Optimization of Non-Coding Regions Improves Protective Efficacy of an mRNA SARS-CoV-2 Vaccine in Nonhuman Primates

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ABSTRACT

The CVnCoV (CureVac) mRNA vaccine candidate for SARS-CoV-2 has recently been evaluated in a phase 2b/3 efficacy trial in humans. CV2CoV is a second-generation mRNA vaccine candidate with optimized non-coding regions and enhanced antigen expression. Here we report a head-to-head study of the immunogenicity and protective efficacy of CVnCoV and CV2CoV in nonhuman primates. We immunized 18 cynomolgus macaques with two doses of 12 ug of lipid nanoparticle formulated CVnCoV, CV2CoV, or sham (N=6/group). CV2CoV induced substantially higher binding and neutralizing antibodies, memory B cell responses, and T cell responses as compared with CVnCoV. CV2CoV also induced more potent neutralizing antibody responses against SARS-CoV-2 variants, including B.1.351 (beta), B.1.617.2 (delta), and C.37 (lambda). While CVnCoV provided partial protection against SARS-CoV-2 challenge, CV2CoV afforded robust protection with markedly lower viral loads in the upper and lower respiratory tract. Antibody responses correlated with protective efficacy. These data demonstrate that optimization of non-coding regions can greatly improve the immunogenicity and protective efficacy of an mRNA SARS-CoV-2 vaccine candidate in nonhuman primates.

INTRODUCTION

In the Phase 2b/3 HERALD trial, CVnCoV (CureVac) mRNA vaccine candidate efficacy against symptomatic COVID-19 was reported to be 48% and 53% in the overall study population and in the 18-60 years of age subgroup, respectively [1]. CV2CoV is a second-generation mRNA vaccine candidate that involves modifications of the non-coding regions that were selected based on an empiric screen for improved antigen expression [2, 3]. Both CVnCoV and CV2CoV are based on RNActive® technology that consists of non-chemically modified, sequence engineered mRNA, without pseudouridine. Both vaccines encode for the same full-length, pre-fusion stabilized SARS-CoV-2 Spike (S) [4] and are encapsulated in lipid nanoparticles (LNP) with identical composition. CV2CoV has been engineered with different non-coding regions flanking the open reading frame (Fig. 1), which have previously been shown to improve transgene expression [3] and protection against SARS-CoV-2 in ACE2 transgenic mice [2].

In this study, we compare head-to-head the immunogenicity and protective efficacy of CVnCoV and CV2CoV against SARS-CoV-2 challenge in nonhuman primates (NHPs).

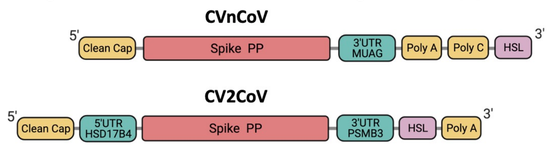
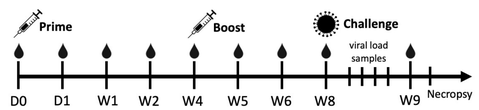


Figure 1. mRNA vaccine design.

EXPERIMENTAL DESIGN

- **mRNA**: CVnCoV and CV2CoV are based on CureVac's RNActive®. Both constructs were encapsulated in lipid nanoparticles (LNP).
- **Nonhuman primates**: Cynomolgus macaques were immunized and challenged as shown in Figure 2. Challenge virus ($1.0x10^5$ TCID50 of SARS-CoV-2 derived from USA-WA1/2020) was administered as 1 ml by the intranasal (IN) route (0.5 ml in each nare) and 1 ml by the intratracheal (IT) route.
- **Immunological assays**: Receptor-binding domain (RBD)-specific binding antibodies were assessed by ELISA [5]. Neutralizing titers were determined via SARS-CoV-2 pseudoviruses neutralization assay [5]. B & T cell data were generated via flowcytometry and ELISpot [6].
- **Virological assays**: Viral titers were determined using subgenomic RT-PCR assay [5].
- **Statistical analysis**: Group comparisons were done via two-tailed nonparametric Mann-Whitney U t test. Correlations were assessed by two-sided Spearman rank-correlation tests.



Groups (N=6)	Vaccines	Antigen	Dose	Route
I	Sham	Sham	Sham	Sham
II	CVnCoV	Full-length Spike (K986P, V987P)	12µg	I.M.
III	CV2CoV	Full-length Spike (K986P, V987P)	12µg	I.M.

Figure 2. NHP vaccine study schema.

RESULTS

IMMUNOGENICITY

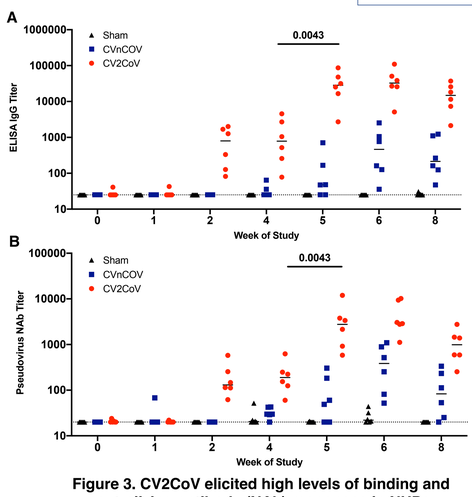


Figure 3. CV2CoV elicited high levels of binding and neutralizing antibody (NAb) responses in NHPs.

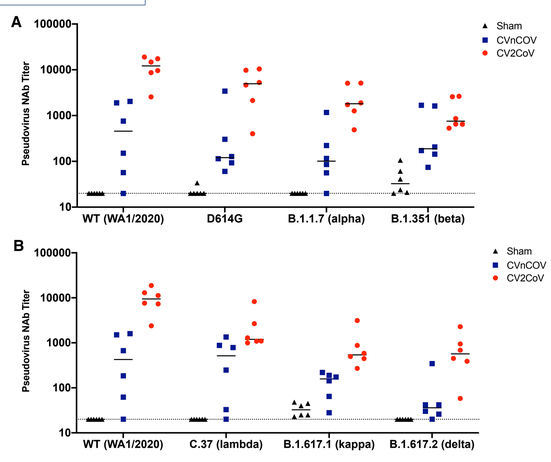


Figure 4. CV2CoV elicited neutralizing antibody responses against various SARS-CoV-2 variants.

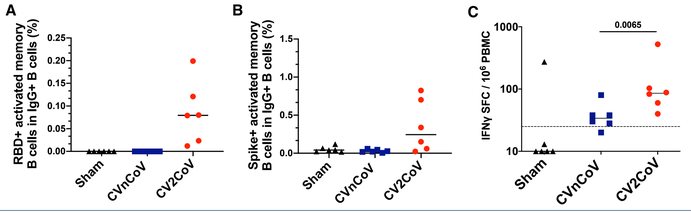


Figure 5. CV2CoV induced memory B cell and T cell immune responses in on day 42.

PROTECTIVE EFFICACY

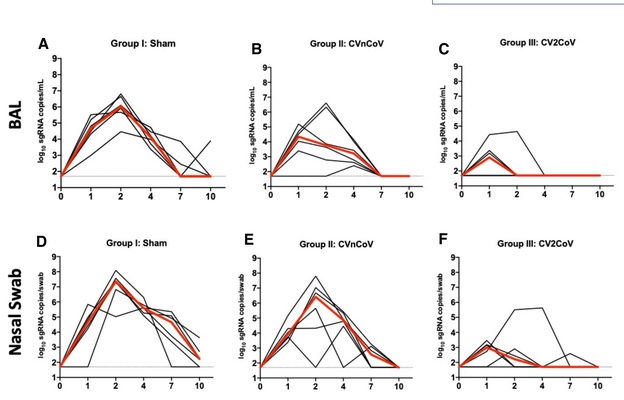


Figure 6. CV2CoV protected NHPs from challenge infection. BAL: Bronchoalveolar lavage

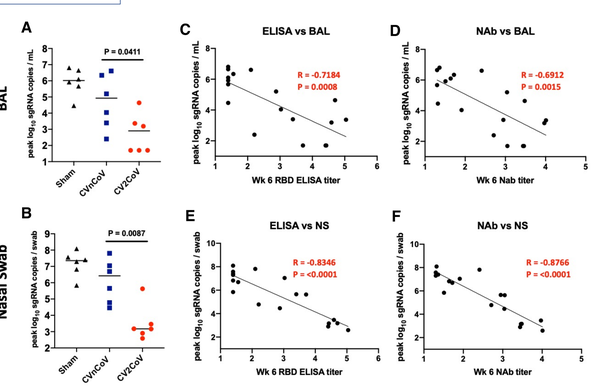


Figure 7. Binding and neutralizing antibody (Nab) titers elicited upon CVnCoV and CV2CoV vaccination correlate with protection against SARS-CoV-2. BAL: Bronchoalveolar lavage; NS: nasal swab

CONCLUSIONS

- The improved, second-generation CV2CoV mRNA vaccine candidate elicited more robust binding and neutralizing antibody titers compared to first-generation CVnCoV vaccine candidate.
- Antigen specific activated memory B cells and T cells were detected primarily post CV2CoV vaccine candidate immunization.
- Bronchoalveolar lavage (BAL) and nasal swab (NS) viral loads demonstrated improved protective efficacy of the CV2CoV vaccine candidate, including protection in the upper respiratory tract.
- ELISA and neutralizing antibody (NAb) titers at week 6 inversely correlated with peak viral loads in BAL and NS.

ACKNOWLEDGMENTS

- We thank Acuitas Therapeutics for LNP formulation. We thank Sarah Gardner, Gabriella Kennedy, and Rachael Edmonston for their generous assistance, as well as Domenico Maione and Marie-Thérèse Martin for critical reviews.
- **Funding Source**: This work was supported by CureVac AG and the German Federal Ministry of Education and Research (BMBF; grant 01KI20703). Development of CV2CoV is carried out in a collaboration of CureVac AG and GSK.

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