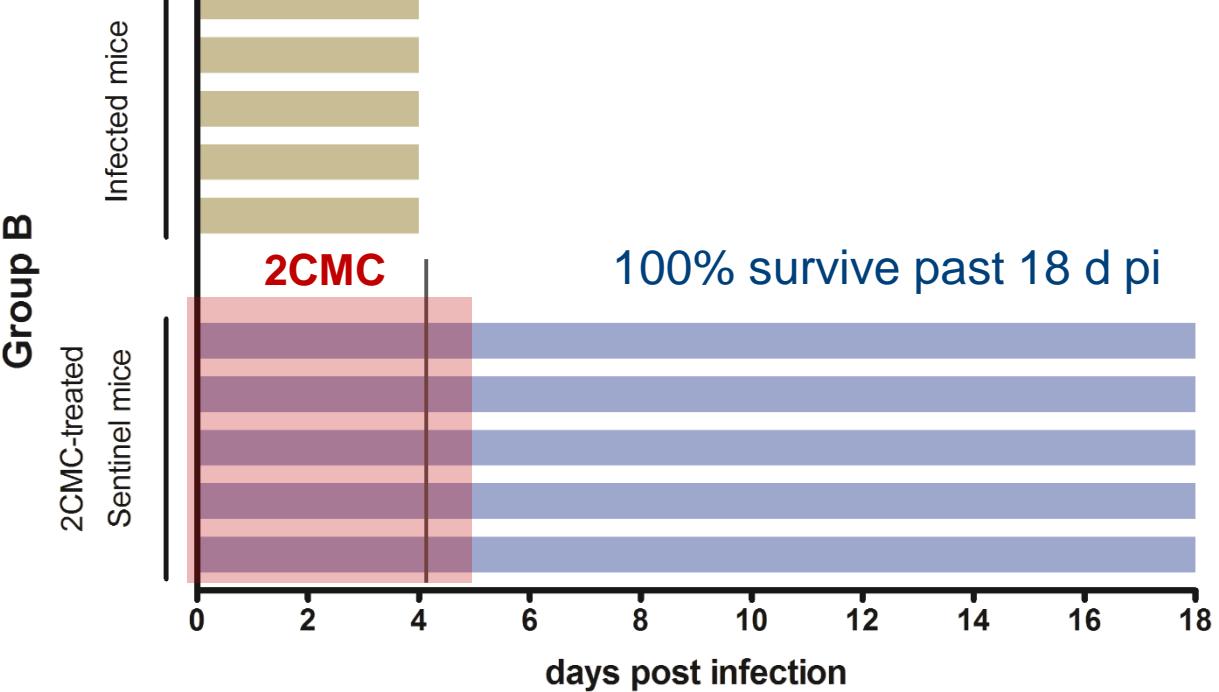
# Prophylaxis with 2CMC prevents transmission



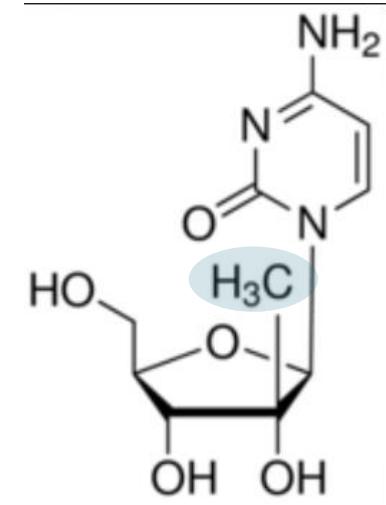
5 infected animals  
+  
5 sentinels (non infected)

2CMC

## Survival

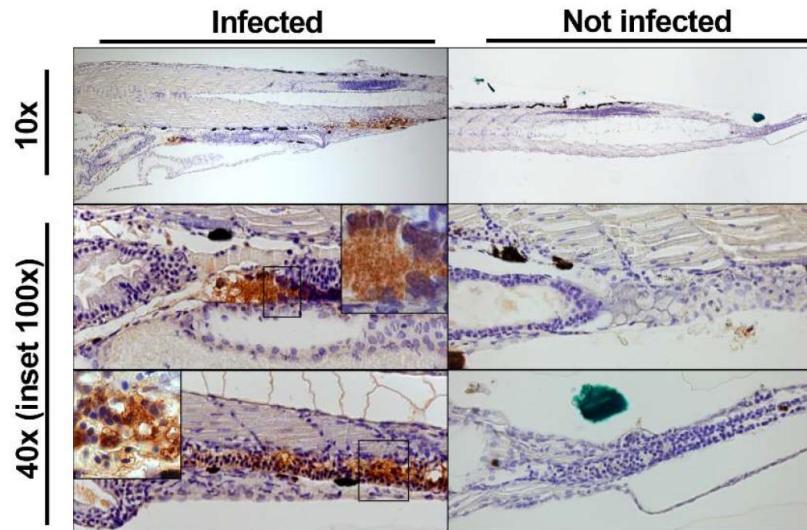
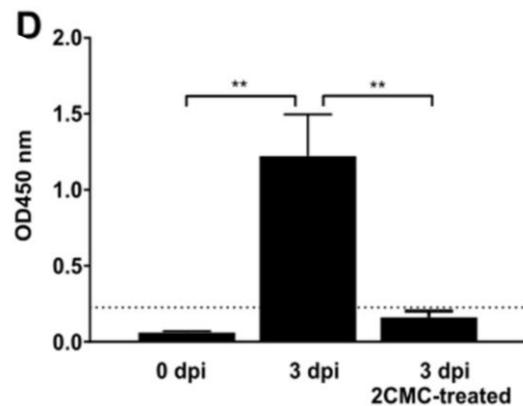
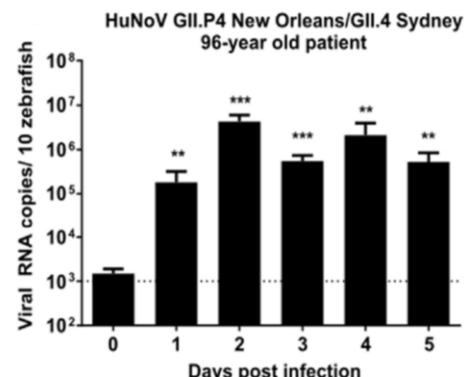
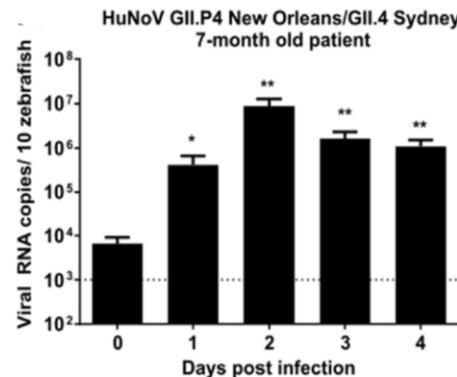
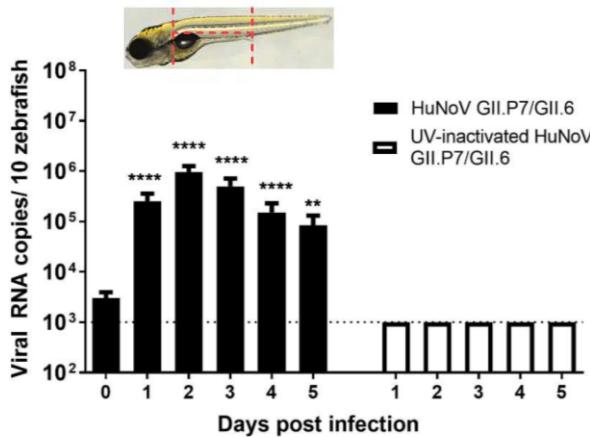


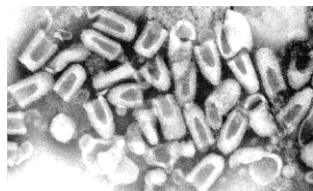
100% survive past 18 d pi



Bedding was replaced at 4 d pi

# huNoV replicates in zebrafish larvae





# Rabies



160 people die of rabies every day, says major new study

---

17 April 2015

A global study on canine rabies, published today (16 April 2015), has found that **160** people die every single day from the disease. The report is the first study to consider the impact in terms of deaths and the economic costs of rabies across all countries. Even though the disease is preventable, the study says that around **59,000** people die every year of rabies transmitted by dogs.



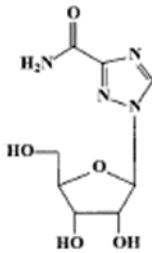


# Hepatitis E virus

- Gt 1-2: exclusively human
- Gt 3-4: zoonosis
  - pig, deer,... (worldwide)



## Chronic infections [gt 3 (gt 4)]



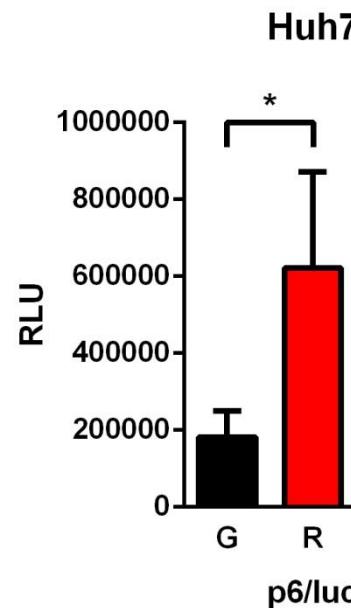
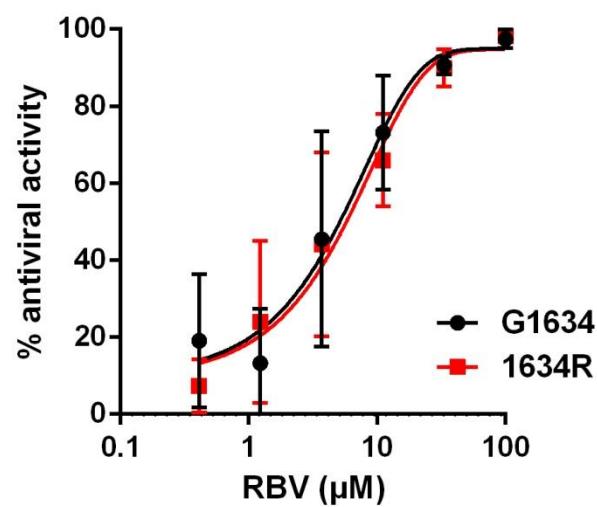
*Photo: A doctor does his rounds in MSF's Hepatitis E ward in Maban County's Batil camp. South Sudan 2013 © Corinne Baker/MSF*

### MSF Responds To Hepatitis E Outbreak In South Sudan Refugee Camps

An epidemic of hepatitis E is escalating across refugee camps in South Sudan's Maban County. To date, Doctors Without Borders/Médecins Sans Frontières (MSF) has treated 3,991 patients in its health facilities in the camps and has recorded 88 deaths, including 15 pregnant women.

# A mutation in the HEV RdRp of transplant patients “resistant” to ribavirin

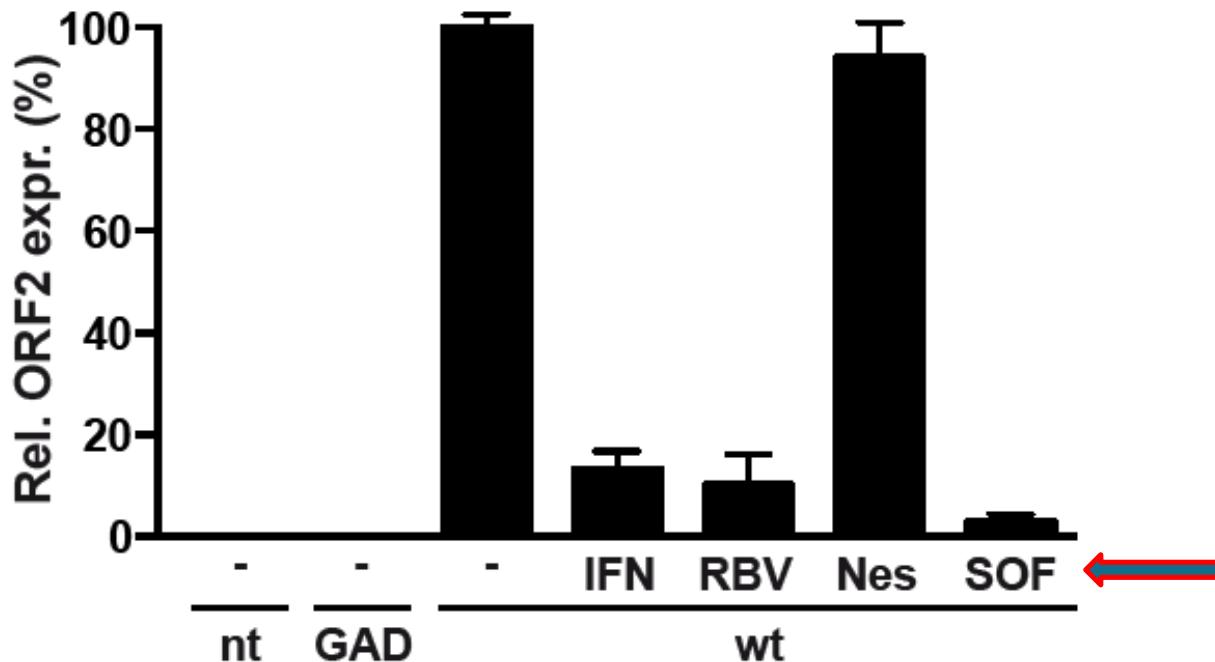
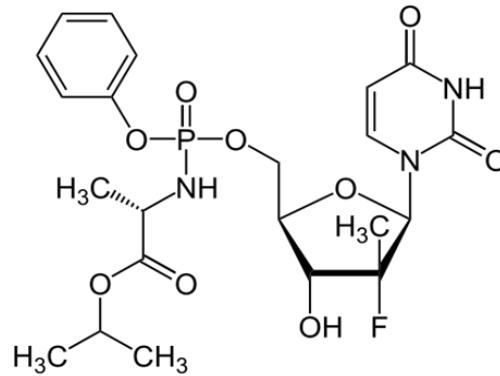
G1634R mutation  
detected in HEV RdRp of  
several patients after long  
term ribavirin treatment



\* p < .05

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# The HCV drug sofosbuvir inhibits HEV



# Conclusion

Antivirals still needed against many viral infections

Excellent molecular targets to be further explored such as

flavivirus NS4B

enterovirus 2C

alphavirus nsP1

Broad-spectrum RNA virus inhibitors needed

pan-genus

pan-family

multi-family

e.g. PI of picorna & noroviruses

T-705-like?

nucleoside analogues

Off-label use (e.g. Sofosbuvir against HEV)

# THANK YOU



## Univ. Utrecht, NL

F. Van Kuppeveld & team

## Univ of Lubeck, DE

R. Hilgenfeld and team

## Univ of Innsburck AT

G. Purstinger & team

## Univ Lausanne, SW

D. Moradpour and team

## CD3/CISTIM, Leuven A.

Marchand

P. Chaltn and team

## Univ. Hannover, DE

H. Wedemeyer & team

[www.antivirals.be](http://www.antivirals.be)

## Univ of Innsburck AT

G. Purstinger & team

## CSC Madrid, ES

MJ. Perez-Perez & team  
A. San-Felix & team

## Pasteur Institute, Paris

H. Bourghy and team  
M. Vignuzzi and team

## Univ. Aix-Marseille, FR

B. Coutard  
E. Decroly  
B. Canard  
X. De Lamballerie  
G. Querrat

## KRICT, Korea

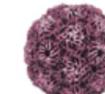
YS Sik & team

## AMC, Amterdam,

K. Wolthers & team

## UTMB, Galveston

P-Y Shi & team  
A. Barrett & N.Bourne



AIROPICO



AGENTSCHAP  
INNOVEREN &  
ONDERNEMEN

## Neyts lab

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