Development of A live attenuated Lassa Fever Vaccine

A multi-Nation Cooperative Project for Local Production

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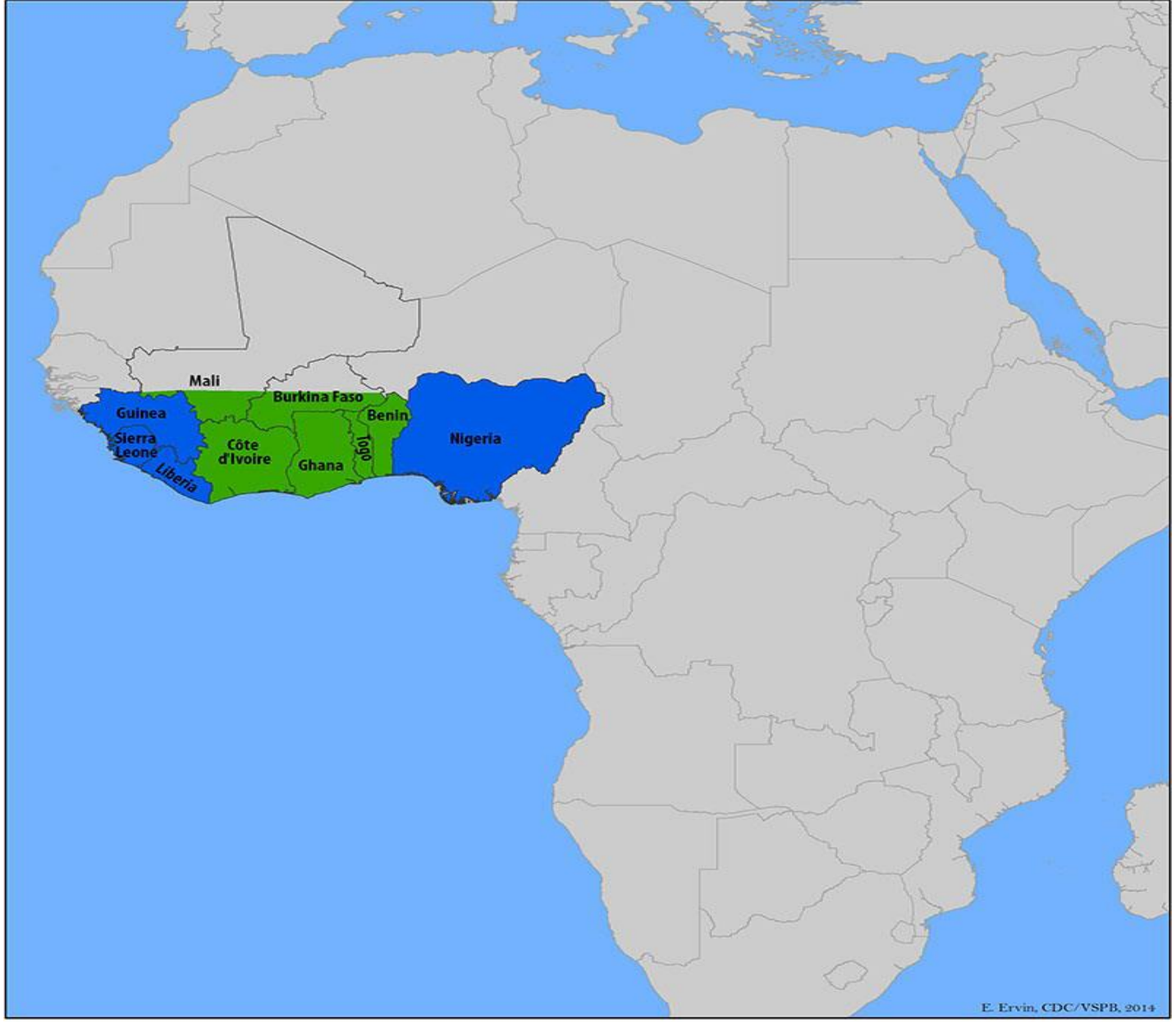
THE EPIDEMIC

Arena Virus causing infection

Virus	Disease
Lassa virus	Lassa fever
Junin virus	Argentine hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever
Guanarito virus	Venezuelan hemorrhagic fever
Sabia	Brazilian hemorrhagic fever



LASSA FEVER DISTRIBUTION MAP

Countries reporting endemic disease and substantial outbreaks of Lassa Fever

Countries reporting few cases, periodic isolation of virus, or serologic evidence of Lassa virus infection

Lassa Fever status unknown

0 240 480 960

Miles

Demographic Info.

at risk- those who live in areas with Mastomys rodents

Lassa carrier, Mastomys Nataliensis

LASSA FEVER IN NIGERIA

History of Lassa Fever Disease

(Modified from a map by Daniel Dalet that is freely available at http://d-maps.com.)

Current outbreak in Nigeria

- From 1st January to 16th September 2018, a total of 2559 suspectedi cases have been reported from 22 states. Of these, 506 were confirmed positive, 10 probable, 2044 negative (not a case)
- Since the onset of the 2018 outbreak, there have been 133 deaths in confirmed cases
- Case Fatality Rate in confirmed cases is 26.3%

Number of HCW affected

- Thirty-nine health care workers have been affected since the onset of the outbreak in seven states –Ebonyi (16), Edo (14), Ondo (4), Kogi (2), Nasarawa (1), Taraba (1) and Abia (1) with ten deaths in Ebonyi (6), Kogi (1), Abia (1), Ondo (1) and Edo (1)
- 82% of all confirmed cases are from Edo (46%), Ondo (23%) and Ebonyi (13%) states

THE NATION
Truth in Defence of Freedom

On: September 17, 2018

Lassa Fever: Delta confirms death, says no cause for alarm

- Last weel the Delta Ministry of Health has confirmed the death of a woman who allegedly manifested symptoms of Lassa fever virus.
- The woman, who is now deceased, had most probably transmitted the infection to 2-HCW who treated her. They, the two patients are currently under medical care and they are responding to treatment, pending the outcome of the test," Okoba said.

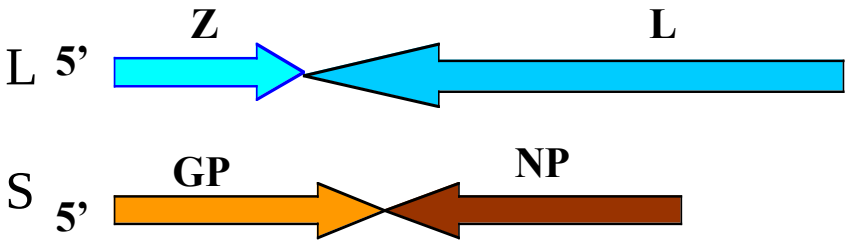
Mapping of the 2018 LF outbreak, Nigeria

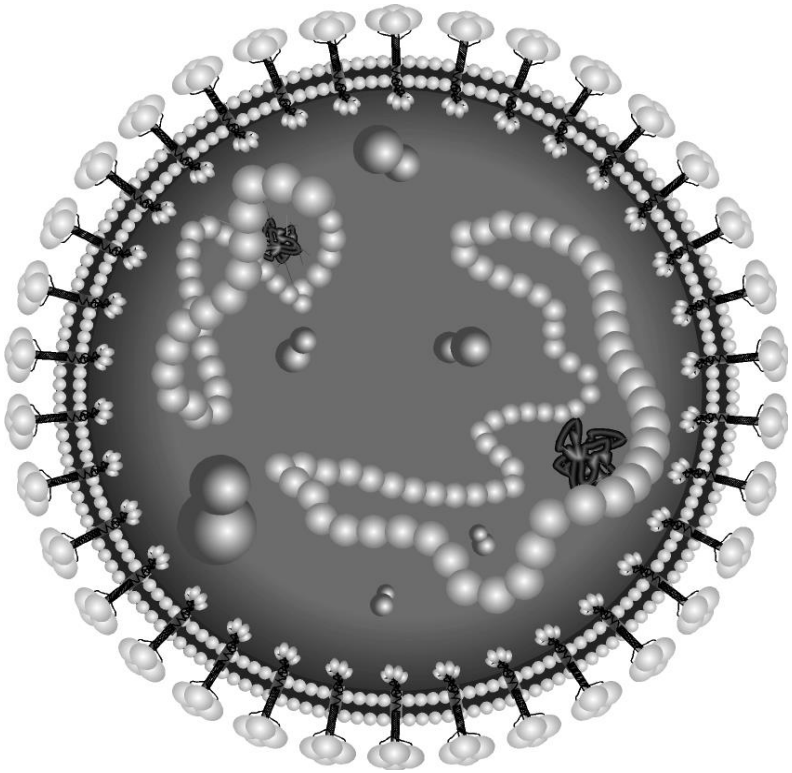
THE VIRUS

Lassa Virus Structure

Family: Arenaviridae, now in the order Bunyavirales

Two ambisense genome segments



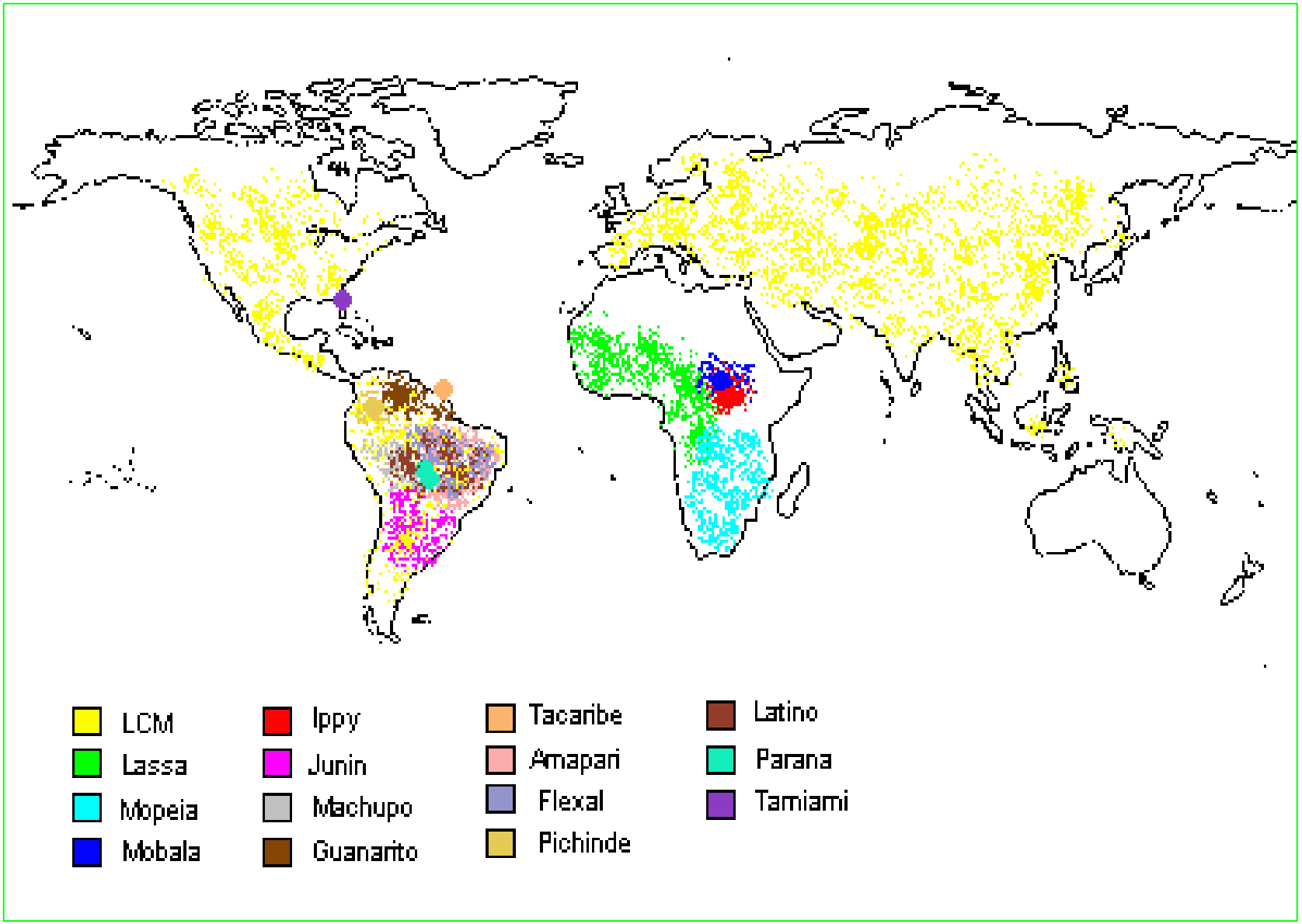


Mopeia Virus, an attenuated relative of Lassa

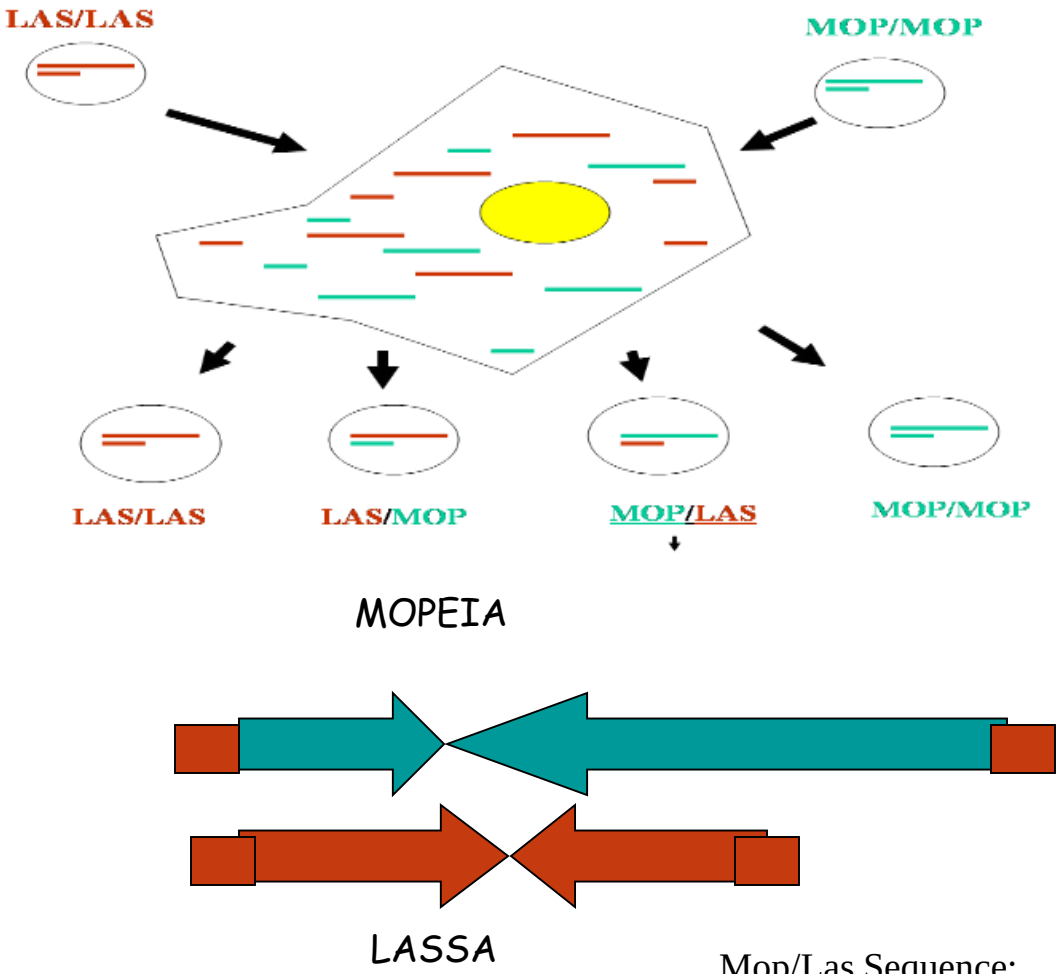
- **Lassa virus** causes up to 300,000 annual infections in West Africa and ~3,000 deaths per year.

- **Mopeia virus** is a close relative of Lassa virus, found in South-Eastern Africa where it does not cause disease. In contrast to Lassa, Mopeia virus is not lethal in guinea pigs and monkeys, and can protect them from LAS challenge, like a natural vaccine.

- **Co-infection of cells with both viruses can produce Mop/Las reassortants.**


LCM
Lassa
Mopeia
Mobala
Ippy
Junin
Machupo
Guanarito
Tacaribe
Amapari
Flexal
Pichinde
Latino
Parana
Tamiami

Reassortment between Lassa and Mopeia Viruses



Mop/Las Sequence:
Moshkoff, Salvato, Lukashevich 2005

THE VACCINE

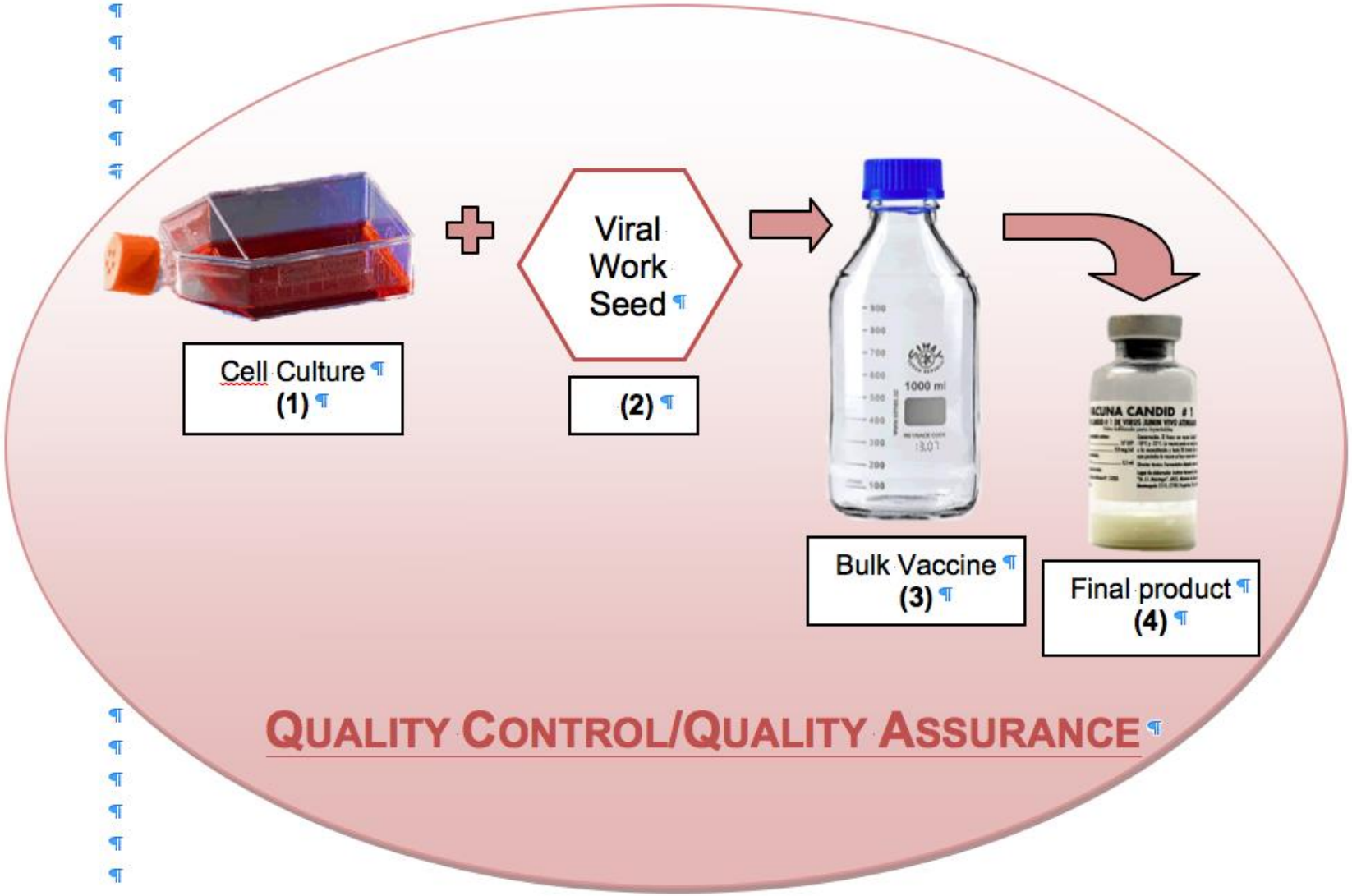
Strategy for vaccine production:

Pre-clinical studies in USA

GMP production in Russia

Tech Transfer to Africa

Process for making AHF vaccine:



Steps in producing Lassa Hemorrhagic fever reassortant live-attenuated Vaccine

Tissue culture

+

72 h

- Can be stored for 3 years at -80° C
- Serum free

Lassa vaccine bulk formulated for lyophilization process

*Sterilization by filtration

* Stock dilution approx. in 10 000 times

Lyophilization and blister filling process

One week for 1 MLN doses

QA/QC release

Final product Lassa vaccine lyophilized + diluent in one blister

Blisters can be stored for 2 years

Facility for making AHF vaccine:

Ana Maria Ambrosio et al.

Cell culture washing and viral inoculation during the manufacture of Candid #1 bulk vaccine

Clinical Trials

Strategy for vaccine clinical trials:

- **Lassa fever is a recurring epidemic, appearing every year during the November to February dry season., fast becoming endemic in many communities of WA**

- **Nigeria has recently been recognized as the richest African country with many natural resources and a dynamic economy.**

- **According to reviews and discussion with WHO (Jan'18) the Mop/Las vaccine is the most broadly cross-protective Lassa vaccine available.**

- **Because Lassa disease is Endemic, recurs annually and because Nigeria can now afford better medicine, it is important to organize proper placebo-controlled clinical trials. Ring vaccinations cannot be the only approach.**

Preclinical evaluation of the safety and immunogenicity of a Mop/Las vaccine candidate

FDA "Two Animal Rule"

(21 *CFR* 314.600 & 21 *CFR* 601.90)

The FDA can approve drugs that are shown to be effective **in two animal models**, without clinical trials for effectiveness, under the following conditions:

- Human efficacy trials are not feasible or ethical
- Efficacy is shown in well understood animal models
- Efficacy is substantiated in multiple species
- Human clinical data on safety, toxicity, and immunogenicity is still required

Diseases that may be affected by the animal rule include:

- Anthrax
- Botulism
- Plague
- Smallpox
- Tularemia
- **Viral hemorrhagic fevers**

Mop/Las Vaccination of Guinea Pigs

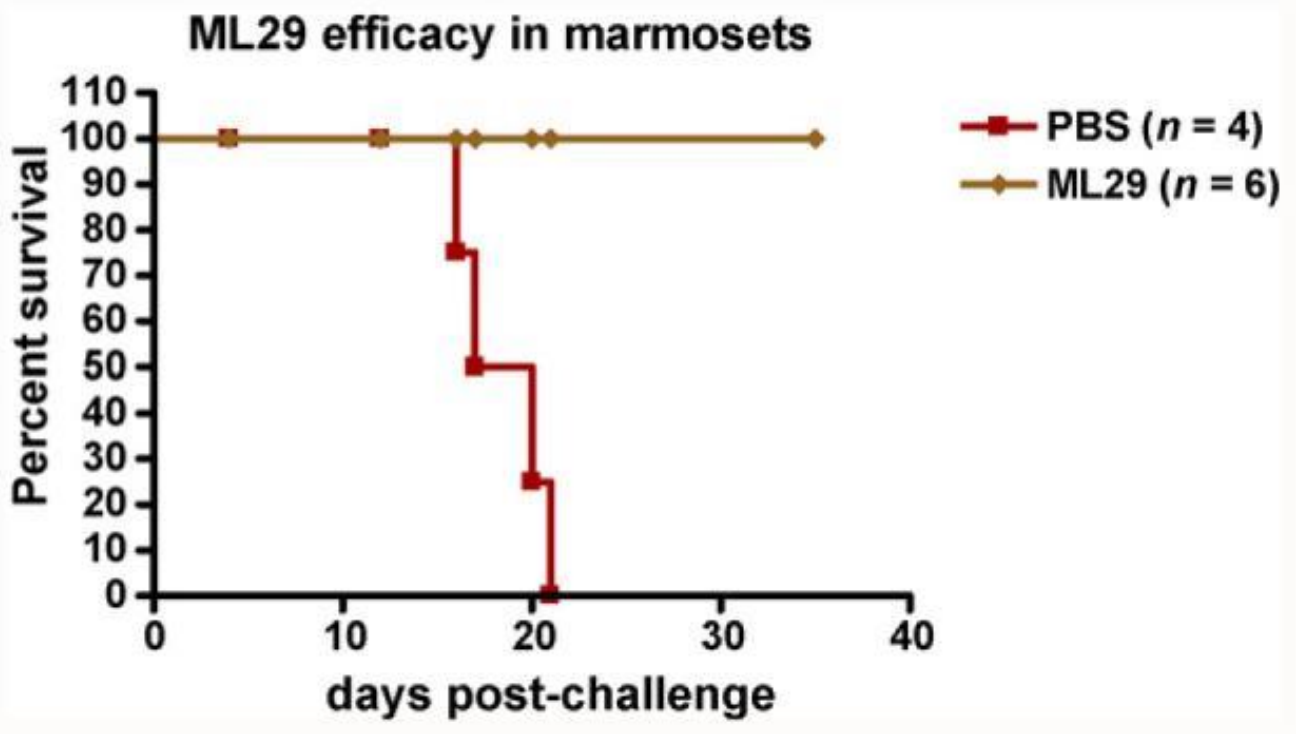
Animal Group	Challenged virus	Dose, PFU	Vacc/chal interval, days [a]	No. survived/ No. infected	Survival, %	Day of death
No vaccination						
1.	LASV-Jo	10e+1	na [b]	0/4	0	15-17
2.	LASV-Jo	10e+3	na	0/5	0	15-16
3.	LASV-803213	10e+3	na	0/5	0	13-15
4.	LCMV-WE	10e+3	na	0/5	0	13-14
The MK18 conventional vaccination (challenge on day 30)						
5. 10e+2	no challenge	na	na	**6/6**	100	na
6. 10e+6	no challenge	na	na	**6/6**	100	na
7. 10e+3	**LASV-Jo**	10e+3	30	**6/6**	100	na
8. 10e+3	**LASV-803213**	10e+3	30	**5/5**	100	na
9. 10e+3	**LCMV-WE**	10e+3	30	**0/6**	0	16-21
The simultaneous vaccination/challenge experiments (challenge on day 0 and 2)						
10a.10e+6	***LASV-Jo***	10e+1	***0***	***5/5***	100	na
10b.10e+6	***LASV-Jo***	10e+1	***2***	***3/5***	60	10[c], 15
11a.10e+6	***LASV-Jo***	10e+3	***0***	***4/4***	100	na
11b.10e+6	***LASV-Jo***	10e+3	***2***	***4/5***	80	10 [c]
12a.10e+2	***LASV-Jo***	10e+3	***0***	***3/4***	75	14
12b.10e+2	***LASV-Jo***	10e+3	***2***	***3/4***	75	16
13. 10e+6	***LASV-803213***	10e+3	***0***	***3/5***	60	12, 17
14. 10e+6	***LCMV-WE***	10e+3	***0***	***0/5***	0	14-16

[a]Animals were s. c. vaccinated with the Mop/Las reassortant (day 0) and challenged simultaneously on day 0, 2, 30 after vaccination. Death or survival past 21 days was set up as an endpoint. Amino acid difference between LASV-Jo and LASV-803213 is the highest within LASV genetic lineages I-IV.

[b]Non applicable.

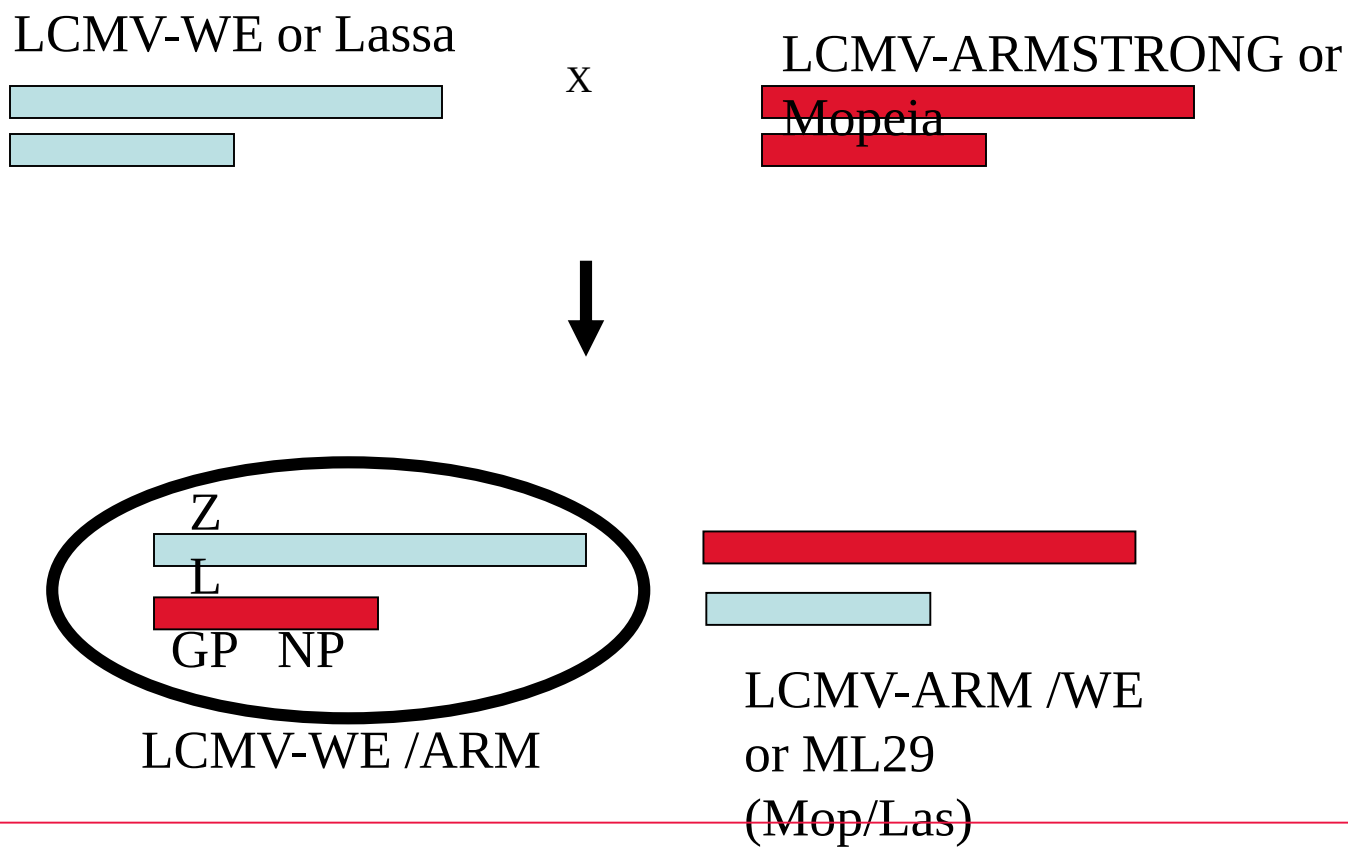
[c]Non-LASV-specific death (inappropriate anesthesia).

Mop/Las Vaccination of Marmosets



•Six animals were s.c. vaccinated with Mop/Las (low dose).
•Four animals were injected with PBS (control).
•On day 30 all animals were challenged with LASV-Josiah (1 × 103 PFU in 0.5 ml).
•During 17–21 days after LASV challenge animals from the control group met euthanasia criteria and were sacrificied.
•Vaccinated animals had no clinical manifestations of disease and were necropsied at the end of the experiment, on day 35 after LASV challenge

Reassortment analysis



Virulence determinants were found on the L RNA

Moshkoff D, Salvato MS, Lukashevich IS. 2007.

Viability of project

Assessment of the Lassa vaccine market in West Africa

Country	Population	Birthrate coefficient (Births per 1,000 population)	Birthrate
Nigeria*	103 127 784	39,9	4 114 799
Ghana	27 414 000	29,6	811 454
Ivory Coast	23 126 000	35,3	816 348
Guinea	10 935 000	39,8	435 213
Benin	10 782 000	40,2	433 436
Togo	7 065 000	36,8	259 992
Sierra Leone	6 513 000	46,2	300 901
Liberia	4 046 000	49,6	200 682
Total population	**193 008 784**	**38,2**	**7 372 824**

**-Population christians (formed 56.8% of the population in Nigeria)*

Scenario: gradual increase of coverage

year	1	2	3	4	5	6	7	8
coverage								
children population	20%	40%	80%	80%	80%	80%	80%	80%
pregnant women	20%	40%	80%	80%	80%	80%	80%	80%
adult population	5%	5%	5%	5%	5%	6%	6%	6%
total*	6%	8%	11%	11%	11%	11%	11%	11%
demand, doses								
children population	1 474 565	2 949 130	5 898 260	5 898 260	5 898 260	5 898 260	5 898 260	5 898 260
pregnant women	1 474 565	2 949 130	5 898 260	5 898 260	5 898 260	5 898 260	5 898 260	5 898 260
adult population	8 913 157	9 091 420	9 273 248	9 458 713	9 647 888	9 840 845	10 037 662	10 238 415
total	11 862 287	14 989 679	21 069 767	21 255 232	21 444 407	21 637 364	21 834 181	22 034 934
price per dose, USD	38,5	38,5	36,6	36,6	36,6	36,6	36,6	36,6
value, USD								
children population	56 770 748	113 541 495	215 728 841	215 728 841	215 728 841	215 728 841	215 728 841	215 728 841
pregnant women	56 770 748	113 541 495	215 728 841	215 728 841	215 728 841	215 728 841	215 728 841	215 728 841
adult population	343 156 535	350 019 666	339 169 056	345 952 438	352 871 486	359 928 916	367 127 494	374 470 044
total	456 698 031	577 102 657	770 626 739	777 410 120	784 329 169	791 386 599	798 585 177	805 927 727

****-WHO Position: it is important to vaccinate most (80 % or more) of the population at risk to prevent transmission in a region with a fever outbreak.***

Please note that Nigerias current population is put at 197,000,000 and West African region is well above 500,000

CEPI criteria for Lassa Vaccine

1. Cheap to produce
2. Product Characterized for FDA approved IND.
    - Chemistry, manufacturing, and controls (CMC) issues in IND applications
3. Broad Cross-protection
4. Capable of rapid or even post-challenge protection.
5. Clear Immune correlates of protection
    - For short-term protection
    - For long-term protection
6. Safe in immune-suppressed people
7. Shelf-life at least a year at room temp.
8. Adaptable to local production

Conclusions about the vaccine:

- **Sequence of the Mop/Las vaccine has been published (Moskoff et al 2006)**
- **Guinea pigs vaccinated with a Mop/Las vaccine experienced sterilizing immunity and complete protection with homologous virus and with the heterologous Nigerian isolate**
- **According to reviews and discussion with WHO (Jan'18) the Mop/Las vaccine is the most broadly cross-protective Lassa vaccine available.**
- **Simultaneous immunization-challenge or challenge 2 days before immunization also protected 60-100% of the animals against both Lassa strains**
- **The vaccination elicits specific immune responses and completely protects Guinea pigs and Marmosets from fatal disease by induction of sterilizing cell-mediated immunity**
- **This vaccine elicits immune responses in SIV-infected monkeys and does not negatively impact their lifespan (Zapata et al 2013)**
- **The Mop/Las reassortant (MK18) is a promising vaccine candidate for Lassa fever**

Conclusion:

- ***Our goals to advance the Mop/Las (MK18) vaccine and transfer technology to Nigeria are in line with the WHO Roadmap for Lassa Fever.***

- ***The WHO task force named the Mop/Las vaccine as one of 3 top vaccine candidates***

- ***The WHO task force recognized the Mop/Las vaccine as the most broadly cross-protective vaccine.***

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FACILITIES

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The End