CV2CoV, an enhanced mRNA-based SARS-CoV-2 vaccine candidate, supports higher protein expression and improved immunogenicity in rats

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Abstract:

More than a year after emergence of the SARS-CoV-2 pandemic, multiple first-generation vaccines are approved and available for vaccination. Still, many challenges remain. The ongoing vaccination programs across the globe suffer from insufficient vaccine supply. The virus is adapting to the human host and novel variants are circulating that are neutralised less efficiently by antibodies raised against ancestral SARS-CoV-2 variants. Here, we describe CV2CoV, a second-generation mRNA vaccine developed for enhanced protein expression and immunogenicity. CV2CoV supports increased levels of protein expression in cell culture compared to our clinical candidate CVnCoV. Vaccination with CV2CoV induces high levels of virus neutralising antibodies with accelerated kinetics in rats. Robust antibody responses are reflected in significant cross-neutralisation of circulating SARS-CoV-2 variants of concern, i.e. B.1.1.7 and B.1.351. Together, these results underline the value of CV2CoV as next-generation SARS-CoV-2 mRNA vaccine.

Introduction

The ongoing COVID19 pandemic creates a worldwide need for fast development of vaccines. By April 2021, thirteen vaccines against SARS-CoV-2 have received authorisation by at least one national regulatory authority [1], and a total of ninety-three vaccine candidates are currently in clinical development [2]. However, the emergence of variants of concern (VOC) of SARS-CoV-2 potentially able to escape pre-existing immunity highlights the need for continuous improvement and adaptation of

vaccines [3 4 5 6 7 8 9]. mRNA vaccines represent a promising technology to meet this need: they are based on a highly versatile, adaptable platform technology [10] whose efficacy as a vaccine against SARS-CoV-2 has been demonstrated [11 12 13 14] resulting in authorized vaccines [1].

CureVac's mRNA technology termed RNActive® [15 16 17 18] is based on non-chemically modified, sequence engineered mRNA. This technology supports high protein expression and moderate activation of innate immune responses [17, 19]. RNActive® vaccines have shown promise in the context of different infectious diseases in both preclinical [20 19 21 22] and clinical [23] studies and provide the basis for the CVnCoV, a lipid nanoparticle (LNP) formulated mRNA vaccine against SARS-CoV-2 encoding for full length Spike (S) protein. Preclinical results have demonstrated CVnCoV's ability to induce robust cellular and humoral immune responses as well as protective efficacy in rodents and non-human primates (NHPs) [24 25]. Phase 1 clinical trials have established CVnCoV's safety and tolerability. Vaccination with 12 µg of CVnCoV resulted in full seroconversion two weeks post injection of the second dose and showed comparable virus neutralising antibody titres to convalescent sera [26]. CVnCoV is currently tested in phase 2b/3 studies that investigate the efficacy, safety, and immunogenicity.

Here, we show the results of our second-generation SARS-CoV-2 vaccine, CV2CoV. This vaccine was developed to further increase vaccine efficacy, thereby setting the stage to tackle future challenges of the SARS-CoV-2 pandemic. These will likely include the requirement for high vaccine-induced immune responses to compensate for reduced virus neutralisation responses and efficacy against different variants [27]. In addition, emerging immune escape variants might necessitate multivalent vaccines, in which each component needs to be efficacious at a lower dose. Beyond this, dose sparing supported by a more immunogenic vaccine might facilitate providing more doses for a worldwide need.

CVnCoV and second generation CV2CoV are sequence optimized, capped, chemically unmodified mRNA-based vaccines that are encapsulated using the same lipid nanoparticle (LNP) technology. Both encode full-length SARS-CoV-2 S protein with pre-fusion stabilising K986P and V987P mutations [28 29]. The vaccines differ in their non-structural elements flanking the open reading frame (ORF), i.e. the 5' and 3' untranslated regions (UTRs) and the 3' tail. These changes were introduced to improve intracellular RNA stability and translation, aimed to increase and prolong protein expression and thereby improve immunogenicity.

Here, we show that the optimised mRNA component in CV2CoV indeed supports higher levels of protein expression in cell culture. Vaccination with CV2Cov in a rat model induces robust levels of

humoral responses that are detectable upon a single injection and that cross-react with distinct SARS-CoV-2 variants.

Results and discussion

Despite promising results of CVnCoV in both preclinical models [24] [25] and early clinical evaluations [26], we have continued the development of our mRNA technology with the aim to create a vaccine against SARS-CoV-2 with enhanced immunogenicity. Here we present results of our preclinical SARS-CoV-2 candidate vaccine CV2CoV that features changes in the non-coding regions compared to our clinical candidate CVnCoV. Flow cytometry-based analysis of protein expression levels of the mRNA components in CV2CoV and CVnCoV in HeLa cells transfected with identical amounts of mRNA demonstrated a significant increase of protein expression upon transfection with CV2CoV compared to CVnCoV mRNA. This effect was evident in both intracellular and cell surface expression of the S protein. CV2CoV mRNA displayed a 3.3 fold increase in the geometric mean in intracellular and a 1.8 fold increase in the geometric mean in cell surface expression compared to CVnCoV mRNA analysed by flow cytometry (Figure 1).

We next characterized the immunogenicity of the second generation vaccine CV2CoV in a small animal model. Wistar rats were vaccinated twice (d0, d21) with five different doses ranging from 0.5 µg - 40 µg of CV2CoV. NaCl vaccinated animals served as negative controls. Rats vaccinated with 0.5 µg, 2 µg or 8 µg of CV2CoV elicited a strong dose dependent binding antibody response directed against the S receptor binding domain (RBD). High values of RBD reactive antibodies without a clear dose response were detectable upon vaccination with 20 µg and 40 µg CV2CoV possibly due to saturating dose levels employed (Figure 2). RBD binding antibodies developed rapidly and were detectable two weeks after a single vaccination in all dose groups (Figure 2A). Both IgG1 and IgG2a titres increased over time and a clear boost effect was detectable upon a second vaccination (Figure 2B and 2C).

Induction of virus neutralising titres in rat sera was assessed in a cytopathic effect (CPE)-based assay with homologue wild-type SARS-CoV-2 evaluating the neutralising dose (ND) 100 of the sera (Figure 3). CV2CoV induced significant, dose dependent neutralising titres two weeks after a single vaccination in all animals vaccinated with a dose of 2 µg or higher (Figure 3A). On day 14, CV2CoV induced homologous titres ranging from 1:42 in the 2 µg group to 1:193 in the 40 µg group. Neutralising titres increased over time and a clear boost effect was detectable (Figure 3B and 3C). The second

vaccination led to a substantial increase in neutralising titres in all dose groups except for two animals in the 0.5 µg group. On day 42 post vaccination, neutralising titres in animals that had received ≥2 µg exceeded the upper range of detection in the assay, i.e. a dilution of 1:5120 (Figure 3C).

In summary, vaccination with CV2CoV induced high, dose dependent levels of RBD binding and virus neutralising antibodies in outbred rats. Antibody responses were detectable in doses as low as 0.5 µg and doses of ≥2 µg resulted in robust antibody levels. Importantly, a single vaccination of ≥2µg was sufficient to induce neutralising titres two weeks post injection. In comparison, preclinical studies in rats, hamsters and NHPs [24] [25] have shown that two vaccinations of CVnCoV are required to induce significant levels of VNTs, providing proof for the enhanced characteristics of CV2CoV.

CV2CoV's ability to induce robust levels of neutralising antibodies was highlighted by its ability to cross neutralise three different SARS-CoV-2 variants. Neutralising titres in the day 42 sera against heterologous SARS-CoV-2 variants, i.e. B.1.1.298, B.1.1.7 and B.1.351 originating from Denmark, the UK and South Africa, respectively, compared to ancestral SARS-CoV-2 (B.1) were analysed. Again, ND100 antibody levels were measured in a comparable CPE based assay system based on wild-type SARS-CoV-2. In the 8 µg dose group, CV2CoV induced median titres of 1:2438 (B.1. (Figure 4A)), 1:4096 (B.1.1.298 (Figure 4B)), 1:1935 (B1.1.7 (Figure 4C)) and 1:806 (B.1.351 (Figure 4D)). Of note, values of 1:4096, mostly present in in the 40 µg groups and in groups tested for neutralisation of B.1.1.298 might be higher since no further dilution step was tested. Overall, sera from CV2CoV-vaccinated animals were able to neutralise B.1.1.298 without detectable decrease. A reduction of neutralising titres were measured against B.1.1.7 and B.1.351, where a median decrease across dose groups 0.5 µg, 2 µg, 8 µg and 20 µg of 1.4 (B.1.1.7) and 3.3 fold (B.1.351) was detected. These values are comparable to or better than previously observed cross-neutralisation [30] [31] [32].

In summary, CV2CoV represents a highly promising second-generation vaccine with improved characteristics, including accelerated induction of neutralising antibody titres two weeks after the first vaccination. Its ability to induce high levels of antibodies against SARS-CoV-2 at low doses supports a scenario in which vaccination with CV2CoV allows dose sparing that might be able to contribute to reduce worldwide vaccine shortage. Moreover, this characteristic allows CV2CoV's application in the context of multivalent vaccines that might become necessary as the virus mutates further. Overall, CV2CoV represents a highly promising candidate for further clinical development to help meet some of the challenges of the SARS-CoV-2 pandemic.

Material and Methods

mRNA vaccines

The mRNA vaccines are based on the RNActive® platform (claimed and described in e.g. WO2002098443 and WO2012019780)) and are comprised of a 5′ cap1 structure, a GC-enriched open reading frame (ORF), 3′ UTR, a vector-encoded polyA stretch and does not include chemically modified nucleosides. CVnCoV contains parts of the 3' UTR of the Homo sapiens alpha haemoglobin gene as 3' UTR followed by a polyA stretch, a C30 stretch and a histone stem loop. CV2CoV further comprises a 5' UTR from the human hydroxysteroid 17-beta dehydrogenase 4 gene (HSD17B4) and a 3' UTR from human proteasome 20S subunit beta 3 gene (PSMB3) followed by a histone stem loop and a polyA stretch. Lipid nanoparticle (LNP)-encapsulation of mRNA was performed by Acuitas Therapeutics (Vancouver, Canada). The LNPs used in this study are particles of ionisable amino lipid, phospholipid, cholesterol and a PEGylated lipid. The mRNA encoded protein is based on the spike glycoprotein of SARS-CoV-2 NCBI Reference Sequence NC_045512.2, GenBank accession number YP_009724390.1 and encodes for full length S featuring $K_{986}P$ and $V_{987}P$ mutations.

In vitro protein expression

For detection of mRNA expression in cell culture, HeLa cells were seeded in 6-well plates at a density of 400.000 cells/well. 24h later, cells were transfected with 2µg of mRNA per well via Lipofection. For this, RNAs were complexed with Lipofectamine 2000 (Life Technologies) at a ratio of 1:1.5 and transfected into cells according to manufacturer's protocol. Protein expression was assessed 24h post transfection. For FACS analysis, cells were fixed and analysed with intact (surface staining) or permeabilised plasma membranes via treatment with Perm/Wash buffer (BD Biosciences, Cat. 554723) (intracellular staining). Specific S protein expression was assessed via staining with Human anti SARS CoV S antibody (CR3022) (Creative Biolabs, Cat. MRO-1214LC) followed by goat anti-human IgG $F(ab')_2$ fragment PE antibody (Immuno Research, Cat. 109-116-097) in a BD FACS Canto II cell analyzer and the FlowJo 10 software.

Animals

Female and male rats (Wistar, 7-8 weeks of age) were provided and handled by Preclinics Gesellschaft für präklinische Forschung mbH, (Potsdam, Germany). The animal study was conducted in accordance with German laws and guidelines for animal protection and appropriate local and national approvals (2347-14-2018 LAVG Brandenburg).

Immunisations

Animals were injected intramuscularly (IM) into the Musculus Gastrocnemius with different doses from 0.5-40 µg LNP-formulated SARS-CoV-2 mRNA (CV2CoV) or NaCl as negative control.

Antibody analysis

SARS-CoV-2 Spike RBD protein specific IgG1 and IgG2a binding antibodies were detected via ELISA. Plates were coated with 1µg/ml of SARS-CoV-2 (2019-nCoV) Spike RBD-His (Sino Biologicals, Cat. 40592-V08B) for 4-5h at 37°C. Plates were blocked overnight in 1% BSA, washed and incubated with serum for 2h at room temperature. For detection, rat sera were incubated with biotin mouse anti-rat IgG1 (Biolegend, Cat. 407404) or biotin mouse anti-rat IgG2a (Biolegend, Cat. 407504), followed by incubation with HRP-Streptavidin (BD, Cat: 554066). Detection of specific signals was performed in a BioTek SynergyHTX plate reader, with excitation 530/25, emission detection 590/35 and a sensitivity of 45.

For the analysis of virus neutralising titres of rat sera shown in Figure 3, serial dilutions of heat-inactivated sera (56°C for 30 min) tested in duplicates with a starting dilution of 1:10 followed by 1:2 serial dilutions were incubated with 100 $TCID_{50}$ of wild type SARS-CoV-2 (strain 2019-nCov/Italy-INMI1) for 1 hour at 37ºC. Every plate contained a dedicated row (8 wells) for cell control which contains only cells and medium, and a dedicated row of virus control which contain only cells and virus. Infectious virus was quantified upon incubation of 100 µl of virus-serum mixture with a confluent layer of Vero E6 cells (ATCC, Cat.1586) followed by incubation for 3 days at 37°C and microscopical scoring for CPE formation. A back titration was performed for each run in order to verify the correct range of $TCID_{50}$ of the working virus solution. VN titres were calculated according to the method described by Reed & Muench. If no neutralisation was observed (MNt <10), an arbitrary value of 5 was reported. Analyses were carried out at VisMederi srl (Siena, Italy).

For the analysis of neutralising titers against the different SARS-CoV-2 isolates shown in Figure 4, sera were pre-diluted 1:16 with DMEM. Three times 100 µl, representing three technical replicates, of this pre-dilution were transferred into a 96 well plate. A log2 dilution was conducted by passaging 50 µl of the serum dilution in 50 µl DMEM, leaving 50 µl of sera dilution in each well. Subsequently 50 µl of the respective SARS-CoV-2 (B.1, B.1.1.298, B.1.1.7 or B.1.351) virus dilution (100 $TCID_{50}$/well) was added to each well and incubated for 1 hour at 37 °C. Lastly, 100 µl of trypsinated VeroE6 cells (cells of one confluent TC175 flask per 100 ml) in DMEM with 2 % penicillin/streptomycin supplementation

was added to each well. After 72 hours incubation at 37 °C, the wells were evaluated by light microscopy. A serum dilution was counted as neutralising in the case no specific CPE was visible. The virus titre was confirmed by virus titration, positive and negative serum samples were included. Analysis were carried out at Friedrich-Loeffler-Institut (Greifswald, Germany).

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Author contributions

N.R., J.S., D.H., S.R., S.O.M. and B.P. conceived and conceptualised the work and strategy. M.T and A.T. were involved in mRNA vaccine design, S.R, N. R. and B.P. designed in vitro and in vivo studies. N.R., J.S., D.H., S.R. analysed and interpreted data. S.R. and N.R. wrote the manuscript. All authors supported the review of the manuscript.

Competing interests

S.R., B.P., N.R., A.T. M.T. and S.O.M are employees of CureVac AG, Tuebingen Germany, a publically listed company developing RNA-based vaccines and immunotherapeutics. All authors may hold shares or stock options in the company. S.R., B.P., N.R., A.T. and M.T. are inventors on several patents on mRNA technology and use thereof.

References

1. VaC. COVID-19 vaccine tracker. 2021.

2. WHO. DRAFT landscape of COVID-19 candidate vaccines. April 2021 [cited]Available from: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

3. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J*, et al.* Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* 2021, **592**(7854)**:** 438-443.

4. PHE. Investigation of novel SARS-COV-2 variant. 2020.

5. Garcia-Beltran WF, Lam EC, St Denis K, Nitido AD, Garcia ZH, Hauser BM*, et al.* Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* 2021.

6. Zhou D, Dejnirattisai W, Supasa P, Liu C, Mentzer AJ, Ginn HM*, et al.* Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* 2021.

7. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B*, et al.* SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat Med* 2021, **27**(4)**:** 622-625.

8. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L*, et al.* Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant. *N Engl J Med* 2021.

9. Dejnirattisai W, Zhou D, Supasa P, Liu C, Mentzer AJ, Ginn HM*, et al.* Antibody evasion by the Brazilian P.1 strain of SARS-CoV-2. *bioRxiv* 2021**:** 2021.2003.2012.435194.

10. Rauch S, Jasny E, Schmidt KE, Petsch B. New Vaccine Technologies to Combat Outbreak Situations. *Front Immunol* 2018, **9:** 1963.

11. Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP*, et al.* Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. *N Engl J Med* 2020, **383**(16)**:** 1544-1555.

12. Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M*, et al.* A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates. *bioRxiv* 2020**:** 2020.2009.2008.280818.

13. Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M*, et al.* Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. *N Engl J Med* 2020.

14. Kalnin KV, Plitnik T, Kishko M, Zhang J, Zhang D, Beauvais A*, et al.* Immunogenicity and efficacy of mRNA COVID-19 vaccine MRT5500 in preclinical animal models. *NPJ Vaccines* 2021, **6**(1)**:** 61.

15. Hoerr I, Obst R, Rammensee HG, Jung G. In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. *Eur J Immunol* 2000, **30**(1)**:** 1-7.

16. Fotin-Mleczek M, Zanzinger K, Heidenreich R, Lorenz C, Thess A, Duchardt KM*, et al.* Highly potent mRNA based cancer vaccines represent an attractive platform for combination therapies supporting an improved therapeutic effect. *J Gene Med* 2012, **14**(6)**:** 428-439.

17. Fotin-Mleczek M, Duchardt KM, Lorenz C, Pfeiffer R, Ojkic-Zrna S, Probst J*, et al.* Messenger RNA-based vaccines with dual activity induce balanced TLR-7 dependent adaptive immune responses and provide antitumor activity. *J Immunother* 2011, **34**(1)**:** 1-15.

18. Kubler H, Scheel B, Gnad-Vogt U, Miller K, Schultze-Seemann W, Vom Dorp F*, et al.* Self-adjuvanted mRNA vaccination in advanced prostate cancer patients: a first-in-man phase I/IIa study. *J Immunother Cancer* 2015, **3:** 26.

19. Lutz J, Lazzaro S, Habbeddine M, Schmidt KE, Baumhof P, Mui BL*, et al.* Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. *NPJ Vaccines* 2017, **2:** 29.

20. Stitz L, Vogel A, Schnee M, Voss D, Rauch S, Mutzke T*, et al.* A thermostable messenger RNA based vaccine against rabies. *PLoS Negl Trop Dis* 2017, **11**(12)**:** e0006108.

21. Schnee M, Vogel AB, Voss D, Petsch B, Baumhof P, Kramps T*, et al.* An mRNA Vaccine Encoding Rabies Virus Glycoprotein Induces Protection against Lethal Infection in Mice and Correlates of Protection in Adult and Newborn Pigs. *PLoS Negl Trop Dis* 2016, **10**(6)**:** e0004746.

22. Petsch B, Schnee M, Vogel AB, Lange E, Hoffmann B, Voss D*, et al.* Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection. *Nat Biotechnol* 2012, **30**(12)**:** 1210-1216.

23. Aldrich C L-RI, Huang KB, et al. First proof-of-concept of a low-dose unmodified mRNA-based rabies vaccine formulated with lipid nanoparticles in human volunteers: a phase 1 trial. *submitted for publication* 2020.

24. Rauch S, Roth N, Schwendt K, Fotin-Mleczek M, Mueller SO, Petsch B. mRNA-based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus-neutralising antibodies and mediates protection in rodents. *NPJ Vaccines* 2021, **6**(1)**:** 57.

25. Rauch S, Gooch K, Hall Y, Salguero FJ, Dennis MJ, Gleeson FV*, et al.* mRNA vaccine CVnCoV protects non-human primates from SARS-CoV-2 challenge infection. *bioRxiv* 2020**:** 2020.2012.2023.424138.

26. Kremsner P, Mann P, Bosch J, Fendel R, Gabor JJ, Kreidenweiss A*, et al.* Phase 1 Assessment of the Safety and Immunogenicity of an mRNA- Lipid Nanoparticle Vaccine Candidate Against SARS-CoV-2 in Human Volunteers. *medRxiv* 2020**:** 2020.2011.2009.20228551.

27. Di Caro A, F C, N P, Nj B, O E, E P*, et al.* SARS-CoV-2 escape mutants and protective immunity from natural infections or immunizations. *Clin Microbiol Infect* 2021.

28. Pallesen J, Wang N, Corbett KS, Wrapp D, Kirchdoerfer RN, Turner HL*, et al.* Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc Natl Acad Sci U S A* 2017, **114**(35)**:** E7348-E7357.

29. Kirchdoerfer RN, Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA*, et al.* Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Sci Rep* 2018, **8**(1)**:** 15701.

30. Wu K, Werner AP, Moliva JI, Koch M, Choi A, Stewart-Jones GBE*, et al.* mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. *bioRxiv* 2021**:** 2021.2001.2025.427948.

31. Tada T, Dcosta BM, Samanovic-Golden M, Herati RS, Cornelius A, Mulligan MJ*, et al.* Neutralization of viruses with European, South African, and United States SARS-CoV-2 variant spike proteins by convalescent sera and BNT162b2 mRNA vaccine-elicited antibodies. *bioRxiv* 2021**:** 2021.2002.2005.430003.

32. Muik A, Wallisch A-K, Sänger B, Swanson KA, Mühl J, Chen W*, et al.* Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *bioRxiv* 2021**:** 2021.2001.2018.426984.

Figures

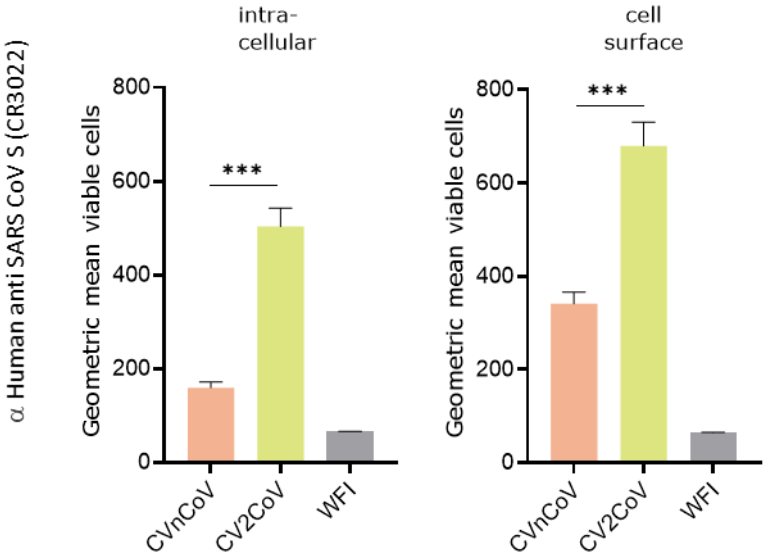

Figure 1: CV2CoV mRNA enhances protein expression. Flow cytometric analysis using an S-specific antibody either with or without membrane permeabilisation allowing detection of intracellular or cell surface bound S protein. Geometric mean fluorescence intensity (GMFI) of transfected HeLa cells are expressed as mean + standard deviation (SD) of duplicate samples of two independent experiments. T-test was used to compare groups.

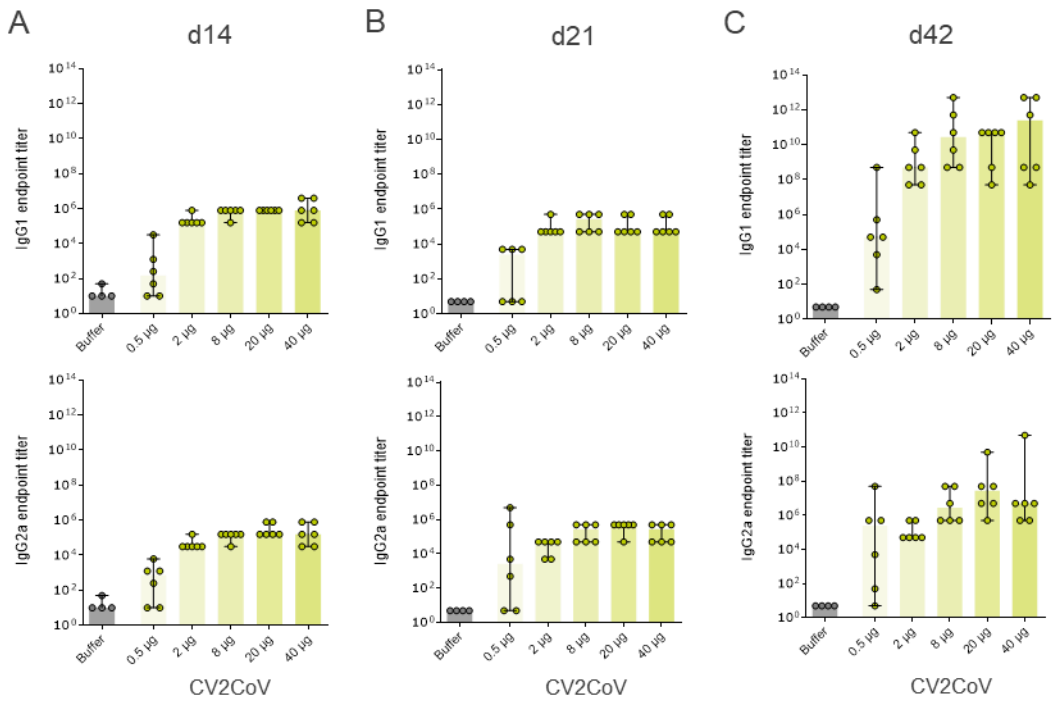

Figure 2: CV2CoV triggers high levels of binding antibody responses in rats. Female and male Wistar rats (n=6/group) were vaccinated IM on day 0 and d21 with five different doses ranging from 0.5 µg – 40 µg of CV2CoV. Wistar rats (n=4) vaccinated with 0.9% NaCl (Buffer) on day 0 and day 21 served as negative control. S_{RBD} protein specific binding antibodies, displayed as endpoint titres for IgG1 and IgG2a in serum upon one vaccination ((A) day 14 and (B) day 21) or two vaccinations ((C) day 42). Each dot represents an individual animal, bars depict the median.

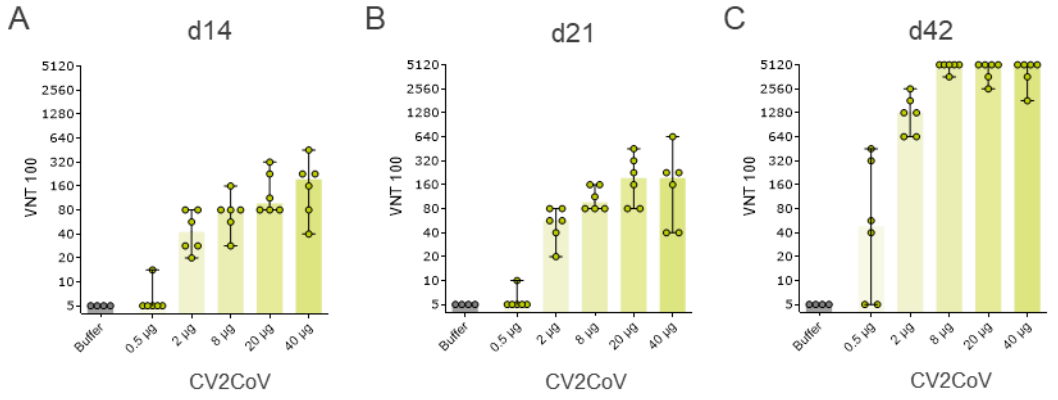

Figure 3: CV2CoV induces high titres of virus neutralising antibodies (VNT) against SARS-CoV-2 in rats. Female and male Wistar rats (n=6/group) were vaccinated IM with five different doses ranging from 0.5 µg – 40 µg of CV2CoV on day 0 and day 21. Animals (n=4) vaccinated with NaCl (Buffer) served as negative controls. SARS-CoV-2 100% neutralization titres (VNT100) in serum samples taken on day 14, day 21 and day 42 were analysed. Highest dilution step was 1:5120. Each dot represents an individual animal, bars depict the median.

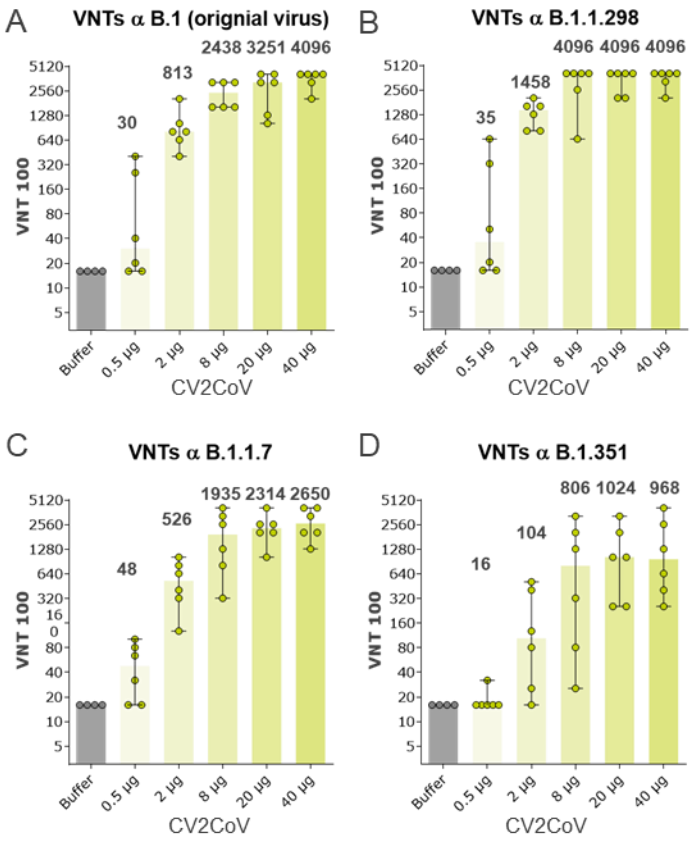

Figure 4: CV2CoV induces high titres of cross neutralising antibodies (VNT) against SARS-CoV-2 variants in rats. Female and male Wistar rats (n=6/group) were vaccinated IM with five different doses ranging from 0.5 µg – 40 µg of CV2CoV on day 0 and day 21. Animals (n=4) vaccinated with NaCl (Buffer) served as negative controls. SARS-CoV-2 100% neutralization titres (VNT100) in serum samples taken on day 42 against distinct SARS-CoV-2 variants were analysed as indicated. Highest dilution step was 1:4096. Each dot represents an individual animal, bars depict the median.