



BioPharma India 2017 Presentation

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Novel Vector and Cell-line for Producing Vaccines

Sementis' SCV Vector and CHO Rescue Cell-Line Technology

An Australian vaccine company building a pipeline of vaccines and immunotherapies

Overview

- Sementis Limited is an Australian unlisted public biotechnology company that was formed in 2011
- The company is applying its proprietary Sementis Copenhagen Vector (**SCV**) platform technology and a **novel production cell substrate** to generate a portfolio of innovative vaccine candidates for prevention of infectious diseases and treatment of allergies and cancers
- The SCV system is a novel vector platform that uses a **multiplication-defective** vaccinia virus to stimulate the full immune response.
- The genes for **disease antigens** are engineered into the SCV genome, which, upon vaccination, elicits a potent immune response to the disease state
- The SCV system enables **scalable** manufacture

Sementis Strategy: Optimizing the SCV technology



Core Technology

- Non-multiplying live viral vector platform (SCV)
- Novel production method (SCV-CHO cell substrate)



Allergies

- Therapeutic vaccination approach
- Desensitization by switching allergen-specific Th2-allergic response to allergen-specific Th1 benign immune response

Peanut

Cat



**Ready for
clinical
development**



**In Pre-clinical
Proof-of-concept
phase**



Cancers

- Therapeutic vaccination approach
- Breaking immunological tolerance to Prostate specific antigens

Prostate



Antigen design phase



Mosquito borne diseases

- Prophylactic Protective vaccines
- Induction of protective immunity

Chikungunya

Zika



Ready for clinical development



The SCV Platform System

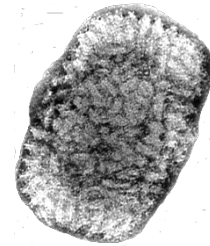
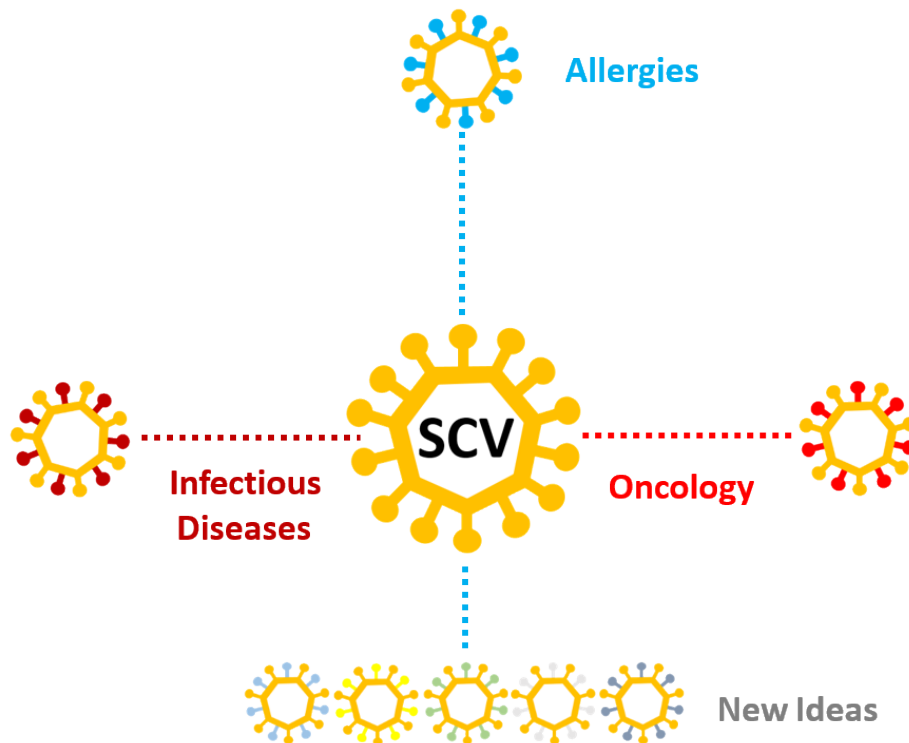
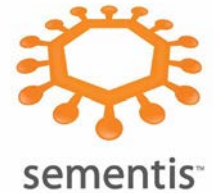
Vector Specifications:

- Highly immunogenic
- Genetically stable
- Easy to genetically manipulate
- Titres to high levels

Cell Substrate specifications:

- Biotechnology friendly
- Cultures to high cell density as suspension cells
- Grows in chemically defined medium

Sementis propriety SCV platform technology



Vaccine Delivery Vehicle (SCV Vector):

Non-multiplying smallpox vaccine that can be engineered to make ANTIGENS from disease targets to raise immunity to that disease

Non-replicating vaccine vector system

Manufacturing Cell Substrate:

The **CHO** biotechnology friendly cell substrate engineered to produce the SCV vector

A first for the production of vectored vaccines!

Principles of the SCV approach

Live viral vector that offers:

- the properties of attenuated vaccine
- unable to multiply upon vaccination providing the added safety of inactivated vaccines
- Accommodate multiple antigens to give the broad spectrum of subunit vaccines

A manufacturing process scalability by using a proprietary genetically engineered suspension cell substrate to produce all SCV vaccines

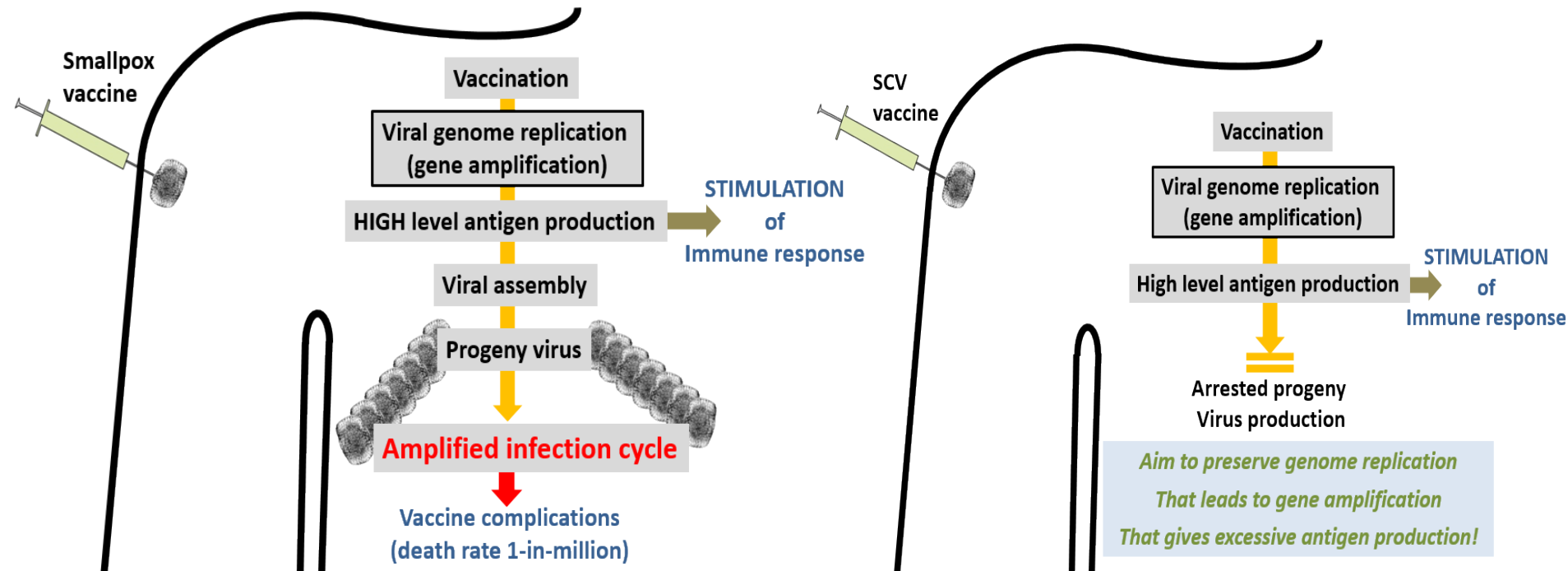
The SCV cell substrate for manufacturing is CHO based – industry gold standard for production of biologicals with the following advantages:

- Biotechnology friendly: bioreactor and processes are standardize to CHO cells
- Fastest growing cell substrate, important for upscaling
- Suspension culture requiring low surface area to volume culturing systems
- Most characterised cell line used for the production of biopharmaceuticals
- Well know and understood by medical control agency around the word (TGA in Australia, FDA in USA, EMEA in Europe, China, Japan, India etc)

How Does SCV Work: originated from Smallpox vaccine

Smallpox Vaccine (Live replication competent)

SCV Vaccine (Live NON-replication competent)



Unlike MVA where it has been attenuated, SCV has not been attenuated it's just lost an essential gene for viral assembly but maintains the immunological potency of the original smallpox vaccine

SCV does not multiply in human and mammalian cells lines



A study was carried to show that SCV does not multiple or propagate in cells derived from key organs of the body after deliberate infection:

	Vaccinia Virus (Parent of SCV)	SCV
Human Bone cell	High Yields of virus (as <i>expected</i>)	No virus production!
Human Lung cells		
Human Kidney cells		
Human Skin cells		
Human Cervical cells		

		Vaccinia	SCV
143B	Human Bone Cells		
MRC-5	Human Lung Cells		
HEK-293	Human Kidney Cells		
A431	Human Skin Cells		
HeLa	Human Cervical Cells		

SCV Technology – Safety

Biodistribution in SCID Mice

Objective

Study the dissemination of SCV (using Sementis' SCV-vectored Chikungunya vaccine as an example SCV (SCV305)) in the absence of a host antiviral immune response when severely immune deficient SCID mice are given a high dose.

In SCID mice the humoral and cellular immune systems fail to mature. Therefore, SCID mice have an impaired ability to make T and B lymphocytes, or activate some components of the complement system and **cannot** efficiently fight vaccinia virus infections.

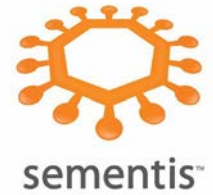
In this study, SCID mice were monitored for:

- loss of body weight and survival
- infectious virus recovery from key organs.

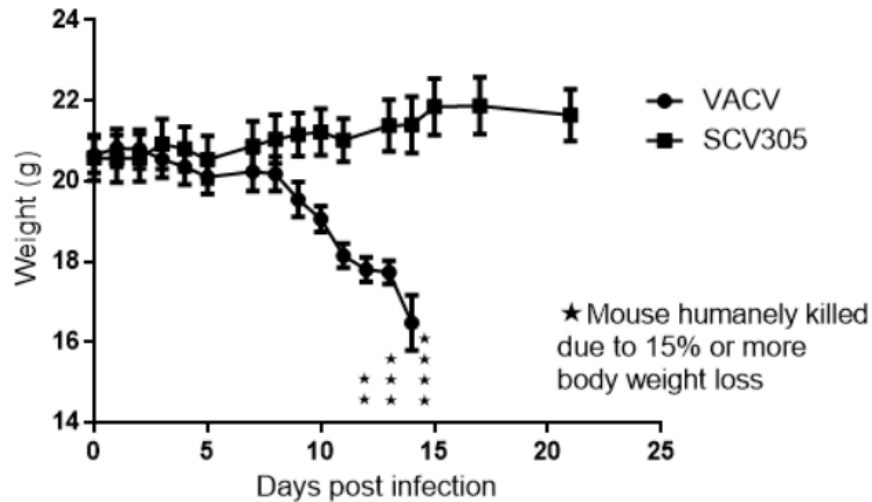
As a positive control, a separate group of SCID mice was infected with a similar dose of replication competent vaccinia virus, Copenhagen strain, known to disseminate in SCID mice.

Test Viruses: VACV-COP at 10^7 pfu/mouse injected IP
SCV (SCV305) at 10^7 pfu/mouse injected IP

SCV Technology – Safety (Biodistribution in SCID Mice)

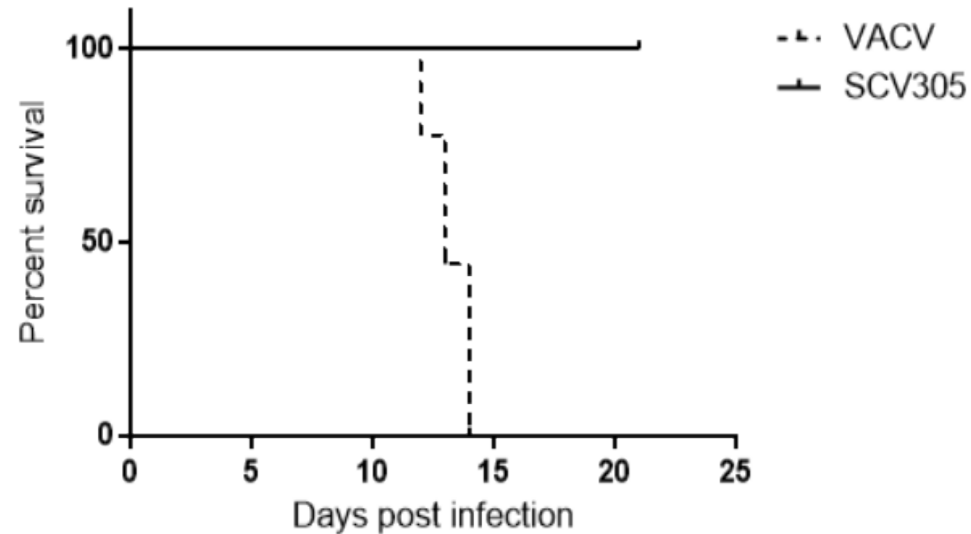


Average Body Weights \pm SEM
(n=9 per treatment group)



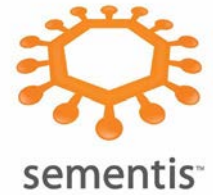
In the absence of an antiviral immune response SCV was unable to cause productive disease.

Survival Plot
(n=9 per treatment group)



In the absence of an antiviral immune response SCV is not pathogenic.

SCV Technology – Safety (Biodistribution in SCID Mice)



Dissemination:

- **SCV** infected mice: **No** live infectious virus could be recovered from Lung, Kidney, Spleens and ovaries
- **Vaccinia virus** infected mice: **high yields** of live infectious virus could be recovery from Lung, Kidneys, Spleens and Ovaries within days are infection

SCV 100 day Observation Group – Pathology Analysis

Health and wellbeing status during the 100 day observation Period:

All mice injected with SCV305 (SCV-CHIKV vaccine) remained healthy and fit during the 100 day observation period, no mice showed signs of illness of any sorts and no deaths occurred in this group.

Pathology Analysis:

At the end of the 100 day observation period all mice were euthanized and sent to SA Pathology in Adelaide for pathology analysis by Prof. John Finnie (Senior Veterinary Pathologist) where tissues were immersion-fixed in 10% neutral buffered formalin, paraffin-embedded, and 6µm sections cut and stained with haematoxylin and eosin. They were then examined by light microscopy and any lesions detected were noted.

Histopathological examination of lung, heart, liver, brain, kidney, spleen and intestinal tract from the 9 mice revealed no significant pathological changes.

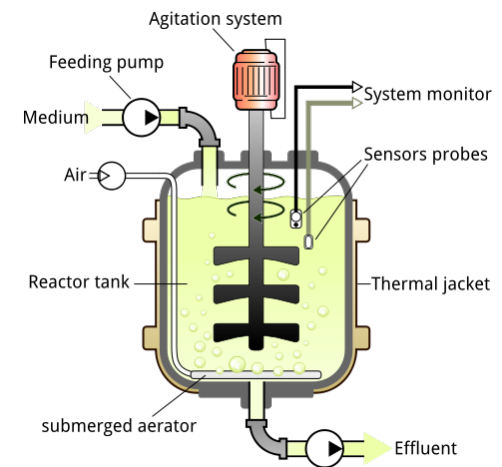
In spleen and lymph nodes, there was a marked paucity of mature lymphoid tissue, consistent with an immunodeficient status of the SCID mice.

SCV Cell Substrate for Manufacturing

Development of a cell substrate for the rescue of progeny virus required to manufacture SCV

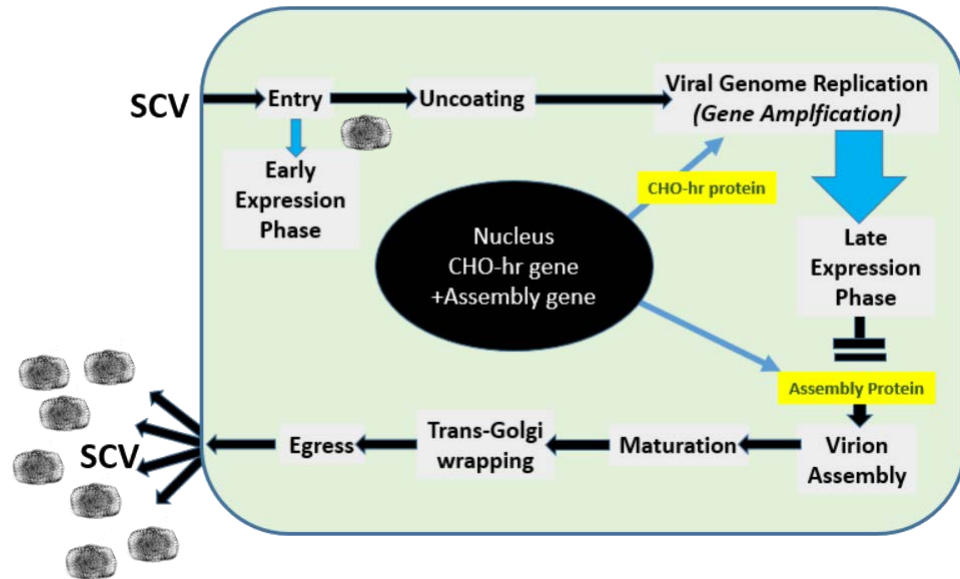
CHO cells were chosen for the basis of constructing a transgenic SCV-rescue cell line for the following reasons:

- ***Yields & Scale up ability***
 - Fast rate of growth
 - High cell density culturing
 - Suspension culture in BioReactors
 - Chemically defined protein-free growth media available
- ***Characterisation***
 - Most characterized cell line
 - Genome recently sequenced by the CHO Consortium
 - Refractory to infection by human viruses
 - Endogenous retroviral like particles are proven to be non-infectious
- ***Regulatory experiences***
 - Well known to medical control agencies such as FDA and EMEA
 - 20 years of proven history as a cell substrate for the production of licensed biological products, eg, monoclonal antibodies, hormones and enzymes
 - Guidelines available for the development of transgenic-CHO cell lines as a cell substrate for biopharmaceutical products



SCV Cell Substrate for Manufacturing

SCV Production CHO Cell Line

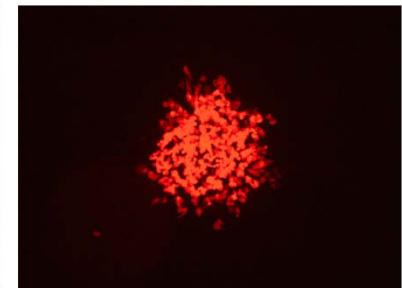


Infection with SCV

(Totally attenuated SCV expressing Red Fluorescent Protein)



CHO



SCV Rescue cell line

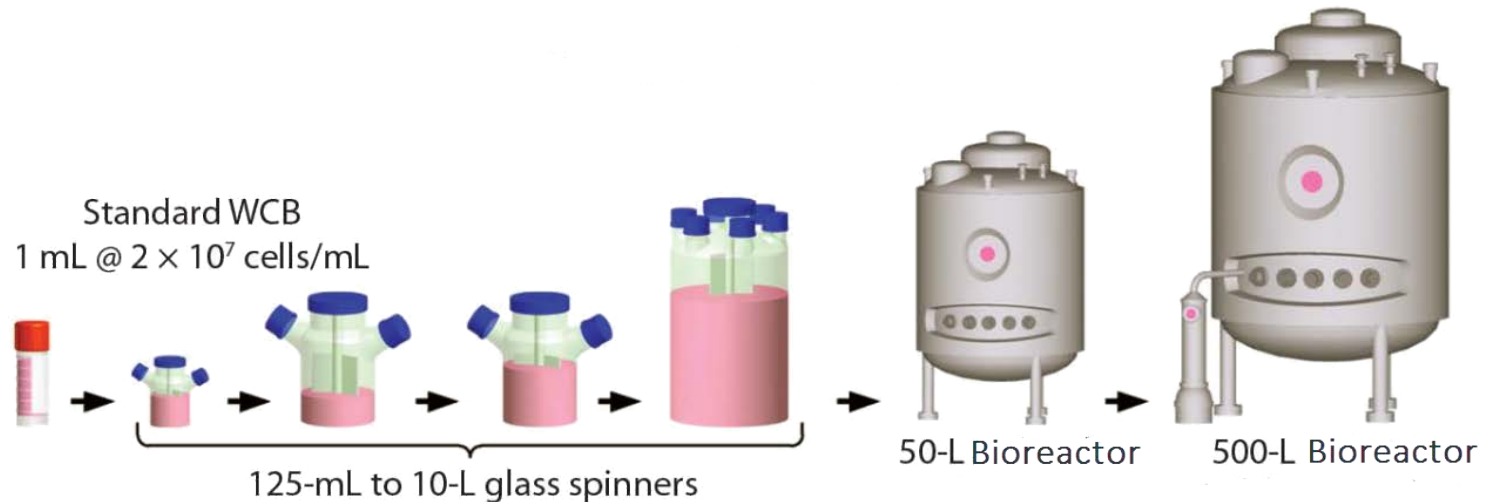
Sementis' SCV-cell substrate for manufacturing was derived from GMP produced CHO-S cell line :

- 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

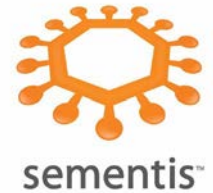
Scaling Up to 500L bioreactor

Upstream Production Process: SCV Vaccines Production From Bioreactors

- Cell expansion rate of 1:10



Up-scaling: expected vaccines yield assuming 100% recovery



BC18A-12:

- Production rate: ~100 pfu per cell
- Experimentally: at an moi of 0.01 pfu/cell at a cell density of 10^6 cells/mL yields ~10,000 pfu per pfu inoculum
 - 5 x 20mL cultures at 10^6 cells/mL infected with 2×10^5 pfu SCV (moi of 0.01 pfu/cell) produced an average yield of 2.4×10^9 pfu (1.2×10^8 pfu/mL)

Predictive Production scenario: infection of 10^7 cells/mL at moi of 0.01 pfu/cell

Production Scale	N° Doses @ 10^8 pfu/dose	N° Doses @ 10^7 pfu/dose
1L	~10,000	~100,000
10L	~100,000	~1,000,000
100L	~1,000,000	~10,000,000
1,000L	~10,000,000	~100,000,000
10,000L	~100,000,000	~1,000 Million

SCV-Chikungunya Vaccine (SCV305)

Mosquito Borne Infectious Disease

How SCV-CHIKV Vaccine (SCV305) Works

SCV-CHIKV
vaccine

Vaccination

Viral genome replication
(gene amplification)

High level CHIKV-antigen production

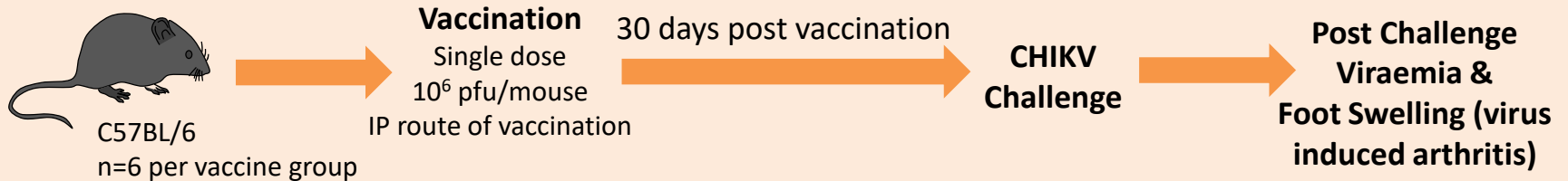
STIMULATION
of anti-CHIKV
Immune response

Arrested progeny
Virus production

Non-infectious
CHIKV-VLP

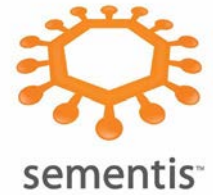
BOOSTING
of anti-CHIKV
Immune response

Preclinical Proof-of-Concept studies

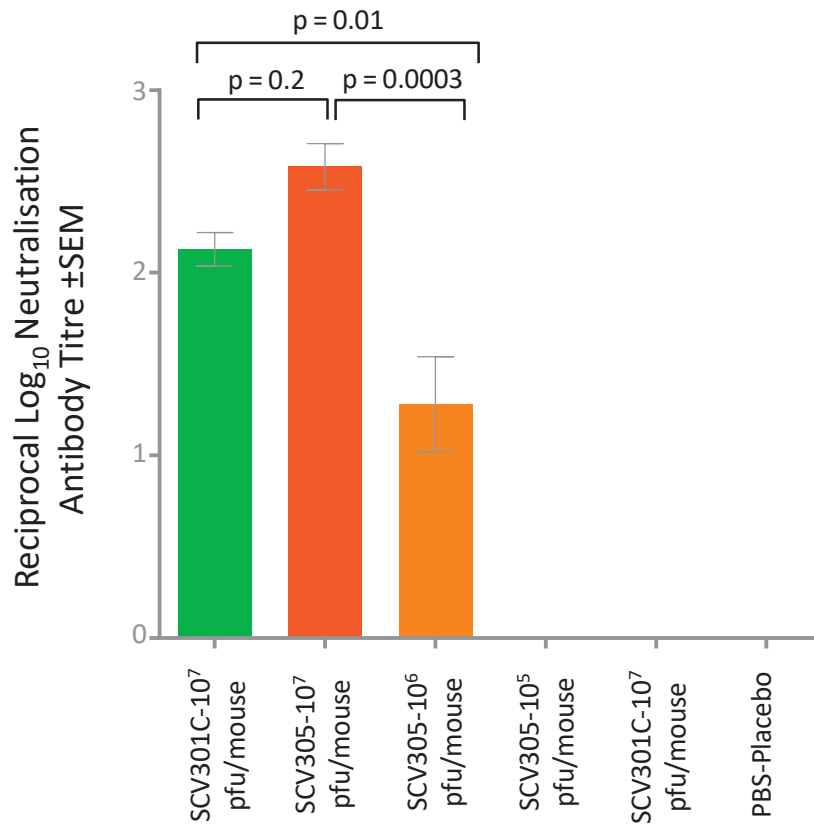


- **Whole-CHIKV-ELISA antibody Measurements:** single dose of 10⁶ produced elevated anamnestic antibody responses 4 weeks post vaccination that persisted 1 year later
- **Micro-Neutralization Assay:** single dose of 10⁶ produced neutralizing antibody detected response 4 weeks after vaccination
- **CHIKV Viraemia:** Single vaccination with 10⁶ pfu prevented CHIKV viraemia post challenging with CHIKV
- **CHIKV induced Arthritis:** Single vaccination with 10⁶ pfu prevented foot swelling at the site of CHIKV challenge
- **CHIKV persistent Infection:** vaccinated mice prevented CHIKV persistence at the site of inoculation one month after challenging as confirmed by the lack of RNA genome detection by RT-real time PCR
- **Long lived antibody secreting cells:** 1 year after vaccination, long lived antibody secreting cells could be detected.
- **Results published in:** Eldi et al (2017) Production of a Chikungunya Vaccine Using a CHO Cell and Attenuated Viral-Based Platform Technology. Molecular Therapy; 25 (10): xxxx

SCV 305 induces neutralizing antibodies with just one vaccination



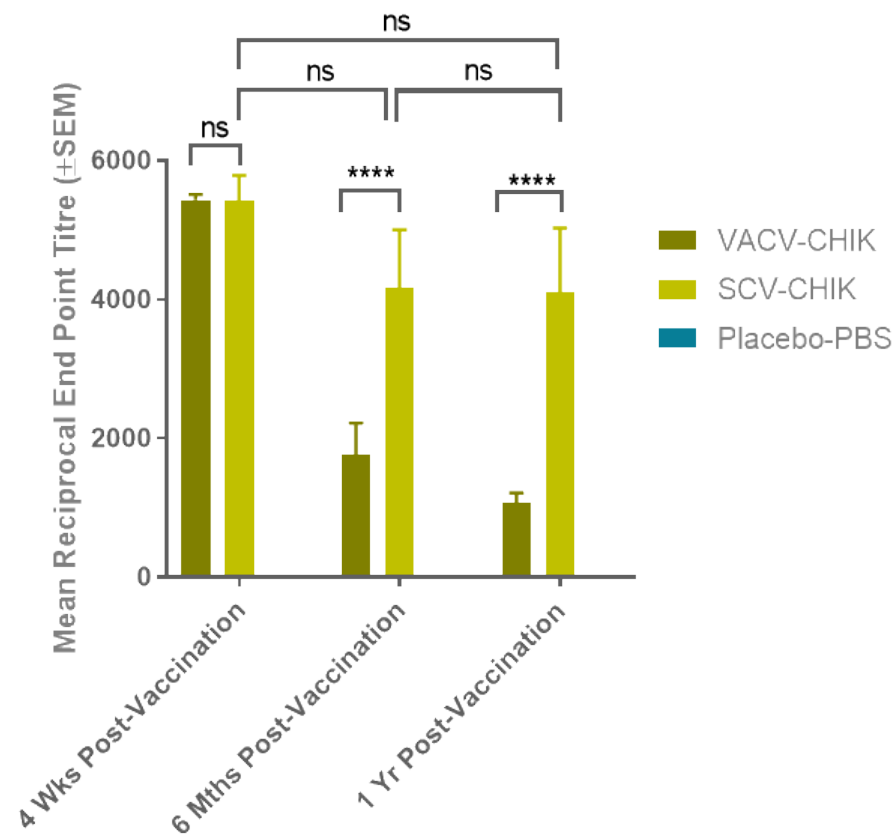
CHIKV neutralising antibody titres at day 31 post vaccination



» Heat inactivated (56 °C for 30 min) serum from each mouse was serially diluted in duplicate in 96 well plates and incubated with 200 uL of 50% cell culture infectivity dose (CCID50) of a strain of CHIKV for 2 h at 37 °C. Vero cells were then added (10⁴/well) and the plates incubated for 5 days. The serum dilution giving 100% protection against cytopathic effect was determined using crystal violet staining.

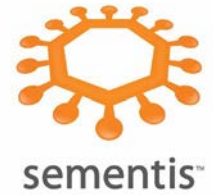
SCV 305 induces long lasting immune responses

Long Term Serum Antibody Titre to CHIKV Whole Virus
(n=6)



Two-Way ANOVA multiple comparison analysis
(ns: not significant; ****: $p<0.0001$)

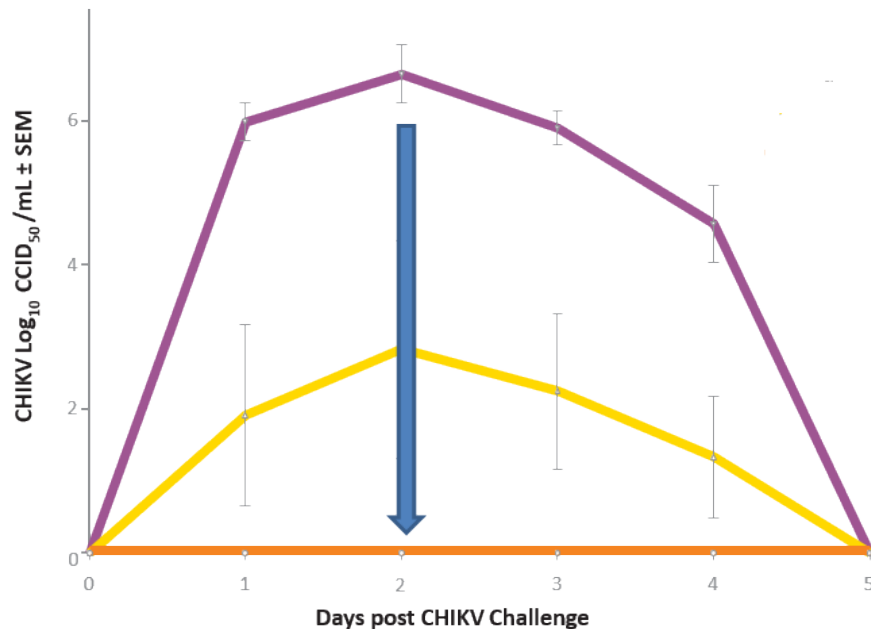
SCV-Chikungunya vaccine (SCV305) protects against viraemia & virus induced arthritis



Immunisation with Chikungunya vaccine protects against Chikungunya virus challenge viraemia

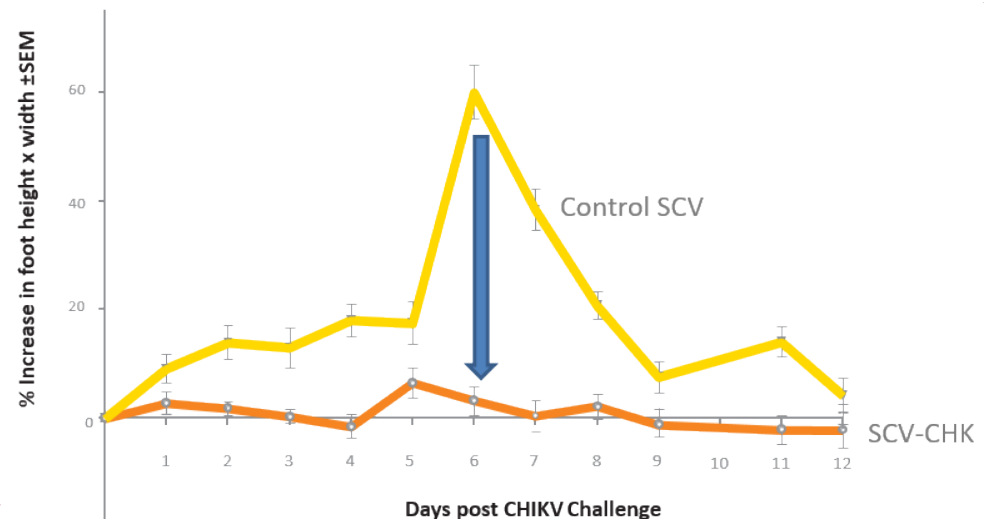
- SCV000- 10^7 pfu/mouse
- SCV305- 10^5 pfu/mouse
- SCV305- 10^6 pfu/mouse

SCV000 = SCV vector only
SCV305 = chikungunya vaccine

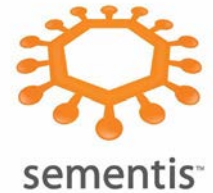


SCV-CHIK vaccine induced foot swelling (arthritis) after Chikungunya virus challenge

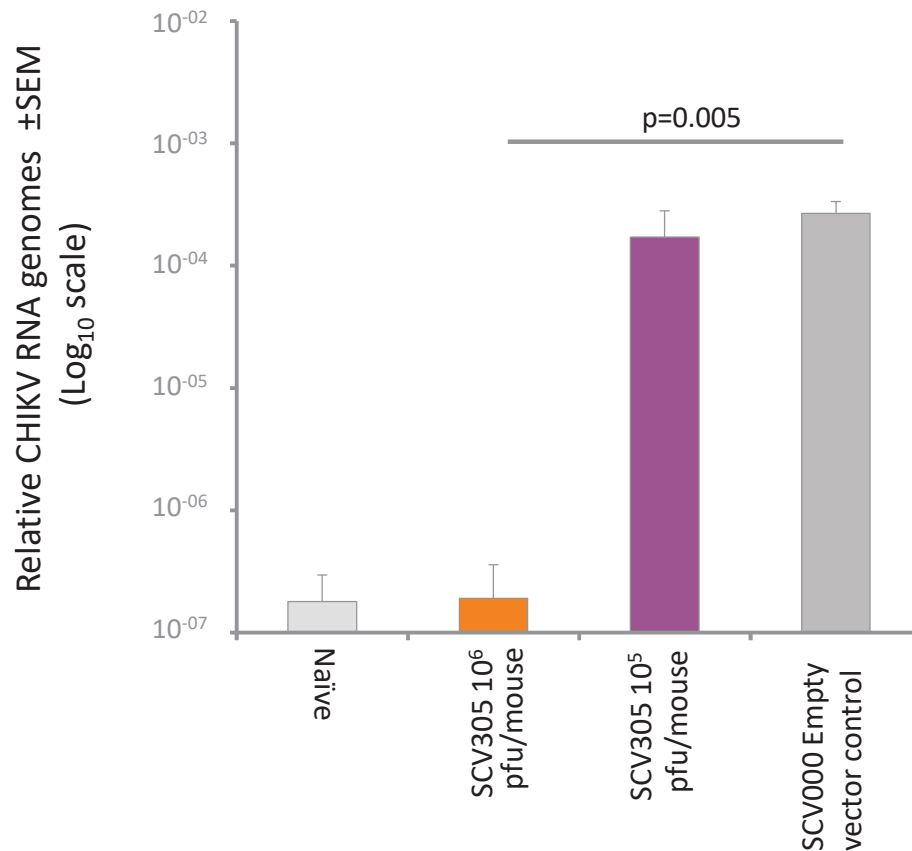
Immunization with SCV305 at 10^6 pfu/mouse was fully protective against Chikungunya virus induced foot swelling



SCV 305 protects against CHIKV persistent infection



Detection of persistent RNA genomes detected in the CHIKV inoculated feet 30 days after challenge (n=6 mice per group)

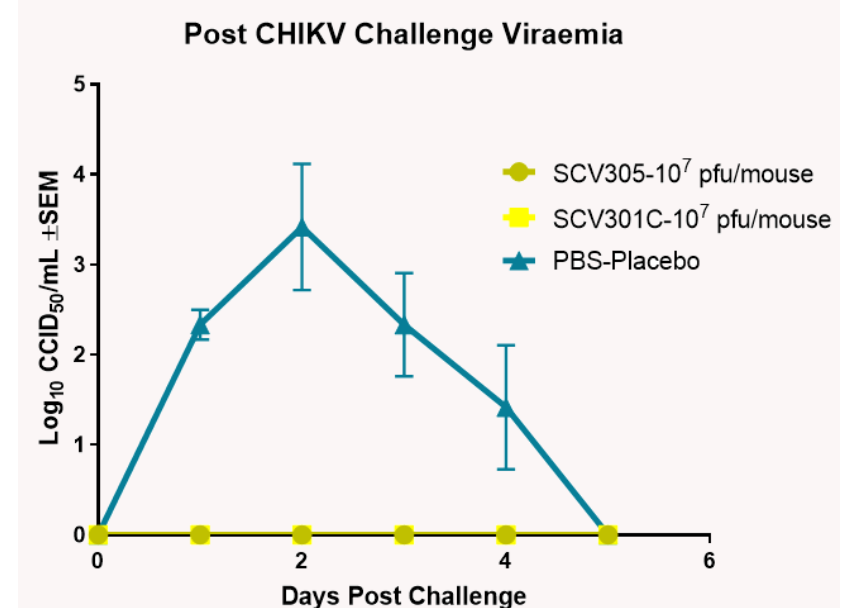
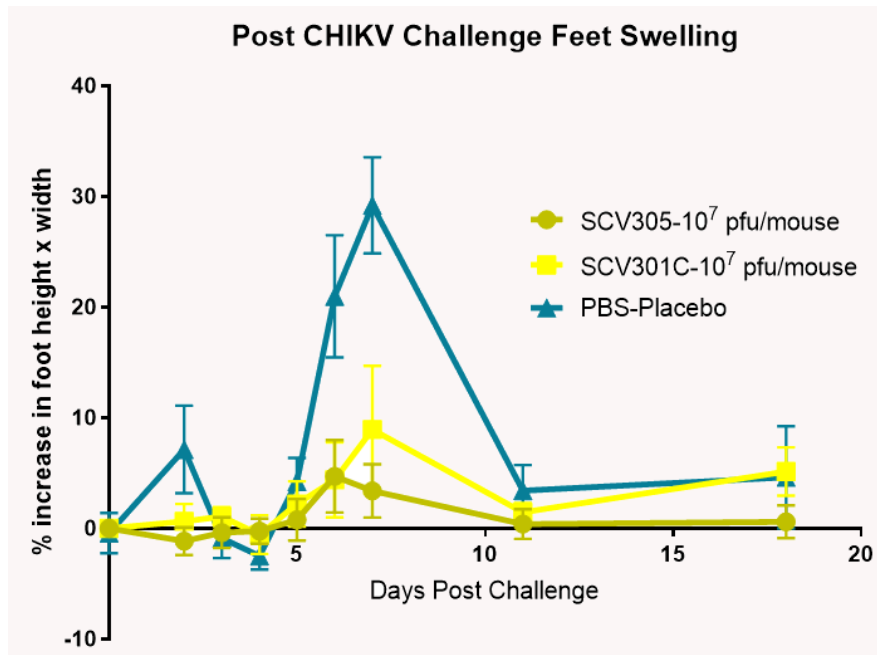


Chikungunya challenge 1 year after vaccination

SCV-CHIK vaccine induced foot swelling (arthritis) and Viraemia after chikungunya virus challenge

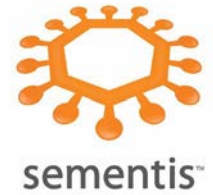
Immunization with SCV-CHIK was fully protective against Chikungunya virus induced foot swelling 1 year after vaccination

Immunisation with SCV-CHIK protects against Chikungunya virus challenge viraemia 1 year after vaccination

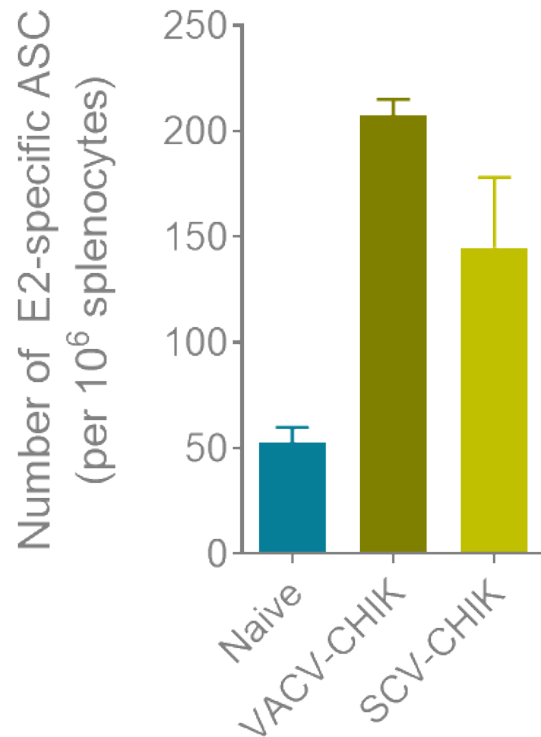


SCV305: SCV-chikungunya vaccine
SCV301C: VACV-chikungunya

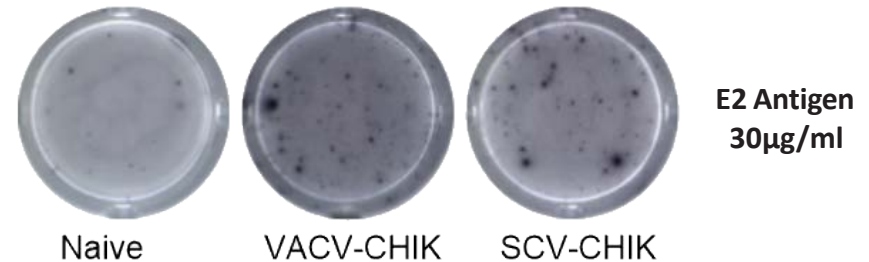
SCV305 (SCV-CHIKV): long lived antibody secreting cells (1 year post vaccination)



Long lived Antibody Secreting Plasma Cells 1 year Post Vaccination (n=3)



Chikungunya E2 Antibody Secreting Cells – IgG ELISPOT



Conclusion

- » B cell populations from SCV305 vaccinated mice contain long-lived plasma cells (ASC).
- » 1 year post-vaccination E2 antigen specific ASC can be detected following a single shot SCV305 vaccination.

Experimental Design

Two groups of C57BL/6 mice (n=3) were vaccinated with 10⁷ PFU of either SCV-CHIK (SCV305) or VACV-CHIK (SCV301C). One Year post-vaccination, the mice were sacrificed and the number of E2- specific long-lived antibody secreting cells (ASC) were analyzed by ELISPOT.

SCV-Chikungunya/Zika Virus Vaccine (SCV1002)

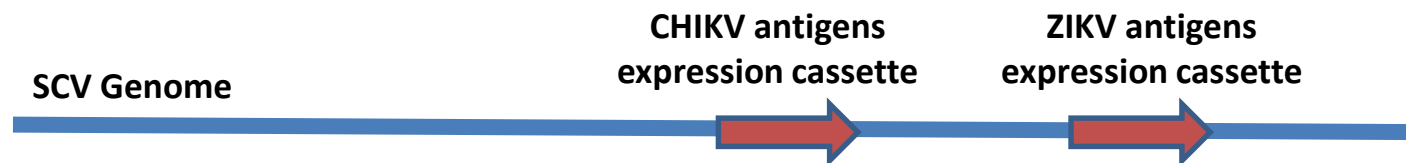
Single Vectored Multi-disease Vaccine

SCV-CHIKV+ZIKV Vaccine Design

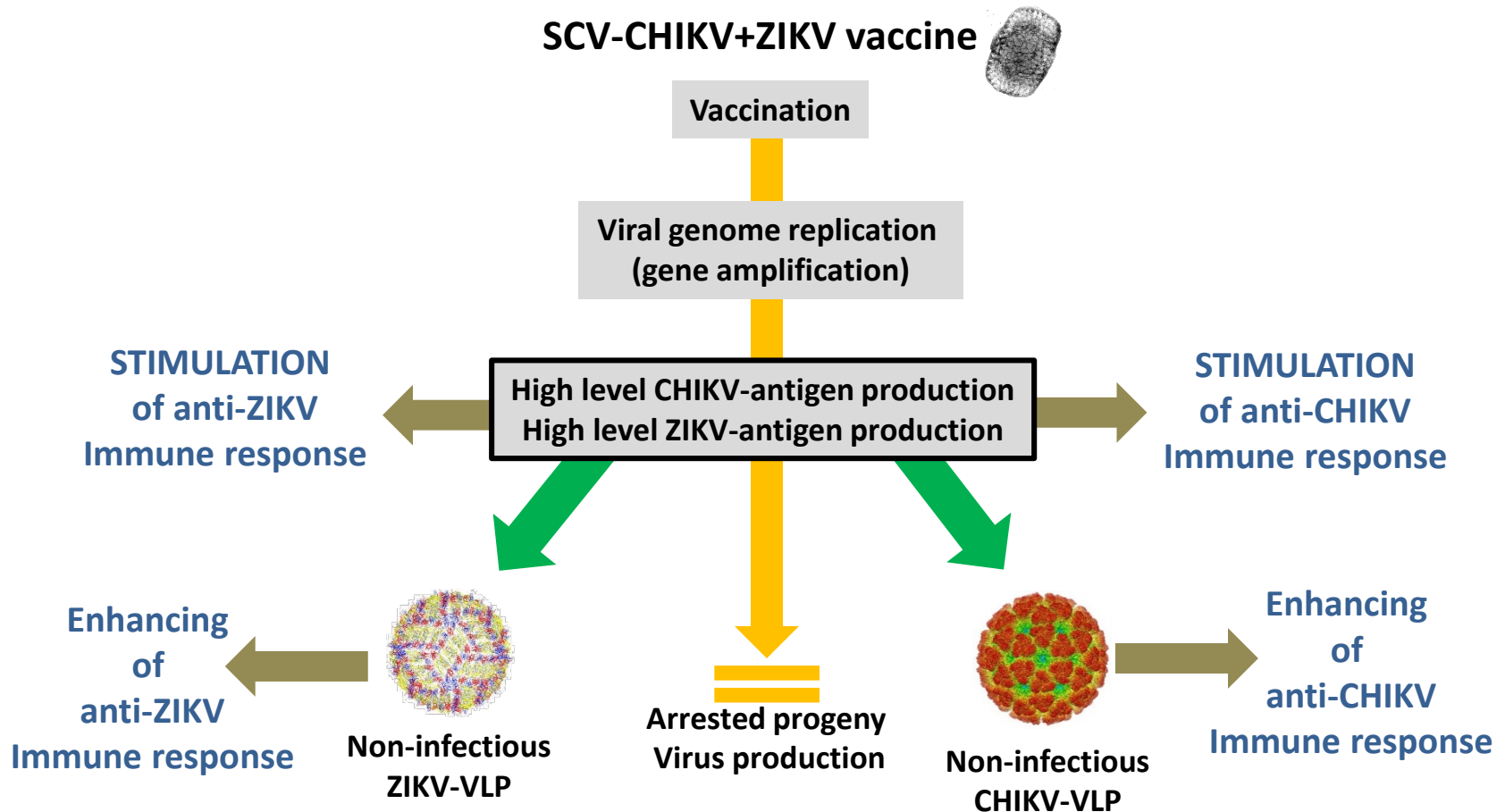
Sementis' single vectored dual chikungunya and Zika virus vaccine: SCV1002

Vaccine design:

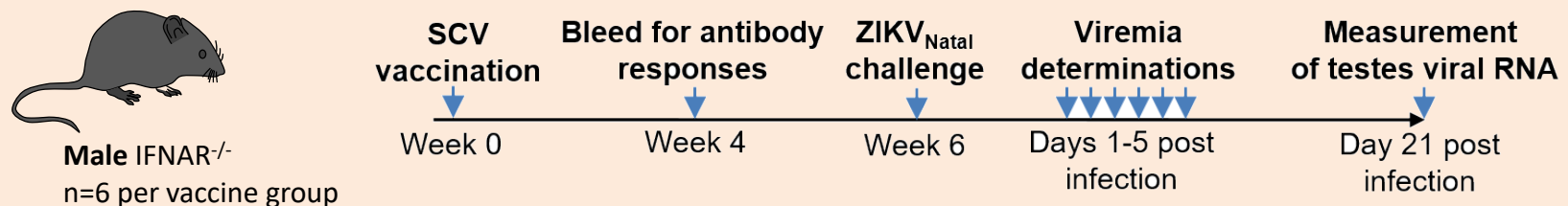
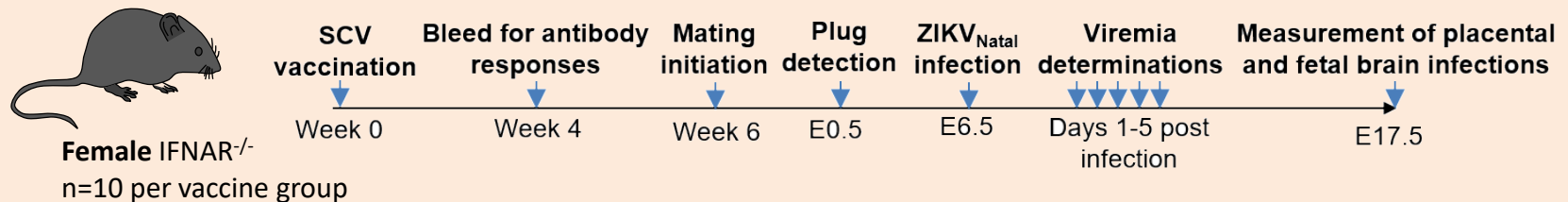
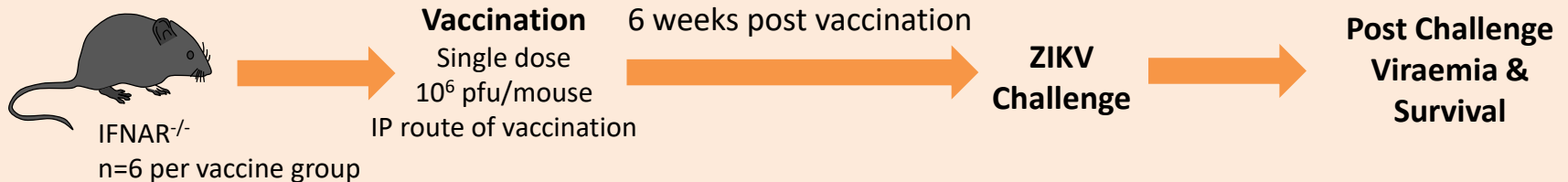
- Contains ZIKV structural proteins expression cassette. The structural protein coding sequences are a representative of the Brazilian strain.
- Contains CHIKV structural proteins expression cassette. The structural protein coding sequences are representative of the Reunion strain.
- Expression of the structural proteins upon vaccination will also lead to VLP formations for ZIKV and CHIKV.



How SCV-CHIKV+ZIKV (SCV1002) Vaccine Works



Preclinical Proof-of-Concept studies



Preclinical Proof-of-Concept studies

- **Whole-CHIKV-ELISA antibody Measurements:** single dose of 10^6 produced elevated anamnestic antibody responses from 2 weeks post vaccination onwards
- **Whole-ZIKV-ELISA antibody Measurements:** single dose of 10^6 produced elevated anamnestic antibody responses from 2 weeks post vaccination onwards
- **CHIKV Micro-Neutralization Assay:** single dose of 10^6 produced neutralizing antibody detected response 4 weeks after vaccination
- **ZIKV Micro-Neutralization Assay:** single dose of 10^6 produced neutralizing antibody detected response 4 weeks after vaccination
- **CHIKV & ZIKV Viraemia:** Single vaccination with 10^6 pfu prevented viraemia post challenging with CHIKV and ZIKV
- **CHIKV induced Arthritis:** Single vaccination with 10^6 pfu prevented foot swelling at the site of CHIKV challenge
- **ZIKV survival from challenging:** Single vaccination with 10^6 pfu resulted in 100% survival after lethal challenge with mouse adapted ZIKV

Preclinical Proof-of-Concept studies

- **ZIKV challenging during pregnancy:** previously vaccinated female mice protect their foetuses from ZIKV infection during pregnancy and thus prevent the risk of new bornes developing congenital Zika Syndrome (microcephaly, brain damage and dysfunction etc)
- **ZIKV infection of testis:** vaccinated male mice prevent infection of their testis after challenging with ZIKV and thus prevent the risk of sexually transmitting ZIKV

Testing for “Antigenic Sin’

- Mice infected with CHIKV → recovery from infection → vaccination with SCV1002 → boosting of CHIKV antibodies and initiation of ZIKV antibodies (no signs of antigen competition or dominance (no antigenic sin)

Results to be published

Conclusion

- SCV platform vaccine delivery vehicle – replication incompetent as proven by in vitro and in vivo infectivity studies (*data published in Eldi et al (2017) Molecular Therapy, October edition*)
- Manufacturing friendly – CHO based cell substrate:
 - Suspension culturing in bioreactor – scalability
 - Culture in chemical defined medium
 - Production yields high
- Applications in vaccination against infectious diseases as exemplified by Sementis' SCV-CHIK vaccine (*data published in Eldi et al (2017) Molecular Therapy, October edition*)
- Applications in single vectored multidisease vaccines for infectious diseases as exemplified by Sementis' dual SCV-CHIK/ZIKA vaccine

Molecular Therapy publication download from:

[http://www.cell.com/molecular-therapy-family/molecular-therapy/abstract/S1525-0016\(17\)30280-0](http://www.cell.com/molecular-therapy-family/molecular-therapy/abstract/S1525-0016(17)30280-0)

Vaccine Industry Practises

Standard Vaccine Preparation Practices	Function	Disadvantages	Sementis Alternative
Aluminium Adjuvants	Immunity stimulator	Excipient (extra component added to the vaccine formulation) <i>Target issue for antivacciners!</i>	Sementis' SCV-vaccines contains no added adjuvants <i>Sementis SCV-vaccine are self adjuvanting, ie, by themselves they are potent at stimulating immunity without the need to add adjuvanting excipients</i>
Formaldehyde	Inactivates pathogens to become vaccines	Formaldehyde is a toxin and needs to be removed after inactivation step <i>Target issue for antivacciners!</i>	Sementis' SCV-vaccines contains no formaldehyde <i>Since Sementis' SCV-vaccines are inherently non-pathogenic, treatment with formaldehyde is not necessary to make the vaccine safe to use</i>
Thimerosal	Mercury based Preservative – stops bacterial and fungal growth	Excipient (extra component added to the vaccine formulation) <i>Target issue for antivacciners!</i>	Sementis' SCV-vaccines contains no Thimerosal <i>Sementis' SCV-vaccines are manufactured under extreme sterile conditions where each batch is rigorously tested for sterility before releasing for use and therefore do not require the addition of preservatives or antibacterial agents</i>

Vaccine Industry Practises

Standard Practices	Function	Disadvantages	Sementis Alternative
Inactivated vaccines	To ensure safety	High risk of incomplete inactivation	<p>ZERO risk as Sementis SCV-vaccines do not require inactivation to be safe</p> <p><i>Sementis' SCV-vaccines are genetically engineered to be non-pathogenic by removing an essential viral-virulence gene to give them the same safety profile of inactivated vaccines</i></p>
Live attenuated vaccines	To produce potent and life long immunity	<p>Reversion of attenuation to virulence</p> <p><i>There have been documented evidence that on a few rare occasions the Polio vaccine had reverted to virulence due to random mutations resulting in disease rather than vaccination</i></p> <p>Live attenuated vaccines that persist after vaccination can lead to autoimmune disease</p> <p><i>Antivacciner concerns relating to autoimmune disease</i></p>	<p>For Sementis' SCV-vaccines the risk of reversion to virulence is ZERO as an essential gene required for virulence has been genetically removed not mutated</p> <p><i>Sementis' SCV-vaccines are genetically engineered to be live but non-pathogenic by removing an essential viral-virulence gene to give them the same safety profile of inactivated vaccines but the potency of live attenuated vaccines</i></p> <p>Sementis' SCV-vaccines do not persist after vaccination as they cannot propagate and thereby eliminating the risk of inducing autoimmune disease</p> <p><i>Sementis' SCV-vaccines have a short life span upon vaccination where infection last approximately 1 day there after residual vaccine is rapidly cleared by the immune system</i></p>

Acknowledgements

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