MVA-vectored multi-antigen COVID-19 vaccines induce protective immunity against SARS-CoV-2 variants spanning Alpha to Omicron in preclinical animal models

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BACKGROUND

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is an airborne virus that has rapidly spread across the world since the beginning of 2020. SARS-CoV-2 infection causes a spectrum of disease from asymptomatic to severe complications, including pneumonia, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), cytokine storm syndrome (CSS) and death. Increasingly contagious variants of concern (VoC) have fueled recurring global infection waves.

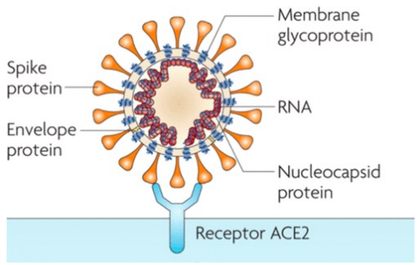

Figure 1: Coronavirus structure and viral receptor ACE2 on the host cell surface (Liu et al., *ACS Cent. Sci., 2020*).

Design of effective vaccines must consider the divergent and rapidly mutating nature of coronavirus spike protein. While a high level of viral escape from neutralizing antibodies exist among the VoCs, the T cell epitopes to spike have remained largely conserved, suggesting the current vaccines may not be rendered completely effective. Our modified vaccinia Ankara (MVA)-VLP vaccine platform combines the safety of a non-replicating virus vector with enhanced immunogenicity of vaccine antigens displayed on the surface of VLPs *in vivo* following immunization. Herein, utilizing the MVA-VLP platform, we tested efficacy of multi-antigen vaccines expressing SARS-CoV-2 S (targeting B.1, B.1.351 and BA.1.1.529), M and envelope (E) in preclinical animal challenge models.

METHODS

1. **Vaccine development:** SARS-CoV-2 genes were inserted into one site between two essential MVA genes, A5R and A6L, under direction of MVA promoter modified H5 (PmH5).
2. **Vaccination:** 6-week-old K18-hACE2 mice were vaccinated intramuscularly with 10^7 PFU of GEO-CM02 vaccine.
3. **Challenge:** Mice were challenged with 10^5 PFU of SARS-CoV-2 (B.1, B.1.351, or BA.1.1.529) by intranasal route on day 56.
4. **Tissue collection:** At days 3 and 6 post infection, the mice were euthanized by cardiac puncture with profusion of 1X PBS. Lungs and brains were collected and flash frozen for further analysis.

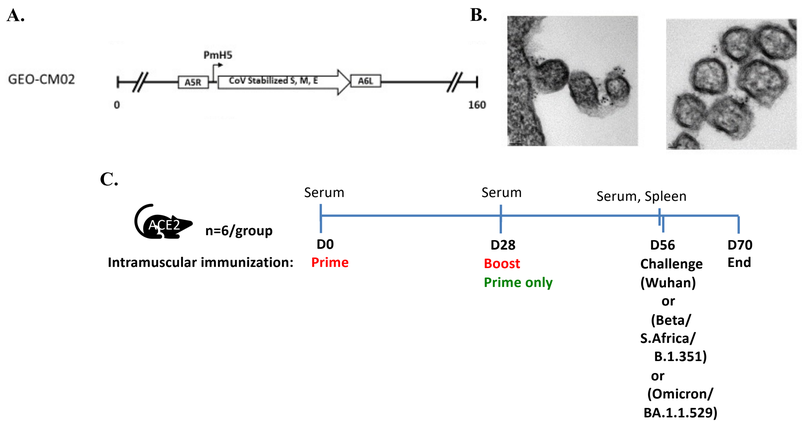

Figure 2: **GeoVax, Inc. SARS-CoV-2 vaccines.** A) Vector construction map. Positions are given in kilobase base pairs in the MVA genome. B) Electron microscopic analysis demonstrating VLP formation. Thin layer cell sections infected with GEO-CM01. Immunogold labeling was carried out using anti-Spike primary antibody (Sino T62) and immunogold secondary. Scale bars in the bottom left of image are 50nm (left and 100nm (right). C) Flow of methods.

Results

1. MVA-vectored multi-antigen SARS-CoV-2 vaccines induce protective immunity against VoC

- Mice vaccinated with GEO-CM02 vaccine induced binding antibodies to Spike protein derived from B.1, B.1.351, or BA.1.1.529 variants at day 43 or 55 post vaccination.
- Mice vaccinated with GEO-CM02 vaccine with prime and boost developed neutralizing antibodies against B.1, B.1.351 and BA.1.1.529 variants, while the prime vaccine resulted in little antibody production.

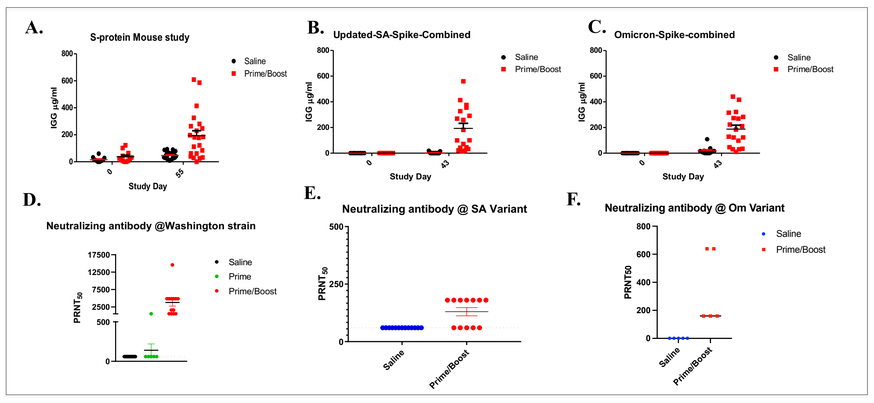

Figure 3. Humoral response following GEO-CM02 vaccination of hACE2 mice. Analyzed by ELISA for binding antibody specific to Spike derived from SARS-CoV-2 (A), Beta variant (B), or Omicron variant (C). Evaluated for neutralization capacity in prime only and prime-boost GEO-CM02 immunized mice against Wuhan (D), Beta variant (E), or Omicron variant (F). Results depicted a 50% plaque reduction neutralization titer (PRNT50).

2. GEO-CM02 vaccine induce protective cellular immunity against VoC

- GEO-CM02 vaccination is capable of producing functional CD4+ and CD8+ T cells while maintaining a Th1 rather than Th2 phenotype.
- Vaccination led to an increase in IFNγ and IL-2 producing CD4+ and CD8+ T cells specific for spike protein.

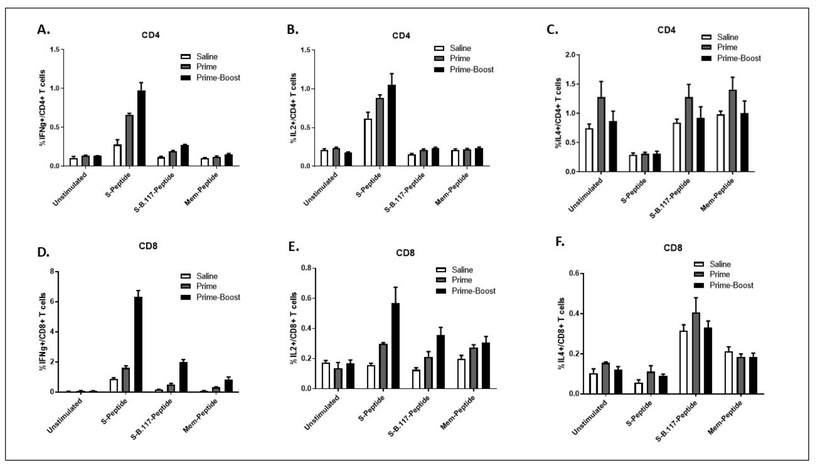

Figure 4. Functional SARS-CoV-2 specific T cells following GEO-CM02 vaccination in K18-hACE2 mice. Cells were stimulated ex vivo with Wuhan (S-peptide), alpha (S-B.1.1.7-peptide) variant spike or membrane (Mem-peptide) then fixed and stained for cell markers CD4 (A, B, C) or CD8 (D, E, F) and intracellular cytokines IFN-γ (A,D), IL-2 (B, E) or IL-4 (C, F) then analyzed by flow cytometry. Data is shown as percent CD4 or CD8 positive for cytokine.

3. Characteristics of K18-hACE2 mice following vaccination and B.1 SARS-CoV-2 challenge

- Vaccinated mice remained healthy with slight weight loss at day 1 post challenge, with recovery, as opposed to saline mice.
- Higher virus titers were observed in lungs of saline mice than the vaccinated mice.

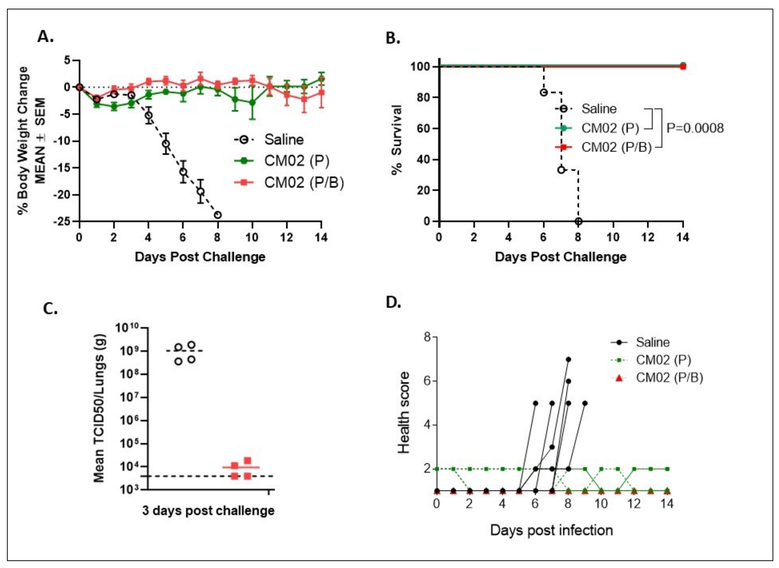

Figure 5. Survival and viral load of mice following challenge. Mice were intranasally infected with 10^5 PFU of B.1 SARS-CoV-2. (A) Body weight, (B) Survival and (D) clinical scores were observed until day 14 post infection. (C) Virus titers were analyzed in the lungs by plaque assay. Each data point represents an individual mouse.

4. Characteristics of K18-hACE2 mice following vaccination and BA.1.1.529 SARS-CoV-2 challenge

- Vaccinated mice remained healthy with slight weight loss at day 1 post challenge, with recovery, as opposed to saline mice.
- Higher virus titers were observed in lungs of saline mice than the vaccinated mice.

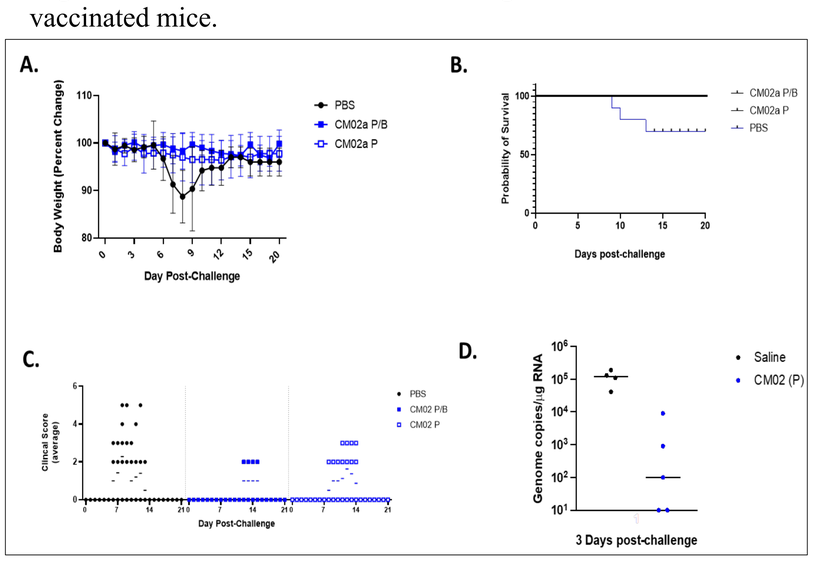

Figure 6. Survival and viral load of mice following challenge. Mice were intranasally infected with 10^5 PFU of BA.1.1.529 SARS-CoV-2. (A) Body weight, (B) Survival and (C) clinical scores were observed until day 14 post infection. (D) Virus titers were analyzed in the lungs by qRT-PCR using the N1 primer. Each data point represents an individual mouse.

5. Decreased viral nucleocapsid protein and leukocytes infiltration in vaccinated mice following BA.1.1.529 challenge

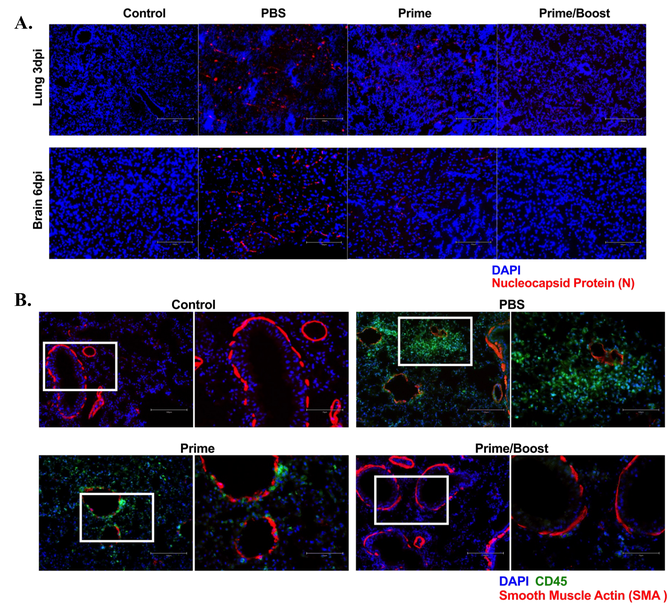

Figure 7. Vaccination decreases N protein expression and CD45 cells in lungs and brain following challenge with BA.1.1.529. (A) Lungs and brain at days 3 and 6 post infection, respectively, are stained with DAPI and N protein. (B) Lungs at day 3 post infection are stained with DAPI, CD45 and SMA.

6. Reduced virus titers in vaccinated mice compared to saline mice

- Mice vaccinated with both prime and boost showed a decrease in the viral RNA levels at days 3 and 6 post challenge in the lungs, brain, nasal turbinates and olfactory bulb compared to the saline group

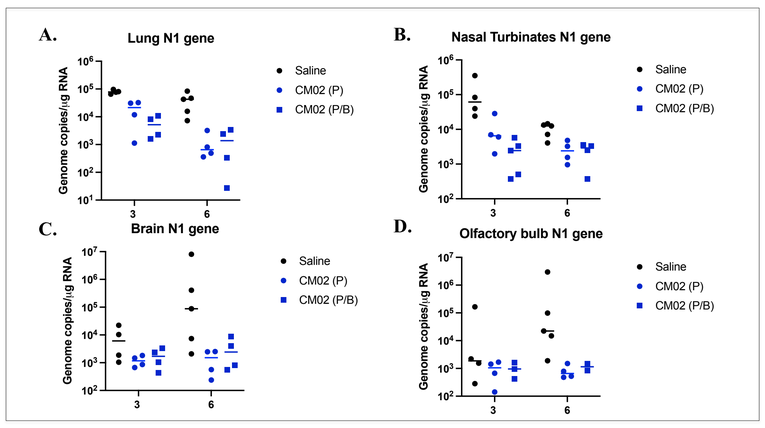

Figure 8. GEO-CM02 vaccine efficacy against BA.1.1.529 SARS-CoV-2. Viral loads were determined by qRT-PCR on tissues collected days 3 and 6 post challenge. Tissues include (A) Lung, (B) Nasal Turbinates, (C) Brain, and (D) Olfactory bulb.

7. Cytokine and chemokine levels in mice following BA.1.1.529 challenge

- Mice vaccinated with GEO-CM02 demonstrated significant decreased protein amounts of IL-6, IP10, MCP1, MIP1α, and MIP1β compared to the saline mice post challenge.
- Mice vaccinated with GEO-CM02 resulted in increased amounts of RANTES compared to the saline mice post challenge

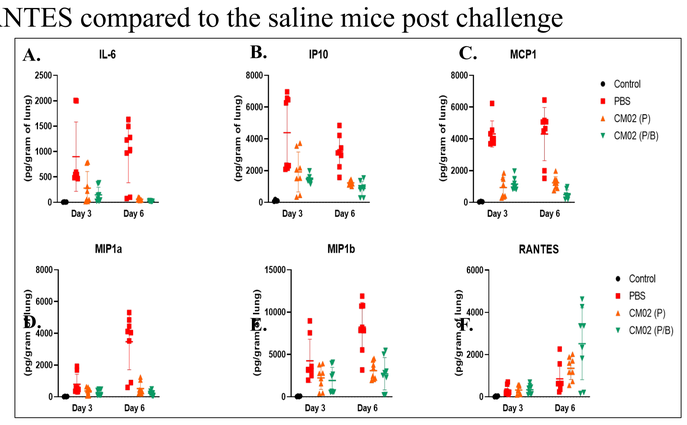

Figure 9. Cytokine and chemokine levels following BA.1.1.529 challenge. Mice were prime-boost immunized with saline or GEO-CM02 then challenge with BA.1.1.529. Cytokines and chemokines in the lungs were analyzed using Luminex. Cytokine includes (A) IL-6, while chemokines include (B) IP10, (C) MCP1, (D) MIP1α, (E) MIP1β and (F) RANTES.

CONCLUSION

- Utilizing the MVA-VLP platform, we tested efficacy of multi-antigen vaccines expressing SARS-CoV-2 S, M and E in preclinical animal challenge models.
- GEO-CM02 vaccine induce protective immunity protects mice from SARS-CoV-2 variants spanning Alpha to Omicron
- Vaccinated mice remained healthy and expressed lower viral loads and an altered immune response compared to the saline mice.

ACKNOWLEDGEMENTS

This work was supported by a grant by GeoVax, Inc.. We thank the members of the GSU High Containment Core and the Department for Animal Research for their assistance with the experiments.