ANIMAL VIRUSES USED FOR VACCINATION OF HUMANS

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Disclosure

• Dr. Monath has current financial interests in
  • Crozet BioPharma LLC
  • US Biologic Inc
  • Public Health Vaccines LLC

• Previous financial interests in:
  • NewLink Genetics Corp
One Health Paradigm

Domestic animals
Transmitting hosts
Dead-end hosts

Wild Animals
Transmitting hosts
Dead-end hosts

Humans
Transmitting hosts
Dead-end hosts
One Health Paradigm

Host range restriction

Dead-end hosts

Inapparent infection

Transmitting hosts

Dead-end hosts

DISEASE

Domestic animals

Transmitting hosts

Humans

Transmitting hosts

Wild Animals

Transmitting hosts

Dead-end hosts
Prevention and control of zoonotic infections by vaccination

Domestic animals
- Dead-end hosts
- Transmitting hosts
  - Rabies

Wild Animals
- Dead-end hosts
- Transmitting hosts
  - Rabies

Humans
- Dead-end hosts
- Transmitting hosts
Prevention and control of zoonotic infections by vaccination

Domestic animals
- Transmitting hosts
  - JE, VEE
  - MERS
  - Hendra
  - Q fever

Wild Animals
- Transmitting hosts
  - Lyme disease
- Dead-end hosts
  - Q fever

Humans
- Transmitting hosts
  - Yellow fever
  - Ebola
  - Hep E
- Dead-end hosts
  - JEV, Q fever

EEE, WEE WN
- Dead-end hosts

Prevention and control of zoonotic infections by vaccination
Reservoir Targeting Vaccine Lyme Disease

E. coli expressing antigen

Osp A Vaccine

Feeding station for Peromyscus
Animal viruses used in human vaccinology

• Animal viruses (bacteria) modified or inactivated to protect against the *homologous* disease agent of humans
  • Vaccines against the corresponding zoonosis
  • Many examples, e.g. anthrax, plague, Q fever, yellow fever, JEV, VEE, TBE, etc.

• Animal viruses (bacteria) used to protect against a *heterologous* disease agent of humans
  • Used directly
    • Naturally attenuated for humans due to restricted host range
    • Cross-protects against the human disease agent (Jennerian vaccine)
  • Used as a vector of the gene/antigen of interest
Animal viruses (bacteria) used to protect against a *heterologous* disease agent of humans

- Naturally attenuated, host range restricted, used directly without modification (*true Jennerian vaccine*)

<table>
<thead>
<tr>
<th>Animal agent</th>
<th>Type</th>
<th>Human indication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium microti</em></td>
<td>Live, host-range restricted</td>
<td><em>M. tuberculosis</em></td>
</tr>
<tr>
<td><em>Mycobacterium vaccae</em></td>
<td>Inactivated, whole cell</td>
<td><em>M. tuberculosis</em></td>
</tr>
<tr>
<td><em>(M. obuense)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinia, MVA</td>
<td>Live, host range restricted</td>
<td>Variola</td>
</tr>
<tr>
<td>Simian, bovine, ovine rotaviruses</td>
<td>Live, host range restricted</td>
<td>Human rotaviruses</td>
</tr>
<tr>
<td>Sendai (mouse parainfluenza-1)</td>
<td>Live, host range restricted</td>
<td>hPIV1</td>
</tr>
<tr>
<td>Langat virus</td>
<td>Live, host range restricted</td>
<td>Tick-borne encephalitis</td>
</tr>
</tbody>
</table>

In red: approved or in registration
Animal viruses (bacteria) used to protect against a heterologous disease agent of humans

- Recombinant technology using animal viruses as a vector

<table>
<thead>
<tr>
<th>Animal agent</th>
<th>Type</th>
<th>Human indication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium bovis</em> (BCG)</td>
<td>Live, recombinant</td>
<td><em>M. tb</em> and multiple other targets</td>
</tr>
<tr>
<td>Bovine rotavirus</td>
<td>Live, bovine-human reassortants</td>
<td><strong>Human rotavirus</strong></td>
</tr>
<tr>
<td>Vaccinia, MVA, canarypox</td>
<td>Live, recombinant (replicating or defective)</td>
<td>Multiple targets</td>
</tr>
<tr>
<td>Chimpanzee adenoviruses (+/- MVA boost)</td>
<td>Live, recombinant (defective)</td>
<td>Ebola, Sudan, HIV, HCV, RSV, rabies</td>
</tr>
<tr>
<td>Vesicular stomatitis</td>
<td>Live, recombinant (replicating)</td>
<td>Ebola, Marburg, Lassa, Nipah, SARS</td>
</tr>
<tr>
<td>Bovine parainfluenza type 3</td>
<td>Live, recombinant (replicating)</td>
<td>RSV, hPIV1-4, hMPV, SARS</td>
</tr>
<tr>
<td>Sendai (mouse parainfluenza-1)</td>
<td>Live, recombinant (replicating)</td>
<td>RSV, hPIV1-4, hMPV, HIV</td>
</tr>
<tr>
<td>Canine distemper</td>
<td>Live, recombinant (replicating)</td>
<td>HIV, rabies</td>
</tr>
<tr>
<td>Newcastle disease virus</td>
<td>Live, recombinant (replicating)</td>
<td>RSV, Influenza, polio, SARS, Ebola</td>
</tr>
<tr>
<td>Flaviviruses (YF, dengue, Langat)</td>
<td>Live, recombinant (replicating or defective)</td>
<td>Dengue, JEV, West Nile (vet.), TBE, Zika, <em>flu</em>, RSV, HIV, rabies, malaria</td>
</tr>
<tr>
<td>Alphaviruses (VEE, CHIK, Sindbis, SFV)</td>
<td>Live, recombinant (replicating, defective)</td>
<td>EEE, WEE, RRV, HIV, SARS, HSV, HPV</td>
</tr>
<tr>
<td>Rabies</td>
<td>Recombinant, defective or inactivated</td>
<td>Marburg</td>
</tr>
<tr>
<td>LCMV</td>
<td>Recombinant, defective</td>
<td>CMV</td>
</tr>
</tbody>
</table>

In red: approved or in registration
Shaded: In clinical development, some with promising results
Advantages of recombinant animal viruses used as vaccines

• Platform technology for multiple indications
• Attenuated by host restriction or defective, single cycle
• Replicating constructs generally provide rapid protection after a single dose
• Memory responses, durable immunity
• No/minimal pre-existing immunity
• Potential for distinguishing natural from vaccine immunity
Recombinant vesicular stomatitis viruses from DNA
(rhabdovirus/viral replication/viral assembly)

NATHAN D. LAWSON*, ELIZABETH A. STILLMAN‡, MICHAEL A. WHITT§, AND JOHN K. ROSE*†§

Departments of *Pathology, ‡Cell Biology, and †Biology, Yale University School of Medicine, New Haven, CT 06510; and §Department of Microbiology and Immunology, University of Tennessee, Memphis, TN 38163
Advantages of VSV as a vector

- Platform technology for multiple indications
- Attenuated by host restriction in humans
  - Wild type virus rare cause of disease in humans
- Replicating, rapid protection after a single dose
- Memory responses, durable immunity
- No/minimal pre-existing immunity
- Potential for distinguishing natural from vaccine immunity
Pseudotyping VSV with Ebola or Marburg virus GP

Complete replacement of VSV G protein (virulence factor, principal antigen) with desired target protein

WHO R&D Blueprint
No presently existing medical countermeasures

- Crimean-Congo haemorrhagic fever
- Ebola virus and Marburg virus disease
- Lassa fever
- Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS)
- Nipah and henipaviral diseases
- Rift Valley fever
- Zika virus disease
- Disease X
Recombinant VSV vaccines against emerging and re-emerging diseases

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Indication, status</th>
<th>Developer (funding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rVSV-ZEBOV (V920)</td>
<td>Ebola, In registration</td>
<td>PHAC, NewLink, Merck (BARDA, DTRA), Profectus</td>
</tr>
<tr>
<td>rVSV-Marburg</td>
<td>Marburg</td>
<td>Public Health Vaccines (BARDA)</td>
</tr>
<tr>
<td>rVSV-Sudan</td>
<td>Sudan</td>
<td></td>
</tr>
<tr>
<td>rVSV-Lassa</td>
<td>Lassa fever</td>
<td>Emergent, Profectus, IAVI (CEPI)</td>
</tr>
<tr>
<td>rVSV-Nipah</td>
<td>Nipah</td>
<td>Public Health Vaccines</td>
</tr>
<tr>
<td>rVSV-CHIK</td>
<td>Chikungunya</td>
<td>Yale (NIAID)</td>
</tr>
<tr>
<td>rVSV-MERS</td>
<td>MERS</td>
<td>Harbin Veterinary Research Institute</td>
</tr>
</tbody>
</table>

VSV explored widely as a vector for other indications and as an oncolytic virus
Strong Preclinical Data Supports VSV as a Vector for Development of Ebola and Marburg Vaccines

- 100% protection against lethal challenge (IM ~1000 LD$_{50}$) in NHPs following a single immunization
- Well tolerated in NHPs, minimal viremia
- Antibody response in 7-14 days
- Antibodies mediate protection
- Protection in NHPs with a single dose given shortly (3-7 days) before or even after challenge, mediated by innate immunity
- Safe, protective in immunodeficient (SHIV-infected) NHPs
- Not neurovirulent after IC inoculation in NHPs
### rVSV as a Platform for Vaccine Development: Complete Protection in Non-human Primate Models

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Dose(s) pfu</th>
<th>No. animals</th>
<th>Seroconversion (ELISA, Neut)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>rVSV-EBOV</td>
<td>3x10^2-1x10^8</td>
<td>45</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>9</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>rVSV-MARV</td>
<td>1x10^7</td>
<td>18</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>rVSV-Lassa</td>
<td>1-3x10^7</td>
<td>21</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>10</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>rVSV-Nipah</td>
<td>1x10^7</td>
<td>3</td>
<td>100%</td>
<td>100% (0% ill)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3</td>
<td>0%</td>
<td>67% (100% ill)</td>
</tr>
</tbody>
</table>

USAMRIID, Merck and NewLink, unpublished data
Feldmann H, unpublished data
Marzi A et al Emerg Infect Dis 215;21:305
Marzi A et al PNAS 2013:110:1893
Prescott J et al Vaccine 2015;33:2823
rVSV-EBOV (V920, Merck) Vaccine

- >18000 people vaccinated in Phase I-III trials
- >750000 people vaccinated in expanded use protocol for outbreak control
- In registration US, EU

![Image of people in protective gear carrying a boat]

- 2014-16: 28,616
- 2018-19: 1,251

![Map of Africa with circles indicating vaccination numbers]
Antibodies mediate immunity by rVSV-EBOV (V920) vaccine

- Mixed effector functions play a role in protection (neutralization and Fc-mediated functional activities)
- Therapeutic efficacy with passive transfer of mAbs having both neutralizing and cell-targeting activities
- Active immunization fully protective despite CD8+ depletion
- Absence of non-survivors makes it difficult to differentiate immune correlates
- Antibody repertoire and epitope specificity in polyclonal response is poorly understood
rVSV-EBOV (V920, Merck) is highly immunogenic in humans across a wide dose range
Phase 1b Clinical Trial (n=512) IgG ELISA

Seroconversion

Geometric Mean Titer (endpoint)

Study day

Study day

Heppner DG et al. Lancet Infect Dis 2017;17:854-866
rVSV-EBOV (V920, Merck) is highly immunogenic across a wide dose range
Phase 1b Clinical Trial (n=512) 60% Plaque-reduction Neutralization

Heppner DG et al. Lancet Infect Dis 2017;17:854-866
Early innate response to rVSV-EBOV (V920) shapes and predicts adaptive humoral response

IP-10 and NK cells strong independent correlate of adaptive response

inflammatory Monocytes, mDCs expressing CD86 counter-regulatory, suppressive effect on adaptive response
rVSV-EBOV Protects NHPs as early as 3 days before, or even briefly, after lethal challenge in absence of detectable adaptive immune response
Protection is not virus-specific and attributable to innate immunity

Marzi A et al. Science. 2015;349:739-42
**rVSV-EBOV (V920, Merck vaccine)**

100% protective by 9 days after vaccination

Phase 3 “Ring Vaccination” trial, Guinea 2015

<table>
<thead>
<tr>
<th></th>
<th>Immediate Vaccination</th>
<th>Delayed Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. individuals (clusters)</td>
<td>2108 (51)</td>
<td>1429 (46)</td>
</tr>
<tr>
<td>Cases EVD</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Attack rate</td>
<td>0%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Vaccine efficacy</td>
<td>100% (p=0.045)</td>
<td></td>
</tr>
</tbody>
</table>

HHS’ BARDA Funds Its First Marburg Virus Vaccine Development

Vaccine Could Address an Important Biodefense and Public Health Threat

To increase national health security against biothreats and protect public health, the U.S. Department of Health and Human Services (HHS) will partner with Public Health Vaccines LLC of Cambridge, Massachusetts, to develop a potential vaccine against Marburg virus. No licensed vaccine for this virus exists today.
Marburg Virus disease
Location, no. cases (case fatality rates)

South Africa
Angola
DRC
2004-2005
252 (90%)
1975
3 (33%)

1998-2000
154 (82%)

1980, 1987
3 (67%)

20 (30%)

Kenya
Uganda
Zimbabwe

Marburg Virus disease
rVSV-Marburg: single dose protects cynos against Marburg Virus

Nipah Virus Disease

- Paramyxovirus
- Sporadic/epidemic
- 10-50 cases/year
- SE Asia, India
- *Pteropus* bat reservoir
- Pigs affected and may be source of human infection
- Encephalitis, vasculitis, pneumonitis
- 70% CFR

- Closely related Hendra virus in Australia
- Commercial subunit Hendra vaccine for horses
- Vaccination of horses protects humans from contact infection
Reverse genetics system for generating rVSV Nipah candidate vaccine
Single dose of rVSVΔG/ZEBOVGP/NiVG elicits strong humoral response and protects African Green Monkeys

Prescott J et al. Vaccine 2015;33:2823
ANIMAL VIRUS VECTOR PLATFORMS FOR VETERINARY APPLICATIONS
Live, replicating recombinant vaccines for veterinary use

- Requirements
  - Host range covers target species
  - Carrying capacity for nonessential foreign gene(s) of interest
  - Immunogenic
  - Safe

- Alphaviruses
- Pox viruses
- Herpes viruses, e.g. pseudorabies (PRV)
- BVDV
- Rhabdoviruses
  - SAD B19-based RABV
  - ???VSV
**Commercial recombinant vectored veterinary vaccines using animal virus vectors**

<table>
<thead>
<tr>
<th>Species</th>
<th>Pathogen</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Feline leukemia</td>
<td>Canary pox</td>
</tr>
<tr>
<td>Cat</td>
<td>Rabies</td>
<td>Canary pox</td>
</tr>
<tr>
<td>Dog</td>
<td>Distemper</td>
<td>Canary pox</td>
</tr>
<tr>
<td>Ferret</td>
<td>Distemper</td>
<td>Canary pox</td>
</tr>
<tr>
<td>Horse</td>
<td>Influenza</td>
<td>Canarypox (MVA, herpes-1)</td>
</tr>
<tr>
<td>Horse</td>
<td>West Nile</td>
<td>Yellow fever chimera, vaccinia</td>
</tr>
<tr>
<td>Poultry</td>
<td>Avian influenza, Newcastle’s, Infectious laryngotracheitis</td>
<td>Fowlpox, Turkey herpesvirus</td>
</tr>
<tr>
<td></td>
<td>Infectious bursal disease</td>
<td>Fowlpox</td>
</tr>
<tr>
<td>Swine</td>
<td>Classical swine fever</td>
<td>Recombinant bovine viral diarrhea chimera</td>
</tr>
<tr>
<td></td>
<td>Circovirus</td>
<td>Canary pox</td>
</tr>
</tbody>
</table>
VSV as a Vector for Animal Vaccines?

• Wide Host range
  – Virtually all mammalian species
• Accommodates large and multiple foreign gene inserts, without affecting replication
• Proven as human viral vector
• Potential for DIVA vaccine
• Potential for mucosal delivery
rVSV-EBOV administered orally, intranasally or by IM injection protects cynos against lethal EBOV challenge

rVSV-EBOV Vaccine for Immunization of Great Apes against Ebola

- Catastrophic loss of >50,000 gorillas and many chimpanzees due to Ebola epizootics 1990s and early 2000s
- Current Ebola outbreak in northeastern DRC threatens gorilla populations including critically endangered Mountain gorillas (*Gorilla beringei*)
- Preparations made to vaccinate habituated gorillas with rVSV-EBOV (V920, Merck) by dart gun

Chimp killed by Ebola, Rep Congo 2014
Walsh P et al. Sci Rep, 2017
The Final (Oral Ebola) Vaccine Trial on Captive Chimpanzees?

Peter D. Walsh¹, Drishya Kurup², Dana L. Hasselschwert³, Christoph Wirblich³, Jason E. Goetzmann³ & Matthias J. Schnell²

Could new oral vaccine technologies protect endangered wildlife against a rising tide of infectious disease? We used captive chimpanzees to test oral delivery of a rabies virus (RABV) vectored vaccine against Ebola virus (EBOV), a major threat to wild chimpanzees and gorillas. EBOV GP and RABV GP-specific antibody titers increased exponentially during the trial, with rates of increase for six orally vaccinated chimpanzees very similar to four intramuscularly vaccinated controls. Chimpanzee sera also showed robust neutralizing activity against RABV and pseudo-typed EBOV. Vaccination did not induce

Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection

Andrea Marzi¹*, Aisling A. Murphy²*, Friederike Feldmann³, Christopher J. Parkins⁴, Elaine Haddock¹, Patrick W. Hanley³, Matthew J. Emery⁵, Flora Engelmann⁶, Ilhem Messaoudi⁷, Heinz Feldmann¹* & Michael A. Jarvis⁸*

Ebola viruses pose significant public health problems due to their high lethality, unpredictable emergence, and localization to the poorest areas of the world. In addition to implementation of standard public health control procedures, a number of experimental human vaccines are being explored as a further means for outbreak control. Recombinant cytomegalovirus (CMV)-based vectors
Nipah Virus Disease

- The 1998 outbreak in Malaysia was concentrated among pig farmers
  - 92% of cases reported contact with pigs
- Compared to controls, persons with Nipah encephalitis were
  - 5.6 X more likely to have close contact with pigs.
- Outbreak ceased following culling over 900,000 pigs
  - Pork industry decimated
- Vaccination of swine a feasible control measure?

Courtesy: S Luby

BBC news
Safety of recombinant VSV constructs for animal species susceptible to VSV disease

- Host range and virulence factors
  - Virus replication dependent on vector (VSV) genes
  - VSV has very wide host range in all mammals
  - Cell/tissue tropism dependent on transgene
Ebola broad species tropism spans 6 mammalian orders

- Bats
- Humans
- Monkeys
- Pigs
- Duiker
- Cats
- Dogs
- Ferrets
- Mice
- Hamsters
- Guinea pigs

Ebola enters mammalian cells through the Niemann-Pick C1 receptor

High sequence homology of NPC-1

3’-N-P-M-GP-L-5’ rVSV-EBOV

3’-N-P-M-G-P-G-L-5’ rVSV-EBOV-NiVG
Henipavirus (Nipah) broad species tropism spans 6 mammalian orders

- Bats
- Humans
- Monkeys
- Pigs
- Horses
- Cats
- Dogs
- Ferrets
- Hamsters
- Guinea pigs

Nipah enters mammalian cells through the ephrin-B2 or ephrin-B3 surface glycoprotein

95-98% sequence homology of ephrin-B2/B3 across mammals

Safety of recombinant VSV constructs for animal species susceptible to VSV disease

• In a study of pigs inoculated (oral, snout) with wild-type VSV or rVSV-ZEBOV, the majority of animals developed VSV-like clinical illness
  – Illness delayed and milder in rVSV-ZEBOV group

• Conclusions
  – rVSV safety for livestock needs careful evaluation in target species, with consideration of the transgene host range

Monath TP et al. Vaccine X 2019:100009
Other Vesiculovirus Vectors

• Closely related viruses with similar genome organization
• Vesiculovirus genus with no known illness in humans or domestic livestock that could serve as vectors
  – Isfahan  -- Jurona  -- Malpais Spring
  – Carajas  -- Maraba  -- Perinet
Conclusions

- One Health Paradigm illustrates opportunities for:
  - Vaccination of animals to prevent certain zoonotic infections of humans
  - Use of animal viruses for design and of vaccines and their application
- Replicating vaccines have advantages for immunogenicity, rapid intervention, durability of protection
- Host range and cell tropism critical to in vaccine design
- Multiple animal virus vectors being clinically tested in humans and some are commercialized or in registration (Pox, Flavi, Rota, VSV)
- VSV a prominent candidate for human vaccines, but safety concerns remain for veterinary applications
- Nonpathogenic VSV-relatives are in development as vectors
Thank you!