Lassa Virus Vaccines

Overview of LASV Vaccine Candidates

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LASV vaccines: factors to consider
Safety in immunosuppressed (HIV/AIDS) patients
LASV vaccines: factors to consider
Safety in pregnant women and children

Lassa fever is especially severe in third trimester of pregnancy:

• Maternal death ~20%
• Fetal loss almost 100%

Hospital study in Nigeria of >300 febrile children showed LASV presence in:

• 5.4% with undifferentiated fever
• 3.9% with convulsions and fever
• 6% with fever but no convulsions

LASV vaccines: factors to consider
Ability to prevent neurological complications and deafness

Symptoms include high fever, malaise, severe sore throat, neurological deficit, mucosal bleeding and effusion

• Long lasting neurological deficit after recovery
• Up to 30% of non-fatal cases result in permanent sudden onset unilateral or bilateral sensorineural deafness
• Ribavirin does not prevent deafness
LASV vaccines: factors to consider
Pre-existing immunity to vectored vaccines
Potential overlap with filovirus outbreaks and vaccine trials
LASV vaccines: factors to consider
Logistical issues

- Efficient, cost-effective manufacturing to produce sufficient vaccine doses
- Sustainable and transferable technology
- Uncomplicated delivery method
- Stability without extensive cold chain requirements
- Minimal number of vaccinations
- Geographic region of use (Africa)

Freetown Lungi International Airport

Rural road, Sierra Leone
# Most Advanced LASV vaccine candidates

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ML29 MOPV/LASV live reassortant
L RNA from Mopeia virus and S RNA from Lassa virus, Josiah strain\textsuperscript{1,2,7}

**Mice:** Adoptive transfer of splenocytes from immune CBA mice protected CBA mice from i.c. challenge\textsuperscript{2} and a single intraperitoneal (i.p.) immunization of CBA/J mice with ML29 protected animals against lethal i.c. challenge\textsuperscript{5}

**Guinea pigs:** A single injection of 10\textsuperscript{3} pfu of ML29 protected strain 13 guinea pigs from 1,000 pfu SC challenge with homologous LASV 30 days after vaccination\textsuperscript{2} and also from a more distant Nigerian strain of LASV\textsuperscript{3}

**Marmosets:** A single injection of ML29 caused low, transient viremia, and low levels of ML29 replication. The vaccine elicited specific immune responses and completely protected marmosets against fatal disease by inducing sterilizing cell-mediated immunity\textsuperscript{4}

**Rhesus macaques:** ML29 infection did not cause detectable disease in uninfected or SIV infected NHP and elicited protective immune responses in a surrogate challenge with LCMV-WE\textsuperscript{6,7}

\textsuperscript{1}Lukashevich 1992. Virology 188:600-605.
\textsuperscript{5}Goicochea et al. 2012. Vaccine 30:1445-1452.
ML29 MOPV/LASV live reassortant
Safety Considerations

- Stable through 12 cell culture passages
- Stability in animals after a single passage showed some sequence changes but not changes in attenuation
- Infection of SIV-infected rhesus macaques did not lead to arenavirus disease, but caused viremia in some of the SIV infected NHP but not in non-SIV infected NHP (protection was not assessed in the immunocompromised NHP)
- ML29 caused transient viremia in marmosets, was detected in some areas of the brain and was recovered from spinal cord
- Shedding detected in 1 of the ML29 infected marmosets, which had viral RNA in oral, rectal and vaginal samples
- Reversion due to reassortment was assessed in guinea pigs by coinfection with homologous LASV or a Nigerian isolate with no increased disease (gene analysis of viruses after coinfection not analyzed)
- Not tested in pregnant NHP
- Safety in humans not assessed
ML29 MOPV/LASV live reassortant
Technical/logistical considerations

Logistical Considerations:
• Single dose conventional needle injection is possible
• Dose required for humans not defined
• Stability likely to have cold chain requirements
• Technology is not amenable to multiagent LASV-filovirus vaccine

New Technology:
• Generate ML29 from infectious clone to stabilize genetic drift and to possibly eliminate ability to reassort
• Engineer S segment for broader immunity
rVSV-vectored GPC
Replication competent VSVΔG/LVGPC\textsuperscript{1,2}

**Guinea pigs**

- Strain 13 guinea pigs vaccinated IP with 10\textsuperscript{6} pfu of VSVΔG/LVGPC were protected from disease after challenge with 10\textsuperscript{4} TCID\textsubscript{50} of LASV strain Josiah as well as with 3 other clade 4 LASV isolates from Liberia, Mali or Nigeria\textsuperscript{3}

- Outbred guinea pigs (n=9-12) were vaccinated with a single dose of VSV-LASVGPC (1x10\textsuperscript{6} PFU) or an irrelevant VSV-based vaccine (VSV-ANDV-GPC) followed by a lethal challenge of GPA-LASV (1x10\textsuperscript{6} TCID\textsubscript{50}) at 7, 14, 25, 189, and 355 days post-vaccination (Safronetz et al. unpublished information).

  - Statistically significant rapid and long-term protection was achieved at all time points with 100% protection at days 7 and 14 post-vaccination. While 83% and 87% protection was achieved at days 25 and 189 post-vaccination, respectively.

  - When guinea pigs were challenged one year after vaccination 71% protection was achieved. None of the control immunized animals survived challenge.

rVSV-vectored GPC
Replication competent VSVΔG/LVGPC$^{1,2}$

**Cynomolgus macaques**
- 4 cynos vaccinated IM with $2 \times 10^7$ pfu of VSVΔG/LVGPC developed humoral and cellular responses to LASV and survived IM challenge (4-weeks after vaccination) with 10,000 pfu of LASV (viremia detected)$^2$
- 3 cynos vaccinated with VSVΔG/LVGPC vaccine were also protected from disease after challenge with a Liberian strain of LASV$^3$
- 3 cynos vaccinated IM with $10^7$ pfu VSVΔG/LVGPC survived IM challenge with LASV 28 days after vaccination and were not viremic$^4$
- These same cynos were vaccinated 90 days after the LASV vaccination with $10^7$ pfu of a similar VSVΔG/EBOV vaccine, and developed antibody responses to EBOV and were protected from disease after an EBOV challenge 28 days later (but the NHP had increased titers to VSV and seroconverted to EBOV VP40$^4$)

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Safety Considerations:

- Platform was already used extensively in Ebola virus outbreak with no serious safety issues.
- Not tested in pregnant NHP, and pregnant and lactating women were excluded from rVSVEBOV clinical trials.
- Not tested in immunocompromised NHP, but rVSVEBOV vaccine did not cause disease in SHIV infected NHP and protected some of them from EBOV challenge.
- rVSVLASV not tested for neurovirulence but rVSVEBOV and rVSVMARV vaccines were safe in NHP neurovirulence model.
rVSV-vectored GPC
Technical/logistical considerations

Logistical Considerations:
• Single dose, conventional needle injection is possible
• Multivalent arenavirus/filovirus DNA vaccines are possible
• Dose required for humans not defined
• Cold chain required
• Pre-existing immunity in overlap regions
• Duration of immunity unknown
LASV DNA vaccine (1 of 3 CEPI candidates (Inovio)
Plasmid DNA, codon optimized GPC (Josiah)\(^1\)

Guinea pigs:
- Strain 13 guinea pigs vaccinated with 100 µg of the LASV DNA vaccine three times at 3-week intervals by dermal electroporation developed neutralizing antibodies and remained healthy and aviremic after SC challenge with 1000 pfu of LASV\(^1\)
- Two vaccinations of strain 13 guinea pigs by dermal electroporation with the LASV and an EBOV DNA vaccine (50 µg or 100 µg) 4 weeks apart provided 100% protection against challenge with LASV (1000 pfu SC), EBOV (1000 pfu IP) or both viruses simultaneously\(^2\)
- Guinea pigs were cross-challenged with LASV or EBOV at 120 days after initial challenge and all remained healthy

Cynomolgus macaques:
- Two vaccinations (10 µg) given 4 weeks apart by dermal electroporation protected 100% of cynos against disease and viremia following IM challenge with 1000 pfu LASV 4-weeks after vaccination
- The LASV DNA vaccine also prevented measureable deafness in cynos

\(^1\)Cashman et al. 2013. Vaccines 1:262-277.
LASV DNA vaccine
Safety considerations

- DNA should be safe in all populations
- Not transmitted person to person or shed into the environment
- Cannot revert to virulence
- No issues with pre-existing immunity
- Plasmid DNA vaccines have been used in more than 100 clinical trials
LASV DNA vaccines (in general)

Technical/logistical issues

Logistical Considerations:
- Well established and approved manufacturing procedures
- Can be quickly and easily modified
- Manufacturing easy and cost effective
- Stable at a wide range of temperatures
- Easy to produce sufficient vaccine quantities
- Multivalent arenavirus and filovirus vaccines are possible
- Probably requires two vaccinations
- Tech transfer of technology/devices required
- Electroporation device needed for delivery (Hand-held battery operated dermal delivery electroporation devices being developed)

New Technology:
- Alternative delivery methods (e.g. Pharmajet Stratis) show promise with other DNA vaccines
- DNA delivery via nanoparticles for timed release could be explored
- Genetic adjuvants to increase and/or skew primary immune response could reduce doses
### CEPI LASV vaccine candidate (2 of 3)

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<th>Vaccine concept</th>
<th>Company</th>
<th>Technology status</th>
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| Measles vector (Themaxyn® platform) | Themis, Inc. (Exclusive license from Institute Pasteur) | • Reverse genetics system used to generate live-attenuated recombinant measles (Schwartz strain) virus  
• Standard measles vaccine as a vaccination vector, which has been in millions of people  
• No LASV publications, but Themaxyn-vectored Chikungunya* and Zika** viruses tested in clinical studies |

**No preclinical or clinical data are disclosed

**General References of technology:**
Other LASV vaccine candidates
tested in animals

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Inactivated Rabies Vaccine platform
LASSARAB – a dual Lassa Fever and Rabies Vaccine

Unpublished information provided by Matthias Schnell, PhD

• Study in Hartley guinea pigs showed that three vaccinations of 30 µg of LASSARAB on days 0, 7, 28 (with adjuvant) protected 80% of animals against death after challenge with $10^4$ pfu LASV 30 days after last vaccination

• High levels of IgG induced with antibodies, but not neutralizing antibodies, correlated with survival of challenged guinea pigs

• LASV RNA remained in some of the challenged animals at study endpoint (50 days after challenge)
MVA-VLP-LASV Vaccine

Unpublished information provided by Farshad Guirakhoo, PhD

CBA/J

- Safety of MVA has been demonstrated in 120,000 subjects including HIV infected patients
- Single dose efficacy shown in animal models with EBOV, and ZIKV vaccines
- CBA/J mice vaccinated once by IM injection of 1x10^7 TCID\textsubscript{50} MVA-VLP-LASV and challenged 14 days later by IC injection of 1x10^3 PFU LASV-ML29, all survived
- Antigen-specific CD4+ and CD8+ T cells were abundant in spleens of immunized animals 11 days after a single IM dose as assessed by IFN-gamma and IL2 expressions in response to LASV GP peptides
- Protection largely mediated by T cell responses
- Collaborations with the Institute of Human Virology and The Scripps Research Institute for developing a “universal MVA-VLP-LASV vaccine” that induces both bNAbs and T cell responses
Next Steps
Addressing key vaccine development issues

Key issues include the lack of:

1. Standardized and well characterized challenge strains
2. Standardized reagents and animal models for assessing and comparing immune responses (especially poor for guinea pigs)
3. Animal modeling and testing with relevant LASV strains
4. Comprehensive understanding of correlates of protective immunity
5. Available BSL4 containment facilities
6. Access to clinical samples
7. Knowledge of the impact of strain diversity on vaccine protection
8. Commercial incentive to develop a vaccine
9. Infrastructure for conducting clinical trials (in some areas)