Supplementary Appendix

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

Supplement to: Normark J, Vikström L, Gwon Y-D, et al. Heterologous ChAdOx1 nCoV-19 and mRNA-1273 vaccination. N Engl J Med. DOI: 10.1056/NEJMc2110716

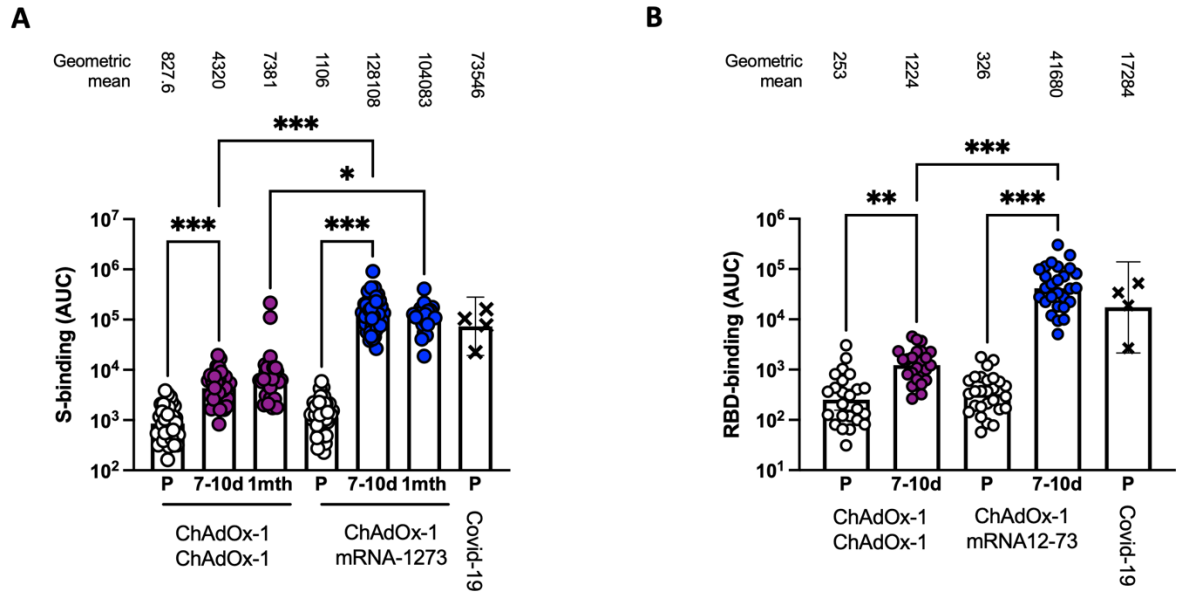
Supplemental information

Supplemental table 1

Characteristics	2 doses ChAdOx-1 (n=37)	1 dose ChAdOx-1 + 1 dose mRNA-1273 (n=51)
Age, median (range)	46 (28-62)	40 (23-59)
Sex no. (%)		
Male	8 (21.6)	12 (23.5)
Female	29 (78.4)	39 (76.5)
BMI, median (IQR)	25.7 (23.6-28.2)	22.9 (21.4-26.1)
Previous COVID-19 infection, n (%)	2 (5.4)*	3 (5.9)*
Comorbidities, n (%)		
Cardiovascular disease	2 (5.4)	0
Ischemic heart disease	1 (2.7)	0
Diabetes Mellitus type II	1 (2.7)	0
Hypertension	6 (16.2)	1 (2)
Asthma	3 (8.1)	7 (13.7)
Rheumatic disease	0	1 (2)
Autoimmune disease	1 (2.7)	1 (2)
Allergy	7 (18.9)	4 (7.8)
Days between doses, median (IQR)	70 (67-76)	84 (82-86)

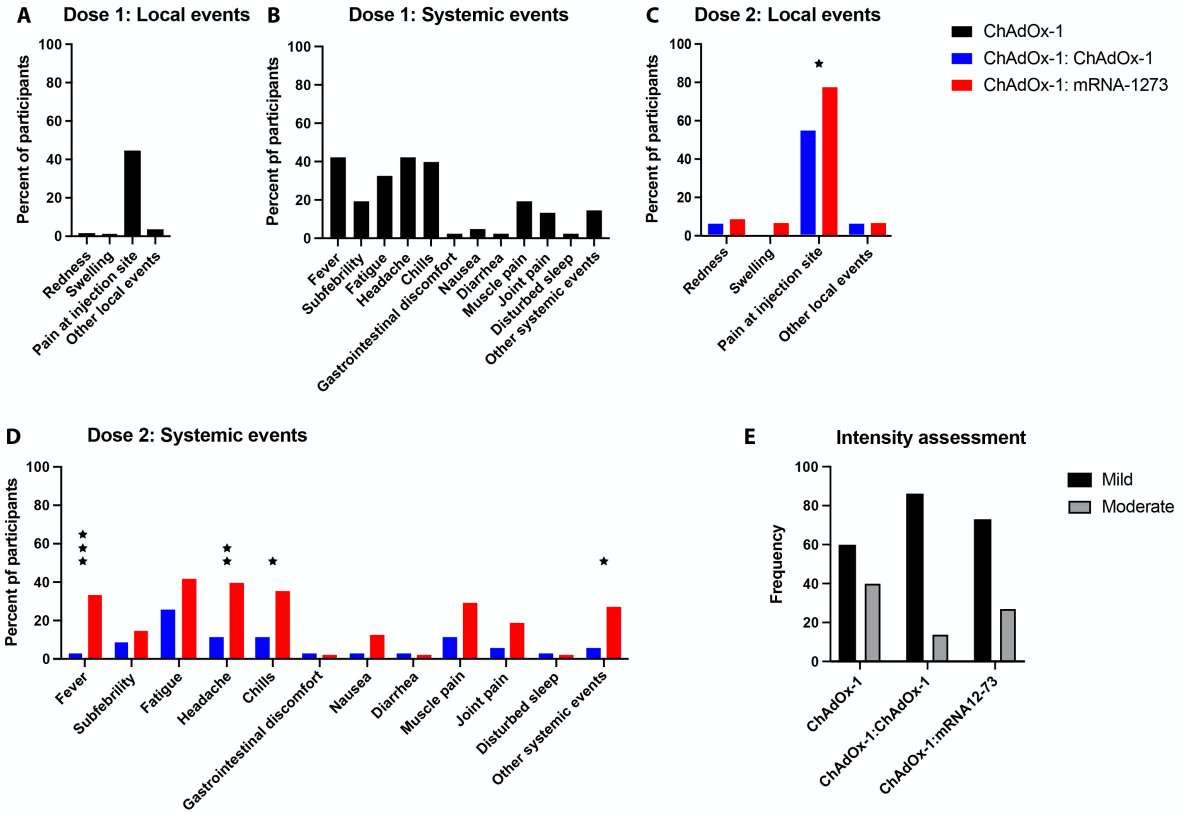
Characteristics of participants. Shown is age, sex distribution, BMI, Covid-19 status, frequency of comorbidities and time between 1st and 2nd dose.

** 5 individuals had reported a historical positive Covid-19-test. Of these, we included 4 as a control group for serological analysis. The remaining individual had not responded on vaccination as previously reported for Covid-19-positive individuals.[1] Since we could not rule out that this individual had received a false positive Covid-19 test, this individual was fully excluded from this study.*



Supplemental figure 1. SARS-CoV-2 S-specific IgG on the day of boost (P), 7-10 days later and 1 month later (panel A). Individual IgG levels were calculated and shown as Area Under the Curve (AUC) pre-boost (open circles) or after a ChAdOx-1 (purple circles) or an mRNA-1273 boost (blue circles). S-specific IgG on day of boost and 7-10 days post boost are shown for 34 individuals that received a ChAdOx-1 boost and 48 individuals that received mRNA-1273 as boost. The 1-month time-point is shown for 28 and 20 individuals from the ChAdOx-1 or mRNA-1273 boost, respectively. RBD-specific IgG on the day of boost and 7-10 days post boost (panel B). RBD-specific IgG is shown for 24 and 27 individuals before and after ChAdOx-1 or mRNA-1273 boost, respectively. As a reference, we included serum responses to S- and RBD- from 4 individuals that were Covid-19-experienced and had received 1 dose of ChAdOx-1 9-12 weeks prior to sampling (crosses).

Supplemental figure 2 – reactogenicity



Supplemental figure 2. Summary of local and systemic adverse events reported 65-87 days after one dose of ChAdOx-1 (Panels A and B) and 7-10 days after a boost with ChAdOx-1 (blue) or mRNA-1273 (red) (Panels C and D). Panel E shows the frequency (percentage) of mild (black) and moderate (gray) adverse events reported 65-87 days after dose 1 of ChAdOx-1 and 7-10 days after boost of either ChAdOx-1 or mRNA-1273. No statistically significant difference (Fisher exact test, *p=0,051*) was detected between the distribution of mild and moderate adverse events after the different boost regimens.

Detailed materials and methods

Trial design and participants

By informed consent, we included 88 healthcare workers at the University Hospital of Northern Sweden in CoVacc, a 4 year follow-up study, to assess safety and immunogenicity of Covid-19 vaccines. All 88 participants had been included in the first two priority groups for Covid-19 vaccination in Sweden, had received one dose of ChAdOx-1 9 to 12 weeks earlier (65-87 days) and were about to receive a second vaccine dose within the regular Swedish vaccination program. Due to the risk of thrombotic thrombocytopenia after ChAdOx1[2], Sweden and several other European countries had changed their recommendations and persons < 65 years of age were offered a mRNA vaccin as booster. Of the 88 health workers, 37 chose to receive ChAdOx-1 while 51 chose to receive mRNA-1273 as their second dose (supplemental Table 1). Five of the 88 had recorded a positive SARS-CoV-2 test prior to vaccine dose 1 and were excluded from assessment of adverse events. Of these, 4 had strong serological responses prior to the booster immunization. This is consistent with previous reports on vaccination of Covid-19 exposed individuals[1]. We included these 4 as a separate positive control group for analysis of serum. The remaining Covid-19 positive individual had not responded differently to vaccination than naïve individuals. This suggested a possibility that the participant may have had a false positive test result during the initial months of the pandemic. Due to this uncertainty, the individual was excluded from analysis of both adverse events and serology. Of the remaining 83, 35 received ChAdOx-1 while 48 received mRNA-1273 as their second dose. Age, sex, BMI and comorbidities of the cohort are shown in supplemental Table 1. All individuals were bled on the day of the booster injection and then again 7-10 days and 1 month after the booster injection of either ChAdOx-1 or mRNA-1273 (median 8 days). This allowed us to specifically assess the potential of mRNA-1273 to boost

immunological memory that had been generated by the ChAdOx-1 prime. For neutralization studies, we also added 4 samples from individuals with prior Covid-19, that had received 1 dose of ChAdOx-1 (n=4) 9-12 weeks previously.

Study oversight and ethical permits

The clinical trial, CoVacc - Immune response to vaccination against Covid-19, an open multicenter phase IV study, was approved by the Swedish Ethics Review Authority (Dnr 2021-00055) and the Medical Products Agency Sweden. The study was registered at European Clinical Trials Database (EUDRACT Number 2021-000683-30) before the first patient was enrolled in the study. Umeå University, Sweden served as trial sponsor and the Clinical Research Center, University Hospital of Northern Sweden was monitoring the study for regulatory compliance. All individuals were included after informed consent and data were stored in accordance with the EU General Data Protection Regulation.

Safety profile

Study participants were at the time of inclusion asked to describe all adverse events experienced after the first injection with ChAdOx-1, and again 7-10 days after inclusion in regard to the booster injection with ChAdOx-1 or mRNA-1273. Adverse events were then classified according to a recent publication[3], with minor modifications. Briefly, the intensity of adverse events was classified by the principal investigator (JN) according to the study protocol as mild, moderate, or severe. Mild adverse events are relatively tolerable and transient in nature but do not affect the subject's normal life. Moderate adverse events cause deterioration of function but does not affect health. The event can be sufficiently

unpleasant and interferes with normal activities but does not completely obstruct them. Severe adverse events cause deterioration of function or work ability or pose a health risk to the subject. There were no severe adverse events identified in this cohort.

Antibody responses

Specific vaccine-induced IgG-responses to the spike (S) protein of SARS-CoV-2 were analyzed by standard ELISA. In brief, 200 ng of produced and purified S-2P or receptor-binding domain RBD protein in PBS were coated to clear flat-bottom Immuno Maxisorp 96-well plates (Thermo Scientific, Waltham, MA, USA) with 200 ng/well and incubated at +4°C overnight, as previously described.[4] Plates were washed one time with 300 µl wash buffer (1xPBS with 0.05% Tween) and incubated with 200 µl blocking buffer (1% non-fat dry-milk in wash buffer) per well for 1 hour at room temperature (RT). After blocking, serum samples and control serum were diluted 1/50 in blocking buffer and seven additional 5-fold serial dilutions and added to plate, incubated at +4°C overnight. Plates were washed 4 times with washing buffer and then incubated with goat-anti human IgG alkaline phosphate conjugated antibody (#A18814, Thermo Scientific) diluted 1/8,000 in blocking buffer for 1 hour at +37°C and then washed 4 times in washing buffer. Additional 100 µl of Phosphatase Substrate 1mg/ml (#S0942, Sigma-Aldrich) dissolved in diethanolamine buffer were added to each well and incubated for 30 min at +37°C. The colorimetric reaction was sopped with 50 µl 3 M NaOH per well and absorbance was measured spectrophotometrically at 405 nm with microplate reader (Tecan Sunrise). For each sample, the Area Under Curve was calculated using a threshold of 0.15 (GraphPad Prism 8.0, San Diego, CA, USA).

Neutralization assays

Authentic SARS-CoV-2 wild-type strain (SARS-CoV-2/01/human/2020/SWE accession no/GeneBank no MT093571.1, provided by the Public Health Agency of Sweden) and B1.351 were grown on VeroE6 cells under BSL3 conditions at the Department of Clinical Microbiology, Umeå University or at Karolinska Institutet, Stockholm, Sweden. For the fluorescent-based neutralization assay, Vero E6 cells (10^4 cells/well) were seeded one day prior to infection in 96 well plates (Greiner CELLSTAR®, Greiner Bio-One, Austria). Then, 50 µl of fourfold serial-diluted serum was pre-incubated with 50 µl of 10,000 plaque-forming units (PFU) per ml of SARS-CoV-2 in serum-free Dulbecco's Modified Eagles Medium (DMEM) + 0.2% penicillin/streptomycin for 30 min at 37 °C. The virus–serum mixtures were added to Vero E6 cells. Eight hours post-infection, the cells were fixed for 40 minutes in 4% formaldehyde. Plates were washed with phosphate buffered saline pH 7.4 (PBS), and then permeabilized with 0.5% Triton X-100 in PBS and 20mM glycine for 10 min at RT, and blocked with PBS + 2% bovine serum albumin for 30 min at RT. Virus-infected cells were stained for 1h with anti-SARS-CoV-2 nucleocapsid protein rabbit monoclonal antibody (Sino Biological, Cat.No. 40143-R001, Wayne, PA, USA) diluted 1:2500 in blocking buffer. Then, a secondary goat anti-rabbit IgG (H+L) Alexa Fluor 488 antibody (Thermo Fisher, Waltham, MA, USA) diluted 1:2500 in blocking buffer was added, incubated for 30 min and then stained with DAPI (0.1 ug/mL in PBS) for 5 min. Fluorescence was quantified using a TROPHOS Plate RUNNER HD® (TROPHOS SA, Marseille, France). The reciprocal half-maximal inhibitory dilution for serum (ID50) was determined using 4-parameter nonlinear regression (GraphPad Prism 8.0, San Diego, CA, USA)

Microneutralization based on Cytopathic Effect (CPE) was performed as previously described.[5] Briefly, heat-inactivated and 3-fold serially diluted serum in Eagle's MEM (Life Technologies) supplemented with 5% FCS (Thermo Fisher Scientific) was mixed with an equal volume of 4,000 50% tissue culture infection dose/ml SARS-CoV-2 (60 µl serum plus 60 µl virus). After 1 h incubation at 37°C / 5% CO2, the mixtures were added to confluent Vero E6 cells seeded on 96-well plates. After 4 days incubation at 37°C / 5% CO2, the cells were inspected for signs of CPE by optical microscopy. Each well was scored as either neutralizing (if <50% of the cell layer showed signs of CPE) or non-neutralizing (if ≥50% CPE was observed). Results are shown as the arithmetic mean of the reciprocals of the highest neutralizing dilutions from the two duplicates for each sample.

Statistical analysis

All individuals that had received one dose of ChAdOx-1 or mRNA-1273 as booster injection were included in statistical analysis for adverse events, except participants previously diagnosed with Covid-19. Statistical analysis of adverse events was carried out with jamovi V. 1.6 (www.jamovi.org). Fischer's exact test was used for group comparison of binomial data. No adjustment for multiplicity was done for safety outcomes. Data were reported as numbers observed (% of the total number of observations), mean with standard deviation (SD) or median with interquartile range (IQR). Statistical significance was set at $p<0.05$. Distribution of longitudinal binding and neutralization data from was defined as non-gaussian by normality and lognormality test (GraphPad Prism 8.0, San Diego, CA, USA). Differences were then analyzed by non-parametric Kruskal-Wallis test ANOVA followed by Dunn's test for correction of multiple comparisons. Adjusted p-values are shown. For comparison of neutralization tests with VOC B.1.351 and SARS-CoV-2 (figure 1B), we instead used Mann-Whitney test. In this

case non-adjusted p-values are shown (GraphPad Prism 8.0, San Diego, CA, USA). Statistical differences are shown as * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

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