



**QIMR Berghofer**  
Medical Research Institute



**THE UNIVERSITY  
OF QUEENSLAND**  
AUSTRALIA



## A vaccinia-based single vector construct multi-pathogen vaccine protects against both Zika and chikungunya viruses

Natalie A. Prow<sup>1,2</sup>, Liang Liu<sup>3</sup>, Eri Nakayama<sup>1,4</sup>, Tamara H. Cooper<sup>3</sup>, Kexin Yan<sup>1</sup>, Preethi Eldi<sup>3</sup>,  
Jessamine E. Hazlewood<sup>1</sup>, Bing Tang<sup>1</sup>, Thuy T. Le<sup>1</sup>, Yin Xiang Setoh<sup>5</sup>, Alexander A Khromykh<sup>2,5</sup>,  
Jody Hobson-Peters<sup>5</sup>, Kerrilyn R. Diener<sup>3,6</sup>, Paul M. Howley<sup>7</sup>, John D. Hayball<sup>3,6</sup> & Andreas Suhrbier<sup>1,2</sup>

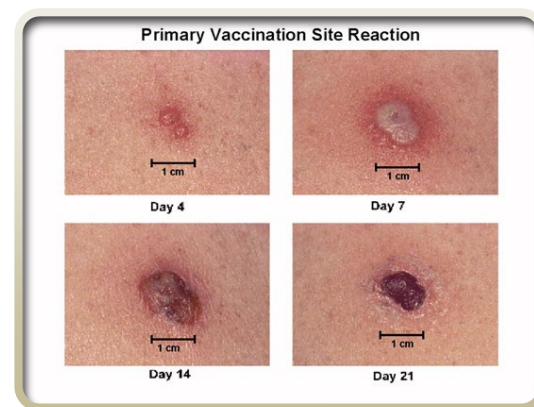
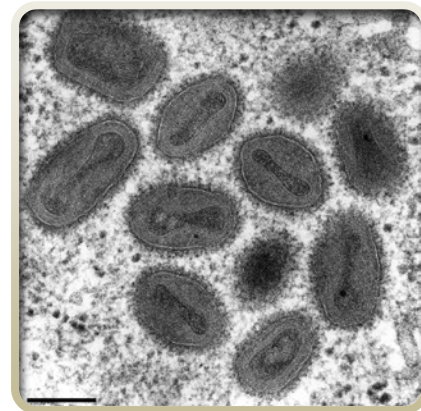


**Dr Natalie Prow**



# Vaccinia virus as a vaccine vector

- **Vaccinia virus (VACV)**
  - VACV vaccination successful eradicated smallpox
  - Good vaccine delivery vector
    - Highly immunogenic – induce life-long immunity (cell mediated and humoral)
    - Large cloning capacity > 25kb
    - Minimal risk of host gene integration
    - **Can induce severe side effect!**



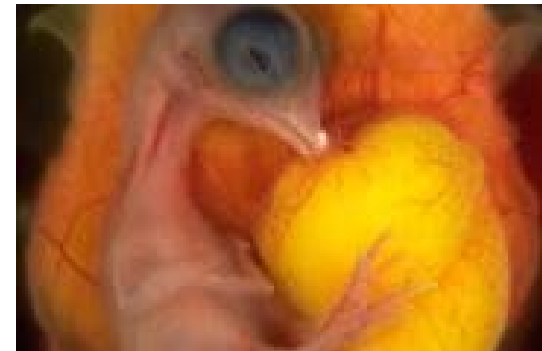
- **Attenuated VACV**

- Modified vaccinia Ankara (MVA)
  - Highly attenuated
  - *IMVAMUNE* – Smallpox vaccine
    - Approved in Canada and in the European Union
    - Phase 3 registration trials concluded in the U.S.
- New York vaccinia (NYVAC)

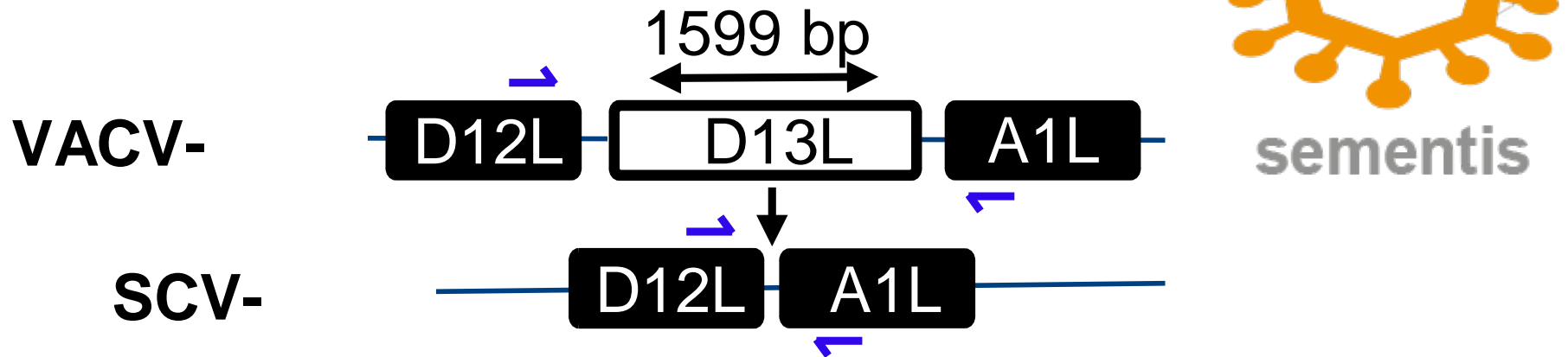


- **Room for improvement**

- Replication-defective by mutating host range factors
- Lower immunogenicity
- Require multiple boosts
- Production cell substrate
  - CEF: primary chicken embryo fibroblasts
  - Vero: adherent cell substrate



## Sementis Copenhagen Vector (SCV)



Remove *D13L* from Copenhagen strain of vaccinia virus (VACV) to produce SCV.

The *D13L* encodes D13 an essential viral assembly protein



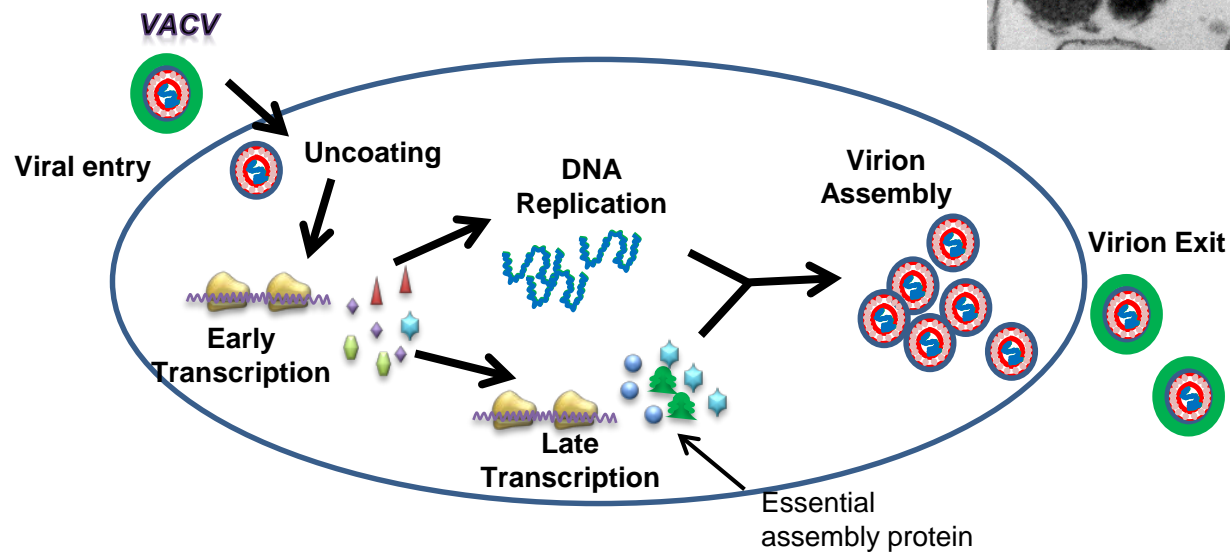
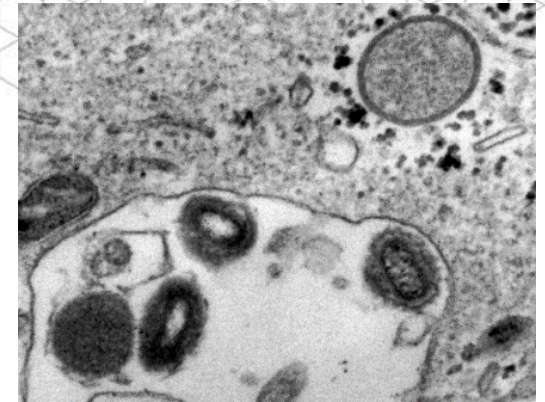
**MVA**; 572 passages,  
many deletions of genes with  
unknown functions

vs

**SCV**; 1 genetically  
defined deletion of a  
gene with a known  
function

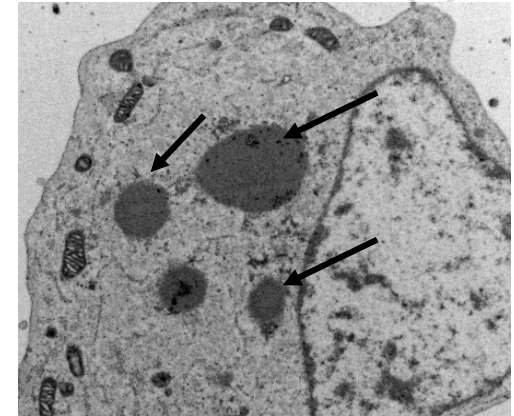
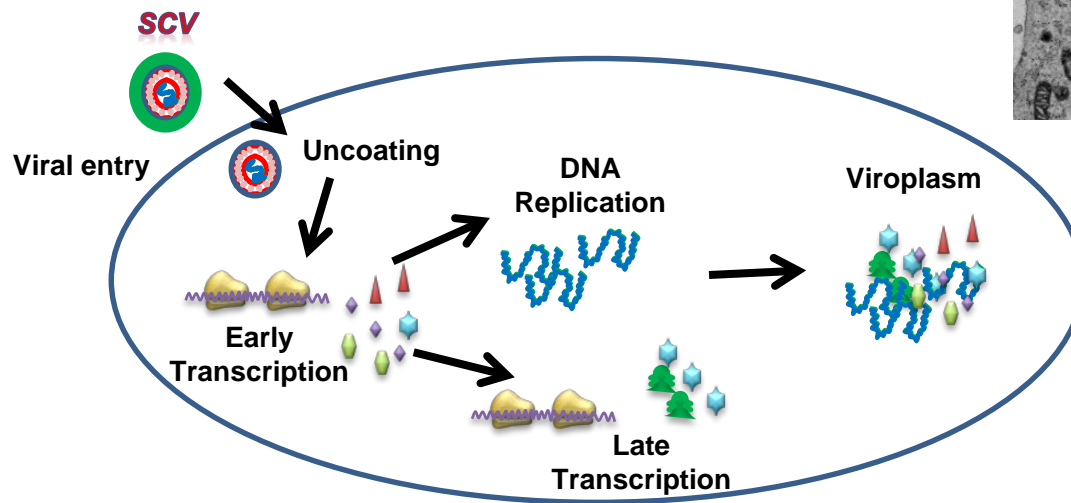
Dr Paul Howley CSO/CEO Sementis Ltd

# VACV replication cycle





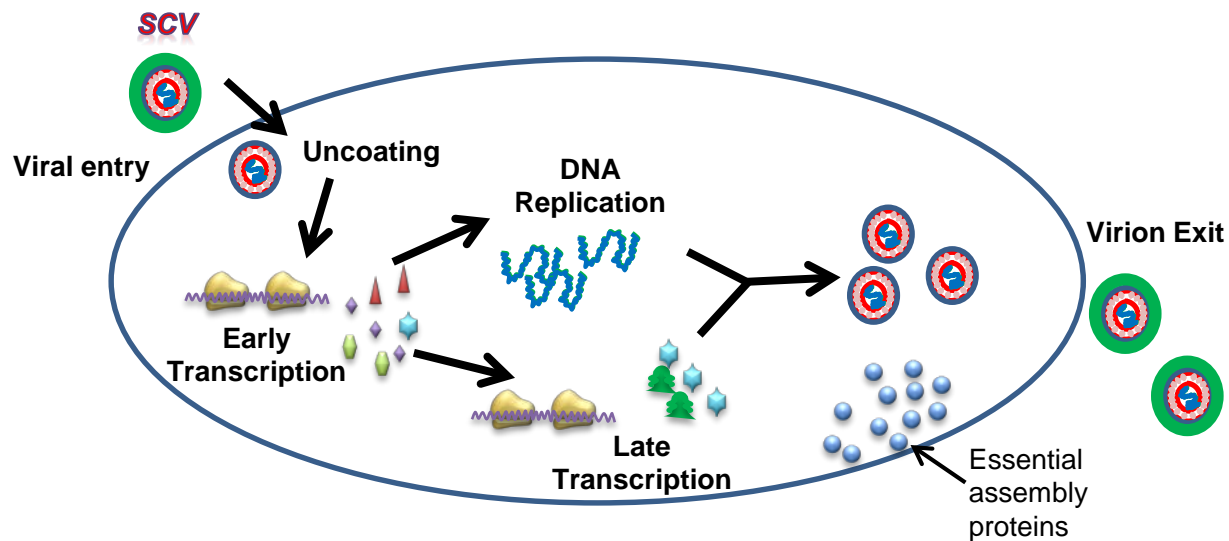
# Concept of SCV platform



**SCV cannot replicate in host cells**

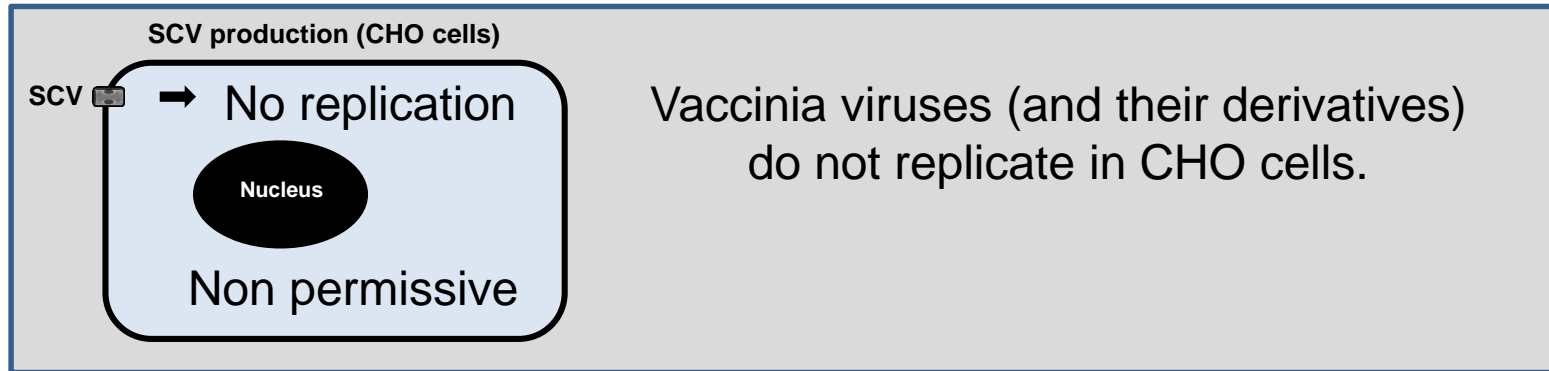
Eldi et al/ Mol Ther. 2017 25(10):2332-2344.

# Production of SCV in Rescue Cell



**SCV RESCUE CELL**

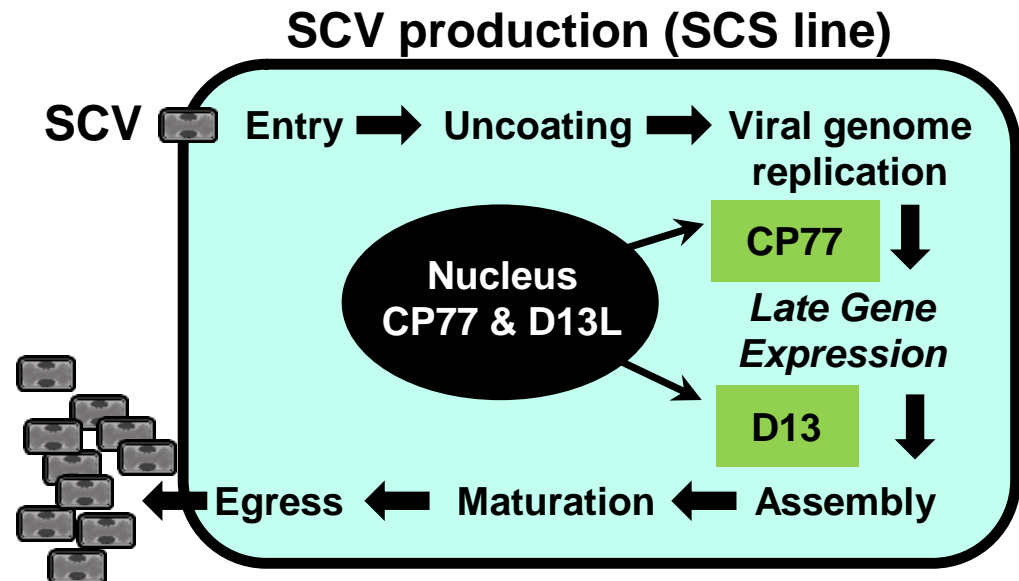
# SCV is manufactured in CHO cells (chinese hamster ovary) Pharma's manufacturing "work-horse"



SCV will replicate in CHO cells  
expressing

-CP77 (host range protein) &  
-D13L (assembly protein deleted  
from SCV).


= SCV Cell Substrate  
(SCS) line





# SCV Rescue cell line (SCS)

- **SCV Rescue cell line**  
**CHO cell --- first choice**

- 
- Bio-pharmaceutical friendly
  - Easy to genetically manipulate
  - High bio-production yields
  - Suspension culture ready
  - Serum-free medium ready
  - Rapid scale-up



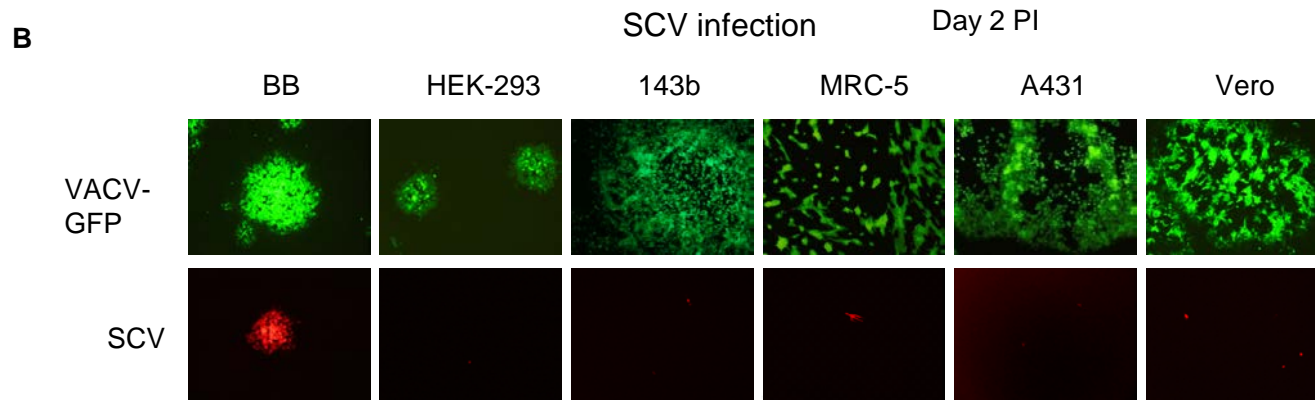
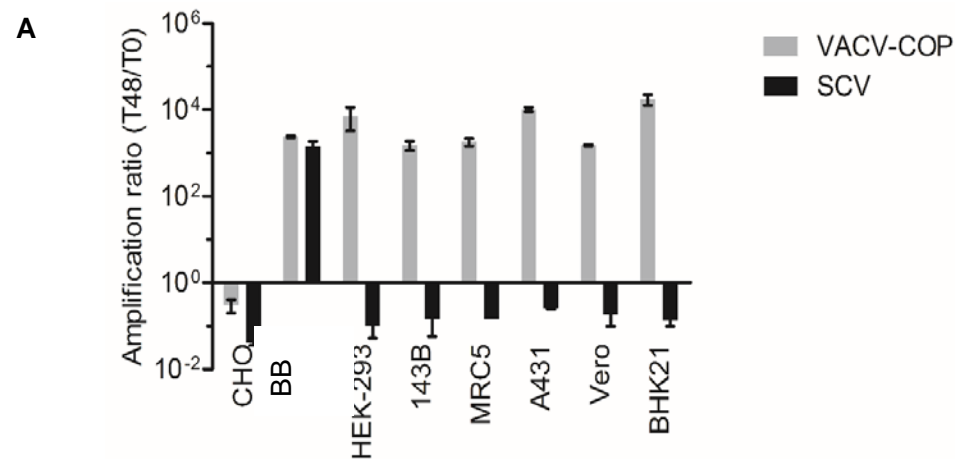


## No more eggs/CEFs!

CHO cell routinely  
used by Pharma for  
production of  
biologics.

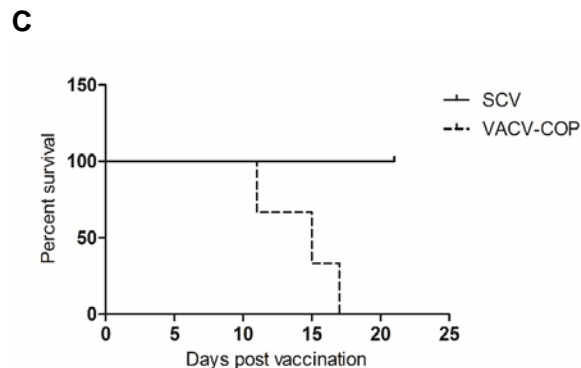
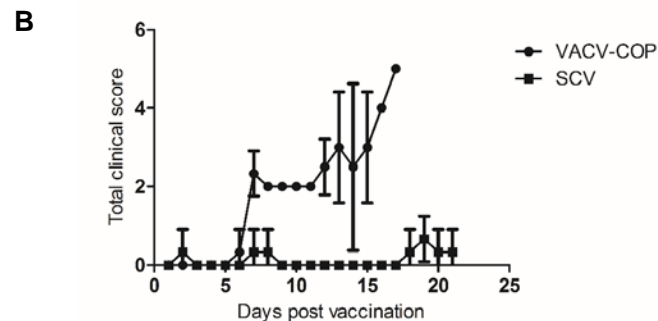
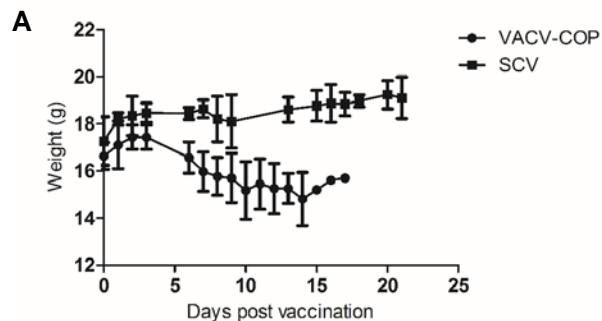
**The NIAID (USA) is  
producing a GMP SCS  
line for SCV production  
funded by the “NIAID  
preclinical services  
program; Master Cell  
Bank” scheme.**

# *In vitro* attenuation of the SCV



## SCV vector Is Safe

# SCV was well tolerated by severe immunocompromised mice (SCID)



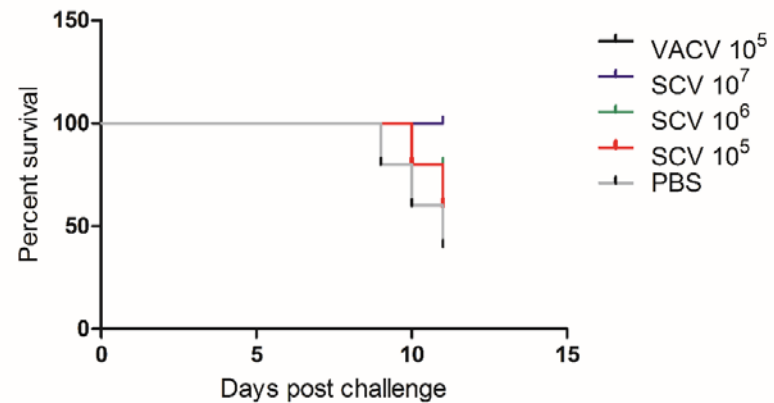
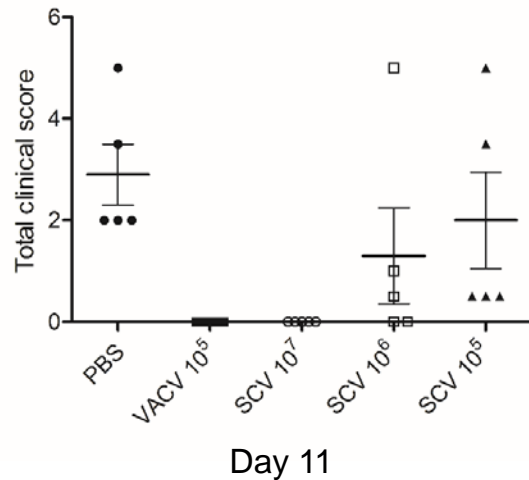
**D**

		Day1		Day3		Day5		Day9		Day15		Day21	
		IVP	PCR	IVP	PCR	IVP	PCR	IVP	PCR	IVP	PCR	IVP	PCR
Lung	VACV	+		+		+		+		+		+	
	SCV	-	+	-	+	-	-	-	-	-	-	-	-
Kidney	VACV	+		+		+		+		+		+	
	SCV	-	+	-	+	-	-	-	-	-	-	-	-
Spleen	VACV	+		+		+		+		+		+	
	SCV	-	+	-	+	-	-	-	-	-	-	-	-
Ovaries	VACV	+		+		+		+		+		+	
	SCV	-	+	-	+	-	+	-	+	-	-	-	-

- IVP: Infectious viral particle.
- +: Infectious viral particle was detected by plaque assay.
- -: No infectious viral particle was detected by plaque assay or viral DNA by PCR assay.

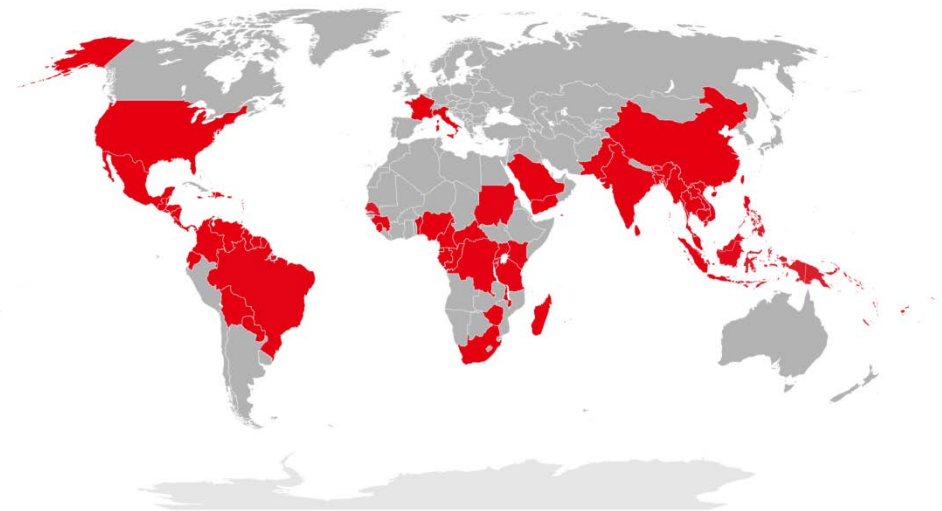
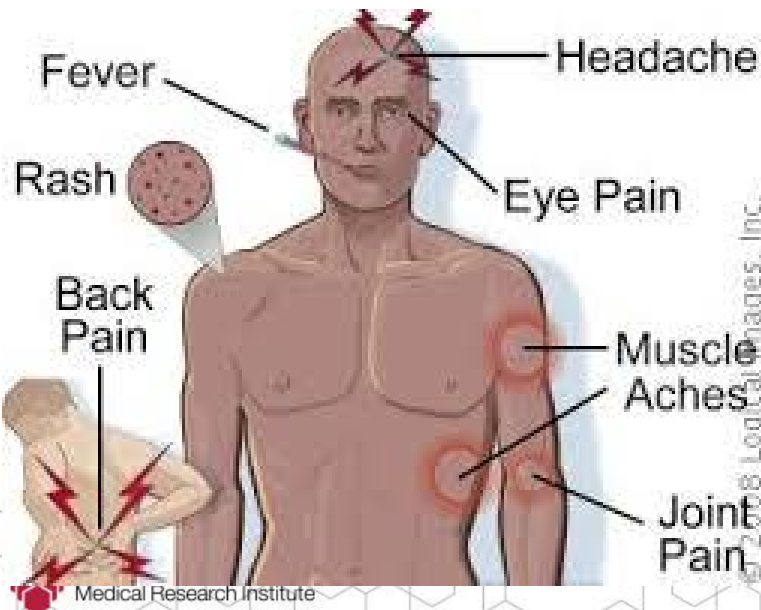
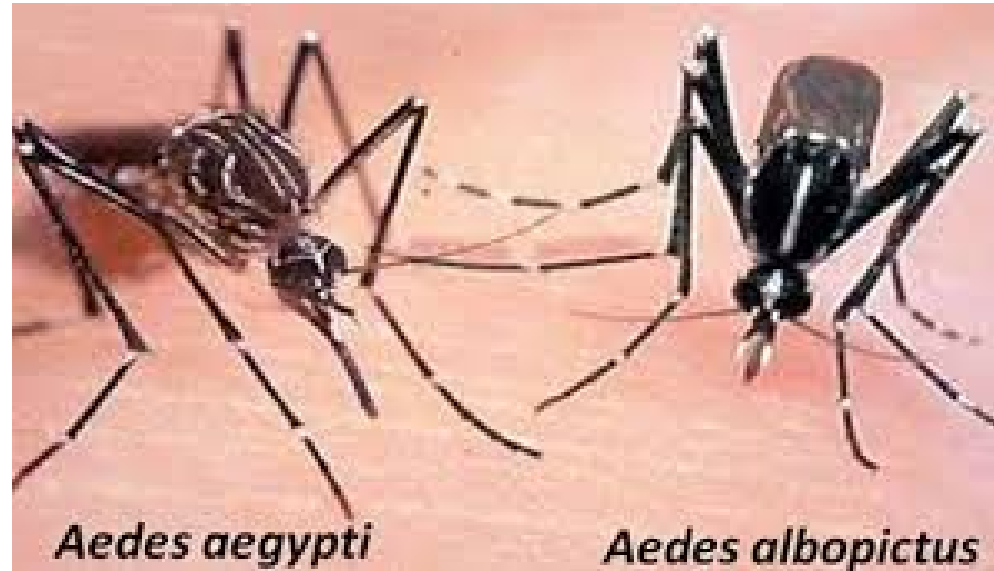
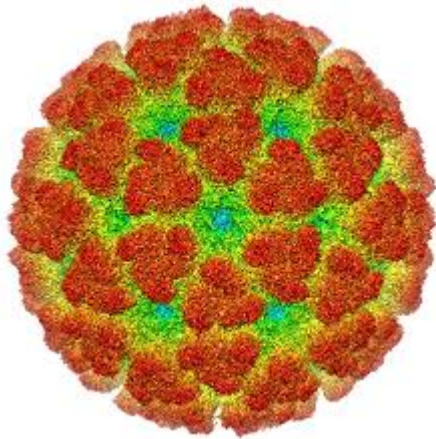
# SCV as Smallpox vaccine

- SCV vaccination protect mice challenge





# The Test Case: Chikungunya virus vaccine





**CHIKV  
reached  
the  
Americas  
in late  
2013**

**As of Dec 2017**

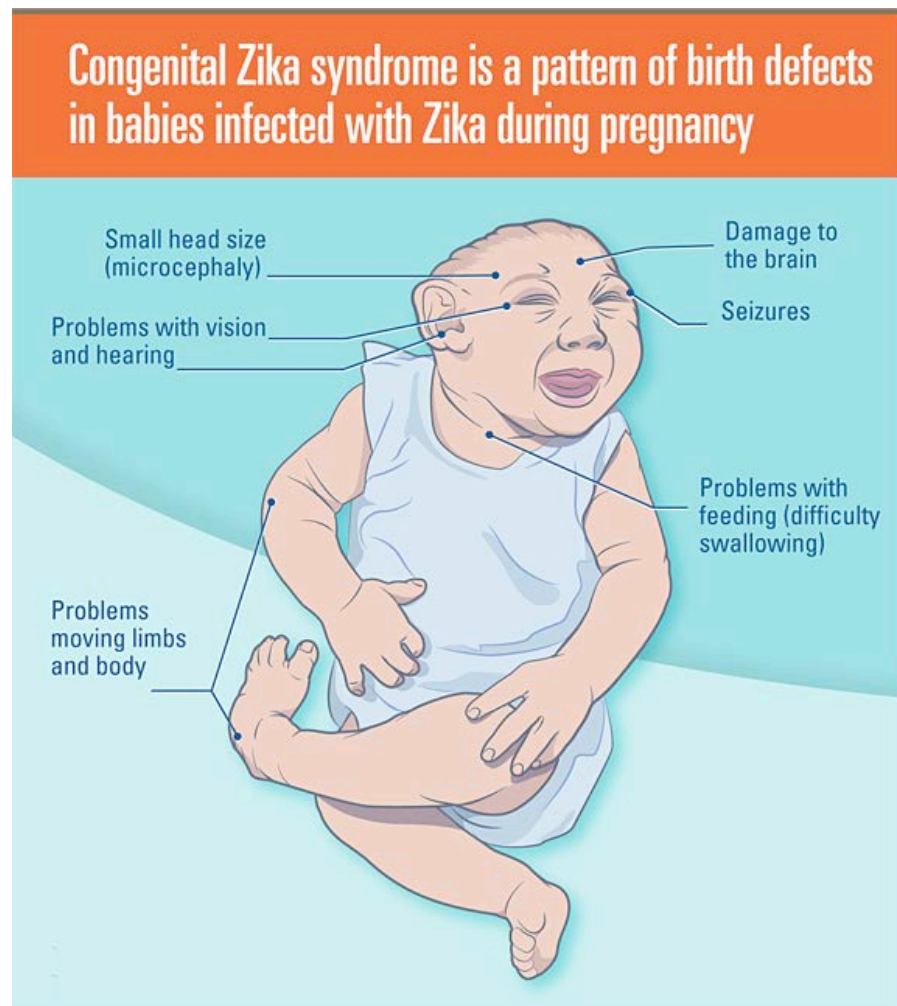
**> 1 million cases**

**>13 deaths**

PAHO WHO

# Zika virus – a new challenge

- *Flaviviridae, Flavivirus*
- Main vector: *Aedes aegypti*
- 500,000 to 1 million infections (Brazil)
- WHO - ZIKV pandemic declared public health emergency of international concern in 2016
- Diverse collection of congenital, neurological symptoms including microcephaly and intrauterine growth restriction (IUGR)
- Predominantly associated with infection during the 1<sup>st</sup> and 2<sup>nd</sup> trimesters





# A vaccinia-based single vector construct multi-pathogen vaccine protects against both Zika and chikungunya viruses

Natalie A. Prow<sup>1,2</sup>, Liang Liu<sup>3</sup>, Eri Nakayama<sup>1,4</sup>, Tamara H. Cooper<sup>3</sup>, Kexin Yan<sup>1</sup>, Preethi Eldi<sup>3</sup>, Jessamine E. Hazlewood<sup>1</sup>, Bing Tang<sup>1</sup>, Thuy T. Le<sup>1</sup>, Yin Xiang Setoh<sup>5</sup>, Alexander A Khromykh<sup>2,5</sup>, Jody Hobson-Peters<sup>5</sup>, Kerrilyn R. Diener<sup>3,6</sup>, Paul M. Howley<sup>7</sup>, John D. Hayball<sup>3,6</sup> & Andreas Suhrbier<sup>1,2</sup>

## WHY TARGET ZIKV & CHIKV IN ONE VACCINE?

- Both ZIKV & CHIKV transmitted by *Aedes aegypti* (*albopictus*)
- ZIKV & CHIKV can be co-transmitted in one mosquito
- ZIKV & CHIKV outbreak zones geographically overlap
- ZIKV & CHIKV often co-circulate
- ZIKV & CHIKV co-infections in one patient well documented
- ZIKV & CHIKV both often confused with dengue.

- Single manufacturing process
- Avoids mixing issues

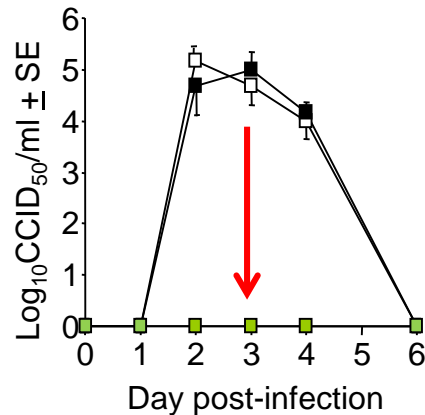
- Reduces “shot burden”



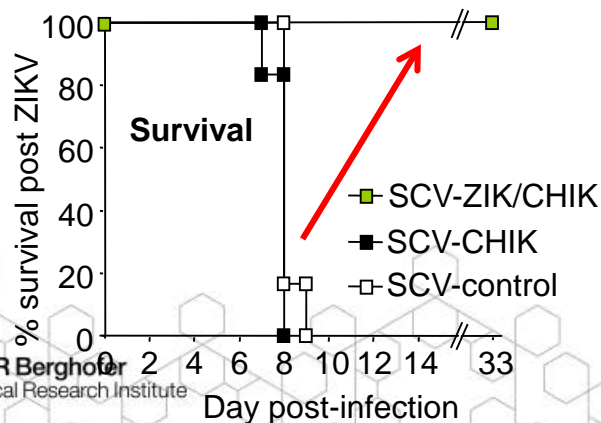
# A single dose of SCV-CHIKV+ZIKV protects against infection with both diseases

- in IFNAR mice

## Protection against viraemia

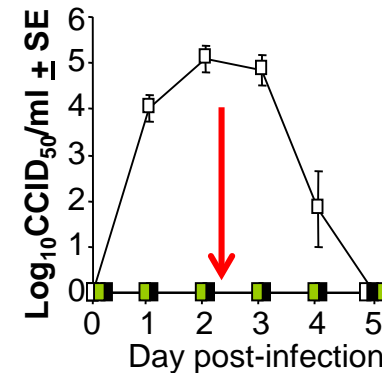


## Protection against lethal ZIKV<sub>MR766</sub>

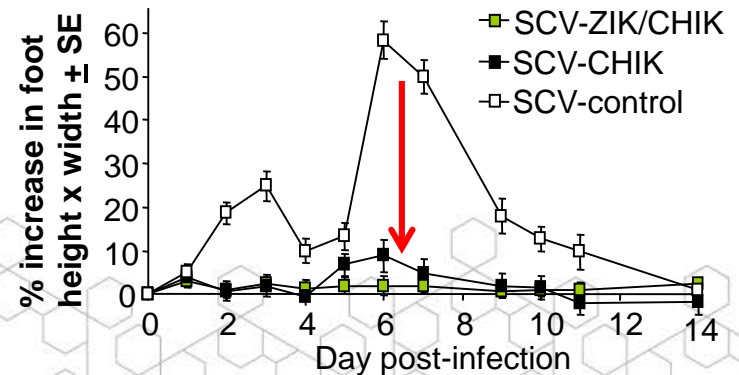


- in C57BL/6 mice

## Protection against viraemia

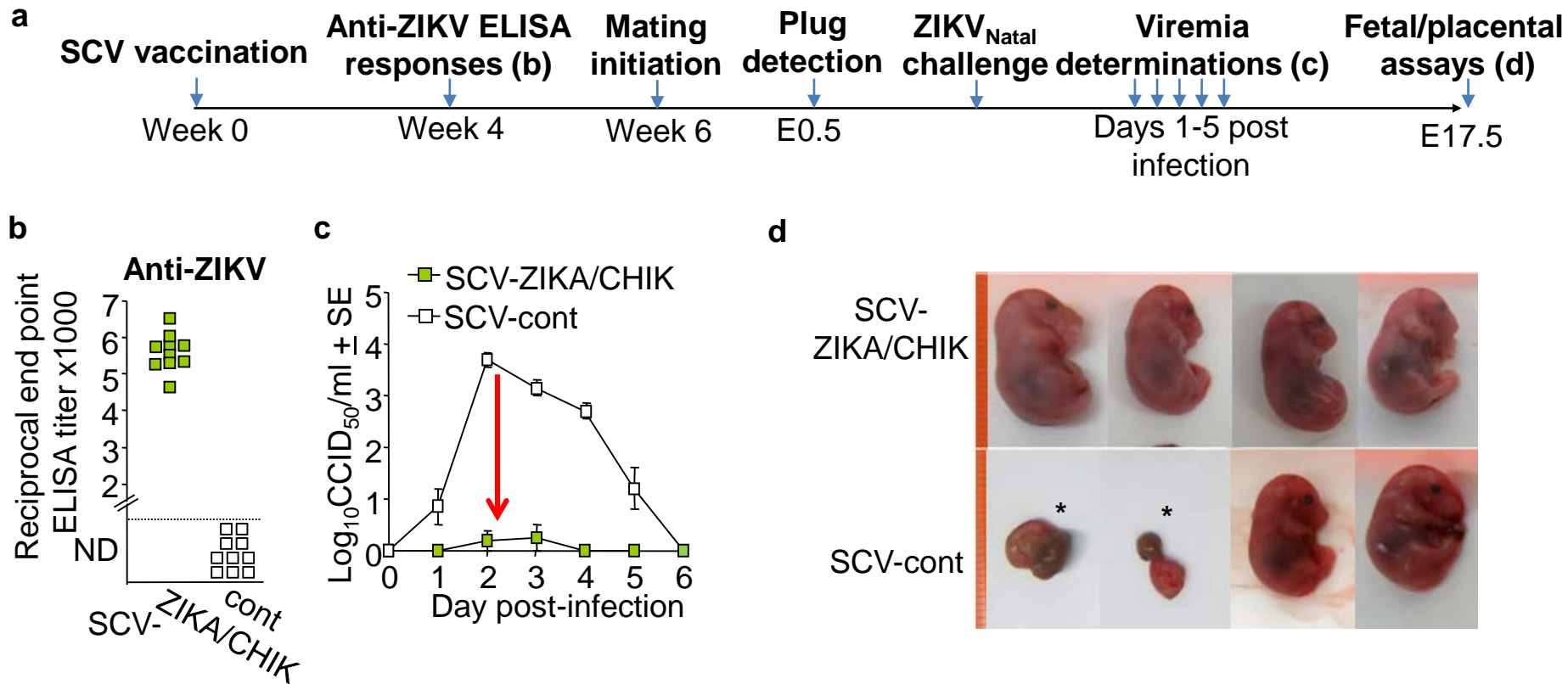


## Protection against CHIKV arthritis



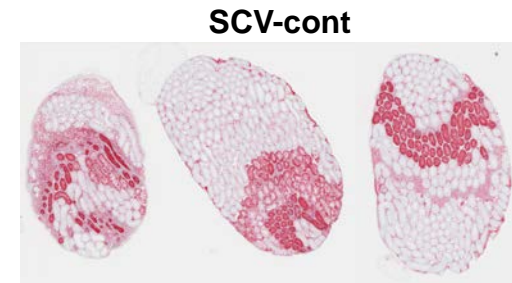
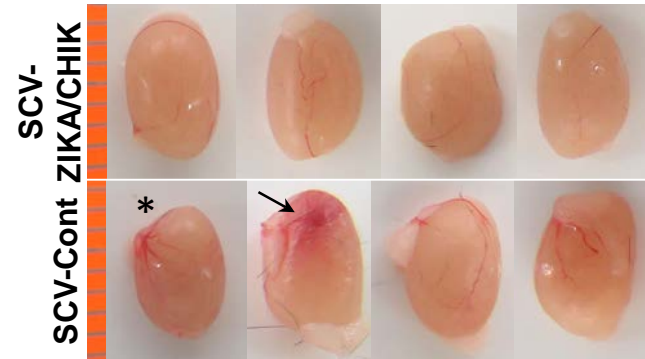
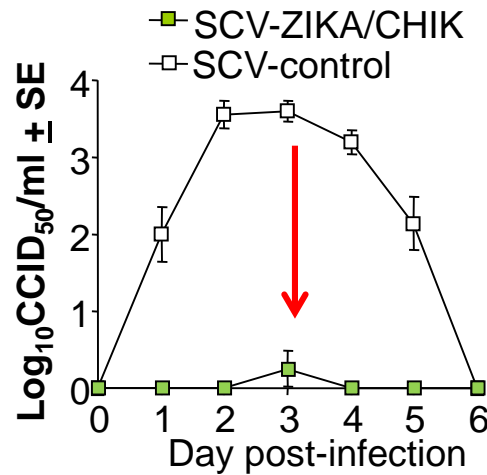
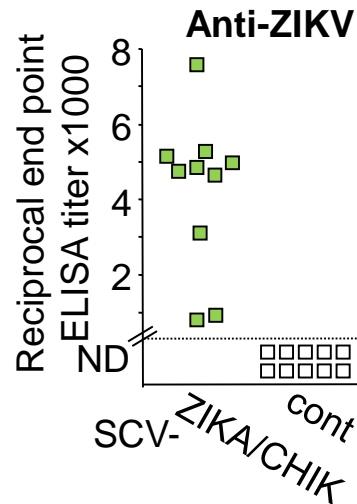


# A single shot vaccination protects against detrimental foetal outcomes



Prow et al., Nat Commun. 2018;9(1):1230.

# A single shot vaccination protects against testicular damage



Prow et al., Nat Commun. 2018;9(1):1230.

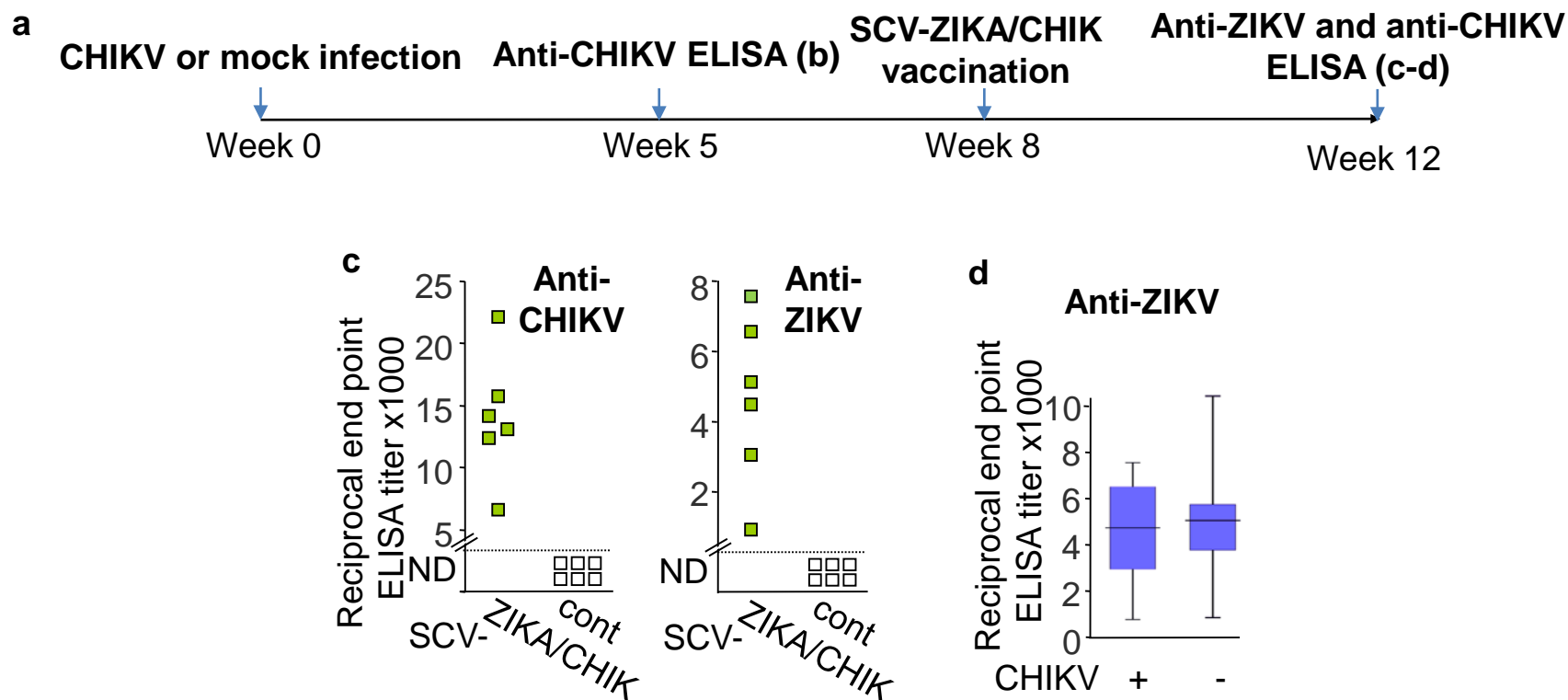


**Prior infection?**

**Vector immunity?**

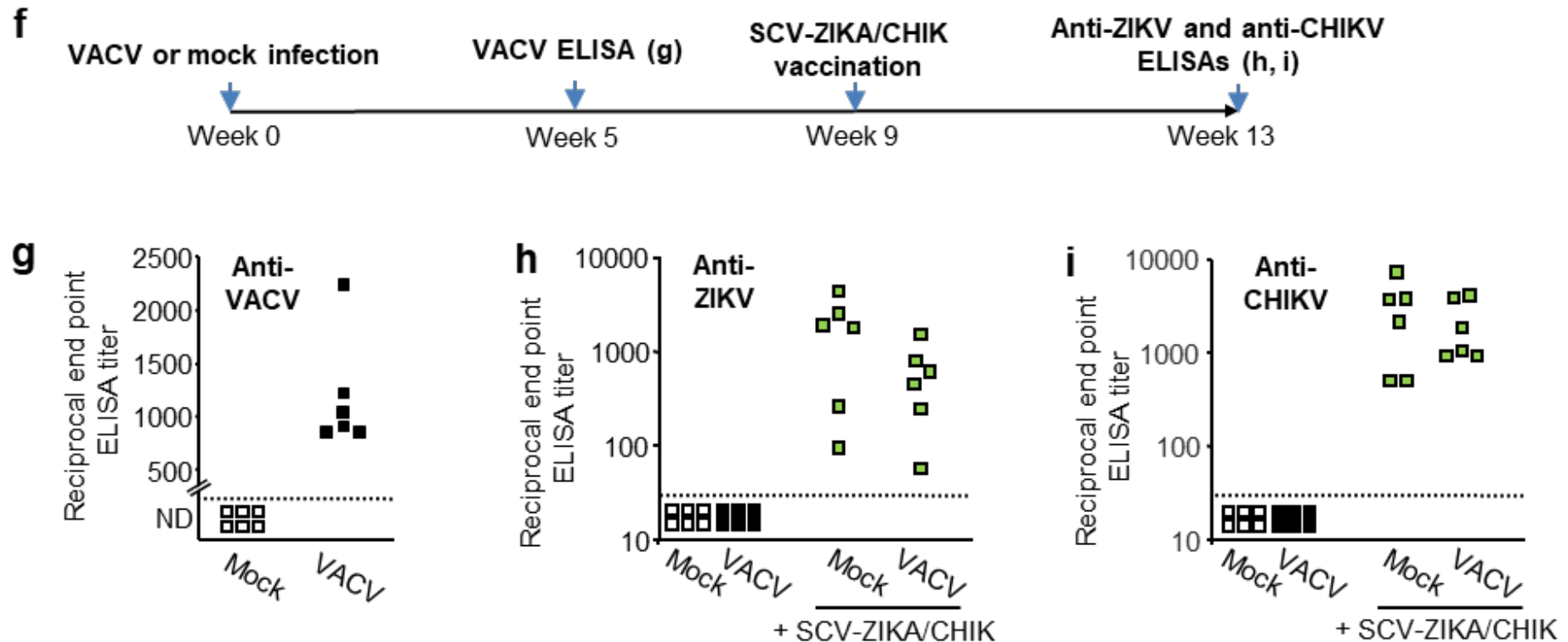
**Antigenic interference?**

# Prior infection does not affect induction of antibody responses post-vaccination



Prow et al., Nat Commun. 2018;9(1):1230.

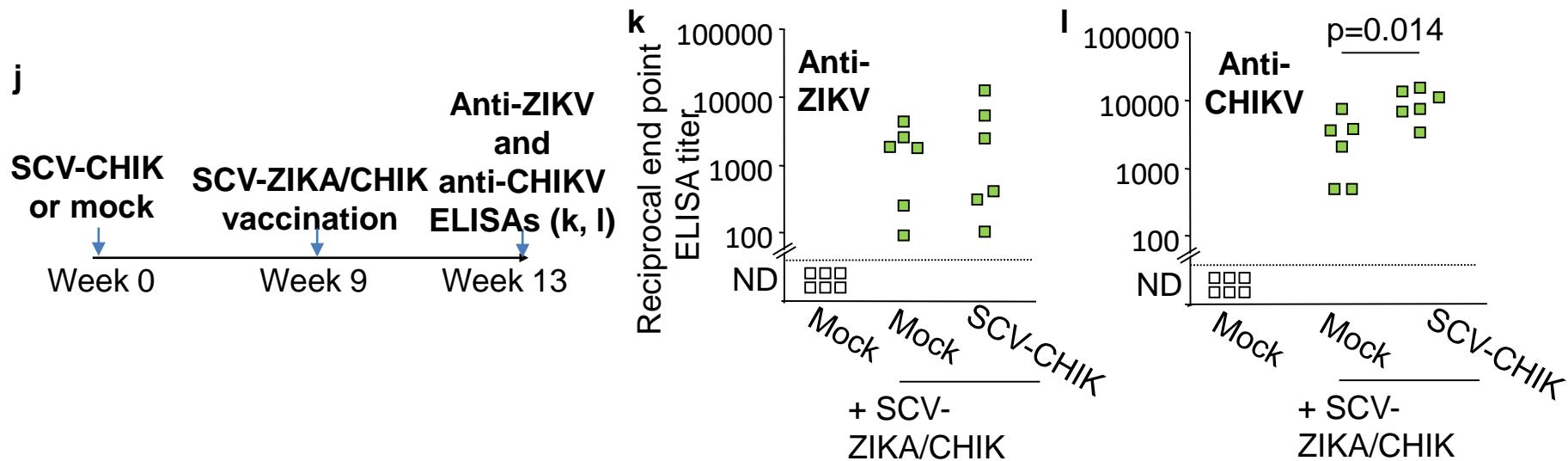
# Prior infection does not affect induction of antibody responses post-vaccination



Prow et al., Nat Commun. 2018;9(1):1230.



# Prior vaccination doesn't prevent induction of antibodies



Prow et al., Nat Commun. 2018;9(1):1230.

**a** SCV vaccination (C57BL/6 females) Anti-ZIKV ELISA responses (b)

Week 0 Week 8

A horizontal timeline with a black arrow pointing from left to right. A blue downward arrow points to the start of the timeline, labeled 'Week 0'. Another blue downward arrow points to the end of the timeline, labeled 'Week 8'.



# Summary

- **SCV represents a safe vaccine platform**
- **SCV can protect against lethal Ectromelia infection**
  - Can act as a smallpox vaccine
- **SCV-ZIKA/CHIK vaccination can afford protection in adult CHIK and ZIKA preclinical models**
- **SCV-ZIKA/CHIKV vaccination can afford protection in Zika pregnancy and testis preclinical models**
- **No evidence of antigenic interference or adverse effects due to vector immunity or prior infection**
- **Multi-disease vaccine is as effective as single vaccine**



## NIAID (USA) is

- producing a **GMP SCS** production cell line (CHO-based) required for vaccine manufacture funded by the “NIAID preclinical services program; Master Cell Bank” scheme.
- Non-human primate (**NHP**) studies funded by “NIAID preclinical services program: Task Order; Zika virus vaccine evaluation in NHPs”. (Southern Research)

# Summary of Non Human Primate (NHP) Study Protocol

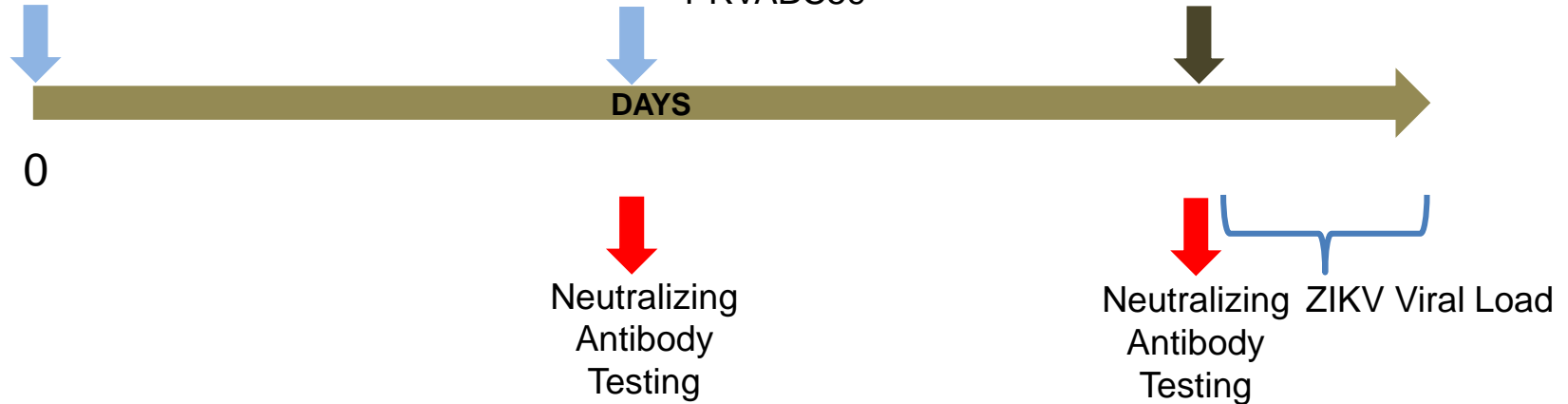
## Vaccination

- SCV-CHIKV-ZIKV
- PRVABC59
- SCV

## Booster Vaccinations

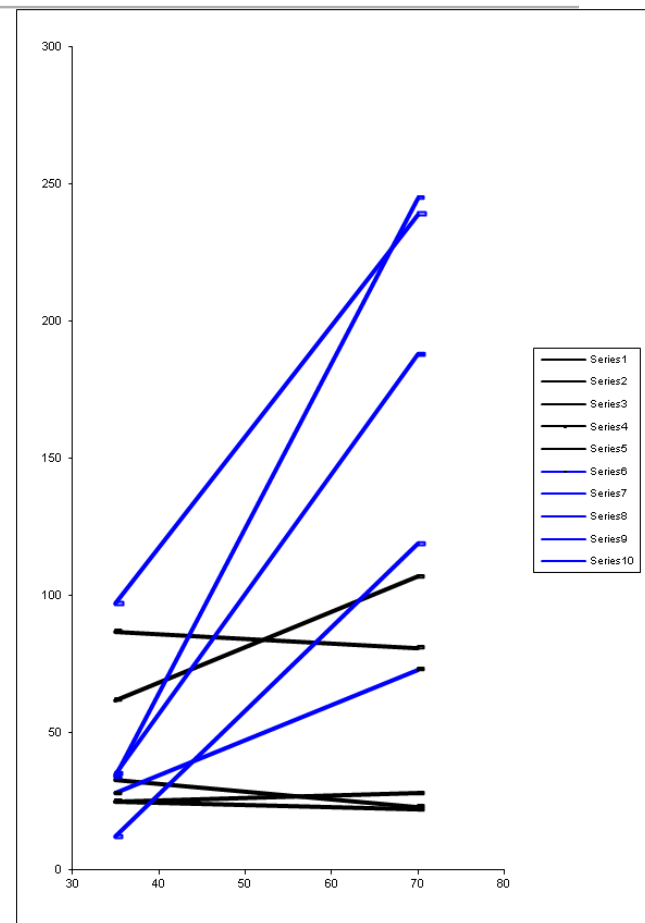
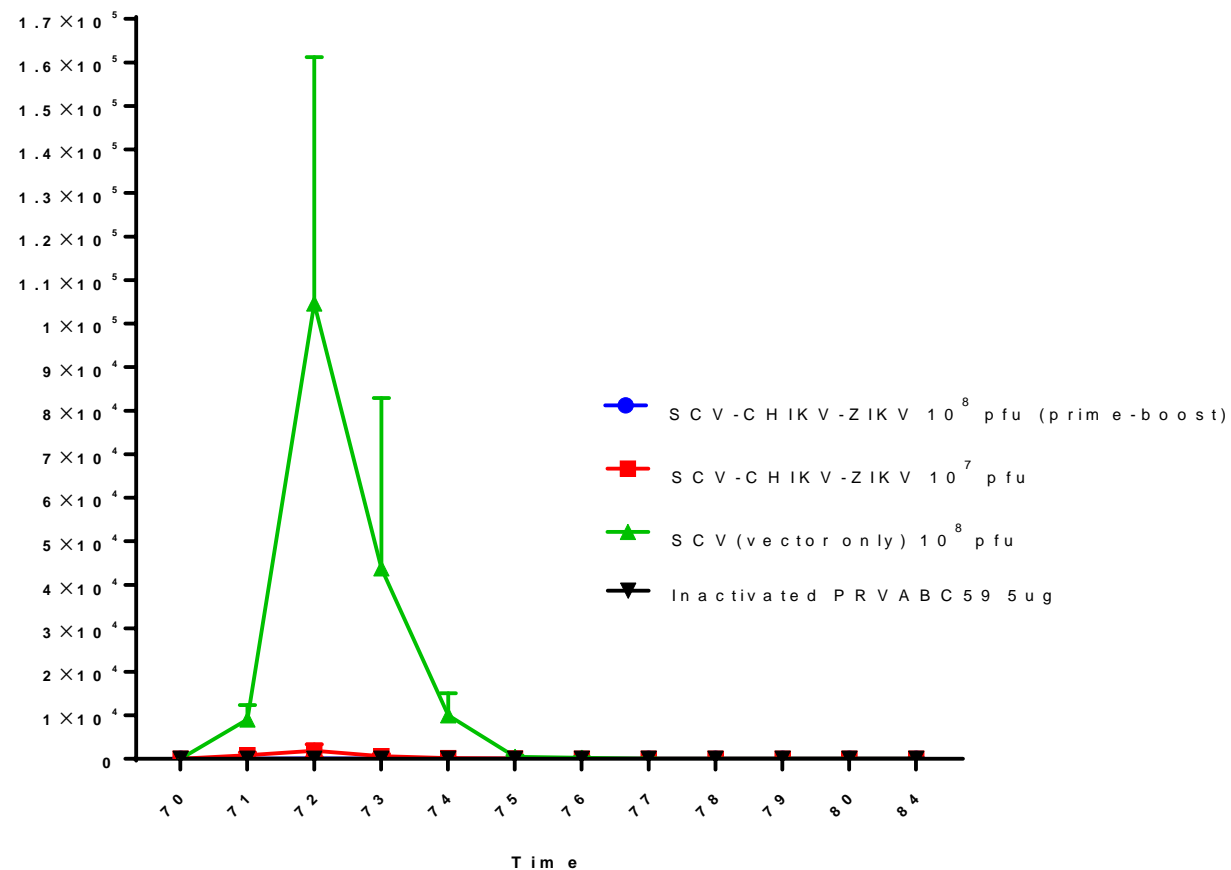
- SCV-CHIKV-ZIKV
- PRVABC59

## ZIKV Challenge



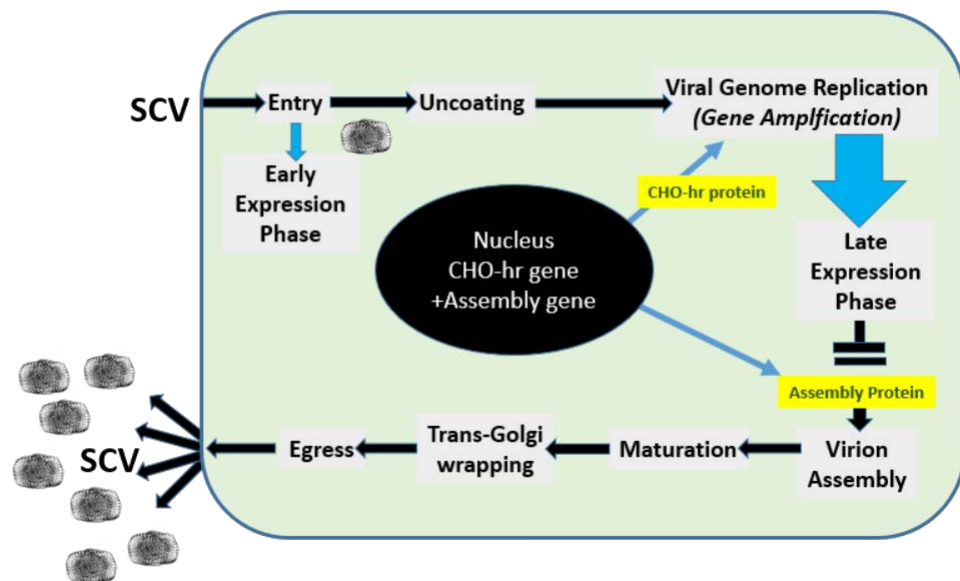


# Preclinical evaluation of SCV-CHIKV-ZIKV in NHP



# SCV Cell Substrate for Manufacturing

## SCV Production CHO Cell Line

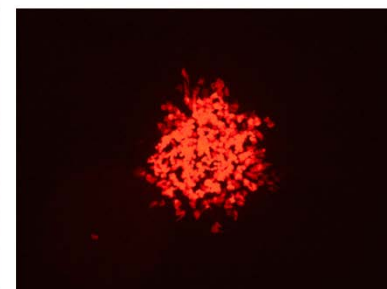


## Infection with SCV

(Totally attenuated SCV expressing Red Fluorescent Protein)



CHO



SCV Rescue cell line

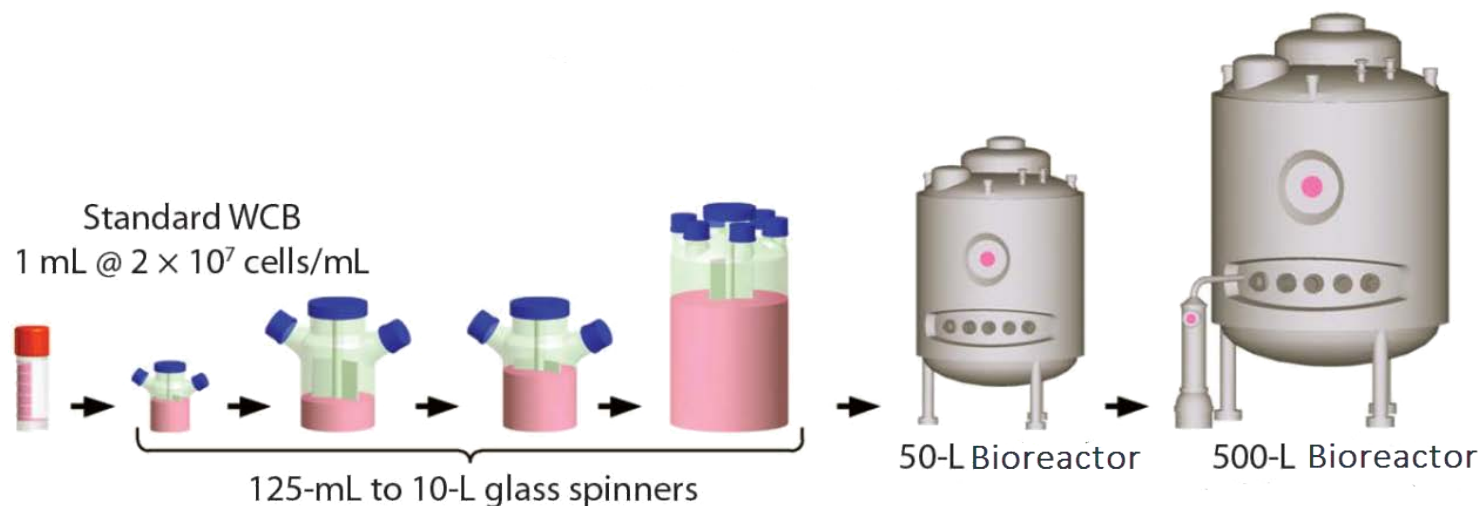
Sementis' SCV-cell substrate for manufacturing was derived from GMP produced CHO-S cell line :

- Sourced as a GMP produced batch of CHO-S from Life Technologies (also known as ThermoFisher Scientific), Cat # A1136401, royalty free, one off licence fee per field, ie, infectious diseases, immunotherapeutics
- Suspension cell line – suitable bioreactor production
- Cultured in serum-free chemically defined medium, eg, CD-CHO medium from Life Technologies, Cat # 10743029

# Scaling Up to 500L bioreactor

## Upstream Production Process: SCV Vaccines Production From Bioreactors

- Cell expansion rate of 1:10



# Up-scaling: expected vaccines yield assuming 50% recovery

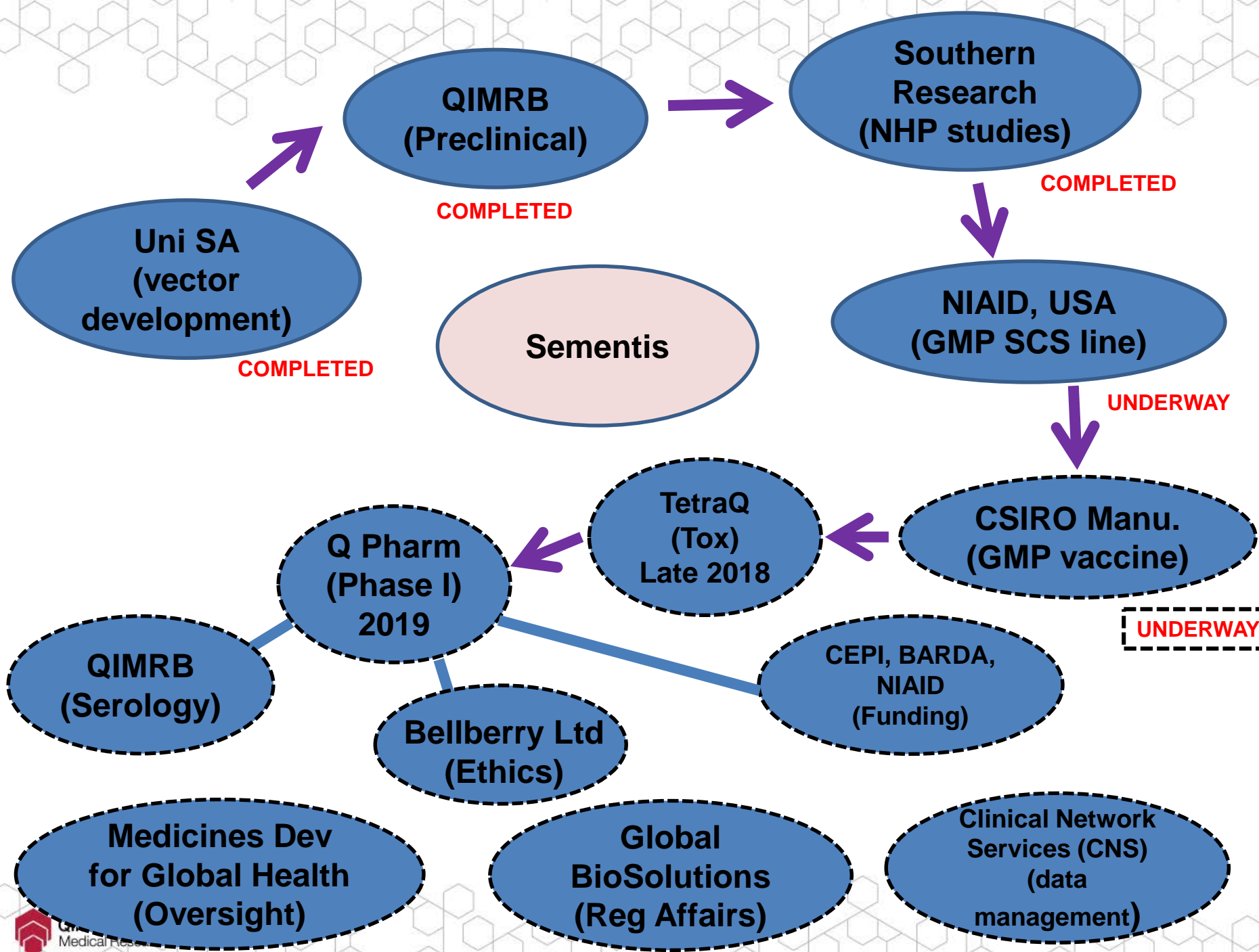
## BC19A-12 (SCV cell substrate derived from GMP-CHO-S cell line)

**Productivity = ~10,000 pfu per 1 pfu of inoculum at 3 days post infection**

(actual  $\bar{x}$  = 12,500)

(cell line efficiency = ~100 pfu/cell (actual  $\bar{x}$  = 125))

Production Scale ( $10^7$ cells/mL)	N° Doses @ $10^8$ pfu/dose	N° Doses @ $10^7$ pfu/dose
1L	~5,000	~50,000
10L	~50,000	~0.5 million
100L	~0.5 million	~5 million
1,000L	~5 million	~50 million



## **QIMR-B**

Andreas Suhrbier  
Bing Tang  
Thuy Le  
Jessamine Hazlewood  
Kexin Yan



John Hayball  
Liang Liu  
Preethi Eldi  
Tamara Cooper

**Sementis Ltd**  
Paul Howley



Kerrilyn Diener



Alex Khromykh  
Yin Xiang Setoh  
Nias Peng



Kevin Walters  
Raj Kalkeri  
Fusataka Koide



**QIMR Berghofer**  
Medical Research Institute



Eri Nakayama

## **Funding**



National Institute of  
Allergy and  
Infectious Diseases



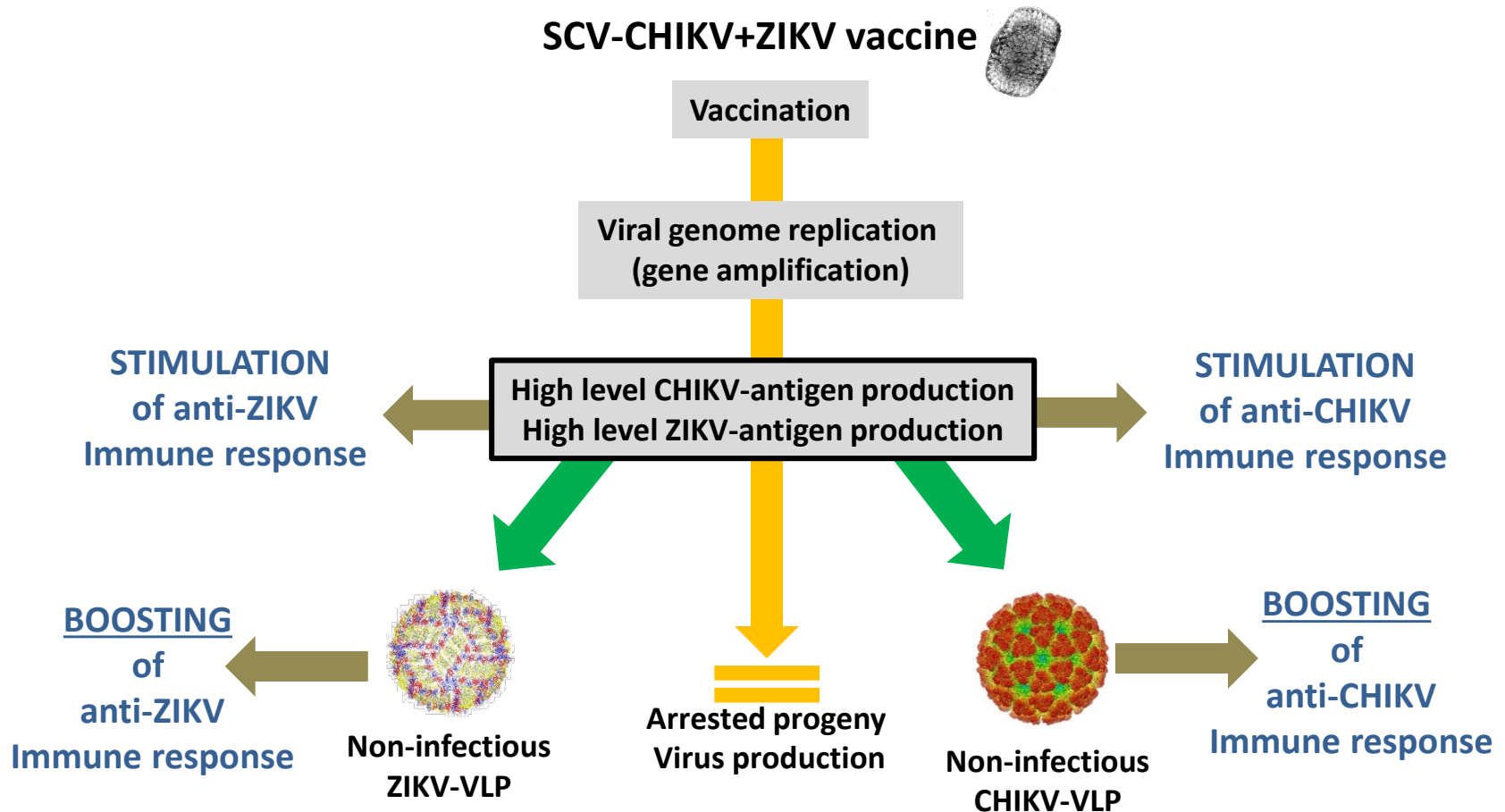
**sementis**



**ADVANCE**  
QUEENSLAND



# How SCV-CHIKV+ZIKV Vaccine Works



# Addressing stability issues with large inserts

