Development of a vaccine candidate against Crimean-Congo Haemorrhagic Fever (CCHF) virus
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TB
Vaccines
Antibiotics
Diagnostics

Toxins
Botulinum
Clostridium
Immunotherapy
Diagnostics

Mening & Pertussis
Correlates
Vaccines

Biosafety
Detection
Decontam
HCAI/vCJD
Training

Animal Models
Efficacy studies
Aerosol
Pathol/ Imaging
Immunology

GxP
Immune Assay
Clinical Trials/NVEC
Assay Validation
Product release
assays

Immune Modulation
Inflammation
Adjuvants

Emerging Diseases
Virology/Influenza
Bacteriology

Diagnostics Technology
Diagnostics
Bio/Molecular

Medical Counter Measures
NIAID
Anthrax

Research

Detection, treatments and vaccines
Crimean-Congo Haemorrhagic Fever (CCHF) virus:

- Severe human infection.
- Fatality rate 30% (9-50%).
- No FDA or European approved vaccine or treatment.
- ACDP - Hazard Group 4 pathogen.
- Reservoired in ticks & wild life mammals, amplified in cattle sheep, goat, camel [No disease in animals]
- Transmission by tick bite or direct / indirect contact with infected blood/body fluids.
CCHF - Clinical Disease

- Incubation period 2-9 days
- Haemorrhagic state develops 3 - 5 days
- Petechial rash / ecchymoses in the skin
- Bleeding from the mucous membranes
  Epitaxisis, Haematuria, Haemoptysis
- Loss of blood pressure - shock
- Death 7-9 days
  [massive bleeding / cardiac arrest]
Re-assortment in CCHF viruses could lead to new viruses and new disease...

Exchange of M segments influence host range

Envelope glycoproteins influence cellular tropism

→ altered pathogenicity

But can we:
… Detect
….Protect against

Chamberlain et al 2005
CCHFV Transmission cycle

Bente et al 2012

Transmission of CCHFV: No disease caused in animals
## Transmission to Health Care Workers

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Primary cases</th>
<th>HCW Contacts</th>
<th>2ary/3ary HCW cases</th>
<th>Exposure</th>
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<tr>
<td>1976</td>
<td>Pakistan</td>
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<td>12</td>
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<td>40</td>
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<tr>
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<td>Iran</td>
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<td>Kenya</td>
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<td>NA</td>
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<tr>
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<td>ND</td>
<td>2</td>
<td>Hospital care</td>
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<td>ND</td>
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<td>2001</td>
<td>Albania</td>
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<td>1</td>
<td>Electrocardiogram</td>
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<td>2002</td>
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<td>154</td>
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<td>2003</td>
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<td>2004</td>
<td>Senegal-France</td>
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<td>Hospital care</td>
</tr>
<tr>
<td>2005</td>
<td>Turkey</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>NA</td>
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</tbody>
</table>

**Total**: 77 primary cases, 494 HCW contacts, 44 2nd/3rd HCW cases.

*ND: not documented; NA: not applicable*
Geographic distribution of CCHF

Hyalomma tick vectors present
Serological evidence and presence of vector
10 – 100 CCHF cases per year
100 and more CCHF cases per year

natural reservoir
wildlife mammals
and birds
Amplicator: cattle,
sheep, goat, camel

CCHF: sporadic ~ 2000 cases/year

Case numbers likely to be an underestimate
Importance of CCHF

1. Spread of vector across Europe.

2. Increased incidence in tourism areas.

Development of a vaccine against CCHF virus
3. Threat is national and international

- CCHF listed in top 10 vector-borne diseases that have the greatest potential to affect European citizens

- WHO Workshop Oman Dec 2015
Importance of CCHF

4. Potential bioweapon

5. Threat to armed forces.

The Washington Times
EXCLUSIVE:
CCHF virus poses new threat to troops

The past and present threat of vector-borne diseases in deployed troops

Development of a vaccine against CCHF virus
No vaccines or antiviral drugs are approved for CCHF by FDA or EMA.

Bulgarian vaccine candidate has major disadvantages:

- Requires live CCHF virus
- Crude preparation (non-standardised homogenisation of mouse brain)
- No efficacy studies, no interest to generate data package since 70s
- Is not acceptable to FDA/MHRA/EMA approval

Alternative approach badly needed for a modern CCHF vaccine that can meet regulatory approval and is proven to be effective.
Development of the vaccine candidate

Our approach: We have used Modified Vaccinia Ankara (MVA) as a viral vector to induce immune responses against an inserted CCHF antigens.

Favourable properties of MVA:
• Human safety history: >100,000 doses in 1970s with no adverse effects.
• Human cells non-permissive.
• Induction of humoral and cellular immunity.
• Industrial GMP established.
• Thermostable.
• Production of recombinant proteins.
• Clear commercial opportunities
  • Vaxgene, OBM, Bavarian Nordic, Jansen/Emergent ➔ all in clinical trials with MVA-based vaccines.
  • Approximately extra 100,000 people vaccinated with no adverse signs.
• Inexpensive, low cost approach
Development of the vaccine candidate

Antigen sequence

GFP for selection of recombinant viruses
N-terminal tPA for secretion & Nab induction
C-terminal V5 for *in vitro* antibody recognition

Wyatt & Moss

Transfer plasmid

MVA genome

MVA permissive cell

GFP+ plaque purification

Recombinant MVA
Choice of CCHF vaccine antigen

Nucleoprotein [NP] (S-segment of CCHFv)
- Highly conserved between CCHFv strains.
- Most immunogenic protein in CCHFv.
- Successfully used for other viruses.

Glycoprotein [GP] (M-segment of CCHFv)
- External envelope spike glycoprotein – readily accessible by antibodies.
- GPs commonly and successfully used for other virus pathogens.

Two vaccine constructs made: MVA-NP and MVA-GP.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Vaccine construct</th>
<th>Protection effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebola virus</td>
<td>Venezuelan equine encephalitis virus replicons</td>
<td>Protection in C57BL/6 mice</td>
</tr>
<tr>
<td></td>
<td>Cytomegalovirus</td>
<td>Protection in mice</td>
</tr>
<tr>
<td>Hantavirus</td>
<td>Recombinant vaccinia virus</td>
<td>Partial protection in Mongolian gerbils</td>
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<tr>
<td>Influenza virus</td>
<td>DNA prime and recombinant adenovirus boost</td>
<td>Protection in mice</td>
</tr>
<tr>
<td></td>
<td>Recombinant adenovirus</td>
<td>Protection in mice</td>
</tr>
<tr>
<td>Lassa virus</td>
<td>Recombinant vaccinia virus</td>
<td>Protection in guinea pigs</td>
</tr>
<tr>
<td>Measles</td>
<td>Recombinant vaccinia virus</td>
<td>Protection from encephalitis in rats</td>
</tr>
<tr>
<td>Pichinde virus</td>
<td>Recombinant vaccinia virus</td>
<td>Delayed mortality in Syrian hamsters</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Raccoon poxvirus</td>
<td>Protection in mice against lethal challenge</td>
</tr>
<tr>
<td>Rift Valley fever virus</td>
<td>DNA vaccine</td>
<td>Partial protection of mice against lethal challenge</td>
</tr>
</tbody>
</table>
Confirmation of antigen expression

Anti-V5 antibody
(expected size of GP-V5 fusion protein = 76.6kDa, positive control protein = 62kDa)

Anti-CCHF rabbit polyclonal sera
(similar post-translational cleavages in MVA-GP to native protein)

(NB: Findings were similar with MVA-NP construct showing positive protein expression)

Development of a vaccine against CCHF virus
Single vs. booster dosing

Balb/C mice, $10^7$ pfu delivered i.m.

Single MVA-NP dose
Double MVA-NP dose
Saline control

Animals culled (n=3/group) at days 3, 8 and 12 post-vaccination for immunogenicity studies.

Antigen-specific T-cell responses made to CCHF NP peptides.
(20mers overlapping by 8aa, two pools containing 31 peptides)

Prime-boost approach gave greater frequencies of Ag-specific T-cells
IFN-γ ELISPOT assay

Solid bars = 129Sv/Ev mice; hatched bars = A129 mice [IFN-α/βR⁻/⁻]

Results for MVA-GP shown.

- Similar responses in 129Sv/Ev and A129 mice were detected.
- Immunogenicity was not evenly distributed across the antigen.
- Responses were specific to the glycoprotein, and similar between mouse strains.
Antibody responses

Western blot

Both MVA-GP and MVA-NP vaccines induced antigen-specific antibodies.
Efficacy studies

No protective effects seen with MVA-NP, but 100% protection from lethal challenge with MVA-GP

⇒ First demonstration of CCHF vaccine efficacy

MVA – GP shows 100% protection against an otherwise lethal CCHFV challenge
Clinical measurements

MVA-GP immunised animals showed no clinical evidence of CCHFv infection post-challenge:
• No loss in weight.
• No significant temperature deviations.
• Clinical signs scored healthy on all occasions.
RT-PCR for CCHFv gene expression (normalised to mouse HPRT gene expression).

Viral loads

Day 32 = 4 days post-challenge
Day 42 = 14 days post-challenge (end of study)

Viral load was significantly lower in MVA-GP vaccinated mice than in control groups.
Histology

Immunostaining

MVA-1974

Immunised A129 mice, 4 days post-challenge

- A few, scattered cells with cytoplasmic staining within the parenchyma.

MVA-GP

- Normal parenchyma.

Spleen

- Frequent, diffuse, positively stained hepatocytes.

Liver

- A few, positively stained cells within an inflammatory cell focus.
Mechanism of Protection

Previous reports and anecdotal evidence point to importance of antibody response in protection


Tishkova, F. et al., *CCHF survivors show strong neutralising antibodies are protected from further infection*. Mikrobiologiya i Virusologiya
Mechanism of Protection

Passive/Adoptive transfer

Immunise mice with MVA-GP

Isolate splenocytes (T-cells) and sera (antibody) from immunised mice

Adoptively transfer splenocytes into naïve mice

Passively transfer sera into naïve mice

Challenge with CCHF virus

Determine survival effects

Preliminary results with MVA – GP show cellular AND antibody responses may be important
Conclusions

- Vaccine is based on CCHF glycoproteins expressed in a viral vector.
- CCHF-specific antibodies and T-cells.
- 100% protection from disease in a pre-clinical model.
- MoA appears to rely on both T cell and antibody

Next steps include:
- NHP pre-clinical data package
- Assess cross neutralisation of CCHFv strains
- Assess prime boost strategies

Buttigieg et al., (2014) PLOS one.9 (3) 91516-28
DNA-based vaccines expressing the CCHFv M segment

Recombinant tobacco leaves expressing $G_N$ and $G_C$

Inactivated virus from cell culture
Canakoglu et al., (2015). PLOS NTD.

CCHF Virus Like Particles:

Recombinant Adenovirus
Feldmannu et al.,

Anti Tick vaccines: Cement & midgut antigen ($Bm86$) partially protective
Labuda et al. 2006 PLOS one 2 (4) e24
Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial

Ana Maria Henao-Restrepo, Ira M Longini, Matthias Egger, Natalie E Dean, W John Edmunds, Anton Camacho, Miles W Carroll, Moussa Doumbia, Bertrand Draguez, Sophie Duraffour, Godwin Enwere, Rebecca Grais, Stephan Gunther, Stefanie Hossmann, Mandy Kader Kondé, Souleymane Kone, Eeva Kuisma, Myron M Levine, Sema Mandal, Gunnstein Norheim, Ximena Riveros, Aboubacar Soumah, Sven Trelle, Andrea S Vicari, Conall H Watson, Sakoba Kéita, Marie Paule Kieny*, John-Arne Røttingen*

Summary

Background A recombinant, replication-competent vesicular stomatitis virus-based vaccine expressing a surface glycoprotein of Zaire Ebolavirus (rVSV-ZEBOV) is a promising Ebola vaccine candidate. We report the results of an interim analysis of a trial of rVSV-ZEBOV in Guinea, west Africa.

100% Efficacy for preventing tertiary cases in ring vaccinations: 16 cases in 21 day vaccine delay compared to 0 for no delay
Vaccine Target

• Healthcare workers in endemic countries
• At risk occupations; abattoirs, farmers
• At risk local population in endemic countries
• International response healthcare workers
• Military personnel
• Farm animals
Acknowledgements

Stuart Dowall
Karen Buttigieg
Stephen Findlay-Wilson
Ola Miloszewska
Emma Rayner
Geoff Pearson
Graham Hall
Roger Hewson

Bernie Moss (NIH)
Linda Wyatt (NIH)
Ali Mirazimi (Karolinska Institute)