A novel vaccinia-based multi-disease vaccine against chikungunya and Zika viruses

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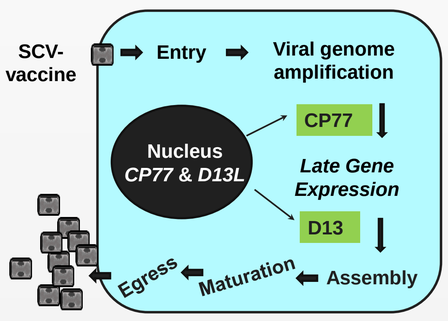
Summary

- Sementis Copenhagen Vector (SCV) is a new poxvirus vaccine vector platform.
- SCV vaccines replicate their genome, but are unable to produce viral progeny *in vivo* due to targeted deletion of *D13L*, which encodes an essential assembly protein.
- SCV vaccines can be manufactured in CHO cells expressing D13 and the host range factor factor CP77.
- Herein we describe SCV-ZIKA/CHIK, a single vector construct vaccine encoding the structural protein cassettes of both Zika virus and chikungunya virus.

Background

- Vaccinia virus (VACV) vaccination successfully eradicated smallpox and a number of recombinant VACV-based vaccine vector systems have been developed.
- The advantages of VACV-based systems include a large transgene payload capacity, minimal risk of host genome integration, induction of robust and long-lived cell-mediated and humoral immune responses, and established manufacturing processes with cold chain-independent distribution capacity.
- A disadvantage of current VACV-based systems is manufacture usually requires primary chicken embryo fibroblasts.
- Chikungunya virus (CHIKV) is an alphavirus transmitted by *Aedes aegypti* responsible for a recent global epidemic causing millions of cases of arthritic disease.
- Zika virus is an emerging flavivirus transmitted by *Aedes aegypti* and is responsible for congenital Zika syndrome caused by infection of foetal brains *in utero*, and is also able to infect testes.
- We have recently reported successful preclinical testing of a SCV-CHIK vaccine, encoding the full structural protein cassette of CHIKV
- (Eldi *et al Molecular Therapy* 2017).

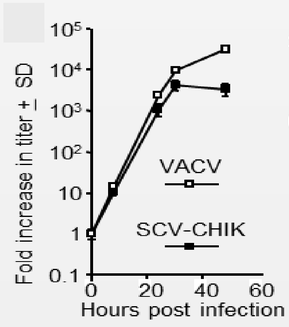
SCV-vaccine production in SCS cells



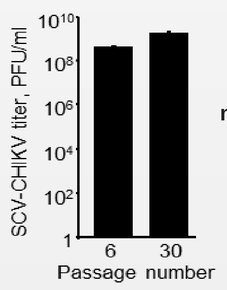
SCV-vaccine production in modified CHO cells; SCV Cell Substrate – SCS cells .

SCS cells express CP77 the host range factor which makes CHO cells permissive for VACV and SCV replication.

SCS cells express D13, the essential assembly protein deleted from SCV. This deletion renders SCV vaccine unable to generate viral progeny in human cells.

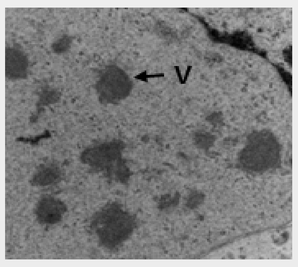


SCV vaccine production in SCS cells (comparison with VACV) illustrating >1000 fold increase in 36 hrs.

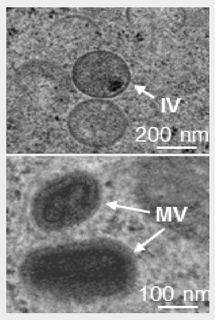


SCV vaccine yield is maintained after 30 passages of SCS cells

SCV-vaccines do not replicate in human cell lines



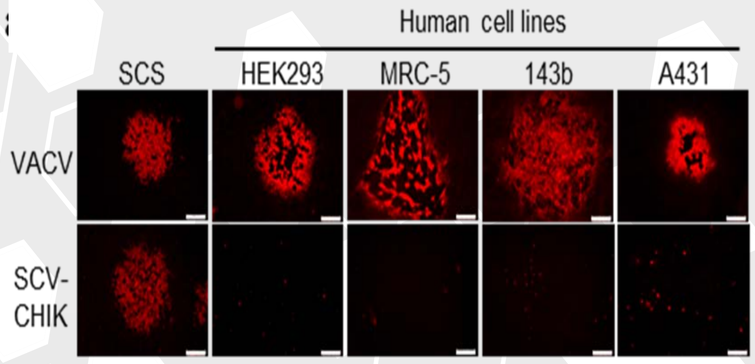
Without D13 SCV is unable to assemble and is arrested at the viroplasma (V) stage



With D13 (in SCS cells) SCV is able to assemble

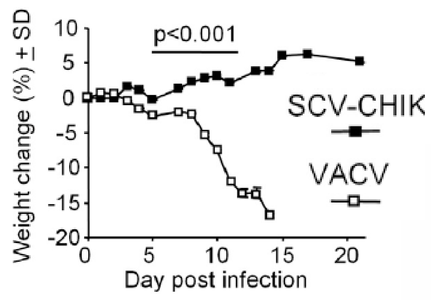
IV - immature virions

MV – mature virions

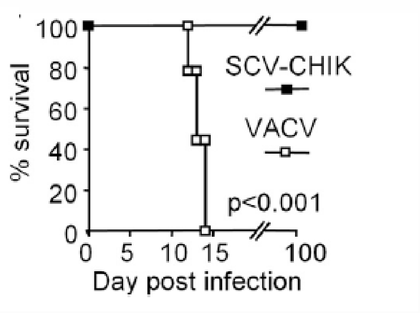


VACV, but not SCV vaccines, are able to generate viral progeny and generate large plaques in human cell lines (Staining by IFA with anti-VACV antibody).

SCV-vaccines are safe in the SCID mouse model of lethal progressive vaccinia

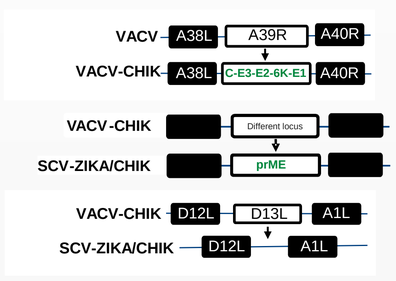


VACV, but not SCV vaccine, results in weight loss in SCID mice

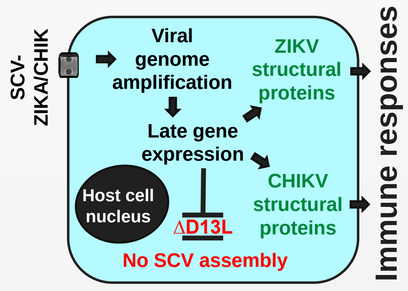


VACV, but not SCV vaccine, results in a lethal infection in SCID mice

SCV-ZIKA/CHIK vaccine design & rationale

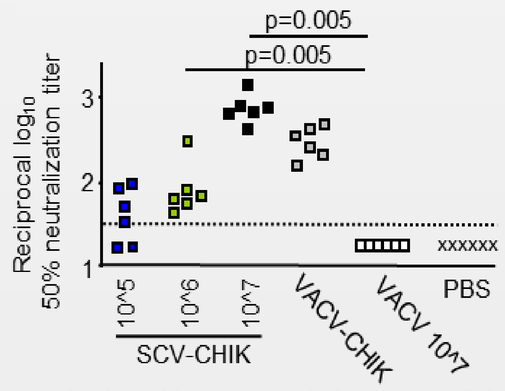


SCV-ZIKA/CHIK construction.
Insertion of structural protein cassette of CHIKV (C-E3-E2-6K-E1) and Zika virus (PrME) and removal of *D13L*.

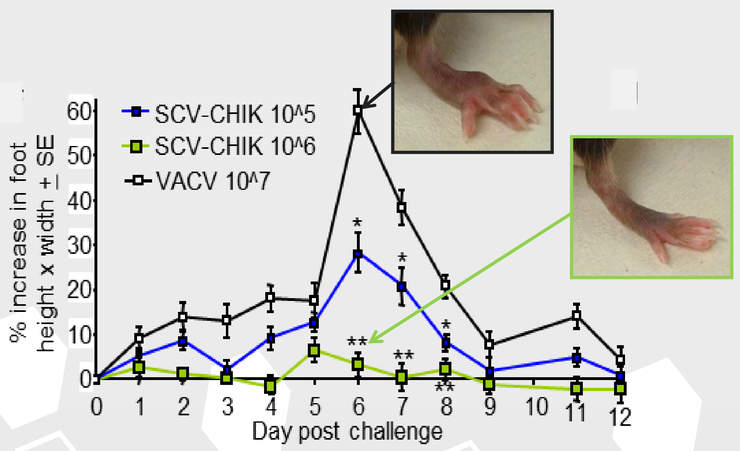


SCV-ZIKA/CHIK vaccination rationale
Infection of host cells, genome amplification (≈10,000 copies), expression of ZIKV and CHIKV immunogens. No viral progeny due to lack of essential D13L assembly protein

SCV-CHIK induces anti-CHIKV antibodies and protects against CHIKV arthritis

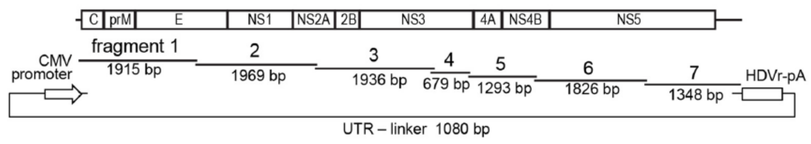


A single vaccination with SCV-CHIK induces neutralising antibody responses to CHIKV; at 10e7 these are comparable with those obtained with replication competent VACV-CHIK.
Similar data was obtained for SCV-ZIKA/CHIK (PCT/AU2017/050879)



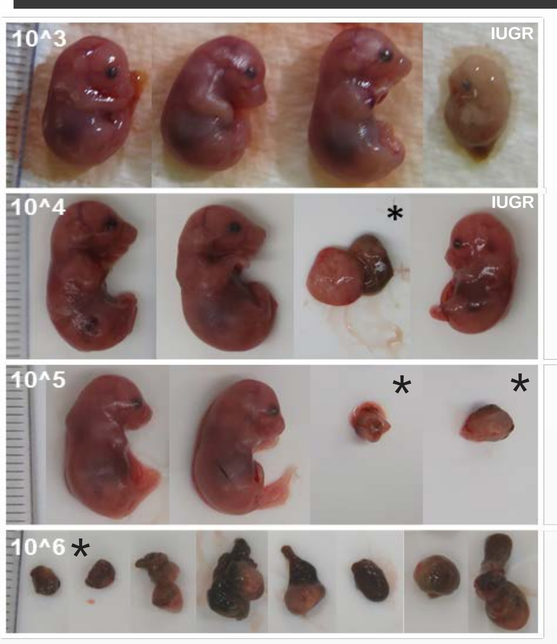
A single vaccination with SCV-CHIK protects against foot swelling (arthritis) in an adult wild-type mouse model.
Similar data was obtained for SCV-ZIKA/CHIK (PCT/AU2017/050879)

De novo synthesis of $ZIKV_{Natal}$, a virus unequivocally associated with microcephaly



***De novo* generation of $ZIKV_{Natal}$ by a modified circular polymerase extension reaction using 8 synthetics overlapping oligonucleotides . Sequence obtained from RNA-Seq of brain from aborted foetus with microcephaly KU527068.**

$ZIKV_{Natal}$ mouse model of foetal brain infection in $IFNAR^{-/-}$ x $IFNAR^{-/-}$ mice



$ZIKV_{Natal}$ foetal infection model.

$ZIKV_{Natal}$ infection of pregnant dams at E6.5 or E12.5; IFNAR-/- females mated with IFNAR-/- male mice (Setoh *et al* 2017).

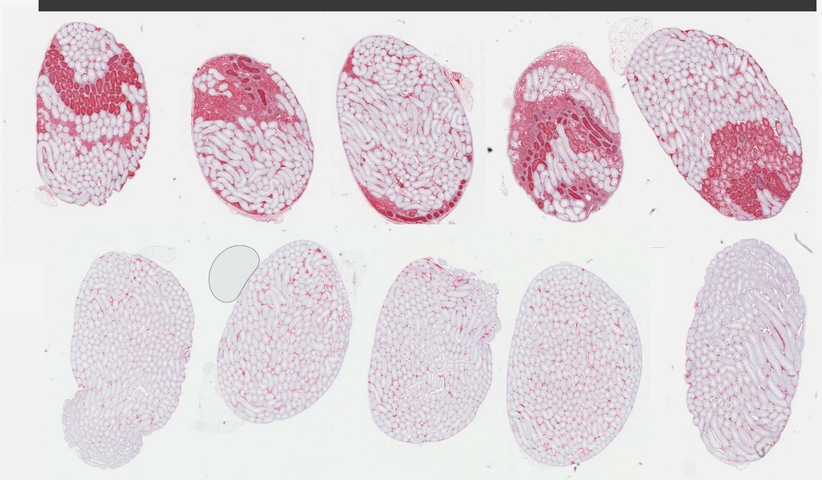
Female mice >8 weeks old show no symptoms after $ZIKV_{Natal}$ infection.

IUGR – intrauterine growth restriction

*** – deformed foetus or foetal placental masses**

SCV-ZIKA/CHIK protects against foetal brain infection in this model (PCT/AU2017/050879)

$ZIKV_{Natal}$ mouse model testes infection in $IFNAR^{-/-}$ male mice



$ZIKV_{Natal}$ infection of testes . Testes are stained by IHC with anti-pan flavivirus antibody 4G2. Top row ZIKA virus infected, bottom row controls. SCV-ZIKA/CHIK protects against testes infection in this model (PCT/AU2017/050879)

Conclusion

To our knowledge this represents the first single vector construct, multi-disease vaccine encoding large polyproteins, which offers both simplified manufacturing and formulation, and reduced "shot burden" for these often co-circulating arboviruses.

We envisage toxicology and safety to be comparable with Modified Vaccinia Ankara (INVAMUNE ®).

REFERENCES AND PATENTS

References:
1. Eldi P, Cooper TH, Liu L, Prow NA, Diener KR, Howley PM, Suhrbier A, Hayball JD. 2017. Production of a chikungunya vaccine using a CHO cell and attenuated viral-based platform technology. *Mol Therapy. (E Pub).*
2. Setoh YX, Prow NA, Peng N, Hugo LE, Devine G, Hazlewood JE, Suhrbier A, Khromykh AA. De Novo generation and characterization of new Zika virus isolate using sequence data from a microcephaly case. mSphere 2(3). pii: e00190-17.

Patents:
SCV-cell substrate: Viral Vector Manufacturing. Inventor: Paul Howley and Liang Liu. PCT/AU2014/050330 (WO 2015/061858).
SCV-dual Chikungunga/Zika virus vaccine: Australian provisional patent;PCT/AU2017/050879.

Peanut hypoallergy vaccine: Immune Modulation. PCT/AU2014/000286 (WO 2014/138824A1).