A new replication-defective, vaccinia-derived, CHO-manufactured, vaccine vector system (SCV) co-expressing chikungunya and Zika virus structural genes is effective in preclinical studies

Dr Natalie Prow
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**ALPHAVIRUSES THAT CAUSE ARTHRITIC DISEASE IN HUMANS**

- Transmitted by mosquito (arboviruses)
- Single stranded positive sense RNA virus, ≈12 kb genomes.
- Symptomatic infections nearly always associated with weeks to months polyarthritis/polyarthralgia.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Occurrence</th>
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<tbody>
<tr>
<td><strong>Chikungunya virus</strong></td>
<td>Large sporadic epidemics every 2-50 years</td>
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<td><strong>Ross River virus</strong></td>
<td>Mean of ≈4,000 cases per annum in Australia. Also an epidemic (1979/80) &gt;60,000 cases</td>
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<tr>
<td><strong>Barmah Forest virus</strong></td>
<td>Mean of ≈ 1000 cases per annum in Australia</td>
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CHIKV EPIDEMIC 2005/6, REUNION ISLAND (FRANCE)

>250 deaths – often elderly with comorbidities and very young

High attack rate

-266,000 cases of CHIKV disease were reported (38% of the population).

Rapid rise in case numbers

- increased to 130,000/month in 4 months
- 45,000 cases during the week of 29 Jan, 2006.

100’s of imported case in France

Eurosurveillance, Volume 11, Issue 34, 24 August 2006
The Indian Ocean/Reunion Island epidemic was associated with a new clade of CHIKV.

E1 Mutation (A226V) allowed efficient CHIKV transmission by Ae. albopictus.

Schuffenecker et al. Plos Med July 2006 3 (7) e263
>1 million CHIKV cases in the Americas 2014

First chikungunya case locally acquired in the United States reported in Florida July 2014
Aedes albopictus; a global invader

An aggressive biter – the BBQ stopper
Disease characterised by acute and chronic symmetrical polyarthritis-polyarthritis.

1) Acute viraemia and arthritis
   - Day 1-14 post infection.
   (Recapitulates viraemia, cytokinemia & arthritis)
   Gardner et al., 2010, J Virol 84(16):8021-32

2) Chronic disease and viral persistence
   - Day 30 post infection.
   (Recapitulates viral RNA & protein persistence, cellular infiltrate and cytokine/ISG profile)
   Poo et al., 2014 PLoS Negl Trop Dis. 8(12):e3354
Adult wild-type mouse model of chikungunya virus infection (viraemia) and disease (foot swelling/arthritis)

Viraemia

Arthritis

Female C57BL/6 mice > 6 weeks old (Gardner et al. 2010. J Virol 84:8021–32)

IRF3/7⁻/⁻ mice die from hemorrhagic shock after CHIKV infection (Rudd et al 2012)
Mouse model of viral persistence and chronic arthritis disease

Persistence of CHIKV RNA

Persistent stimulation of IRGs

Persistent detectable cellular infiltrates (arthritis);

quantifiable using Aperio system

Persistence of capsid antigen (d 30 pi)
Vaccination

Antigen expression

Antigen-specific immune responses

Nucleus

No viral progeny

Manufacture

Antigen expression

Viral assembly on D13

Nucleus

D13

Vaccine stocks
Rationale for the Sementis Copenhagen Vector (SCV) vaccine platform technology

Vaccinia virus infection (host cell)

VACV

Entry → Uncoating → Viral genome amplification → Late Gene Expression → Maturation → Assembly → Egress

SCV infection (host cell)

SCV

Entry → Uncoating → Viral genome amplification → Late Gene Expression

SCV-CHIK production (SCS line)

SCV-CHIK

Entry → Uncoating → Viral genome amplification → Late Gene Expression → Maturation → Assembly → Egress

SCV-CHIK vaccination (host cell)

SCV-CHIK

Entry → Uncoating → Viral genome amplification → Late Gene Expression

Immune response → CHIKV structural proteins → No SCV assembly
Construction and *in vitro* characterisation of the SCV-CHIK vaccine
Production and morphology of SCV-CHIK in SCS cells

**Figure a:** Western blot analysis showing protein expression in CHO and SCS cells.

**Figure b:** Time-lapse images and quantification of plaque diameter for SCV-CHIK and VACV in CHO-D13L and CHO-CP77 cells. Black bars: SCV-CHIK, white bars: VACV. Statistically significant difference (p < 0.001).

**Figure c:** Graph showing fold increase in titer over time for SCV-CHIK and VACV.

**Figure d:** Graph indicating SCV-CHIK titer in BHK-21 cells after different passage numbers.

**Figure e:** Electron micrographs of SCV-CHIK in BHK-21 and SCS cells, highlighting virus particles (V), intracellular vesicles (IV), and membranous vesicles (MV).
SCV-CHIK replication defect in human cell lines and attenuated pathology

SCID mice vaccinated
A single shot SCV-CHIK induces antibodies and protects against CHIKV infection and disease.

**Figure a**: Bar graph showing the reciprocal CHIKV E2-specific endpoint titer x 1000 + SE for different groups.

**Figure b**: Reciprocal log10 50% neutralization titer with significance levels (p=0.005).

**Figure c**: IgG2c and IgG1 absorbance at 405 nm + SE.

**Figure e**: Viral load, log10 (CID50/ml) + SE.

**Figure f**: % increase in foot height x width + SE.

**Figure g**: Normalized CHIKV RNA + SE with significance level (p=0.005).
Conclusions

• Innovative vaccinia virus-derived vaccine platform (SCV)

• Multiplication deficient by targeted deletion of the essential viral assembly gene, D13L

• SCV cell substrate line developed for SCV vaccine production in CHO cells

• Benefit - scale up capacity for manufacturing

• Single shot vaccination of SCV-CHIK provided protection against CHIKV challenge, preventing both viraemia and arthritis
1947 – 1<sup>st</sup> isolation from rhesus monkeys in Zika Forest, Uganda (MR766 prototype)

1948 – 1<sup>st</sup> isolation from *Aedes africanus* mosquito

1952 – 1<sup>st</sup> recorded human infection, Nigeria

2007 – 1<sup>st</sup> major human outbreak, Yap Islands, Micronesia

2013-14 – French Polynesian outbreak

>25,000 people infected

2015 (ongoing) – Latin American outbreak

1.6 million people infected in Americas

185 people infected
Why the panic?

- Could be as many as 500,000 to 1 million Zika infections in Brazil
- Only 1 in 5 develop clinical symptoms
- 4,231 suspected microcephaly cases are still being investigated (March 9, 2016)
- Zika virus is spreading rapidly
- Confirmed non-vector modes of transmission
- No vaccines or approved therapies
- Little is known about the virus, transmission etc
Zika virus – transmission cycle

Transmission via
1. Mosquito
2. Mother to baby *in utero*
3. Sexual transmission
4. Blood transfusion
Neurological birth defects

**Microcephaly**

- Symptoms include below-average head size
- Often caused by failure of brain to grow at normal rate
- Head circumference measuring **less than 31.5-32cm** at birth
- Affects **25,000 children** in US each year

Source: ADAM, WHO
Development of a single vectored SCV-CHIK/ZIKA vaccine

Need for preclinical mouse models
De novo generation of infectious Zika_Natal by CPER

### A

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>C</td>
<td>prM</td>
<td>E</td>
<td>NS1</td>
<td>NS2A</td>
<td>2B</td>
<td>NS3</td>
</tr>
<tr>
<td>fragment 1</td>
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<td>3</td>
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<td>1915 bp</td>
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### B

<table>
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<tr>
<th>Days post transfection</th>
<th>Supernatants</th>
<th>Cell lysate</th>
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<tbody>
<tr>
<td>6</td>
<td>1000 bp</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000 bp</td>
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<tr>
<td>10</td>
<td>1000 bp</td>
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<tr>
<td>10</td>
<td>1000 bp</td>
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</tr>
</tbody>
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### C

- ZIKV_Natal
- α-NS1
- α-dsRNA
- Mock

### D

- ZIKV_Natal
- ZIKV_MR766

### E

<table>
<thead>
<tr>
<th>Virus titer, log_{10} pfu/mL ± SE</th>
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<tr>
<td>Vero</td>
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<tr>
<td>A549</td>
</tr>
<tr>
<td>WT MEF (MOI=0.1)</td>
</tr>
<tr>
<td>WT MEF (MOI=1)</td>
</tr>
<tr>
<td>IFNAR−/− MEF (MOI=0.1)</td>
</tr>
<tr>
<td>C6/36 (MOI=0.05)</td>
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</tbody>
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ZIKV\textsubscript{Natal} infection of IFNAR\textsuperscript{-/-} and IRF7\textsuperscript{-/-} mice

**A** IFNAR\textsuperscript{-/-}

- ■ ZIKV\textsubscript{Natal}
- □ ZIKV\textsubscript{MR766}

**B** Percent survival

- ■ ZIKV\textsubscript{Natal}
- □ ZIKV\textsubscript{MR766}

**C** IRF7\textsuperscript{-/-}

- ■ ZIKV\textsubscript{Natal}
- □ ZIKV\textsubscript{MR766}
Characterisation of ZIKANatal in vivo

Viraemia, log$_{10}$ CCID$_{50}$/ml ± SE

Day post infection

IFNAR$^{-/-}$

Percent survival

0 10 20 30
Day post infection

ZIKV$_{Natal}$ $10^3$ CCID$_{50}$ s.c. 4 weeks
ZIKV$_{Natal}$ $10^6$ CCID$_{50}$ s.c. 8-12 weeks
ZIKV$_{Natal}$ $10^4$ CCID$_{50}$ i.p. 8-12 weeks
ZIKV$_{Natal}$ $10^5$ CCID$_{50}$ i.p. 8-12 weeks

Testes virus titer
log$_{10}$ CCID$_{50}$/g ± SE
Pregnancy model steps: a brief overview

- **Timed matings**
- **Conception (indicated by plug)**
- **Weigh mothers to track pregnancy**

**Pregnant IFNAR1−/− mice**
(early or mid pregnancy)
> 8 weeks of age

Cull mice at E17.5 and harvest foeti and maternal placenta

Take daily blood samples for viraemia

Subcutaneously infect mothers at E6.5 or E12.5 with $10^3$-$10^6$ CCID$_{50}$ ZIKV$_{Natal}$
Pregnancy outcomes of $\text{ZIKV}_{\text{Natal}}$-infected dams

A

$10^3$

$10^4$

$10^5$

$10^6$

B

C

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

**Vaccine design:**
- Contains ZIKV PrME polyprotein structural protein expression cassette. The structural protein coding sequences are a representative of the Brazilian strain ZikaSPH2015 (Genbank: KU321639).
- Contains CHIKV 26S polyprotein structural protein expression cassette. The structural protein coding sequences are representative of the Reunion strain 06_21 (Genbank: AM258992).
- Expression of the structural proteins upon vaccination will also lead to VLP formations for ZIKV and CHIKV.
How SCV-CHIKV+ZIKV (SCV1002) Vaccine Works

SCV-CHIKV+ZIKV vaccine

Vaccination

Viral genome replication (gene amplification)

High level CHIKV-antigen production
High level ZIKV-antigen production

STIMULATION of anti-ZIKV Immune response

STIMULATION of anti-CHIKV Immune response

BOOSTING of anti-ZIKV Immune response

Non-infectious ZIKV-VLP

Arrested progeny Virus production

Non-infectious CHIKV-VLP

Non-Confidential
Preclinical testing of SCV1002 still ongoing!

- *De novo* generation of a clinically relevant isolate, unequivocally associated with congenital Zika syndrome using a circular polymerase extension reaction protocol

- Established fetal brain infection model in IFNAR-/-
  - Inc intrauterine growth restriction

- No clinical symptoms in dams

- Established male testis infection model

- Testing a single vectored SCV-CHIK/ZIKA in CHIKV and Zika adult mouse models

- Ongoing testing in fetal brain and testis infection models

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* *contributed equally*