Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Wu K, Werner AP, Koch M, et al. Serum neutralizing activity elicited by mRNA-1273 vaccine. N Engl J Med. DOI: 10.1056/NEJMc2102179

SUPPLEMENTAL APPENDIX

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Methods

Animal Studies

Experiments in animals were performed in compliance with National Institutes of Health (NIH) regulations and with approval from the Animal Care and Use Committee of the Vaccine Research Center. Female and male Indian-origin rhesus macaques (12 of each sex; age range, 3 to 6 years) were sorted according to sex, age, and weight, and then stratified into groups. Animals were vaccinated intramuscularly at week 0 and at week 4 with either 30 or 100 µg of mRNA-1273 in 1 ml of 1× phosphate-buffered saline (PBS) into the right hind leg. At week 8 (4 weeks after the second vaccination), sera were collected for immunoassay analyses.

Clinical Trial

Humans were immunized with 100 µg mRNA-1273 on a prime-boost schedule and sera was collected 7 days post-boost (day 36). Study protocols and results are reported previously[1].

Lentiviral-based Pseudovirus Neutralization

To produce SARS-CoV-2 pseudotyped lentivirus, a codon-optimized CMV/R-SARS-CoV-2 S (Wuhan-1, GenBank: MN908947.3) plasmid was constructed and subsequently modified via site-directed mutagenesis to contain the D614G mutation. Additional spike mutations were implemented into the D614G backbone (i.e. N501Y, E484K, N439K, and other combinations akin to those of the B.1.1.7 and B.1.351 variants). Pseudoviruses were produced by the co-transfection of plasmids encoding a luciferase reporter, lentivirus backbone, and the SARS-CoV-2 S genes into HEK293T/17 cells (ATCC CRL-11268), as previously described[2]. Additionally, a human transmembrane protease serine 2 (TMPRSS2) plasmid was co-transfected to produce pseudovirus[3]. Neutralizing antibody responses in sera were assessed by PsVN assay, with slight modifications to what was previously-described[1]. Briefly, heat-inactivated serum was serially diluted 4-fold 8x in duplicate, then mixed with appropriately titrated pseudoviruses and incubated at 37°C and 5% CO2 for roughly 45 minutes. 293T-hACE2.mF cells were diluted to a

concentration of 7.5 x 10^4 cells/mL and suspended in DMEM (Gibco) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin and immediately added to the serum-pseudovirus mixtures[4]. Seventy-two hours later, cells were lysed and luciferase activity (in relative light units (RLU)) was measured. Percent neutralization was normalized considering uninfected cells as 100% neutralization and cells infected with pseudovirus alone as 0% neutralization. IC_{50} titers were determined using a log(agonist) vs. normalized-response (variable slope) nonlinear regression model in Prism v8 (GraphPad).

Recombinant VSV-based Pseudovirus Neutralization

Codon-optimized full-length spike protein of the original Wuhan isolate (D614), D614G, or the indicated spike variants listed in Tables 1 and 2 were cloned into pCAGGS vector. To make SARS-CoV-2 full-length spike pseudotyped recombinant VSV-ΔG-firefly luciferase virus, BHK-21/WI-2 cells (Kerafast, EH1011) were transfected with the spike expression plasmid and subsequently infected with VSVΔG-firefly-luciferase as previously described (Whitt, 2010). For neutralization assay, serially diluted serum samples were mixed with pseudovirus and incubated at 37 Celsius for 45 minutes. The virus-serum mix was subsequently used to infect A549-hACE2-TMPRSS2 cells for 18 hr at 37 Celsius before adding ONE-Glo reagent (Promega E6120) for measurement of luciferase signal (relative luminescence unit; RLU). The percentage of neutralization is calculated based on RLU of the virus only control, and subsequently analyzed using four-parameter logistic curve (Prism 8).

Statistical Analysis

Wilcoxon matched-pairs signed rank test was used for the statistical analysis.

Figures

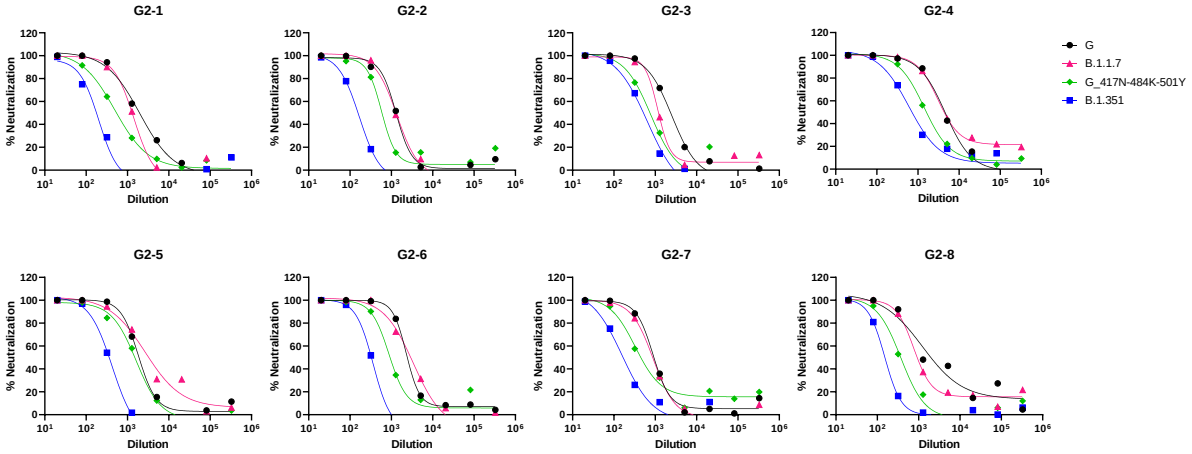

Figure S1. Neutralization curves of human sera in the VSV-based pseudovirus neutralization assay. Phase 1 participants were immunized with 100 µg mRNA-1273 on a prime-boost schedule, and sera were collected 7 days post-boost. Neutralization was measured against recombinant VSV-based pseudovirus incorporating the B.1.351 variant full-length spike protein. Each graph represents an individual participant, as indicated. Each data point is an average from three replicate wells.

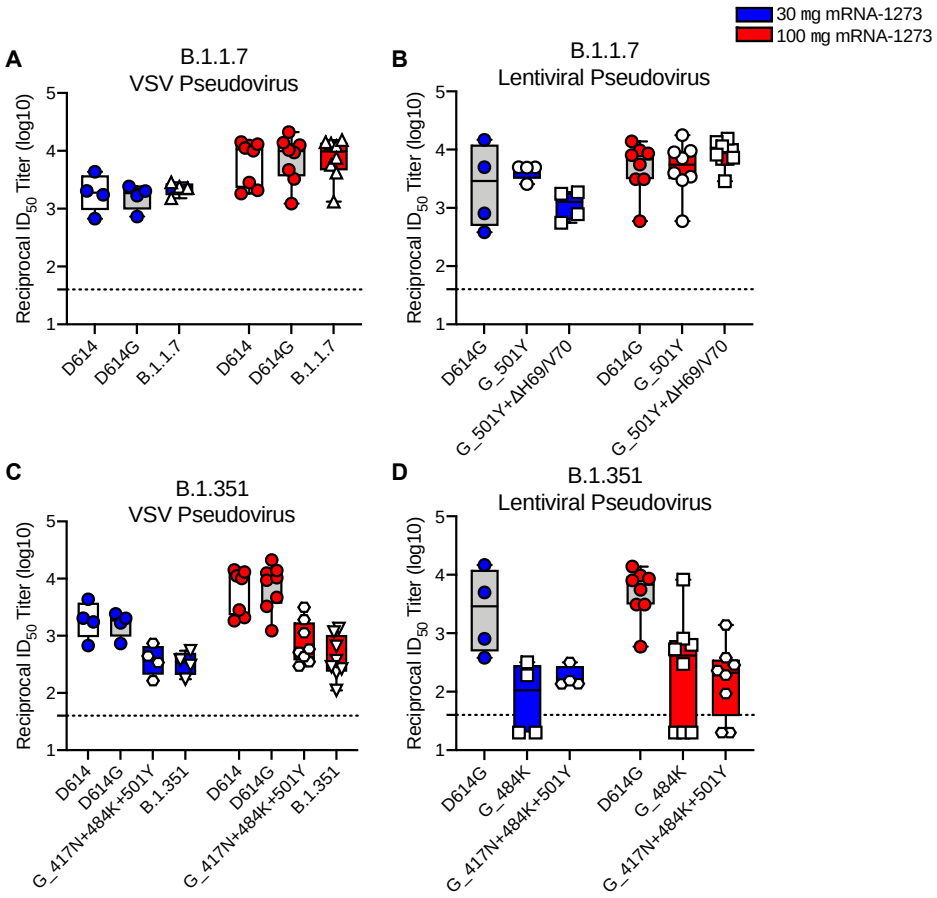

Figure S2. Neutralization of B.1.1.7 and B.1.351 SARS-CoV-2 pseudoviruses by serum from mRNA-1273-immunized NHPs. Rhesus macaques (NHPs) were immunized with 30 (blue) or 100 µg (red) mRNA-1273 on a prime-boost schedule, and sera were collected 4 weeks post-boost. Neutralization was measured by recombinant VSV-based pseudovirus neutralization assay (A, C) or lentiviral pseudovirus neutralization assay (B, D). The assays incorporated full-length Spike protein of the original D614, D614G, or the indicated Spike variants present in the B.1.1.7 variant (A, B) or B.1.351 variant (C, D). Min to max box plots, with the box from 25-75% and the median value denoted by the line. The horizonal dotted lines indicate the lower limit of quantification (LLOQ). G=D614G

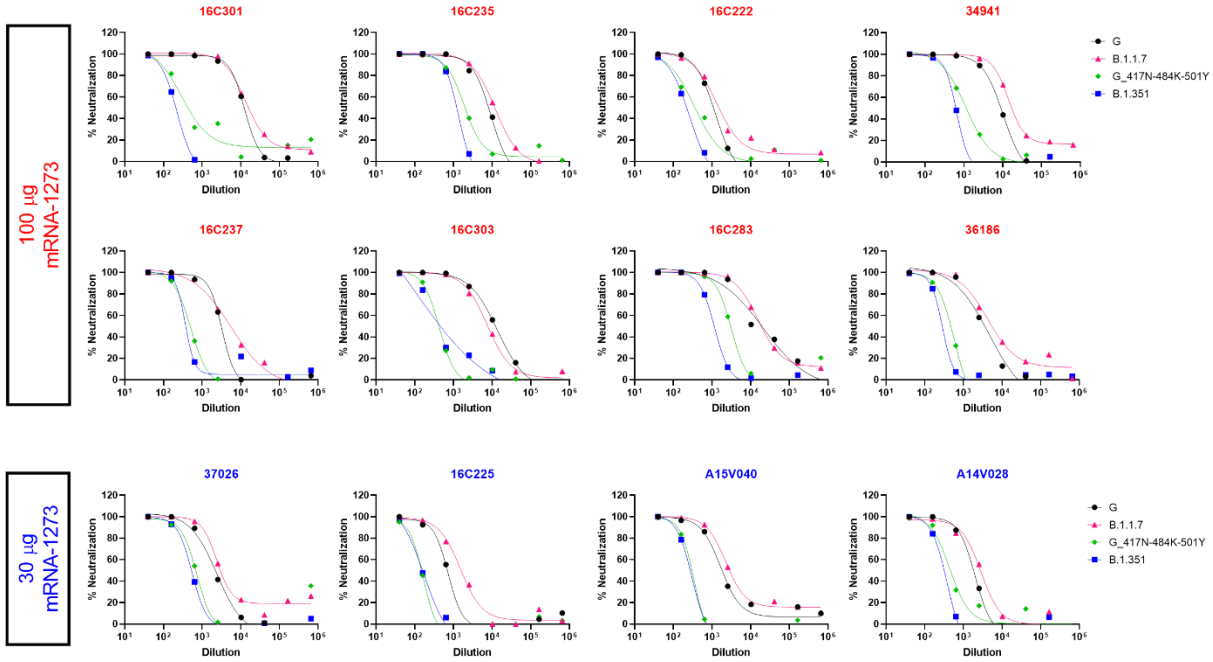

Figure S3. Neutralization curves of NHP sera in the VSV-based pseudovirus neutralization assay. Rhesus macaques (NHPs) were immunized with 30 (blue animal ID) or 100 µg (red animal ID) mRNA-1273 on a prime-boost schedule, and sera were collected 4 weeks post-boost. Neutralization was measured against recombinant VSV-based pseudovirus incorporating the B.1.351 variant full-length spike protein. Each graph represents an individual animal as indicated by identification code. Each data point is an average from three replicate wells.

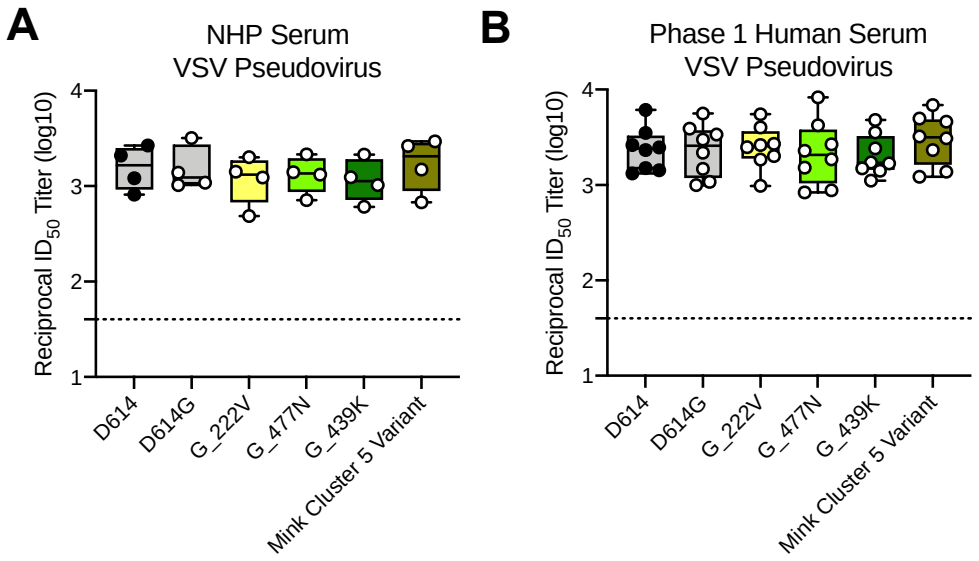

Figure S4. Ability of mRNA-1273 immune sera from NHPs and humans to neutralize SARS-CoV-2 pseudoviruses representing early variants. (A) Rhesus macaques (NHPs) were immunized with 30 µg mRNA-1273 on a prime-boost schedule, and sera were collected 4 weeks post--boost. (B) Phase 1 trial participants were immunized with 100 µg mRNA-1273 on a prime-boost schedule, and sera were collected 1 week post-boost. Neutralization was measured by a recombinant VSV-based SARS-CoV-2 pseudovirus neutralization assay incorporating full-length spike protein of the Wuhan isolate (D614) or the indicated spike variants (D614G, A222V-D614G, S477N-D614G, N439K-D614G, mink cluster 5 variant). Min to max box plots, with the box from 25-75% and the median value denoted by the line. The horizonal dotted lines indicate the lower limit of quantification (LLOQ=40). G=D614G.

Table

Variant Name	Amino Acid Changes in Spike
20E (EU1)	A222V-D614G
20A.EU2	S477N-D614G
N439K-D614G	N439K-D614G
Mink Cluster 5 Variant	ΔH69ΔV70-Y453F-D614G-I692V-M1229I
B.1.1.7 (a.k.a., 20I/501Y.V1, VOC 202012/01)	ΔH69ΔV70-ΔY144-N501Y-A570D-D614G-P681H-T716I-S982A-D1118H
B.1.351 (a.k.a., 20H/501Y.V2)	L18F-D80A-D215G-ΔL242ΔA243ΔL244-R246I-K417N-E484K-N501Y-D614G-A701V
P.1 (a.k.a., 20J/501Y.V3)	L18F-T20N-P26S-D138Y-R190S-K417T-E484K-N501Y-D614G-H655Y-T1027I-V1176F
B.1.427/B.1.429 (a.k.a., 452R.V1, CAL.20C)	Version 1 (v1): S13I-W152C-L452R-D614G
	Version 2 (v2): S13I-P26S-W152C-L452R-D614G
B.1.1.7 + E484K	ΔH69ΔV70-ΔY144-E484K-N501Y-A570D-D614G-P681H-T716I-S982A-D1118H

Table S1. Spike mutations in SARS-CoV-2 variants evaluated in this study.

Contributions

Conceptualization, K.W., A.P.W., B.S.G., A.C., K.S.C., R.A.S, and D.K.E.; methodology, K.W., A.P.W., S.B.B, W.S., J.M., N.J.S., and K.S.C.; formal & statistical analysis, K.W., A.P.W., and K.S.C.; writing—original draft preparation, R.A.S. and D.K.E; writing—review and editing, K.W., A.P.W., G.S.J., H.B., N.J.S., B.S.G., A.C., K.S.C., R.A.S., and D.K.E. All authors have read and agreed to the published version of the manuscript.

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Disclosures

K. Wu, G. Stewart-Jones, M. Koch, A. Choi, H. Bennett, A. Carfi, and D. Edwards are employed by ModernaTX, Inc. and hold equities from the company, B. S. Graham and K. S. Corbett are inventors on the following Patent Applications: EP Patent Application 17800655.7 filed 13 May 2019, entitled “Prefusion coronavirus spike proteins and their use”; US Patent Application 16/344,774 filed 24 April 2019 entitled “Prefusion coronavirus spike proteins and their use” [HHS Ref. No. E-234-2016-1-US-03]; US Provisional Patent Application 62/972,886 filed 11 February 2020 entitled “2019-nCoV Vaccine”.

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