Zika virus mouse model

Johan Neyts
University of Leuven, Belgium
www.antivirals.be

KU LEUVEN

WHO Zika Research Meeting, Geneva March 3-5th 2016

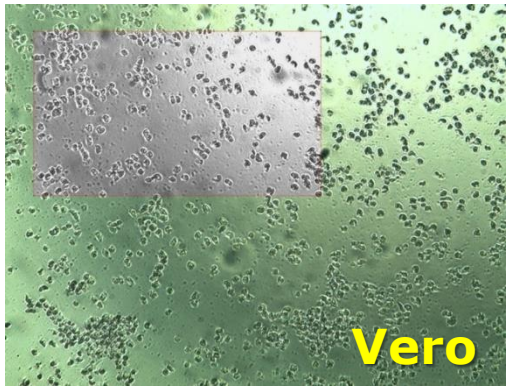
Collaboration in the fight against Dengue fever

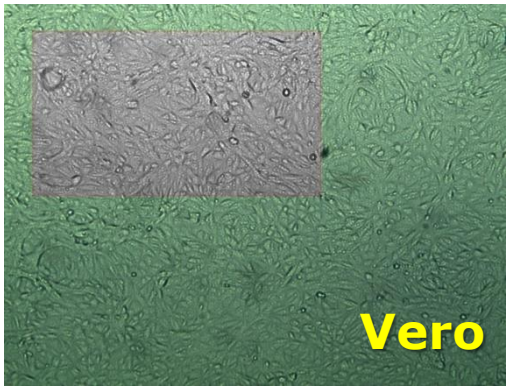
The Rega Instituut of the KU Leuven, the Wellcome Trust and Janssen are together joining the fight against Dengue fever (breakbone fever). In August 2013 they concluded a collaboration agreement for the development of a treatment or preventive medicine against Dengue fever virus infection.

The Dengue virus is potentially life-threatening. It is a virus spread by mosquitoes that infects hundreds of millions of people every year, chiefly in the developing countries. At present there is still no approved vaccine or specific treatment for the disease. The Rega Instituut and Janssen are pooling their expertise and resources in a joint research program. The Wellcome Trust will finance Rega Instituut's research activities. Janssen will obtain the possibility of taking out an exclusive, worldwide license for the development and commercialization of compounds that could be discovered within the context of this research program.

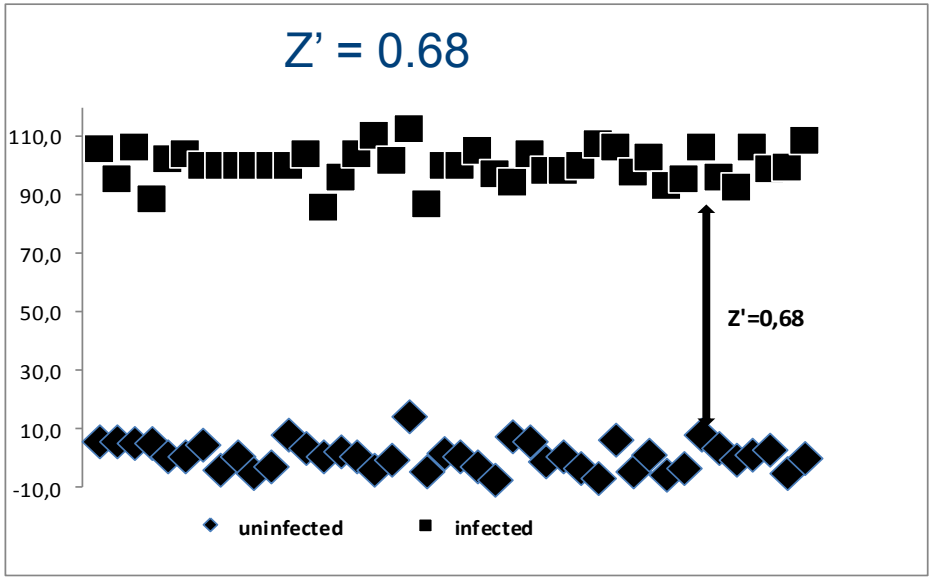
This collaboration is another example of Janssen's efforts to combat contagious diseases with a still largely unanswered medical need.

ZIKV induced cytopathic effect





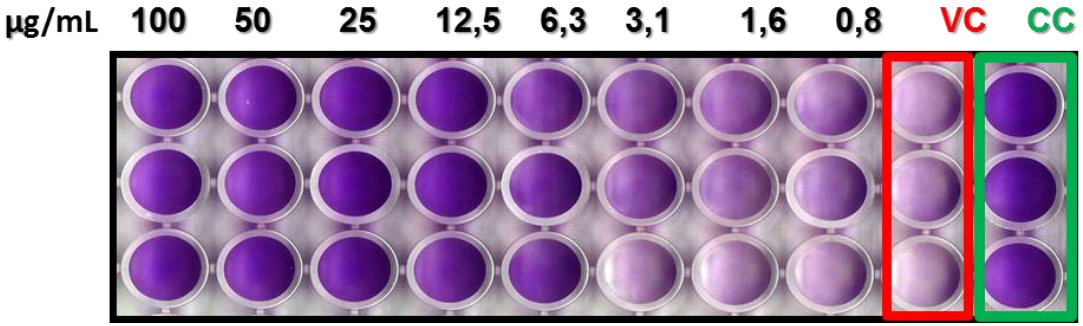
Strain MR766



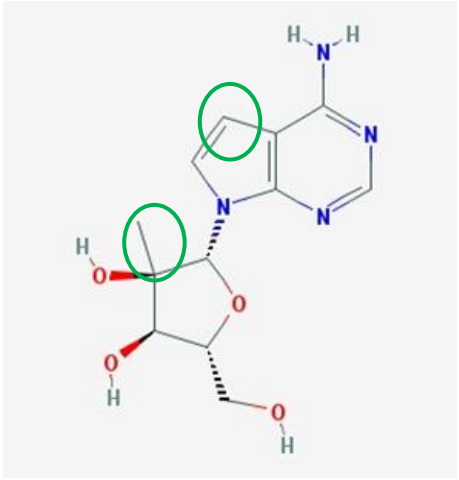
KU LEUVEN

Antiviral activity of 7DMA, *in vitro*

- Antiviral assay

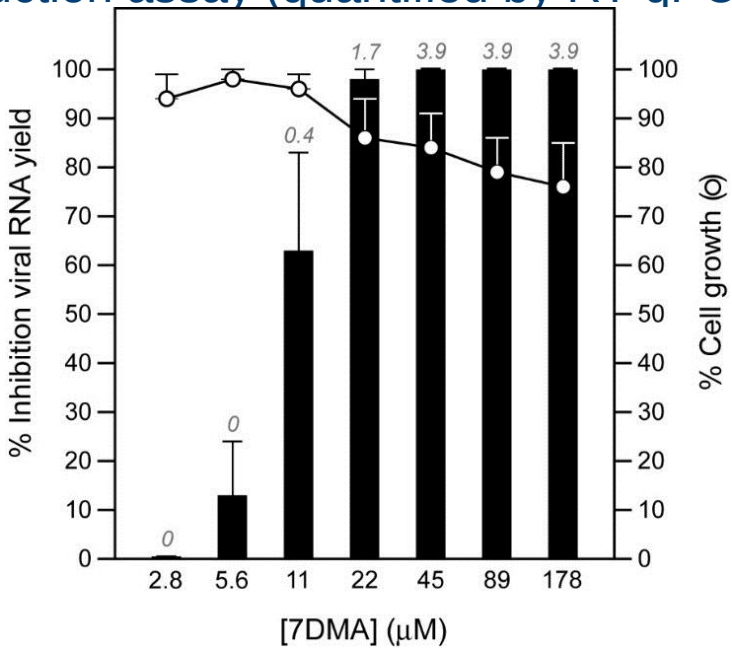


Also established for 384 well plate format



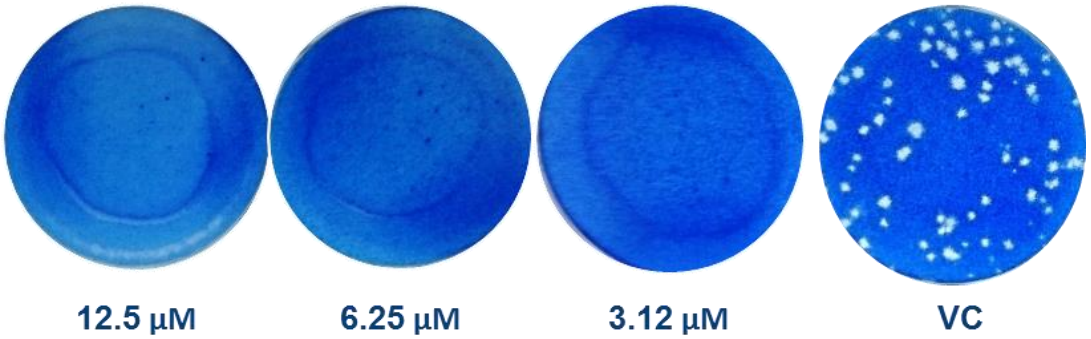
7-deaza-2'-*C*-methyladenosine

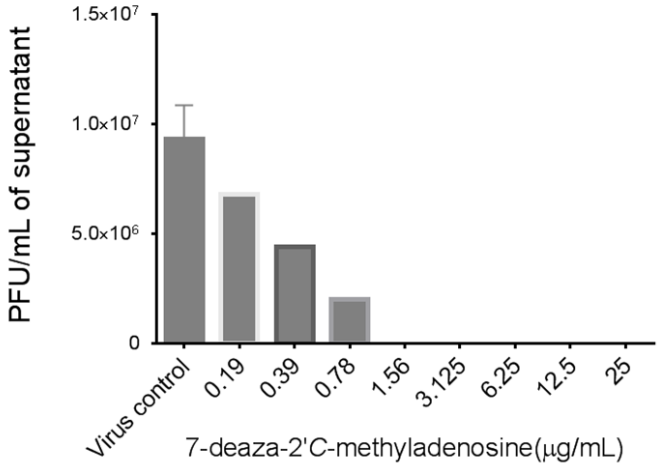
- Virus yield reduction assay (quantified by RT-qPCR)



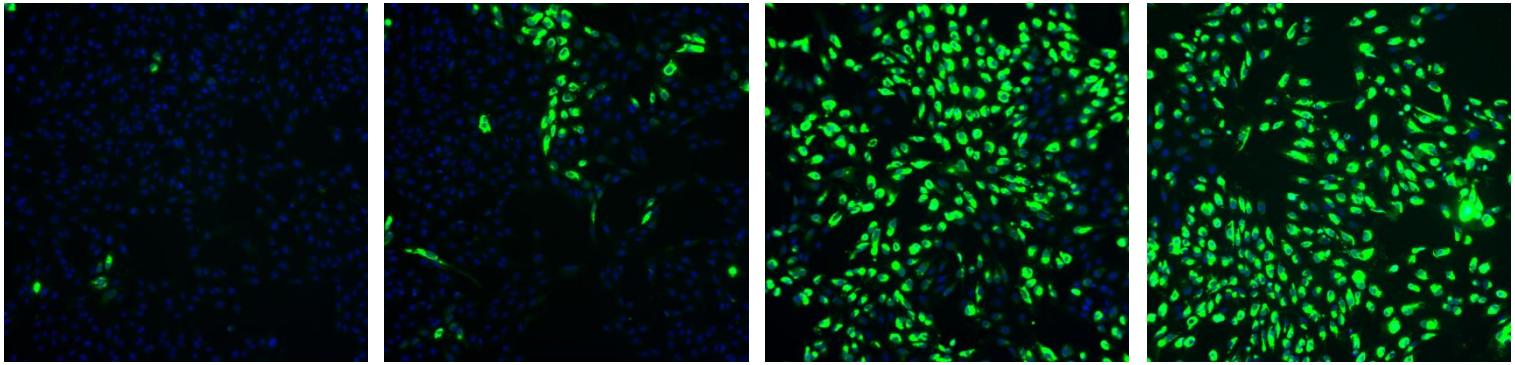
- **EC_{50} = 9.6 ± 2.2 µM**

Plaque reduction & viral antigen expression





Immunofluorescence assay using anti-Flavivirus Group Antigen Antibody, clone D1-4G2-4-15



11 µM 7DMA | **5.6 µM 7DMA** | **2.8 µM 7DMA** | **Virus control**

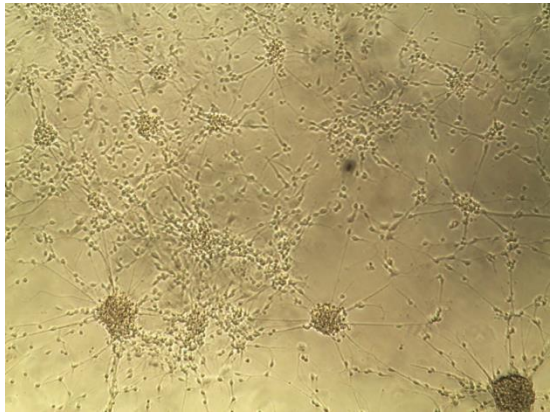
EC50 = 5.7 ± 2.2 µM

KU LEUVEN

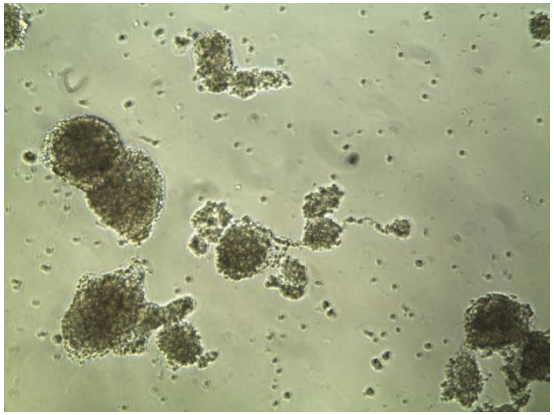
ZIKV infection in stem-cell derived immature cortical neurons

Induced pluripotent stem cell (iPSC)-derived cortical neuron cells (immature) infected with Zika strain MR766 (MOI 0.1)

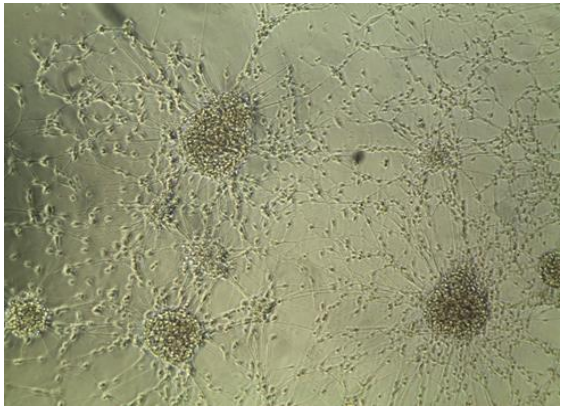
uninfected



Infected (day 6 p.i.)



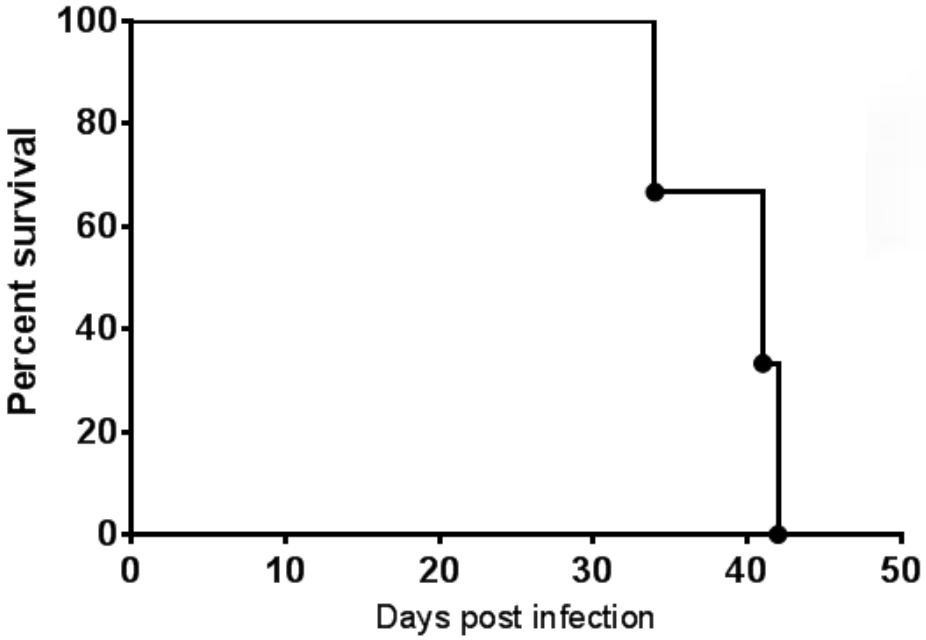
Infected + 7DMA (day 6)



KU LEUVEN

Infection of SCID mice with ZIIKV

pilot studies

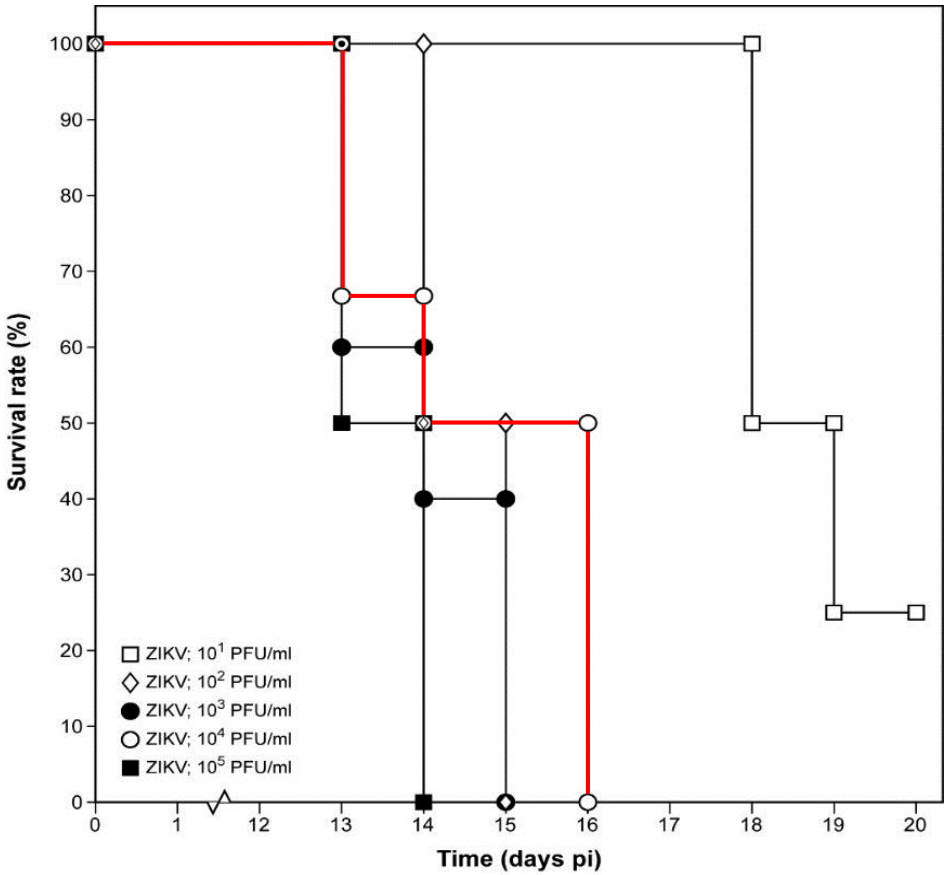


10^4 PFU/mL (200 µL), ip

MDE = 40.0 ± 4.4 d pi

Infection of AG129 mice with ZIKV

pilot studies



IFN alfa, beta, gamma receptor KO

10^4 PFU/mL (200 µL), ip

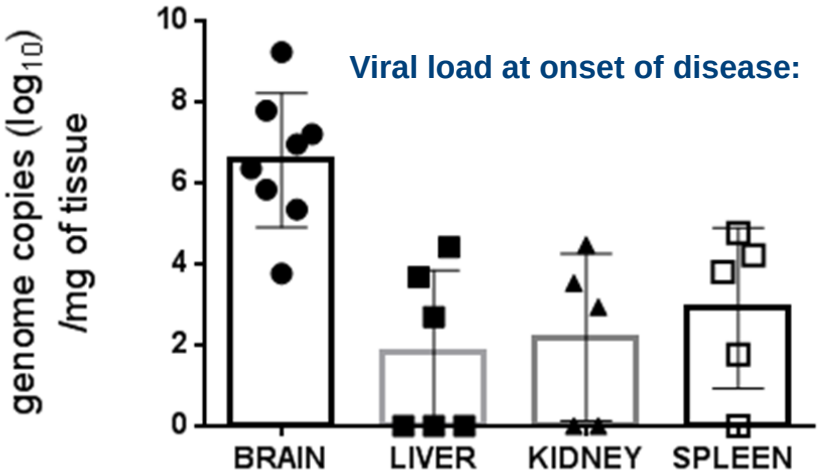
MDE = 15.0 ± 1.5 d pi

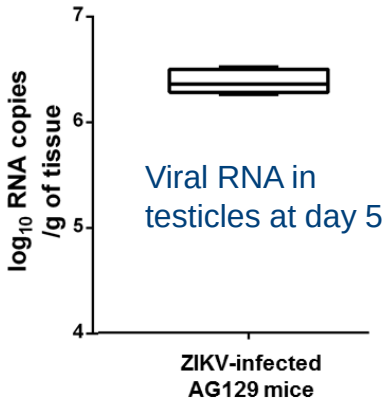
Symptoms	Days post infection	Affected/Total
paralysis of lower limbs	12-16	18/18
weight loss	13-16	18/18
hinged back	13-16	18/18
inflammation of eyes/conjuctivitis	14-16	2/18

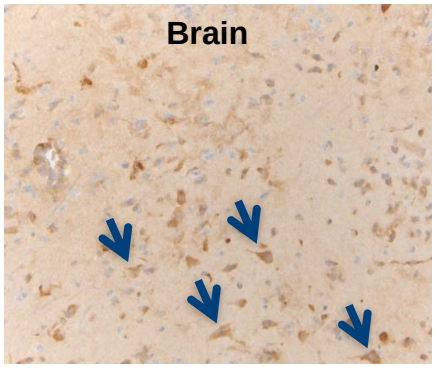
KU LEUVEN

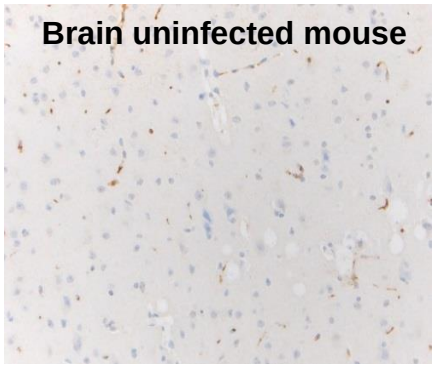
Zmurko *et al.*, submitted - bioRxiv

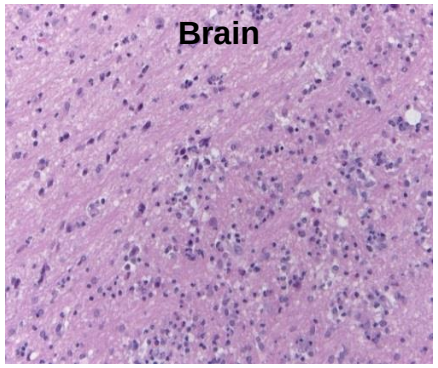
Infection of different organs & brain

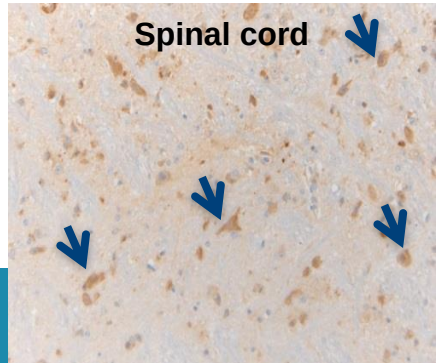


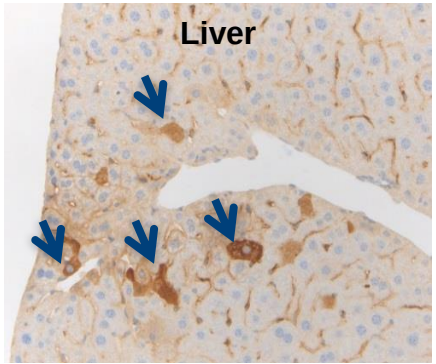


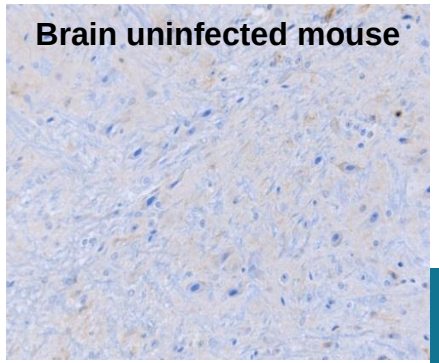






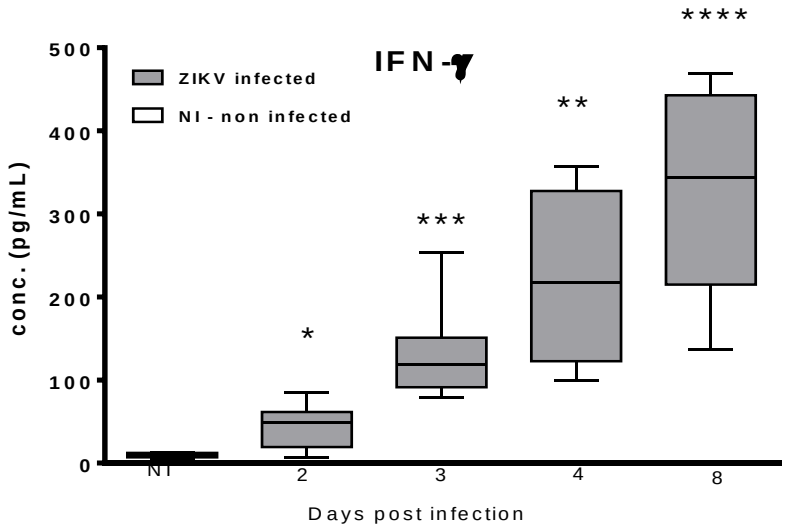


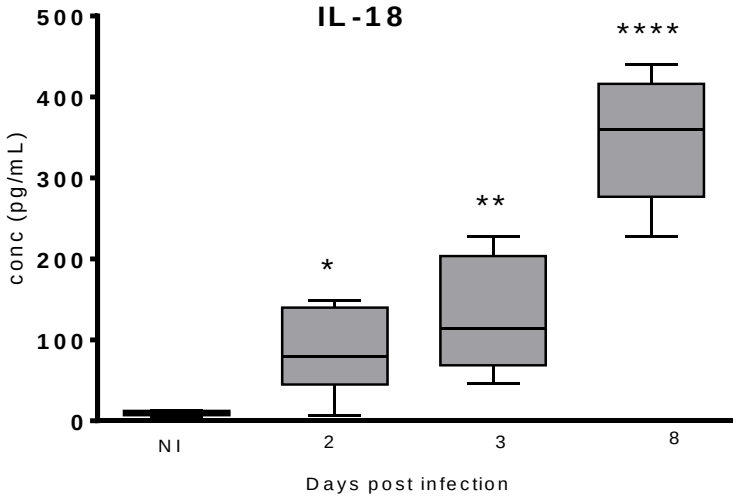




KU LEUVEN

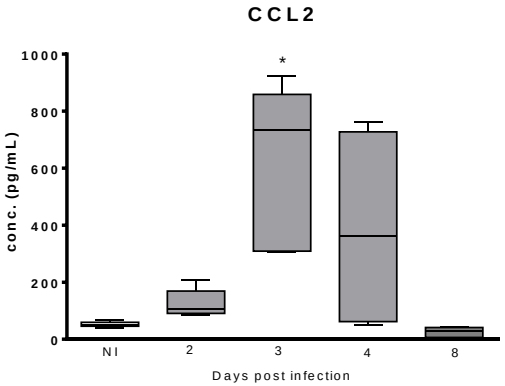
Expression of pro-inflammatory cytokines and chemokines induced by ZIKV infection

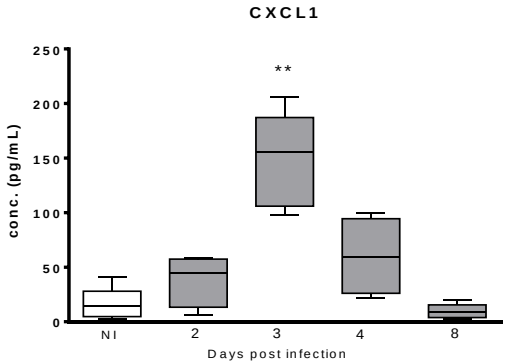


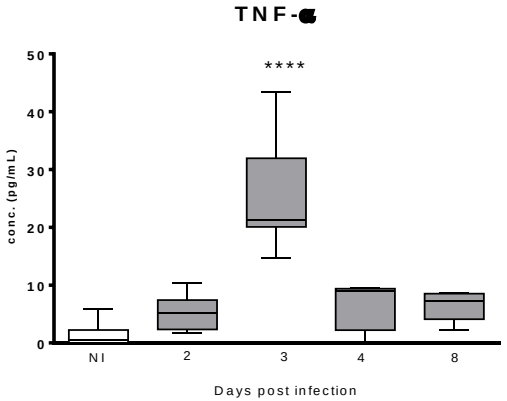


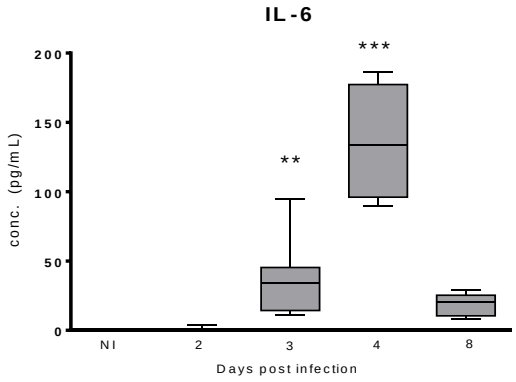
In particular, **IFN-γ** and **IL-18** were increased systematically during the course of infection.

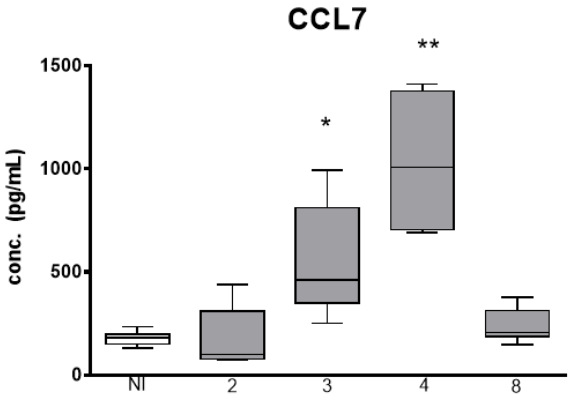
Levels of pro-inflammatory cytokines and chemokines

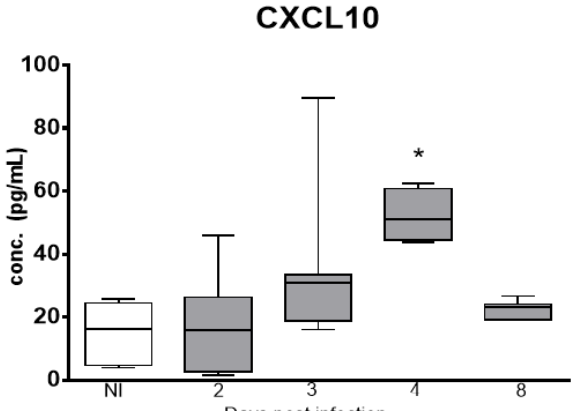










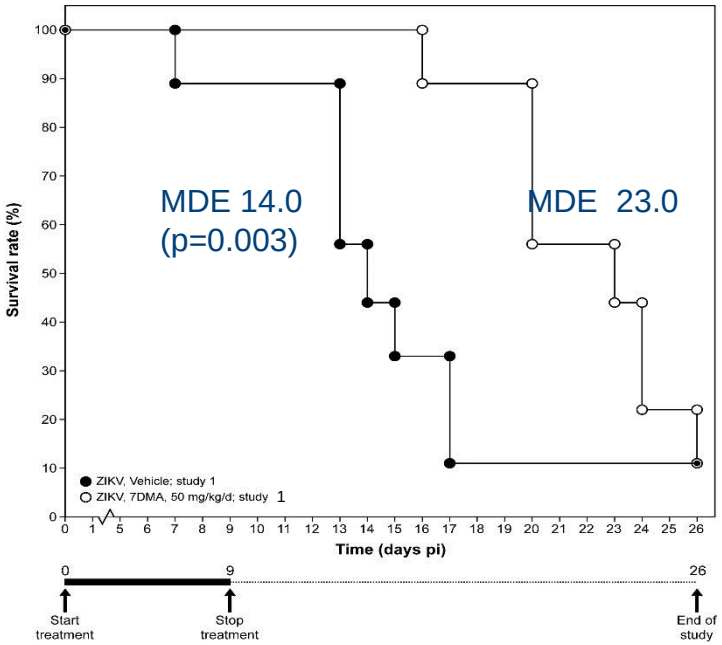


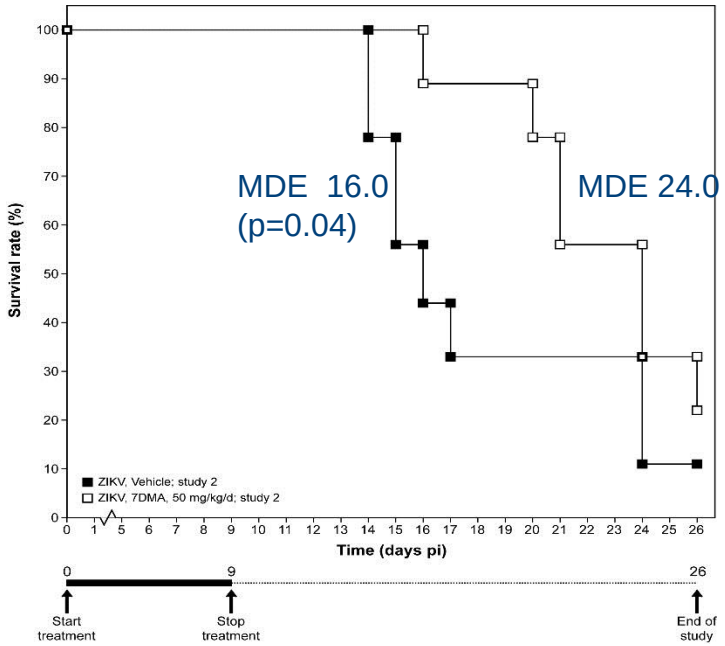
Infection with ZIKV resulted in the induction of pro-inflammatory cytokines (IFN-γ, IL18, TNF-α, IL-6) and chemokines (CCL2, CCL5, CCL7, CXCL1, CXCL10) in serum of AG129 mice.

KU LEUVEN

Zmurko *et al.*, submitted - bioRxiv

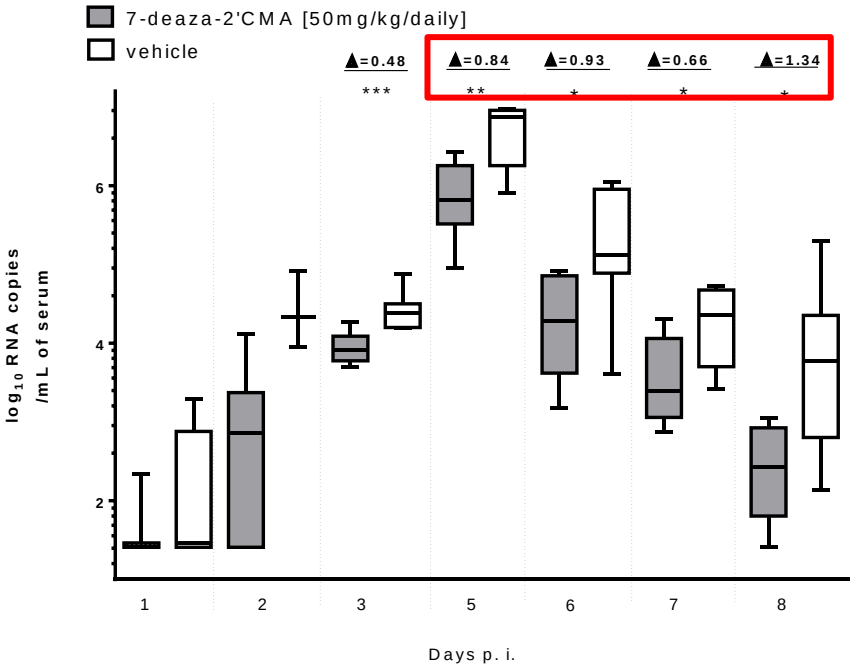
7DMA delays ZIKV induced disease



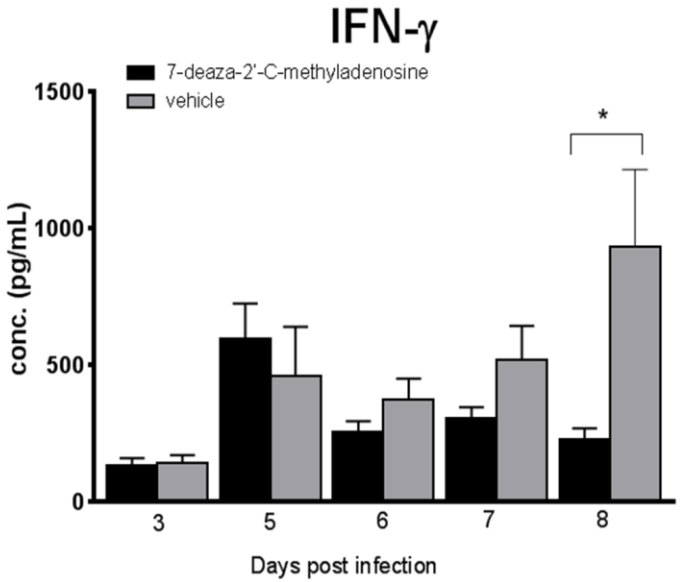


7DMA (50mg/kg/day, QD, for 10 days)

7DMA reduces viremia



Significant reduction of viral RNA load detected in serum: ~1$\log_{10}$ reduction at d5-d7 pi



Significant reduction in serum levels of IFN-γ at d8 pi

KU LEUVEN

Cold Spring Harbor Laboratory

bioRxiv beta

THE PREPRINT SERVER FOR BIOLOGY

New Results

The viral polymerase inhibitor 7-deaza-2'-C-methyladenosine is a potent inhibitor of in l vitro Zika virus replication and delays disease progression in a robust mouse infection model

Joanna Zmurko, Rafael E Marques, Dominique Schols, Erik Verbeken, Suzanne J.F. Kaptein, Johan Neyts

doi: http://dx.doi.org/10.1101/041905

KU LEUVEN

The YFV vaccine is safe and highly effective but thermostable and there is a shortage

Max Theiler
Nobelprize

Subculturing YFV Asibi strain >200 times in mice and cell culture: YFV-17D strain (1937)

↓

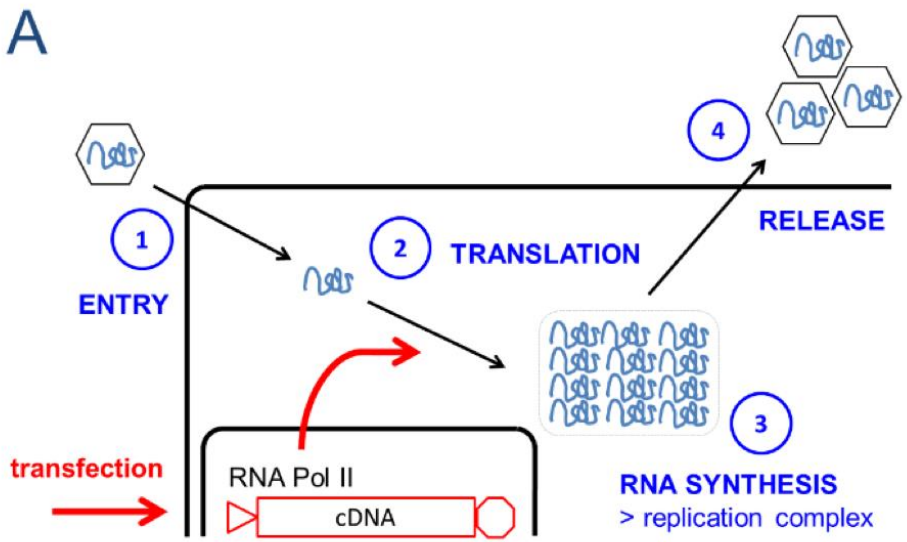
Vaccination with >10^6 pfu of YFV-17D: >99.5% protection

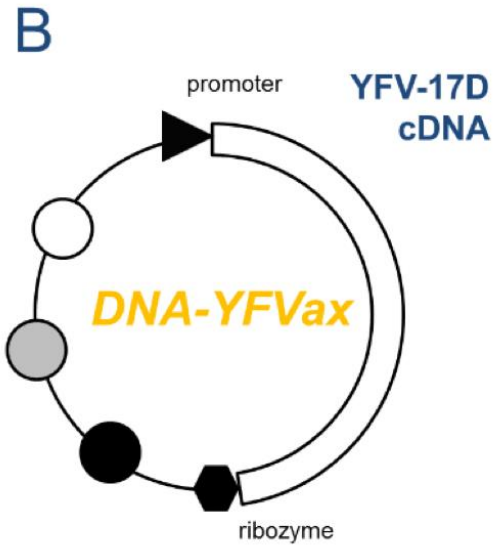
Sanofi Pasteur

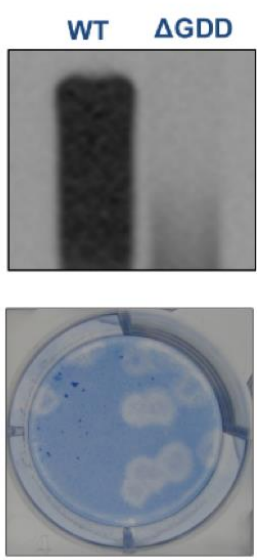
Bio-Manguinhos, Brasil

KU LEUVEN

DNA-YFVax technology: Replication of YFV-17D can be efficiently launched from plasmid DNA





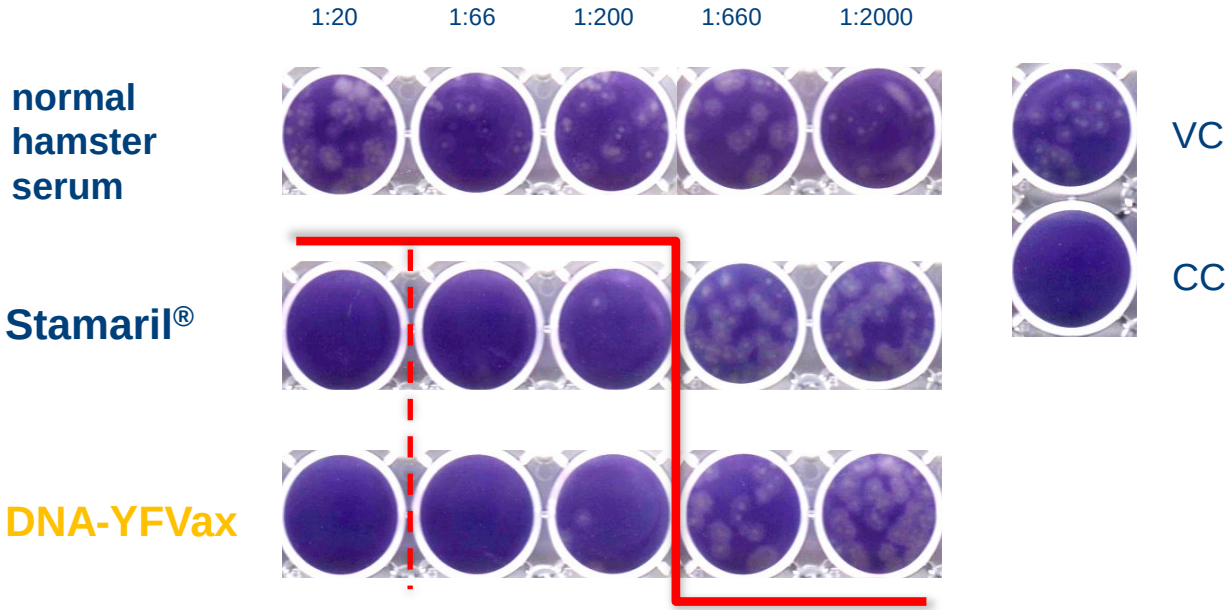


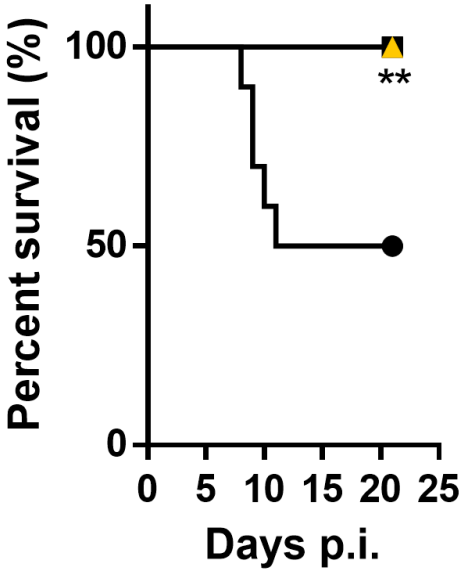
YFV-17D cloned in a BAC

ΔGDD − replication deficient mutant with lethal deletion in the viral polymerase gene

KU LEUVEN

DNA-YFVax as effective as the commercial vaccine



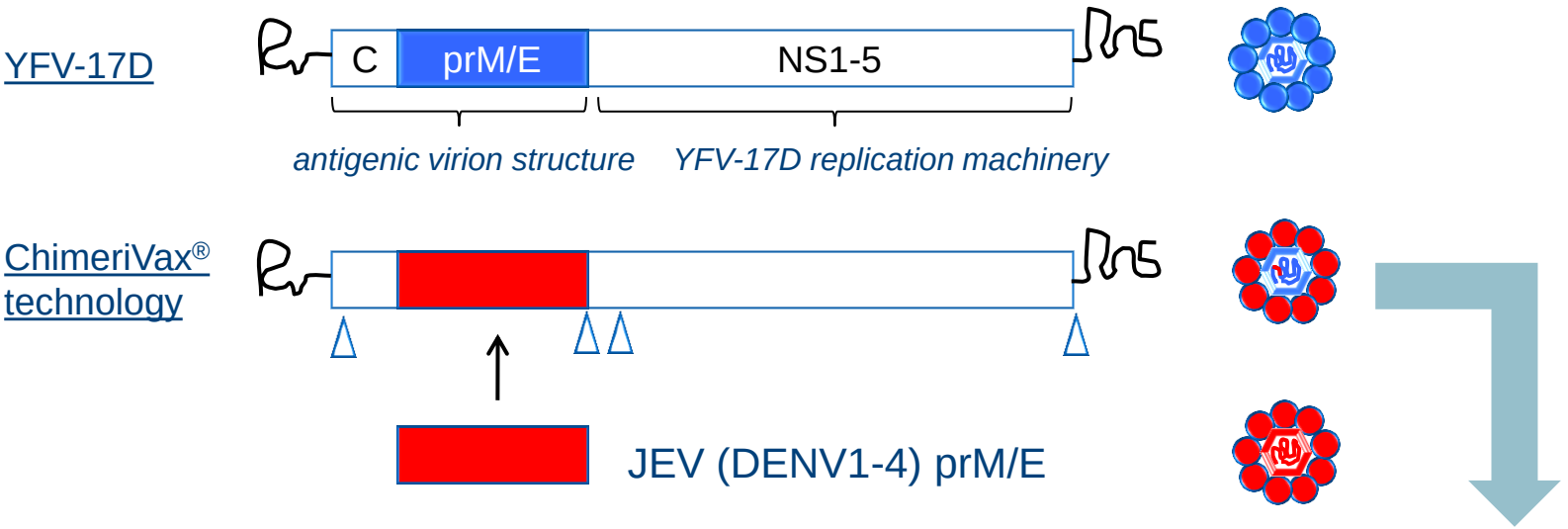


Plaque reduction neutralization test (PRNT) titers exceed log neutralization indices (LNI) that are considered immune correlates of protection* by > 40-fold.

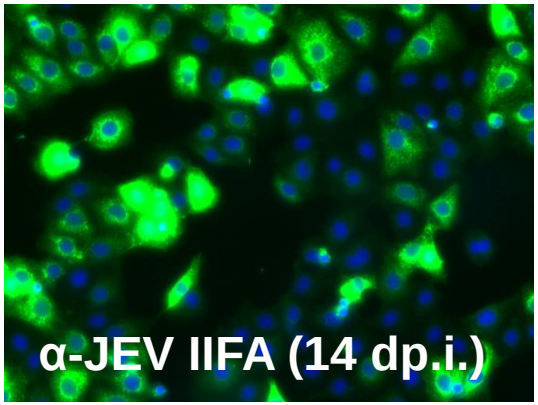
KU LEUVEN

** WHO Wkly Epidemiol Rec 2013*

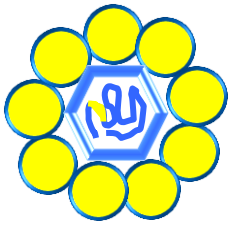
DNA-YFVax approach can be used for other flavivirus vaccines viruses



POC:



Ongoing:



DNA-ZIKVax

KU LEUVEN

Conclusion

Assays available for HTS (in 384well plate format) and validation

AG129 ZIKV model validated for antiviral studies
- whether sexual transmission occurs
- study effect of infection on pubs

AG129 ZIKV model can likely also be used to initially assess vaccine efficacy

DNA-YFVax technology may allow to easily produce heat stable flavivirus vaccines as efficient as the parent vaccines

Pan-flavivirus drugs are needed that can be used for the treatment/prophylaxis of dengue, zika, JEV….

Acknowledgements

Neyts-lab Leuven
Joanna Zmurko
Rafael Elias
Carolien Dekeyser
Leen Delang
Suzanne Kaptein
Rafael Elias
Niraj Mishra
Dieudonné Buh Kum
Kai Dallmeier

Stem cell Institute Leuven
Nicky Helsen
Tine Tricot,
Catherine Verfaillie

Univ. Logan, Utah
Justin Julander

NIAID
Heather Greenstone
Christina Cassetti