BSL-4 and BSL-3 facilities of the Galveston National Laboratory

Modified mRNA-based vaccines against filoviruses and Lassa virus

WHO mRNA Technology Transfer Hub

Cape Town, South Africa

April 20, 2023

Alex Bukreyev

University of Texas Medical Branch

Galveston National Laboratory

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The Bukreyev Laboratory

The laboratory works with highly pathogenic and emerging viral infections in the following areas:

- Development of vaccines and investigation of the mechanisms of their protective effects.
- Investigation of dysregulated immune response to Ebola virus at the epigenetic level.
- In-depth characterization of antibody responses to viral infections in humans.
- Development of therapeutic human monoclonal antibody treatments and investigation of the mechanisms of their protective effects.
- Comparative immunology of bats as a reservoir of emerging viral infections.

An important component of our research is work with live viruses under containment in Galveston National Laboratory, which is part of UTMB.

Galveston National Laboratory
https://www.utmb.edu/gnl

<u>The viruses we work with</u>

- Ebola
- Marburg
- Lassa
- Andes and Sin Nombre (New World hantaviruses)
- Crimean-Congo hemorrhagic fever virus
- Respiratory syncytial virus

Email for collaborations and vacancies in the lab inquiries: alexander.bukreyev@utmb.edu

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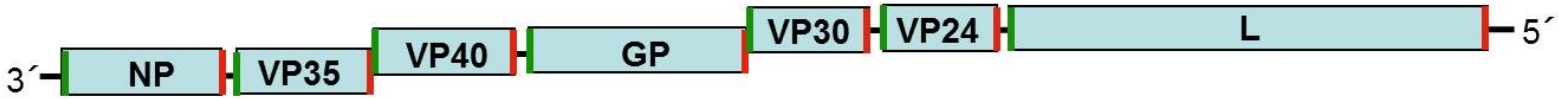
Ebola Zaire virus

Virion: mean length 1,200 nm, diameter 80 nm

Envelope
Lipid bilayer
Glycoprotein **(GP)**
Matrix **(VP40)** protein
Second matrix protein **VP24**

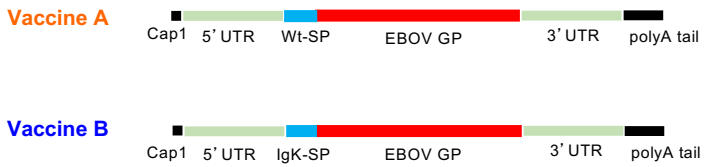
Nucleocapsid (diameter 50 nm)
RNA genome
Nucleoprotein **(NP)**
Phosphoprotein **(VP35)**
Transcription activation factor **(VP30)**
Large polymerase **(L)** protein



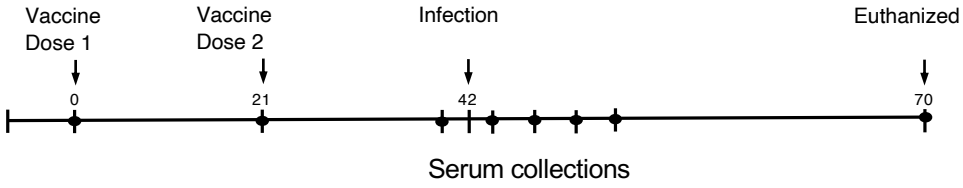
Single strand, negative-sense RNA genome of 18,959 nt

Ebola virus

1-methylpseudouridine modified RNA LNP-formulated vaccine



The study design



Hartley guinea pigs
Vaccine dose level : 20 μg
Challenge: 1,000 PFU of guinea pig-adapted Ebola virus

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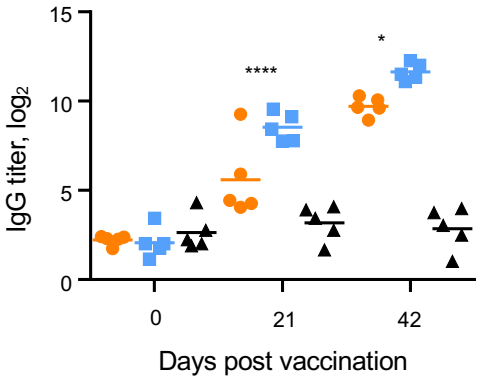
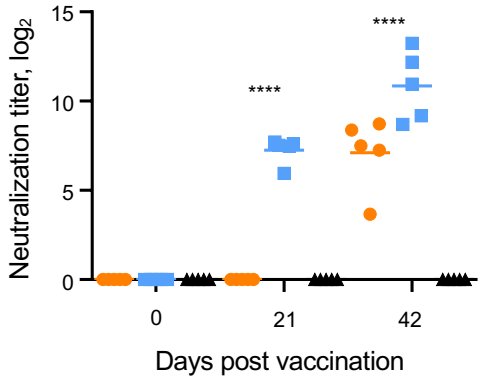
Antibody responses

Binding antibodies



Neutralizing antibodies



Vaccine A

Vaccine B

Meyer et al., *JID,* 2018

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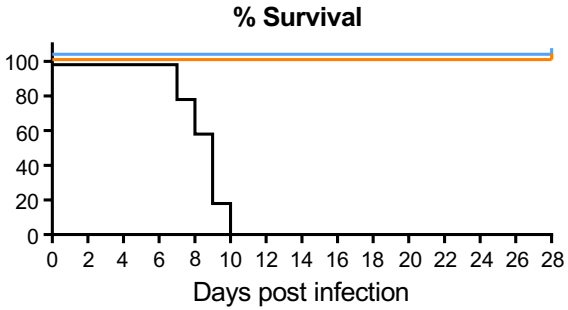
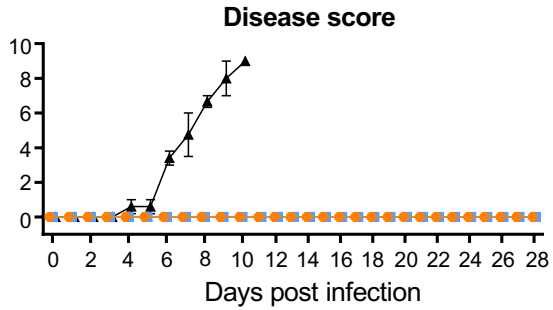
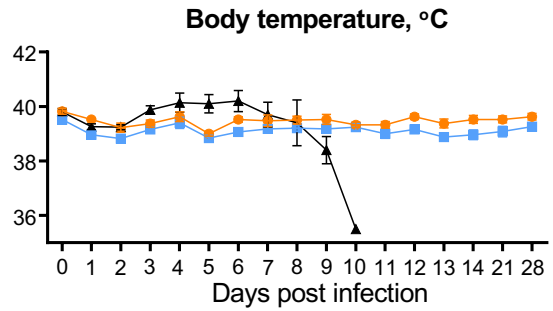
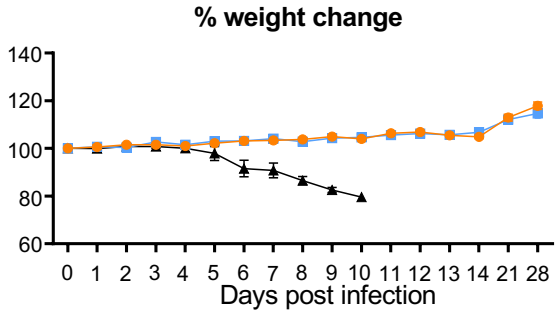
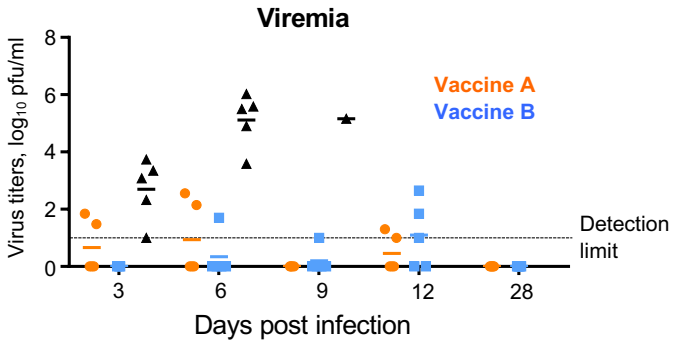
Protection against challenge











Meyer et al., *JID,* 2018

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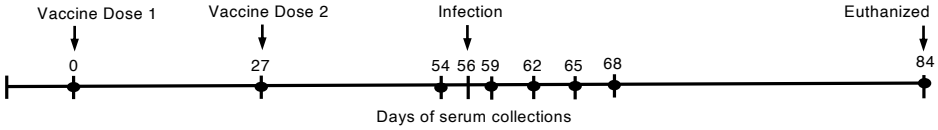
Marburg virus

1-methylpseudouridine modified RNA LNP-formulated vaccine

Species Marburg includes two viruses which are equally pathogenic for human (previously known and “lineages of Marburg virus): Marburg and Ravn

Testing of Marburgvirus mRNA vaccines:
- Marburg virus vaccine against guinea pig-adapted Marburg
- Ravn virus vaccine against guinea pig-adapted Ravn

The study design



Hartley guinea pigs
Vaccine dose level : 40 µg
Challenge: 1,000 PFU of guinea pig-adapted Marburg virus

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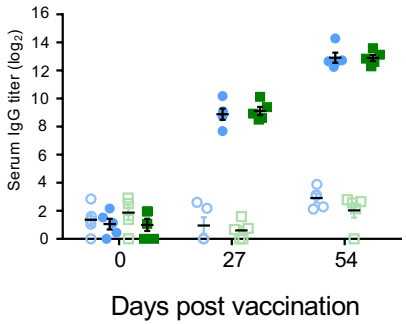
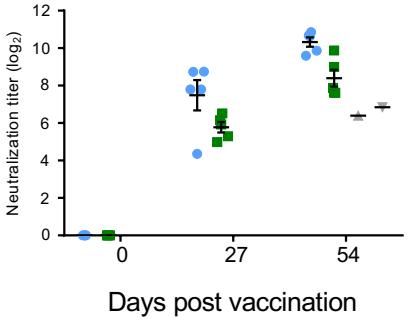
Antibody responses

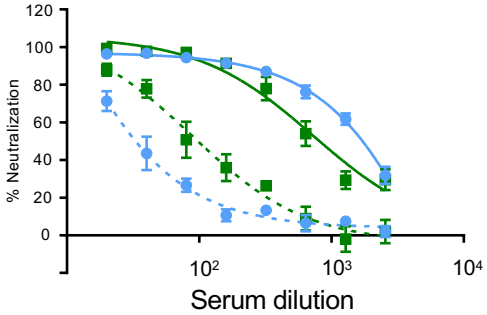
Binding antibodies



Neutralizing antibodies



Cross-neutralizing antibodies



- Ravn control group
- Ravn vaccine group
- Marburg control group
- Marburg vaccine group

Vaccine dose level: 40 µg

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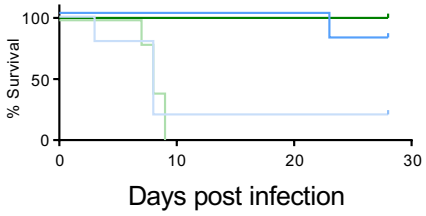
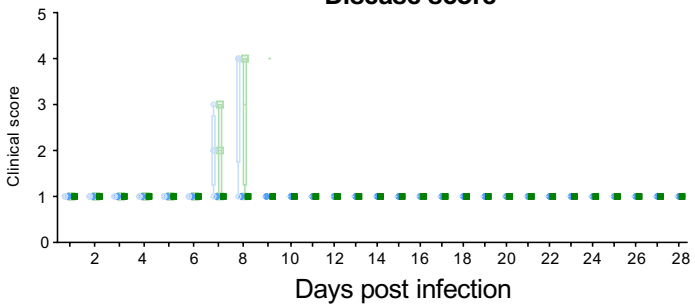
Protection against Marburg challenge

The survival curve

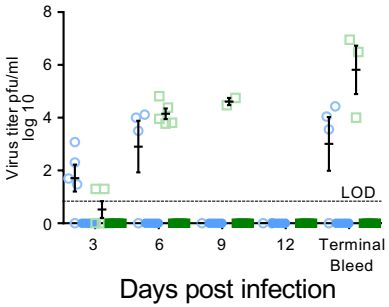


Disease score

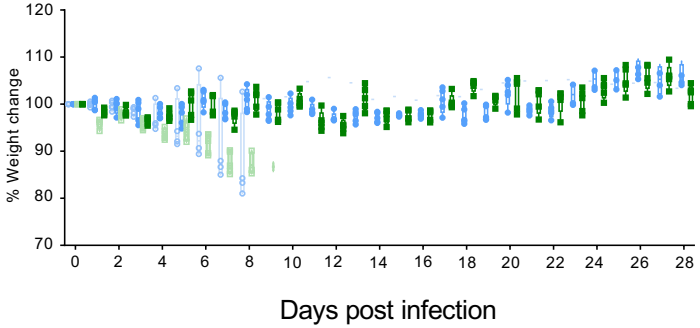


Ravn control group
Ravn vaccine group
Marburg control group
Marburg vaccine group

Viremia



Weight change



Note: One animal in group B (vaccinated) was euthanized on day 23 due to a severe leg injury in the cage. This animal did not have Marburg virus disease.

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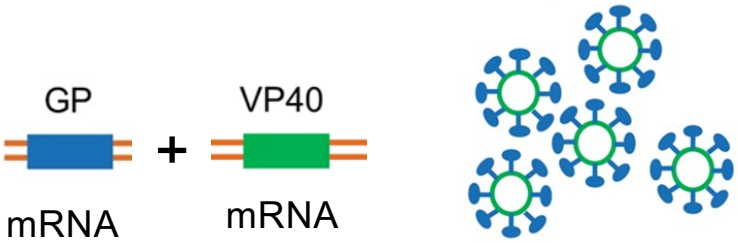
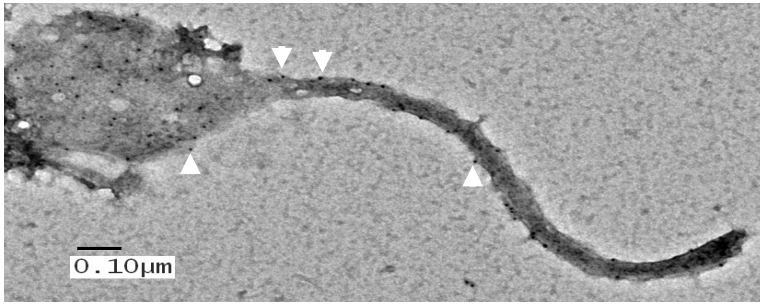
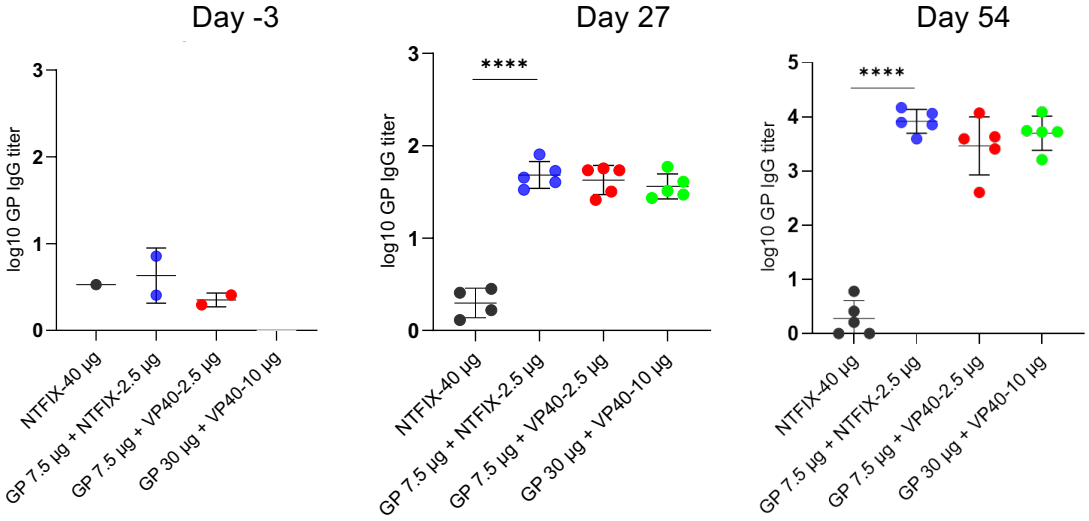
VLP approach



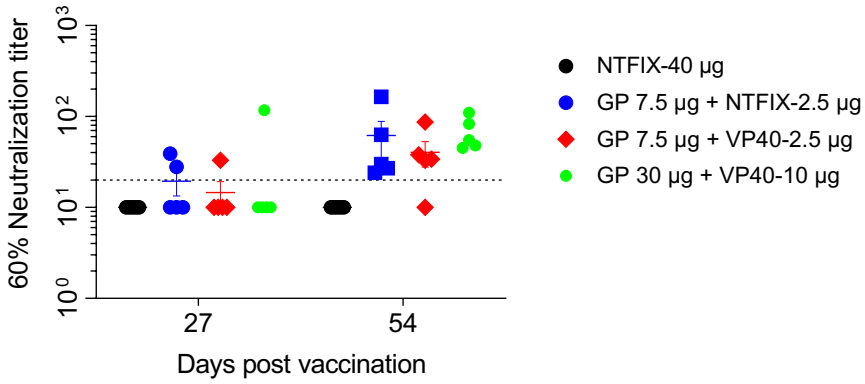
Marburg VLP: Anti GP-gold and negative staining EM



GP-binding antibodies (ELISA)



Marburg virus-neutralizing antibodies



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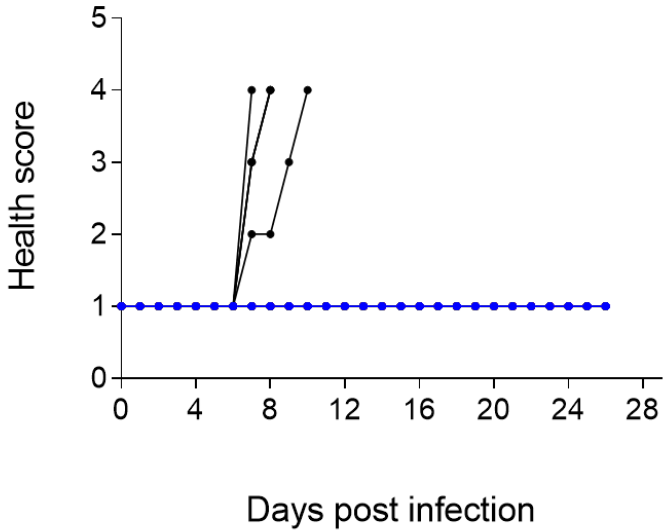
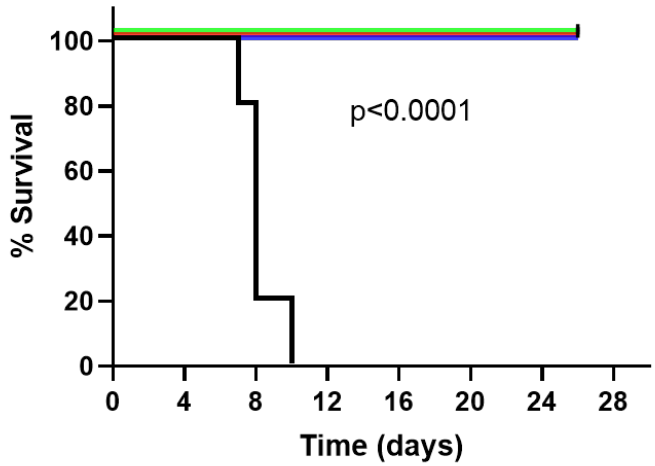
mRNA vaccine protects from death and disease caused by Marburg virus





NTFIX-40 µg
GP 7.5 µg + NTFIX-2.5 µg
GP 7.5 µg + VP40-2.5 µg
GP 30 µg + VP40-10 µg

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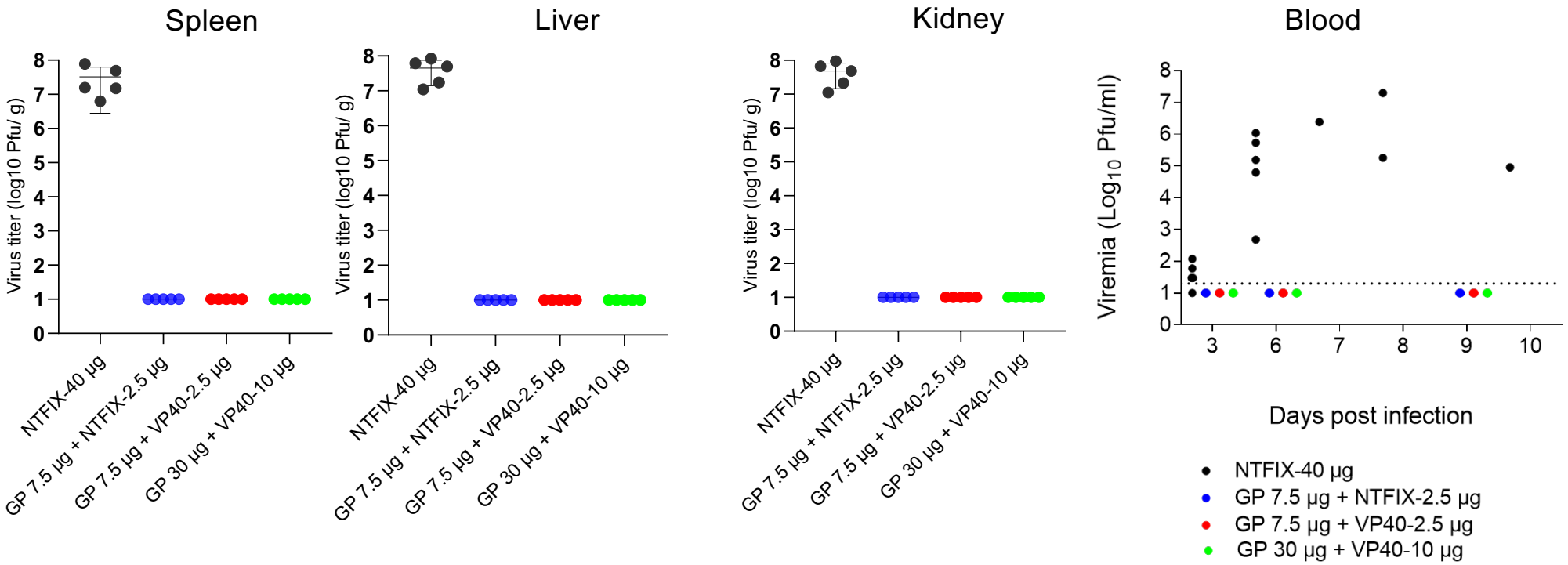
mRNA vaccine protects from Marburg viral load



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mRNA vaccines against Ebola and Marburg viruses

- Excellent immunogenicity with induction of virus-neutralizing antibody responses.

- Excellent protection in the guinea pig models.

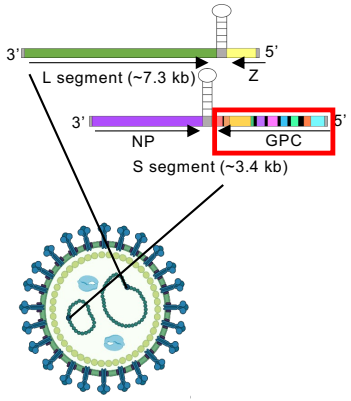
- The advantage of the Marburg VLP approach could not be demonstrated due to equally high protective efficacy of the vaccines.

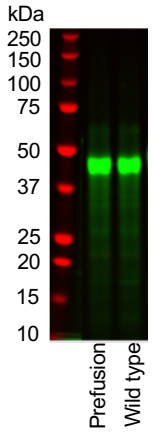
- Testing of the lower doses of the Marburg vaccines with and without VP40 is in preparation.

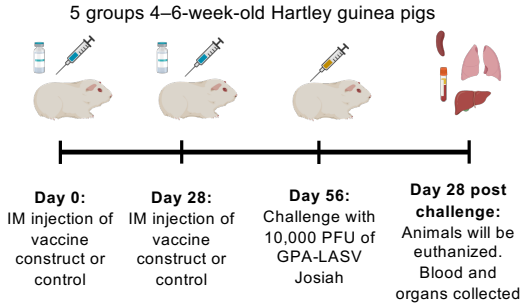
- The Bukreyev lab and Moderna prepare testing of the Marburg, Ebola and Lassa mRNA vaccines in NHPs. If protective, this will be followed by phase I clinical trials.

mRNA vaccine against Lassa virus

Prefusion conformation-stabilized* and non-stabilized versions of GPC







*Hastie et al., 2017, *Science*

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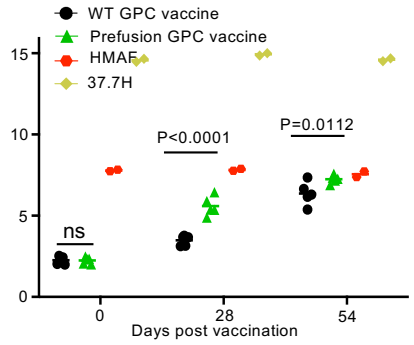
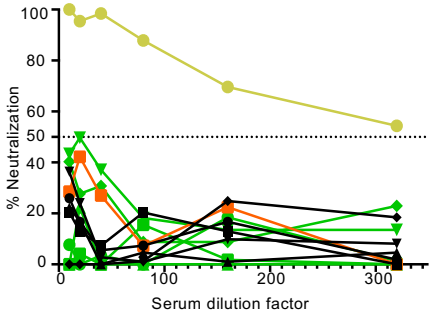
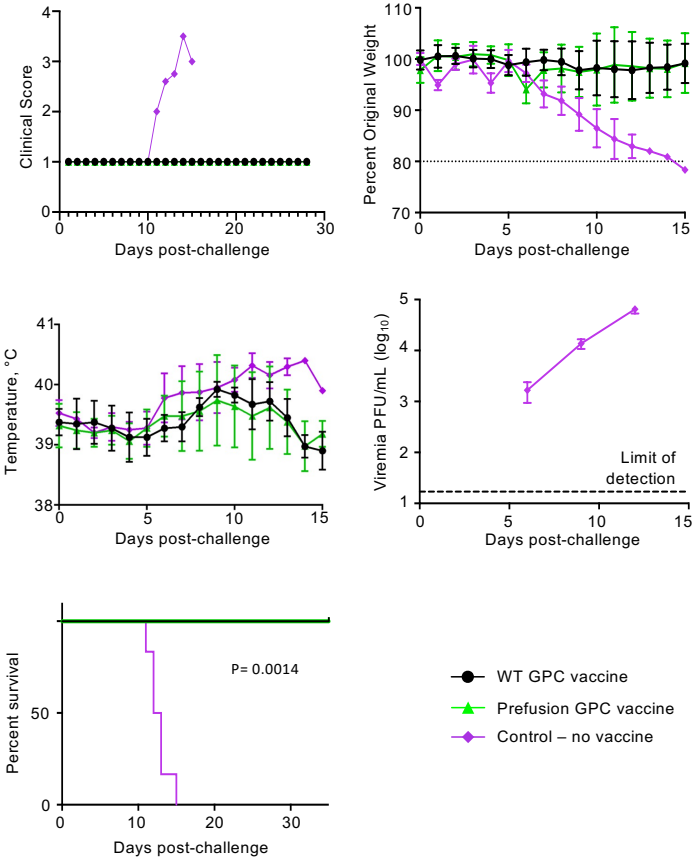
Binding antibody responses



Neutralizing antibody responses





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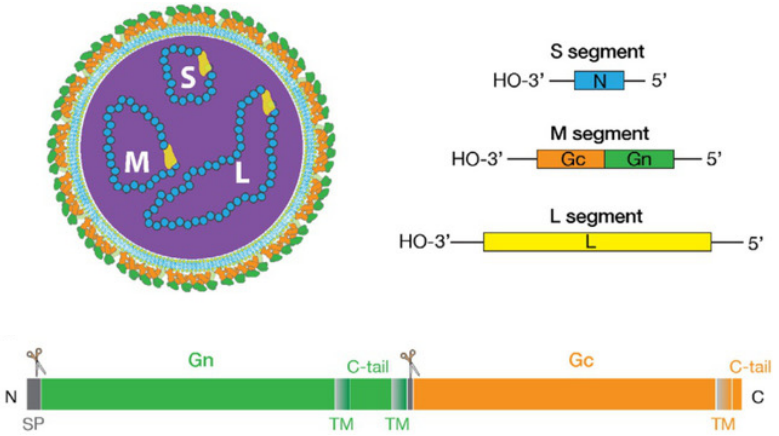
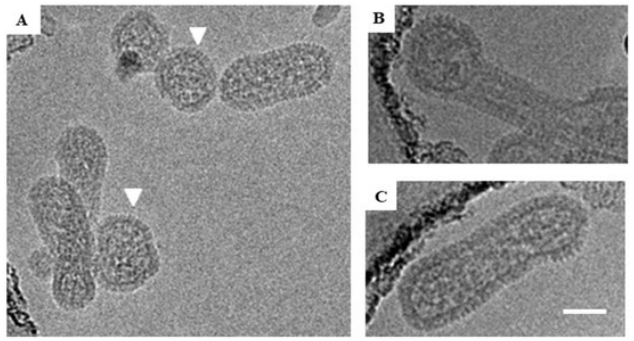
Andes virus

A human outbreak in Argentina in 2019 caused 34 infections including 11 deaths

- Tri-segmented RNA genome of negative polarity
- The protective antigen: GPC, 1,138 amino acids
- Proteolytically cleaved to Gn and Gc



PMID: 31439149



Cryo-EM images of representative ANDV particles. (A) Both round and tubular viral particles are shown in the image. Round particles are indicated by white triangles. (B) Irregular particle with both round and tubular portions. (C) Tubular particle. Scale bar = 100 nm. Amar Parvate, PMID: PMID: 31527500

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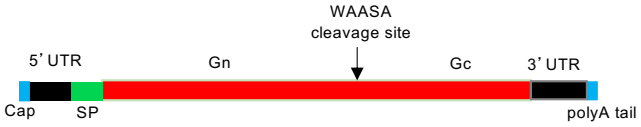
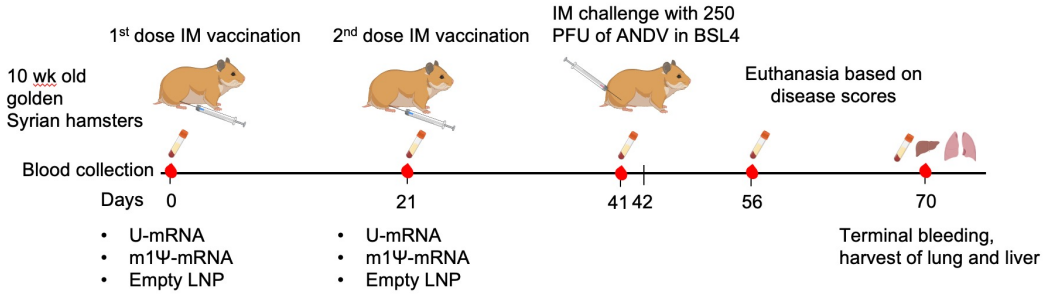
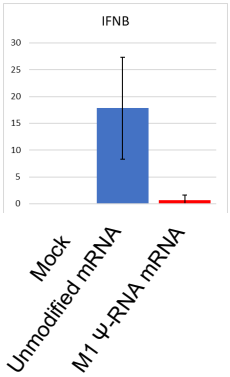
Immunogenicity and protective efficacy in golden Syrian hamsters

Monocistronic linear mRNA platform

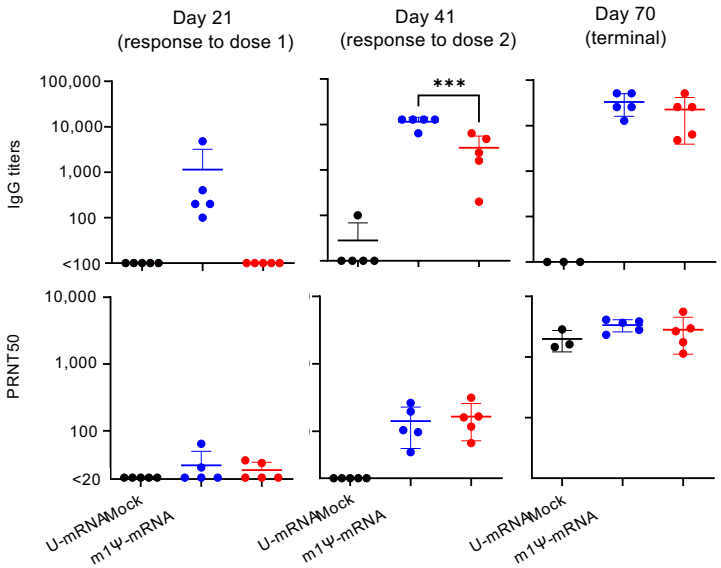




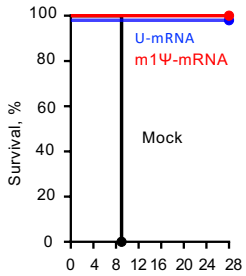
M1Ψ modification of mRNA disables innate immune response in transfected A549 cells



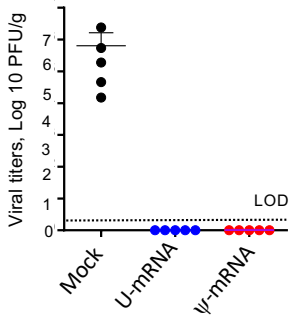
Antibody responses



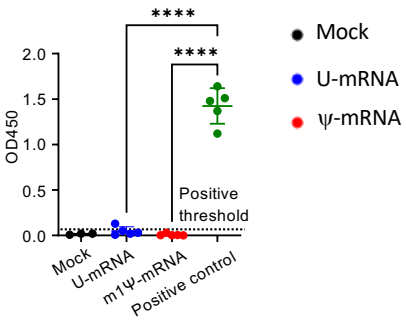
Survival



Viral load in liver after challenge



Antibody response To ANDV N protein



Vaccine dose level: 25 µg

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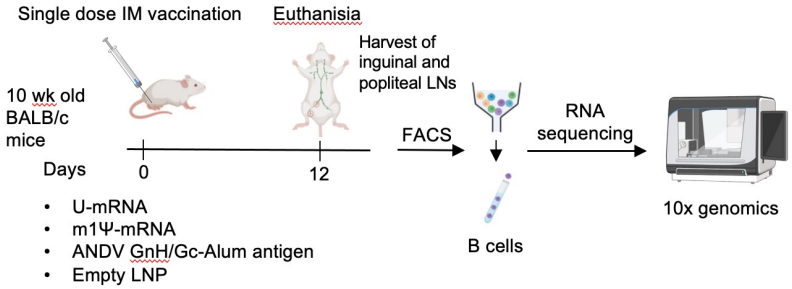
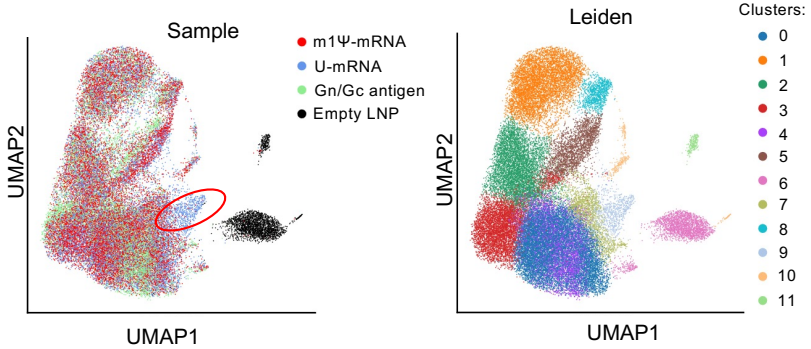
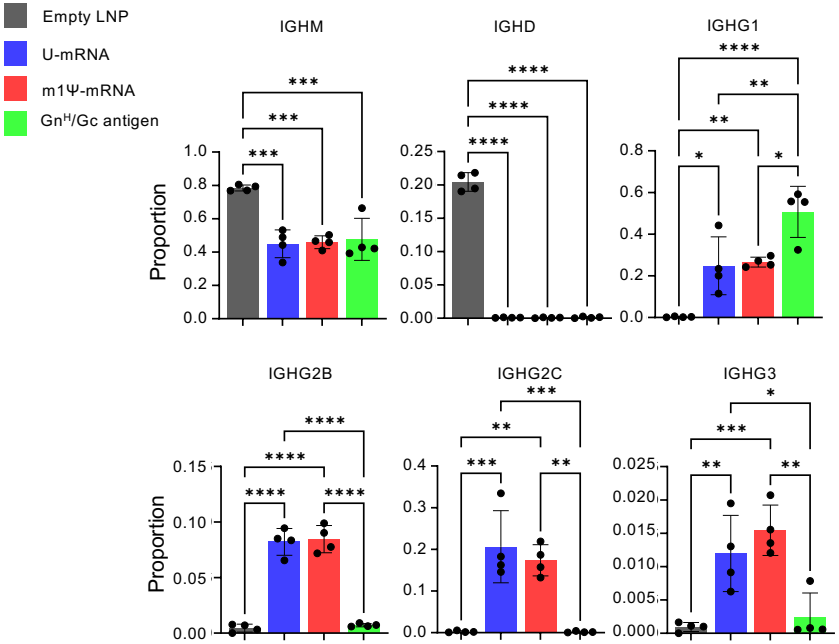


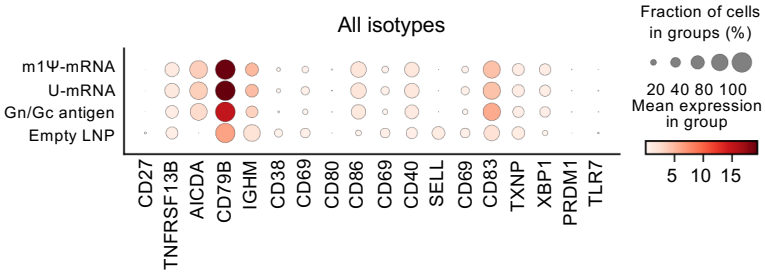
Two-dimensional UMAP projection of B cell single-cell RNA-seq profiles across all vaccination groups



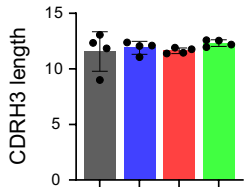
Proportions of cells expressing the C region of immunoglobulin heavy chain



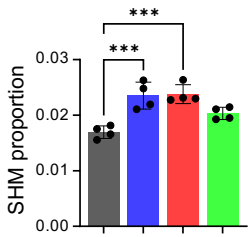
Mean expression of B cell activation and plasma cell markers across the groups.



Average lengths of antibody heavy chains CDR3 regions



Average proportion of somatic hypermutation in CDRH3



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mRNA vaccine against Andes virus

- To our knowledge, first direct side-by-side comparison of non-modified and modified mRNA platforms.
- M1 Ψ modification of uridine blocked induction of IFN-β, IFN-λ and multiple cytokines.
- Despite that, M1 Ψ modification had only a modest effect the expression of GnCc in transfected cells.
- The two vaccine platforms induced comparable germinal center responses and comparable neutralizing antibody responses.
- The two vaccines platform induced “sterilizing” protection against lethal challenge with Andes virus.
- IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines. Regulation of inflammation in response to RNA vaccines is different in mouse and human cells such as in the case of IL-1 and IL-1Ra-mediated response (PMID: 35332327).

Problem: Future pandemics caused by RNA viruses

Emerging Vaccines for Emerging Viruses

EMERVAX

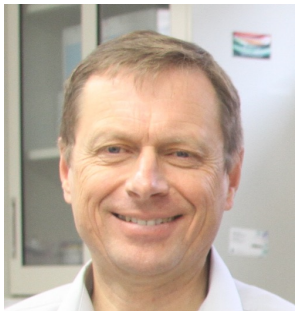
EMERVAX OVERVIEW

- Epidemics/pandemics caused by emerging viral infections will increase
- Emervax was founded in 2021 to address this concern
- Strong leadership team (science/business) and formidable advisory board
- Emervax is establishing a global network of collaborators and partners (Inst Pasteur in Dakar Senegal; Afrigen)
- Emervax: unique expertise and innovation for mRNA vaccine development

Team: Emervax Leadership

Emerging Vaccines for Emerging Viruses

EMERVAX



Alex Bukreyev, PhD

Co-Founder and President

Mariano A. Garcia-Blanco, MD, PhD

Co-Founder, Chief Scientific Officer, and Chair, Advisory Board

Thomas Forest Farb-Horch

Member, Board of Directors

Co-founder, President & CEO, Thrive Bioscience

Senior executive & founder of 10 companies (>35 yrs)

Four multi-billion dollar exits

Pei-Yong Shi, PhD

VP for Research Collaborations

Professor and Vice-President for Research Innovation, UTMB

Previously Director of Biology at Novartis Institute of Tropical Diseases (Singapore)

Critical role in the FDA approval of the Pfizer COVID-19 vaccine

Peter Weinstein, PhD, JD

Intellectual property and business development

Founder and Chief Executive Officer of Entralta

Previously Senior Patent Counsel at Baxter Healthcare Corporation

www.emervax.com

EMERVAX: Partners and Networks

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Institut Pasteur de Dakar (Senegal)

- mRNA production facility supported by the WHO
- Expertise in pathogen surveillance and vaccine clinical trials

Afrigen Biologics and Vaccines

- Cape Town-based biotechnology company
- Supported by WHO for mRNA technology transfer

University of Texas Medical Branch Galveston National Laboratory

The Bukreyev lab

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Ivan Kuzmin
Ruben Soto-Acosta
Philipp Ilinykh
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