DESIGN OF A VESICULAR STOMATITIS VIRUS-BASED VACCINE ENCODING RECEPTOR-BINDING DOMAIN OF SARS-CoV AND MERS-CoV SPIKE PROTEIN

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BACKGROUND

GENERAL INFORMATION [1,2]

- *Severe acute respiratory syndrome-related coronavirus* (SARS-CoV) and *Middle East respiratory syndrome-related coronavirus* (MERS-CoV) belong to the *Coronaviridae* family (CoVs).
- They are enveloped viruses with a positive-sense single-stranded RNA genome, which can infect a wide variety of animals, including humans, causing severe respiratory diseases (e.g., SARS and MERS).
- Human-to-human transmission is the most common route.
- There are **NO** specific treatments or vaccines available.
- Both diseases are considered **severe emerging infectious diseases** of the 21st century.

HYPOTHESIS & OBJECTIVE

A successful immunization against SARS-CoV and MERS-CoV can be achieved in mice by developing a **vaccine** based on recombinant vesicular stomatitis viruses (rVSVs) which express the RBD of the S protein of each coronavirus.

The aim of this study is to generate a vaccine that confers sustained, protective immunity to animal models against SARS-CoV and MERS-CoV, with the intention of progressing to safe clinical trials.

VESICULAR STOMATITIS VIRUS (VSV) [3,4]

- VSV is an enveloped virus enclosing a nonsegmented, negative-sense single-stranded RNA.
- It belongs to the *Rhabdoviridae* family and it naturally infects livestock, but rarely humans.
- Recombinant VSV is one of the most promising vaccine platforms.

WHY IS IT USED AS A VECTOR?

✓ Low risk of pre-existing immunity.

✓ Replication in almost all mammalian cell lines.

✓ Strong induction of immune response.

✓ Used as an attenuated vector→ deletion of the viral glycoprotein (G) gene.

✓ G protein can be replaced for other virus glycoproteins.

S PROTEIN AND RECEPTOR-BINDING DOMAIN [2]

- The spike protein (S) of human CoVs recognizes and binds to the host cell receptor. It is a surface-located glycoprotein formed by a signal peptide and two subunits: S1 and S2 **(Fig. 1)**.
- The receptor-binding domain (RBD) is located in the **S1 subunit (Fig. 1)**. RBD is responsible for the interaction with the cell receptor. SARS-CoV and MERS-CoV RBDs are **highly** similar.

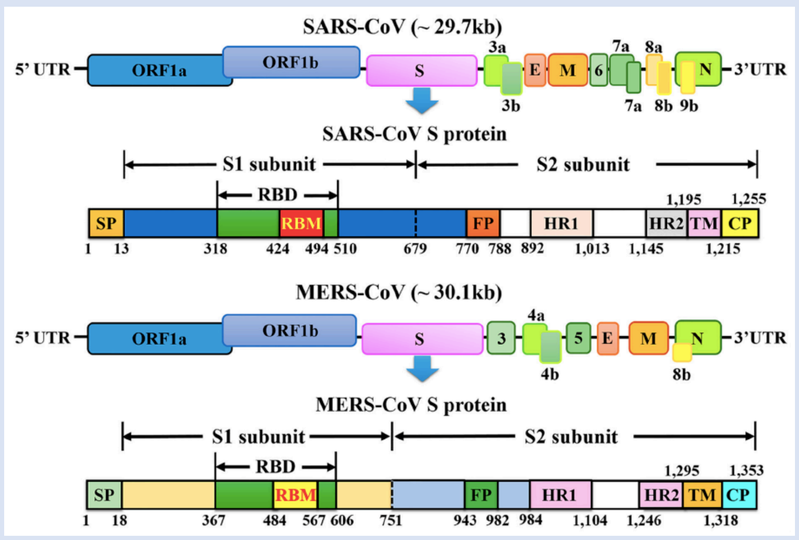

Figure 1. Representation of the genome organization and functional domains of S protein (in pink) for SARS-CoV and MERS-CoV. The RBD of the S protein is showed in green for both viruses and the subunits are indicated with brackets. The numbers refer to the amino acid. *Image retrieved from [2].*

MATERIAL & METHODS

1 VECTOR CONSTRUCTION AND rVSV RECOVERY [5,6]

- SARS-CoV and MERS-CoV RBD regions are subcloned into **pVSVFL(+)** under the control of T7 polymerase promoter, resulting in the plasmids **pVSV-RBD$_{SARS}$** and **pVSV-RBD$_{MERS}$**, respectively.
- pVSVFL(+) is a plasmid DNA construct that expresses the complete VSV positive-strand (antigenomic) RNA, and it is used as the carrier for the two exogenous proteins.
- **rVSV-RBD/S** (SARS-CoV), **rVSV-RBD/M** (MERS-CoV) and wild-type (WT) rVSV vectors are recovered in BHK-21 cell cultures.

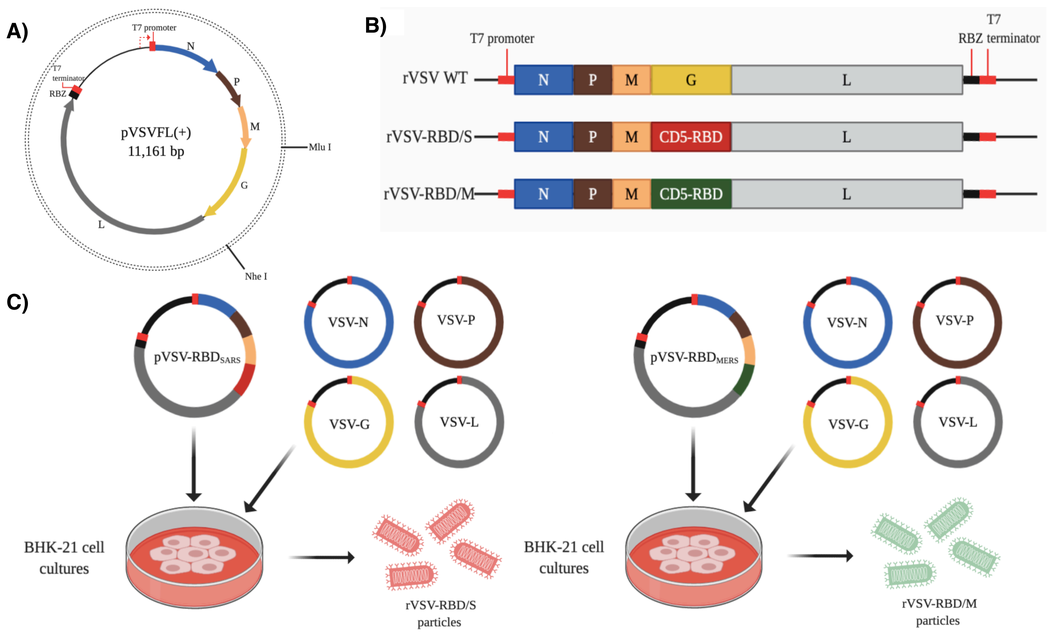

Figure 2. **Generation of rVSV vaccines. A)** Representation of pVSVFL(+) designed by Lawson et al. (1995) [5]. Genes encoding VSV proteins (N, P, M, G and L) are shown as arrows. RE sites are shown in the outer circles. RBZ= ribozyme. **B)** Sequences of the three rVSV vectors: WT rVSV, rVSV-RBD/S and rVSV-RBD/M. The CD5 signal peptide will be ligated to the 5' end of the RBD fragments. VSV glycoprotein (G) gene will be excised by RE digestion. **C)** Overview of rVSV recovery, consisting of a co-transfection of vaccinia-T7 infected cells with pVSV-RBD$_{SARS}$ and/or pVSV-RBD$_{MERS}$, along with a set of helper plasmids expressing the VSV N, P, G and L proteins. The length of each gene is not represented accurately. *All illustrations are created in BioRender.com.*

2 IMMUNOFLUORESCENCE ASSAY AND TITRATION OF rVSV VECTORS [6,7]

Detection of SARS/MERS-CoV RBD expression is done by indirect immunofluorescence. rVSV vectors (including wild-type rVSV) are titrated by real-time quantitative PCR.

3 ANIMAL VACCINATION AND SAMPLE COLLECTION [7,8]

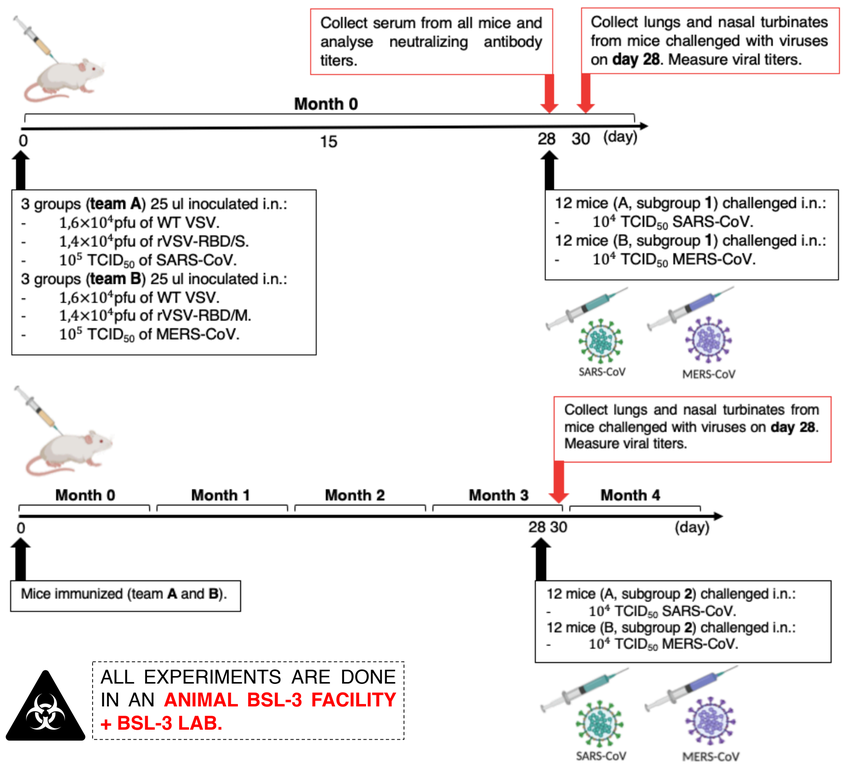

Figure 3. Chronogram of mice vaccinations, sample collection and virus challenges. Text enclosed in red describes the sample collection. *Created in Microsoft PowerPoint 2019 and BioRender.com.*

4 NEUTRALIZING ANTIBODY ASSAY [7,8]

- **SARS/MERS-CoV**-neutralizing antibody titers are determined based on the serum dilution which viral cytopathic effects are inhibited in 50% of the wells.
- **VSV**-neutralizing antibody titers→ highest dilution of serum without evidence of VSV infection.

5 PASSIVE PROTECTION STUDY [7,8]

Inoculate sera from immunized mice to non-immunized mice:

- rVSV-RBD/S, rVSV-RBD/M or wild-type rVSV immune sera.
- SARS-CoV, MERS-CoV immune sera (C+), or naïve mice sera (C-).

EXPECTED RESULTS

1. Mice inoculated with rVSV-RBD/S, rVSV-RBD/M and WT rVSV→ **HIGH** VSV-neutralizing antibody titers.
2. rVSV-RBD/S or rVSV-RBD/M immunization→ It will generate a **STRONG** immune response against virus infection.
3. Lung and nasal turbinate samples→ **LOW/UNDETECTABLE** virus titers after immunization with vaccines.
4. Inoculation of rVSV-RBD/S-, rVSV-RBD/M- or SARS-CoV/MERS-CoV antisera→ **PRESENCE** of passive protection after virus challenge.

DISSEMINATION PLAN

The results, when published in a scientific journal, will be presented to these conferences:

AMERICA

ANNUAL CONEFRENCE ON VACCINOLOGY RESEARCH

EUROPE

INTERNATIONAL CONFERENCE ON VACCINE RESEARCH

ASIA

VACCINE CONGRESS
(Vaccine Journal, Elsevier)

Relevant references

[1] Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia: MERS, SARS and coronaviruses. Respirology. 2018 Feb;23(2):130–7. **[2]** Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, et al. From SARS to MERS, Thrusting Coronaviruses into the Spotlight. Viruses. 2019 Jan 14;11(1):59. **[3]** Kapadia SU, Simon ID, Rose JK. SARS vaccine based on a replication-defective recombinant vesicular stomatitis virus is more potent than one based on a replication-competent vector. Virology. 2008 Jun;376(1):165–72. **[4]** Rauch S, Jasny E, Schmidt KE, Petsch B. New Vaccine Technologies to Combat Outbreak Situations. Frontiers in Immunology [Internet]. 2018 Sep 19 [cited 2018 Dec 14];9. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2018.01963/full **[5]** Lawson ND, Stillman EA, Whitt MA, Rose JK. Recombinant vesicular stomatitis viruses from DNA. Proc Natl Acad Sci U S A. 1995 May 9;92(10):4477–81. **[6]** Du L, He Y, Wang Y, Zhang H, Ma S, Wong CKL, et al. Recombinant adeno-associated virus expressing the receptor-binding domain of severe acute respiratory syndrome coronavirus S protein elicits neutralizing antibodies: Implication for developing SARS vaccines. Virology. 2006 Sep;353(1):6–16. **[7]** Kapadia SU, Rose JK, Lamirande E, Vogel L, Subbarao K, Roberts A. Long-term protection from SARS coronavirus infection conferred by a single immunization with an attenuated VSV-based vaccine. Virology. 2005 Sep;340(2):174–82. **[8]** Subbarao K, McAuliffe J, Vogel L, Fahle G, Fischer S, Tatti K, et al. Prior Infection and Passive Transfer of Neutralizing Antibody Prevent Replication of Severe Acute Respiratory Syndrome Coronavirus in the Respiratory Tract of Mice. Journal of Virology. 2004 Apr 1;78(7):3572–7.