A rationally designed broadly-protective Lassa Fever Vaccine as an effective tool for fighting Lassa fever epidemics and endemicity

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Lassa Fever

- Viral hemorrhagic fever caused by Lassa Virus, an Old world Arenavirus
- Estimated annual incidence as high as 300,000 cases
- Approximately 10,000 annual deaths (~ 1-2% case fatality rate)
- Enzootic in multimammate (Mastomys natalensis)mice
- Ribavirin is used as primary treatment with mixed success
LF endemicity has potential for extension

2018 Nigerian LF Outbreak

- 3498 suspected LF cases
  - 633 confirmed positive
  - 20 probable
- 171 deaths from confirmed cases
  - 27.0% case fatality rate
- 45 Health care worker cases

https://ncdc.gov.ng
The Virus
Arena Virus causing infection

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lassa virus</td>
<td>Lassa fever</td>
</tr>
<tr>
<td>Junin virus</td>
<td>Argentine hemorrhagic fever</td>
</tr>
<tr>
<td><strong>(Candid #1 vaccine)</strong></td>
<td></td>
</tr>
<tr>
<td>Machupó virus</td>
<td>Bolivian hemorrhagic fever</td>
</tr>
<tr>
<td>Guanarito virus</td>
<td>Venezuelan hemorrhagic fever</td>
</tr>
<tr>
<td>Sabia</td>
<td>Brazilian hemorrhagic fever</td>
</tr>
</tbody>
</table>
Lassa Virus Structure

Family: Arenaviridae, now in the order Bunyavirales

Two ambisense genome segments

L 5' → Z → L

S 5' → GP → NP
Philogentic Tree Showing Genetic diversity and lineages of LFV

LASV Genetic Diversity

• High LASV genetic diversity, up to 25% and 32% for the S and L RNA segments, respectively

• LASV genetic diversity is a great challenge for the design of a universal LF vaccine

• ML29 is the only current vaccine candidate with demonstrated efficacy against Clade IV and Clade II


Kafetzopoulou LE et al, 2019, Science
THE VACCINE:
Rationally designed Mop/Las reassortant, clone ML29
2017 WHO Target Product Profile (TPP) for LASV vaccine

- Prefered preventive use vs. “emergency” (LF is endemic disease)
- WHO-acceptable safety/reactogenicity
- Injectable single-dose regimen
- ≥ 70% efficacy against LASV lineages I to IV
- Confers long-lasting (≥ 5 years) protection
- Acceptability for WHO pre-qualification (cheap, easy for production and application in countries with poor infrastructures)
### Advanced LASV vaccine candidates tested in “proof-of-concept” efficacy trials in NHPs (based on peer-reviewed publications)

<table>
<thead>
<tr>
<th>Vaccine candidate</th>
<th>LASV vaccine antigen formulation</th>
<th>Vaccine regiment</th>
<th>Efficacy against LASV/JOS$^a$</th>
<th>Efficacy against LASV/NIG$^b$</th>
<th>Viremia after challenge$^c$</th>
<th>Correlates of protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant vaccinia virus</td>
<td>GPC (JOS) NP GPC&amp;NP</td>
<td>Single vaccination, at 4 sites, total 1x10³ PFU, ID</td>
<td>88% 20% 100%</td>
<td>ND</td>
<td>Low-moderate</td>
<td>CMI</td>
</tr>
<tr>
<td>Reassortant MOPV/LASV, ML29 (JOS)</td>
<td>GPC&amp;NP (JOS)</td>
<td>1 dose, 1x10³ PFU, SC</td>
<td>100%</td>
<td>100%</td>
<td>&lt;LD</td>
<td>Sterilizing CMI</td>
</tr>
<tr>
<td>rVSVΔG/LVGP C</td>
<td>GPC (JOS)</td>
<td>1 dose, 1-6x10³ PFU, IM</td>
<td>100%</td>
<td>ND</td>
<td>Low, transient</td>
<td>nAbs? CMI?</td>
</tr>
<tr>
<td>YF17D/LASV</td>
<td>GPC (JOS)</td>
<td>2 doses, 1x10⁷ FFU, SC</td>
<td>20%</td>
<td>ND</td>
<td>Moderate-High</td>
<td>ND</td>
</tr>
<tr>
<td>VEEV-TC83 RNA replicon particles</td>
<td>GPC (JOS&amp;LP)$^d$</td>
<td>2 doses, 1x10⁷, SC</td>
<td>80%</td>
<td>20%</td>
<td>Moderate</td>
<td>ND</td>
</tr>
<tr>
<td>MOPEVAC$_{LASV}$</td>
<td>GPC (JOS)</td>
<td>1 dose, 6x10⁶ PFU/dose, SC</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>nAbs, CMI</td>
</tr>
<tr>
<td>DNA</td>
<td>GPC (JOS)</td>
<td>2 immunizations, 20 mg DNA at 4 sites, ID electroporation</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>nAbs?</td>
</tr>
</tbody>
</table>

Lukashevich IS et al., 2019 *F1000Res*
LASV Vaccine Design:
Rational Approach (e.g., ML29) vs. Platform-Based Approach (e.g., rVSV, ChAdOx1, DNA)

- LASV L RNA is major factor pathogenicity/attenuation and adaptation to mammalian hosts
- 80% LASV-exposed individuals with sub-clinical or flu-like symptoms are susceptible to re-infection with LASV, but have long-term protection against progressing LF disease
- LF survivors had strong memory CD4+ T-cell responses against conserved epitopes in NP and GP2 from LASV/JOS and Nigerian LASV strains
- ML29 presents LASV NP- and GPC-derived epitopes to the MHC molecules in the most effective way to induce robust cross-protective T cell responses
- Anti-NP T cell response at early stage effectively controls infection and contribute to cross-protective immunity
- NP-mediated IFN type I suppression is a common feature of pathogenic and non-pathogenic mammalian arenaviruses related to the establishment persistence in rodents
LCMV and LASV Virulence Maps to the L RNA

ARM/ARM

ARM/WE

WE/WE

WE/ARM

MOP/MOP

MOP/LAS

LAS/LAS

LAS/MOP

Riviere Y et al., 1985
Riviere Y & Oldstone MB, 1986
Lukashevich IS, 1992
Lukashevich IS et al., 2005
Protective Efficacy of Vv-expressed LASV Proteins in NHPs (cynomolgus & rhesus)
Contribution of LASV NP to strong adaptive immunity

Expression of GPC is necessary for protection
The GPC&NP provided the highest protection
No neutralizing Abs
Cynos and rhesus induced similar responses

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Survivors</th>
<th>%</th>
<th>Viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP1 or GP2</td>
<td>0</td>
<td>0</td>
<td>High</td>
</tr>
<tr>
<td>GPC</td>
<td>6/7</td>
<td>85</td>
<td>Moderate</td>
</tr>
<tr>
<td>NP</td>
<td>3/11</td>
<td>27</td>
<td>High, Low</td>
</tr>
<tr>
<td>GPC+NP</td>
<td>5/6</td>
<td>83</td>
<td>Low</td>
</tr>
<tr>
<td>GPC&amp;NP</td>
<td>3/3</td>
<td>100</td>
<td>Barely detectable</td>
</tr>
</tbody>
</table>

Fisher-Hoch et al., 2000
McCormick & Fisher-Hoch, 2002
Reassortant ML29 is a Lead Vaccine Candidate Against LF

Safe & Immunogenic in all Animal models including SIV-Rhesus macaques

ML29-specific mutations (upper panel) are represented by bars. Asterisks indicate non-conservative substitutions. Predicted structure of GP2 (low panels); the K272E mutation (marked by star) located between two fusion domains of GP2 introduces two negatively charged groups and results in refolding and exposure of transmembrane and cytoplasmic domains of ML29/Jos GP2 (right panel) (http://www.sbg.bio.ic.ac.uk/phyre2/html/).

ML29 is Genetically Stable In Vitro and In Vivo:

- Limited Population Diversity (quasi-species)
- No Reversions to Parental Sequences

(A) Plaque Phenotype & Lethality in Mice

<table>
<thead>
<tr>
<th>Virus</th>
<th>P2</th>
<th>P6</th>
<th>P12</th>
</tr>
</thead>
<tbody>
<tr>
<td>LASV</td>
<td>LP,&lt;50%</td>
<td>LP,&lt;50%</td>
<td>LP,&lt;50%</td>
</tr>
<tr>
<td>MOPV</td>
<td>SP,90-100%</td>
<td>SP,90-100%</td>
<td>SP,90-100%</td>
</tr>
<tr>
<td>ML29</td>
<td>SP,&lt;50%</td>
<td>SP,&lt;50%</td>
<td>SP,&lt;50%</td>
</tr>
</tbody>
</table>

(B) SNPs detected in ML29

<table>
<thead>
<tr>
<th>Host/Genes</th>
<th>GP</th>
<th>NP</th>
<th>Z</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>Marmoset</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>CBA/J</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>C57BL6</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Vero</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

(C) Host-specific SNPs

<table>
<thead>
<tr>
<th>Animal</th>
<th>GPC</th>
<th>NP</th>
<th>RdRp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>I252</td>
<td>M179L</td>
<td>L266L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D341G</td>
<td>L494L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R551K</td>
<td>H1572Y</td>
</tr>
<tr>
<td>Marmoset</td>
<td>I252L</td>
<td>I252M</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>I252M</td>
<td>R59R</td>
<td>T223A</td>
</tr>
</tbody>
</table>

Host-specific SNPs in ML29 recovered from vaccinated animals: rhesus macaques (black), marmosets (yellow), and mice (red). Green, mutations found in marmosets and mice. Common SNPs are in green.

(A), Phenotypic Stability of ML29 during passages in Vero cells. LP and SP, large and small plaques, respectively; %, lethality in suckling mice after IC inoculation. (B), SNPs detected in ML29 recovered from different hosts; (C), Host-specific SNPs found in Rhesus, marmosets, and in mice.

Zapata JC et al., 2014
Summary from ML29 Pre-clinical Development

✓ Safe in all available animal models including SIV-infected Rhesus macaques mimicking HIV-infected individuals

✓ Well-tolerated to tested doses, $1 \times 10^2$ – $1 \times 10^6$ PFU

✓ Highly protective against challenge with homologous (LASV/Jos) and distantly-related LASV/NIG strains from clade II

✓ Genetically stable during passages in vitro and in vivo

✓ Easy to manufacture in Vero cells (yield 6-7 logs, 1 ml of harvested culture medium = 1000 doses)

✓ Stable at 4 C and RT during 1 week (observation period)
ML29 Deficiency:

no FDA-required track records on all passages of ML29

Solution:

Reverse Genetics to rescue ML29 from cDNA clones and generate recombinant ML29 (rML29)
Rescue of rML29 from cDNA clones

Day 1 plasmid transfection

Day 6 post-transfection: virus detection in TCS

Immunofluorescence using virus-specific antibodies

No virus

Rescued virus: rML29

Trans-acting factors protein expression plasmids

pCAGGS NP

pCAGGS L

pPol-I v/cRNA expression plasmids

hPol-I L

hPol-I S
Characterization of rML29. **Left panel.** Growth kinetics in cultured cells. Vero cells were infected (moi = 0.001) with either ML29 biological isolate (bi) or with the rescued rML29. At the indicated hours p.i., virus titers in TCS were determined by immune focus assay. **Middle and Right panel.** Adult B6 mice (N = 8/group including males and females) were infected (i.c. 10^3 ffu/mouse) with either ML29bi or rML29 or mock-infected (PBS), and monitored daily for clinical symptoms (weight loss, middle panel) and lethality on day 16 (right panel). LCMV/ARM was used as a control (100% lethality) in the lethality assay.
ML29 Reverse Genetics: To secure Safety and expand Cross-Reactivity

✓ Improve manufacturing and control of Master and Working Virus stocks

✓ Assess contribution of ML29-specific mutations in attenuation

✓ Secure safety profile

✓ Pan-LASV vaccine capabilities using tri-segmented RNA technology (e.g., r3ML29/LP, rML29/EBOV-GP, adjuvants)
Conclusions:

- Reassortant technology was used for rational design of MOPV/LASV reassortants as promising LASV vaccine candidates.

- One of the selected MOPV/LASV reassortant, biological clone ML29, is the most pre-clinically studied LASV vaccine candidates.

- Unique feature of ML29 are: safety in all tested LF animal models including SIV-immunocompromised Rhesus macaques.

- Induction of cell-mediated sterilizing immunity responsible for full protection of NHPs against challenge with LASV/Jos (lineage IV) and LASV/NIG from lineage II. ML29 is the only vaccine candidate with documented efficacy against Nigerian strains.

- Low protection dose, $1 \times 10^3$ PFU, and thermostability are another unique features of ML29.

- Recent rescue of ML29 from cDNA clones provides powerful reverse genetics tools to further facilitate manufacture and control of recombinant ML29 vaccine and approval process. We have documented that both, bML29 and rML29, shared safety and immunogenicity features.

- Recombinant ML29 is well-positioned to meet WHO TPP criteria for pan-LASV vaccine for West Africa.
Our Goal:

Our goal is to advance the rML29 vaccine development and conduct Phase I-II clinical trials in Nigeria in line with the WHO Roadmap for Lassa Fever.
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